ANNUAL REVIEWS

Annual Review of Plant Biology

Nitrate Transport, Signaling, and Use Efficiency

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Annu. Rev. Plant Biol. 2018. 69:85–122

First published as a Review in Advance on March 23, 2018

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

https://doi.org/10.1146/annurev-arplant-042817-040056

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Keywords

nitrate transporter, nitrate signaling, nitrogen-use efficiency, NUE, transceptor, NRT1, NPF, NRT2

Abstract

Nitrogen accounts for approximately 60% of the fertilizer consumed each year; thus, it represents one of the major input costs for most nonlegume crops. Nitrate is one of the two major forms of nitrogen that plants acquire from the soil. Mechanistic insights into nitrate transport and signaling have enabled new strategies for enhancing nitrogen utilization efficiency, for lowering input costs for farming, and, more importantly, for alleviating environmental impacts (e.g., eutrophication and production of the greenhouse gas N_2O). Over the past decade, significant progress has been made in understanding how nitrate is acquired from the surroundings, how it is efficiently distributed into different plant tissues in response to environmental changes, how nitrate signaling is perceived and transmitted, and how shoot and root nitrogen status is communicated. Several key components of these processes have proven to be novel tools for enhancing nitrate- and nitrogen-use efficiency. In this review, we focus on the roles of NRT1 and NRT2 in nitrate uptake and nitrate allocation among different tissues; we describe the functions of the transceptor NRT1.1, transcription factors, and small signaling peptides in nitrate signaling and tissue communication; and we compile the new strategies for improving nitrogen-use efficiency.

Contents

1. INTRODUCTION

Nitrate and ammonium are two major nitrogen sources for plants. Under aerobic conditions, nitrate is the predominant form of nitrogen in the soil. To be incorporated into amino acids, nitrate has to be reduced and converted into nitrite by nitrate reductase in the cell cytosol, and nitrite is then reduced and converted into ammonium by nitrite reductase in plastids or chloroplasts. Both nitrite and excess ammonium are toxic to plants. Therefore, at the cellular level, these assimilation steps need to be well coordinated in response to nitrogen supply and nitrogen demand, as well as carbon status, which provides the scaffold for amino acid synthesis. Unlike ammonium, nitrate can be stored in large quantities in vacuoles and be retrieved when needed. Thus, in order to decide how much nitrate will be assimilated to support growth, how much nitrate will be stored in vacuoles for later use, and how much nitrate will be allocated to other tissue, plants must first effectively monitor levels of external nitrate supply, then respond to nitrogen demand from their different tissues, and finally, systematically communicate this information to activate the metabolic enzymes and transporters through transcription or posttranscriptional regulation. How do plants integrate multiple streams of internal and external information to regulate nitrate assimilation and nitrate translocation in different tissues to optimize plant growth without causing toxic effects and wasting resources? To answer this fascinating question, we need to characterize the individual components involved and then reassemble the pieces to reveal the entire machinery.

Molecular studies of nitrate assimilation were initiated as early as 1988 (19, 26). These studies have been intensively reviewed (25) and are not included in this article. Studies of nitrate transport at the molecular level began in 1993 (149). At first, most of these studies focused on nitrate uptake. Nevertheless, in the past decade, many studies have elucidated how nitrate is distributed among different tissues and how plant growth is affected by these translocations in response to environmental changes. Several textbooks emphasize that nitrate is mainly transported in xylem and that only organic nitrogen amino acids are transported in phloem. Nevertheless, studies of several nitrate transporters have shown that phloem nitrate transport plays an important role in regulating nitrate distribution and plant growth, so the textbooks need to be corrected.

Nitrate signaling at the molecular level is only a recent research avenue as the nitrate signaling components (e.g., receptors, kinases, transcription factors) were not identified until this decade (14, 55, 57), but great progress has been made in a short period of time. With increasing numbers of transporters and signaling components being identified and their functional and regulatory mechanisms elucidated, we can start to understand the strategies plants have evolved to make the best use of the nitrate available to them. This information can contribute to new approaches for enhancing nitrogen utilization efficiency by transgenics.

2. NITRATE TRANSPORT

Transmembrane proteins are required for absorption of nitrate from the external environment and for its transportation and translocation among cells, tissues, and organs. Four protein families including NITRATE TRANSPORTER 1 (NRT1)/PEPTIDE TRANSPORTER (PTR) family (NPF), NRT2, CHLORIDE CHANNEL (CLC) family, and SLOWLY ACTIVATING ANION CHANNEL are involved in nitrate transport. This review focuses on current studies of NPF and NRT2 transporters in *Arabidopsis thaliana* and rice (*Oryza sativa*) (see **Table 1**).

NRT1: NITRATE TRANSPORTER 1 family; subsequently known as NPF

NPF: NITRATE TRANSPORTER 1/PEPTIDE TRANSPORTER (NRT1/PTR) family

NRT2: NITRATE TRANSPORTER 2 family

2.1. Physiological Functions of NPF Transporters

Most NPF transporters display low nitrate affinities, but CHL1/NRT1.1/AtNPF6.3 and OsNRT1.1B/OsNPF6.5 display dual affinities. There are 53 and 93 NPF genes in *Arabidopsis*

Table 1 Summary of identified NPF transporters in *Arabidopsis* and rice, their substrates, and their functions in planta

Clade	New name	Locus	Old name	Spatial locations of gene expression	In vitro substrates (system)	Function in planta	Reference(s)
Arabidopsis thaliana							
1	AtNPF1.1	*At3G16180*	NRT1.12	Companion cells of the major veins in expanded leaves	NO_3^- (oocyte) ABA, GA, JA-Ile (yeast)	Nitrate allocation to young leaves	21, 56
	AtNPF1.2	*At1G52190*	NRT1.11	Companion cells of the major veins in expanded leaves	NO_3^- (oocyte) GA, JA-Ile (yeast)	Nitrate allocation to young leaves	21, 56
2	AtNPF2.3	*At3G45680*	NA	Root pericycle	NO_3^- (liposome) GA (yeast)	Root-to-shoot nitrate transport under salinity	21, 147
	AtNPF2.4	*At3G45700*	NA	Root stele	Cl^- (oocyte) GA, JA-Ile (yeast)	Xylem loading of chloride under salinity	21, 82
	AtNPF2.5	*At3G45710*	NA	Root cortex	Cl^- (oocyte, yeast) ABA, GA (yeast)	Chloride efflux from the root	21, 83
	AtNPF2.6	*At3G45660*	NA	ND	GA, JA-Ile (yeast)	ND	21
	AtNPF2.7	*At3G45650*	NAXT1	Root cortex	NO_3^- (liposome) GA, JA-Ile (yeast)	Nitrate efflux in roots under acidic conditions	21, 135
	AtNPF2.9	*At1G18880*	NRT1.9	Companion cells of root phloem	NO_3^-, GLS (oocyte)	Phloem loading of nitrate in root	105, 156
	AtNPF2.10	*At3G47960*	GTR1	Anthers and filaments, mesophyll cells, lateral root branching points	GLS, NO_3^-, GA, JA-Ile (oocyte) GA, JA-Ile (yeast)	GLS translocation to seeds JA-Ile translocation to undamaged leaf	3, 21, 63, 105, 127

(*Continued*)

Table 1 (*Continued*)

Clade	New name	Locus	Old name	Spatial locations of gene expression	In vitro substrates (system)	Function in planta	Reference(s)
	AtNPF2.11	*At5G62680*	GTR2	Major veins of leaves, lateral root branching points	GLS, NO_3^-, GA (oocyte)	GLS translocation to seeds	3, 105, 127
	AtNPF2.12	*At1G27080*	NRT1.6	Vascular tissues of the silique and funiculus	NO_3^- (oocyte) GA (yeast)	Nitrate transport to embryo	1, 21
	AtNPF2.13	*At1G69870*	NRT1.7	Phloem of the minor veins in the old leaves	NO_3^-, GLS (oocyte) GA, JA-Ile (yeast)	Nitrate remobilization from old to young leaves	21, 33, 105
	AtNPF2.14	*At1G69860*	NA	ND	GLS (oocyte)	ND	105
3	AtNPF3.1	*At1G68570*	Nitr	Minor veins, hypocotyl, junction of anther and filament, root endodermis	NO_3^-, NO_2^-, GA (oocyte) GA, and JA-Ile (yeast)	Nitrite accumulation in leaves GA accumulation and responses	21, 25, 119, 141, 145
4	AtNPF4.1	*At3G25260*	AIT3	ND	ABA, GA, JA-Ile (yeast) GA (oocyte)	ND	21, 64, 127
	AtNPF4.2	*At3G25280*	AIT4	ND	ABA, GA (yeast)	ND	21, 64
	AtNPF4.5	*At1G27040*	AIT2	ND	ABA (yeast)	ND	64
	AtNPF4.6	*At1G69850*	NRT1.2/ AIT1	Root hairs and root epidermis	NO_3^- (oocyte) ABA (yeast, insect cell)	Nitrate uptake in roots ABA-mediated inhibition of seed germination	21, 62, 64, 65
5	AtNPF5.1	*At2G40460*	NA	ND	ABA, GA, JA-Ile (yeast)	ND	21
	AtNPF5.2	*At5G46050*	PTR3	ND	Dipeptides (yeast) ABA, GA (yeast)	Pathogen response	21, 66, 67
	AtNPF5.3	*At5G46040*	NA	ND	ABA (yeast)	ND	21
	AtNPF5.5	*At2G38100*	NA	ND	NO_3^- (oocyte)	Nitrogen accumulation in embryo	80
	AtNPF5.6	*At2G37900*	NA	ND	GA (yeast)	ND	21
	AtNPF5.7	*At3G53960*	NA	ND	ABA, GA, JA-Ile (yeast)	ND	21
	AtNPF5.10	*At1G22540*	NA	ND	NO_3^- (oocyte)	ND	80
	AtNPF5.11	*At1G72130*	NA	Vascular stele of roots and leaves	NO_3^- (oocyte)	Vacuolar nitrate efflux (together with NPF5.12 and NPF5.16)	54

(*Continued*)

Table 1 (*Continued*)

Clade	New name	Locus	Old name	Spatial locations of gene expression	In vitro substrates (system)	Function in planta	Reference(s)
	AtNPF5.12	*At1G72140*	NA	Vascular stele of roots and leaves	NO_3^- (oocyte)	Vacuolar nitrate efflux (together with NPF5.11 and NPF5.16)	54
	AtNPF5.16	*At1G22550*	NA	Vascular stele of roots and leaves	NO_3^- (oocyte)	Vacuolar nitrate efflux (together with NPF5.11 and NPF5.12)	54
6	AtNPF6.2	*At2G26690*	NRT1.4	Petiole	NO_3^- (oocyte)	Nitrate storage in petiole	22
	AtNPF6.3	*At1G12110*	NRT1.1	Emerging leaves and young stipules; style, stigma, and anthers; guard cells; epidermis near root tips; cortex or endodermis in mature roots	NO_3^-, auxin (oocyte)	Nitrate uptake in root Lateral root growth by nitrate-regulated auxin transport	50, 61, 75
	AtNPF6.4	*At3G21670*	NRT1.3	Mesophyll, cortical cells of stems, pedicels, sepals, hypocotyl	Unknown	Polyamine resistance	148
7	AtNPF7.2	*At4G21680*	NRT1.8	Xylem parenchyma cells of roots and leaves	NO_3^- (oocyte)	Nitrate retrieval from xylem	85
	AtNPF7.3	*At1G32450*	NRT1.5	Xylem-pole pericycle	NO_3^- (oocyte)	Xylem loading of nitrate	89
8	AtNPF8.1	*At3G54140*	PTR1	Phloem and phloem parenchyma cells of leaves; vascular tissues of roots, sepals, and stamens	Dipeptides (oocyte, yeast) JA-Ile	Peptide uptake by root	21, 30, 52, 70
	AtNPF8.2	*At5G01180*	PTR5	Pollen, seeds	Dipeptides (oocyte, yeast) ABA, GA, JA-Ile (yeast)	Peptide transport into germinating pollen	21, 52, 70
	AtNPF8.3	*At2G02040*	PTR2	Embryos	Dipeptides (yeast)	Late flowering and altered seed development	19, 124
Oryza sativa							
2	OsNPF2.2	*Os12g44100*	OsPTR2	Xylem parenchyma cells	NO_3^- (oocyte)	Root-to-shoot nitrate transport Vascular development	87

(*Continued*)

Table 1 (*Continued*)

Clade	New name	Locus	Old name	Spatial locations of gene expression	In vitro substrates (system)	Function in planta	Reference(s)
	OsNPF2.4	*Os03g48180*	NA	Root epidermis, xylem parenchyma, phloem companion cells in roots and shoots	NO_3^- (oocyte)	Acquisition and long-distance transport of nitrate	159
4	OsNPF4.1	*Os11g12740*	SP1	ND	Unknown	Rice panicle size	86
6	OsNPF6.5	*Os10g40600*	OsNRT1.3/ NRT1.1B	Root hairs, epidermis, vascular tissues	Unknown	Nitrate-use divergence between rice subspecies	57, 60
7	OsNPF7.2	*Os02g47090*	NA	Root sclerenchyma, cortex, stele	NO_3^- (oocyte)	Intracellular nitrate allocation in roots	59
	OsNPF7.3	*Os04g50950*	OsPTR6	Stems, lateral roots	Dipeptide (yeast)	Nitrogen allocation and grain yield	112
8	OsNPF8.1	*Os01g04950*	OsPTR7	Leaves, node I, roots	Unknown	Dimethylarsenate accumulation in rice grain	146
	OsNPF8.9	*Os03g13274*	OsNRT1	Root epidermis, root hairs	NO_3^- (oocyte)	Unknown, but OsNPF8.9b with 6 TMs has high-affinity nitrate transport activity	34, 88
	OsNPF8.20	*Os06g49250*	OsPTR9	Leaves, panicles; young main root tips and cortical fiber cells of lateral roots	Unknown	NH_4^+ uptake; nitrogen assimilation, growth, and grain yield	38

Abbreviations: ABA, abscisic acid; AIT, ABA-importing transporter; GA, gibberellin; GLS, glucosinolate; GTR, GLS transporter; JA-Ile, jasmonoyl-isoleucine; NA, not applicable; NAXT, NITRATE EXCRETION TRANSPORTER; ND, not determined; Nitr, nitrite transporter; NPF, NITRATE TRANSPORTER 1 (NRT1)/PEPTIDE TRANSPORTER (PTR) family; SP, short panicle; TM, transmembrane.

