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A contemporary view of nitrate signaling and control

Ch. Aruna Kumari and Sameena Begum

Abstract
Since amino acids and nucleotides are primarily made up of nitrogen (N), it is essential for plant growth, development, and stress responses. The two types of nitrogen that plants can absorb from the soil are nitrate and ammonium. In most crop soils, nitrate is the main type of nitrogen. Nitrate transporters move the absorbed nitrate from the root to other organs. Nitrate sensing stimulates signaling pathways that have an impact on local and systemic molecular, metabolic, physiological, and developmental responses. In the past ten years, significant advancements in our understanding of nitrate and other N nutritional responses have been made thanks to the development of genomics technologies and genetic tools. Furthermore, it has been crucial to find new components using methods that exploit the natural polymorphisms seen in divergent individuals of a single species. Our comprehension of how nitrate signaling affects biological procedures in plants still has some limitations. Therefore, understanding the intricate mechanisms behind nitrate signaling, control and interaction with hormones is the main goal of this review paper.

Keywords: Nitrate signaling, amino acids, nitrogen

Introduction
One of the primary nitrogen (N) sources for plants, nitrate, is crucial for the control of gene expression, metabolism, growth, and development (Wang *et al*., 2018) [68]. Natural environments often have soil nitrate concentrations of less than 1 mM, whereas fertilized agriculture soils can have concentrations as high as 70 mM (Reisenauer, 1966) [55]. Nitrate (NO_3-), is the principal source of N in well-aerated soils (Crawford and Forde, 2002) [9]. Additionally, the most plentiful source of N in agricultural soils is nitrate (Owen and Jones, 2001) [53]. Typically, agricultural soils have nitrate concentrations between 1 and 5 mM. (Owen and Jones, 2001) [53]. This supply is unstable since runoff might potentially deplete it and the nitrate ion is extremely mobile in the soil solution due to the primarily negative charge of ground particles (Miller and Cramer, 2004) [51]. The loss of soil nitrate is also influenced by biotic processes including microbial denitrification and plant uptake (Crawford and Glass, 1998) [10]. To adapt to changing N concentrations in the soil, plants have developed sophisticated mechanisms. Exogenous nitrate treatment stimulates lateral root extension, encouraging root colonization of nitrate-rich soil patches as the root architecture adapts to this changing environment (Gojon *et al*., 2009; Zhang and Forde, 1998) [23, 73]. Plants cultivated in N-sufficient settings have fewer lateral roots than those grown in low N conditions, a strategy that allows N foraging only when this nutrient is rare. However, plants subjected to long-term N treatments exhibit a distinct behaviour (Gifford *et al*., 2008) [18].
To balance N supply and demand within the plant, this local root nitrate acquisition must be coordinated with systemic signals (Ruffel *et al*., 2011) [57]. In addition to serving as a nutrient, nitrate also serves as a regional and systemic signal that links uptake to plant growth and development (Alvarez *et al*., 2012) [2]. According to Vidal and Gutiérrez (2008) [61] and Wang *et al*. (2003) [70], nitrate causes alterations in the transcription of genes involved in acquiring nitrogen (N), assimilating nitrate, producing the reducing equivalents required for N metabolism, producing carbon (C), and a variety of other processes. Thus, nitrate has a wide range of effects on plant growth, including promoting seed germination, controlling shoot growth, and delaying blooming (Vidal *et al*., 2014; Walch-Liu *et al*., 2006; Yuan *et al*., 2016) [62, 63, 72]. Nitrate Reductase (NR) reduces nitrate inside the cell, and Nitrite Reductase further reduces the nitrite product to ammonia (NiR). The GS/GOGAT cycle uses the ammonia produced to combine it with the amino acid glutamate to produce glutamine (Krapp, 2015; Marschner, 2012; Xu *et al*., 2012) [38, 48, 71]. The assimilated N is converted into more amino acids or biomolecules in subsequent processes (Krapp, 2015) [38]. A delicate equilibrium between N and C must be achieved by the plant since the incorporation of inorganic N into

