Review Article

Molecular Frameworks of Nitrogen Response in Plants: A Review

ABSTRACT

Nitrogen is a crucial element for all living organisms especially plants which rely on substantial nitrogen quantities to sustain their growth and productivity. From the rhizosphere plants absorb nitrogen in the form of nitrate (NO_3^-), ammonium (NH_4^+), or organic nitrogen (amino acids and urea). Plants exhibit an array of sensing and adaptive mechanisms to respond to the diverse nitrogen nutrition conditions which include morphological and physiological responses. Two primary systems govern nitrogen uptake in plants: the High-affinity transport system (HATS) and Low-affinity transport system (LATS). Nitrate transporters fall into two categories, Nitrate Transporter 1 (*NRT1*) and Nitrate Transporter 2 (*NRT2*) transporters, Chloride Channel Family (*CLC*) transporters and Slow Anion Associated Channel Homologs (*SLAC/SLAHs*). The ammonium transporter family includes Ammonium Transporter 1 (*AMT1*) and Ammonium Transporter 2 (*AMT2*) transporters. The uptake of organic nitrogen is facilitated through amino acid and urea uptake and transport systems. In fluctuating environmental conditions, plants employ nitrogen response mechanisms to fine-tune homeostasis. A comprehensive understanding of these regulatory mechanisms holds the potential to yield valuable insights for the development of crops with enhanced nitrogen use efficiency.

Keywords: Nitrogen response, Nitrogen use efficiency, Nitrate uptake, Ammonium uptake, Nitrate transporters, Ammonium transporters

1. INTRODUCTION

Nitrogen plays a crucial role in the vitality of all living organisms, serving as a building block for essential biomolecules such as proteins, nucleic acids and secondary metabolites. Plants, in particular, heavily rely on substantial quantities of nitrogen to support their growth and productivity. However, the acquisition of nitrogen by plants poses a limiting factor in both natural and agricultural ecosystems. In the early twentieth century, the development of Haber–Bosch process revolutionized ammonia synthesis, facilitating mass production of nitrogen fertilizers. Since then, extensive applications of nitrogen fertilizers have been pivotal in boosting crop production, contributing to the sustenance of approximately half of the world's population by enhancing crop productivity [1][2]. Therefore, an understanding of how plants perceive diverse nitrogen sources and adapt their physiological and developmental processes to varying nitrogen supplies is vital for the maintenance and enhancement of current crop production.

The collective adaptive responses of plants to different nitrogen nutrition conditions constitute the nitrogen response, a crucial element of the regulatory network governing plant growth. Numerous physiological, molecular, and genetic studies have been conducted to characterize the nitrogen response and unravel its underlying mechanisms. Investigations into plant responses to nitrate have been extensive, given its prominence as the primary nitrogen resource in oxidative terrestrial ecosystems. An inclusive exploration of nitrogen responses in plants, considering nitrate, ammonium, and organic nitrogen as nitrogen sources, will contribute significantly to advancing our understanding of nitrogen response regulation. This knowledge, in turn, holds the potential to refine crops, enhancing their nitrogen use efficiency and ultimately improving global agricultural practices.

2. NITROGEN RESPONSE

Plants typically acquire nitrogen in various forms such as nitrate, ammonium and organic nitrogen (including amino acids and urea) from the rhizosphere. They possess multiple layers of sensing and adaptive mechanisms to respond to the availability of these nutrients. These adaptive responses, collectively known as ‘nitrogen responses,’ include morphological and physiological responses that enable plants to effectively uptake nitrogen and adapt to spatiotemporal fluctuations in nitrogen abundance within the field [3]. Roots absorb different nitrogen forms, and specific transporters allocate them to various tissues, triggering distinct nitrogen responses in those specific tissues [4].

2.1 Morphological responses

Nitrogen sources in our fields exhibit uneven distribution due to localized fertilizer application and the spatially heterogeneous presence of soil microorganisms engaged in nitrification or denitrification activity [5]. In response to this uneven soil distribution of nitrogen, plants actively sense nitrogen sources and modify their root architecture to efficiently exploit the available nitrogen sources [6]. A prominent adaptation is the notable augmentation of lateral root growth and axial root/primary root in response to a localized source of nitrate or ammonium [3].

