





Review Article

Nutrient-dependent regulation of symbiotic nitrogen fixation in legumes

Yanlin Ma ¹, Chengbin Xiao ², Jianquan Liu ¹ and Guangpeng Ren ^{1,*}¹State Key Laboratory of Herbage Improvement and Grassland Agro-Ecosystem, College of Ecology, Lanzhou University, No. 222 South Tianshui Road, Lanzhou 730000, China²MOE Key Laboratory of Cell Activities and Stress Adaptations, School of Life Sciences, Lanzhou University, No. 222 South Tianshui Road, Lanzhou 730000, China*Corresponding author. E-mail: rengp@lzu.edu.cn

Abstract

Mineral nutrients are essential for plant growth and development, playing a critical role in the mutualistic symbiosis between legumes and rhizobia. Legumes have evolved intricate signaling pathways that respond to various mineral nutrients, selectively activating genes involved in nodulation and nutrient uptake during symbiotic nitrogen fixation (SNF). Key minerals, including nitrogen, calcium, and phosphorus, are vital throughout the SNF process, influencing signal recognition, nodule formation, the regulation of nodule numbers, and the prevention of nodule early senescence. Here, we review recent advancements in nutrient-dependent regulation of root nodule symbiosis, focusing on the systemic autoregulation of nodulation in nitrate-dependent symbiosis, the roles of nodule inception-like proteins, and the function of essential nutrients and their associated transporters in legume symbiosis. Additionally, we discuss several key research areas that require further exploration to deepen our understanding of nutrient-dependent mechanisms in SNF.

Introduction

Legumes interact with compatible rhizobia to form specialized root-derived organs, known as nodules, where atmosphere nitrogen (N_2) is converted into ammonia (NH_3) for use by the host plant. This process is called symbiotic nitrogen fixation (SNF). The beneficial symbiotic relationship between legumes and rhizobia revolves around the exchange of nutrients [1–3]. Plants provide carbon (C) in the form of carbohydrates produced through photosynthesis to the rhizobia, which in return fix N_2 from the air for the plant [2, 4]. To effectively manage this symbiosis, host plants must tightly regulate SNF, including controlling the number of nodules, in order to balance their nitrogen (N) needs with C input [5, 6]. Legumes have developed sophisticated sensing and signaling systems to monitor environmental N levels and regulate SNF accordingly [7–9]. The autoregulation of nodulation (AON) pathway specifically controls nodule numbers in response to rhizobial infection. Additionally, nodule inception (NIN)-like proteins (NLPs) inhibit nodule formation in N-sufficient environments to conserve resources [10, 11] (Fig. 1).

In addition to C, plants supply mineral nutrients necessary for bacterial metabolism to their symbiotic partners [2, 12]. These mineral nutrients are delivered to rhizobia through roots, nodule vascular systems, and symbiosome membranes [2, 3, 13]. Transporters and enzymes play critical roles in this intricate nutrient exchange between nodules and host plants, as well as between host cells and symbionts. This is evidenced by the high expression levels of genes associated with metabolism and nutrient transport in mature nodules [14–16]. Effective SNF requires more than just a low-N environment. It also depends on calcium-mediated early

symbiotic signal transduction between rhizobia and legumes, efficient phosphate uptake and translocation, the availability of various metal nutrients essential for plant and bacteroid growth and metabolism. Additionally, a precise N metabolism pathway is crucial for the success of the symbiotic relationship (Fig. 1).

In this review, we present an updated summary of recent advancements in understanding how essential mineral nutrients regulate SNF in legumes, building on existing knowledge. We explore the molecular mechanisms underlying nitrate regulation of nodulation, the roles of key nutrients in legume symbiosis, and the function of mineral nutrient transporters within root nodules. By elucidating the roles of critical regulators and their associated networks in nutrient-dependent SNF, this review aims to offer insights that could guide future research efforts in sustainable agricultural practices.

Molecular mechanisms of AON in NO_3^- -induced control of nodulation

The molecular mechanism of AON is a complex signaling system in legumes that regulates nodule formation to optimize N fixation and overall growth [6, 17, 18]. AON involves long-distance signals from developing nodules to the shoots and feedback from shoots to roots, which suppresses further nodulation [19, 20] (Figs 1 and 2). This system integrates both local and systemic signals to balance nodule numbers with environmental and nutritional conditions [1, 17, 21] (Fig. 2).

AON is mediated by CLAVATA3/ESR-related (CLE) peptides produced in response to rhizobia and high N levels [8, 22–27].

Received: 15 August 2024; Accepted: 11 November 2024; Published: 26 November 2024; Corrected and Typeset: 1 March 2025

© The Author(s) 2025. Published by Oxford University Press on behalf of Nanjing Agricultural University. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

