Novel Aspects of Nitrate Regulation in Arabidopsis

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Nitrogen (N) is one of the most essential macronutrients for plant growth and development. Nitrate (NO₃⁻), the major form of N that plants uptake from the soil, acts as an important signaling molecule in addition to its nutritional function. Over the past decade, significant progress has been made in identifying new components involved in NO₃⁻ regulation and starting to unravel the NO₃⁻ regulatory network. Great reviews have been made recently by scientists on the key regulators in NO₃⁻ signaling, NO₃⁻ effects on plant development, and its crosstalk with phosphorus (P), potassium (K), hormones, and calcium signaling. However, several novel aspects of NO₃⁻ regulation have not been previously reviewed in detail. Here, we mainly focused on the recent advances of post-transcriptional regulation and non-coding RNA (ncRNAs) in NO₃⁻ signaling, and NO₃⁻ regulation on leaf senescence and the circadian clock. It will help us to extend the general picture of NO₃⁻ regulation and provide a basis for further exploration of NO₃⁻ regulatory network.

Keywords: nitrate regulation, leaf senescence, circadian clock, post-transcriptional regulation, non-coding RNA

INTRODUCTION

Nitrogen (N) is a macronutrient required by many important biological processes in plants and also a limiting factor for crop production in the agricultural system. Most crops such as maize and wheat absorb nitrate (NO₃⁻) as the main N forms. NO₃⁻ uptake, transport, and assimilation are the principal processes for NO₃⁻ utilization and have long been studied. Besides its nutritional role, NO₃⁻ can also serve as a signaling molecule that modulates plant growth and development. During the last decade, great progress has been made on NO₃⁻ regulation in root system architecture, shoot growth, seed dormancy, flowering time, as well as crosstalk with other signals (hormone, calcium) and nutrients (P and K) (Footitt et al., 2011, 2013; Bouguyon et al., 2015, 2016; Riveras et al., 2015; Yan et al., 2015; Yuan et al., 2016; Li et al., 2017; Lin and Tsay, 2017; Gaudinier et al., 2018; Gras et al., 2018; Du et al., 2019; Hu et al., 2019; Medici et al., 2019). Some crucial components involved in NO₃⁻ signaling have been identified, such as ANR1, NRT1.1, NLP6/7, TCP20, LBD37/38/39, TGA1/4, SPL9, bZIP1, NAC4, NRG2, and NIGT/HRS1.
Post-transcriptional Regulation of Nitrate

The importance of post-transcriptional modifications of genes associating with a wide range of plant responses has been already described in plants (Kim et al., 2008; Kwon et al., 2009; Berr et al., 2010; Nelissen et al., 2010). However, the role of post-transcriptional modifications in N regulation is still poorly understood. The factor IWS1 has been shown to be involved in several aspects of transcription, like transcription elongation, recruitment of chromatin-remodeling factors, mRNA processing and export, and post-transcriptional histone modification (Krogan et al., 2002; Yoh et al., 2007, 2008; Zhang et al., 2008). Lepechin’s group found that IWS1 represses NRT2.1 transcription in response to high N supply, which is associated with an IWS1-dependent accumulation of histone H3 lysine 27 trimethylation (H3K27me3) (Nimura et al., 2011). The result suggests that IWS1 represses gene expression by specifically promoting H3K27me3 methylation under high N conditions. This is the first evidence demonstrating that post-transcriptional chromatin modifications regulate N acquisition. More recently, Coruzzi’s group reported that histone methyltransferase SET DOMAIN GROUP8 (SDG8) mediates genome-wide changes of H3K36me3 after NO$_3^-$ treatments, resulting in the altered expression of its target genes, RNA processing, and physiological responses (Li et al., 2020).

More epigenetic marks, such as other histone modifications (e.g., histone acetylation), chromatin landscape, and DNA methylation need to be investigated in the future to gain a comprehensive understanding of chromatin regulation in NO$_3^-$ signaling.