amino acids need a carbon (C) skeleton. Early studies demonstrated that nitrate absorption fluctuates depending on the supply of photosynthates, imposing diurnal oscillations. Numerous nitrate-controlled genes' expression is found to be regulated by C metabolites, according to molecular and computational research (Palenchar *et al*., 2004) [54]. In a 150 bp tract that is sensitive to nitrate, N metabolites, and sucrose, Girin *et al*. (2007) [19] discovered the N- and C-dependent transcriptional regulation of a nitrate transporter (Girin *et al*., 2007) [19]. Additionally, N metabolites govern the circadian clock master regulator CCA1's transcription, which in turn controls the transcription of genes involved in N assimilation (Gutiérrez *et al*., 2008) [61]. This creates a link between the circadian clock and N nutrition. Additionally, genome-wide studies showed that nitrate promotes the expression of other genes involved in other metabolic pathways, such as the pentose phosphate pathway, in addition to genes associated with N assimilation (Wang *et al*., 2003) [70]. The physiological significance of nitrate-induced expression of metabolic enzyme genes has not yet been clarified, with the exception of genes relevant to N absorption. In fact, nitrate is a significant signaling molecule in addition to being food. Nitrate transport and nitrate signaling are the main topics of this review.

Nitrate transport

The initial step in the process of nitrate absorption in plants is the uptake of nitrate by roots from the soil (Glass, 2009) [22]. Nitrate levels in agricultural soils vary greatly and range from 1 to 10 mM. Plants have created three unique nitrate absorption systems, with two having strong affinities and the third having low affinities for nitrate, to deal with this significant variance in nitrate content (Kiba, 2012; Krapp, 2011) [32, 37]. Regarding the induction by nitrate and the operational concentration range of nitrate in the soil, these absorption mechanisms are different from one another. While the constitutive high-affinity transport system (also known as cHATS) is expressed consistently, the inducible high-affinity transport system (iHATS) is highly activated in the presence of nitrate (Glass *et al*., 2013) [20]. The iHATS has a substantially greater capacity for nitrate uptake despite the cHATS's strong affinity for nitrate [Km values of 6-20 M compared to 13-79 M for the iHATS; (Krapp, 2011)] [37]. When the external concentration rises (>1 mM), the low-affinity transport system (LATS) enters the picture. While in some plants this system does not need to have been exposed to nitrate beforehand, in others the expression is increased when there is a supply of nitrate. From plants, the genes for nitrate transporters have been cloned and studied (Forde, 2000) [16]. According to their sequence analysis, these newly discovered genes have been divided into the NRT1 and NRT2 nitrate transporter families (Crawford, 2002; Glass, 2013) [9, 20]. NRT2 has high-affinity (M nitrate) transporters, whereas NRT1 has low-affinity (mM nitrate) transporters. However, it has been discovered that one transporter, AtNRT1, is a dual affinity transporter (Wang *et al*., 2012) [69]. There are reports on the rice OsNRT1 low affinity transporter gene, which has been cloned and functionally described (Li *et al*., 2010) [44].

Nitrate Transport Control

The balance between the two opposing fluxes—influx from apoplasm to cytoplasm and efflux in the other direction—determines the rate of nitrate absorption, and transport is a tightly controlled process (Fernandez, and Galvan, 2007) [15]. Numerous internal and external signals can affect how quickly nitrate is absorbed by roots. The nitrate itself is the main inducer. In plants that are either given another source of nitrogen or are deprived of nitrate, a basal level of nitrate absorption with both high and low affinity systems is present. The uptake rate rises after exposure to nitrate. When plants are given high quantities of nitrate and ammonium, nitrate uptake diminishes (feedback inhibition). It has been demonstrated that ammonium inhibits influx rather than stimulating efflux, and iHATS are more severely impacted than cHATS or LATS. Additionally, supplying amino acids as the only source of nitrogen for plant growth strongly inhibits the uptake of NO_3. Circadian rhythms decreased carbon, and shoot nitrogen needs all have an impact on the uptake (Crawford and Forde, 2002) [2].

Primary uptake can also take place in leaves, which is a route that is crucial for epiphytes and for incorporating foliar fertilizer applications. Nitrate may be instantly transformed into ammonium and amino acids once within the plant cell. Alternately, the nitrate could be long-distance transported to the leaves for reduction by being temporarily stored in the plant's root system or injected into the xylem. When there is an insufficient external supply of nitrogen, the high vacuolar concentration of nitrate can act as a source of nitrogen or contribute to the overall osmoticum. According to reports, nitrate is also lost to the soil via efflux across the plasma membrane. Depending on the plant's kind, developmental stage, and external nitrate concentration, the nitrate can be decreased in either the leaves or the roots (Faure *et al*., 2001) [14]. Root, shoot, and leaves are the primary storage organs.