(**Figure 1**) and rice, respectively. Plants uniquely host huge numbers of NPF genes. Although in this review we report their role in transporting divergent substrates, our knowledge of their specific functions is still limited. Unless specifically indicated in the text, most of these NPF transporters are localized in the plasma membrane.

2.1.1. Nitrate uptake. Nitrate is the major nitrogen source for most land plants. Nitrate concentrations in soil fluctuate considerably over short distances and in the short term. Plants actively take up nitrate from the environment through a proton/nitrate-coupled mechanism. Several NPF members in *Arabidopsis* and rice are involved in nitrate uptake. The physiological functions of these NPF members are summarized below and their spatial expression patterns are listed in **Table 1**.

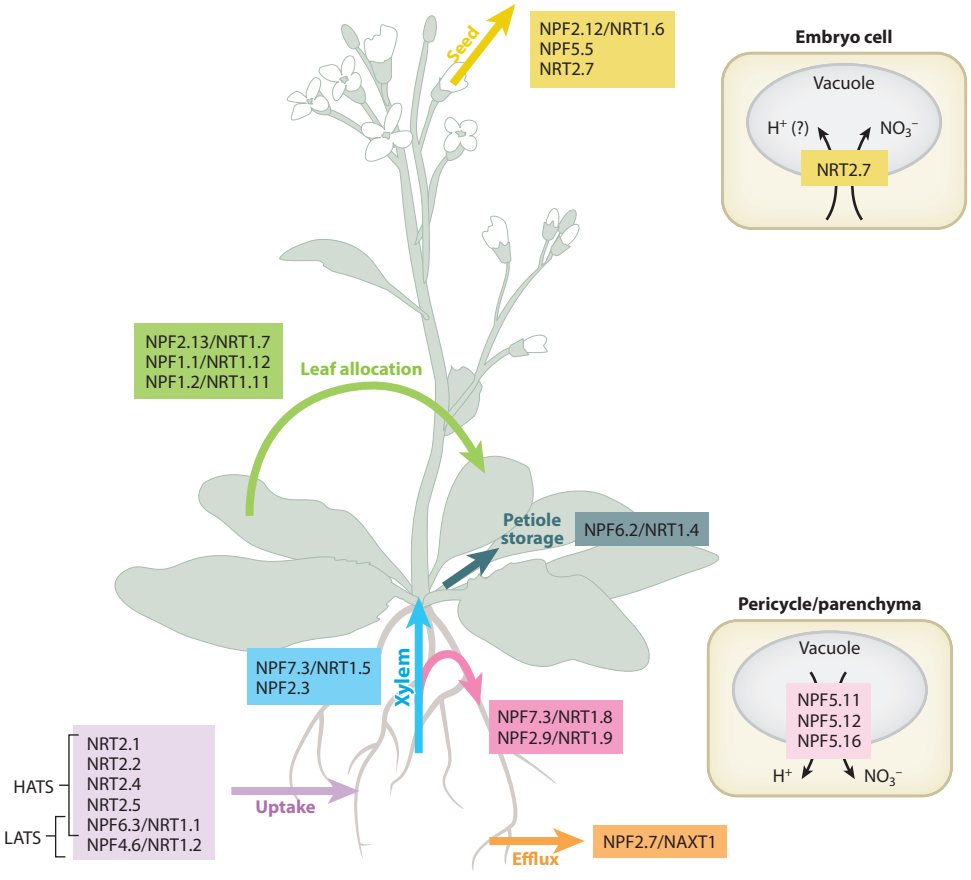


Figure 1

Physiological functions of *Arabidopsis* NPF nitrate transporters, showing roles in nitrate uptake and efflux from soil, root-to-shoot transport, nitrate allocation among leaves, and seed development. Abbreviations: HATS, high-affinity transport system; LATS, low-affinity transport system; NAXT, NITRATE EXCRETION TRANSPORTER; NPF, NITRATE TRANSPORTER 1 (NRT1)/PEPTIDE TRANSPORTER (PTR) family; NRT, NITRATE TRANSPORTER.

2.1.1.1. Arabidopsis NPF6.3 is a dual-affinity nitrate transporter. AtNPF6.3 (also known as CHLORATE RESISTANT 1, CHL1, or NRT1.1) was the first nitrate transporter involved in nitrate uptake in higher plants to be cloned (61, 149). Mutant *npf6.3* is defective in both low- and high-affinity nitrate uptake. When expressed in *Xenopus* oocytes, NPF6.3 displays dual-affinity nitrate transport activity (92), and the switch between the two affinities is modulated by phosphorylation at threonine residue 101 (Thr101) of NPF6.3 (94). To take up nitrate efficiently, NPF6.3 can change its affinity to nitrate in response to external nitrate concentrations (55). According to the crystal structures of NPF6.3, phosphorylation at Thr101 may decouple its dimer form (115, 143). When external nitrate is abundant, dephosphorylated NPF6.3 forms a dimer with low structural flexibility and functions as a low-affinity nitrate transporter. When environmental nitrate is limited, NPF6.3 is phosphorylated to decouple the dimer and increase flexibility, allowing NPF6.3 to operate as a high-affinity nitrate transporter.

Nitrogen-use efficiency (NUE): an indicator to evaluate the efficiency of external nitrogen uptake and internal nitrogen utilization by plants

2.1.1.2. AtNPF4.6/NRT1.2 mediates constitutive low-affinity nitrate uptake. Unlike NPF6.3, NPF4.6 is a pure low-affinity nitrate transporter and is constitutively expressed (62). *NPF4.6* antisense transgenic plants exhibit low nitrate uptake at the low-affinity range of nitrate, suggesting that NPF4.6 mediates low-affinity nitrate uptake in *Arabidopsis*. As discussed below, NPF4.6 also can transport abscisic acid (ABA) (65).

2.1.1.3. OsNRT1.1B/OsNPF6.5 is responsible for the difference in nitrogen-use efficiency between indica and japonica rice cultivars. Given the importance of rice to half the world's human population, the functions of NPF in rice have also been intensively analyzed. Ten of the 93 NPF genes in rice have been characterized, although some have unknown substrates. *OsNRT1.1B/OsNPF6.5*, one of the closest orthologs of *AtNPF6.3* in rice, also encodes a dual-affinity nitrate transporter and mediates nitrate uptake and root-to-shoot transport (57). Interestingly, a single-nucleotide polymorphism resulting in a Thr327Met substitution between the *indica* and *japonica* cultivars is responsible for enhanced nitrate uptake, root-to-shoot transport, nitrate assimilation, and better nitrogen-use efficiency (NUE) in *indica*. These results suggest that marker-aided molecular breeding to modify nitrate transporters is a feasible way to enhance NUE in plants.

2.1.1.4. OsNPF2.4 participates in low-affinity nitrate acquisition. OsNPF2.4 mediates not only nitrate acquisition but also root-to-shoot nitrate transport and nitrogen remobilization from source to sink organs (159). Unexpectedly, OsNPF2.4 may also have an indirect effect on root-to-shoot partitioning of potassium (K^+) because it is impaired in *osnpf2.4* mutants (159).

2.1.1.5. OsNRT1/NPF8.9 has two splicing forms. *OsNRT1/NPF8.9* was the first rice NPF gene to be characterized (88). The spatial expression pattern of OsNRT1 suggests that it may participate in nitrate uptake. OsNRT1.1b is another spliced form of OsNRT1 mRNA (34). To avoid confusion, we refer to OsNRT1.1b as OsNPF8.9b and OsNRT1 as OsNPF8.9a in this review. *OsNPF8.9b* encodes a protein with six transmembrane domains and exhibits nitrate uptake activity at 0.25 mM NO_3^- in oocytes. Furthermore, overexpression lines of *OsNPF8.9a* and *OsNPF8.9b* using a ubiquitin promoter show increased shoot dry weight (34).

2.1.2. Root-to-shoot transport. Once nitrate is taken up into root cells, it can be assimilated or stored in roots or transported to shoots. The capacity for root-to-shoot nitrate transport varies among plant species and is regulated by environmental conditions (**Figure 2**).

2.1.2.1. AtNPF7.3/NRT1.5 mediates xylem loading of nitrate. AtNPF7.3/NRT1.5 is a transporter with nitrate efflux activity and is expressed in xylem-pole pericycle cells (89). In *npf7.3* mutants, less nitrate is transported to shoots, resulting in a lower shoot-to-root ratio of nitrate content, and less nitrate is detected in xylem sap (85, 89), indicating that NPF7.3 mediates xylem loading of nitrate in root pericycle cells.

2.1.2.2. AtNPF7.2/NRT1.8 mediates nitrate retrieval from xylem and participates in stress tolerance. *AtNPF7.2/NRT1.8* is expressed in xylem parenchyma cells, and it is induced by nitrate and by various abiotic and biotic stresses, such as cadmium (Cd^{2+}), salt, cold, and pathogen infection (85). In *npf7.2* mutants, more nitrate is found in xylem sap. Under Cd^{2+} treatment, expression of *NPF7.2* is highly induced, and more nitrate as well as Cd^{2+} is transported to shoots in *npf7.2* mutants, resulting in a nitrate-dependent Cd^{2+}-hypersensitive phenotype. Therefore, the activity of NPF7.2 under stress can enhance plant stress tolerance by retaining nitrate in roots

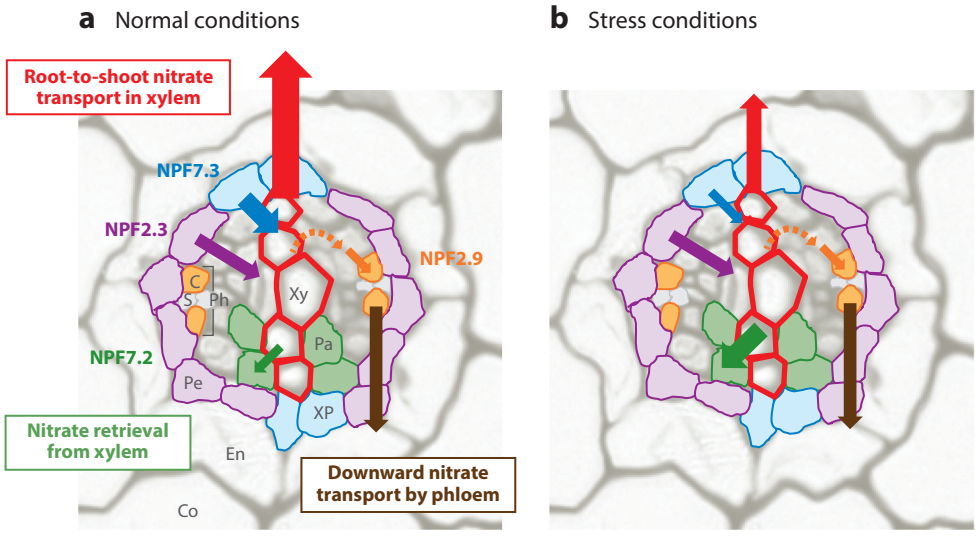


Figure 2

Regulation of root-to-shoot nitrate transport under normal and stress conditions in *Arabidopsis*. Arrows indicate nitrate flow, with arrow size indicating the relative size of the effect in panel *a* versus panel *b*. Colors of protein names match the cell type in which the protein is expressed (if applicable); an arrow of the same color indicates flow mediated by that protein. The dashed arrows indicate proposed or uncertain flow. (*a*) Under normal conditions, root-to-shoot nitrate transport is active (*large red arrow*). Xylem loading of nitrate is facilitated by NPF7.3 (*blue arrow*) and partially negatively regulated by NPF2.9-mediated phloem loading (*orange arrow*) and downward nitrate transport (*brown arrow*). (*b*) Under stress conditions, lower expression of NPF7.3 and higher expression of NPF7.2 repress root-to-shoot nitrate transport (*small red arrow*), which mediates nitrate retrieval from xylem (*green arrow*). NPF2.3, a constitutively expressed nitrate transporter in pericycle cells (*purple*), maintains a certain amount of nitrate transport to shoots (*purple arrow*). Abbreviations: C, companion cell; Co, cortex; En, endodermis; NPF, NITRATE TRANSPORTER 1 (NRT1)/PEPTIDE TRANSPORTER (PTR) family; Pa, xylem parenchyma; Pe, pericycle; Ph, phloem; S, sieve element; XP, xylem-pole pericycle; Xy, xylem.

