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Review

The Utilization and Roles of Nitrogen in Plants

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Abstract: Nitrogen (N) is an essential mineral element for plants and is the main component of protein, nucleic acid, phospholipid, chlorophyll, hormones, vitamins and alkaloids. It is involved in all stages of plant growth and development. Low-N stress seriously hinders plant growth and reduces yield and quality. Plants have evolved a series of elaborate regulatory mechanisms for N uptake and assimilation to cope with different soil N states. After N is absorbed and utilized by plants, it plays important regulatory roles in phytohormones, microRNA (miRNA), root development and mycorrhizal symbiosis to cope with environmental stress. Here, we highlight the research progress on the regulation mechanisms for N absorption and assimilation. Then, we emphasize the regulation roles of N regarding hormone signals, miRNA, lateral root growth, drought resistance, anthocyanin synthesis and mycorrhizal symbiosis. A thorough understanding of N uptake, utilization and interaction with other biological processes is helpful to improve N use efficiency and to breed plants with "less-input-more-output".

Keywords: nitrogen; utilization; drought stress; lateral root; hormone; mycorrhizal; miRNA

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1. Introduction

As an essential macronutrient, the content of nitrogen (N) is second only to carbon and oxygen in the dry weight of plants. N is the primary constituent of proteins, nucleic acids, phospholipids, chlorophyll, hormones, vitamins and alkaloids, and participates in all stages of plant growth and development [1–3]. In natural ecosystems and agricultural production systems, N deficiency is often one of the important limiting factors, because soil erosion seriously aggravates soil poverty, resulting in a reduction in soil N. Forest ecosystems may contain some fast-growing tree species, which require large amounts of N. When N absorbed by plants cannot meet their normal growth and development, low-N stress occurs. In response to low-N stress, plants will degrade their own proteins to achieve N reuse. However, this often results in decreased photosynthesis, accelerated leaf senescence and a slowing down of biomass accumulation, which then causes irreversible damage to the plants [4]. A large amount of N fertilizer is used in order to increase yield and only 30%–50% of the applied fertilizer N is actually captured by plants [5], resulting in low N use efficiency (NUE). The extensive application of N fertilizer not only increases the cost of crop production but also causes serious environmental problems such as the eutrophication of surface water sources, nitrate pollution of drinking water and soil acidification. Therefore, determining how to improve plant NUE has become a critical point of focus that must be solved for the sustainable development of current agriculture practices.

Nitrogen has an important effect on the uptake of water from soil by plants. A plant's acquisition of N sources (mainly nitrate and ammonium N) is generally a function of root

uptake. The effect of inorganic N sources (nitrate and ammonium N) on water uptake varies with plant type. N and water exert both synergistic and antagonistic effects on plant growth [6,7]. The effect of N fertilizer application on plant growth under drought stress is influenced by the plant species, N fertilizer absorption preferences, drought stress severity, N fertilizer application levels and the treatment time. The N content in plants is also associated with the ability of the antioxidant system to remove reactive oxygen species (ROS). Shi et al. [8] reported an enhancement in antioxidant enzyme activities, including superoxide dismutase (SOD) activity and glutathione (GSH) content, under normal N supply conditions compared with a low-N treatment, effectively inhibiting ROS accumulation and consequently reducing drought stress damage to plants. N can also induce the expression of hormone metabolism genes in root systems, suggesting its ability to act as a signaling molecule via hormone metabolism [9].

The role of N in plants is twofold; namely, it provides a large amount of essential N element for plants and serves as a signal substance that participates in a wide range of plant developmental processes. This review focuses on the absorption, assimilation and regulation of two inorganic N sources in plants, as well as the interaction between N and drought resistance, lateral root growth, hormone signals, anthocyanin synthesis, mycorrhizal symbiosis and microRNA (miRNA) regulation. The information presented here on the regulation of the N metabolism can be usefully adopted in research on the regulation of N uptake and utilization in plants, and it provides theoretical knowledge for the efficient application of N fertilizers to crops to cope with biotic and abiotic stresses caused by low-N stress.

2. Study of the Mechanism of N Uptake and Assimilation in Plants

Plants have evolved different absorption systems for inorganic N, including nitrate transporters and ammonium transporters, with extremely complex regulatory mechanisms in order to adapt to the varying types and concentrations of N in the external environment.

2.1. Research Progress on NO_3^- Uptake in Plants

Nitrate transporters in plants can be classified into the nitrate transporter 1/peptide transporter family (NRT1/PTR), nitrate transporter 2 family (NRT2), chloride channel (CLC) and the slow anion channel-associated 1/slow anion channel homologues (SLAC1/SLAH) based on the structure, location and function of the transporter [10,11].

Plant nitrate transporters (NRTs) play key roles in NO_3^-–N uptake by roots, as well as long-distance transport and redistribution in roots and stems [7,12]. NO_3^-–N transport systems in plants can be divided into two types, including low-affinity transport systems (LATS, >1 mM) and high-affinity transport systems (HATS, 1 μm–1 mM). In addition, each transport system can be categorized as cLATS/cHATS and iLATS/iHATS, depending on whether gene expression is induced by the NO_3^-–N concentration in the soil [1,13]. Plant *NRT* genes are mainly divided into three families, namely, NRT1/PTR, NRT2 and NRT3. Since NRT1 and PTR are closely related in terms of the gene structure and function, the nitrate transporter 1/peptide transporter family (NRT1/PTR Family) is denoted as NPF [14]. A total of 53 NRT1 and seven NRT2 members in *Arabidopsis thaliana* (hereafter, *Arabidopsis*) have been identified. NAR2 is the accessory/partner protein of NRT2, which forms a complex with NRT2 then maintains NRT2 protein stability [11]. NRT1 not only transports nitrate N but also transports a variety of substrate molecules, indicating that the NRT1 protein plays an important role in all aspects of plant growth and development [11]. Among them, some NRT1 family transporters and all NRT2 family transporters exhibit the capacity to transport NO_3^-–N (Figure 1). The first identified NRT member in *Arabidopsis* was *AtNRT1.1* or *CHL1* [15], which is a double-affinity transporter and possesses transport capacity for both high and low NO_3^-–N concentrations [16].