The lateral root growth exhibit complexity and depends on the nitrate and ammonium concentrations in the soil. Optimal stimulation of lateral root growth occurs in the presence of moderate concentrations of nitrate and ammonium, approximately 0.5 mM and 0.8 mM, respectively. However, elevated concentrations exceeding 5 mM for nitrate and 10 mM for ammonium inhibit lateral root growth in Arabidopsis [7]. The nitrogen nutrient status also influences root architecture by regulating primary root elongation as evidenced in maize, where nitrogen starvation consistently stimulated axial root growth [8].

Nitrogen-deficient conditions promote aerenchyma formation in the root cortex enabling them to adapt to diverse nitrogen nutrient conditions by modifying both root architecture morphology and internal root structure. Root cortical aerenchyma formation results from cell death and the modulation of internal nitrogen metabolite levels, rather than being solely resulted by external nitrate or ammonium levels [9].

2.2 Physiological responses

Various physiological processes in distinct plant tissues are intricately influenced by environmental nitrogen nutrient conditions or intracellular nitrogen status [10]. A primary example is the regulation of nitrogen uptake activity. Under nitrogen-deficient conditions, the activity of both nitrate and ammonium uptake in plants is enhanced. Conversely, when nitrogen fertilizers are applied to plants facing nitrogen-starved conditions, there is a transient expansion of nitrogen uptake potential, achieved by upregulating the expression of genes associated with nitrate and ammonium uptake [11]. These complex modulations might be a strategy to conserve energy and acquire nitrogen at an adequate level in response to prevailing nitrogen conditions. These physiological adaptations in nitrogen uptake occur within minutes or hours, in contrast to the morphological changes of roots, which necessitate several days.

Another example of a physiological response lies in the mutual interplay between photosynthesis and nitrogen assimilation, as indicated by the strong correlation between leaf nitrogen content and the CO_2 assimilation rate [12]. The ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) content is significantly influenced by the nitrogen supply [13]. Conversely, photosynthesis provides the energy and carbon skeletons necessary for nitrogen assimilation [14]. Nitrogen nutrients play a pivotal role in promoting the expression of photosynthesis-related genes [15], whereas soluble sugars contribute to the expression of genes related to nitrate assimilation [16]. This mutual interplay between photosynthesis and nitrogen assimilation underscores the crucial role of nitrogen nutrition in biomass production and crop yield.

The impact of nitrogen nutrition on the life cycle and the transition from the vegetative to the reproductive stage in plants is a subject of debate due to inconsistent findings. In wheat and maize, nitrogen fertilizer application has been linked to an advancement in the transition to the reproductive stage, while low nitrogen availability has been associated with a delay [17][18]. Conversely, Vidal *et al*.

[19] proposed that under low-nitrogen conditions, there is an earlier transition from the vegetative to the reproductive stage in Arabidopsis, accompanied by alterations in the expression levels of genes related to flowering. However, conflicting results emerged from a meta-analysis conducted by [20] on wheat and barley, as they could not establish a clear correlation between nitrogen fertilizer application and the timing of flowering/heading. Therefore, the regulation of the reproductive stage transition by nitrogen nutrients remains unclear, suggesting that this transition may be influenced in collaboration with other factors, such as the presence of additional nutrients.

Later [21] studied the molecular basis of flowering in Arabidopsis and its connection to nitrogen application. They demonstrated the flowering time variations in response to altered nitrogen levels that are mediated by two key factors: ferredoxin-NADP$^+$-oxidoreductase (*FNR*1) and the blue-light receptor cryptochrome 1 (*CRY1*). The expression of both genes is triggered by low nitrogen levels, and mutants with loss-off function exhibit insensitivity to altered N concentration.

2.3 Molecular aspects of nitrogen uptake and transport

Plants have distinct uptake systems to maximize uptake efficiency in a wide range of external nitrate concentrations. These systems feature diverse properties to accommodate variations in nitrate/ammonium uptake capacity [22]. The two primary systems in plants are High-affinity transport system (HATS) and Low-affinity transport system (LATS). The HATS facilitates absorption at low (<0.5 mM) external nitrate/ammonium concentrations. Under low nutrient concentration, HATS scavenge ions and allow plants to maintain a normal uptake rate. Conversely, the Low affinity transport system (LATS) allows transport in high (>0.5 mM) external nitrate/ammonium concentration. In high nutrient concentrations, LATS is activated, resulting in increased uptake along increasing nitrate gradient [23]. The specificity of nutrient transporters corresponds to the form of nitrogen available in the soil, and the molecular mechanisms governing the utilization of these three distinct forms of nitrogen will be further discussed.