Nitrate signaling

In addition to being a necessary nutrient, nitrate also acts as a signaling molecule to drive leaf growth, control the formation of lateral roots, and coordinate the expression of genes associated with nitrate (Alboresi *et al*., 2005; Walch-Liu *et al*., 2000; Zhang and Forde, 2000) [1, 76, 74]. (Wang *et al*., 2000) [64]. These latter years have seen significant advancements in our understanding of the later reaction, sometimes known as the major nitrate response. With reference to outstanding previous reviews on the other facets of nitrate signaling, we concentrate on the molecular participants in the primary nitrate response in this study (Bouguyon *et al*., 2012; Wang *et al*., 2012) [4, 69]. The initial nitrate response of numerous proteins, including nitrate transporters and absorption enzymes, is regulated by nitrate in order for the plant to use them. This so-called primary nitrate response involves the fast (within minutes) nitrate regulation of up to 1000 genes' expression (Marchive *et al*., 2013, Vidal *et al*., 2013) [47, 60].

Several investigations use nitrate reductase null mutants (Wang *et al*., 2004) [65] and mutants of the nitrate sensor NPF6.3 (NRT1.1/CHL1) to distinguish between direct molecular reactions to nitrate and responses to nitrite (Wang *et al*., 2007) [66] and general responses to N supply (Wang *et al*., 2009) [67]. Additionally, it has been demonstrated that the components for nitrate signaling and nitrate-responsive transcription pre-exist in plant cells regardless of whether nitrate is present or absent. These genes, which encode nitrate reductase and nitrite reductase, respectively, are known to be a nitrate-inducible expression of NIA and NII genes (Gowri *et al*., 1992) [24].

Genes involved in amino acid and nucleic acid biosynthesis, transcription and RNA processing, ribosome and hormone biosynthesis, reductant supply, and trehalose metabolism also respond within 3-360 minutes of nitrate induction, in addition