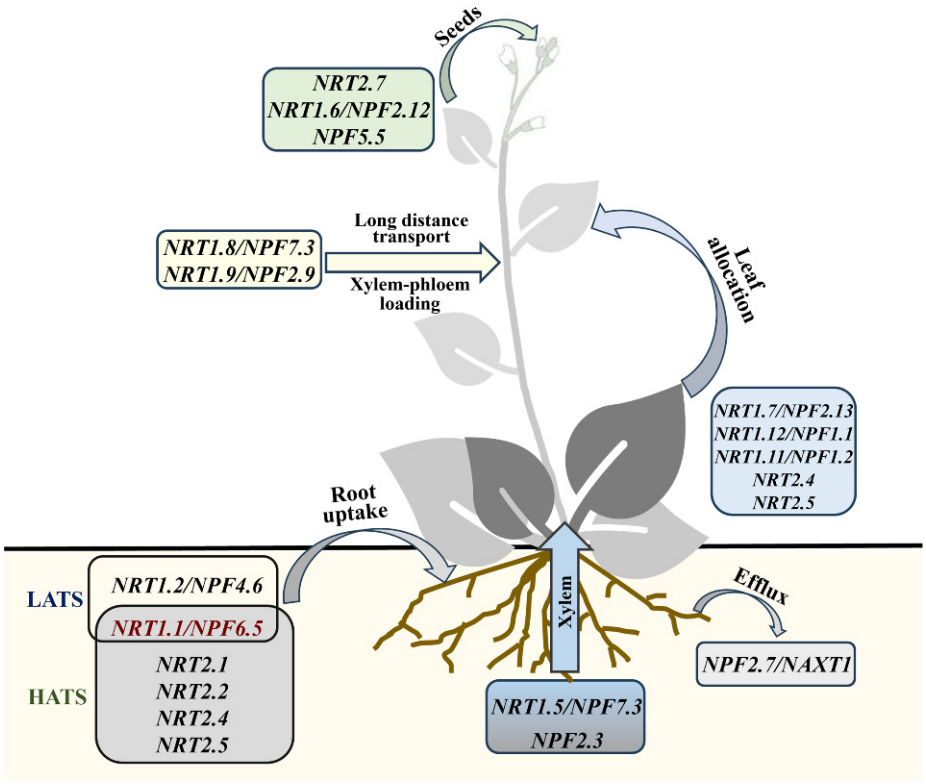


Figure 1. Role of NPF and NRT2 families in nitrogen uptake and utilization of *Arabidopsis*. HATS, high-affinity transport system; LATS, low-affinity transport system.

CLC transporters are a class of chloride and other anion channel proteins localized in organelles or cytoplasmic membranes. Such proteins generally include 10–12 transmembrane domains and C-terminal and N-terminal regions located in the cytoplasm. There are generally three highly conserved amino acid sequences related to anion-specific recognition among different transmembrane domains [17]. In *Arabidopsis*, the CLC transporter family includes seven members, denoted as a–g [18]. CLCa is located on the vacuolar membrane and can store nitrate in the vacuole of plants while transporting chloride ions in the reverse direction [19,20]. Recent studies showed that nucleotides and phospholipids can regulate the CLCa activity of *Arabidopsis* in different physiological scenarios [18]. CLCb is also localized in the vacuolar membrane. Research on *Xenopus oocytes* has demonstrated that CLCb also has the function of Cl^-/NO_3^- reverse cotransport [21]. Shi et al. [22] showed that under low-N conditions, HRS1 HOMOLOG2 (*HHO2*) expression was down-regulated and negatively regulated *CLCb*, resulting in the up-regulated expression of *CLCb*, which promoted vacuolar NO_3^- efflux, improved NUE and thus improved plant adaptability to low N.

SLAC1/SLAH slow anion channel transporters are localized in the plasma membrane of plant guard cells and are closely related to ABA-regulated stomatal closure. *Arabidopsis* has five SLAC1/SLAH family members, namely *SLAC1* and *SLAH1–SLAH4*, all of which contain 10 helical domains [23]. *SLAH2* is expressed in root column cells and may mediate nitrate transport from the root to the shoot [24]. Following changes in the endogenous ABA signal induced by environmental changes, SLAC1 and SLAH3 have been reported to exhibit transport activity that regulates the efflux process of chloride ions and nitrate, which subsequently leads to stomatal closure [25]. SLAC1 shows a similar transport capacity for chloride and nitrate, while a stronger transport capacity has been observed for SLAH3 [26]. Recent studies have revealed that NRT1.1 and SLAH3 can form a functional unit to regulate the nitrate-dependent alleviation of ammonium toxicity in *Arabidopsis* [27].

2.2. Research Progress on NH_4^+ Uptake in Plants

Ammonium transporters (AMTs) are responsible for the uptake of NH_4^+-N from soil [28]. The first ammonia transporter, denoted as AtAMT1.1, was isolated by Ninnemann in 1994 [29]. The AMT gene family in plants consists of two subfamilies, AMT1 and AMT2. The number of members in each subfamily varies with the plant species. For example, in *Arabidopsis*, the AMT1 and AMT2 families include five and two members, respectively [30]. The AMT1 family of poplar includes six members, while the AMT2 family includes eight members. The AMT1 and AMT2 families of rice include three and seven members, respectively [31]. The protein sequences of the AMT1 and AMT2 members are distinct, yet the high-level structures of the proteins are similar and contain 9–11 transmembrane structural regions, while the tertiary structures are also very similar [32]. The expression of AMT family members varies depending on the plant tissues and organs. This may be a result of their different functions [33]. In *Arabidopsis*, AMT1.1 and AMT1.3 are localized on the cell membrane of the root epidermis and cortex and occupy approximately 30% of the high-affinity absorption system, respectively [34]. Overexpression of *OsAMT1.1* can significantly improve crop NH_4^+-N uptake and yield under low NH_4^+-N conditions. Moreover, the NO_3^--inducible promoter region on 5′UTR of *OsNAR2.1* can promote the expression of *OsAMT1.1* and increase the grain yield of *pOsNAR2.1:OsAMT1.1* transgenic plants compared with the wild type and *pUbi:OsAMT1.1* transgenic plants [35]. AMT1.2 is localized in the root cortex and is mainly responsible for the transportation of ammonium ions to the vascular system or the absorption of ammonium ions from non-protoplasts of roots. *AMT1.4* is specifically expressed in plant flowers, and its primary function is transporting ammonium ions to pollen to maintain pollen activity [36]. *AMT2.1* is expressed in numerous plant tissues, including the root tips, cortex and vascular bundles, yet its absorption capacity for ammonium ions in the soil is weak [37].