2.3.1 Nitrate

NO_3^- concentrations in soil solutions exhibit variability, prompting plants to evolve regulatory systems for NO3- transporters to adapt to this fluctuation [24]. Nitrate is absorbed from the soil into the roots by different nitrate transporters. During the past two decades, at least four families of transporters functioning in NO_3^- transport have been identified that participate in nitrate uptake, distribution or storage in plants. They are Nitrate Transporter1/Peptide Transporter Family (NPF) transporters, Nitrate Transporter 2 (NRT2) transporters, Chloride Channel Family (CLC) transporters and Slow Anion Associated Channel Homolog (SLAC/SLAH) [25].

2.3.1.1 Nitrate Transporter1/Peptide Transporter Family (NPF) transporters

The first NO_3^- transporter family identified in plants is the Nitrate Transporter1/Peptide Transporter Family (NPF) transporter. This family includes a substantial number of genes, categorized into 8 to 10 subfamilies. The NRT family of transporters is mainly responsible for the low-affinity transport system (LATS) of NO_3^-, except for *NRT1.1* which functions as a dual affinity transporter [23].

2.3.1.2 Nitrate Transporter 2 (NRT2) transporters

The second NO_3^- transporter family in plants is NRT2 comprising at least seven members in Arabidopsis [26] and five in the rice genome [27]. Many members of the NRT2 family are incapable of independently transporting NO_3^-; they require a partner protein, NAR2 (nitrate assimilation-related protein). When the NO_3^- concentration in the external medium is low, the HATS depend on the activity of the NRT2 family [28]. *NRT2* transcript abundance was found to be higher during the day and decreased at night, resembling the fluctuation of root sugar levels, suggesting the role of root sugar levels in the diurnal expression pattern of *NRT2* [29].

2.3.1.3 Chloride Channel Family (CLC) transporters

Chloride Channel Family (CLC) transporters are found across various kingdoms, with seven members identified in Arabidopsis. The *CLC* gene in *Arabidopsis thaliana* (*AtCLC*) is particularly expressed in the tonoplast of guard cells. This tonoplast is located $2NO_3^-/1H^+$ antiporter and plays a critical role in NO_3^- accumulation in the vacuole [30]. Phosphorylation of the vacuolar anion exchanger *AtCLCa* is essential for the stomatal response to abscisic acid [31].

2.3.1.4 Slow Anion Associated Channel Homolog (SLAC/SLAH) transporters

The SLAC/SLAH family comprises five members in *Arabidopsis thaliana*, likely encoding S-type anion channels in guard cells and probably participating in stomatal closure [32]. Among them, SLAH2 is expressed in the root stele, probably aiding in facilitating NO_3^- transport from root to shoot [33].

Among all of these transporter families, *Nitrate transporter 1.1* (*NRT1.1*) is the first one isolated [34] and the most extensively studied in the plant kingdom. It is also known as CHL1 or NPF 6.3 and is expressed in the epidermal cells of root tips and cortical and endodermal cells of mature roots [35]. *NRT1.1* regulates other NO_3^- uptake systems, stimulates root proliferation by NO_3^-, relieves seed dormancy and facilitates the activity of phytohormones.

Sun *et al.* [36] elucidated the crystal structure of the plant dual-affinity nitrate transporter *NRT1.1*. The *NRT1.1* comprising 12 membrane-spanning segments, facilitates proton-coupled nitrate transport activity in the plants. *NRT1.1* is essential for both high and low affinity nitrate absorptions in Arabidopsis. *NRT1.1* shares sequence homology with members of the NRT1/PTR family and was initially shown to be a low-affinity nitrate transporter. Subsequent findings revealed that plants with *nrt1.1* mutation were also defective in high affinity nitrate uptake and concluded it as a dual affinity transporter [37].