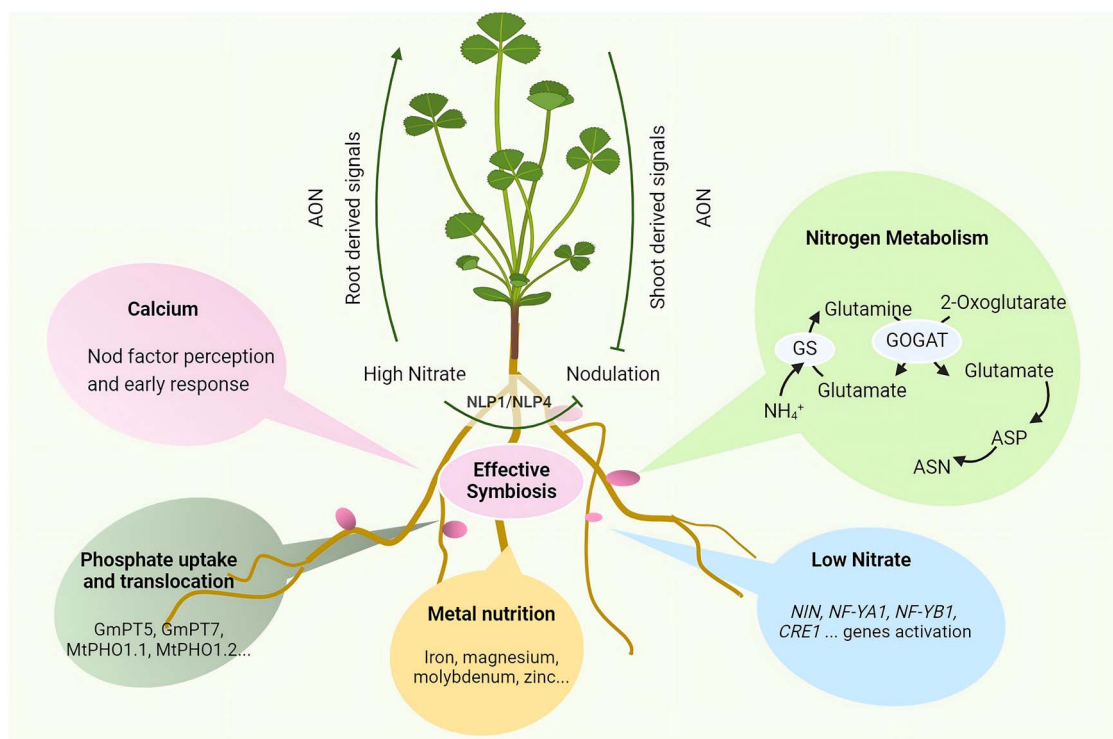


Figure 1. Overview of nutrient effects on SNF. Under low N conditions, rhizobium nodulation factors stimulate the expression of *NIN*, which in turn upregulates downstream genes like *NF-YA1*, *NF-YB1*, and *CRE1*, crucial for rhizobial infection and nodule formation. Calcium acts as a macronutrient in legumes, serving as a secondary messenger connecting nodulation factors perception at the plasma membrane with changes in calcium signaling. Legumes require high levels of inorganic phosphate for nodule growth, facilitated by phosphate uptake and translocation. The phosphate transporters *GmPT5*, *GmPT7*, *MtPHO1.1*, and *MtPHO1.2* influence phosphorus uptake into nodules, subsequently affecting both nodule size and number. Macronutrients like sulfate and magnesium, along with micro-nutrients such as iron, molybdenum, and zinc, also play significant roles in nodule formation and N fixation. N metabolism is crucial for effective symbiosis, where ammonium produced through SNF is transported back to the plant and converted into glutamine and glutamate by *GS* and *GOGAT*. In indeterminate nodules, glutamine and glutamate are further converted into *ASP* and *ASN*. The AON system tightly regulates nodule number to balance N fixation with other developmental processes. This signaling pathway involves root-derived signals, shoot receptors, and shoot-derived inhibitors, enabling root-shoot-root communication to determine the optimal number of nodules. Abbreviations: *ENOD40*, early nodulation genes; *NIN*, nodule inception, *NF-YA1*, nuclear factor-Y subunit A-1, AON, autoregulation of nodulation. *GS*, glutamine synthetase. *GOGAT*, glutamate synthase. Images were created with BioRender (<https://biorender.com>).

These peptides travel from roots to shoots via xylem, where they activate receptors that inhibit nodule formation through the shoot-derived inhibitor signal [28–31]. This process involves the *CLE/SUNN-HAR1/miR2111/TOO MUCH LOVE (TML)* network, which downregulates *miR2111* and increases *TML* gene expression to limit nodules [22, 32, 33]. High N conditions further suppress nodulation via the *NLP/CLE/SUNN* pathway [8], while cytokinin (*CK*) produced in the shoot, regulated by the *CLE-RS1/2-HAR1* module, also acts as a negative regulator of nodule formation [34] (Fig. 2). Additionally, the *CRA2* pathway positively regulates nodules under low N, with *CRA2* acting as a receptor for *CEP1*, integrating signals to maintain balance between lateral root and nodule formation [35–37] (Fig. 2). These findings underscore the complex interplay of systemic and local signals in controlling legume nodulation, ensuring that nodule numbers are finely tuned to environmental conditions and plant requirements.

NLPs mediate nitrate inhibition of nodulation

RWP-RK domain-containing NLP transcription factors play a pivotal role in regulating nitrate-inhibited nodulation under high N conditions. In *Medicago truncatula*, *NLP1* is essential for this process, directly binding to the *CLE35* promoter to activate its expression, thereby inhibiting nodulation [9]. Grafting experiments have further revealed the complex interaction

between positive regulation by *CRA2* and negative regulation by *SUNN* in the shoot, combined with *NLP1*'s inhibitory activity in the root. This interaction influences nitrate-induced rhizobial infection, nodule development, and nitrogenase activity, ultimately determining nodule numbers [9]. *CRA2* positively regulates nodule formation by modulating the expression of *CEP1/2*, while further research indicates that *NLP1* directly targets the *CEP1* promoter, suppressing *CEP1* expression in the presence of nitrate [38]. In addition to these findings, recent study has shown that in *Glycine max*, *GmNLP1b* and *GmNLP4a* bind to the promoters of nitrate-induced *CLE* peptide genes, *GmNIC1a* and *GmNIC1b*, activating their expression in response to nitrate and inhibiting nodulation [39]. These discoveries highlight the intricate interplay between systemic and local signals in nitrate-mediated nodulation inhibition. They also underscore the complex regulatory mechanisms through which NLPs mediate nitrate-induced inhibition of nodulation, demonstrating how legumes fine-tune their symbiotic interactions to adapt to varying N availability.

The molecular mechanism of nitrate-induced nodule senescence

Nitrate-induced nodule senescence is another critical factor in the inhibition of SNF under high soil N conditions. Elevated nitrate levels suppress N fixation and disrupt nodule metabolism