2.3. Nitrogen Assimilation in Plants

The assimilation process of NO_3^- typically includes the following steps: (i) the absorption of NO_3^- and reduction to nitrite; (ii) reduction to NH_4^+; (iii) NH_4^+ synthesis to glutamine (Gln) under the action of glutamine synthetase (GS); (iv) the glutamate synthetase (GOGAT) catalytic formation of glutamate (Glu); and (v) conversion to the synthesis of other amino acids used by plants (Figure 2). Nitrate reductase (NR), which catalyzes the reduction of NO_3^-–N to NO_2^-–N in plants, is a rate-limiting enzyme induced by NO_3^-–N concentrations [38,39]. NR also has a sensitive response to abiotic stress [40]. Nitrite reductase (NiR) is mainly found in plant chloroplasts and catalyzes the reduction of NO_2^- to NH_4^+. The activity of NiR is affected by the supply of NO_2^-. Reduced NH_4^+ is catalyzed by GS and subsequently combines with Glu to form Gln by consuming ATP. The NH_4^+ utilized in the above process mainly comes from two sources: direct root absorption and NO_3^- reduction. GS is generally observed in plants in two forms, namely GS1 in the cytoplasm and GS2 in the plastid [41,42]. In plants, glutamate dehydrogenase (GDH) regulates the synthesis and decomposition of Glu by catalyzing the reversible reactions of amination/deamination [10]. Masclaux-Daubresse et al. [43] revealed that GDH is mainly responsible for the deamination of Glu, while the GS/GOGAT pathway acts as the dominant synthesis pathway of Glu. Many genes are involved in the regulation of enzyme activity related to N metabolism, such as *NR*, *NiR* and *GS* [44].

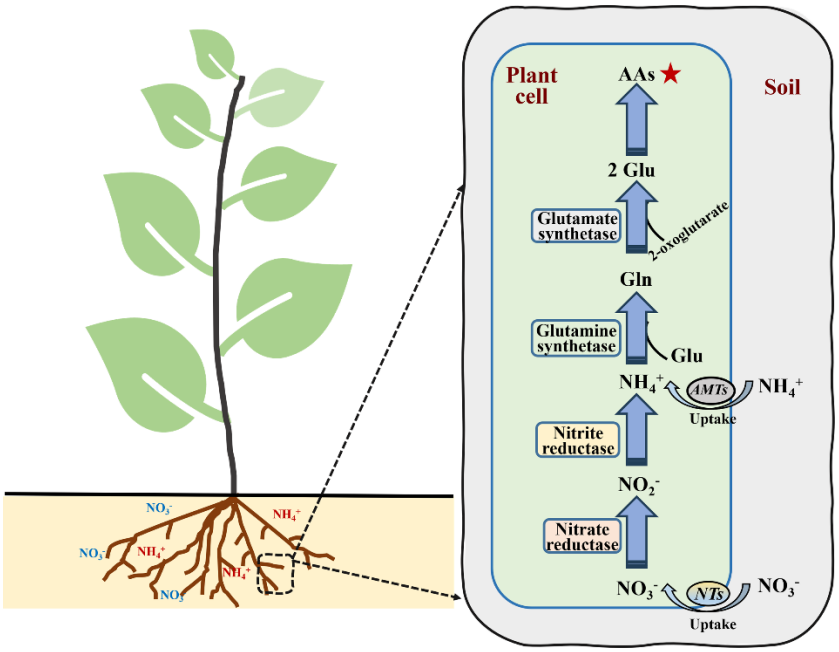


Figure 2. The process of nitrogen assimilation in plants. Gln: glutamine; Glu: glutamate; AAs: amino acids; NTs: nitrate transporters; AMTs: Ammonium transporters. The star that the products of nitrogen assimilation are amino acids (AAs).

3. Research Progress on Plants' N Utilization and Regulation

The N transporters, assimilation enzymes and signal transduction in plants are regulated by transcriptional regulation in response to changes in available N concentrations. Posttranscriptional modification and calcium- and phosphorylation-related signaling cascades are all critical posttranscriptional regulatory pathways [45]. The metabolic pathways of carbon metabolism and hormones also undergo corresponding changes to adapt to the regulation of N metabolism and the plant growth process [46]. The double-affinity nitrate transporter NRT1.1 in *Arabidopsis* is a signal transduction protein with a nitrate sensing function. An NRT1.1-induced nitrate response in plants is required for the normal expression of nitrate-sensitive genes in plants [47]. NRT1.1 shows dual-affinity nitrate transport ability, which is due to the phosphorylation and dephosphorylation of Thr101, and nitrate signals promote this change. When Thr101 phosphorylates, NRT1.1 shows high-affinity nitrate transport ability; when Thr101 dephosphorylates, it shows low-affinity nitrate transport ability [48]. Previous studies have demonstrated that NRT1.1 may not be the only nitrate receptor, and NRT2.1 could potentially be an additional nitrate sensor mediating root growth, yet its role in nitrate signaling remains unclear [49].

Numerous transcription factors (TFs) related to N metabolism have been identified in plants [50–53]. In particular, the nitrate signaling pathway and the role of calcium ions as downstream second messengers of nitrate signaling in plant roots and stems are well understood. Recent research has identified NIN-like protein 7 (NLP7) as a transcription factor of the N signaling pathway and also as a nitrate receptor [12]. In addition to NLP7, other TFs in plants that regulate the expression of genes related to N metabolism include *Arabidopsis* Regulated 1 (ANR1), LOB Domain-Containing proteins (LBD37/38/39), Squamosa Promoter Binding Protein-Like 9 (SPL9), Basic Leucine-Zipper 1 (bZIP1), NAC Domain-Containing Protein 4 (NAC4), TGA1/TGA4, NLP2, Teosinte Branched1/Cycloidea/Proliferating Cell Factor 20 (TCP20), CIPK23 and Antares Regulatory Gene 2 (NRG2) [46,54,55]. BES1, a brassinosteroid TF, is strongly induced and bound to the promoters of *NRT2.1* and *NRT2.2* directly, which then promote their expression under nitrate-deficient conditions [56]. NRT1.1B-NLP3-mediated NO_3^-–N signal transduction can regulate the utilization of ammonium, thus endowing rice with the ability to reduce ammonium toxicity and adapt to high-ammonium conditions [57]. In addition,

ABI2, a member of the protein phosphatase 2C family, and CBL1, a calcium sensor, also exert regulatory effects on NRT1.1 nitrate transport [58]. CIPK8 is specifically involved in nitrate response under high-N conditions and acts as a positive regulator, while CIPK23 is specifically involved in nitrate response under low-N conditions through the direct phosphorylation of $NRT1.1^{Thr101}$ [59]. eIF4E1 is upstream of *NRT1.1* in the nitrate signal transduction pathway and can regulate nitrate signal transduction by regulating *NRT1.1* at the translation and transcription levels. The overexpression of eIF4E1 can significantly promote plant growth and increase yield and N use efficiency [60].