(45, 85). Interestingly, the functions of NPF7.2 and NPF7.3 are antagonistic, and expressions of NPF7.2 and NPF7.3 are contrastingly regulated upon stress treatments (85, 164).

AtNPF7.2 and AtNPF7.3 show antagonistic stress responses and both proteins are involved in stress-initiated nitrate allocation to roots (SINAR). Abiotic stress affects root-to-shoot nitrate transport by regulating the expression of *AtNPF7.2* and *AtNPF7.3* (16, 85). Under salt and Cd^{2+} treatment, expression of *AtNPF7.2* for xylem nitrate retrieval is upregulated, but expression of *AtNPF7.3* for xylem loading is downregulated, which results in SINAR as more nitrate is kept in roots in response to these stresses (164). Mutants of *atnpf7.3* show enhanced stress tolerance but decreased root growth under nonstressed conditions, suggesting that SINAR is a trade-off between plant growth and environmental acclimation (164).

Stress-initiated nitrate allocation to roots (SINAR): a phenomenon that enhances the stress tolerance of plants by accumulating nitrate in roots

2.1.2.3. AtNPF2.3 is important for root-to-shoot nitrate translocation under salinity. AtNPF2.3 facilitates root-to-shoot nitrate transport under salt stress to prevent excessive SINAR (147). Disruption of *NPF2.3* decreases xylem flux of nitrate to shoots under salt stress (147). *NPF2.3* is constitutively expressed, but it only contributes to root-to-shoot nitrate translocation under conditions of salinity, probably because the activity of NPF7.3 is too strong to reveal the contribution of NPF2.3 under normal conditions. The differing functional properties (pH

preferences) and different spatial expression patterns (pericycle for AtNPF2.3 versus xylem-pole pericycle for AtNPF7.3) of these two transporters may ensure optimal xylem loading of nitrate under different conditions.

2.1.2.4. AtNPF2.9/NRT1.9 participates in phloem loading of nitrate and negatively regulates root-to-shoot nitrate transport. *AtNPF2.9/NRT1.9* is expressed in root companion cells (156). In *npf2.9* mutants, upward xylem transport of nitrate is enhanced, but downward phloem nitrate transport is reduced, resulting in a higher shoot-to-root ratio of nitrate content under high-nitrate conditions. Interestingly, *npf2.9* mutants show enhanced plant growth under high-nitrate conditions (156). The trade-off in growth advantage for this mutant is becoming less responsive to the light necessary for enhancing plant growth. These data indicate that phloem transport of nitrate in roots regulates root-to-shoot xylem transport of nitrate and that this regulation affects plant growth.

2.1.2.5. Roles of AtNPF5.11, AtNPF5.12, and AtNPF5.16 in vacuolar nitrate efflux. AtNPF5.11, AtNPF5.12, and AtNPF5.16, localized in tonoplast, were proposed to mediate nitrate efflux from vacuoles and to regulate nitrate partitioning between roots and shoots (54). In *npf5.11 npf5.12 npf5.16* triple mutants, more root-fed $^{15}NO_3^-$ is translocated to shoots. However, *NPF5.12* overexpression lines show a similar phenotype, with less nitrate being retained in roots under nitrogen starvation compared with wild type (54). These results suggest that NPF5.11, NPF5.12, and NPF5.16 may modulate nitrate redistribution between roots and shoots by mediating nitrate efflux from vacuoles to the cytosol.

2.1.2.6. OsNPF2.2 is involved in root-to-shoot nitrate transport and vascular development. *OsNPF2.2* mutants present opposing root-to-shoot nitrate-partitioning phenotypes under normal and nitrate starvation conditions (87). In addition, programmed cell death in some xylem vessels is blocked in *npf2.2* mutants, resulting in abnormal vascular development (87). Therefore, OsNPF2.2 participates in root-to-shoot nitrate transport and vascular development.

2.1.3. Leaf nitrate allocation. Once transported to shoots, nitrate is stored or assimilated. Depending on the developmental stages of the leaves, nitrogen demand and the capacity for nitrate storage and assimilation are altered. Therefore, nitrate allocation among different tissues is a key step for efficient nitrate utilization in higher plants.

2.1.3.1. AtNPF6.2/NRT1.4 mediates nitrate storage in the petioles. *AtNPF6.2/NRT1.4* is highly expressed in the petiole and midrib of leaves (22). Compared with the wild type, less nitrate accumulates in the petiole, but more nitrate is detected in the leaf blade of *npf6.2* mutants, suggesting that NPF6.2 is involved in nitrate storage of the petiole and regulates leaf nitrate homeostasis. Moreover, *npf6.2* mutants exhibit wider leaf morphology than wild-type leaves, suggesting that leaf nitrate homeostasis may affect leaf development (22).

2.1.3.2. AtNPF1.1/NRT1.12 and AtNPF1.2/NRT1.11 redistribute nitrate into developing tissues. Although it had long been assumed that only xylem transports nitrate, several recent studies have shown that phloem nitrate transport has an important function in nitrate homeostasis and plant growth. AtNPF2.9/NRT1.9 (Section 2.1.2.4), AtNPF1.1/NRT1.12, AtNPF1.2/NRT1.11, and AtNPF2.13/NRT1.7 (Section 2.1.3.3) are good examples of the importance of phloem nitrate transport. Root-to-shoot nitrate transport is mediated by xylem, and the xylem stream is driven by transpiration. Therefore, expanded leaves receive more nitrate from xylem than do smaller

developing leaves. A study of AtNPF1.1/NRT1.12 and AtNPF1.2/NRT1.11 demonstrated that redistribution of nitrate from larger expanded leaves to the youngest tissues is important for plant growth (56). Both *NPF1.1* and *NPF1.2* are expressed in the companion cells of the major veins in expanded leaves. In *npf1.1 npf1.2* double mutants, more root-fed $^{15}NO_3^-$ is detected in mature and larger leaves but less in the youngest tissues, suggesting that NPF1.1 and NPF1.2 are involved in diverting root-derived nitrate into phloem in the major vein of mature and expanded leaves for redistribution to the youngest tissues. Furthermore, unlike wild-type plants, these double mutants do not show enhanced plant growth when the external nitrate concentration is increased. In summary, these findings suggest that NPF1.1 and NPF1.2 are required for xylem-to-phloem nitrate transfer to redistribute nitrate into developing tissues and satisfy their high nutrient demand, and that this redistribution of nitrate is important for efficient utilization of ample nitrate to promote plant growth.

2.1.3.3. AtNPF2.13/NRT1.7 remobilizes nitrate from old into young leaves under nitrogen starvation. When there is no external nitrate supply, efficient remobilization of stored nitrate is important for sustaining plant growth. AtNPF2.13/NRT1.7 is responsible for this process by mediating source-to-sink remobilization of leaf nitrate during nitrogen starvation (33). *AtNPF2.13* is expressed in phloem of the minor veins of old leaves and in the distal parts of transition leaves, according to the source strength of the tissues. Disruption of *NPF2.13* reveals that more nitrate accumulates in old leaves and less nitrate is detected in the phloem exudates of old leaves, suggesting that NPF2.13 facilitates outward nitrate transport by phloem loading in the source leaves. Upon nitrogen starvation, *npf2.13* mutants show retarded growth, indicating that remobilizing excess nitrate from old leaves to feed young developing leaves is important for plants to sustain vigorous growth under nitrogen starvation. Interestingly, expression of *NPF2.13* is regulated by photoperiod, with levels of *NPF2.13* transcripts gradually increasing during the light period and maximal levels being attained in the early part of the dark period, suggesting that NPF2.13-mediated nitrate remobilization via phloem occurs under normal conditions when nitrate assimilation and transpiration are low.

Because the function of NPF2.13 is more important under nitrogen deficiency, a regulatory circuit of the microRNA miR827 and *NITROGEN LIMITATION ADAPTATION* (*NLA*), which encodes a RING-type ubiquitin ligase, further secures its abundance (95). NPF2.13 and NLA interact with each other on the plasma membrane, and in vitro and in vivo evidence has revealed that NPF2.13 can be ubiquitinated by NLA and then degraded. Interestingly, under nitrogen deficiency, NLA is decreased in an miR827-dependent manner (95). Taken together, these results suggest that NPF2.13 is increased during nitrogen limitation owing to NLA reduction so that source-to-sink (old leaf to young leaf) nitrate remobilization via phloem is enhanced to sustain plant growth.

To maximize the growth of young leaves when external nitrate supply is sufficient, AtNPF1.1 and AtNPF1.2 reallocate xylem-borne nitrate in the major vein of mature leaves to feed developing leaves (**Figure 3**). However, when there is no external nitrate supply, AtNPF2.13 remobilizes excess nitrate stored in old leaves to the minor vein of old leaves to fulfill the nitrogen demand of young leaves. Although xylem is the main mediator of long-distance nitrate transport, phloem is required for fine-tuning nitrate redistribution into demanding tissues.

2.1.4. Seed development and nitrogen storage. Organic nitrogen, such as from amino acids and peptides, is generally considered the major nitrogen source for seed development. Nevertheless, nitrate can also accumulate in seeds and affect seed dormancy, development, and nitrogen

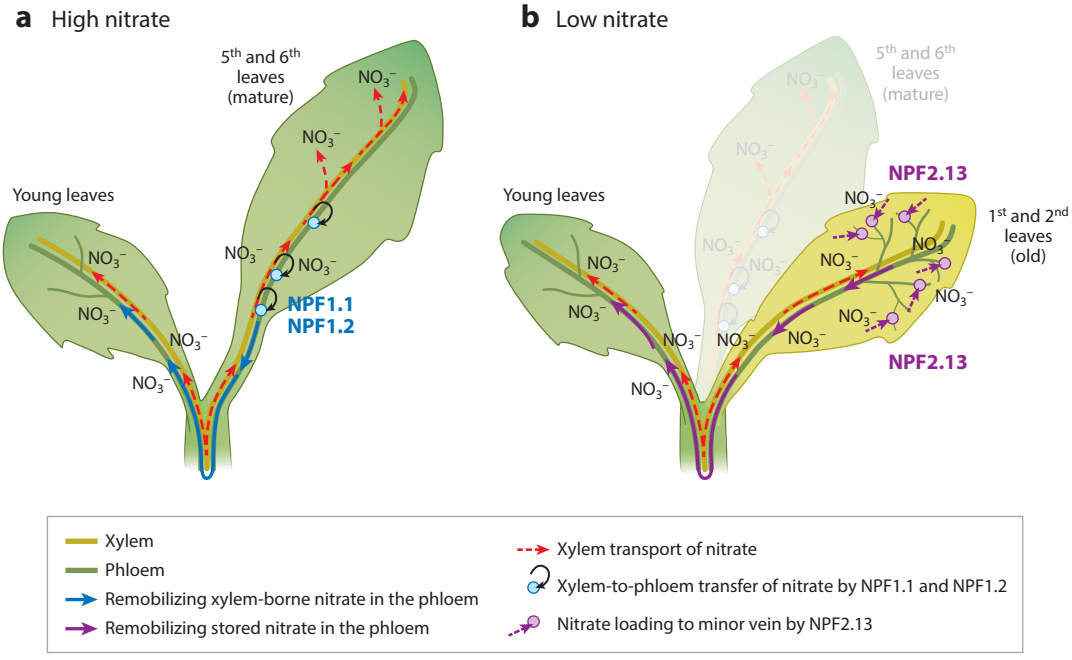


Figure 3

Regulation of leaf nitrate allocation in response to external nitrate (NO_3^-) supply in *Arabidopsis*. NPF1.1, NPF1.2, and NPF2.13 play different roles in nitrate allocation. (*a*) When nitrate supply is sufficient, xylem-borne nitrate (the major nitrate source for leaf growth at that time) is redistributed by NPF1.1 and NPF1.2 in phloem of the major vein in mature leaves (such as fifth and sixth leaves) to feed developing young leaves. (*b*) When nitrate supply is limited, stored nitrate in old leaves (such as first and second leaves) is remobilized by NPF2.13 in phloem of the minor vein to support the nitrogen demand in young leaves. Abbreviation: NPF, NITRATE TRANSPORTER 1 (NRT1)/PEPTIDE TRANSPORTER (PTR) family.

storage. Two *Arabidopsis* NPF genes that are involved in seed development and nitrogen storage are described below.

2.1.4.1. AtNPF2.12/NRT1.6 delivers nitrate into developing embryos. Expression of *AtNPF2.12/NRT1.6* is detectable in the vascular tissues of the silique and funiculus, and it is induced after pollination (1). Disruption of *NPF2.12* results in lower nitrate accumulation in mature seeds and a higher seed abortion rate, suggesting that delivery of nitrate from maternal tissues to developing embryos is mediated by NPF2.12 and is important for seed development. The defects of aborted seeds in *npf2.12* mutants are found mainly in the suspensor cells at the one- or two-cell stage of embryo development. This study also revealed that nitrate is crucial for early embryo development and that NPF2.12 is required for delivering nitrate into developing embryos (1).