RNA processing is important for the post-transcriptional regulation of gene expression, and is a rate-limiting step in the expression of proteins. The CPSF30 gene encodes the cleavage and polyadenylation specificity factor, which forms a larger protein (CPSF30-L) with 65 kDa and a smaller protein (CPSF30-S) with 28 kDa. Both proteins possess three characteristic C3H zinc finger motifs and act as an RNA-binding protein and an endonuclease (Addeppalli and Hunt, 2007). The CPSF30-L contains an additional YT512-B Homology (YTH) domain along with the three zinc finger motifs (Li et al., 2017). By using forward genetics, the CPSF30-L gene was cloned and found that it plays a crucial role in NO$_3^-$ signaling, transport, and assimilation in Arabidopsis (Li et al., 2017). Molecular and genetic assays revealed that CPSF30-L works upstream and regulates the expression of NRT1.1, while it functions independently in NLP7 in NO$_3^-$ signaling. FIP1, another RNA-binding protein, is also a core component of a pre-mRNA processing complex (Forbes et al., 2006). Further research showed that FIP1 interacts with CPSF30-L and regulates NO$_3^-$ signaling and assimilation (Wang et al., 2018b). Both CPSF30-L and FIP1 modulate the expression of NO$_3^-$ regulatory genes CIPK8 and CIPK23 and influence the alternative polyadenylation (APA) of the 3’-UTR of NRT1.1 mRNA (Li et al., 2017; Wang et al., 2018b). These results indicate that CPSF30-L and FIP1 may modulate NO$_3^-$ signaling through influencing the APA processing of NRT1.1.

RNA can be chemically modified with many different reactions, among which methylation is one of the most well studied modifications. N$^6$-methyladenosine (m$^6$A) is a pivotal internal mRNA modification, which plays crucial roles in plant growth and development by regulating gene expression at the post-transcriptional level. Recently, it has been reported that the EVOLUTIONARILY CONSERVED C-TERMINAL REGION2 (ECT2) acts as an m$^6$A reader depending on the presence of the YTH domain (Wei et al., 2018; Yue et al., 2019). As CPSF30-L contains an YTH domain, whether it also serves as the m$^6$A reader to regulate NO$_3^-$ signaling needs further investigation.

There are over 100 types of RNA modifications (Roundtree et al., 2017; Bailey-Serres et al., 2020), yet our knowledge about their occurrence and functions are still limited. One thing is certain though: post-transcriptional regulation of gene expression is much more intricate than previously thought. Unraveling the functions and underlying mechanisms of RNA modifications are essential to understand the roles of NO$_3^-$ regulatory genes and will provide valuable insights into post-transcriptional regulation of NO$_3^-$.
Nitrate and ncRNAs