4. Regulation Roles of Nitrogen in Plants

NUE is an important agronomic indicator. Generally, NUE can be defined as the crop yield per unit of N uptake from soil. Another evaluation method of NUE is the usage index, which is expressed as the ratio of the aboveground biomass to the aboveground N concentration and usually used to indicate the NUE at the vegetative growth stage [61]. Plant N status can affect many biological processes that then change the NUE of plants.

4.1. Nitrogen and Hormone Signals

Ethylene acts as an important cellular signal to mediate a range of adaptive responses to external N status, and the biosynthesis and signaling of ethylene are regulated by N availability and N forms [62]. The N-triggered ethylene signal can regulate a number of morphological and physiological responses in plants, including the root system architecture (RSA), N uptake and translocation, ammonium toxicity, anthocyanin accumulation and premature senescence [63]. Moreover, ethylene also plays an important role in nodulation formation and N fixation in legumes [64]. Chen et al. [2] showed that the expression of 1-aminocylopropane-1-carboxylic acid (ACC) synthase gene *ACS2/6/7/11* and ACC oxidase gene *ACO2* in the ethylene biosynthetic pathway were up-regulated by ammonium as the sole N source (5 mM NH_4^+) and down-regulated by nitrate as the sole N source (5 mM NO_3^-). Plants can typically produce ethylene under both low- and high-N conditions. When plants are transferred from high-N to low-N conditions (10 mM to 0.2 mM NO_3^- media), the expression of EIN3 is induced for the rapid production of ethylene [65]. When plants are transferred from low- to high-N conditions (0.1 mM to 10 mM NO_3^- media), the expression of ACS2/4/5/6/7/8/11 and ACO1/2 is up-regulated to produce a surge in ethylene emission [66]. Previous work has revealed that ethylene production induced by excessive N is often accompanied by negative effects such as cell death, leaf necrosis and growth inhibition, while ethylene production induced by N deficiency generally plays a positive role in plant adaptive response [67]. Comparing the natural variation in ethylene production in *Arabidopsis* under nitrate-deficient conditions indicates that regulating the ethylene pathway can improve NUE [68].

Auxin (IAA) is an important plant endogenous hormone and plays a key role in the growth and development of plants [69]. Ma et al. [70] found that environmental N availability directly regulates the expression of TAA1 and its homologs TAR1 and TAR2, which are fundamental in the first step of IAA synthesis. The authors also determined that high concentrations of auxin can inhibit the growth of primary roots and promote the formation of lateral roots. This is of great significance for the response of plants to low-N stress. This positive regulatory mechanism plays an important role in cucumber resistance to low-N environments. Krouk et al. [71] proved that nitrate can regulate auxin transport by means of NRT1.1, as the NRT1.1 and AUX/LAX proteins from different transporter families (PTR and ATF1, respectively) share common amino acid transporters. *MdATG5a* can positively regulate N uptake under low-N conditions by increasing the accumulation of IAA in apple roots, suggesting an important link between N uptake and IAA [4]. In addition, Chen et al. [72] proved the close relationship between the auxin receptor gene *OsTIR1* and the uptake and utilization of N. This indicates that auxin plays an important role in N uptake and utilization. The F-box protein 3 (AFB3), which belongs to a group of F-box receptors for IAA, was found to be the only IAA receptor gene rapidly induced by

NO_3^-, and specifically in the main root tip and pericycle. This suggests that NO_3^- not only modulates IAA gradients in roots, through NRT1.1 dual NO_3^-/IAA transport activity, but also increases IAA sensitivity of the roots through stimulation of AFB3 expression [1]. The aforementioned findings from previous studies demonstrate the interaction between N and auxin, yet the specific regulatory mechanism requires further research.

Abscisic acid (ABA), also known as a plant stress hormone, plays an important role in plant growth and can inhibit lateral root development [73]. High nitrate concentrations can promote an increase in available ABA content in *Arabidopsis* by stimulating the conversion of abiotically active ABA–glucose ester (ABA-GE) to biologically active ABA [74]. The low affinity nitrate transporter NRT1.2/NPF4.6/AIT1 mediates ABA uptake in *Arabidopsis* seeds during germination and post-germination growth. Thus, the N signal can relieve the inhibitory effect of ABA on seed germination by reducing ABA levels [75]. NLP8 in nitrate signaling has also been observed to participate in the regulation of the ABA metabolism by directly binding to the promoter of CYP707A2, a key enzyme gene for ABA decomposition [76]. Su et al. [77] found that SnRK2s, key protein kinases in ABA signaling, play an important role in root growth and nitrate uptake in *Arabidopsis* under low-N stress. Under N starvation stress, SnRK2s in plants phosphorylate NRT1.1 to regulate nitrate transport activity and mediate plant tolerance to low-N stress. This reveals a new mechanism of ABA signaling for the balance of stress adaptation and growth in plants under low-N stress, and it expands the regulatory network of plants responding to low-N stress. Furthermore, the resupply of nitrate after N starvation in wheat can induce ABA accumulation in roots through activating the ANR1/BG1 pathway, and as a consequence, ABA can up-regulate NRT2/NAR2 gene expression through Wabi5 [78]. These results suggest that ABA can also regulate nitrate uptake and signal transduction through transcriptional regulation and post-translational modification under low-N stress.

Cytokinins (CTKs) act as important signaling molecules in long-distance communication between organs in systemic growth regulation in response to N conditions. N-dependent isopentenyl transferase (IPT) expression accumulates N^6-(Δ^2-isopentenyl) adenine (iP)- and trans-zeatin (tZ)-type CTK species in the roots and shoots, then activates CTK signaling [79]. Previous research on *Arabidopsis* has shown that the expression of IPT3, a key enzyme in the first step of CTK synthesis, is induced by N. An NRT1.1-mediated primary N signal induces a strong expression of IPT3 in roots and a weak expression in stems. This indicates the close relationship between nitrate and CTK synthesis in plants, particularly in roots, through the activation of IPT3 [80]. Interestingly, the transcription factor TCP20 has been reported to participate in nitrate–cytokinin–auxin signaling. Guan et al. [81] revealed that lateral root growth was inhibited under a heterogeneous nitrate concentration environment and promoted under low-N conditions in a root split experiment, highlighting the root foraging deficiency of this phenotype. However, TCP20 inhibits the CTK metabolism-related gene ARR4/6/7 and auxin AUX/IAA13, 16 and 27, which consequently reduces auxin signaling [82]. These results suggest that TCP20 may act as an intermediate node in the nitrate–cytokinin–auxin signaling network.