to genes involved in nitrate uptake and assimilation. In fact, a thorough analysis of the time course of the early nitrate responses in the roots of young seedlings revealed that the earliest responses (3–9 min) involve genes and functions necessary to create the conditions for plants to use or reduce nitrate, such as ribosomes and the oxidative pentose phosphate (OPP) pathway, the latter of which provides reductants for nitrate assimilation. It was determined by comparing early nitrate-specific responses (up to 9 min) with hormone-regulated genes that interactions with other signals, like hormones, take place after an early nitrate-specific response (Krouk *et al*., 2010a) [41]. This nitrate signal-induced, incredibly quick gene expression response is still complicated. Early expression alterations frequently change quickly. For instance, just three genes showed a steady rise in expression over the first 20 min following the injection of nitrate to N-starved Arabidopsis seedlings (Castaings *et al*., 2011) [7]. In subsequent time points after nitrate delivery, many of the early triggered genes are down-regulated and are hence undetectable in samples. Now that we are aware of these early responsive genes, we need to learn more about how they contribute to nitrate signaling. Identification of cis-elements sensitive to nitrate One might assume that the abundance of nitrate-regulated genes would make it simple to pinpoint the promoter elements involved in nitrate's role in transcriptional control. However, nitrate-responsive promoter elements haven't been found in any of the current global transcriptome investigations. It may be challenging to identify a consensus sequence involved in this extensive reprogramming of transcription in response to a signal as important as N supply because the nitrate response combines a number of molecular players who act in cascades or synergy and interact with other signaling cascades. Individual nitrate-inducible gene promoters have, however, been examined. Reporter genes were given nitrate-inducible expression by the promoters of the Arabidopsis nitrate reductase-encoding genes (NIA1 and NIA2) (Lin *et al*., 1994) [46], and further investigation of these promoters using linker scanning mutagenesis resulted in the definition of a nitrate-responsive cis-element (Hwang *et al*., 1997) [30]. Another nitrate-responsive sequence (NRE) was revealed to be both essential and sufficient for nitrate-responsive transcription in the Arabidopsis nitrite reductase (NII) promoter (Konishi and Yanagisawa, 2010) [34]. The NRE is a pseudopalindromic sequence of 43 base pairs that consists of two half-sites separated by a non-conserved spacer sequence of 10 base pairs. Although the distal half-site plays a major role, both sites are required for complete nitrate stimulation of the expression of the Arabidopsis NII gene in planta (Konishi and Yanagisawa, 2010) [34]. A -glucuronidase (GUS) reporter gene construct's nitrate-inducible expression is mediated by NRE and is not dependent on protein synthesis. Several dicotyledonous and monocotyledonous NII promoters contain NRE-like sequences, but no additional nitrate-regulated genes have been found to contain them. For instance, it was discovered that the NRT2.1 promoter's 150 bp region, which lacks similarity to the NRE, is adequate to mediate nitrate induction and N metabolites' suppression. The 5′-flanking regions of nitrate-regulated genes do not always contain nitrate-responsive sequence motifs, which is an interesting fact. 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The circumstances surrounding the nitrate induction studies may account for the apparent discrepancy between Konishi and Yanagisawa's (2011) [35] finding that the NIA2 and NIA1 promoter sequences, which are 2.7 kb and 1.9 kb, respectively, did not direct nitrate-inducible expression. Factors controlling the main nitrate response during transcription. The significant changes in gene expression in response to a nitrate signal are anticipated to be mediated by transcription factors on a molecular level. Recently, NIN-like proteins (NLPs) have emerged as master regulators of nitrate signaling. Identification of transcription factors implicated in nitrate signaling has advanced in recent years (Konoshi and Yanagisawa, 2013; Marchive *et al*., 2013) [36, 47]. These RWP-RK transcription factors are related to the NIT2 protein, which controls the expression of nitrate reductase in Chlamydomonas, and the NIN (nodule inception) protein, which is involved in the initial stages of the N-regulated symbiosis between rhizobia and legume roots in *Lotus japonicus* (Schauser *et al*., 1999) [77]. (Camargo *et al*., 2007) [78]. One of the nine members of the Arabidopsis NIN-like family, NLP7, was discovered to play a role in the reactions to nitrate and N famine because nlp7 mutants showed a constitutive N starvation phenotype that may have been brought on by faulty N signaling (Castaings *et al*., 2009) [6]. In fact, NLP7 interacts to 851 genes in response to nitrate, preferentially binding around the transcriptional start site of the target genes, as demonstrated by Marchive *et al*. (2013) [47] using a ChIP-chip technique. This gene set is particularly rich in genes associated with N metabolism, the OPP pathway, sulphur and carbon metabolism, as well as regulatory proteins like transcription factors. Nearly all of the previously identified nitrate signaling genes, including ANR1 (Zhang and Forde, 1998) [73], LBD37/38 (Rubin *et al*., 2009) [56], CIPK8 (Hu *et al*., 2009) [28], and NPF6.3 (NRT1.1/CHL1; Ho *et al*., 2009) [28], were found among the bound genes. The NLP7-immunoprecipitated sequences did not, however, appear to be strongly enriched for any evident DNA-binding motifs. In the same experimental setup as the ChIP, 91 of these 851 genes showed an attenuated nitrate response in the nlp7 mutant context. The deregulation of direct NLP7 targets affects the control of nitrate across the entire genome. In actuality, NLP7 loss of function causes transcriptome alterations that go beyond the genes it directly regulates. These findings collectively identify NLP7 as the upper hierarchical coordinator of nitrate responses. The quick activation of NLP7 by the nitrate signal is another justification for the critical role it plays. In the nucleus of numerous tissues involved in N transport, the NLP7 protein is found (e.g. root hairs, emerging lateral roots, and stem vascular tissues). However, N deficiency causes nuclear NLP to relocate to the cytosol. Within minutes after replenishing nitrate following N deprivation, NLP7 was moved into the nucleus. This relocalization is nitrate-specific and unaffected by transcriptional control. It is possible to replicate the effects