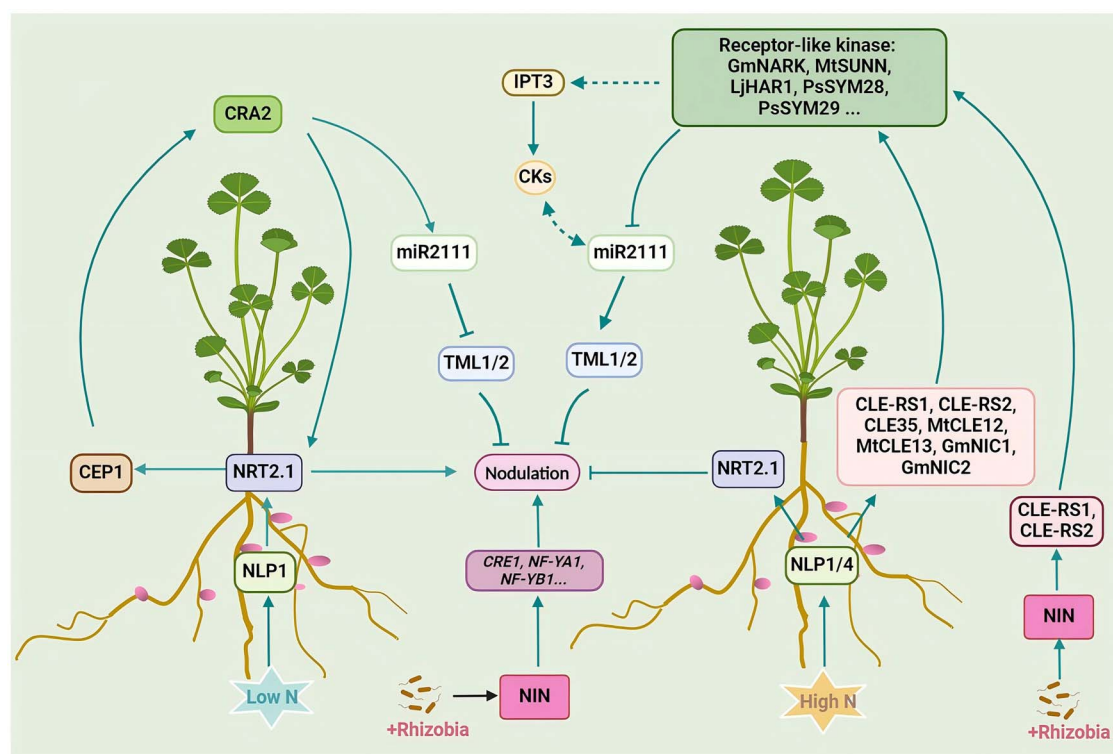


Figure 2. A general summarized model of rhizobia and NO_3^- regulation in legumes: local and AON pathways for nodulation control. AON involves a long-distance signaling process that coordinates root and shoot responses. Under low N conditions, MtCEP1 expression induces systemic MtNRT2.1 expression through MtCRA2 in the shoot. MtNLP1 also activates low-level MtNRT2.1 expression, further promoting nodulation and MtCEP1 expression. NFs induce the activation of NIN, which upregulates genes essential for rhizobial infection and nodule formation, including NF-YA1 and CRE1. Rhizobial infection activates NIN expression, leading to the synthesis of CLE-RS1 and RS2 peptides. Under high N conditions, MtNLP1 activates MtNRT2.1 expression, increasing NO_3^- uptake and suppressing nodulation. MtNLP1 also activates MtCLE35 and represses MtCEP1, systemically regulating nodulation. High NO_3^- levels activate NRSYM1 (NLP4), inducing CLE-RS2 and RS3 peptide synthesis. CLE-like peptides (CLE-RS1, CLE-RS2, CLE35, MtCLE12, MtCLE13, GmNIC1, GmNIC2) are thought to be transported from root to shoot, where they are sensed by receptor-like kinases such as GmNARK, MtSUNN, LjHAR1, PsSYM28, and PsSYM29. The CLE-like peptides and receptor-like kinases form receptor-ligand complexes, reducing miR2111 expression in the shoot. This decreases miR2111 levels in the root, increasing the transcript level of its target, TML. TML encodes an F-box protein that negatively regulates nodule formation on roots. CKs also act as shoot-derived inhibitors in the AON pathway. LjIPT3 is involved in CK biosynthesis in shoots and inhibits nodule formation, depending on the LjCLE-RS1/2-LjHAR1 signaling pathway. Images were created with BioRender (<https://biorender.com>)

by reducing antioxidant content and nitrogenase activity, accelerating senescence [40, 41]. This process is marked by nodule color changes, increased reactive oxygen species, and damage to symbiosomes and bacteroids [42–45]. High nitrate also impairs carbon allocation to nodules and heightens oxygen diffusion resistance, further compromising nodule function [40, 46]. Although similar to natural senescence, nitrate-induced senescence progresses more rapidly, with its molecular pathways gradually being uncovered. The NAC-type transcription factor LjNAC094 has been identified as a key promoter of nitrate-induced root nodule senescence [47]. Acting downstream of LjNLP1 and LjNLP4, LjNAC094 regulates the expression of senescence-associated genes in response to nitrate [47]. Additionally, in soybean, nitrogen-associated NAPs (SNAPs) transcription factors SANP1/2/3/4 play critical roles in mediating nitrate-induced inhibition of nitrogenase activity and acceleration of nodule senescence [48]. These transcription factors reprogram the nodule transcriptome in response to N by directly regulating a subnetwork of senescence-associated genes and transcriptional regulators, including TFs in the NAC, WRKY, and ethylene responsive factor (ERF) families [48]. These findings elucidate the connection between nitrate signaling and nodule senescence progression.

Essential nutrients and related transporters in the legume symbiosis

Nitrogen

NO_3^- transport

NO_3^- and NH_4^+ are the primary inorganic N sources for plants, serving as essential nutrients and signaling molecules that regulate plant growth and metabolism [49, 50]. Nitrate transporters (NRT) from the NRT1 and NRT2 families enable root cells to uptake NO_3^- through high- and low-affinity systems, depending on NO_3^- availability [51, 52]. Key transporters like *Arabidopsis* NRT1.1 and *M. truncatula* NRT1.3 function as dual-affinity transporters, regulating NO_3^- uptake based on soil NO_3^- concentration [53, 54]. AtNRT1.1, the first plant NO_3^- transporter identified, was initially linked to NO_3^- uptake and transport [55]. Subsequent studies revealed that AtNRT1.1 can switch its NO_3^- transport activity between high- and low-affinity states depending on its phosphorylation status [56, 57]. AtNRT1.1 serves as an NO_3^- sensor, activating NO_3^- response pathways independently of its NO_3^- uptake function [56].

Several NRT1/peptide transporter family (NPF) proteins, such as MtNPF1.7 and LjNPF8.6, play significant roles in SNF in *M. truncatula* and *Lotus japonicus* [58–61]. MtNPF1.7, previously

Table 1. Summary of identified major nutrients transporters for SNF in different legume species.