Whole-genome sequencing analyses have identified thousands of ncRNAs playing vital roles in numerous biological processes in plants (Laporte et al., 2007; Rymarquis et al., 2008). Several microRNAs (miRNAs) and a long ncRNA (lncRNA) have been reported to function in NO$_3^-$ regulation in Arabidopsis and crops (Miin-Feng et al., 2006; Vidal et al., 2010; Liu et al., 2019; Table 1). In Arabidopsis, miR167 targets two auxin responsive factors ARF6 and ARF8. The miR167/ARF8 module regulates lateral root growth in response to NO$_3^-$ by controlling a group of NO$_3^-$-responsive genes (Miin-Feng et al., 2006; Gifford et al., 2008). The miR393 and AFB3 can respond to NO$_3^-$ by integrating internal organic N signals, external NO$_3^-$ availability and root auxin sensitivity to control root architecture (Vidal et al., 2010). Upon N starvation, the expression of miR160, miR164, miR780, miR826, miR842, miR846, and miR5090 is induced, whereas the expression of miR169, miR171, miR395, miR397, miR399, miR408, miR827, and miR857 is repressed (Xu et al., 2011; Liang et al., 2012; He et al., 2014; Li et al., 2016). Among them, miR160, miR167, and miR171 can be responsible for the development of root systems under N-starvation conditions (Liang et al., 2012). Moreover, miR826 and miR5090 suppress the expression of ALKENYL HYDROXALKYL PRODUCING 2 (AOP2), which is involved in the modification of the glucosinolate side chain. Overexpression of miR826 and miR5090 results in less glucosinolate and enhanced tolerance to N starvation (He et al., 2014). Overexpression of miR169 reduces N uptake and total N content by decreasing the expression of NRT1.1 and NRT2.1 and thus promotes N starvation-induced early leaf senescence (Zhao et al., 2011; Figure 1A). In addition, the miR5640 post-transcriptionally regulates the expression of its target AtPPC3 in response to NO$_3^-$ (Vidal et al., 2013). The N-responsive miRNA miR156 works upstream of SPL9, a SBP-box transcription factor (TF) identified to control the genes involved in the NO$_3^-$ primary response (Krouk et al., 2010), implying that the miR156/SPL9 module functions in NO$_3^-$ regulation. MiRNAs have also been found to be responsive to N limitation in crops (Xu et al., 2011; Trevisan et al., 2012). MiR169 is up-regulated in maize but down-regulated in rice under N starvation condition (Xu et al., 2011; Li et al., 2016). Two novel putative miR169 species miRC10 and miRC68 are reported to play major roles in the adaptation to NO$_3^-$ limitation in maize seedlings (Zhao et al., 2013). Furthermore, OsmiR444a plays multiple roles including NO$_3^-$-dependent root growth, NO$_3^-$ accumulation and phosphate-starvation responses in rice (Yan et al., 2014). Chengcai Chu’s group reported that OsmiR3979 functions as a key regulator to optimize root growth in response to NO$_3^-$. It targets LOC Os06g40330 and LOC Os01g59660, encoding MYB family TFs, and cleaves them at the predicted cleavage sites (Jeong et al., 2011; Li et al., 2016). Those results suggest that miRNAs play critical roles in NO$_3^-$ regulation by modulating the expression of their target genes.

Long non-coding RNAs are a class of ncRNAs with a length longer than 200 nucleotides and poor protein-coding potential (Pang et al., 2006; Ponting et al., 2009). A multitude of lncRNAs have been identified by using next-generation sequencing during the last several years, but only a few have been characterized (Xin et al., 2011; Liu et al., 2012; Meng et al., 2012). It has been reported that lncRNAs play crucial roles in flowering, phosphate starvation, polar auxin transport, photomorphogenesis, mRNA alternative splicing, and plant immune response (Francozorrilla et al., 2007; Amor et al., 2009; Swiezewski et al., 2009; Heo and Sung, 2011; Bardou et al., 2014; Seo et al., 2017). Several papers described changes of lncRNA responding to N treatments or N deficiency in Populus and maize (Vidal et al., 2013; Alvarez et al., 2014; Chen M. et al., 2016; Lv et al., 2016). However, the role of lncRNAs in N regulation remains largely unclear. Our group found six NO$_3^-$-dependent lncRNAs using RNA-seq technology and validated by qRT-PCR. Further investigation revealed that the TCONS_00005120 (T5120), showing the highest induction by NO$_3^-$, could regulate NO$_3^-$ response and assimilation.