2.1.4.2. AtNPF5.5 is involved in nitrogen storage in embryos. AtNPF5.5 affects nitrogen accumulation in the *Arabidopsis* embryo (80). The *NPF5.5* transcript can be detected in embryos and total nitrogen levels in *npf5.5* mutant embryos is reduced compared with the wild type. Subcellular localization and tissue-specific expression patterns of NPF5.5 require further investigation to understand how they affect nitrogen accumulation in the embryo and whether they disrupt embryo development or seed germination.

2.1.5. Stomatal regulation. Stomatal movement is highly regulated by multiple pathways to reduce excess water loss and maintain CO_2 uptake for photosynthesis. The role of nitrate as an osmoticum affecting stomatal opening was elucidated through study of *AtNPF6.3/NRT1.1* (51), which is expressed in guard cells. In the presence of nitrate, *npf6.3* mutants show reduced stomatal apertures and a lower transpiration rate in response to light compared with wild-type plants, resulting in enhanced drought tolerance.

2.1.6. Versatile functions of NPF transporters. In addition to transporting nitrate, there is growing evidence that the substrates of NPF transporters may be very diverse. Having miscellaneous substrates suggests that NPF transporters have versatile functions in plant growth and development. A related review about the substrate specificity of *Arabidopsis* NPFs has been published (24). Below, we highlight the latest findings.

2.1.6.1. Peptides. All NPF homologs in nonplant organisms transport dipeptides. Three *Arabidopsis* NPFs—NPF8.1/PTR1, NPF8.2/PTR5, and NPF8.3/PTR2—show dipeptide transport activity in both *Xenopus* oocytes and yeast systems, but no nitrate transport activity; *NPF5.2/PTR3* also complements a yeast dipeptide uptake mutant (20, 30, 41, 52, 66, 70, 80, 120, 124, 138). However, NPF8.4/PTR4 and NPF8.5/PTR6, in the same subgroup, have not shown dipeptide transport activity (157). Both NPF8.1 and NPF8.2 are localized in the plasma membrane, whereas NPF8.3, NPF8.4, and NPF8.5 are localized in tonoplast. NPF8.1 and NPF8.2 participate in peptide uptake into roots and pollen, respectively, whereas NPF8.3 is involved in flowering control and seed development (30, 70, 137). *NPF5.2* is induced by stress and participates in defense against bacterial pathogens. The relationship between peptide transport activity and phenotypes of late flowering and pathogen susceptibility remains to be elucidated. In rice, OsNPF7.3/PTR6 mediates peptide transport and plays a role in regulating total nitrogen content and plant growth (36, 37, 112).

2.1.6.2. Chloride ions. Two NPF transporters in *Arabidopsis*, AtNPF2.4 and AtNPF2.5, display chloride efflux activity (82, 83). *NPF2.4* is expressed mainly in the root stele and mediates root-to-shoot transfer by loading chloride into xylem. In contrast, *NPF2.5* is expressed in root cortical cells and mediates chloride efflux from the root. Upon high salt treatment, *NPF2.4* is downregulated, whereas *NPF2.5* is upregulated. Taken together, NPF2.4 and NPF2.5 function in reducing chloride content in shoots upon salt stress.

2.1.6.3. Potassium. In addition to exhibiting a nitrate-related phenotype (Section 2.1.2.1), *npf7.3* mutants also display K^+-related phenotypes, including lateral root development and leaf senescence (100, 166). Four different research groups have shown that root-to-shoot K^+ transport is defective in *npf7.3* mutants, but the influence of nitrate on defects of K^+ partitioning in *npf7.3* mutants is controversial (32, 84, 89, 100, 166). With the use of yeast complementation, NPF7.3 does not show K^+ transport activity (32), but NPF7.3-expressing oocytes display pH-dependent K^+ efflux activity, indicating that NPF7.3 may be a proton-coupled H^+/K^+ antiporter for K^+ loading into xylem (84).

2.1.6.4. Hormones. Increasing evidence shows that NPFs also transport plant hormones. In addition to oocyte functional assays, a screening analysis using yeast two-hybrid systems expressing receptors and coreceptors of specific hormones has found that several NPFs mediate transport of ABA, gibberellins (GAs), and jasmonoyl-isoleucine (JA-Ile) (21, 64). Interestingly, some NPFs transport both nitrate and hormones. Below, we discuss the interaction between nitrate and other substrates.

2.1.6.5. Auxin. AtNPF6.3/NRT1.1 displays auxin transport activity that can be inhibited by nitrate in oocytes (75). Nitrate-regulated auxin transport by NPF6.3 modulates lateral root growth in response to different external nitrate concentrations. When there is little or no nitrate, NPF6.3 functions as an auxin transporter to prevent auxin accumulation in the lateral root tips, resulting in cessation of lateral root elongation. Auxin transport by NPF6.3 is inhibited in the presence of nitrate, so lateral roots elongate as a result of auxin accumulation in the lateral root tips, revealing an interaction between nutrient and hormone signaling during plant development (75). Interestingly, nitrate not only represses auxin transport activity but also affects accumulation of NPF6.3 transcripts and protein. Nitrate promotes transcription as well as mRNA accumulation of *NPF6.3* in the epidermis, cortex, and lateral root primordia. Nevertheless, nitrate represses NPF6.3 protein accumulation specifically in the lateral root primordia, leading to further auxin accumulation there to promote lateral root growth (9).

2.1.6.6. Abscisic acid. AtNPF4.6/NRT1.2/AIT1, AtNPF4.5/AIT2, AtNPF4.1/AIT3, and AtNPF4.2/AIT4 have been identified as ABA transporters in yeast two-hybrid screenings (64). An *npf4.6* mutant has exhibited an ABA-insensitive phenotype in seed germination (64, 65). Nitrate, another substrate of NPF4.6, does not affect the ABA transporter activity of NPF4.6 and NPF4.1 (65), but it does alleviate ABA-mediated inhibition of seed germination in both the *npf4.6* mutant and the wild type (65). Thus, NPF4.6 is not involved in interactions between nitrate and ABA signals. However, the *npf4.6* mutant seems more responsive to nitrate-mediated enhancement of seed germination.

2.1.6.7. Gibberellins. AtNPF3 was originally identified as a nitrate/nitrite transporter that regulates nitrite accumulation in leaves (119, 141). It was further identified as a GA transporter affecting GA accumulation in root endodermis (145). Although in *Xenopus* oocytes GA transport activity of NPF3 is not affected by nitrate (145), NPF3 mediates GA-stimulated hypocotyl elongation only under low-nitrate conditions (27), which suggests that NPF3 could serve as a crosstalk modulator for GA and nitrate in hypocotyl elongation.

2.1.7. Glucosinolates and other secondary metabolites. Glucosinolates (GLS) are a group of nitrogen- and sulfur-rich secondary metabolites found mainly in Brassicaceae. These compounds function in herbivore defense for plants, but benefits to human health have also been described (78). Five *Arabidopsis* NPF transporters—NPF2.10/GTR1, NPF2.11/GTR2, NPF2.9/NRT1.9, NPF2.14, and NPF2.13/NRT1.7—display GLS transport activity. NPF2.10 and NPF2.11 also display low-affinity nitrate transport that has no effect on GLS transport (105). Translocation of GLS to seeds is almost abolished in an *npf2.10 npf2.11* double mutant. In addition to nitrate and GLS, NPF2.10 also mediates transport of GAs and JA-Ile in oocytes and yeast cells, and *npf2.10* mutant displays GA- and JA-related phenotypes (63, 105, 127). Analysis of the mutant indicates that NPF2.10 is involved in JA/JA-Ile translocation from damaged to undamaged leaves during wounding responses (63). However, GAs, but not JA, can rescue a stamen defect in *npf2.10* mutant (127). It remains to be determined whether nitrate can affect NPF2.10-mediated GA- and JA-related functions in planta.

Monoterpene indole alkaloids (MIAs) are specialized metabolites with anticancer activity produced across six plant families (108). The multiple steps of MIA biosynthesis in the medicinal plant *Catharanthus roseus* take place in different cell types and in different intracellular compartments. CrNPF2.9, an NPF in *C. roseus*, mediates the export of an MIA intermediate from vacuoles in the epidermis for subsequent reactions (116).

The huge number of NPFs has puzzled plant biologists. Why do plants have so many NPF transporters? They are definitely not all nitrate or peptide transporters. Whereas the NPFs in yeast, fruit fly, and human display peptide transport activity, those in plants have evolved different substrate specificities and functions, such as nitrate acquisition, hormone translocation/homeostasis, metabolite compartmentalization, and plant defense. Indeed, it would not be surprising if new substrates for NPFs are identified in future. Characterization of the biological functions of NPF genes and the physiological interplay among their multiple substrates will help us understand why this large transporter family and their versatile functions have evolved in plants.

2.2. Physiological Functions of NRT2 Transporters

In general, NRT2s likely display high-affinity nitrate transport activity, but some display only low-affinity transport activity when expressed in oocytes (42). The *Arabidopsis* genome includes seven NRT2 genes and two NAR2 genes; the larger rice genome has only four NRT2 genes and two NAR2 genes. NAR2 forms a complex with NTR2 and is required for plasma membrane targeting and for maintaining NRT2 protein stability. A yeast two-hybrid assay and biomolecular fluorescence complementation study showed that all six NRT2s except for AtNRT2.7 interact with NAR2.1 in the plasma membrane (73). Indeed, when NAR2 is coexpressed in *Xenopus* oocytes, the nitrate transport activities of AtNRT2.1, AtNRT2.2, AtNRT2.5, OsNRT2.1, and OsNRT2.3a are greatly enhanced (39, 73). Nevertheless, NAR2 is not required for the nitrate transport activities of AtNRT2.4 or OsNRT2.3b (39, 69). We review and discuss the physiological functions of NRT2 below.

2.2.1. Four AtNRT2 transporters are involved in nitrate uptake. It has long been well documented that *AtNRT2.1* and *AtNRT2.2* are two linked genes responsible for high-affinity nitrate uptake in *Arabidopsis* (15, 40). AtNRT2.4 and AtNRT2.5 are also involved in high-affinity nitrate uptake, but their contribution is revealed only under nitrogen starvation (69, 81). After a long period of starvation, expression of *AtNRT2.5* is highly induced, and it becomes the major transporter for high-affinity uptake (81). The spatial expression patterns of *AtNRT2.1*, *AtNRT2.4*, and *AtNRT2.5* also differ; *AtNRT2.1* is mainly expressed in the older part of the main root, *AtNRT2.4* in the younger part of the primary root and the distal region of lateral roots, and *AtNRT2.5* in the root hair zone of primary and lateral roots (69, 81). It will be interesting to determine whether nitrate affinity is another diverse property among these three NRT2 transporters, as a transporter with higher affinity may be necessary for plants to cope with infertile soils after long-term starvation. *AtNRT2.4* and *AtNRT2.5* are also expressed in phloem of the major and minor veins of shoots, respectively, and under certain conditions or given particular genetic mutations, they affect nitrate content in shoot phloem (69, 81).

2.2.2. AtNRT2.7 regulates seed nitrate content and dormancy. *AtNRT2.7* is specifically expressed in seeds and is the only NRT2 transporter located in tonoplast (23). Seed nitrate content of *nrt2.7* mutants is reduced by 35–70%, suggesting that NRT2.7 is responsible for loading nitrate into vacuoles. NRT2.7 also has an unknown function in proanthocyanidin accumulation or oxidation; although the proanthocyanidin phenotype is not correlated with seed nitrate content, the seed coat of *nrt2.7* mutants is pale brown.

2.2.3. AtNRT2.1, AtNRT2.5, and AtNRT2.6 are involved in plant-microbe interactions. An *nrt2.6* mutant shows no nitrate-related phenotype but is more sensitive to attack by the phytopathogenic bacterium *Erwinia amylovora* owing to reduced production of reactive oxygen species

(28). Independently of such production and of nitrate uptake, *nrt2.5* and *nrt2.6* mutants abolish plant growth stimulated by the rhizospheric bacterium *Phyllobacterium brassicacearum* STM196 (68). An *nrt2.1* mutant shows reduced susceptibility to the bacterial pathogen *Pseudomonas syringae* owing to reduced susceptibility to the bacterially secreted toxin coronatine (11). Therefore, the mechanisms involved are not identical for different NRT2 transporters and different microbes.

