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. Crop production is greatly influenced by nitrogen consumption efficiency and a significant amount of nitrogen fertilizers is used to increase yield. Approximately half of N fertilizers are not utilized by the crops and are lost to the environment by polluting water sources or by releasing pollutants into the atmosphere. 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 the 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 (N) 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 N to support their growth and productivity. However, the acquisition of N by plants poses a limiting factor in both natural and agricultural ecosystems. In the early twentieth century, the development of the 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]. Most of the N is lost to the environment by polluting water sources or by releasing pollutants into the atmosphere. The agriculture sector is estimated to be responsible for 60 per cent of the projected increase in N pollution by 2050, which is expected to be 150 per cent more than in 2010[2]. Nitrogen compounds that can be hazardous to the environment and human health are ammonia, nitrates, and nitrogen oxides (N_2O), which are formed from fertilizers [3][4]. Additionally, methane is released by rice fields and ruminant livestock farms, whereas greenhouse crop production emits gases such as nitrous oxide (N_2O), carbon dioxide (CO_2), and methane (CH_4). Hence, it is crucial to comprehend how plants perceive different nitrogen sources and adjust their physiological and developmental processes to varying nitrogen supplies, which is vital for the maintenance and enhancement of crop production.

The combined adaptive responses of plants to different nitrogen nutrition conditions constitute the nitrogen response, a crucial element of the regulatory network governing plant growth. Numerous molecular, physiological, and genetic studies have been conducted to define the nitrogen response and unravel its underlying mechanisms. Extensive research has been carried out to examine plant reaction to nitrate, given its prominence as the primary nitrogen source 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 from the rhizosphere in various forms such as nitrate, organic nitrogen (including amino acids and urea) and ammonium. They possess several layers of sensing and adaptive mechanisms to react to the available nutrients. These adaptive responses, collectively known as ‘nitrogen responses,’ involve morphological and physiological adjustments that enable plants to effectively absorb nitrogen and acclimate to spatial and temporal variations in nitrogen abundance within the field [5]. Roots absorb different nitrogen forms, and specific transporters allocate them to various tissues, triggering distinct nitrogen responses in those specific tissues [6].

2.1 Morphological responses

Nitrogen sources in our fields exhibit uneven distribution owing to targeted fertilizer application and the presence of soil microorganisms engaged in nitrification or denitrification processes [7]. In response to this irregular nitrogen distribution in soil, plants actively detect nitrogen sources and modify their root architecture to efficiently utilize the accessible nitrogen supplies [8]. 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 [5].

The complexity of lateral root growth depends on the concentration of nitrate and ammonium in the soil. Optimal stimulation of lateral root growth occurs when there are moderate concentrations of nitrate (~0.5 mM) and ammonium (~0.8 mM). However, elevated concentrations exceeding 5 mM for nitrate and 10 mM for ammonium inhibit lateral root growth in Arabidopsis [9]. The nitrogen nutrient status also influences root architecture by regulating primary root elongation, as observed in maize, where nitrogen deficiency consistently stimulates axial root growth [10].

Under nitrogen-deficient conditions, aerenchyma formation in the root cortex is enhanced, allowing plants to adapt to varied nitrogen nutrient conditions by modifying both the morphology of root architecture and internal root structure. The formation of root cortical aerenchyma arises from cell death and the adjustment of internal nitrogen metabolite levels, rather than being solely influenced by external nitrate or ammonium levels [11].

2.2 Physiological responses

Various physiological processes in distinct plant tissues are intricately influenced by intracellular nitrogen status as well as environmental nitrogen nutrient conditions [12]. A primary example is the regulation of the nitrogen uptake process. Under nitrogen-deficient conditions, the process 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 [13]. These intricate adjustments may serve as a strategy to conserve energy and ensure an adequate nitrogen level in response to prevailing conditions. Such physiological adaptations in nitrogen uptake occur rapidly, within minutes or hours, in contrast to the morphological changes of roots, which necessitate several days.

