

# Nitrate signaling and use efficiency in crops

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## ABSTRACT

Nitrate ( $\text{NO}_3^-$ ) is not only an essential nutrient but also an important signaling molecule for plant growth. Low nitrogen use efficiency (NUE) of crops is causing increasingly serious environmental and ecological problems. Understanding the molecular mechanisms of  $\text{NO}_3^-$  regulation in crops is crucial for NUE improvement in agriculture. During the last several years, significant progress has been made in understanding the regulation of  $\text{NO}_3^-$  signaling in crops, and some key  $\text{NO}_3^-$  signaling factors have been shown to play important roles in  $\text{NO}_3^-$  utilization. However, no detailed reviews have yet summarized these advances. Here, we focus mainly on recent advances in crop  $\text{NO}_3^-$  signaling, including short-term signaling, long-term signaling, and the impact of environmental factors. We also review the regulation of crop NUE by crucial genes involved in  $\text{NO}_3^-$  signaling. This review provides useful information for further research on  $\text{NO}_3^-$  signaling in crops and a theoretical basis for breeding new crop varieties with high NUE, which has great significance for sustainable agriculture.

**Keywords:** nitrate signaling, nitrogen use efficiency, nitrate-regulatory gene, nitrate regulation, crop

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## INTRODUCTION

Nitrogen (N) is an essential nutrient for plant growth and has profound effects on crop productivity. During the last several decades, large amounts of chemical N fertilizer have been applied to fields to obtain high crop yields (Godfray et al., 2010; Liu et al., 2013). However, only 30–40% of the applied N can be absorbed by crops, and a large portion is lost to the environment, resulting in severe environmental pollution and ecological damage (Good et al., 2004; Coskun et al., 2017; Li et al., 2017). N use efficiency (NUE) is a complex agronomic trait that describes the efficiency of N uptake and utilization by crops. It mainly includes N uptake efficiency (NUpE) and N utilization (assimilation) efficiency (NUtE) (Raun and Johnson, 1999; Good et al., 2004; Xu et al., 2012). Improving crop NUE is the key to solving these problems. The processes of N uptake and utilization are finely controlled by complex and delicate regulatory networks. Therefore, it is important to elucidate the regulatory factors and underlying mechanisms in order to promote sustainable agriculture.

Nitrate ( $\text{NO}_3^-$ ) is the predominant form of N absorbed by most crops such as maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), and barley (*Hordeum vulgare* L.) (Crawford and Glass, 1998). Plants absorb  $\text{NO}_3^-$  mainly through  $\text{NO}_3^-$  transporters (NRT1 and NRT2 families), and it can then be translocated, stored, or assimilated into amino acids by  $\text{NO}_3^-$  assimilation proteins (NR, NiR, GS, and GLN) (Vidal and Gutierrez, 2008; Girin et al., 2014).  $\text{NO}_3^-$  also functions as an important signaling molecule to regulate plant growth and development. According

to the time period when plants respond to  $\text{NO}_3^-$ ,  $\text{NO}_3^-$  signaling can be divided into short-term and long-term effects (Medici and Krouk, 2014; Zhao et al., 2018b). More and more regulators involved in short- and long-term  $\text{NO}_3^-$  signaling have been identified, and some have been found to coordinately regulate multiple processes, including  $\text{NO}_3^-$  transport and assimilation, root system architecture, and interactions of plant and environmental factors to affect yield and NUE (Alboresi et al., 2005; Wilkinson et al., 2007; Krouk et al., 2010; Zhao et al., 2018b). Therefore, key regulators involved in  $\text{NO}_3^-$  signaling may play important roles in the NUE of crops.

Over the past decade, an increasing number of factors that function in  $\text{NO}_3^-$  signaling have been identified, mainly in the model plant *Arabidopsis*, and several fine review papers have summarized these advances (Wang et al., 2012, 2018b; Mu and Luo, 2019). Great progress has also been made in the last few years in identifying important regulators of crop  $\text{NO}_3^-$  signaling, although this work has not been reviewed to date. In addition, scientists have discovered some critical factors that contribute to crop NUE, and several excellent review papers have recently summarized these findings (Xu et al., 2012; Han et al., 2015; Li et al., 2017; Wang et al., 2018b, 2020b; Yu et al., 2022). However, no recent reviews have focused on the functions of  $\text{NO}_3^-$  signaling regulators in the NUE of crops. Here, we summarize major advances in  $\text{NO}_3^-$  signaling of crops from three

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aspects: short-term signaling, long-term signaling, and the impact of environmental factors. We also review the functions of NO<sub>3</sub><sup>-</sup>-signaling regulators in the NUE of crops. This review provides important information for regulation research on crop NO<sub>3</sub><sup>-</sup> signaling and a theoretical basis for improving the NUE of crops.

## SHORT-TERM NITRATE SIGNALING

Short-term NO<sub>3</sub><sup>-</sup> signaling is defined as the primary nitrate response (PNR) (Medici and Krouk, 2014; Liu et al., 2019). The PNR is a series of early events that occur in plants after NO<sub>3</sub><sup>-</sup> provision and cause changes in the expression of thousands of genes related to NO<sub>3</sub><sup>-</sup> metabolism (Stitt, 1999; Scheible et al., 2004). A few key components of the regulation of NO<sub>3</sub><sup>-</sup> signaling in crops have been discovered in the last several years.

### NRT1.1

In *Arabidopsis*, NRT1/PTR FAMILY 6.3/NITRATE TRANSPORTER 1.1 (*AtNPF6.3/NRT1.1*) has been reported to be the first NO<sub>3</sub><sup>-</sup> sensor, and it plays an essential role in both NO<sub>3</sub><sup>-</sup> signaling and metabolism (Tsay et al., 1993; Liu and Tsay, 2003; Ho et al., 2009; Wang et al., 2009). *OsNRT1.1A* (*OsNPF6.3*), *OsNRT1.1B* (*OsNPF6.5*), and *OsNRT1.1C* (*OsNPF6.4*) are the rice homologs of *AtNRT1.1* (Wang et al., 2018a). In the *osnrt1.1b* mutant, the induction of *OsNIA1* and *OsNIA2* after NO<sub>3</sub><sup>-</sup> treatment is greatly repressed, indicating that *OsNRT1.1B* functions in NO<sub>3</sub><sup>-</sup> signaling (Hu et al., 2015). *OsNRT1.1A* is located mainly on the vacuole membrane. Unlike *OsNRT1.1B*, *OsNRT1.1A* functions mainly in upregulating the expression of N utilization-related genes under NO<sub>3</sub><sup>-</sup> and ammonium conditions (Wang et al., 2018a). In maize, *ZmNPF6.6* and *ZmNPF6.4* have been found to transport NO<sub>3</sub><sup>-</sup> and chloride (Wen et al., 2017). However, whether both genes are involved in NO<sub>3</sub><sup>-</sup> signaling has not been reported. In wheat, 331 homologous NPF genes have been identified (Wang et al., 2020a). Again, which of these wheat homologs regulates NO<sub>3</sub><sup>-</sup> signaling remains unknown.