Transporter category	Name	Ion	Sublocalization	Reference
Nitrate and ammonium transporter	LjNRT2.4	NO ₃ ⁻	PM	7
	Mt/LjNRT2.1	NO ₃ ⁻	PM	65, 66
	MtNRT1.3	NO ₃ ⁻	PM	53
	MtNPF7.6	NO ₃ ⁻	PM	63
	LjNPF3.1	NO ₃ ⁻	PM	64
	LjNPF8.6	NO ₃ ⁻	SM	7, 61
	LjAMT1;1	NH ₄ ⁺	PM	67
LjAMT1;2	NH ₄ ⁺	PM	68, 69	
Phosphorus	GmPT5	PO ₄ ³⁻	PM	70
	GmPT7	PO ₄ ³⁻	PM	71
	MtVPT2/3	PO ₄ ³⁻	unknow	72
	MtPHO1.1	PO ₄ ³⁻	PM/	73
	MtPHO1.2	PO ₄ ³⁻	PM/Golgi	73
Potassium	GmHAK5	K ⁺	PM	74
	LjKUP	K ⁺	PM	75
Magnesium	GmMGT4	Mg ²⁺	PM	76
	GmMGT5	Mg ²⁺	PM	76
Sulfate	LjSST1	SO ₄ ²⁻	SM	77
	MtESN2	SO ₄ ²⁻	PM	78
Iron	GmVTL1a	Fe ²⁺	SM	79, 80
	MtFPN2	Fe ²⁺	SM	81
	LjSEN1	Fe ²⁺	SM	82
	MtVTL4/8	Fe ²⁺	SM	83
	GmYSL7	Fe ²⁺	SM	84–86
	MtYSL3	Fe ²⁺	PM	87
	GmDMT1	Fe ²⁺	SM	88
	MtNRAMP1	Fe ²⁺	PM	89
Molybdenum	MtMOT1.2	MoO ₄ ²⁻	PM	90
	MtMOT1.3	MoO ₄ ²⁻	PM	91
Zinc	MtZIP1	Zn ²⁺	SM	92
	MtZIP6	Zn ²⁺	PM	93
	MtYSL3	Zn ²⁺	PM	87
	MtMTP2	Zn ²⁺	ER	94
Copper	MtCOPT1	Cu ²⁺	PM	95
Citrate	LjMATE1	Citrate	PM	96
	LjMATE67	Citrate	PM/SM	97

Abbreviations: plasma membrane, PM; symbiosome membrane, SM; nuclear envelope, NE; endoplasmic reticulum, ER

glutamine and glutamate via the glutamine synthetase (GS)-glutamate synthase (GOGAT) pathway [99]. This process begins when NH₄⁺ binds to glutamic acid in an ATP-dependent reaction catalyzed by GS, forming glutamine [99] (Fig. 1). GOGAT then catalyzes the conversion of glutamine and 2-oxoglutarate to produce glutamic acid, completing the primary assimilation of ammonium [99] (Fig. 1). N export patterns vary with the host plant. Temperate legumes like *M. truncatula* and pea develop indeterminate nodules and mainly reduce N₂ to amides such as glutamine and asparagine. Whereas, tropical legumes like *G. max* and *L. japonicus*, which form determinate nodules, use N₂ to produce ureides like allantoin and allantoic acid, which are then transported to the stems and leaves through the nodule's vascular system [100]. The ureides are essential for synthesizing critical biomolecules like amino acids, nucleotides, and chlorophylls. The suppression of NADH-GOGAT expression in *M. sativa* decreases nodule amino acid levels and negatively affects SNF [101]. Additionally, the enzyme glutamine phosphoribosyl pyrophosphate

amidotransferase (PRAT) initiates ureide synthesis through purine oxidation, and inhibiting PvPRAT3 reduces ureide production, impacting SNF [102]. *G. max* nodulin 26 (Nod26), a member of the aquaporin superfamily, is the major component of the symbiosome membrane (SM) enclosing N-fixing bacteroids in root nodules [103–105]. Nod26 promotes efficient N assimilation and prevents potential ammonia toxicity by binding to the conserved C-terminal domain of GS [106].

Ammonium is the main product of SNF. The transport and assimilation of NH₄⁺ are critical processes in the plant-rhizobium interaction. LjAMT1;1, encoding a high-affinity NH₄⁺ transporter, is expressed in both the infection zone and vascular tissue of *L. japonicus* nodules [67]. Inhibiting LjAMT1;1 expression partially impairs N-fixing activity in nodules and increases nodule number compared to control plants, suggesting that LjAMT1;1 is involved in modulating NH₄⁺ homeostasis in nodules [67]. In *G. max*, the transport of reduced allantoin and allantoic acid from nodules relies on a urea permease, UPS1 [107]. Repression of *GmUPS1*–1

and *GmUPS1–2* expression in nodules leads to an accumulation of ureides and affects N translocation from nodules to shoots [107]. By expressing a common bean *UPS1* transporter in the cortex and endodermis cells of soybean nodules, it was found that N transfer from nodules to the shoot and seed development were significantly increased [108]. Additionally, the number of transgenic nodules increased, and the nitrogenase activity per nodule was also enhanced, indicating that transporter function in N export from nodules is a key step for enhancing atmospheric N fixation and nodule function, as well as for improving shoot N nutrition and seed development in legumes [108].

Phosphorus

Phosphorus (P), a vital component of nucleic acids, amino acids, phospholipids, and secondary metabolites, plays a crucial role in photosynthesis, energy conversion, and maintain enzyme activity, all of which are essential for plant growth and development [109, 110]. In legumes, the formation of nodules is an energy-intensive process that necessitates a significant amount of P [111–113] (Fig. 1). A deficiency in P can have a detrimental effect on nodule initiation, leading to a reduction in nodule number, size, and activity, ultimately hindering SNF [113, 114]. Adequate P supply has been shown to enhance *G. max* nodulation by increasing both nodule number and size [115]. Therefore, maintaining an appropriate level of P in nodules is crucial for overall plant growth and biological N fixation.

P acquisition in plants is mediated by P transporters. Inorganic phosphate (Pi) transport in rhizobia is crucial for efficient SNF. The regulation of P transporters is essential for plant adaptation to low-P stress [116, 117]. The P transporter family protein PHT1/PT is primarily responsible for the uptake of Pi from the soil and its mobilization within plants, maintaining Pi homeostasis [117, 118]. The *G. max* gene *GmPT5* encodes a high-affinity phosphate transporter that transports Pi from roots to nodules, particularly under limited P conditions [70] (Figs 1 and 3). Another nodule-localized phosphate transporter, *GmPT7*, absorbs Pi from the external environment and transfers it from the nodule cortex to the fixation zone, participating in SNF in legumes [71] (Fig. 3). Similarly, over-expression of the rice (*O. sativa*) phosphate transporter gene *OsPT2* in *G. max* enhances both N fixation and NH_4^+ assimilation under P-deficiency conditions [119]. Vacuolar phosphate transporters (VPTs) modulate P adaptation and play essential roles in mutualistic rhizobium–legume symbiosis by regulating long-distance Pi transport. Mutations in *MtVPT2* and *MtVPT3* result in cytosolic Pi deficiency in nodules, reducing nodule number and nitrogenase activity under different phosphate conditions [72]. Therefore, *MtVPT2* and *MtVPT3* are crucial for maintaining a stable cytosolic Pi level in the fixation zone of the nodule under low-phosphate stress, as well as for regulating nitrogenase activity and phosphate homeostasis in root nodules [72].