The dual-affinity function of NRT1.1 is primarily regulated through phosphorylation modification at a key threonine residue, Thr101 [37]. Positioned on the intracellular side between the third and fourth transmembrane helix of NRT1.1, Thr101 plays a pivotal role. Phosphorylation of Thr101 by the CIPK23 kinase enzyme [38] transforms NRT1.1 into a high-affinity nitrate transporter, while in its unphosphorylated state, it functions as a low-affinity transporter. Mutations on Thr101, prevent phosphorylation, and effectively convert the dual-affinity transporter into either a monophasic low-affinity or high-affinity transporter [39]. This regulatory mechanism of NRT1.1 enables rapid adaptation to changing nitrate levels.

Maghiaoui *et al.* [40] based on their studies in Arabidopsis understood that *NRT1.1* transceptor coordinately controls auxin biosynthesis and transport to regulate root branching in response to nitrate. In Arabidopsis, the *NRT1.1* nitrate transceptor suppresses the lateral root development under low nitrate availability by promoting basipetal auxin transport out of the lateral root primordia region. In addition, *NRT1.1* also serves as a negative regulator of the *TAR2* auxin biosynthetic gene expression in the root stele, indicating its potential to repress local auxin biosynthesis. Furthermore, *NRT1.1* exerts a negative influence on the expression of the *LAX3* auxin influx carrier. The findings suggest that the *NRT1.1* transceptor coordinately controls several auxin-associated processes crucial for lateral root primordia development. Consequently, *NRT1.1* plays an integrated role in regulating the nitrate response of root system architecture.

The regulation of cytokinin levels involves the participation of nitrate sensor *NRT1.1,* thus influencing root elongation under both normal and nitrogen deficit conditions. The mutation of the *NRT1.1* gene led to a reduction in cytokinin levels in the roots of chl1-5 mutants [41]. Previously it was established that *NRT1.1* plays a role in the regulation of flowering time in Arabidopsis. Teng *et al*. [42] used genetic and molecular methods to investigate the key flowering pathway in which *NRT1.1* may be involved. Mutant alleles of *CO* and *FLC*, the two crucial components in the flowering pathway were introduced into *NRT1.1* defective mutant. Upon introducing the *CO* mutation into chl1-5 plants, *CO* transcription levels remained unchanged. However, *FLC* loss of function could rescue the late flowering phenotype of the chl1-5 mutant, with a significant increase in *FLC* expression levels in the *NRT1.1* defective mutant.

In the chl1-5flc-3 double mutant plants, FT expression levels were restored when the FLC mutation was introduced into chl1-5 plants. It was also found that the up-regulation of *FLC* transcripts in the chl1-5 mutant plants was not related to nitrate availability. This study suggested that *NRT1.1* affects flowering time by interacting with the *FLC* dependent flowering pathway to influence its target gene *FT.*

Furthermore, *NRT1.1* may be included in an additional signaling pathway that represses the expression of *FLC* in a nitrate-independent manner.

Calcium ions play a crucial role in nitrate signaling facilitated by *NRT1.1*. Nitrate triggers calcium transient in the cytosol and nucleus. The mechanism involving phospholipase C (PLC), inositol 1,4,5-trisphosphate (IP3) and the external or internal sources responsible for nitrate-triggered calcium increase remains unclear. These alterations are anticipated to impact the expression of nitrate-related genes, and thus *NRT1.1* acts as a nitrate sensor that regulates the expression of other genes related to nitrate uptake [43].

2.3.2 Ammonium

Although the average NH_4^+ concentration of soil is often lower than that of nitrate, NH_4^+ serves as the predominant source of nitrogen available for plant nutrition [44]. The uptake of NH_4^+ by roots involves the Ammonium transporter family (AMT) transporters which are categorized into AMT1 and AMT2 transporter families. In *A. thaliana* six families of AMT type NH_4^+ transporters, with all AMT genes except *AMT1.4*, being expressed in roots [45]. Both HATS and LATS for NH_4^+ uptake are found in plant roots [46]. Transporters of *AMT1.1, AMT1.3,* and *AMT1.5*, expressed in the plasma membrane of epidermis cells and root hairs, are responsible for the NH_4^+ symplastic pathway [45]. Furthermore, NH_4^+ can also bypass the root cells through the apoplastic transport pathway and enter the root symplast as mediated by *AMT1.2* [47].