### Transcription factor (TF) NLPs

Increasing evidence has shown that *Arabidopsis* NIN-LIKE PROTEIN 7 (NLP7) plays a central role in the regulation of NO<sub>3</sub><sup>-</sup> signaling, and there has been great research interest in its regulatory mechanisms (Guan et al., 2017; Liu et al., 2017; Mu and Luo, 2019; Zhao et al., 2018b). *OsNLP3* is the closest rice homolog to *AtNLP7*, and the *osnlp3* mutant shows reduced expression of *OsNRT2.1*, *OsNIA1*, and *OsNir1* after NO<sub>3</sub><sup>-</sup> treatment (Chardin et al., 2014). Moreover, *OsNLP3* can directly bind to NO<sub>3</sub><sup>-</sup> response element (NRE)-like *cis*-elements in the promoters of *OsNIA1*, *OsNIA3*, *OsNRT1.1B*, *OsNRT2.4*, and *OsGRF4* and activate their expression (Zhang et al., 2022). *OsNLP4* also binds to the promoter of *OsNir* and activates its expression (Yu et al., 2021). These results indicate that *OsNLP3/4* are involved in regulating NO<sub>3</sub><sup>-</sup> signaling. Furthermore, *OsSPX4*, a phosphate (Pi)-signaling repressor, can interact with *OsNLP3* to inhibit its NO<sub>3</sub><sup>-</sup>-induced cytoplasmic–nuclear shuttling (Hu et al., 2019). In the presence of NO<sub>3</sub><sup>-</sup>, *OsNRT1.1B* is able to promote the ubiquitination and degradation of *OsSPX4* by recruiting the E3 ubiquitin ligase NBIP1 (NRT1.1B interacting protein 1). Degradation of *OsSPX4* then releases *OsNLP3* to shuttle from the cytoplasm to the nucleus, thereby activating

the NO<sub>3</sub><sup>-</sup> response (Hu et al., 2019). Thus, the *OsNRT1.1B*–*SPX4*–*NLP3* module plays important roles in regulating NO<sub>3</sub><sup>-</sup> signaling from the cell membrane to the nucleus. Interestingly, *OsNRT1.1A* can promote the localization of *OsNLP3* and *OsNLP4* in the nucleus in the absence of NO<sub>3</sub><sup>-</sup>, implying that *OsNRT1.1A* may regulate NO<sub>3</sub><sup>-</sup> signaling by promoting cytoplasmic–nuclear shuttling of NLPs (Wang et al., 2018a) (Figure 1). In maize, *ZmNLP3.1/5/6/8* can bind to NRE elements in the promoters of NO<sub>3</sub><sup>-</sup> response-related genes to activate their expression, thereby regulating NO<sub>3</sub><sup>-</sup> signaling (Cao et al., 2017; Wang et al., 2018c, 2020c; Zhao et al., 2018b) (Figure 2A). In *Triticeae* crops, only barley *HvNLP2* has been found to function in the regulation of NO<sub>3</sub><sup>-</sup> signaling by binding to and activating the expression of *HvNIR1*, *HvNIR*, and *HvNRT2.1* (Gao et al., 2022). Functional characterization of other NLPs in NO<sub>3</sub><sup>-</sup> signaling in rice, maize, and barley, as well as NLPs in other crops, has not yet been reported.

### Other factors

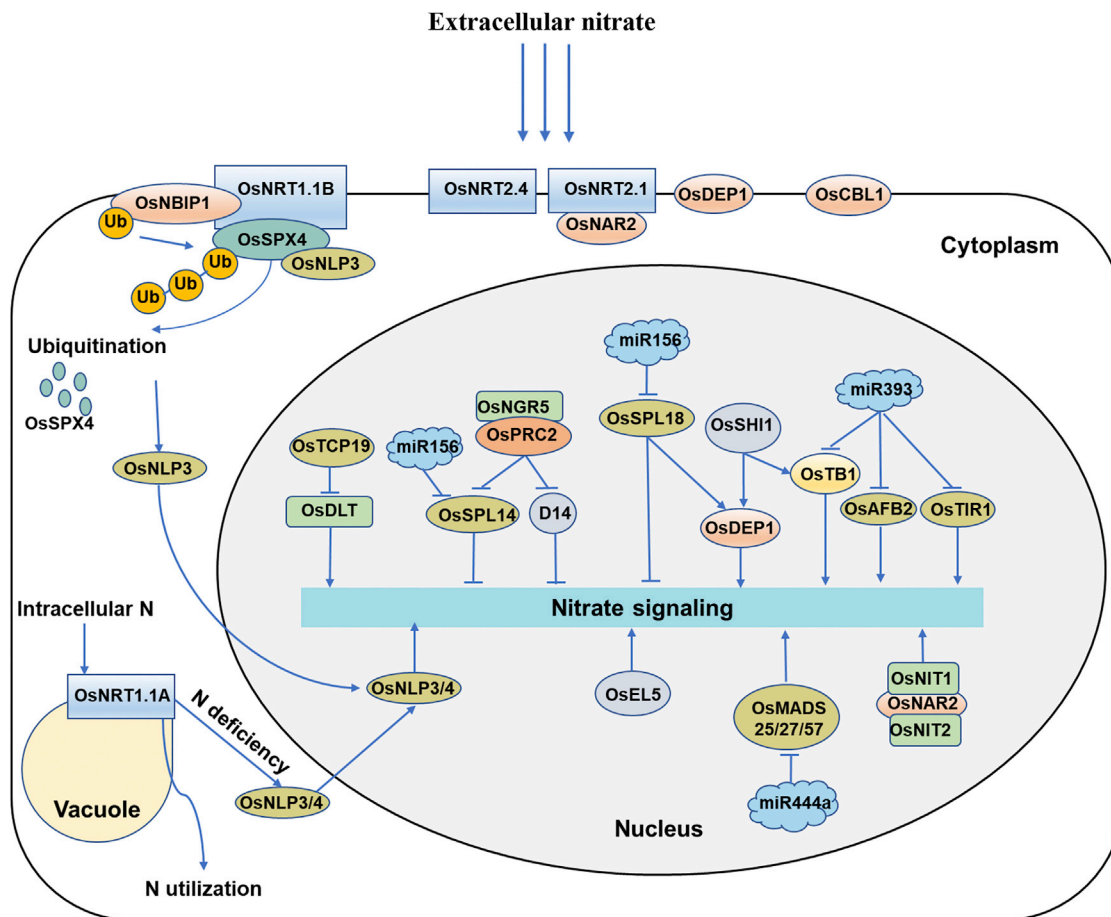
In addition to the NRT1.1s and NLPs described above, a few key factors have been identified as regulating the short-term NO<sub>3</sub><sup>-</sup> response in crops. Calcium (Ca<sup>2+</sup>) serves as an essential nutrient and a signaling molecule, and CALCINEURIN B-LIKE proteins (CBLs) are a unique group of Ca<sup>2+</sup> sensors that have been found to participate in various stress responses and plant nutrient acquisition (Tang et al., 2020). After NO<sub>3</sub><sup>-</sup> treatment in rice, the expression of *OsNRT2.1*, *OsNRT2.2*, and *OsNAR2.1* is decreased in the *oscb1* mutant, suggesting that *OsCBL1* is required for the regulation of NO<sub>3</sub><sup>-</sup> signaling (Hu et al., 2021) (Figure 1). Chromatin-remodeling complexes (CRCs) play essential roles in plant growth and development (Sarnowska et al., 2016; Song et al., 2021). *ZmCHB101* is the core subunit of the SWI/SNF-type ATP-dependent CRCs in maize (Meng et al., 2020). In the absence of NO<sub>3</sub><sup>-</sup>, *ZmCHB101* binds to the NREs of *ZmNRT2.1* and *ZmNRT2.2*, antagonizing the binding of *ZmNLP3.1* to the NREs. In the presence of NO<sub>3</sub><sup>-</sup>, the binding affinity of *ZmCHB101* for the NREs is decreased, resulting in increased binding of *ZmNLP3.1* to the NREs (Wang et al., 2018c; Meng et al., 2020) (Figure 2A).