Phosphate starvation response 1 (PHR1), a constitutively expressed MYB-domain transcription factor, binds to a cis-element in its target genes, regulating their expression and promoting Pi uptake by directly inducing the expression of *PHOSPHATE-TRANSPORTER1* (*PHT1*) [120, 121]. The PHR1-PHT1 module is a key regulatory component in the plant P signaling pathway [118]. Multiple *GmPHR*-*GmPHT1* modules operate in both infected and noninfected nodule tissues, with each *GmPHR* targeting multiple *GmPHT1*s and vice versa. These overlapping modules regulate Pi homeostasis, nodule initiation, and development. Over-expression of *GmPHR1* boosts *GmPHT1;11* expression, increasing nodule Pi content and size, underscoring the importance of the PHR-PHT1 module in nodules [122].

Proteins related to Pi homeostasis, such as *GmSPX8*, *GmSPX5*, *MtPHO1.1*, and *MtPHO1.2*, are essential for *G. max* nodulation. *GmSPX8*, a nucleus-localized SPX protein, is vital for nodule development and N fixation under low P conditions [123]. Overexpression of *GmSPX8* increases nodule number, weight, and nitrogenase activity, enhancing N and P content in P-deficient environments, whereas suppression impairs these processes [123]. *GmSPX8* interacts with *GmPTF1*, further promoting nodule development and N fixation [123]. Both *GmSPX5* and *GmSPX8* exhibit a predominant expression in nodules and show an increase in expression levels when phosphorus (P) deficiency occurs. The overexpression of either of these proteins leads to an increase in both the number and weight of nodules, which in turn results in enhanced N and P content within the nodules [123, 124]. *GmNF-YC4*, which is a transcription factor belonging to the nuclear factor Y family, interacts with *GmSPX5*, enhancing its binding to downstream gene promoters and supporting nodule development and function [124]. In *M. truncatula*, the PHO1 family members *MtPHO1.1* and *MtPHO1.2*, found in the plasma membrane and Golgi, respectively (Table 1, Fig. 3), play a vital role in the transport of Pi from the infected nodule cells to the bacteroids [73].

Potassium

Potassium (K) plays a vital role in plant responses to abiotic stress, particularly in regulating osmotic pressure, turgor, and ion homeostasis [125–127]. K transport is crucial during root nodule formation and development, with nodules showing heightened sensitivity to ionic stress, likely due to ion homeostasis disruptions in infected cells. In *M. truncatula*, studies reveal significant declines in K levels within symbiosomes and vacuoles of infected cells over the cell life cycle, potentially disturbing K homeostasis [128]. Low-temperature scanning electron microscopy and X-ray microanalysis highlight substantial reductions in K during nodule maturation and senescence, correlating with the mislocalization or partial loss of key Shaker K channels, *MtAKT1* and *MtSKOR/GORK*, impairing K balance and nodule function [128]. K availability is also critical for SNF, as it enhances ATP production and electron flow, thereby supporting N fixation [129]. Additionally, K mediates the transfer of carbohydrates from the host plant to the nodules, modulating C input and N output, which further affects the N fixation process [2, 129, 130]. In *L. japonicus*, the K transporter *LjKUP*, highly expressed during late nodule development, is localized to the plasma membrane [75]. Similarly, in soybean, the K transporter *GmHAK5*, highly expressed in vascular tissues of roots and nodules, facilitates K translocation from roots to nodules [74]. Therefore, K is essential for nodule development, ion homeostasis, and efficient SNF in legumes.

Calcium

Calcium (Ca) is integral to plant nutrition and signal transduction, functioning as a key component of cell walls and membranes, as well as an intracellular second messenger [131]. In legume–rhizobia symbiosis, Ca is crucial throughout preinfection, infection, and nodule development [132–134]. Ca deficiency, particularly in acidic soils, disrupts nodule formation and growth by impairing rhizobial multiplication and root infection [135, 136]. The interaction between Ca and boron under stress conditions enhances SNF efficiency [137]. Additionally, Ca acts as a secondary messenger in Nod factor signaling, modulating gene expression during early symbiotic events [133, 138, 139]. Ca^{2+} -ATPases and Ca-dependent protein kinases are essential for active Ca transport and regulation within the symbiosome, underscoring its critical role in nodulation and SNF [140, 141].

Sulfur

Sulfur (S) is the fourth essential nutrient element for plants after N, P, and K. It is a component of the amino acids cysteine and methionine, glutathione, plant chelating agents, coenzyme A, and other S compounds, and it participates in important physiological and biochemical reactions in plants [142, 143]. In legumes, S supply is positively correlated with SNF, and S deficiency can cause three additional main effects: fewer nodules, inhibition of SNF, and inhibition of nodule metabolism [144, 145]. Sulfate transporters facilitate the absorption and transport of sulfate [143]. In *L. japonicus*, the sulfate transporter SST1 is located on the SM and mediates sulfate transport from the plant cell cytoplasm to intracellular rhizobia [2, 77]. Additionally, *Early Senescent Nodule 2* (*MtESN2*) in *M. truncatula* encodes a protein of the sulfate transporters family, expressed in both roots and nodules. This protein localizes to the plasma membranes of infected and uninfected cells within the transition and N-fixing zones of nodules [78] (Fig. 3). The *Mtesn2* mutant exhibits significantly reduced nodule size and nitrogenase activity, with nodules showing early senescence under symbiotic conditions [78].

Iron

Iron (Fe) is critical for SNF due to its role in Fe-dependent proteins, including leghemoglobin, ferroproteins, nitrogenase, cytochrome, and hydrogenases [146–150]. Fe is required at all stages of nodulation and SNF in legumes, though its impact varies by species [149]. In *Lupinus angustifolius*, Fe deficiency impairs both nodule initiation and development, whereas in crops like soybean, peanut, and common bean, it primarily affects later stages of nodule maturation [148, 151]. The requirement for Fe in legume–rhizobia symbiosis is higher than in nonleguminous plants, and Fe deficiency reduces nodule number, size, and N fixation efficiency [149, 152, 153]. In *Phaseolus vulgaris*, nodule Fe concentration correlates positively with N fixation rates [152]. To compensate for Fe deficiency, soybean initiates adaptive responses such as increased H⁺ secretion and Fe(III) reductase activity to enhance Fe uptake from the soil [154].