4.2. Nitrogen Metabolism and miRNA Regulation

miRNA plays an important regulatory role in numerous plant growth stages and can regulate plant response to low-N stress [83,84]. The sequencing of two wheat small RNA libraries proved that many target genes were predicted to be involved in the process of N metabolism [85]. Studies have shown that N deficiency has an impact on the elongation of the primary root and the growth of the lateral root of miR393-overexpressing plants. Furthermore, miR393 can be induced by nitrate to regulate the auxin receptor gene in *Arabidopsis* roots [86]. Yu et al. [87] reported that *Osa-MIR169*o overexpression will contribute to increased NUE. ARF6 and ARF8, the target genes of miR167, regulate the lateral root development of plants. Thus, the down-regulation of miR167 and the up-regulation of ARF8 will induce lateral root development [88]. However, IAR3, another target gene of miR167, can hydrolyze indole-3-acetic acid-alanine to auxin, and miR167 can affect plant growth

by regulating IAR3 [89]. The up-regulation of miR167 expression has been reported under low-N conditions, and changes in miR167 precursor genes vary with tissue type [90]. The expression levels of miR169 and miR398 can increase under low-N conditions, which may regulate the expression of the target gene ARF8 and control the lateral root structure, while miR169 can also shear HAP2-1, a gene that regulates nodule primordium differentiation. This shows that miR169 is significantly down-regulated in *Arabidopsis* under low-nitrate stress, while its target gene NFYA family is significantly up-regulated [91]. Cheng et al. [92] also concluded that the miR169c–*NFYA10* module confers tolerance to low-N stress in *Betula luminifera*. The overexpression of miR444 can increase NO_3^- accumulation and increase NRTs gene expression under high-N conditions, but it reduces the ability of plants to transport NO_3^- from old leaves to new leaves and ultimately affects the plant's ability to adapt to N deficiency [93]. Other research demonstrated the applicability of miRNA171a as a simple sequence repeat (SSR) candidate marker to discriminate contrasting genotypes for NUE in wheat [94].

4.3. Nitrogen Regulates Lateral Root Growth

The imbalanced distribution of nutrient elements in the soil, particularly essential elements such as N and phosphorus, affects the morphogenesis of plant roots [95–97]. Research on *Arabidopsis* has shown that plants can sense and respond to different concentrations of nitrate and subsequently regulate the density and length of their lateral roots through their interaction with various hormones, thus enabling more efficient usage of nitrate N [98,99]. The Arabidopsis ABF2 and ABF3 TFs can directly regulate most of the nitrate response genes in the root endodermis cells. At the same time, the growth and development of lateral roots in response to nitrate could be regulated by the ABA signal, which establishes a relationship between the nitrate signal, ABA pathway and lateral root development [100]. In addition, ammonium transporters play an important role in the synergistic regulation of rice crown root formation as well as the response to ammonium N [101]. Under low N conditions, NRT1.1 promotes coreceptor QSK1 phosphorylation, transmits signals to PM ATPase AHA2, controls the activation of its phosphorylation site and inhibits the phosphorylation rate. This subsequently leads to changes in proton pump activity and the regulation of lateral root growth [102].

Nitrate can affect root development by regulating the polar transport and signaling of auxin [103]. Krouk et al. [71] revealed that NRT1.1 not only transports nitrate but is also involved in the uptake of auxin. In particular, NRT1.1 represses lateral root growth during times of low nitrate availability by promoting the transport of basipetal auxin out of these roots. The phosphorylation of the AtNRT1.1 protein is involved in the regulation of lateral root development and pattern-triggered immunity (PTI) response in *Arabidopsis* under low nitrate concentrations. At low nitrate concentrations, the AtNRT1.1 protein promotes the basal transport of auxin and reduces the accumulation of auxin in lateral roots, thus inhibiting the growth of lateral roots. Under high nitrate concentrations, auxin transport mediated by the AtNRT1.1 protein is inhibited, and the accumulation of auxin in the lateral roots is enhanced, which promotes the generation of lateral root primordia and the growth of new lateral roots [104]. Bouguyon et al. [105] demonstrated that high concentrations of nitrate down-regulated the expression level of AtNRT1.1 proteins in lateral roots through post-transcriptional levels. Therefore, the AtNRT1.1 protein has a low content in the lateral roots and exhibits a poor basipetal auxin transport ability under high nitrate concentrations. Together, these two effects promote the germination and growth of the lateral roots. In addition to auxin, cytokinin, ethylene, abscisic acid and other phytohormones are also involved in the nitrate-regulated lateral root growth process [106].

In addition to its role in co-regulating lateral root development with auxin, the AtNRT1.1 protein also regulates lateral root growth through its downstream CpKS–NLP7–*ANR1* (*Arabidopsis nitrate regulated 1*) signal pathway to adapt to different nitrate supply conditions in the outside world. The MADS-box transcription factor *ANR1* is the first identified key factor in the nitrate regulation of lateral root development. The overexpression of

ANR1 can up-regulate the number and length of lateral roots of *Arabidopsis* across different nitrate concentrations. Under high nitrate concentrations, the down-regulation of ANR1 expression results in a phenotype with arrested lateral root development [107], while under sufficient N, nitrate stimulates the expression of ANR1 in the lateral root primordial and other sites in an AtNRT1.1-dependent manner, which consequently induces the occurrence of lateral roots [108]. Analysis has revealed the ability of AtNLP7 to bind to the promoter of the *ANR1* gene and regulate its expression [109]. When nitrate is deficient, the SENSITIVE-TO-PROTON-RHIZOTOXICITY1 (STOP1) transcription factor accumulates in the nucleus of lateral root primordia cells and directly regulates the expression of NRT1.1 in *Arabidopsis* (Figure 3). Zhang et al. [106] also found that the phosphorylation status of the AtNRT1.1 protein T101 can control lateral root development by regulating its endocytosis and signal transduction capacity, thereby affecting auxin transport and *ANR1* gene expression.