## LONG-TERM NITRATE SIGNALING

Long-term NO<sub>3</sub><sup>-</sup> signaling refers to NO<sub>3</sub><sup>-</sup> regulation of plant growth and development over a longer period of time and involves processes such as root development, tillering, and flowering (Krapp et al., 2014; Liu et al., 2019; O'Brien et al., 2016). Following the identification of important players involved in long-term NO<sub>3</sub><sup>-</sup> signaling in *Arabidopsis* (Lin and Tsay, 2017; Wang et al., 2018b; Zhao et al., 2018b; Jia and von Wirén, 2020), significant advances have been made in this area for crops during the last several years.

### Root development

Root system architecture directly affects the absorption and utilization of nutrients as well as plant growth. Root growth and development are regulated by NO<sub>3</sub><sup>-</sup> availability. The MADS-box family TF *AtANR1* was the first gene identified in NO<sub>3</sub><sup>-</sup> regulation of root growth in *Arabidopsis*, and overexpression of *AtANR1* increases lateral root (LR) growth in the presence of NO<sub>3</sub><sup>-</sup> (Zhang and Forde, 1998). In rice, *OsMADS25/27/57* have also been



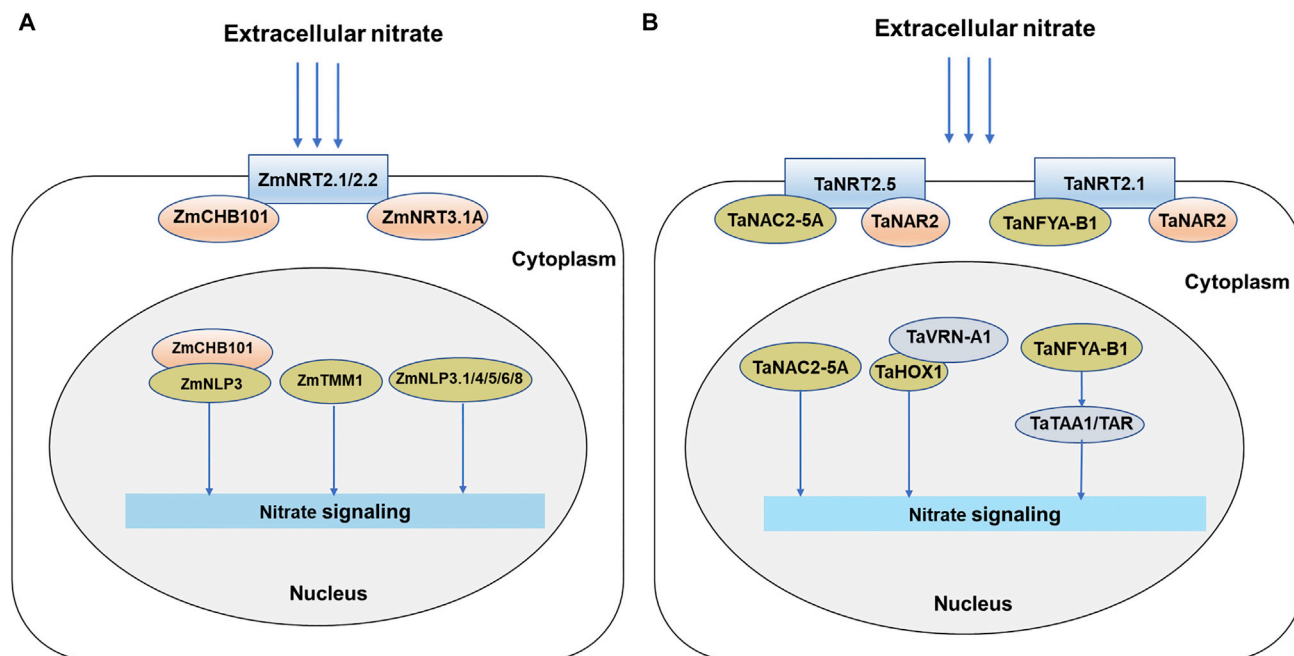
**Figure 1. Schematic representation of factors that regulate nitrate signaling in rice.**

Blue boxes indicate nitrate transporters. Brown ellipses indicate transcription factors. Blue cloud shapes indicate microRNA. Other shapes indicate other nitrate signaling regulators. Arrows indicate positive regulation, and blunted lines indicate negative regulation.