Primary nitrate response (PNR): a rapid nitrate-induced transcriptional response that regulates 1,000 genes in *Arabidopsis*

Transceptor: a transporter that can also function as a receptor

Root foraging: roots promote growth in nitrate-rich sites but inhibit growth in nitrate-deficient sites

3. NITRATE SIGNALING

Nitrate serves not only as a nitrogen source but also as a signaling molecule that regulates numerous processes including gene expression, root architecture, shoot development, seed germination, and flowering (90, 103, 107). Nitrate regulates a transcriptional response called the primary nitrate response (PNR) (29, 47), which is induced by nitrate and does not require de novo protein synthesis (47, 153). This rapid response can induce gene expression in minutes, reaching a peak at approximately 30 min. Induction levels of PNR genes depend on nitrate concentrations; low (less than 1 mM) and high concentrations induce corresponding levels of the PNR (58). PNR genes include the nitrate assimilation genes *NITRITE REDUCTASE* (*NIR*) and *NITRATE REDUCTASE 1* and *2* (*NIA1* and *NIA2*); nitrate transporter genes in the *NRT1* and *NRT2* families; and genes involved in the pentose phosphate pathway, glycolysis, and trehalose-6-P metabolism that provide the carbon scaffold and endow reducing power (133, 152). Another well-characterized response is nitrate-regulated root growth, which involves lateral root initiation, lateral root elongation, root hair growth, and primary root growth. To acquire nitrate efficiently, local and systematic nitrate signaling needs to be integrated through long-distance communication to orchestrate root growth in response to the uneven nitrate concentration in soil. Several players involved in the nitrate signaling pathway have been identified, including the nitrate transceptor, calcium signaling, kinases, transcription factors, and various peptides and proteins. Here, we discuss the network of regulators in three phases: perception, signal transduction (**Figure 4**), and long-distance communication (**Figure 5**).

3.1. Perception

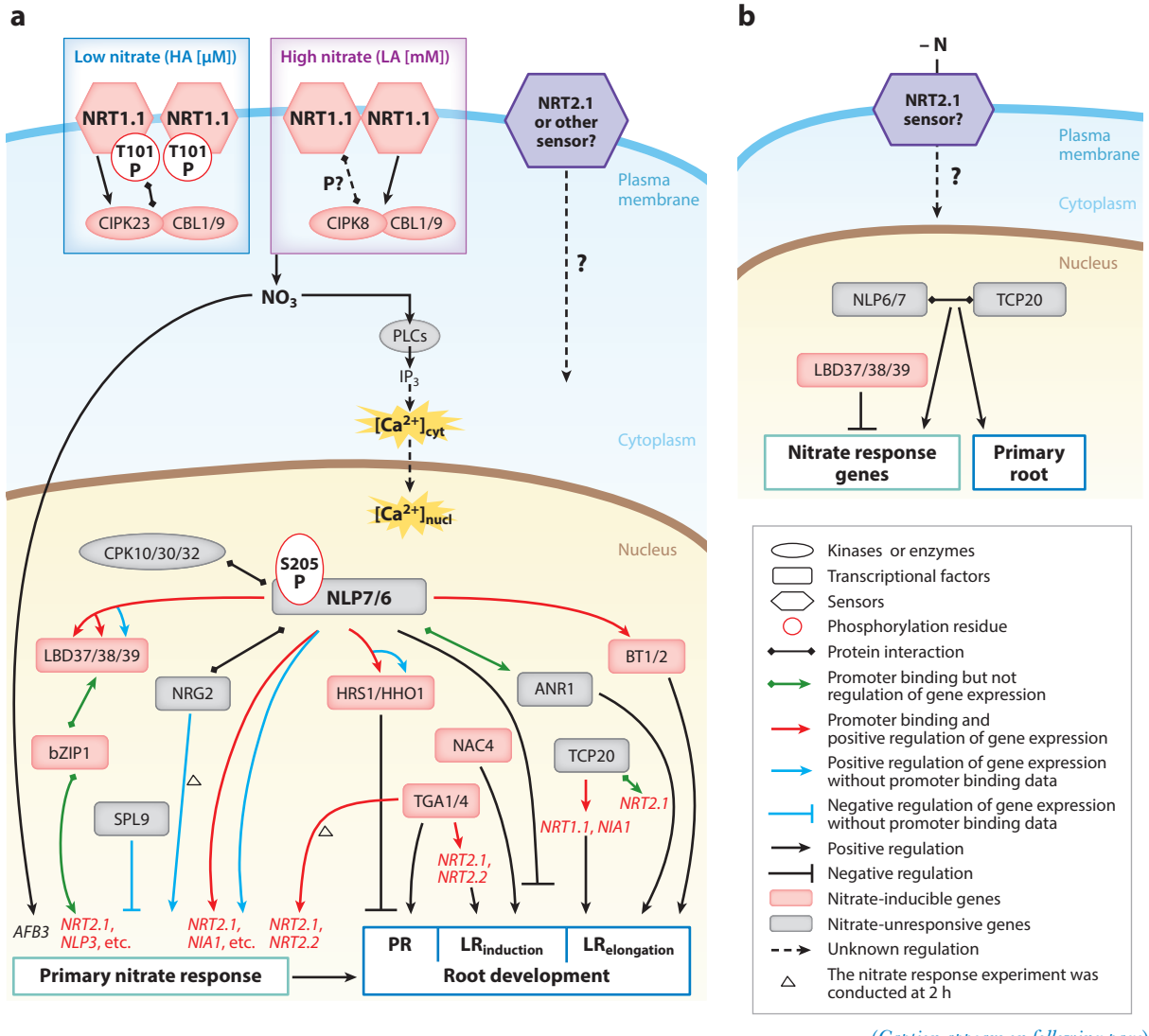
As the first step of nitrate signaling, external nitrate is perceived by the dual-affinity transceptor NRT1.1 (CHL1/NPF6.3) (92, 149). Owing to the PNR and nitrate root-foraging defect observed in the NRT1.1 mutant *chl1-5*, NRT1.1 may participate in nitrate signaling (102, 123, 154). However, it is difficult to determine whether this defect is a direct result of nitrate sensing or an indirect result of deficient uptake. Fortunately, another mutant *chl1-9* (NRT1.1^{P492L}, in which the proline 492 residue is mutated to leucine) has revealed the nitrate-sensing function of NRT1.1. The NRT1.1^{P492L} mutant lacks nitrate uptake ability but still displays a typical PNR as in the wild type, indicating that nitrate uptake activity is decoupled from the nitrate-sensing function and that NRT1.1 directly participates as a sensor (55). Furthermore, because NRT1.1^{P492L} is localized both on the plasma membrane and intracellularly, targeting to the plasma membrane may not be necessary for NRT1.1 to trigger the PNR (8).

As a dual-affinity transporter, NRT1.1 can switch between high and low affinities in response to external nitrate concentrations through phosphorylation and dephosphorylation at the Thr101 site. When external nitrate is low, NRT1.1 is phosphorylated at Thr101, which converts it into a high-affinity transporter and triggers a low-level nitrate response. When nitrate concentration is high, NRT1.1 is dephosphorylated, rendering it a low-affinity transporter and triggering a high-level nitrate response. Two CALCINEURIN B-LIKE (CBL)-INTERACTION PROTEIN KINASES (CIPKs) interact with NRT1.1 and differentially regulate nitrate signaling.

As a positive regulator, CIPK8 specifically engages in low-affinity responses (58), whereas the negative regulator CIPK23 specifically engages in high-affinity responses by phosphorylating NRT1.1 at the Thr101 site in response to low nitrate (55). Recently, a study of the crystal structure of NRT1.1 has suggested that the large central loop between transmembrane domains 6 and 7 could be the docking site for other proteins to interact with NRT1.1 (143).

NRT1.1 regulates not only the PNR but also the root-foraging process. To absorb nitrate more efficiently from heterogeneous soil, plants promote lateral root growth at the nitrate-rich side while inhibiting lateral growth at the nitrate-poor side. Differing nitrate concentrations are sensed through NRT1.1. On the nitrate-rich side of a split-root system, NRT1.1 is required for lateral root proliferation by upregulating ARABIDOPSIS NITRATE-REGULATED 1 (ANR1) to promote lateral root growth (123). On the nitrate-poor side, NRT1.1 can moderate auxin levels and meristem activity to repress lateral root growth (101). The ABA-insensitive mutant *abi2*

Split-root system: one side of a root system is grown under conditions of high nitrogen and the other side is grown under low nitrogen, reflecting heterogeneity of nitrate concentrations



(*Caption appears on following page*)

Figure 4 (*Figure appears on preceding page*)

Nitrate signaling pathway. (*a*) Nitrate is sensed by the transceptor NRT1.1 under both high and low concentrations. CIPK8 regulates NRT1.1 under high-nitrate conditions, whereas CIPK23 phosphorylates the transceptor under low-nitrate conditions. Then, through a calcium-dependent or calcium-independent pathway, nitrate signaling triggers downstream nitrate responses. Calcium accumulation requires PLC enzymatic activity, which is accompanied by IP_3 accumulation. When calcium accumulates in the nucleus, NLP7 is phosphorylated by CPKs and is retained in the nucleus to regulate nitrate responses. NLP7 acts as a master regulator to directly regulate other transcription factors involved in nitrate signaling. These transcription factors function in nitrate responses (crosstalk between them is shown). The nitrate response of root development includes primary root growth, lateral root induction, and lateral root elongation. (*b*) In the absence of nitrate, starvation signaling triggers a nitrate-related gene response through interaction between NLP7 and TCP in the nucleus to promote primary root growth. LBDs function as negative regulators of nitrate-related genes. Abbreviations: AFB, AUXIN SIGNALING F-BOX PROTEIN; ANR, ARABIDOPSIS NITRATE REGULATED; BT, BTB and TAZ DOMAIN PROTEIN; bZIP, BASIC LEUCINE ZIPPER; CBL, CALCINEURIN B-LIKE PROTEIN; CIPK, CBL-INTERACTION PROTEIN KINASE; CPK, CALCIUM-SENSOR PROTEIN KINASE; cyt, cytoplasm; HA, high-affinity range; HHO, HRS1 HOMOLOG; HRS, HYPERSENSITIVITY TO LOW PI-ELICITED PRIMARY ROOT SHORTENING; IP_3, INOSITOL 1,4,5-TRISPHOSPHATE; LA, low-affinity range; LBD, LATERAL BOUNDARY DOMAIN-CONTAINING PROTEIN; LR, lateral root; N, nitrate; NAC, NAM-ATAF-CCUC DOMAIN-CONTAINING PROTEIN; NLP, NIN-LIKE PROTEIN; NRG, NITRATE REGULATORY GENE; NRT, NITRATE TRANSPORTER; nucl, nucleus; P, phosphorylation; PLC, PHOSPHOLIPASE C; PR, primary root; S205, serine residue 205; SPL, SQUAMOSA PROMOTER BINDING PROTEIN-LIKE; T101, threonine residue 101; TCP, TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTOR; TGA, TGACG MOTIF-BINDING FACTOR.

also exhibits the nitrate root-foraging phenotype, probably via dephosphorylation of CBL1 and CIPK23 to modulate NRT1.1, suggesting crosstalk with the stress hormone ABA (79).

In addition to NRT1.1, other nitrate sensors may exist because the deletion mutant of NRT1.1, *chl1-5*, does not always entirely abolish the PNR. When plants are starved before nitrate induction, the *chl1-5* mutant still exhibits a low degree of the PNR (58). Studies show that NRT2.1 may be a sensor for root development (2, 6, 142), but its exact function in nitrate signaling has yet to be clarified. Whether other sensors can detect internal nitrate concentrations also remains to be ascertained.

3.2. Signal Transduction: Calcium and Transcription Factors

After nitrate is sensed by NRT1.1, the signal needs to be transmitted to the nucleus and magnified through cytosolic regulators. Two CIPKs and their partner CBL reveal that calcium may participate in nitrate signaling. A recent study also showed that calcium is the secondary messenger linking the nitrate signal to downstream regulators (74, 93).

3.2.1. Calcium. In plants, calcium signaling participates in responses to biotic and abiotic stress, nodulation, circadian rhythms, and polar tip growth (31). The involvement of calcium in nitrate signaling was first described 30 years ago in maize and barley. mRNAs of *NITRATE REDUCTASE* and *NITRITE REDUCTASE* do not accumulate in response to nitrate when treated with the calcium chelator EGTA or the calcium channel blocker La^{3+} (128, 140). Using aequorin reporter lines, Riveras et al. (125) observed calcium accumulation in cytoplasm in response to nitrate and dependence on NRT1.1. Interestingly, when nitrate was treated with the inhibitors EGTA and La^{3+}, some but not the entire PNR was affected, indicating that there are two pathways regulating the PNR; one is a calcium-dependent pathway regulating *NRT2.1* and *TGACG MOTIF-BINDING FACTOR 1* (*TGA1*), and the other is a calcium-independent pathway regulating *AUXIN SIGNALING F-BOX 3* (*AFB3*) (125). Liu et al. (93) revealed how the calcium signal is transmitted downstream by three CALCIUM-SENSOR PROTEIN KINASES (CPKs): CPK10, CPK30, and CPK32. They showed that nitrate induces both calcium accumulation in the nucleus and rapid