Another physiological response involves the interplay between photosynthesis and nitrogen assimilation. Leaf nitrogen content correlates strongly with CO_2 assimilation rate [14]. Nitrogen supply significantly influences the content of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) [15]. Conversely, photosynthesis provides the energy and carbon skeletons essential for nitrogen assimilation [16]. Nitrogen nutrients play a pivotal role in promoting the expression of photosynthesis-related genes [17], whereas soluble sugars contribute to the expression of genes related to nitrate assimilation [18]. This interplay between photosynthesis and nitrogen assimilation emphasizes the critical role of nitrogen nutrition in biomass production and crop yield.

The impact of nitrogen nutrition on the life cycle of plants, particularly the transition from the vegetative to the reproductive stages, is a subject of debate due to inconsistent findings. In wheat and maize, nitrogen fertilizer application is linked to an advancement in the transition to the reproductive stage, while low nitrogen availability is associated with a delay [19]. However, Vidal *et al*. [20] 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 of flowering-related genes. A meta-analysis on wheat and barley [21] produced conflicting results, failing to establish a clear correlation between nitrogen fertilizer application and the timing of flowering/heading. Thus, the regulation of transition to the reproductive stage by nitrogen nutrients remains unclear, suggesting potential collaboration with other factors, such as additional nutrients.

In Arabidopsis, a study was conducted to explore the molecular basis of flowering and its connection to nitrogen application [22]. The researchers 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 [23]. The two primary systems in plants are the 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 concentrations, 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 [24]. 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 and examples for each distinct form of N are given in Table 1.

2.3.1 Nitrate

NO_3^- concentrations in soil solutions exhibit variability, prompting plants to evolve regulatory systems for NO_3^- transporters to adapt to this fluctuation [25]. 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) [26].

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 [24].

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 [27] and five in the rice genome [28]. 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 [29]. *NRT2* transcript abundance was found to be higher during the day and lower at night, resembling the fluctuation of sugar levels in the root, suggesting the role of root sugar levels in the diurnal expression pattern of *NRT2* [30].

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 [31]. Phosphorylation of the vacuolar anion exchanger *AtCLCa* is essential for the stomatal response to abscisic acid [32].

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 [33]. Among them, SLAH2 is expressed in the root stele, probably aiding in facilitating NO_3^- transport from root to shoot [34].

Among all of these transporter families, *Nitrate transporter 1.1* (*NRT1.1*) is the first one isolated [35] 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 [36]. *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.* [37] 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 [38].

The dual-affinity function of NRT1.1 is primarily regulated through phosphorylation modification at a key threonine residue, Thr101 [38]. 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 [39] 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 [40]. This regulatory mechanism of NRT1.1 enables rapid adaptation to changing nitrate levels.

Maghiaoui *et al.* [41] 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.

NRT1.1 has two homologues, NRT1.1a and NRT1.1b. Among these NRT1.1b has a role in the recruitment of microorganisms. For example, among rice types, indica type was found to have better nitrogen use efficiency than japonica. The NRT1.1b serves as a sensor and nitrate transporter that has been associated with variations in the nitrogen use efficiency between indica and japonica cultivars. The NRT1.1b regulates the difference in the root microbiota of indica and japonica types and helps in the recruitment of N-metabolizing soil-borne bacteria [42].

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 [43]. Previously it was established that *NRT1.1* plays a role in the regulation of flowering time in Arabidopsis. Teng *et al*. [44] 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 [45].

Table 1. Examples of N-responsive genes in various crops for three distinct forms of N

Form of N	**Gene family**	**Genes**	**Examples**	**References**
Nitrate	Nitrate transporter 1 (NRT)/Nitrate peptide transporter (NPF)	*NRT1.1a* *NRT1.1b*	*Arabidopsis thaliana* *Oryza sativa*	[46] [47]

	Nitrate transporter 2	*NRT2*	*Oryza sativa* *Brassica napus* *Populus tomentosa* *Solanum lycopersium* *Glycine soja*	[48] [49] [50] [51] [52]
	Chloride Channel transporters	*CLC*	*Arabidopsis thaliana*	[31]
	Slow Anion Associated Channel Homolog transporters	*SLAC/SLAH*	*Arabidopsis thaliana*	[33]
Ammonium	Ammonium transporters 1	*AMT1*	*Arabidopsis thaliana* *Solanum lycopersium* *Oryza sativa* *Malus hupehensis*	[53] [54] [55] [56]
	Ammonium transporters 2	*AMT2*	*Manihot esculenta* crantz	[57]
Organic N	Lysine histidine transporter, Amino acid permease, Proline transporters, Degradation of Urea, Major intrinsic proteins	*LHT1* *AAP* *ProT* *DUR3* *MIPs*	*Arabidopsis thaliana*	[58] [59] [60]

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 [61]. 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*, are expressed in roots [53]. Both HATS and LATS for NH_4^+ uptake are found in plant roots [62]. 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* [63].