found to regulate root growth in response to NO<sub>3</sub><sup>-</sup> (Yu et al., 2015; Chen et al., 2018; Huang et al., 2019). Under different NO<sub>3</sub><sup>-</sup> conditions, *OsMADS25* overexpression can promote primary root (PR) and LR growth (Yu et al., 2015). Overexpression of *OsMADS27*, however, inhibits PR elongation and increases LR number and length under 10 mM NO<sub>3</sub><sup>-</sup> conditions (Chen et al., 2018). The *osmads57* mutant shows reduced total root length after treatment with low NO<sub>3</sub><sup>-</sup> due to shorter seminal and adventitious roots. Further investigation has revealed that mutation of *OsMADS57* inhibits the expression of *CYCLINB1;1* (*OsCYCB1;1*) and induces the polar transport of auxin toward and within the roots, thereby repressing meristematic cell proliferation (Huang et al., 2019). In addition, *OsmiR444* has been reported to target *OsMADS23*, *OsMADS27a*, *OsMADS27b*, and *OsMADS57*. Overexpression of *OsmiR444a* represses the expression of these *OsMADS* genes and root growth under NO<sub>3</sub><sup>-</sup> supply (Yan et al., 2014). In maize, local supply of NO<sub>3</sub><sup>-</sup> can induce the expression of *ZmTMM1*, a truncated MIKC-type MADS-box TF, to promote LR growth (Liu et al., 2020). Although *TaANR1* has been identified in wheat, its function in NO<sub>3</sub><sup>-</sup>-regulated root development has not been reported (Lei et al., 2018). These results suggest that the functions of *ANR1* homologs in regulating root growth are highly conserved, but the underlying

mechanisms may be diverse in different species (Figures 1 and 2, Table 1).

N deficiency promotes plant root development and thus increases N uptake from soil. Some *Arabidopsis* *NRT2* genes are mainly expressed in the roots and play a central role in regulating LR development under conditions of NO<sub>3</sub><sup>-</sup> limitation (Remans et al., 2006). In rice, overexpression of *OsNRT2.1* increases total root length, including that of seminal and adventitious roots, under 0.5 mM NO<sub>3</sub><sup>-</sup> conditions (Naz et al., 2019). The expression of auxin transporter genes *OsPIN1a/b/c* and *OsPIN2* also increased significantly in *OsNRT2.1*-overexpressing lines, implying that *OsNRT2.1* may modulate NO<sub>3</sub><sup>-</sup>-dependent root elongation by regulating auxin transport in roots. In addition, knockdown of *OsNAR2.1*, a partner of *OsNRT2.1*, suppresses LR formation under low NO<sub>3</sub><sup>-</sup> conditions (Yan et al., 2011; Song et al., 2020). Further research has found that *OsNAR2.1* can directly interact with NITRILASE 1 and 2 (*OsNIT1* and *OsNIT2*). In the *osnar2.1* mutant, the expression of *OsNIT1* and *OsNIT2* is decreased under NO<sub>3</sub><sup>-</sup> supply, thereby repressing the expression of *OsPIN1c* and *OsPIN1d* to alter the distribution of auxin in roots. Thus, *osnit1* and *osnit2* as well as *osnar2.1* show reduced PR length and LR density in the presence



**Figure 2. Schematic representation of factors that regulate nitrate signaling in maize and wheat**

The schematic representation of nitrate signaling regulators in maize (A) and wheat (B). Blue boxes indicate nitrate transporters. Brown ellipses indicate transcription factors. Other shapes indicate other nitrate signaling regulators. Arrows indicate positive regulation.

of NO<sub>3</sub><sup>-</sup> (Müller, 2020; Song et al., 2020). These results suggest that *NRT2.1/NAR2.1/NITs* play a critical role in modulating root development and in the crosstalk with auxin regulation in response to NO<sub>3</sub><sup>-</sup>. Moreover, *OsNRT2.4* has been reported to be expressed mainly in the base of LR primordia, and mutation of *OsNRT2.4* inhibits LR number and length under 0.25 mM and 2.5 mM NO<sub>3</sub><sup>-</sup> conditions (Wei et al., 2018) (Figure 1 and Table 1).

As well as the genes mentioned above, several other factors, such as TFs, ubiquitin ligase, and CRCs, have also been reported to participate in root development. Rice *OsNLP1* promotes root elongation, as mutation of this gene can significantly reduce root length under different NO<sub>3</sub><sup>-</sup> conditions (Alfatih et al., 2020). Maize *ZmNLP3.1/6/8* and barley *HvNLP2* can also increase PR length and LR number when overexpressed in *Arabidopsis* under low- and high-NO<sub>3</sub><sup>-</sup> conditions (Cao et al., 2017; Gao et al., 2022; Wang et al., 2018c; Yu et al., 2016) (Figure 2A and Table 1). In addition, the rice ubiquitin ligase *OsEL5* is involved in the maintenance of root meristematic viability and LR formation by participating in cytokinin-mediated N effects (Koiwai et al., 2007; Mochizuki et al., 2014). Moreover, low N can enhance strigolactone biosynthesis and signaling, which in turn reduce *OsPIN1b*-mediated auxin polar transport from shoot to root and attenuate seminal root growth (Sun et al., 2014b, 2018). In maize, the knockout mutant *zmchb101* shows accelerated root growth and increased biomass under low NO<sub>3</sub><sup>-</sup> conditions, suggesting that *ZmCHB101* may negatively regulate root development in response to NO<sub>3</sub><sup>-</sup> (Meng et al., 2020) (Figure 2A and Table 1). In wheat, RNAi lines of *TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS1/TRYPTOPHAN AMINOTRANSFERASE-RELATED* (*TaTAA1/TAR*) show reduced

LR growth under both low- and high-N conditions (Shao et al., 2017) (Figure 2B and Table 1). Furthermore, overexpression of the wheat NUCLEAR FACTOR-Y TF *TaNFYA-B1* upregulates expression of *TaTAR2-A1*, *TaTAR2-B1*, and *TaTAR2-D1* in roots, thus increasing LR growth (Qu et al., 2015).

### Tillering

Increased tillering can contribute to high crop yields, and NO<sub>3</sub><sup>-</sup> is one of the important factors that regulate crop tillering. In rice, the *indica* *NADH/NADPH-DEPENDENT NITRATE REDUCTASE 2* (*OsNR2*) promotes NO<sub>3</sub><sup>-</sup> uptake via feed-forward interaction with *OsNRT1.1B* and increases effective tiller number in *japonica* rice. The *japonica* *OsNR2* also functions in increasing tiller number but not to the extent seen in the *indica* *OsNR2*-overexpression lines (Gao et al., 2019).