Typically imported into the nodule as ferric citrate via the xylem, Fe must then traverse several cell layers to reach the infected cells [147, 155]. This transport occurs through both symplastic and apoplastic routes [155]. Specifically, two multidrug and toxic compound extrusion (MATE) transporters LjMATE1 and MtMATE67 play important roles in delivering Fe to the nodule infection zone, thus maintaining Fe homeostasis in nodules [96, 97]. Additionally, MtNRAMP1, an Fe transporter in the cytoplasmic membranes of infected root nodule cells, facilitates apoplastic Fe uptake, promoting SNF [89] (Fig. 3). Moreover, nodule-specific vacuolar iron transporter-like 4 (MtVTL4) and MtVTL8, particularly MtVTL8, the closest homolog to LjSEN1, serve as main Fe transport proteins to bacteroids in *M. truncatula* [83] (Fig. 3). In *G. max*, the GmVTL1a transporter functions in the N fixation region of root nodules, transporting Fe across the symbiotic membrane to bacteroids [79] (Fig. 3). Furthermore, ferrous Fe uptake by infected cells is facilitated by the nodule-specific protein Ferroportin2 (MtFPN2) in *M. truncatula*, located in symbiosomes in the interzone and early-fixation zone, delivering Fe to N-fixing bacteroids [81]. Similarly, *GmDMT1* (*G. max* divalent metal transporter 1), a member of the NRAMP/Dmt1 family, is highly expressed during the initial stages of N fixation in developing *G. max* nodules. It functions as a nodule-enhanced divalent metal transporter, facilitating the transport of ferrous Fe across the peribacteroid membrane [88]. The yellow stripe-like (YSL) family of metal transporters is essential for transporting Fe from roots to

nodules. For instance, MtYSL3, located in the plasma membranes of vascular cells in roots and nodules, as well as cortical nodule cells, facilitates the delivery of Fe and Zn to N-fixing nodule cells, playing a critical role in SNF [87]. Recent research has highlighted the roles of YSL7 proteins in symbiotic nodulation in legumes [3, 84–86]. Specifically, MtYSL7, a clade III YSL protein, localizes to the plasma membranes of cells within the root pericycle, nodule cortex, and vasculature [85] (Fig. 3). It functions in oligopeptides transport, thereby maintaining iron (Fe) and copper (Cu) homeostasis in nodules, which is crucial for SNF [85]. Similarly, GmYSL7 in *G. max*, specifically situated in the SM, regulates nodule development and nitrogenase activity by transporting oligopeptides to symbiosomes [86]. Additionally, GmYSL7, highly expressed in multiple nodule cells, responds to changes in Fe levels, facilitating Fe accumulation in *G. max* nodules, thereby influencing nodule development and N fixation activity [84].

Magnesium

Magnesium (Mg), the most abundant divalent cation in cells, is crucial as an activator of numerous enzymes and is essential for various physiological processes in plants, including photosynthesis, carbon metabolism, and protein synthesis [156, 157]. During SNF, the rhizobium nitrogenase relies on Mg-ATP hydrolysis to transfer electrons from the Fe protein to the Mo–Fe protein [158]. While Mg does not directly affect nodule formation, it influences nodule development by optimizing carbohydrate distribution and facilitating the transfer of carbohydrates from shoots to nodules. Mg specifically regulates carbon and N transport and exchange within nodules [3, 159]. Specifically, Mg accumulation in the inner cortex of *G. max* nodules activates the activity of the β -1,3-glucanase GmBG2 in inner cortical cells, leading to callose decomposition, increased plasmodesmata permeability, and enhanced carbon-N exchange [76]. Leguminous plants transport Mg to nodules either by unloading it from vascular tissues or by directly absorbing it through the nodule cortex. GmMGT4 and GmMGT5, members of the *G. max* Mg Transporter (MGT) family located on the plasma membrane of the inner cortex within the nodule, together facilitate Mg absorption and accumulation in cortical cells [76] (Fig. 3). Disruption of *GmMGT4* and *GmMGT5* inhibits nodule growth, increases callose accumulation in cortical cells, decreases sugar content, and reduces N export rates, highlighting their critical roles in maintaining Mg homeostasis and supporting effective symbiosis [76].

Molybdenum

Effective SNF require a steady supply of molybdenum (Mo), a component of the Fe–Mo cofactor of nitrogenase [160, 161]. Mo plays a crucial role in molybdoenzymes such as nitrogenase, xanthine dehydrogenase, and nitrate reductase, facilitating electron transfer during SNF [162]. In *Bradyrhizobium japonicum*, the high-affinity ModABC transport system mediates molybdate uptake, essential for efficient N fixation, indicating molybdate transport across the symbiosome membrane [163]. Additionally, molybdate is required for the Mo cofactor (Moco), which supports essential metabolic processes, including nitrate assimilation, purine metabolism, and sulfite detoxification [160, 162, 164]. In *M. truncatula*, two Mo transporters, MtMOT1.2 and MtMOT1.3, are crucial for Mo uptake into nodules [90, 91]. MtMOT1.2 is located in the plasma membrane of endodermal cells surrounding vascular vessels in nodules, facilitating Mo uptake and distribution within nodule cells [90] (Fig. 3). MtMOT1.3, located in the plasma membranes of infected cells, transports MoO₄²⁻ into nodule cells, essential for synthesizing functional nitrogenase [91] (Fig. 3).