The ammonium transporters, specifically *AMT1.1, AMT1.2* and *AMT1.3* have been identified as major contributors, accounting for approximately 90 per cent of the total high-affinity uptake of NH_4^+ in Arabidopsis roots [45]. Recent advancements in research reveal that these AMTs play pivotal roles in various physiological processes. They are involved in transporting ammonium from symbiotic fungi to plants, mediating ammonium acquisition from the soil solution, facilitating the transfer of ammonium from roots to shoots, and participating in ammonium transfer within leaves. Furthermore, these ammonium transporters contribute to processes such as ammonium acquisition in reproductive organs, enhancing resistance to plant diseases through ammonium transport, and supporting root development [48].

2.3.3 Organic nitrogen

Organic nitrogen uptake as well as inorganic nitrogen acquisition, contributes to the complexity of plant N nutrition. In the soil, organic nitrogen exists predominantly in the form of amino acids, urea, peptides, and proteins. However, soil microorganisms break down peptides and proteins into their constituent amino acid units through the secretion of proteases.

Amino acid uptake is mediated by specific transporters, including lysine histidine transporter 1 (LHT1), amino acid permease 1 (AAP1), amino acid permease 5 (AAP5) and proline transporters 2 (ProT2) [49][50]. Each transporter exhibits distinct specificity and affinity for amino acid uptake and transport. Transporters like LHT1 and AAP5 play essential roles in soil amino acid uptake in cultivated and natural ecosystems. These two transporters complement each other in affinity spectra and perform different roles in amino acid uptake. LHT1 is mainly involved in the uptake of acidic and neutral amino acids, whereas AAP5 is involved in the uptake of basic amino acids [51].

The plant kingdom features two types of urea-transporting proteins: DUR3 (Degradation of Urea) orthologs and the Plasma Membrane localized major intrinsic proteins (MIPs). DUR3, identified as a high-affinity urea transporter, functions as a urea/H^+ symporter for urea uptake. *AtDUR3*, a member of sodium-solute symporter superfamily in Arabidopsis, is predicted to contain 14 transmembrane-spanning domains [52]. Some subfamilies of the MIPs mediate passive urea transport [53].

2.4 Coordination of nitrogen and phosphorus responses

Levels of nitrogen source levels in the soil exert a significant influence on uptake of other nutrients. Nitrogen response in plants is not only modulated by nitrogen availability but also influenced by responses to other nutrients, such as phosphorus. The availability of phosphorus is positively affected by the rate of nitrogen source uptake, and molecular factors that facilitate the coordination of nitrogen and phosphorus responses are explored in this context.

One such factor is Nitrogen Limitation Adaptation (NLA), a ubiquitin E3 ligase in Arabidopsis that mediates degradation of the plasma membrane localized phosphate transporter *PHT1.4*, thereby negatively regulating phosphate uptake [54]. A small ubiquitin-related modifier (SUMO) E3 ligase in Arabidopsis, along with its homolog in rice, has been found to impact both nitrogen and phosphorus homeostasis [55]. Hypersensitivity to Low Pi-Elicited Primary Root Shortening1 (HRS1) and its close homolog (HRS1 homolog) are nitrate-inducible genes in Arabidopsis, that play a role in suppressing primary root growth in the absence of phosphate. This inhibitory effect on root growth becomes more pronounced when a medium containing nitrate is used [56].

Another important factor coordinating nitrogen and phosphorus responses is a rice microRNA, miR444a. Expression of miR444a is induced under conditions of nitrogen and phosphorus limitation. Overexpression of miR44a results in elevated levels of nitrate and phosphate, accompanied by increased expression levels of both nitrate and phosphate transporter genes. The overexpression of rice miR444a rendered plants insensitive to applied nitrate and phosphate starvation, particularly in terms of lateral root formation, further highlighting its role in maintaining nitrogen and phosphorus homeostasis [57].

3. CONCLUSION

Plants deploy intricate sensing and adaptive mechanisms to respond to fluctuating nitrogen nutrient concentrations in the soil. The nitrogen response is a complex interplay of various factors, mutually influenced by responses to other nutrients. These mechanisms form the basis for fine-tuning nitrogen homeostasis amidst fluctuating nitrogen conditions. A comprehensive understanding of these nitrogen responses and their regulatory mechanisms holds the promise of providing insights into the development of crops with enhanced nitrogen use efficiency. While much remains to be uncovered about the potential regulation of the nitrogen response by nitrogen containing organic metabolites, future investigations into these mechanisms will be instrumental in elucidating how the current knowledge can be translated into applications in crop production.

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