NITROGEN-MEDIATED TILLER GROWTH RESPONSE 5 (*OsNGR5*) is a positive regulator of rice growth and development in response to N (Wu et al., 2020). It interacts with POLYCOMB REPRESSIVE COMPLEX 2 (PRC2) to regulate the expression of *DWARF14* (*D14*) and *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 14* (*OsSPL14*) by mediating histone methylation (H3K27me3) modification levels, thereby regulating rice tillering and bud outgrowth in the presence of N. *OsNGR5* is also a target of the gibberellin (GA) receptor GIBBERELLIN INSENSITIVE DWARF1 (*GID1*), and GA can promote degradation of *OsNGR5* protein. Further research has revealed that the plant-growth-inhibitory factor DELLA can interact with *OsNGR5*, competing with *OsGID1* at the binding site to inhibit GA-mediated degradation of *OsNGR5* and thus increase *OsNGR5* stability (Wu et al., 2020). Therefore, the GA-GID1-NGR5 and DELLA-NGR5



Gene	Species	Gene family	Roles in nitrate signaling	Reference
OsCBL1	Rice	Calcineurin B-like protein	Short-term signaling	Zhang et al. (2019)
OsDEP1	Rice	Heterotrimeric G protein	Long-term signaling	Sun et al. (2014a), 2014b;
OsEL5	Rice	Ubiquitin ligase	Long-term signaling	Koiwai et al. (2007); Mochizuki et al. (2014)
OsMADS25/27/57	Rice	MADS-box	Long-term signaling	Chen et al. (2018); Huang et al. (2019); Xu et al., 2018
OsmiR156	Rice	microRNA	Long-term signaling	Jiao et al. (2010); Xie et al. (2006); Yuan et al. (2019)
OsmiR393	Rice	microRNA	Long-term signaling	Li et al. (2016)
OsmiR444a	Rice	microRNA	Long-term signaling	Yan et al. (2014)
OsNAR2.1	Rice	A partner of OsNRT2.1	Long-term signaling	Chen et al. (2017); Song et al. (2020)
OsNGR5	Rice	APETALA2-domain transcription factor	Long-term signaling	Wu et al. (2020)
OsNhd1	Rice	v-myb avian myeloblastosis viral oncogene homolog (MYB) transcription factor	Long-term signaling	Zhang et al. (2021)
OsNIT1/2	Rice	Nitrilases	Long-term signaling	Müller (2020); Song et al. (2020)
OsNLP3/4	Rice	NiN-like protein	Short- and long-term signaling	Hu et al. (2019); Yu et al. (2021)
OsNR2	Rice	NADH/NADPH-dependent nitrate reductase	Long-term signaling	Gao et al. (2019)
OsNRT1.1A	Rice	Nitrate transporter 1/peptide transporter	Long-term signaling	Fan et al. (2016a), 2016b; Wang et al. (2018a)
OsNRT1.1B	Rice	Nitrate transporter 1/peptide transporter	Short- and long-term signaling	Fan et al. (2016a), 2016b; Hu et al. (2019)
OsNRT2.1/2.4	Rice	Nitrate transporter 2	Long-term signaling	Naz et al. (2019); Wei et al. (2018)
OsSHI1	Rice	Short internodes family	Long-term signaling	Duan et al. (2019)
OsSPL14/18	Rice	Squamosa promoter-binding protein-like protein	Long-term signaling	Jiao et al. (2010); Yuan et al. (2019)
OsTCP19	Rice	Teosinte branched 1, cycloid-proliferating cell factor (TCP)	Long-term signaling	Liu et al. (2021)
ZmCHB101	Maize	SWI/SNF-type ATP-dependent CRCs	Short- and long-term signaling	Meng et al. (2020)
ZmTMM1	Maize	Truncated MIKC-type MADS-box	Long-term signaling	Liu et al. (2020)
ZmNLP3.1/5/6/8	Maize	NiN-like protein	Short- and long-term signaling	Cao et al. (2017); Ge et al. (2020); Wang et al. (2018b)
HvNLP2	Barley	NiN-like protein	Short- and long-term signaling	Gao et al. (2022)
TaANR1	Wheat	MADS-box	Long-term signaling	Lei et al. (2018)
TaNAC2-5A	Wheat	Nitrate-inducible and cereal-specific NAM, ATAF, and CUC (NAC) transcription factor	Long-term signaling	He et al. (2015)
TaNfYA-B1	Wheat	NUCLEAR FACTOR Y transcription factor	Short- and long-term signaling	Qu et al. (2015)
TaTAR1	Wheat	Tryptophan aminotransferase of Arabidopsis1/Tryptophan aminotransferase-related gene	Long-term signaling	Shao et al. (2017)
TaVRN-A1	Wheat	MADS-box	Long-term signaling	Lei et al. (2018)

**Table 1. Summary of nitrate-regulatory genes involved in short- and long-term nitrate signaling in crops**

molecular modules may synergistically improve the growth and maintain the semi-dwarfing and high-yield characteristics of rice (Figure 1 and Table 1).

Tillering and panicle branching are important determinants of plant architecture and yield in rice. Overexpression of *DENSE AND ERECT PANICLE1* (*OsDEP1*) can increase tiller number and panicle branching under high N supply (Sun et al., 2014a). *OsSPL14*, also known as IDEAL PLANT ARCHITECTURE1 (*IPA1*), can directly activate the expression of *OsDEP1* and *TEOSINTE BRANCHED1* (*OsTB1*) to regulate tiller outgrowth and panicle branching (Duan et al., 2019). Rice *SHORT INTERNODES1* (*OsSHI1*) can repress the expression of *OsSPL14* to alter plant architecture. In addition, overexpression of *OsSPL18* can increase the expression of *OsDEP1* to regulate panicle development (Yuan et al., 2019). Sequence analysis implies that *OsSPL14* and *OsSPL18* are targets of *OsmiR156*, and overexpression of *OsmiR156* results in decreased expression of *OsSPL14/18*. The reduced expression of *OsSPL14* leads to decreased tiller number, whereas *osspl18* mutants show increased tiller number (Jiao et al., 2010; Xie et al., 2006; Yuan et al., 2019). Another microRNA, *OsmiR393*, targets and suppresses the expression of *OsTB1* and the two auxin receptor genes *OsAFB2* and *OsTIR1* under NO<sub>3</sub><sup>-</sup> conditions, which affects the transport of auxin and finally controls tiller growth (Li et al., 2016). In addition, *OsTCP19* can directly bind to and repress the activity of the tiller-promoting gene *DWARF AND LOW-TILLERING* (*DLT*) and negatively regulate tillering in the presence of N (Liu et al., 2021). Further investigation has revealed that a 29-bp insertion and/or deletion in the *OsTCP19* promoter confers a differential transcriptional response and variation in tillering in response to N among rice varieties (Figure 1 and Table 1). In wheat, overexpression lines of the NO<sub>3</sub><sup>-</sup>-INDUCIBLE AND CEREAL-SPECIFIC NAM, ATAF, AND CUC (NAC) TF *TaNAC2-5A* show significantly higher tiller numbers under low-N conditions (He et al., 2015), suggesting that *TaNAC2-5A* is a key gene for NO<sub>3</sub><sup>-</sup>-regulated tillering.