Table 1: Characterized ncRNAs involved in NO$_3^-$ regulation.

| Gene name | Targets | Species | Reference |
|-----------|---------|---------|-----------|
| miR156    | SPL9    | Arabidopsis | Krouk et al., 2010 |
| miR160    | Auxin response factors | Arabidopsis | Liang et al., 2012 |
| miR167    | ARF6, ARF8 | Arabidopsis | Miin-Feng et al., 2006 |
| miR169    | CCAAT-binding transcription factor | Arabidopsis, Maize, Rice | Zhao et al., 2011 |
| miR171    | SCL transcription factors | Arabidopsis | Liang et al., 2012 |
| miR393    | AFBS    | Arabidopsis | Vidal et al., 2010 |
| miR5090   | AOP2    | Arabidopsis | He et al., 2014 |
| miR6840   | PPC3    | Arabidopsis | Vidal et al., 2013 |
| miR826    | AOP2    | Arabidopsis | He et al., 2014 |
| miR827    | NLA     | Arabidopsis, Maize | Liu et al., 2017 |
| miR846    | Jacalin lectin family | Arabidopsis | Xu et al., 2011 |
| miR444a   | MKC-type MADS-box genes | Rice | Yan et al., 2014 |
| miR979    | IAA3    | Rice | Li et al., 2016 |
| miR159a.1 | LOC Os06g40330, LOC Os01g59660 | Rice | Jeong et al., 2011; Li et al., 2016 |
| IncRNA T5120 | Unknown | Arabidopsis | Liu et al., 2019 |
Molecular and genetic assays showed that NLP7 directly binds to the promoter of T5120 to regulate its expression. In addition, the expression of T5120 was modulated by NRT1.1 (Liu et al., 2019). This is the first attempt to investigate the role of IncRNAs in NO\textsuperscript{3} signaling and provides a fundamental base for discovering more IncRNAs functioning in NO\textsuperscript{3} regulation.

**Nitrate and Leaf Senescence**

Leaf senescence is a developmental process accompanying resource rearrangement (Havé et al., 2017). It occurs in a coordinated manner, starting with the inhibition of leaf expansion, and then the induction of metabolic changes followed by degradation of organic substances and nutrient remobilization (Soltabayeva et al., 2018). Some environmental stresses can induce early leaf senescence, among which N deficiency tends to promote leaf senescence to meet the demand of N in young and developing tissues (Aguera et al., 2010).

Although it is previously known that N deficiency can accelerate leaf senescence (Schildhauer et al., 2008; Gregersen et al., 2013), the underlying mechanisms are still poorly understood. It has been found that NRT1.5 functions in transporting NO\textsuperscript{3} from the roots to the shoots (Lin et al., 2008) and the expression of NRT1.5 is strongly induced by leaf senescence in *Arabidopsis* (Van Der Graaff et al., 2006). Jiming Gong’s lab has recently reported that NRT1.5 suppresses the leaf senescence induced by NO\textsuperscript{3} (but not nitrogen) starvation (Meng et al., 2016) and this suppression is independent of the NO\textsuperscript{3} transport function of NRT1.5. Further analyses showed that foliar K\textsuperscript{+} levels decreased in nrt1.5 when exposed to NO\textsuperscript{3} starvation and adding K\textsuperscript{+} could restore the early leaf senescence phenotype of nrt1.5 plants, suggesting that NRT1.5 may increase foliar K\textsuperscript{+} levels to suppress NO\textsuperscript{3} starvation-induced leaf senescence (Meng et al., 2016; Figure 1A). Lately, it was found that the expression of NRT1.5 could be positively modulated by CPSF30-L (Li et al., 2017) and suppressed by FIP1 (Wang et al., 2018b). Therefore, it would be interesting to investigate if both genes participate in NO\textsuperscript{3} starvation-induced leaf senescence (Figure 1A).