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 [53]. 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 [64].

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) [58]. 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 [59].

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 the sodium-solute symporter superfamily in Arabidopsis, is predicted to contain 14 transmembrane-spanning domains [65]. Some subfamilies of the MIPs mediate passive urea transport [60].

2.4 Coordination of nitrogen and phosphorus responses

Levels of nitrogen sources in the soil exert a significant influence on the uptake of other nutrients. Nitrogen response in plants is not solely governed by its availability but is also impacted by response 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 [66]. 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 [67]. 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 [68].

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 [69].

2.5 Role of Microbes in Nitrogen uptake and transport

Some of the N-responsive genes help in the regulation of rhizomicrobiome and this molecular regulatory network of plant-microbe interactions could improve crop production [70]. Microbiomes have a great role in structuring plant phenotype by induction of more lateral roots for efficient absorption of nutrients and thus help in increasing NUE in crops [71]. An increase in root surface area, root hairs, and lateral roots are significant factors in changing the rhizosphere microbial community, and this is the primary mechanism through which nutrient-uptake-related genes regulate rhizosphere microbes [72]. It was also found that plant functional genes have a role in the recruitment of rhizomicrobiome by secretion of volatile compounds by the crop. Recent studies showed that the NRT1.1b expression determines the NUE of japonica and indica rice [42]. By regulating root cell transporter protein activity, secreting root exudates such as secondary metabolites, organic acids, and hormones and thereby regulating plant nutrient utilization and altering root environmental conditions (eg. soil pH, O_2 partial pressure, and carbon source). Specific genes related to nutrient uptake and transport can affect the composition of the rhizomicrobiome [73].

3. CONCLUSION

Plants deploy intricate sensing and adaptive mechanisms to respond to fluctuating N concentrations in the soil. The efficient working of N uptake and transporter genes helps the crops cope with environmental fluctuations. The N response is a complex interplay of various factors and is also mutually influenced by other nutrients. These mechanisms form the basis for fine-tuning N homeostasis amidst fluctuating nitrogen conditions. A comprehensive understanding of these N 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 N response by N-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.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

AUTHOR'S CONTRIBUTIONS

Elizabeth Jose wrote the first draft of the manuscript. Dr. Soni K.B., Dr. Swapna Alex, Dr. Shalini Pillai P., Dr. Beena R. and Dr. Roy Stephen scrutinized and corrected the manuscript. All authors read and approved the final manuscript.

REFERENCES

1. Francis B, Aravindakumar CT, Brewer PB, Simon S. Plant nutrient stress adaptation: A prospect for fertilizer limited agriculture, Environ. Experimental Botany. 2023; 213: 105431 DOI: 10.1016/j.envexpbot.2023.105431.

2. Martínez-Dalmau J, Berbel J, Ordóñez-Fernández R. Nitrogen fertilization. A review of the risks associated with the inefficiency of its use and policy responses. Sustainability. 2021; 13:5625. DOI:10.3390/su13105625

3.Fesenfeld LP, Schmidt TS, Schrode A. Climate policy for short-and long-lived pollutants. Nat. Clim. Chang. 2018; 8:933–936.

4.Liu M, Huang X, Song Y, Tang J, Cao J, Zhang X et al. Ammonia emission control in China would mitigate haze pollution and nitrogen deposition; but worsen acid rain. Proc. Natl. Acad. Sci. 2019;116:7760–7765.

5. Ueda Y, Konishi M, Yanagisawa S. Molecular basis of the nitrogen response in plants. Soil Sci. Plant Nutr. 2017; 63(4):329-341.