### Flowering time

Flowering time has a critical impact on crop yield. Recent studies have shown that NO<sub>3</sub><sup>-</sup> concentrations affect plant flowering time and that lower NO<sub>3</sub><sup>-</sup> can promote flowering (Lin and Tsay, 2017). In rice, overexpression of *OsNRT1.1A* upregulates the expression of *HEADING DATE 3A* (*Hd3a*), *EARLY HEADING DATE1* (*Ehd1*), and *RICE FLOWERING LOCUS T 1* (*RFT1*), which are known to promote flowering, resulting in an early-flowering phenotype (Wang et al., 2018a). N-MEDIATED HEADING DATE-1 (*Nhd1*) can directly activate the expression of *OsHd3a* and negatively regulate the expression and activity of *OsFd-GOGAT*, thus balancing flowering time and photoperiod in rice (Zhang et al., 2021) (Figure 1 and Table 1). Wheat *TaVRN-A1* can interact with *TaANR1*, and mutations in both *TaVRN-A1* and *TaANR1* result in delayed heading after N fertilization (Lei et al., 2018) (Figure 2B and Table 1). *osnhd1* mutant and *TaANR1*-RNAi lines show delayed flowering time and a longer growth period (Lei et al., 2018; Zhang et al., 2021; Li et al., 2022). In general, early plant flowering is closely related to shortened growth period and reduced yield (Roux et al., 2006; Lu et al., 2014; Zhao et al., 2018a). However, *OsNRT1.1A*-overexpression lines exhibit both earlier flowering and high yields. Therefore,

*OsNRT1.1A* has great potential for overcoming the antagonism between early flowering and high yield, enabling the breeding of new rice varieties in which yield and flowering time are well balanced.

## INFLUENCE OF ENVIRONMENTAL FACTORS ON NITRATE SIGNALING

NO<sub>3</sub><sup>-</sup> signaling within plants can be affected by various biotic and abiotic factors, and plants have evolved sophisticated mechanisms to coordinate both NO<sub>3</sub><sup>-</sup> signaling and other signaling pathways to adapt to the changing environment. Recently, several critical genes involved in regulating the signaling of both NO<sub>3</sub><sup>-</sup> and other nutrients, including Pi and potassium (K<sup>+</sup>), have been identified in crops (Li et al., 2014; Hu et al., 2019). In rice, *OsNRT1.1B* plays an important role in regulating NO<sub>3</sub><sup>-</sup> signaling (Wang et al., 2020b). It can also interact with *OsSPX4* to promote ubiquitination and degradation of *OsSPX4* by recruiting NBIP1. This then causes the Pi-signaling TF *OsPHR2* to translocate into the nucleus and initiate the expression of Pi utilization genes, resulting in highly coordinated utilization of N and Pi (Hu et al., 2019). *OsCBL1* is not only involved in NO<sub>3</sub><sup>-</sup> signaling but also forms a complex with *OsCIPK23* to enhance *OsAKT1*-mediated K<sup>+</sup> uptake (Li et al., 2014). These findings may provide insights into synergistically improving the uptake efficiency of N, Pi, and K<sup>+</sup> in rice.

The rhizobial and arbuscular mycorrhizal symbioses are very important for efficient plant uptake of nutrients from the environment (Wang et al., 2021). Rhizobia can convert atmospheric N into ammonium for absorption and utilization by legumes, and in turn, rhizobia can obtain nutrients and carbon sources from the plants to maintain growth (Müller, 2021). Advances in our understanding of symbiotic N fixation have been well summarized in several excellent review papers (Oldroyd et al., 2011; Udvardi and Poole, 2013; Ferguson et al., 2019; Yang et al., 2022). However, few genes involved in coordinately regulating NO<sub>3</sub><sup>-</sup> signaling and the rhizobial symbiosis have been identified to date. In the presence of NO<sub>3</sub><sup>-</sup>, *Medicago* MtNLP1 can enter the nucleus to form a complex with the NODULE INCEPTION (NIN) TF protein, thereby inhibiting expression of two downstream genes, *CRE1* and *NF-YA1*, which are essential for nodule development (Lin et al., 2018). This ultimately leads to inhibition of nodule formation and N fixation. In addition, low NO<sub>3</sub><sup>-</sup> induces the production of C-TERMINALLY ENCODED PEPTIDE (*MtCEP1*) signaling peptides in roots (Mohd-Radzman et al., 2016). MtCEP1 promotes accumulation of *miR2111* through the receptor COMPACT ROOT ARCHITECTURE 2 (*MtCRA2*), resulting in repression of TOO MUCH LOVE (*MtTML*) target transcripts and increased nodulation in the absence of rhizobia (Mohd-Radzman et al., 2016; Gautrat et al., 2020). Furthermore, MtNLP1 can bind to the half NRE (hNRE) in the *MtCEP1* promoter to repress its expression, resulting in reduced nodule formation in the presence of NO<sub>3</sub><sup>-</sup> (Luo et al., 2022). Interestingly, MtNLP1 can also bind to the NRE in the *MtCLE35* promoter to activate its expression, and its product is then transported to the shoot to activate SUPER NUMERIC NODULES (SUNN) systemic pathways and further restrict nodule number under NO<sub>3</sub><sup>-</sup> conditions (Luo et al., 2021). All the results above suggest that there is NLP1-NO<sub>3</sub><sup>-</sup>-mediated inhibition of nodulation via systemic and

local regulatory pathways in *Medicago*. The arbuscular mycorrhizal fungi (AMF) symbiosis is the result of the co-evolution of plants with mycorrhizal fungi in the long-term survival process, and it can increase plant uptake of soil nutrients, especially Pi (Montesinos-Navarro et al., 2012). Recent work has shown that AMFs can induce the expression of the NO<sub>3</sub><sup>-</sup> transport genes *OsNPF2.2/PTR2*, *OsNPF1.3*, *OsNPF6.4*, and *OsNPF4.12* to improve N uptake in rice (Drechsler et al., 2018), but the mechanisms by which AMFs and NO<sub>3</sub><sup>-</sup> signaling affect one another remain elusive. Recently, *OsNRT1.1B* and *OsCIPK2* have been found to modulate the root microbiome in rice to increase N utilization (Khan et al., 2019; Zhang et al., 2019). Clearly, rhizosphere microorganisms strongly influence NO<sub>3</sub><sup>-</sup> signaling and utilization, although the underlying mechanisms require further investigation.