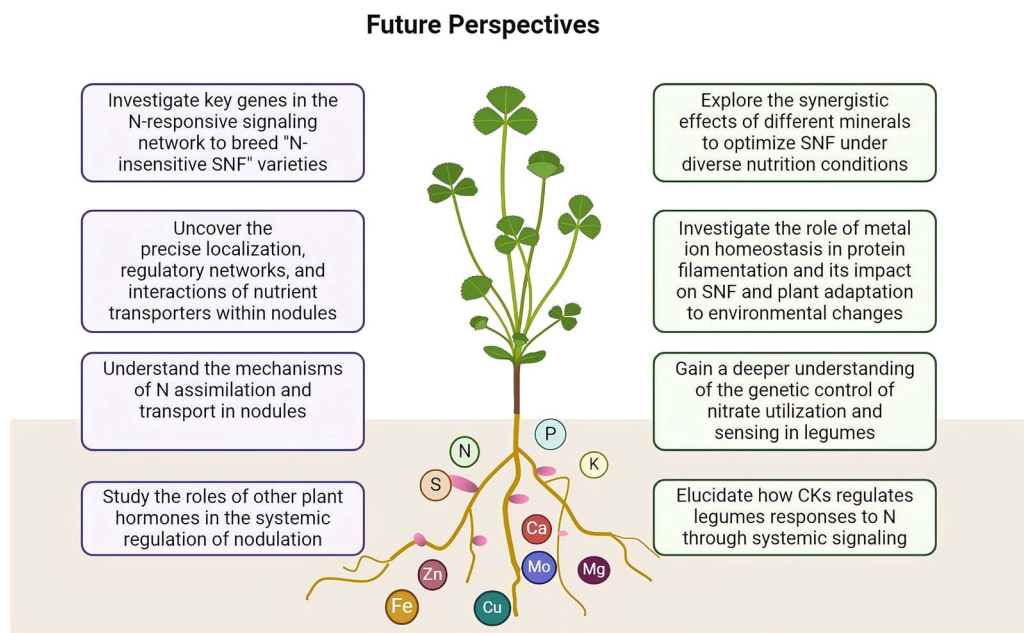


Figure 4. Outlooks in enhancing N Fixation and nutrient uptake in legumes

Zinc

Zinc (Zn) is essential for numerous proteins and enzymes, playing key roles in chlorophyll biosynthesis and auxin production [3]. It stabilizes zinc-finger transcription factors, ensuring accurate DNA interactions for gene regulation [165, 166]. In legume root nodules, Zn is integral to homocitrate synthase, a metalloenzyme responsible for homocitrate production, a precursor in the formation of the iron-molybdenum cofactor of nitrogenase, which is crucial for SNF [2, 167]. Since most rhizobia lack the ability to synthesize homocitrate, they rely on their host plants for its production via the *FEN1* gene [167]. Additionally, Zn supports Cu-Zn superoxide dismutase, an enzyme that reduces oxidative stress in nodules, preserving nodule health and nitrogenase activity [3, 168]. *GmZIP1*, a Zn transporter specific to *G.max* symbiosis, is located in the peribacteroid membranes of root nodule cells, aiding in Zn uptake within symbiosomes [92] (Fig. 3). Conversely, *MtZIP6*, located in the plasma membranes of N-fixing nodule cells, facilitates Zn uptake from the apoplast [93] (Fig. 3). Additionally, *MtMTP2*, an intracellular Zn efflux transporter, regulates nodule development and bacteroid differentiation [94]. Collectively, *MtZIP6* and *MtMTP2* are critical to the maintenance of Zn homeostasis in nodules, constituting integral components of the Zn transport system essential for effective SNF. Recently, a pivotal study has elucidated the role of Zn as an intracellular second messenger, mediating the conversion of soil N nutrients into Zn concentration signals within root nodules [169]. This process is integral for linking environmental changes to N homeostasis through the regulation of the transcriptional regulator *FIXATION UNDER NITRATE* (*FUN*). Zn modulates the transition of *FUN* from an inactive filamentous megastructure to an active transcriptional regulator, thereby optimizing nodule function in response to varying N conditions [169]. Under low N conditions, elevated Zn levels within nodules promote the formation of inactive *FUN* filaments, facilitating efficient N fixation. Conversely, in high N environments, decreased Zn concentrations trigger the dissociation of these filaments and activation of *FUN*, orchestrating multiple pathways to suppress N fixation and induce nodule senescence [169]. This mechanism highlights the potential of

FUN in enhancing legume tolerance to soil NO_3^- , improving the efficiency of fixed N delivery, minimizing fertilizer dependence, and advancing sustainable agricultural practices.

Copper

Copper (Cu) is critical for electron transfer in photosynthesis, respiration, and carbohydrate metabolism, as well as regulating free radicals through Cu-Zn superoxide dismutase [3, 170]. In legume nodules, Cu is essential for cytochrome *cbb₃* oxidase activity, facilitating the microaerobic respiration of rhizobial bacteroids, a process vital for SNF [2, 171, 172]. Additionally, Cu supports bacterial tyrosinase, which is important for rhizobial survival [3, 173]. Copper transport from host cells to bacteroids is mediated by nodule-specific transporters like *MtCOPT1* [95], while copper chaperones such as *NCC1* enhance intracellular Cu delivery [174], ensuring the functionality of N-fixing enzymes like cytochrome oxidase and nitrogenase.

Influence of soil nutrient availability and pH on N fixation in legumes

N fixation in legumes is significantly influenced by soil nutrient availability and pH. In P-deficient soils, P fertilization enhances N fixation by promoting root growth and extensive nodule development [175]. However, low soil pH negatively affects rhizobial survival and activity, reducing N fixation efficiency [176, 177]. Acidic conditions also increase the solubility of toxic elements like aluminum and manganese, which further inhibit root growth and nodule formation [178]. Legumes perform best in soils with a pH between 6.0 and 7.5; when pH drops below 5.5, the availability of essential nutrients such as Ca, Mg, and P decreases, adversely affecting nodulation and N fixation [176, 179]. Liming is often used in acidic soils to raise pH and improve nutrient availability, creating more favorable conditions for N fixation [180]. Mo deficiency, exacerbated in acidic environments, can further impair N fixation due to its role in the nitrogenase enzyme complex [160, 181]. The distribution and abundance of soil nutrients directly regulate N fixation by affecting nodule formation,

rhizobial activity, and plant health. Nutrient deficiencies and unfavorable soil conditions, such as low pH or poor aeration, substantially hinder N fixation, while well-balanced nutrition and optimal environmental conditions support robust legume–rhizobia symbiosis, maximizing N fixation [182, 183]. Understanding these dynamics is crucial for optimizing N fixation in legume-based agriculture.

Conclusion and future directions

Mineral nutrients are essential for the symbiosis between legumes and rhizobia. This review examines recent research advancements in the complex signaling pathways that respond to these nutrients, activating genes involved in nodulation and nutrient uptake during SNF. Findings indicate that the efficiency of SNF in legume root nodules, facilitated by rhizobia, is influenced by the host plant's genetic background and the availability of essential mineral nutrients (N, P, K, Ca, Mg, S, Fe, Zn, Cu, Mo). Each nutrient plays a unique role in N fixation mechanisms. While significant progress has been made in understanding their individual contributions, comprehensive knowledge of their coordinated regulation is still lacking. Future research should focus on exploring the synergistic effects of these minerals and developing integrated strategies to optimize SNF under various environmental conditions (Fig. 4). We propose two key research directions related to mineral nutrients that require further enhancement:

(1) Investigating nitrate and hormonal interactions

N availability, particularly in the forms of nitrate (NO_3^-) and ammonium (NH_4^+), critically modulates SNF efficiency. Low NO_3^- levels promote SNF, whereas high levels inhibit it [6, 184]. Specific transporters from the NRT1 and NRT2 families regulate nitrate uptake and distribution, directly influencing SNF [16, 65, 185]. Understanding the genetic control of NO_3^- utilization and sensing in legumes is essential for improving biological N fixation and NUE. Additionally, further exploration is needed regarding the role of plant hormones, such as CKs, in regulating nodulation and responses to N and C levels in conjunction with mineral nutrients (Fig. 4).