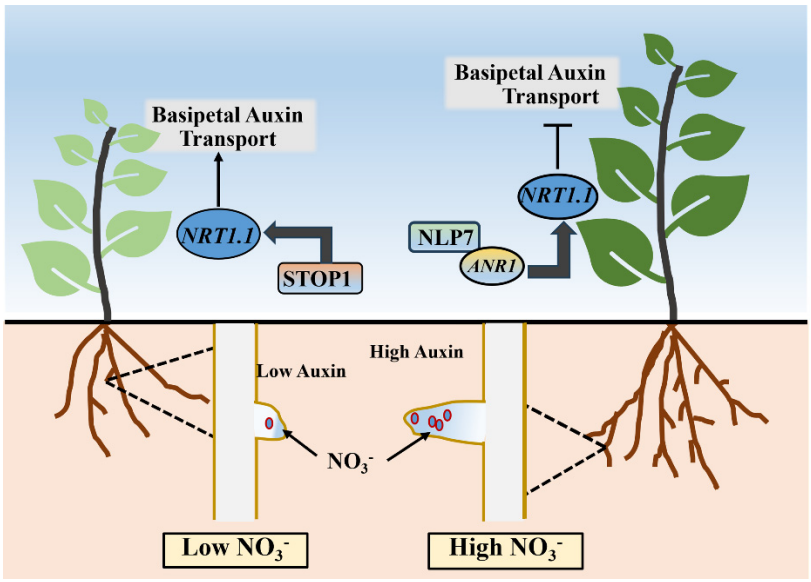


Figure 3. The *NRT1.1*-mediated nitrate-dependent lateral root development pathway in plants.

4.4. Nitrogen and Drought Resistance

The effect of the N source on water uptake by plant roots varies with the type of source [110]. Guo et al. [111] reported that, when subjected to drought stress, the photosynthetic rate of rice with NH_4^+-N as the only N source decreases, and the root growth is less inhibited. Previous research has revealed that plants' water use efficiency showed an increase with the application of N fertilizer, which contributes to ROS scavenging and cell membrane maintenance, consequently increasing water absorption [112]. Furthermore, the application of N fertilizer can alleviate nutrient stress, maintain plant physiological activities, promote biomass accumulation and avoid damage caused by both water and nutrient stress [113]. The sufficient application of N fertilizer is able to alleviate the inhibitory effect of drought on root growth, particularly for diameters < 0.2 mm, thus promoting plant resistance to drought stress [8]. Drought stress enhances plants' N allocation process in leaves, while N application reduces this trend [6]. Moderate nitrate supply can alleviate apple drought stress by maintaining the photosynthetic capacity of seedlings [114]. Recent studies have shown that calcium-dependent protein kinase 6 (CPK6) regulates *Arabidopsis* drought stress resistance by altering the phosphorylation of nitrate transporter AtNRT1.1 under high N conditions [7]. The overexpression of a DREB-like transcription factor, *GmTDN1*, from soybean (*Glycine max* L. Merr.) can enhance the resistant ability of two winter wheat (*Triticum aestivum* L.) varieties, Shi4185 and Jimai22, to drought and low-N stress by improving photosynthesis, osmotic regulation, ROS scavenging, drought stress-related genes and nitrate transporter *NRT2.5* expression [115].

Drought stress significantly inhibits plant growth and development mainly because it affects plant N metabolism and the NO_3^-–N signaling [110,116]. A recent study proved that TFs involved in ABA signaling could affect N metabolism and NO_3^-–N responses, and, meanwhile, NO_3^-–N signaling might affect the expression of ABA and drought resistance genes [117]. The opening process of plant stomata also requires the participation and regulation of N, and the content of ABA in leaves will decrease under high-N conditions, which is conducive to the opening of the plant stomata. Shi et al. [8] demonstrated that under drought stress, an appropriate N element supply could increase the ABA content in the leaves of *Catalpa bungei* and improve drought tolerance. In plants, the target-of-rapamycin (TOR) kinase plays an important role in N signals and drought stress, which can phosphorylate the ABA receptors PYL. When drought stress occurs, TOR was inhibited by ABA-activated SnRK2s phosphorylate to deal with adversity. Meanwhile, N signals can trigger the small GTPase Rho-related protein 2 (ROP2) in plants, which will activate TOR. Thus, the ROP2–TOR module may perceive and conduct N signals to promote plant growth [118,119]. ABA directly suppresses NO_3^-–N signal perception and transport [117]. NO_3^-–N is sensed and transported by NRT1.1 (a transporter and sensor, also known as CHL1 or NPF6.3) to plant root cells [59], while ABA signaling can oversee the activity of NRT1.1 through the phosphatase ABI2. During this regulatory process, ABI2 dephosphorylates the CBL1–CIPK23 complex, which regulates phosphorylation and subsequently inhibits NRT1.1 [58] (Figure 4). ABF2 and ABF3 play an important role in N response by means of regulating a series of genes related to NO_3^-–N response. ABF2 and ABF3 also have important roles in drought resistance; therefore, those two TFs may act as the main contact channel integrating NO_3^-–N, ABA and drought response signals [100]. *OsABF1* also can regulate the expression of genes responding to both drought stress and N status, which links gene expression to phenotypic responses under low-N conditions and drought stress [120].

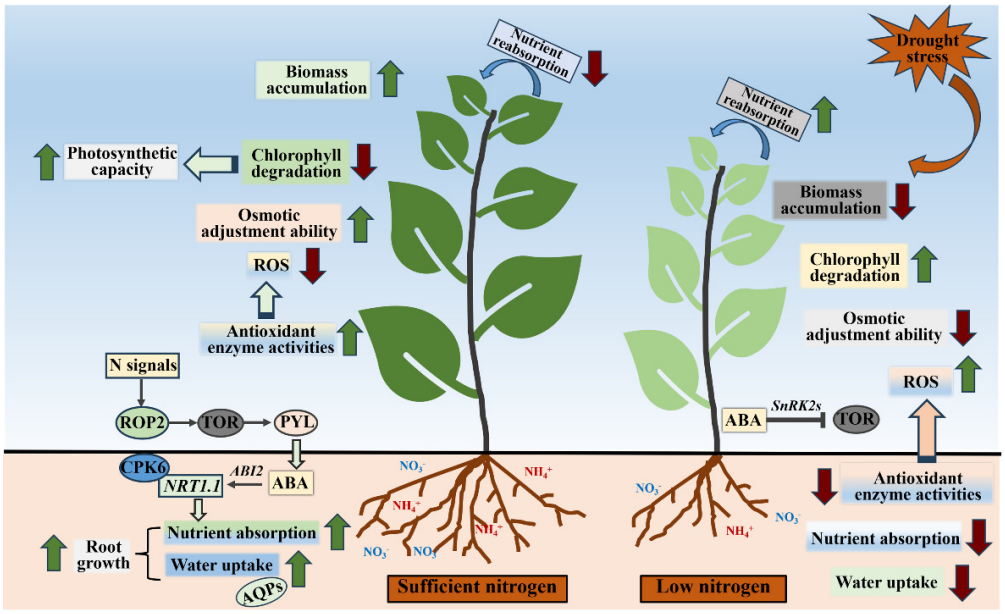


Figure 4. Mitigation effect of sufficient nitrogen supply on drought stress in plants.