6. Zayed O, Hewedy OA, Abdelmoteleb A, Ali M, Youssef MS, Roumia AF, et al. Nitrogen Journey in Plants: From Uptake to Metabolism, Stress Response, and Microbe Interaction. Biomolecules. 2023;13(10):1443.

7. Hodge A. The plastic plant: root responses to heterogeneous supplies of nutrients. New Phytol. 2004; 162:9 - 24.

8. Lopez G, Ahmadi SH, Amelung W, Athmann M, Ewert F, Gaiser T, et al. Nutrient deficiency effects on root architecture and root-to-shoot ratio in arable crops. Front. Plant Sci. 2023; 13:1067498. DOI: 10.3389/fpls.2022.1067498

9. Lima JE, Kojima S, Takahashi H, Von-Wirén N. Ammonium triggers lateral root branching in Arabidopsis in an Ammonium Transporter 1.3 - dependent manner. Plant Cell. 2010; 22:3621-3633.

10. Chun L, Mi G, Li J, Chen F, Zhang F. Genetic analysis of maize root characteristics in response to low nitrogen stress. Plant Soil. 2005; 276: 369-382.

11. Abiko T, Obara, M. Enhancement of porosity and aerenchyma formation in nitrogen-deficient rice roots. Plant Sci. 2014; 215:76-83.

12. Nacry P, Bouguyon E, Gojon A. Nitrogen acquisition by roots: physiological and developmental mechanisms ensuring plant adaptation to a fluctuating resource. Plant Soil. 2013; 370:1–29.

13. Gu R, Duan F, An X, Zhang F, Von-Wirén N, Yuan L. Characterization of AMT-mediated high-affinity ammonium uptake in roots of maize (*Zea mays* L.). Plant Cell Physiol. 2013; 54:1515–1524.

14. Makino A. Photosynthesis, grain yield, and nitrogen utilization in rice and wheat. Plant Physiol. 2011; 155:125–129.

15. Makino A, Osmond B. Effects of nitrogen nutrition on nitrogen partitioning between chloroplasts and mitochondria in pea and wheat. Plant Physiol. 1991; 96:355–362.

16. Griffiths CA, Paul MJ, Foyer CH. Metabolite transport and associated sugar signalling systems underpinning source/sink interactions. Biochim. Biophys. Acta Bioenerg. 2016; 1857:1715–1725.

17. Martin T, Oswald O, Graham IA. Arabidopsis seedling growth, storage lipid mobilization, and photosynthetic gene expression are regulated by carbon: nitrogen availability. Plant Physiol. 2002; 128: 472–481.

18. Melo-Oliveira R, Oliveira IC, Coruzzi GM. Arabidopsis mutant analysis and gene regulation define a nonredundant role for glutamate dehydrogenase in nitrogen assimilation. Proc. Natl. Acad. Sci. 1996; 93: 4718–4723.

19. Gungula DT, Kling JG, Togun AO. CERES-Maize predictions of maize phenology under nitrogen-stressed conditions in Nigeria. Agron. J. 2003; 95:892–899.

20. Vidal EA, Moyano TC, Canales J, Gutiérrez RA. Nitrogen control of developmental phase transitions in Arabidopsis thaliana. J. Exp. Bot. 2014; 65:5611–5618.

21. Hall AJ, Savin R, Slafer GA. Is time to flowering in wheat and barley influenced by nitrogen? : a critical appraisal of recent published reports. Eur. J. Agron. 2014; 54:40–46.

22. Yuan S, Zhang ZW, Zheng C, Zhao Z, Wang Y, Feng L et al. Arabidopsis cryptochrome 1 function in nitrogen regulation of flowering. Proc. Natl. Acad. Sci.2016; 113:7661–7666.

23. Ye JY, Tian WH, Jin CW. Nitrogen in plants: from nutrition to the modulation of abiotic stress adaptation. Stress Biol. 2022; 7;2(1):4. DOI: 10.1007/s44154-021-00030-1.

24. Crawford NM, Glass ADM. Molecular and physiological aspects of nitrate uptake in plants. Trends Plant Sci.1998; 3:389–395.