## NUE OF CROPS

NUE is a complicated agronomic trait that involves the multiple interconnected steps of NO<sub>3</sub><sup>-</sup> absorption, assimilation, translocation, and remobilization. Previous reviews on NUE have focused mainly on the key genes that function in these processes (Han et al., 2015; Li et al., 2017; Wang et al., 2018b). However, no reviews have focused specifically on NUE in terms of NO<sub>3</sub><sup>-</sup> signaling regulators. More and more data indicate that many factors involved in NO<sub>3</sub><sup>-</sup> signaling play important roles in regulating crop NUE, and we have therefore summarized advances in this area.

In rice, several NO<sub>3</sub><sup>-</sup> transporter genes have been found to play important roles in the regulation of NUE in addition to modulating NO<sub>3</sub><sup>-</sup> signaling. Phylogenetic analysis shows that *OsNRT1.1B* diverged between *indica* and *japonica* subspecies (Hu et al., 2015). *OsNRT1.1B-indica* can improve tiller number, grain yield, and NUE, and plants overexpressing *OsNRT1.1B-indica* grow better and show a greater NUE than plants overexpressing *OsNRT1.1B-japonica* under both high- and low-N conditions. These results indicate that *OsNRT1.1B-indica* plays a more important role in NUE than *OsNRT1.1B-japonica*. Furthermore, *japonica* plants carrying *OsNRT1.1B-indica* exhibit significantly increased tiller number, grain yield, and NUE, suggesting that *OsNRT1.1B-indica* can improve the NUE of *japonica* rice (Hu et al., 2015; Fan et al., 2016a). *OsNRT1.1B* can also interact with *indica* *OsNR2* to synergistically improve grain yield and NUE of *japonica* rice (Gao et al., 2019). *OsNRT1.1A* can dramatically increase grain yield and NUE by improving panicle size (Wang et al., 2018a), and transgenic lines overexpressing *OsNRT2.1* or *OsNAR2.1* show increased grain yield and NUE (Araus et al., 2016; Chen et al., 2016, 2017). Nevertheless, rice NUE was decreased in plants overexpressing *OsBT2*, a member of the *BRIC-A-BRAC/TRAMTRACK/BROAD* gene family. The *bt1bt2* double mutant displays higher expression of *OsNRT2.1* and *OsNRT2.4* and 20% higher NUE under low-N conditions (Araus et al., 2016). *OsNAR2.1* can also interact with *OsNRT2.3a*, and co-overexpression of *OsNAR2.1* and *OsNRT2.3a* increased grain yield and NUE by 24.6% and 28.56%, respectively (Feng et al., 2011; Yan et al., 2011; Liu et al., 2014; Chen et al., 2020). Functional analysis indicates that overexpression of *OsNRT2.3b* can increase N uptake and improve grain yield and NUE of rice (Fan et al., 2016b; Li et al., 2017).

Besides the *OsNRTs*, several other rice NO<sub>3</sub><sup>-</sup>-signaling regulators have also been found to participate in affecting NUE. *OsDEP1*-overexpressing plants show increased N uptake and assimilation, resulting in improved harvest index and grain yield (Huang et al., 2009; Sun et al., 2014a). *OsSPL18* can bind to the promoter of *OsDEP1* and increase its expression to regulate panicle architecture (Yuan et al., 2019). The *osspl18* mutant exhibits reductions in grain width and thickness, panicle length, and grain number, resulting in decreased yield, suggesting that *OsSPL18* positively regulates grain yield in rice (Xie et al., 2006; Yuan et al., 2019). By contrast, rice *osspl14* mutants show increased tiller number and lodging resistance and enhanced grain yield, indicating that *OSSPL14* acts as a negative regulator of rice growth and yield (Jiao et al., 2010). Overexpression of *OsNGR5* can repress the expression of *OsSPL14* and *D14* to enhance rice tiller bud outgrowth, thereby increasing tiller number, grain yield, and NUE (Wu et al., 2020). In addition, both *OsNLP3* and *OsNLP4* can bind to the promoters and activate the expression of downstream N assimilation-related genes to increase tiller number, yield, and NUE (Wu et al., 2021; Yu et al., 2021; Zhang et al., 2022).

Recently, studies on NO<sub>3</sub><sup>-</sup>-signaling regulators involved in modulating NUE in barley, maize, and wheat have also been reported successively. Barley *HvNLP2* and maize *ZmNLP6/8* can increase the expression of NR-regulated genes to enhance NR activity and amino acid content, thereby increasing grain yield and NUE (Cao et al., 2017; Gao et al., 2022). *zmnlp5* mutants accumulate less N in the ear leaves and seed kernels under N deficiency, implying that *ZmNLP5* may take part in improving NUE of maize (Ge et al., 2020). In wheat, *TaNAC2-5A* has been found to directly bind to the promoters and activate the expression of *TaNRT2.1-6B*, *TaNRT2.5-3B*, *TaNPF7.1-6D*, and *TaGS2-2A* to affect NO<sub>3</sub><sup>-</sup> transport and assimilation, resulting in increased grain yield and NUE (He et al., 2015; Li et al., 2020). Overexpression of *TaNFYA-B1* also increases wheat grain yield and NUE by increasing spike number under low-N conditions (Qu et al., 2015). In addition, *TaANR1*-RNAi lines exhibit fewer fertile tillers and decreased grain yield, whereas *TaVRN-A1*-RNAi lines show increased plant biomass after N application (Lei et al., 2018). This result implies that *TaVRN-A1* may function oppositely to *TaANR1* in regulating wheat grain yield and NUE, even though both proteins can interact with each other. Moreover, *TaHOX1* (a homeobox protein involved in wheat heading date) can also interact with *TaVRN-A1*, and plants overexpressing *TaHOX1* show lower grain yield, implying that *TaHOX1* has a repressive effect on yield (Lei et al., 2018).

All the NO<sub>3</sub><sup>-</sup>-signaling regulators that function in affecting NUE characterized to date in rice, wheat, and maize are shown in Table 2. They modulate important plant characteristics including growth, grain development, and tiller number to improve crop yield and NUE.