In contrast, Friedrich et al. [121] showed that increasing N application under drought conditions can promote the growth of above-ground plant components, enhance leaf transpiration and accelerate water loss, which are not conducive to plant resistance to drought stress. In addition, enhanced N application in beech (*Fagus sylvatica*) has been observed not to be conducive to root growth, preventing the plant from effectively absorbing water from the soil and consequently promoting drought stress damage to the plant. In this case, reducing the application rate of N fertilizer can be beneficial to plant resistance under

drought stress [122]. Therefore, the effects of N application on plant response to drought stress need to be further studied in different species.

4.5. Nitrogen Regulates Anthocyanin Synthesis

Anthocyanins belong to a class of flavonoids that play an important role in both biotic and abiotic stresses in plants [123]. These substances typically accumulate in the vacuoles of plants and give plant organs a pink to purple color, which is useful for pollination and seed dispersal [124]. In addition, anthocyanins are important ROS scavengers, and thus anthocyanin-rich fruits are favored by consumers [125,126]. N is also an important factor regulating anthocyanin synthesis in plants [127,128]. Low-N conditions generally promote anthocyanin accumulation, while high N inhibits anthocyanin synthesis and accumulation in plants [129–131]. RNA interference of proline-rich protein 6 (*MdPRP6*) proves that plant *MdPRP6* negatively regulates low-N tolerance by affecting anthocyanin synthesis and IAA concentration in apple (*Malus domestica*) roots [96]. In *Arabidopsis*, nitrate reduces anthocyanin accumulation mainly by inhibiting PRODUCTION OF ANTHOCYANIN PIGMENT (*PAP*) gene expression. Members of the LATERAL ORGAN BOUNDARIES DOMAIN (LBD) family, which are induced by nitrate, act as negative regulators of AtPAPs to regulate the accumulation of anthocyanin [132]. DELLAs can interact with PAP1 and enhance the transcriptional activity of PAP1, which plays an important role in the accumulation of anthocyanins induced by N deficiency and the regulation of plant adaptation to low-N stress [126]. MYB genes are involved in the anthocyanin accumulation process induced by nitrate at the transcriptional level. Research has revealed that the MYB10 transcription factor plays an important role in the color formation of red-fleshed apple and can improve nitrate uptake efficiency by transcriptionally activating the expression of *MdNRT2.4-1* [133]. Wang et al. [134] found that the transcriptional and posttranslational modulation of MdMYB1 (a master regulator of anthocyanin biosynthesis and fruit coloration) occurred in response to nitrate treatment, which consequently degraded via a ubiquitin-dependent pathway in response to nitrate, ultimately inhibiting anthocyanin synthesis in apple.

4.6. Nitrogen and Mycorrhizal Symbiosis

Mycorrhizas are mutualistic symbionts formed by mycorrhizal fungi in soil and plant roots and are universally observed in nature [135]. Ectomycorrhizal (ECM) fungi can produce exocellular hydrolytic enzymes then obtain small organic N-bearing molecules from soil organic matter [136]. Arbuscular mycorrhizas (AMs) are the most widely distributed endophytic mycorrhizas in nature and play an important role in the rhizosphere of plants [137,138]. AM symbiosis can effectively improve the N response of N-source absorption and utilization by host plants, transferring N between fungi and host plants, as well as between different plants via hyphal bridges [135]. ECM fungi, to some extent, will help plants access organic N from soil organic matter and transfer it to host plants [139]. Interestingly, soil N availability in AM forests is higher than that in ECM forests. This may be attributed to higher N decomposition due to the greater nitrification rates facilitated by lower acidity and higher ammonium pools in AM forests compared to ECM forests [140]. The root mycorrhizal community will also change with the mycorrhizal inoculum species and the soil's available N content [141,142].

Arbuscular mycorrhizal fungi (AMFs) have a system for absorbing N from the soil, and three AMT genes (*GintAMT1*, *GintAMT2* and *GintAMT3*) have been identified from *Rhizophagus irregularis* [143]. *GintAMT1* and *GintAMT2* are expressed in both extraradical and intraradical mycelium, and they are involved in the NH_4^+ absorption of AMFs and recovery of NH_4^+ loss at the symbiotic interface due to fungal metabolism [144]. *GintAMT3* is highly expressed only in intraradical mycelium, and its expression is regulated by matrix concentration and carbon source [145]. The process of N absorption from peri-arbuscular space to symbiotic plants mainly occurs through N transporters, which are located in plants' peri-arbuscular membranes, such as the AMF-induced NO_3^- transporter OsNPF4.5, the amino acid transporter LjLHT1.2 and the NH_4^+ transporter LjAMT2;2 [146–149]. The

expression level of NPF4.5 homologs in maize (*Zea mays*) and sorghum (*Sorghum bicolor*) is also induced after AMF treatment, demonstrating that the NO_3^- absorption process is highly activated by AM symbiosis [146]. Saia et al. [150] found that AMFs can significantly increase the expression of *NRT1.1*, *AMT1;2* and *AMT2;1* in durum wheat (*Triticum durum* Desf.). In addition, ZmAMT3;1, which is induced by AMFs and specifically expressed in cortical cells, can absorb 68%–74% of the N transported from AMFs to maize plants [151]. The extracorporeal hyphae of AMFs can expand the absorption of nutrients and develop the root system of mycorrhizal plants. This also promotes the absorption and utilization of N (Figure 5). Moreover, leguminous plants inoculated with mycorrhizal and N-fixing microorganisms can form root nodules while developing mycorrhizal symbionts. This trinity symbiosis system provides sufficient N nutrition for host plants.