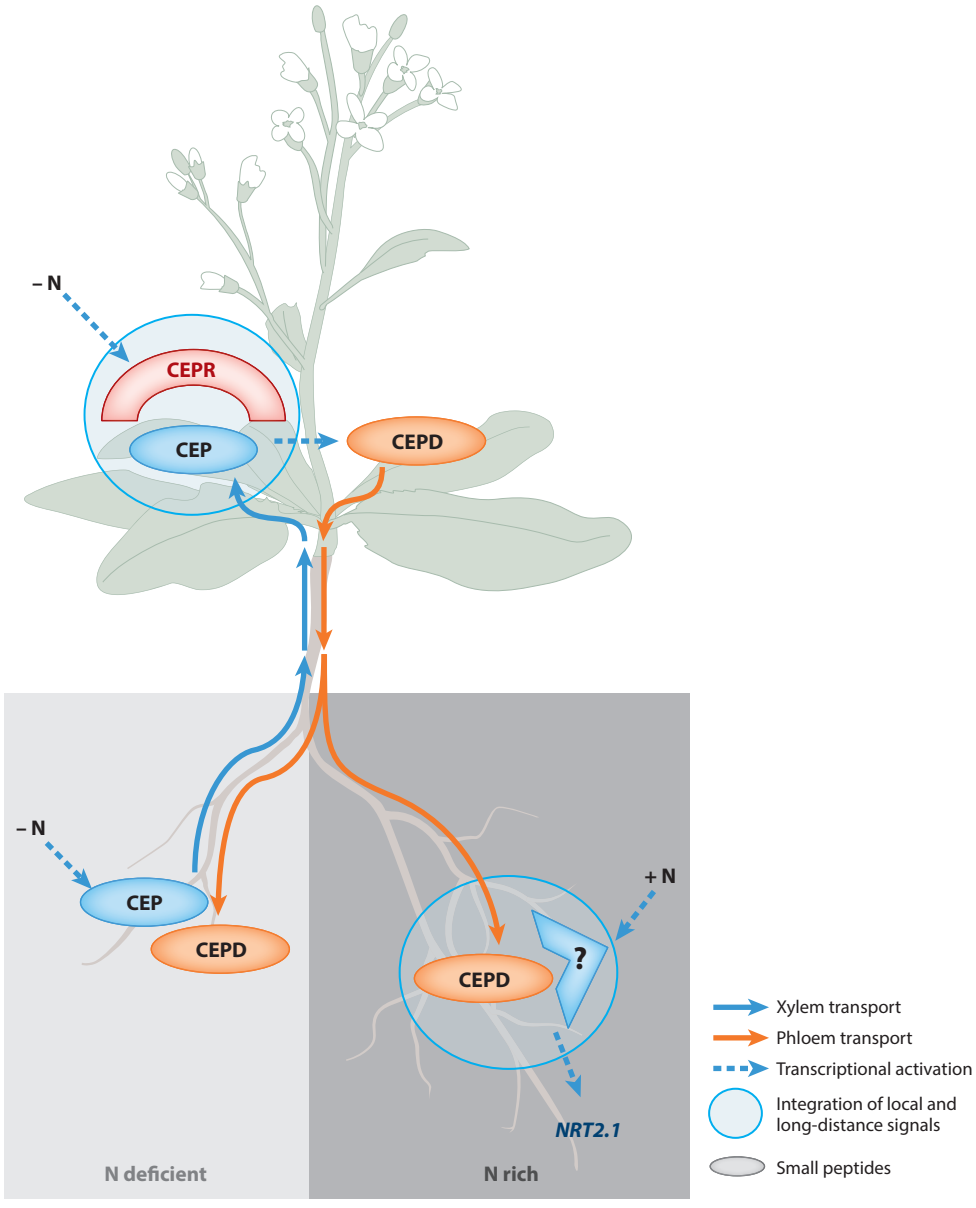


Figure 5

Long-distance communication for N signaling mediated by CEP, CEPR, and CEPD. CEP1 is upregulated by local N deficiency in the root, and transported to the shoot via xylem. When shoots also experience N shortage, the receptors CEPR1 and CEPR2 are expressed. They then perceive root-derived CEP1 in leaf phloem, leading to induction of the nonsecreted small signaling peptides CEPD1 and CEPD2. CEPD1 and CEPD2 are transported equally to both N-deficient and N-rich sites of the root. When CEPD1 and CEPD2 are integrated with the local N-rich signal, NRT2.1 is induced, leading to enhanced nitrate acquisition. Abbreviations: CEP, C-TERMINALLY ENCODED PROTEIN; CEPD, CEP DOWNSTREAM; CEPR, CEP RECEPTOR; N, nitrogen; NRT, NITRATE TRANSPORTER.

nuclear translocation of CPKs. Upon interaction in the nucleus, CPKs can phosphorylate NIN-LIKE PROTEIN 7 (NLP7), which is the master regulator of the PNR. Phosphoproteomic analysis has shown that serine 205 of NLP7 is phosphorylated. The dephosphorylated Ser205A mutant of NLP7 is not retained in the nucleus and cannot rescue the root phenotype of *nlp7*, indicating that calcium functions as a secondary messenger in response to nitrate and then regulates CPK activity to control nuclear retention of NLP7 (93). This finding prompts interesting questions such as whether CPKs can also phosphorylate NLP7 in the cytoplasm to regulate its nuclear import (74) and whether NRT1.1 is also regulated by calcium through CIPK or CBL.

Nitrate response *cis*-element (NRE): a short sequence in the promoter region of genes that can be bound by transcription factors in response to nitrate

3.2.2. Transcription factors. Signals need to be transmitted to the nucleus to regulate gene expression. Several transcription factors involved in the nitrate response have been characterized. Among these, NLP7, BASIC LEUCINE ZIPPER 1 (bZIP1), TGA1, and TEOSINTE BRANCHED 1/CYCLOIDEA/PROLIFERATING CELL FACTOR 1–20 (TCP20) directly interact with nitrate-related target genes. Detailed properties of these transcription factors are discussed below.

3.2.2.1. NLP7 is a master regulator in nitrate signaling. As the master regulator of the PNR, NLP7 could regulate other transcription factors to broadly influence nitrate signaling. NLP7 was first identified by its homology to NITRATE ASSIMILATION REGULATORY PROTEIN (NIT2), which activates NIA1 in response to nitrate in *Chlamydomonas* (12), and by its homology to NODULE INCEPTION (NIN), which activates nodule inception development in legumes (131). In *Arabidopsis*, *nlp7* mutants exhibit an impaired PNR and display a starvation-like root phenotype manifested as a longer primary root and higher lateral root density even in the presence of nitrate (14). Gene expression of *NLP7* is not regulated by nitrate, but it can shuttle into and be retained in the nucleus in response to nitrate (97). ChIP-chip analysis shows that the master regulator NLP7 binds to the promoters of 851 genes when nitrate is present. Among them, 100 genes, including nitrate transporters, nitrate assimilation genes, and transcriptional factors, are nitrate inducible and regulated by NLP7 (97).

Arabidopsis possesses nine members of the NLP family, all of which contain the N-terminal nitrate response region, a RWP-RK DNA-binding domain, and a PB1 protein-protein interacting domain (72, 132). NLP6, the closet homolog to NLP7, represses the PNR when fused to a repressor SUPRD (carboxy-terminal 30 amino acids of SUPERMAN) domain (71). NLP6 also shows nitrate-induced nuclear retention (48). However, the repressive effect of the NLP6-SUPRD line may be caused by inhibition of all NLPs. Thus, the individual role of NLP6 needs to be further clarified. In addition, NLP8 promotes seed germination in response to nitrate by activating CYP707A2, an ABA catabolic enzyme, revealing nitrate signaling crosstalk with ABA signaling in seed germination (161).

Apart from nitrate induction, the NLP family might also participate in nitrate starvation signaling. NLP7 induces nuclear retention in the presence of nitrate to trigger downstream target genes. In the nucleus, NLP7 or NLP6 also interacts with TCP20 under nitrate-free conditions and regulates expression of *NRT1.1*, *NIA1*, and *NIA2*, suggesting that NLPs may also be involved in the nitrate starvation response (48). It will be interesting to establish whether other NLPs play a role in either nitrate or nitrogen starvation signaling because most can bind to the nitrate response *cis*-element (NRE) of NIR and NIA1 (71). Furthermore, NLPs may interact with different family members through the PB1 domain to regulate nitrate or nitrogen starvation responses that are gene, concentration, and time dependent.

3.2.2.2. NRG2 functions in the nitrate response. NRG2 (NITRATE REGULATORY GENE 2) belongs to the bZIP family. NRG2 was identified through genetic screening using a nitrate-responsive promoter fused with a YFP reporter. NRG2 is a positive regulator involved in the nitrate response, partially through regulating expression of *NRT1.1*. NRG2 can interact with NLP7 in the nucleus, but nitrate-induced nuclear retention of NLP7 is not dependent on NRG2 (160). Peak PNR activity usually occurs within 1 h. However, a study of NRG2 recorded the nitrate response 2 h after induction (160), which might be past the peak period of the PNR. It will be interesting to determine whether NRG2 and NLP7 target to the same *cis*-element of PNR genes.

3.2.2.3. LBD37, LBD38, and LBD39 function as negative regulators in the nitrate starvation response. LBD37, LBD38, and LBD39 (LATERAL BOUNDARY DOMAIN-CONTAINING PROTEIN 37, 38, and 39) are negative regulators of anthocyanin biosynthesis. Their mutants show constitutive accumulation of anthocyanin even under nitrogen- and NO_3-sufficient conditions. Comparison between two LBD overexpression lines and the wild type in nitrate-rich and nitrate-depleted conditions showed that LBD37 and LBD38 act more profoundly as repressors of *NRT1.1*, *NRT2.1*, and *NIA1* under nitrate-deficient conditions (98, 126). LBDs are also upregulated by nitrate and NLP7 (97). It will be interesting to determine how LBDs function differently in the presence or absence of nitrate and nitrogen.

3.2.2.4. bZIP1 rapidly and catalytically regulates the nitrate response. bZIP1 integrates light and nitrate signaling (106). As shown by a TARGET (transient assay reporting genome-wide effects of transcription factors) methodological approach, bZIP1 acts through a hit-and-run mechanism that allows transient binding to target genes. However, it does not directly regulate expression of nitrate-related genes such as *NRT2.1*, *NLP3*, *LBD38*, and *LBD39*. Thus, bZIP1 may act as a catalytic transcription factor, recruiting other factors to rapidly regulate nitrate response genes (114).

3.2.2.5. SPL9 is a potential hub in the nitrate response. SPL9 (SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 9) was identified by high-resolution time-course transcriptomics analysis. In silico analysis predicts that SPL9 may act as a hub to affect the nitrate-driven gene network. Overexpression of *SPL9* affects expression levels of only *NIR* and *NIA2* (76).

3.2.2.6. ANR1 functions in lateral root elongation. *ANR1* is a MADS-box gene and was the first transcription factor identified in the nitrate response. It is responsible for lateral root elongation in nitrogen-rich conditions (165). Expression of *ANR1* is induced in lateral primordia and root tips, acting downstream of NRT1.1 to promote lateral root elongation in a split-root system on the nitrate-rich side (43, 123). Interestingly, ANR1 was bound by NLP7 (97), but whether it acts downstream of NLP7 is not known.

3.2.2.7. TGA1 and TGA4 function in root architecture. TGA1 and TGA4 belong to the bZIP family and were first discovered through a bioinformatics approach. TGA1 can bind directly to the promoter regions of *NRT2.1* and *NRT2.2*, and both TGA1 and TGA4 regulate expression of *NRT2.1* and *NRT2.2* (2, 167). Expression of both *TGA1* and *TGA4* is upregulated by nitrate and occurs downstream of NRT1.1 and the calcium signal (125). The phenotype of the *tga1 tga4* double-knockout mutant indicates that TGA1 and TGA4 function in promoting primary root growth, lateral root initiation and emergence, and root hair density in a nitrate-dependent manner. TGA1 and TGA4 may act upstream of NRT2.1 and NRT2.2 to regulate lateral root initiation but downstream of NRT1.1 to regulate root hair growth (2, 13).

3.2.2.8. NAC4 functions in promoting lateral root number in crosstalk with auxin. Nitrate induction of *NAC4* (NAM-ATAF-CCUC DOMAIN-CONTAINING PROTEIN) is controlled by AFB3 and is dependent on auxin signaling (151). In contrast, nitrate induction of *AFB3* occurs downstream of NRT1.1 in the calcium-independent pathway (125). NAC4 participates in nitrate-induced enhancement of lateral root density, but it does not affect primary root growth. The roles of NRT1.1, AFB3, and NAC4 reveal how nitrate signaling is integrated with auxin signaling to regulate lateral root growth.

3.2.2.9. HRS1 and HHO1 repress primary root growth in crosstalk with phosphate deficiency. HRS1/NIGT1 (HYPERSENSITIVITY TO LOW PI-ELICITED PRIMARY ROOT SHORTENING 1/NITRATE-INDUCIBLE GARP-TYPE TRANSCRIPTIONAL REPRESSOR 1) and its closest homolog HRS1 HOMOLOG 1 (HHO1) belong to the GARP family. HRS1 was first identified as a regulator involved in phosphate signaling because the overexpression line displays hypersensitivity to phosphate deficiency in terms of primary root shortening, whereas the double-knockout mutant *hrs1 hho1* (but not the single mutants) shows the opposite phenotype (91, 99). The phosphate-related primary root phenotype of *hrs1 hho1* can be observed only when nitrate is present, indicating that HRS1 and HHO1 integrate nitrate and phosphate signaling (99). Indeed, expression of *HRS1* and *HHO1* is highly induced by nitrate downstream of NRT1.1 and NLP7 (97) and is also induced by phosphate deficiency. NIGT1, the homolog of HRS1 in rice, represses its own expression after nitrate induction, suggesting that it functions as a repressor during negative-feedback regulation (130). Interestingly, in *Arabidopsis*, nitrate-related genes such as *NRT1.1* and *NRT2.4* are also downregulated in the overexpression line and upregulated in the double mutant, suggesting that HRS1 and HHO1 may play a negative role in the nitrate response (99).