Nitrogen limitation adaptation encodes a RING E3 ubiquitin ligase. A previous study showed that the nla mutant could not accumulate anthocyanin and instead exhibited early senescence phenotype under N deficiency condition (Peng et al., 2008). Recently, Wenxue Li’s group confirmed this result and found that the content of \(^{15}\text{N}\) in the young leaves of *nla* mutant is significantly higher than that of wild-type (WT), indicating that NLA decreases translocation of N from the old leaves to the young leaves (Liu et al., 2017). It has been reported that NRT1.7 works in transporting NO\textsuperscript{3} from the old to young leaves as less \(^{15}\text{NO}_3\) spotted onto old leaves is remobilized to young leaves in nrt1.7 mutant compared with WT (Fan et al., 2009). Further investigation demonstrated that NLA directly interacts with NRT1.7 and promotes NRT1.7 degradation in vivo to repress early senescence. The NLA gene is a target of miRNA827. N limitation strongly repressed the NLA protein abundances in...
WT, while the loss of function of miRNA827 abolished the repression of NLA protein and down-regulated the NRT1.7 protein abundance under N limitation condition, indicating that N deficiency-induced repression of NLA is dependent on miRNA827 (Liu et al., 2017; Figure 1A).

An extensive reprogramming of gene expression has been found to be involved in leaf senescence to meet the complex biochemical and structural changes. Up to now, about 100 TFs that belong to about 20 different families (in particular, bZIP, WRKY, NAC, C2H2 zinc finger, MYB, and AP2-EREBP) have been described to play important roles in leaf senescence (Guo et al., 2004; Buchanan-Wollaston et al., 2005; Ay et al., 2014). ORESARA1 (ORE1, also known as NAC92) is a key factor that promotes age-dependent leaf senescence by regulating the expression of hundreds of senescence-associated genes (SAGs). These SAGs function mainly in the breakdown of nucleic acids and proteins, and the transport of sugar in Arabidopsis (Balazadeh et al., 2010a; Matallana-Ramirez et al., 2013). Nam-Hai Chua's group showed that ORE1 interacts with NLA and UBPI2/13 (ubiquitin-specific protease 12/13) to regulate leaf senescence (Park et al., 2014, 2018, 2019). Under N sufficient condition, ORE1 is polyubiquitinated by NLA and its E2 conjugase PHO2 (UBC24), and then degraded by 26S proteasomes leading to delayed leaf senescence (Park et al., 2018). But when plants were exposed to N-deficient conditions, UBPI2/UBP13 antagonize the action of NLA to maintain ORE1 homeostasis by promoting the deubiquitination of ORE1 (Park et al., 2019). The elevated ORE1 levels activate the expression of downstream SAGs such as BFN1, RNS3, NYE1, VNI2, SAG29, and SINA1 resulting in early leaf senescence (Park et al., 2019; Figure 1A). Those results provide us the post-translational regulation of N deficiency-induced leaf senescence. In addition, miR164 regulates age-induced leaf senescence by targeting ORE1 mRNA cleavage (Kim et al., 2009; Balazadeh et al., 2010b). But miR164 does not regulate the expression of ORE1 at transcriptional levels under N-starvation condition (Liang et al., 2015; Park et al., 2018).

HY5 is a key regulator of photomorphogenesis (Lee et al., 2007). About 30% of the senescence-related regulatory genes are predicted as putative targets of the HY5, such as AP2-EREBP, NAC, and WRKY (Breeze et al., 2011; Ay et al., 2014), suggesting that HY5 may play an important role in the regulatory network of leaf senescence. Interestingly, HY5 has been found to activate the expression of NIA1, NIA2, and NRT2.1, but inhibit the expression of NRT1.1 (Jonassen et al., 2009a,b; Chen X. et al., 2016), implying that HY5 may be involved in NO3− regulation. Recently, the light-signaling protein FAR-RED ELONGATED HYPOCOTYL3 (FHY3) was reported to negatively regulate salicylic acid (SA) biosynthesis and light-mediated leaf senescence (Tian et al., 2020). Both the fhy3 mutant and WRKY28-overexpressing plants exhibited early leaf senescence under high (Red/Far-Red) R: FR light conditions. FHY3 could directly bind to the promoter of WRKY28 to repress its expression. These results indicate that the FHY3-WRKY28 module prevents leaf senescence under high R: FR light conditions (Tian et al., 2020). It was reported previously that FHY3 and HY5 physically interact with each other and co-regulate the expression of CONSTITUTIVE PHOTOMORPHOGENESIS1 (COP1), a multifunctional E3 ubiquitin ligase, in response to photomorphogenic UV-B (Huang et al., 2012). Further investigation is needed to determine if FHY3, COP1, and HY5 function in N starvation-induced leaf senescence.