25. Aluko OO, Kant S, Adedire OM, Li C, Yuan G, Liu H et al. Unlocking the potentials of nitrate transporters at improving plant nitrogen use efficiency. Front. Plant Sci. 2023; 14:1074839. DOI: 10.3389/fpls.2023.1074839

26. Fan X, Naz M, Fan X, Xuan W, Miller AJ, Xu G. Plant nitrate transporters: from gene function to application. J. Exp. Bot. 2007; 68(10):2463–2475.

27. Miller AJ, Fan X, Orsel M, Smith SJ, Wells DM. Nitrate transport and signalling. J. Exp. Bot. 2007; 58: 2297-2306.

28. Feng H, Yan M, Fan X, Li B, Shen Q, Miller AJ, Xu G. Spatial expression and regulation of rice high-affinity nitrate transporters by nitrogen and carbon status. J. Exp. Bot. 2011; 62:2319–2332.

29. Williams LE, Miller AJ. Transporters responsible for the uptake and partitioning of nitrogenous solutes. Annu. Rev. Plant Biol. 2001; 52:659-688.

30. Matt P, Geiger M, Walch-Liu P, Engels C, Krapp A, Stitt M. The immediate cause of the diurnal changes of nitrogen metabolism in leaves of nitrate-replete tobacoo: a major imbalance between the rate of nitrate reduction and the rates of nitrate uptake and ammonium metabolism during the first part of the light period. Plant Cell Environ. 2001; 24:177-190.

31. Bergsdorf E, Zdebik AA, Jentsch TJ. Residues Important for Nitrate/Proton Coupling in Plant and Mammalian CLC Transporters. J. Biol. Chem. 2009; 284:11184-11193.

32. Wege S. Angeli AD, Droillard M, Kroniewicz L, Merlot S, Cornu D et al. Phosphorylation of the vacuolar anion exchanger *AtCLCa* is required for the stomatal response to abscisic acid. Sci Signal. 2014; 7(333):65-76.

33. Geiger D, Maierhofer T, Al-Rasheid KA, Scherzer S, Mumm P, Liese A et al. Stomatal closure by fast abscisic acid signaling is mediated by the guard cell anion channel SLAH3 and the receptor RCAR1. Sci. Signal. 2011; 4:32-40.

34. Maierhofer T, Lind C, Hüttl S, Scherzer S, Papenfuß M, Simon J et al. A single-pore residue renders the Arabidopsis root anion channel SLAH2 highly nitrate selective. Plant Cell. 2014; 26:2554–2567.

35. Tsay YF, Schroeder JI, Feldmann KA, Crawford NM. The herbicide sensitivity gene CHL1 of Arabidopsis encodes a nitrate-inducible nitrate transporter. Cell. 1993; 72:705-713.

36. Huang N, Chiang C, Crawford NM, Tsaya Y. CHLl Encodes a Component of the Low-Affinity Nitrate Uptake System in Arabidopsis and Shows Cell Type-Specific Expression in Roots. Plant Cell. 1996; 8:2183-2191.

37. Sun J, Bankston JR, Payandeh J, Hinds TR, Zagotta WN, Zheng N. Crystal structure of the plant dual-affinity nitrate transporter NRT1.1. Nat. 2014; 507:73-77.

38. Liu KH, Tsay YF. Switching between the two action modes of the dual-affinity nitrate transporter CHL1 by phosphorylation. EMBO J. 2003; 22:1005–1013.

39. Ho CH, Lin SH, Hu HC, Tsay YF. CHL1 functions as a nitrate sensor in plants. Cell. 2009; 138:1184–1194.

40. Sun J., Zheng N. Molecular Mechanism Underlying the Plant NRT1.1 Dual-Affinity Nitrate Transporter. Front. Physiol. 2015; 6:386.

41. Maghiaoui A, Bouguyon E, Cuesta C, Perrine-Walker F, Alcon C, Krouk G, Benkova E, Nacry P, Gojon A, Bach L. The Arabidopsis NRT1.1 transceptor coordinately controls auxin biosynthesis and transport to regulate root branching in response to nitrate. J. Exp. Bot. 2020;71(15):4480-4494.