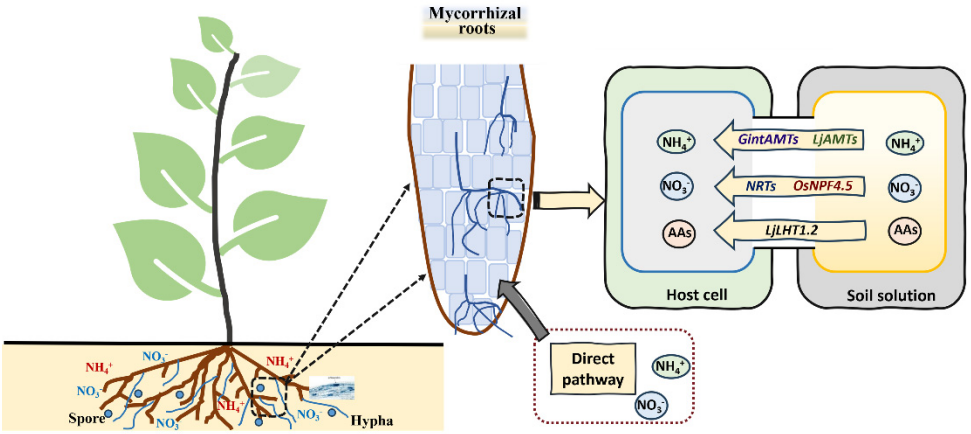


Figure 5. The nitrogen uptake by plants in mycorrhizal roots. Mycorrhizal plants can absorb both NO_3^- and NH_4^+, as well as organic N forms, such as amino acids (AAs), via either the direct pathway or the mycorrhizal pathway.

4.7. Nitrogen and Rhizobium–Legume Symbiosis

Legume roots can form symbiotic nodules with rhizobia. Then, bacteria fix and reduce N_2 to NH_4^+, which will be assimilated into amino acids by plants. Some abiotic stresses may reduce symbiotic activity and lead to plant N deficiency. Then, systemic signaling may compensate for the N deficit by stimulating symbiotic root N foraging. Furthermore, N also plays an important role in rhizobium–legume symbiosis. The autoregulation of nodule number (AON) is also affected by plant N demand. Under N deficiency, the symbiotic plant will release the AON repression signal to increase the nodule number [152]. NO_3^-–N application can reduce symbiotic N fixation via changing nodule gene expression and activating local and long-distance (systemic) responses [153]. Some TFs, such as NLPs, regulate the NO_3^-–N inhibition nodulation formation process [154]. Some members of the N uptake and transport gene family have been reported to be associated with nodule induction in legumes, such as *Medicago truncatula NPF7.6*, *Lotus japonicus NRT2.4* and *AMT1;1* [155–157].

5. Conclusions and Future Perspectives

Nitrogen (N) fertilizer application increases forests' productivity and enhances the carbon sequestration of forest stands. However, overuse of N fertilizers increases the release of nitrous oxide (N_2O), a greenhouse gas more harmful to global warming than CO_2 [158]. Therefore, it is urgent to improve the NUE of forest ecosystem to reduce the application of N fertilizer. With global environmental changes, plants in forest ecosystems will not only suffer from low-N stress in the growth process, but this will also be accompanied by other stresses. It is important to gain a comprehensive understanding of plant N uptake and its regulation of other physiological processes. This review focused on the absorption, assimilation and regulation of N in plants, and it subsequently explained the interaction between N and hormone signals, miRNA regulation, lateral root growth,

drought resistance, anthocyanin synthesis and mycorrhizal symbiosis (Figure 6). The in-depth understanding of N's uptake and regulation of other physiological processes and stresses can improve forest ecosystem NUE and reduce the environmental pollution caused by excessive N application.

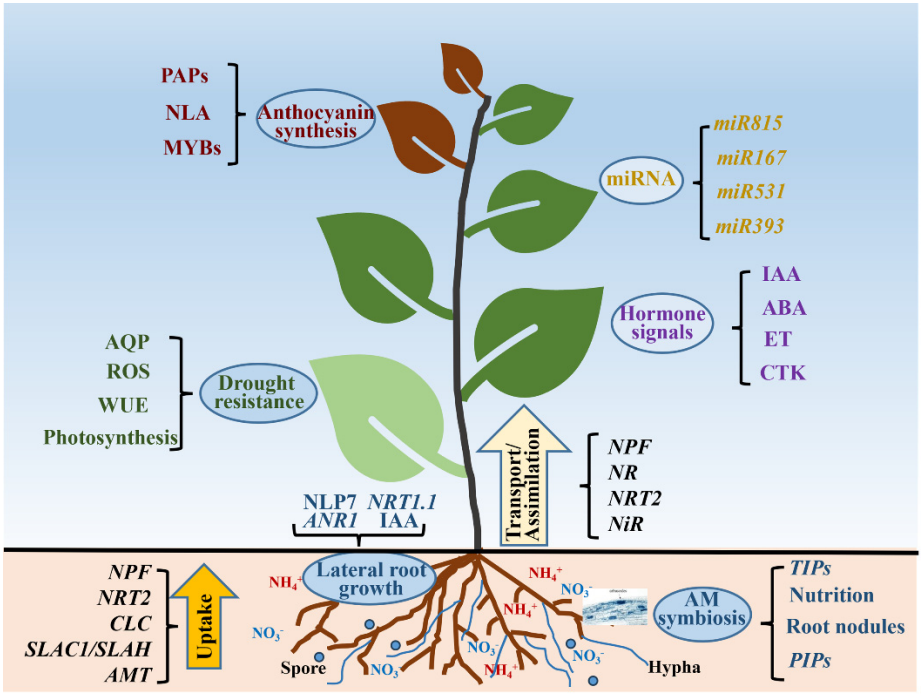


Figure 6. Uptake and some functions of nitrogen in plants. AQP: aquaporin; ROS: reactive oxygen species; WUE: water use efficiency; AM: arbuscular mycorrhiza; IAA: auxin; ABA: abscisic acid; ET: ethylene; CTK: cytokinins; TIPs: plasma membrane intrinsic proteins; PIPs: plasma membrane intrinsic proteins; NPF: nitrate transporter 1/peptide transporter family; NRT2: nitrate transporter 2 family; CLC: chloride channel; SLAC1/SLAH: slow anion channel associated 1/slow anion channel homologues; AMT: ammonium transporter.

The improvement of plant NUE can be considered from the following perspectives: (1) Use reverse genetic strategies to identify new regulators for plant responses to N starvation stress based on the phenotypes of primary root elongation and lateral root genesis. (2) Depend on increasingly sophisticated whole-genome sequencing technologies to expand the scope of research on plant responses to N signals and provide optimal gene resources for breeding. (3) Through further research on the relationship between N signaling and a variety of abiotic stresses, improve plant NUE by allowing plants to adapt to increasingly severe environmental challenges.

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