## Perspectives

Improving crop NUE is the most effective way to reduce the application of N fertilizer and the resulting environmental pollution. However, only limited advances have been made in achieving high NUE in crops, as the molecular mechanisms and networks of NO<sub>3</sub><sup>-</sup> signaling are still poorly understood. Although some

Gene	Species	Gene effects	Reference
OsBT2	Rice	Regulating NUE	Araus et al. (2016)
OsDEP1	Rice	Improving harvest index and grain yield	Sun et al. (2014a), 2014b
OsNAR2.1	Rice	Improving grain yield and NUE	Chen et al. (2017)
OsNGR5	Rice	Increasing tiller number, grain yield, and NUE	Wu et al. (2020)
OsNLP3/4	Rice	Increasing tiller number, grain yield, and NUE	Wu et al. (2021); Yu et al. (2021); Zhang et al. (2022)
OsNR2	Rice	Increasing effective tiller number, grain yield, and NUE	Gao et al. (2019)
OsNRT1.1A	Rice	Increasing panicle size, grain yield, and NUE	Fan et al. (2016a), 2016b
OsNRT1.1B	Rice	Improving tiller number, grain yield, and NUE	Fan et al. (2016a), 2016b; Hu et al. (2019); Zhang et al. (2019)
OsNRT2.1	Rice	Improving grain yield and NUE	Chen et al. (2016); Chen et al. (2017); Araus et al. (2016)
OsNRT2.3a	Rice	Improving grain yield and NUE	Chen et al. (2020); Feng et al. (2011); Liu et al. (2014); Yan et al. (2011)
OsNRT2.3b	Rice	Improving grain yield and NUE	Fan et al. (2016b); Li et al. (2017)
OsNRT2.4	Rice	Improving grain yield and NUE	Araus et al. (2016); Wei et al. (2018)
OsSPL18	Rice	Inhibiting grain width and thickness but increasing panicle length and grain number	Yuan et al. (2019)
OsSPL14	Rice	Increasing lodging resistance and grain yield	Jiao et al. (2010)
ZmNLP6/8	Maize	Increasing growth, yield, and NUE	Cao et al. (2017)
HvNLP2	Barley	Increasing growth, yield, and NUE	Gao et al. (2022)
TaANR1	Wheat	Increasing grain yield	Lei et al. (2018)
TaHOX1	Wheat	Regulating biomass and grain yield	Lei et al. (2018)
TaNAC2-5A	Wheat	Increasing root growth and grain yield	He et al. (2015); Li et al. (2020)
TaNFYA-B1	Wheat	Increasing spike number and grain yield	Qu et al. (2015)
TaVRN-A1	Wheat	Inhibiting plant biomass	Lei et al. (2018)

**Table 2. Summary of nitrate-signaling regulators involved in NUE in crops**

key NO<sub>3</sub><sup>-</sup>-signaling regulators have been successfully characterized as described above, these efforts are just the start of deciphering the sophisticated NO<sub>3</sub><sup>-</sup> regulation in crops. Many more novel genes and regulatory mechanisms remain to be identified.

AtNRT1.1 is the first and the only one NO<sub>3</sub><sup>-</sup> sensor and plays a powerful role in regulating NO<sub>3</sub><sup>-</sup> signaling and transport as well as in transporting auxin in *Arabidopsis* (Ho et al., 2009; Mounier et al., 2014; Wang et al., 2020b). In rice, *OsNRT1.1B* has also been found to play a central role in regulating NO<sub>3</sub><sup>-</sup> signaling and plant growth, yield, and NUE (Hu et al., 2015, 2019; Fan et al., 2016a; Zhang et al., 2019). However, gene(s) like *AtNRT1.1* that can transport NO<sub>3</sub><sup>-</sup> and regulate NO<sub>3</sub><sup>-</sup> signaling in other crops such as wheat and maize need to be identified.

The complex regulation of NO<sub>3</sub><sup>-</sup> signaling in plants is influenced by various environmental factors. Recently, several critical regulators have been shown to play important roles in the crosstalk between crop NO<sub>3</sub><sup>-</sup> signaling and that of other nutrients such as Pi and K<sup>+</sup> (Li et al., 2014; Hu et al., 2019). With the development and application of metagenomic sequencing techniques, a number of factors like *OsNRT1.1B* and *OsCIPK2* have been found to regulate the adaptation of root microbiota to the soil environment and to coordinate the uptake of different nutrients (Khan et al., 2019; Zhang et al., 2019). However, research in this area is very preliminary, and further in-depth study is needed. Moreover, the relationships between NO<sub>3</sub><sup>-</sup> signaling and other environmental factors such as light, temperature, drought, and salt have not been reported, and this topic also requires further investigation. Research on the NO<sub>3</sub><sup>-</sup>



regulation of crop development, including germination, root growth, tiller number, flowering, senescence, and seed development, is still in its infancy or even absent. All of the above subjects require more efforts to identify key genes involved in regulating the crosstalk between NO<sub>3</sub><sup>-</sup> signaling and other factors in the environment and the plants themselves.

Forward genetics techniques such as QTL and GWAS have been successfully used to identify novel genes involved in NO<sub>3</sub><sup>-</sup> signaling in rice (Huang et al., 2009; Hu et al., 2015; Gao et al., 2019; Wu et al., 2020) but not yet in other crops like wheat and maize. Recently, reverse genetics has also been gradually applied to identify crop NO<sub>3</sub><sup>-</sup> regulators such as *ZmNLP3.1/6/8* and *HvNLP2* (Cao et al., 2017; Wang et al., 2018c; Gao et al., 2022). Compared with forward genetics, reverse genetics has been proven to be more accurate, efficient, and time saving for characterizing the roles of homologous genes in regulating NO<sub>3</sub><sup>-</sup> signaling in crops. In addition, systems biology is a powerful approach and has been successfully used to identify key factors in NO<sub>3</sub><sup>-</sup> signaling in *Arabidopsis*, although it is not being used in crops. Therefore, according to the characteristics of different crops, comprehensive use of these techniques to identify more NO<sub>3</sub><sup>-</sup> regulatory genes in crops is very important for accelerating our understanding of NO<sub>3</sub><sup>-</sup> signaling regulation.

Compared with our understanding of NO<sub>3</sub><sup>-</sup> signaling in the model plant *Arabidopsis*, much less is known in crops. More endeavors are needed to identify and characterize novel genes involved in regulating NO<sub>3</sub><sup>-</sup> signaling as well as the crosstalk between NO<sub>3</sub><sup>-</sup> and other environmental factors. Following the application of advanced techniques, including multi-omics and gene editing, the NO<sub>3</sub><sup>-</sup>-regulatory mechanisms and networks in crops will be clearly elucidated. This will lay a solid foundation for evaluating the potential of these genes and taking full advantage of them in breeding new crop varieties with high NUE.

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## AUTHOR CONTRIBUTIONS

All work was performed in collaboration. Y.W. and Y.G. wrote the original draft and prepared the figure. Y.W., Y.G. and S.Q. designed and finalized the manuscript. The authors have read and agreed to the published version of the article.

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