42. Zhang J, Liu YX, Zhang N, Hu B, Jin T, Xu H et al. NRT1.1B is associated with root microbiota composition and nitrogen use in field-grown rice. Nat Biotechnol. 2019; 37:676–684. DOI:10.1038/s41587-019-0104-4

43. Korobova AV, Akhiyarova GR, Fedyaev VV, Farkhutdinov RG, Veselov SY, Kudoyarova GR. Participation of nitrate sensor NRT1.1 in the control of cytokinin level and root elongation under normal conditions and nitrogen. Moscow Univ. Biol. Sci. Bull. 2020; 74:221-226.

45. Liu K, Diener A, Lin Z, Liu C, Sheen J. Primary nitrate responses mediated by calcium signalling and diverse protein phosphorylation. J. Exp. Bot. 2020;71(15):4428–444.

44. Teng Y, Liang Y, Wang M, Mai H, Ke L. Nitrate transporter 1.1 is involved in regulating flowering time via transcriptional regulation of flowering locus C in *Arabidopsis thaliana*. Plant Sci. 2019; 284:30-36.

46. Jian S, Liao Q, Song H, Liu Q, Lepo JE, Guan C et al. NRT1.1-Related NH_4^+ Toxicity Is Associated with a Disturbed Balance between NH_4^+ Uptake and Assimilation. Plant Physiol. 2018;178(4):1473-1488. DOI: 10.1104/pp.18.00410.

47. Hu B, Jiang Z, Wang W, Qiu Y, Zhang Z, Liu Y *et al.* Nitrate–NRT1.1B–SPX4 cascade integrates nitrogen and phosphorus signalling networks in plants. Nat. Plants. 2019; 5:401–413. Doi:10.1038/s41477-019-0384-1

48. Naz M, Luo B, Guo X, Li B, Chen J, Fan X. Overexpression of Nitrate Transporter *OsNRT2.1* Enhances Nitrate-Dependent Root Elongation. Genes (Basel). 2019;10(4):290. Doi: 10.3390/genes10040290.

49. Du RJ, Wu ZX, Yu ZX, Li PF, Mu JY, Zhou J et al. Genome-wide characterization of high-affinity nitrate transporter 2 (NRT2) gene family in *Brassica napus*. Int J Mol Sci. 2022; 23(9):4965.

50. Zhao L, Chen P, Liu P, Song Y, Zhang D. Genetic effects and expression patterns of the nitrate transporter (NRT) gene family in *Populus tomentosa*. Front Plant Sci. 2021; 12:661635.

51. Akbudak MA, Filiz E, Cetin D. Genome-wide identification and characterization of high-affinity nitrate transporter 2 (NRT2) gene family in tomato (*Solanum lycopersicum*) and their transcriptional responses to drought and salinity stresses. J Plant Physiol. 2022; 272:153684.

52. You H, Liu Y, Minh TN, Lu H, Zhang P, Li W et al. Genome-wide identification and expression analyses of nitrate transporter family genes in wild soybean (*Glycine soja*). J Appl Genet. 2020; 61(4):489–501.

53. Yuan L, Loqué D, Kojima S, Rauch S, Ishiyama K, Inoue E et al. The organization of high-affinity ammonium uptake in Arabidopsis roots depends on the spatial arrangement and biochemical properties of AMT1-type transporters. Plant Cell. 2007; 19:2636–2652.

54. Filiz E, Akbudak MA. Ammonium transporter 1 (AMT1) gene family in tomato (*Solanum lycopersicum* L.): Bioinformatics, physiological and expression analyses under drought and salt stresses. Genomics.2020;112(5):3773-3782. DOI: 10.1016/j.ygeno.2020.04.009.