Although N starvation induces senescence in plants, it has also been reported that senescence is reversible in some cases after the resupply of N (Schildhauer et al., 2008). Mueller-Roemer's group further performed transcriptome analysis to investigate the N resupply induced reversal of senescence in Arabidopsis. Their results showed that the senescence program was tuned by the N status, indicating that plants undergoing senescence retain the capacity to sense and respond to the N availability (Balazadeh et al., 2014). Crops such as maize (Girardin et al., 1985), rice (Wang et al., 2012), or barley (Schildhauer et al., 2008), have also been found to have the ability to stop or even reverse the senescence induced by N deficiency when sufficient N is resupplied. However, the underlying mechanism still remains unclear. It is nevertheless evolutionarily important to have a regulatory system capable of monitoring and integrating such environmental stimuli.

Nitrate and Circadian Clock

The circadian clock is one of the most central endogenous factors that allow organisms to synchronize internal biological activities with the external environment. There is increasing evidence that circadian clock regulates N assimilation (Tucker et al., 2004; Cookson et al., 2005; Gutiérrez et al., 2008; Teng et al., 2017). It has been shown that organic N such as Glu or Gln can serve as signals to regulate the expression of N-related genes in plants (Oliveria and Coruzzi, 1999). Coruzzi's group performed a transcriptome analysis on plants treated with organic N (Gutiérrez et al., 2008). By using systems biology, they found that the center clock gene CCA1 could regulate the expression of organic N-regulated genes. Further investigation revealed that CCA1 could directly bind to the promoters of the glutamine synthetase gene (GLN1.3) and the glutamate dehydrogenase gene (GDH1) to regulate their expression. Moreover, the phase response curve analysis exhibited that organic N could influence the CCA1 phase. These data indicate that organic N may regulate circadian rhythms through CCA1-mediated mechanism (Gutiérrez et al., 2008). Another group found that low N conditions increased the amplitudes of CCA1, LHY, and TOC1 transcripts throughout the circadian cycle, whereas high N conditions decreased the amplitudes of these genes in WT plants (Yuan et al., 2016). Further study showed that CCA1 influence nitrate reductase activity by binding to the CCA1-binding site in the promoters of NIA1 and NIA2 in vitro and activating the NIA1 expression while repressing NIA2 expression (Teng et al., 2017). These results provide basic insight for the link between N and circadian clock (Figure 1B).

It has been reported that TCP20 binds to the promoters of NIA1, NRT1.1, NRT2.1 and acts in systemic NO3− signaling that directs NO3− foraging in Arabidopsis roots (Guan et al., 2014). Furthermore, TCP20 can physically interact with NLP6/7 and ARF8, and their interactions are required for activating
the expression of the G2/M cell-cycle marker gene CYCB1;1 to regulate lateral root initiation and growth (Guan et al., 2017; Fan et al., 2019a,b). Interestingly, TCP20 has also been found to be a circadian clock factor that can interact with LIGHT-REGULATED WD1 (LWD1). Both TCP20 and LWD1 can bind to the promoter of CCA1 to activate its expression (Wu et al., 2016). These results imply that TCP20 may be involved in connecting the N signaling with circadian clock.