of nitrate by using the nuclear export-inhibiting medication leptomycin B. Thus, it was proposed that nitrate directly or indirectly inhibits NLP7's export from the nucleus through an as-of-yet unidentified mechanism, causing a fast nuclear accumulation in response to the signal (Marchive *et al*., 2013) [47]. Furthermore, a yeast one-hybrid (Y1H) screening employing the 43bp NRE provided significant evidence for the primary involvement of NLPs in nitrate signaling (Konishi and Yanasigawa, 2013) [36]. It has been shown that all nine NLPs bind to the NRE in yeast, indicating that all NLPs may also bind to this component in plants. Different NLPs bind to the two components of the palindromic NRE sequence with distinctly different specificities. By generating an NLP6-EAR fusion construct, which converts an activator into a dominant chimeric repressor and is thus ideally adapted to research gene families with potential redundant activities, the importance of NLPs for the main nitrate response was examined (Hiratsu *et al*., 2003) [27]. In fact, NLP7's predicted nuclear export motif is found at the protein's N-terminus, and NLP6 also has a relatively comparable sequence. No doubt, other transcription factors contribute to the major nitrate response in addition to NLPs. Squamosa promoter-binding-like protein 9 (SPL9) was predicted to be a participant in the primary nitrate response using a systems approach. During a nitrate resupply kinetic, SPL9 overexpression altered the expression of sentinel genes that are controlled by nitrate (Krouk *et al*., 2010b) [42]. Additional information regarding SPL9's effects is not yet available. Other nitrate signaling transcription factors, such as the LOB domain-binding proteins LDB37/38/39 or the MADS box transcription factor ANR1 (Zhang and Forde, 1998) [78], are involved in the regulation of nitrate-related traits but have not yet been demonstrated to be active in the primary nitrate response. Several well-known nitrate-induced genes are frequently used in investigations as so-called sentinel genes for the initial nitrate reaction. Remembering that the major nitrate response is a complicated alteration in global gene expression that includes a variety of response patterns, including extremely early induction, transitory induction, repression, etc., is undoubtedly significant (compare Krouk *et al*., 2010b; Marchive *et al*., 2013) [42, 47]. It is possible that the selected sentinel gene might not accurately represent the entire main nitrate response. Additionally, depending on the experimental conditions, the age of the plants, etc., nitrate supply to plants may have an impact on the transcriptome, and a specific sentinel gene may be regulated by additional parameters. One of the nitrate signaling pathway's unmet needs is calcium. One of the most extensively researched second messengers in cell signaling is calcium, and plants are not an exception (Dodd *et al*., 2010) [12]. For a wide range of cellular and plant responses in plants, including stomatal aperture, biotic stress, abiotic stress, nodulation, circadian clock, polar tip growth, and self incompatibility (reviewed by (Dodd *et al*., 2010) [12]. The first evidence of a relationship between calcium and NO_3 was found in the detached leaves of barley and maize (Sakakibara *et al*., 1997; Sueyoshi *et al*., 1999) [58, 59]. Independent of de novo protein synthesis, NO_3 - treatments increase the expression of the genes for nitrate reductase (NR), nitrate reductase (NiR), and plastidic glutamine synthase (GS2), and glutamate synthase (GOGAT) (Sakakibara *et al*., 1997) [58]. However, in detached maize leaves prepared with EGTA or La3+, mRNA for these genes does not assemble to the same amount in response to NO_3 - treatments (Sakakibara *et al*., 1997) [58]. In a different investigation utilizing barley leaves that had been removed, a similar outcome was attained. NR and NiR gene expression in response to NO_3 treatments was dramatically reduced when leaves were pretreated with La3+ (Sueyoshi *et al*., 1999) [59]. Other than these first findings, the function of calcium in the NO_3 - signaling pathway was not thoroughly investigated until recently. *In-vivo* cytoplasmic calcium alterations in response to NO_3 treatments were observed by Riveras *et al*. (2015) [79] using Arabidopsis reporter lines that expressed aequorin in the cytosol. This study strengthened earlier research by demonstrating that NPF6.3 (CHL1/NRT1.1) activity is necessary for the buildup of cytoplasmic calcium brought on by NO_3. Additionally, it was suggested that phospholipase C (PLC) activity was upstream of calcium changes and downstream of NPF6.3 (CHL1/NRT1.1) by the use of the phospholipase C inhibitor U73122 and measurements of inositol 1,4,5 triphosphate (IP3). According to this research, NO_3