55. Wu XX, Yuan DP, Chen H, Kumar V, Kang SM, Jia B, Xuan YH. Ammonium transporter 1 increases rice resistance to sheath blight by promoting nitrogen assimilation and ethylene signaling. Plant Biotechnol. J. 2022; 20(6):1085-1097. DOI:10.1111/pbi.13789

56. Li J, Yu M, Li H, Yang G, Huang L, Hao Y. Functional Characterization of Ammonium Transporter MhAMT1;2 in *Malus hupehensis*. Horticulturae. 2023; 9(4):434. DOI: 10.3390/horticulturae9040434

57. Jinze X, Yu W, Tingting Z, Chengcai P, Yiyin J, Yang Z, Xingyu J. Genome-wide identification, expression profiling, and functional analysis of ammonium transporter 2 (AMT2) gene family in cassava (*Manihot esculenta Crantz*). Front. Genetics. 2023; 14:1145735. DOI:10.3389/fgene.2023.1145735

58. Yang G, Wei Q, Huang H, Xia, J. Amino acid transporters in plant cells: A brief review. Plants. 2020; 9:967.

59. Svennerstam H, Jamtgard S, Ahmad I, Huss-Danell K, Nasholm T, Ganeteg U. Transporters in Arabidopsis roots mediating uptake of aminoacids at naturally occurring concentrations. New Phytol. 2011; 191:459-467.

60. Witte CP. Urea metabolism in plants. Plant Sci. 2011;180: 431-438.

61. Esmaeilzadeh-Salestani K, Bahraseman MRS, Tohidfar, M, Khaleghdoust B, Keres I, Mõttus A, Loit E. Expression of AMT1;1 and AMT2;1 is stimulated by mineral nitrogen and reproductive growth stage in barley under field conditions. J. Plant Nutrition. 2023; 46(7):1246-1258, DOI: 10.1080/01904167.2022.2067764

62. Glass ADM, Britto DT, Kaiser BN, Kinghorn JKR, Kronzucker HJ, Kumar A, Okamoto M, Rawat S, Siddiqi MY, Unkles SE, Vidmar JJ. The regulation of nitrate and ammonium transport systems in plants. J. Exp. Bot. 2002; 53(370):855–864.

63. Wang M, Shen Q, Xu G, Guo S. New insight into the strategy for nitrogen metabolism in plant cells. Int. Rev. Cell Mol. Biol. 2014; 310:1-37.

64. Hao D, Zhou J, Yang S, Qi W, Yang K, Su Y. Function and Regulation of Ammonium Transporters in Plants. Int. J. Mol. Sci. 2020; 21:3557-3578.

65. Kojima S, Bohner A, Von-Wiren N. Molecular Mechanisms of Urea Transport in Plants. J. Membrane Biol. 2006; 212:83–91.

66. Park BS, Seo JS, Chua NH. Nitrogen Limitation Adaptation recruits PHOSPHATE2 to target the phosphate transporter PT2 for degradation during the regulation of Arabidopsis phosphate homeostasis. Plant Cell. 2014; 26:454–464.

67. Wang H, Sun R, Cao Y, Pei W, Sun Y, Zhou Y et al. OsSIZ1, a SUMO E3 ligase gene, is involved in the regulation of the responses to phosphate and nitrogen in rice. Plant Cell Physiol. 2015; 56:2381–2395.

68. Medici A, Marshall-Colon, A, Ronzier E, Szponarski W, Wang R, Gojon A et al. AtNIGT1/HRS1 integrates nitrate and phosphate signals at the Arabidopsis root tip. Nat. Commun. 2015; 6:6274- 6280.

69. Yan Y, Wang H, Hamera S, Chen X, Fang R. MiR444a has multiple functions in the rice nitrate-signaling pathway. Plant J. 2014; 78:44–55.

70. Liu Q, Cheng L, Nian H, Jin J, Lian T. Linking plant functional genes to rhizosphere microbes: a review. Plant Biotechnol. J. 2023;21(5):902-917. Doi: 10.1111/pbi.13950.

71. Zhao B, Jia X, Yu N, Murray JD, Yi K, Wang E. Microbe-dependent and independent nitrogen and phosphate acquisition and regulation in plants. New Phytol. 2023. Doi: 10.1111/nph.19263.

72. Yu P, He X, Baer M, Beirinckx S, Tian T, Moya YAT, et al. Plant flavones enrich rhizosphere Oxalobacteraceae to improve maize performance under nitrogen deprivation. Nat. Plants 2021; 7:481-499

73. Liu Y, Evans SE, Friesen ML, Tiemann LK. Root exudates shift how N mineralization and N fixation contribute to the plant-available N supply in low fertility soils. Soil Biol. Biochem. 2022; 165:108541.