3.2.2.10. TCP20 functions in root foraging. TCP20 was identified from a yeast one-hybrid screen using the NRE region of *NRT2.1* and *NIA1*. TCP20 directly binds to the promoters of *NRT1.1*, *NRT2.1*, and *NIA1*. During root foraging, TCP20 participates in systematic nitrate signaling to enhance lateral root growth on the nitrate-rich side of a split-root system and to reduce root growth on the nitrate-poor side. Unlike how TCP20 reacts to systematic signaling, NLP7 reacts to local nitrate signals that function independently during TCP20 and NRT1.1 nitrate-induced lateral root induction (49).

TCP20 also reacts to local signals to regulate expression of *NRT1.1* and *NIA1* on the nitrate-poor side in a split-root system but not on the nitrate-rich side (49). TCP20 interacts with NLP6 or NLP7 in the nucleus, but only under nitrate-free conditions. It also acts in the same pathway as NLP6 and NLP7 to induce *NRT1.1*, *NIA1*, and *NIA2* and to repress *CYCLIN-DEPENDENT PROTEIN KINASE CYCB1;1* (a cell-cycle progression gene). The effect on *CYCB1;1* expression may be responsible for the primary root growth defect observed in *tcp20* mutants (48). It will be interesting to elucidate how TCP20 has a distinct function in systematic and local nitrate signaling and how TCP20 can change its function between high- and low-nitrate conditions.

3.2.2.11. BT1 and BT2 comprise a central hub regulating nitrogen-use efficiency. BT2 (BTB and TAZ DOMAIN PROTEIN 2) has been identified as a regulator under the control of light, sucrose, hormones, and nitrate (96). On the basis of a bioinformatics approach, BT2 has been identified as a potential central hub for the NUE gene network. BT1 is the closest homolog of BT2. Phenotypes of the double mutant and overexpression lines show that only under nitrate-limiting conditions do BT1 and BT2 act as negative regulators to repress plant growth and nitrate-use

efficiency. BT1 and BT2 repress expression of *NRT2.1* and *NRT2.4* and thereby reduce nitrate uptake under nitrate-limiting conditions (4). Expression of *BT1* and *BT2* is induced by nitrate and regulated by NLP6-SUPRD. Indeed, NLP6 and NLP7 can directly bind to the NRE region in the promoter of *BT1* and *BT2* (129). Overexpression of BT2 can rescue the growth defect of the NLP6-SUPRD line. The double mutant *bt1 bt2* shows reduced lateral root length only under high-nitrate conditions, suggesting that BT1 and BT2 play a positive role in plant growth under these conditions. It will be interesting to determine whether BT1 and BT2 target different sets of genes under nitrogen-sufficient and nitrogen-deficient conditions to effect opposing impacts on plant growth.

3.3. Shoot-to-Root and Root-to-Shoot Communication

External nitrate status and internal nitrate and nitrogen status or demands need to be delicately integrated through root-to-shoot, shoot-to-root, and even cell-to-cell communication to intricately regulate nitrate assimilation and plant development. Nitrate is distributed heterogeneously in the soil. As a result, some parts of a root system may experience low nitrate, whereas the rest can be located in a nitrate-rich zone. This patchy distribution of nitrate can be experimentally reproduced with lab-based split-root assays to determine local and systemic signaling. Such experiments reveal that a nitrogen-demand signal is transmitted from the low-nitrogen side of the root system to the high-nitrogen side to enhance lateral root proliferation, *NRT2.1* expression, and nitrate uptake (123).

3.3.1. A CEP1-CEPR1/2-CEPD1/2 relay enhances nitrate acquisition in nitrogen-rich patches. Studies of the relay of C-TERMINALLY ENCODED PEPTIDE 1 (CEP1), CEP RECEPTOR 1 and 2 (CEPR1 and CEPR2), and CEP DOWNSTREAM (CEPD) have revealed multiple layers of integration between local and systemic signals in roots and shoots to orchestrate both the soil microenvironment and internal demand, thereby stimulating nitrate acquisition in the nitrate-rich patch. The secreted small signaling peptide CEP1 is upregulated by local nitrogen deficiency in roots and translocated to the shoots via xylem to notify shoots about the root nitrogen status (144). In the leaf, root-derived CEP1 is concentrated within leaf vascular bundles and reaches phloem cells where CEPR1 and CEPR2, which are leucine-rich repeat receptor kinases, are expressed (109). Expression of *CEPR1* and *CEPR2* is also upregulated by local nitrogen deficiency in a CEP1-independent manner (109). Thus, the root-derived nitrogen starvation signal needs to be reinforced and bolstered by a local nitrogen starvation signal in the shoot to trigger the next responsive step. This linkage represents the first step of communication between local and systemic signals on nitrogen status.

When CEP1 is perceived by CEPR1 and CEPR2 in leaf phloem, expression of the nonsecreted small signaling peptides CEPD1 and CEPD2 (in the class III glutaredoxin family) is induced (109). CEPD1 and CEPD2 are then equally translocated via phloem to both the high- and low-nitrogen sites of a split-root system. However, CEPD1-mediated upregulation of *NRT2.1* occurs only at the nitrogen-rich site of that split-root system (109). As a result, to ensure energy nitrate acquisition is not wasted at the nitrogen-depleted site, *NRT2.1* is induced only when the CEPD1- or CEPD2-mediated systematic nitrogen-demand signal is well integrated with the local nitrogen-rich signal. This crosstalk is the second step of communication between local and systemic signals.

In roots, shoot-derived CEPD1 is predominantly found in phloem. Thus, other players may be necessary to mediate communication between phloem and epidermal cells where *NRT2.1* is mainly expressed and nitrate is acquired from the soil. With several potential derivative routes, the relay of CEP1-CEPR1/2-CEPD might not be a simple linear one.

3.3.2. CLE-CLV1/HAR1 peptide-receptor pathways inhibit root development and nodulation in a nitrogen-dependent manner. Expression of *CLE1*, *CLE3*, *CLE4*, and *CLE7*, each of which encodes secreted signaling peptides of the CLAVATA3/EMBRYO-SURROUNDING REGION (CLE3)-related family, are induced under nitrogen-deficient conditions (5). Overexpression of these small signaling peptides inhibits the emergence of lateral roots. The receptor kinase CLAVATA1 (CLV1) is required for CLE3-mediated inhibition of lateral root development. In roots, *CLE3* is mainly expressed in pericycle cells, whereas *CLV1* is expressed in phloem company cells, suggesting that they function in a non-cell-autonomous way and that either cell-to-cell or long-distance communication is required. In fact, *CLV1* is also expressed in shoots, but it remains to be determined whether root-to-shoot-to-root communication is required for this signaling module to restrict root development under nitrogen-deficient conditions.

In *Lotus japonicus*, the peptide-receptor CLE-RS-HAR1 signaling pathway is involved in root-to-shoot-to-root negative-feedback control of nodulation (77, 104, 111). Earlier-formed nodules or rhizobia infection induces expression in roots of *CLE ROOT SIGNAL 1* (*CLE-RS1*) and *CLE-RS2* by the RWP-RK-containing transcription factor NIN to systematically inhibit further nodule formation. In addition, the leucine-rich repeat receptor kinase HYPERNODULATION ABERRANT ROOT 1 (HAR1) in shoots is required for the inhibitory effect of CLE-RS1 and CLE-RS2 (111, 139), indicating that root-to-shoot-to-root long-distance communication is involved. The same long-distance signaling pathway is responsible for nitrate-mediated inhibition of nodulation (110).

4. NITRATE- AND NITROGEN-USE EFFICIENCY

Production of nitrogen fertilizer consumes 1–2% of global energy, but crops use only 30–50% of applied nitrogen. Negatively charged nitrate cannot be easily preserved in the negatively charged soil matrix and it is quickly leached out, causing severe environmental problems (e.g., eutrophication and emission of the greenhouse gas nitrous oxide). To reduce the input cost of farming and to alleviate the impact of eutrophication, enhancing NUE is an urgent issue in agriculture. NUE is a complicated agronomic trait involving the multiple interconnected steps of assimilation, transport, and signaling. Indeed, many transgenic approaches have shown that manipulating the expression of genes involved in nitrogen transport, assimilation, and signaling can enhance crop growth or grain yield (see **Table 2**).

Several studies have shown that manipulation of nitrogen-assimilation enzymes is a feasible strategy for improving crop NUE. For example, GLUTAMINE SYNTHETASE (GS) incorporates ammonium into glutamine through the GS-GOGAT (Glutamine synthetase-Glutamate synthase) cycle, a crucial step for converting inorganic nitrogen into organic nitrogen in plants. Expression of *OsGS1* driven by a *ubiquitin* promoter in rice and expression of *SbGln* driven by a *35S* promoter in sorghum can lead to increased grain yield (10, 150).

By contrast, overexpression of transporters in the NRT1 (NPF) and NRT2 families also enhances NUE. For example, overexpression of rice *OsNRT2.3b* not only enhanced nitrate and iron uptake but also improved yield under both low- and high-nitrogen conditions in the field (35). Transporters are usually expressed specifically in certain tissues or cells. There can be negative effects on NUE and yield when they are expressed constitutively and ubiquitously in most tissues. For instance, introducing *OsNRT2.1* driven by a *ubiquitin* promoter into rice led to decreased NUE and grain yield, but introducing *OsNRT2.1* driven by the nitrate-inducible promoter *OsNAR2.1p* had the opposite effect (18). Therefore, for some transporters, a specific promoter is required for genetic manipulation to improve crop NUE.

Table 2 Transgenic approaches to improve plant growth or grain yield

Gene name and product	Promoter	Target plant	Physiological trait enhanced	Biomass increase	Improved yield	N condition	Growth condition	Reference(s)
Transporter								
AtSTP13 Hexose transporter	*CaMV 35S*	*Arabidopsis*	<*NRT2.2*>, [NO_3], [N], [amino acid]	75%	NA	HN: 9 mM NO_3	Plate	134
AtAVP1 Vacuolar pyrophosphatase 1	*CaMV 35S*	Lettuce	<*LsNRT2.1*>, {H^+-ATPase}, {H^+-ATP hydrolysis}	83% in $HN_{Pot, 0.8}$ 32% in $HN_{Pot, 0.4}$ 55% in $MN_{Pot, 0.2}$ 43% in $MN_{Pot, 0.1}$ 40% in LN_{Pot}	21% in $HN_{Field, 93}$ 49% in $HN_{Field, 70}$ 38% in $MN_{Field, 46}$ 45% in $MN_{Field, 23}$ 37% in LN_{Field}	HN_{Pot}: 0.4, 0.8 g N; HN_{Field}: 70, 93 kg N/ha MN_{Pot}: 0.1, 0.2 g N; MN_{Field}: 23, 46 kg N/ha LN_{Pot}: 0 g N; LN_{Field}: 0 kg N/ha	Pot Field	113
OsPTR6 PTR/NRT1 transporter	*Ubiquitin*	Rice	<*OsAMT1.1*>, <*OsAMT1.2*>, <*OsAMT1.3*>, {Glutamine synthetase}, [N]	$(17–166\%)_{SDW}$ in HN $(33–120\%)_{SDW}$ in LN $(22–177\%)_{RDW}$ in HN	NA	HN: 5 mM NH_4, 5 mM NO_3, 2.5 mM NH_4NO_3 LN: 0.2 mM NH_4, 0.2 mM NO_3	Hydro	36
OsAMT1;1 Ammonium transporter	*Ubiquitin*	Rice	$\{NH_4\}_{uptake}$, [NH_4], [Glutamine]	24% in HN↓ 33% in MN_{30}	21% in MN_{300} 36% in MN_{30}	HN: 3,000 μM NH_4 MN: 30, 300 μM NH_4 LN: 0.3, 3 μM NH_4	Hydro	122
OsNRT1.1B-indica Nitrate transporter	*CaMV 35S/ OsNRT1.1B*	Rice	NUE_{yield}	NA	9–11% in HN 26–30% in LN	HN: 200 kg N/ha LN: 100 kg N/ha	Field	57
OsNRT2.1 Nitrate transporter	*Ubiquitin*	Rice	$[N]_{leaf}$, $[N]_{culm}$, ANUE↓	13% in HN 15% in LN	17% in HN↓ 19% in LN↓	HN: 139 kg N/ha LN: 84 kg N/ha	Field	18
	OsNAR2.1		$[N]_{panicle}$, $[N]_{culm}$, ANUE	23% in HN 30% in LN	21% in HN 22% in LN			

(*Continued*)

Table 2 (*Continued*)