During last several years, scientists have demonstrated the roles of the circadian clock genes in the regulation of leaf senescence in plants (Sakuraba et al., 2014; Song et al., 2014; Kim et al., 2018; Zhang et al., 2018). PIF4/PIF5 promote dark-induced leaf senescence by directly activating the expression of ORE1 and indirectly activating the expression of ETHYLENE INSENSITIVE 3 (EIN3) in ethylene signaling and bZIP factors ABA INSENSITIVE 5 (ABI5) and ENHANCED EM LEVEL (EEL) in ABA signaling (Sakuraba et al., 2014; Song et al., 2014). ELF3 inhibits leaf senescence by repressing the activity of PIF4/PIF5 (Sakuraba et al., 2014; Figure 1C). The transcript levels of ORE1 and its repressor miR164 show circadian rhythmic patterns (Kim et al., 2018). PRR9 can activate the expression of ORE1 directly or suppress the expression of miR164 to promote dark-induced leaf senescence (Kim et al., 2018; Figure 1C). In addition, elf4 and lux show obviously early leaf senescence than WT in darkness (Sakuraba et al., 2014; Kim et al., 2018). The circadian evening complex (ELF3-ELF4-LUX) can repress jasmonate-induced leaf senescence by directly binding to the promoter of MYC2, a key activator of jasmonate-induced leaf senescence (Zhang et al., 2018; Figure 1D). Ubiquitin-specific protease genes UBP12 and UBP13 are regulated by circadian clock and they in turn regulate circadian clock and photoperiodic flowering through GI and CO, which extends our understanding of deubiquitination in circadian clock and photoperiodic flowering regulation at posttranslational level (Cui et al., 2013). These results demonstrate a strong interplay between the circadian clock and leaf senescence. However, if circadian clock is involved in N deficiency-induced senescence remains unknown.

CONCLUSIONS AND PERSPECTIVES

During last several years, research on NO$_3^-$ regulation has expanded to multiple regulatory levels such as post-transcriptional modulations and also to some new developmental processes such as leaf senescence and circadian clock. The progress on these fields has started to shed light on better understanding the complex NO$_3^-$ regulatory network. However, we still lack an integrated view on how NO$_3^-$ regulates these processes and the full picture of NO$_3^-$ regulatory network is far from complete.

Post-transcriptional regulation of NO$_3^-$ has attracted more attention recently and gained further insights into NO$_3^-$ regulation. The C3H-type zinc finger motif-containing protein CPSF30-L has been characterized to function in NO$_3^-$ signaling (Li et al., 2017). The C3H-type zinc finger protein family contains 68 members and whether other members of this family participate in NO$_3^-$ regulation needs to be investigated. Although ncRNAs have been found to be involved in the regulation of multiple biological processes, the underlying mechanisms still remain elusive, especially in NO$_3^-$ signaling. The leaf senescence is finely tuned by N status. However, our understanding of the mechanisms that N availability affects leaf senescence is fragmentary and more efforts are still needed. One interesting avenue for future research is to screen mutants without early senescence symptoms under N-deficient conditions to identify the key components. In addition, systems biology may be used to globally reveal novel regulatory genes involved in N deficiency-induced leaf senescence. The core circadian genes CCA1, LHY, TOC1 are the main factors that connect circadian clock and NO$_3^-$ regulation reported so far. Further studies are needed to find out more genes involved in circadian clock and NO$_3^-$ regulation and explore their intrinsic mechanisms. The application of translatomics, structural biology and other new technologies will surely promote the clarification of the mechanisms that NO$_3^-$ regulates plant development. Notably, the major part of the results therein resumed have been obtained in Arabidopsis, which although being an excellent model for studying NO$_3^-$ signaling and providing useful fundamental knowledge for the scientific community, is quite different from crops (especially monocots), for which specific and targeted research projects are required. With the identification of more novel NO$_3^-$ regulators and decryption of NO$_3^-$ regulatory networks, as well as combined with scientific and rational fertilization management, we will effectively improve NUE and reduce the environmental pollution caused by loss of N fertilizers.

AUTHOR CONTRIBUTIONS

HF and YW wrote the manuscript. SXQ, SDQ, and NX provided assistance for further modification of manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.