is recognized by the transceptor NPF6.3 (CHL1/NRT1.1) and initiates a PLC activity, which causes a rise in cytoplasmic calcium. Some key responder genes, such as NRT2.1 and TGA1, must change in gene expression in order for this calcium signal to occur. Not all NO_3 - sensitive genes rely on this signaling pathway, as would be predicted. For instance, the transceptor NPF6.3 (CHL1/NRT1.1) is necessary for the activation of the auxin receptor AFB3, while PLC and calcium are not required. The NPF6.3 (CHL1/NRT1.1) NO_3 - transceptor's numerous signaling pathways branching downstream are consistent with these findings. Phosphorylation of proteins in nitrate signaling Changes in protein phosphorylation state is one of the immediate effects of an increase in cytosolic calcium (Sanders *et al*., 1999) [80]. Years ago, protein phosphatase and tyrosine protein kinase inhibitors were used to address the significance of protein phosphorylation for NO_3 - signaling (Sueyoshi *et al*., 1999) [59]. The NO_3 - dependent induction of NR and NiR in barley leaves is greatly hampered in the presence of the inhibitors. For the CBL1-CIPK23 and CBL9-CIPK23 protein complexes to activate K+ TRANSPORTER 1 *in vivo*, calcineurin B-like protein 1 (CBL1) and CBL9 must also be phosphorylated (Hashimoto *et al*., 2012) [26]. Up to 38 proteins can change their phosphorylation status in NO_3 - deprived whole seedlings, according to more recent untargeted phosphoproteomic studies. Functionally speaking, the majority of the proteins found in this study belong to basic metabolic pathways. Engelsberger and Schulze (2012) [13] found 589 proteins that were differently phosphorylated following NO_3 treatments in nitrogen-starved Arabidopsis seedlings. Fast sensitive proteins, such as GPI anchored proteins, receptor kinases, and transcription factors, fall into one of two types. The second group relates to proteins that are involved in hormone metabolism, central metabolism, and protein synthesis and breakdown. Uncertainty still exists regarding how NO_3-induced calcium changes are detected and how this signal is translated to phosphorylate target proteins. These phosphoproteomic investigations, which indicate putative protein effectors for phosphorylation alterations in response to N, revealed an intriguing overrepresentation of kinases and phosphatases among the proteins with changes in their phosphorylation pattern. This group of protein kinases included receptor-like kinases, MAP kinases, Snf1-related protein kinases, calcium-dependent protein kinases, and calcineurin-B like (CBL)-CBL-interacting protein kinase (CIPK) kinases. Both CIPK8 and CIPK23 are potential elements of the calcium-dependent signalling pathway that

have been previously linked to NO_3-signaling (Ho *et al*., 2009) [28].

In low NO_3 - concentrations, CIPK23 is known to phosphorylate the NO_3 - transceptor NPF6.3 (CHL1/NRT1.1), negatively inhibiting the primary NO_3 - response (Ho *et al*., 2009) [28]. In contrast, ABA INSENSITIVE 2 (ABI2) is the sole known target of CIPK8 (Ohta *et al*., 2003). However, it has been demonstrated that this kinase functions as a positive regulator in the low-affinity NO_3 - response (Hu *et al*., 2009) [29].

Nitrate sensors

Similar to animal cells, plant cells detect environmental cues and respond to them through receptors that communicate biochemical data to the nucleus, where alterations in gene expression take place, via protein-protein interactions. Thus, the plant must recognise nitrate as a signal molecule at the cellular level. Many scenarios are conceivable. First, a protein that is linked to the membrane may recognize the external presence of nitrate. In contrast, the parameter that is felt can be the intracellular nitrate level, either in the cytosol or in other cellular structures like the storage vacuole. Another idea is that nitrate fluxes are sensed by proteins that transport or metabolize nitrate, similar to how hexokinase is thought to sense sugar fluxes (Hanson and Smeekens, 2009) [25]. A mutation at the NPF6.3 (NRT1.1/CHL1) locus was present in one of the isolated mutants with decreased YFP expression upon nitrate feeding (nrg1). 113 genes, including those involved in nitrate assimilation, energy metabolism, and the pentose phosphate pathway, were impacted by nitrate regulation in the nrg1 mutant (Wang *et al*., 2009) [67]. The intriguing discovery of an NPF6.3 mutant (chl1-9) with a P492L mutation between the 10th and 11th trans membrane domains, which is impaired for nitrate transport but functional for transducing the nitrate signal, supports the role of NPF6.3 as a so-called nitrate transceptor (transporter-receptor) (Ho *et al*., 2009) [29]. As a result, the dual-affinity nitrate transporter NPF6.3 is able to detect changes in a variety of nitrate concentrations in the soil and cause various levels of transcriptional responses.

Transcriptome analyses of the npf6.3 (nrt1.1/chl1-5) mutant revealed the presence of two nitrate-inducible protein kinases (CIPK8 and CIPK23), which are members of the family of CBL-interacting protein kinases. Low nitrate availability causes CIPK23 to phosphorylate NPF6.3 at T101, which attenuates the main response (Ho *et al*., 2009) [28]. NPF6.3 is not phosphorylated in cipk23 mutants, which results in high levels of initial transcriptional response in the presence of little nitrate. This suggests that the high-affinity response is negatively regulated by CIPK23. However, the targets of CIPK8, which is a positive regulator of the low-affinity response, are not known (Hu *et al*., 2009) [29].

Conclusion

In order to give a comprehensive understanding of N-nutrient/metabolite sensing and responses in plants, it will be crucial to elucidate the many nitrate-sensing systems and comprehend their spatiotemporal cross-talk at the cell-specific, organ-specific, and organism level.

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