Gene name and product	Promoter	Target plant	Physiological trait enhanced	Biomass increase	Improved yield	N condition	Growth condition	Reference(s)
OsNRT2.3b Nitrate transporter	*CaMV 35S*	Rice	$\{N\}_{uptake}$, $\{Fe\}_{uptake}$, $\{P\}_{uptake}$, NUE_{yield}	NA	40% in HN 41% in LN	HN: 102 kg N/ha LN: 51 kg N/ha	Field	35
OsNAR2.1 Nitrate associate protein	*OsNAR2.1*	Rice	<*OsNRT2.1*>, <*OsNRT2.2*>, <*OsNRT2.3a*>, $\{N\}_{uptake}$, [N], ANUE	25%	22%	139 kg N/ha	Field	17
OsNPF7.3 Nitrate/peptide transporter	*CaMV 35S*	Rice	<*OsGS1.1*>, [Amino acid], [N], [Protein], NUE_{yield}	21%	6%	34 kg N/ha	Field	37
PsAAP1 Amino acid permease 1	*AtAAP1*	Pea	$\{N\}_{uptake}$, $[\text{Amino acids}]_{leaf}$, NUE_{yield}	$(13\%)_{SDW}$ in HN $(11\%)_{SDW}$ in MN $(22\%)_{SDW}$ in LN	17% in HN 17% in MN 40% in LN	LN: 2 g NH_4NO_3 MN: 4 g NH_4NO_3 HN: 8 g NH_4NO_3	Pot	118
N assimilation related								
HvAlaAT Alanine aminotransferase	*btg26*	Canola	$\{NO_3\}_{uptake}$	$(5–29\%)_{SDW}$ in HN_{Pot} $(33–75\%)_{SDW}$ in LN_{Pot}	33% in HN_{Field} 42% in LN_{Field}	HN_{Pot}: 5 mM urea; HN_{Field}: 168 kg N/ha LN_{Pot}: 0.5 mM urea; LN_{Field}: 56 kg N/ha	Pot Field	46
HvAlaAT Alanine aminotransferase	*OsAnt1*	Rice	$\{N\}_{uptake}$, $[N]_{shoot}$	51%	75%	NO_3 + NH_4NO_3 + urea	Pot	136
EcAsnA Asparagine synthetase A	*pMAC*	Lettuce	[Asparagine], [Aspartic acid], [Glutamine], [Chlorophyll a], [Protein]	39%	NA	0.24 g NH_4NO_3	Pot	44
EcAAT/OsAAT1-3 Aspartate aminotransferase	*CaMV 35S*	Rice	$[NO_3]_{leaves\ of\ OsAAT1/2}$ $[\text{Amino acid}]_{seed\ of\ OsAAT1/2\ and\ EcAAT}$	NA	NA	NA	NA	168

(*Continued*)

Table 2 (*Continued*)

Gene name and product	Promoter	Target plant	Physiological trait enhanced	Biomass increase	Improved yield	N condition	Growth condition	Reference(s)
OsGS1;2 Glutamine synthetase	*Ubiquitin*	Rice	$[N]_{spikelet}$, UtE	NA	40% in HN 50% in HN→LN	HN: 10 mM KNO_3 LN: 3 mM KNO_3	Pot	10
DvGS1/2 Glutamine synthetase	*CaMV 35S*	*Arabidopsis*	[N], [Protein]	$(35–55\%)_{FW}$ in HN $(22–58\%)_{FW}$ in LN	17% in HN of DvGS1 114% in LN of DvGS2	HN: 9 mM KNO_3 LN: 2 mM KNO_3	Hydro	169, 170
SbGln1 Glutamine synthetase	*CaMV 35S*	Sorghum	NA	$(108\%)_{SDW}$ in HN	28% in HN	HN: 12 mM NO_3 + 2 mM NH_4 LN: 4 mM NO_3	Pot	150
Transcription factor								
OsENOD93-1 Early nodulin 93-1	*Ubiquitin*	Rice	[Amino acid]	$(11\%)_{SDW}$ in HN $(14\%)_{SDW}$ in LN	14% in HN 21% in LN	HN: 10 mM NO_3 LN: 3 mM NO_3	Pot	7
TaNAC2-5A NAC transcription factor	*Ubiquitin*	Wheat	<*NRT2.1*>, <*NRT2.2*>, <*NPF7.1*>, <*NPF7.2*>, <GS2>, $[N]_{shoot}$, $\{NO_3\}_{uptake}$	$(14\%)_{SDW}$ in HN_{Pot} $(13\%)_{SDW}$ in LN_{Pot}	11% in HN_{Field} 8% in LN_{Field}	HN_{Pot}: 100 mg $Ca(NO_3)_2$/kg soil HN_{Field}: 84 kg N/ha LN_{Pot}: 10 mg $Ca(NO_3)_2$/kg soil LN_{Field}: 0 kg N/ha	Field Pot	53
	CaMV 35S	*Arabidopsis*	<*NPF4.6*>, <*NPF7.3*>, <*NRT2.1*>, $\{NO_3\}_{uptake}$	$(4\%)_{PRL}$ in HN $(13\%)_{PRL}$ in LN	NA	HN: 6 mM KNO_3 LN: 0.2 mM KNO_3	Plate	
TaNFYA-B1 NFY transcription factor	*Ubiquitin*	Wheat	<*TaNRT1.1*>, <*TaNRT2.1*>, <*TaPHT1.1–7/9*>, $\{NO_3\}_{uptake}$	NA	13% in HN 19% in LN	HN: 84 kg N/ha LN: 0 kg N/ha	Field	121

(*Continued*)

Table 2 (*Continued*)

Gene name and product	Promoter	Target plant	Physiological trait enhanced	Biomass increase	Improved yield	N condition	Growth condition	Reference(s)
AtTGA4 bZIP transcription factor	*CaMV 35S*	*Arabidopsis*	[N]	$(59–62\%)_{RL}$	NA	0.15 mM NH_4NO_3	Plate	167
TabHLH1 bHLH-type transcription factor	*CaMV 35S*	Tobacco	<*NtNRT2.2*>, {SOD}, {CAT}, {POD}, [N]	$(44–90\%)_{SDW}$	NA	LN: 0.1 mM N	Pot	162
NLP7 NIN-like protein 7	*CaMV 35S*	*Arabidopsis*	<*NRT1.1*>, <*NRT2.1*>, <*GS2*>, <*NIA1, 2*>, <*NIR1*>, <*NLA*>, <*LBD37/38*>, {NR}, $\{N\}_{uptake}$, {photosynthesis}, {carbon assimilation}, [Glutamate], [Glutamine], [NO_3], [Protein]	$(39\%)_{FW}$ in HN $(50\%)_{FW}$ in MN $(50\%)_{FW}$ in LN	NA	HN: 10 mM NO_3 MN: 3 mM NO_3 LN: 1 mM NO_3	Plate	163
	Tobacco	<*NtNRT2.1*>, <*NtGln1–5*>, <*NtNii1/2/4*>	$(20\%)_{FW}$ in HN $(17\%)_{FW}$ in MN $(17\%)_{FW}$ in LN	NA				
AtBT2 BTB protein	*CaMV 35S*	*Arabidopsis*	<*NRT2.1*>↓, <*NRT2.4*>↓, $\{NO_3\}_{uptake}$↓, aNUE↓	$(50\%)_{Early\text{-}stage\ growth}$↓	NA	0.5 mM KNO_3	Pot	3

(*Continued*)

Table 2 (*Continued*)

Gene name and product	Promoter	Target plant	Physiological trait enhanced	Biomass increase	Improved yield	N condition	Growth condition	Reference(s)
ZmDof1 DNA binding with one finger	*Ubiquitin*	Wheat	{PEPC}, NUE_{rbcs1}	40% in HN_{Hydro}↓ 19% in LN_{Hydro}↓	0% in HN_{Field} 0% in LN_{Field}	HN_{Hydro}: 15 mM KNO_3 HN_{Field}: 95.3 kg N/ha LN_{Hydro}: 0.3 mM KNO_3 LN_{Field}: 53.8 kg N/ha	Hydro Field	117
	rbcS1			12% in HN_{Hydro} 19% in LN_{Hydro}	45% in HN_{Field} 45% in LN_{Field}			
Other								
MbSnRK1 Snf1 protein kinase	*CaMV 35S*	Tomato	{Photosynthesis}, $\{N\}_{uptake}$, [N]	(11 and 17%)$_{SDW}$ (14 and 44%)$_{FDW}$	NA	NA	Pot	155
GmATG8c Autophagy-related genes	*CaMV 35S*	*Arabidopsis*	[N]	(41%)$_{FW}$ HN (50%)$_{FW}$ HN→LN	6–22% in HN	HN: 1/2 Hoagland's solution LN: N-free solution	Pot	158

Abbreviations: ANUE, agricultural NUE [measurement: (grain yield − grain yield of zero N plot)/N supply]; aNUE, agronomic NUE (measurement: seed number per plant/N applied); ATG, autophagy-related gene; BT2, BTB and TAZ domain protein 2; btg26, *Brassica* turgor gene 26, a gene expressed specifically in root; CAT, catalase; FDW, fruit dry weight; FW, fresh weight; HN, high nitrogen; Hydro, hydroponic system; LBD, LATERAL BOUNDARY DOMAIN-CONTAINING PROTEIN; LN, low nitrogen; MN, medium nitrogen; N, nitrogen; NA, not applicable; NIA, nitrate reductase; NIR, nitrite reductase; NLA, nitrogen limitation adaptation; NPF, nitrate transporter 1 (NRT1)/peptide transporter (PTR) family; NR, nitrate reductase; NUE_{yield}, nitrogen-use efficiency (measurement: yield/nitrogen supplied); OsAnt1, promoter of aldehyde dehydrogenase gene; PEPC, phosphoenolpyruvate carboxylase; PRL, primary root length; pMAC, hybrid promoter of fragments of mannopine synthetase and 35S enhancer; POD, peroxidase; rbcS1, rubisco subunit 1 promoter; RDW, root dry weight; RL, root length; SDW, shoot dry weight; SOD, superoxide dismutase; UtE, utilization N use efficiency (measurement: spikelet yield/shoot nitrogen content).

Angle brackets indicate gene expression, square brackets indicate content, curly brackets indicate activity, down arrow indicates decrease, and right arrow indicates shift to; blue-colored text indicates a decrease of the trait.

Over the past decade, more and more transcription factors involved in regulating nitrogen and nitrate signaling have been identified. Manipulating some of these transcription factors in various crops could increase yield (**Table 2**) and have a broader impact on the expression of several genes involved in nitrogen uptake, transport, and assimilation. For example, expression of the nitrate transporters *NRT2.1*, *NRT2.2*, *NPF7.1*, and *NPF7.2*, as well as GS2, is simultaneously increased in NAC2-overexpressing wheat (53). Expression of *NRT1.1*, *NRT2.1*, *NIA*, *NIR1*, and GS2 is increased in NLP7-overexpressing *Arabidopsis* (163). However, in the case of *BT2* overexpression, growth inhibition under low-nitrogen conditions is due to repression of two nitrate transporter genes, *NRT2.1* and *NRT2.4*, and no growth inhibition was found under normal nitrogen conditions (4). This finding suggests that care must be exercised when manipulating transcription factors, as their effects might rely on additional factors and growth conditions.

Several of the cases presented in **Table 2** were performed in pot or hydroponic systems. The real challenge is to establish whether growth behavior observed in the laboratory can be extended to field tests and eventually become adopted agricultural practices. Moreover, for some of the cases, improvements were found in either high-nitrogen or nitrogen-limited conditions, suggesting that multiple strategies need to be integrated to enhance plant growth under the full range of nitrogen statuses. For example, to fully boost NUE, manipulation of the genes involved in nitrogen transport, assimilation, signaling, and regulation of the nitrogen/carbon balance needs to be stacked to maximize NUE. To avoid deploying genetically modified organisms in the field, marker-aided molecular breeding can be used to improve NUE. The single-nucleotide polymorphism identified in *OsNRT1.1B/NPF6.5* could be a good example for applying this approach (57). With the identification of more new components of nitrate transport and signaling, as well as the development of new techniques such as CRISPR/Cas, combined with good practices of fertilizer application and field management, it could be feasible in the near future to reduce the consumption of nitrogen fertilizer and simultaneously maintain or even increase crop yield.

SUMMARY POINTS

1. Nitrate transporters in the NPF and NRT2 family regulate different layers of nitrate movement within plants, including nitrate acquisition, root-to-shoot transport, and leaf allocation.
2. The nitrate transceptor NRT1.1 functions as a dual-affinity transporter and nitrate sensor in response to various nitrate concentrations by using phosphorylation and dephosphorylation to switch between dual-affinity binding and to induce different nitrate response levels.
3. *Arabidopsis* NPF genes play diverse roles in plant development and growth owing to various substrates.
4. Understanding the physiological functions of NPF genes in plants will provide us with a new vision for improving food supply.
5. Calcium is a secondary messenger that transmits nitrate signaling from the NRT1.1 sensor downstream by activating CPKs that phosphorylate NLP7 (which is then retained in the nucleus) to trigger the nitrate response.
6. NLP7 is the main regulator in the nitrate response, and together with other transcription factors, it coordinates a complex gene network regulating this response as well as crosstalk with other signaling pathways.

7. Manipulating genes involved in nitrogen uptake/transport, assimilation, and signaling can be a feasible approach to improving NUE.

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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93. Reveals how calcium regulates CPKs in response to nitrate as a secondary messenger to transmit signals to downstream NLP7.

94. Demonstrates that the dual-affinity transport activity of NPF6.3/CHL1 is mediated by phosphorylation at the Thr101 residue.

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105. Identifies NPFs as glucosinolate transporters.

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