(2) Enhancing molecular understanding of legume–rhizobia symbiosis

Rhizobia induce nodule formation and differentiate into bacteroids, which convert atmospheric N_2 into NH_3 . However, the metabolic mechanisms controlling C and N allocation in bacteroids involving in mineral nutrients are not fully understood. Insights into the molecular mechanisms of fixed N transport and assimilation that are related to mineral nutrients could enhance the effectiveness of legume–rhizobia symbiosis. Recent advancements in single-cell RNA sequencing and proteomics offer new opportunities to investigate nutrient transporter functions. Furthermore, developing 'N-insensitive SNF' leguminous crop varieties could sustain high N fixation efficiency under heavy fertilization, mitigating the inhibitory effects of excess N on nodulation and SNF (Fig. 4).

In summary, while significant progress has been made in understanding the molecular mechanisms underlying SNF and nutrient transport in legumes, the regulatory networks governing these processes require further investigation. Focusing on nutrient transporters and signaling pathways will be crucial for advancing our knowledge of legume–rhizobia symbiosis and improving SNF efficiency in sustainable agriculture.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (No. 32301477 to Y.M.) and (No. 32370224 to G.R.).

Author contributions

M.Y.L. and X.C.B. prepared the manuscript including text and figures in this review. R.G.P. and L.J.Q. gave valuable discussion and instructions. All authors read and approved the manuscript.

Data availability

All data supporting the findings of this review are available within the article.

Conflict of interest statement

The authors declare that they have no conflict of interest.

References

- Gautrat P, Laffont C, Frugier F. et al. Nitrogen systemic signaling: from symbiotic nodulation to root acquisition. *Trends Plant Sci.* 2021;**26**:392–406
- Udvardi M, Poole PS. Transport and metabolism in legume–rhizobia symbioses. *Annu Rev Plant Biol.* 2013;**64**:781–805
- Li Y, Liu Q, Zhang D-X. et al. Metal nutrition and transport in the process of symbiotic nitrogen fixation. *Plant Commun.* 2024;**5**:100829
- Yurgel SN, Kahn ML. Dicarboxylate transport by rhizobia. *FEMS Microbiol Rev.* 2004;**28**:489–501
- Mortier V, Holsters M, Goormachtig S. Never too many? How legumes control nodule numbers. *Plant Cell Environ.* 2012;**35**:245–58
- Ferguson BJ, Mens C, Hastwell AH. et al. Legume nodulation: the host controls the party. *Plant Cell Environ.* 2019;**42**:41–51
- Valkov VT, Sol S, Rogato A. et al. The functional characterization of LjNRT2.4 indicates a novel, positive role of nitrate for an efficient nodule N_2 -fixation activity. *New Phytol.* 2020;**228**:682–96
- Moreau C, Gautrat P, Frugier F. Nitrate-induced CLE35 signaling peptides inhibit nodulation through the SUNN receptor and miR2111 repression. *Plant Physiol.* 2021;**185**:1216–28
- Luo Z, Lin J-s, Zhu Y. et al. NLP1 reciprocally regulates nitrate inhibition of nodulation through SUNN-CRA2 signaling in *Medicago truncatula*. *Plant Commun.* 2021;**2**:100183
- Lin JS, Li X, Luo Z. et al. NIN interacts with NLPs to mediate nitrate inhibition of nodulation in *Medicago truncatula*. *Nat Plants.* 2018;**4**:942–52
- Liu J, Bisseling T. Evolution of NIN and NIN-like genes in relation to nodule symbiosis. *Genes.* 2020;**11**:777
- Roy S, Liu W, Nandety RS. et al. Celebrating 20 years of genetic discoveries in legume nodulation and symbiotic nitrogen fixation. *Plant Cell.* 2020;**32**:15–41
- Udvardi MK, Day DA. Metabolite transport across symbiotic membranes of legume nodules. *Annu Rev Plant Physiol Plant Mol Biol.* 1997;**48**:493–523
- Mergaert P, Kereszt A, Kondorosi E. Gene expression in nitrogen-fixing symbiotic nodule cells in *Medicago truncatula* and other nodulating plants. *Plant Cell.* 2020;**32**:42–68
- Prell J, Poole P. Metabolic changes of rhizobia in legume nodules. *Trends Microbiol.* 2006;**14**:161–8

16. Pellizzaro A, Alibert B, Planchet E. *et al.* Nitrate transporters: an overview in legumes. *Planta*. 2017;**246**:585–95
17. Caetano-Anolles G, Gresshoff PM. Plant genetic control of nodulation. *Ann Rev Microbiol*. 1991;**45**:345–82
18. Delves AC, Mathews A, Day DA. *et al.* Regulation of the soybean-rhizobium nodule symbiosis by shoot and root factors. *Plant Physiol*. 1986;**82**:588–90
19. Ferguson BJ, Indrasumunar A, Hayashi S. *et al.* Molecular analysis of legume nodule development and autoregulation. *J Integr Plant Biol*. 2010;**52**:61–76
20. Magori S, Kawaguchi M. Long-distance control of nodulation: molecules and models. *Mol Cells*. 2009;**27**:129–34
21. Yang J, Lan L, Jin Y. *et al.* Mechanisms underlying legume-rhizobium symbioses. *J Integr Plant Biol*. 2022;**64**:244–67
22. Mortier V, den Herder G, Whitford R. *et al.* CLE peptides control *Medicago truncatula* nodulation locally and systemically. *Plant Physiol*. 2010;**153**:222–37
23. Nishida H, Handa Y, Tanaka S. *et al.* Expression of the CLE-RS3 gene suppresses root nodulation in *Lotus japonicus*. *J Plant Res*. 2016;**129**:909–19
24. Okamoto S, Ohnishi E, Sato S. *et al.* Nod factor/nitrate-induced CLE genes that drive HAR1-mediated systemic regulation of nodulation. *Plant Cell Physiol*. 2009;**50**:67–77
25. Soyano T, Hirakawa H, Sato S. *et al.* Nodule inception creates a long-distance negative feedback loop involved in homeostatic regulation of nodule organ production. *Proc Natl Acad Sci*. 2014;**111**:14607–12
26. Lim CW, Lee YW, Hwang CH. Soybean nodule-enhanced CLE peptides in roots act as signals in GmNARK-mediated nodulation suppression. *Plant Cell Physiol*. 2011;**52**:1613–27
27. Reid DE, Ferguson BJ, Gresshoff PM. Inoculation- and nitrate-induced CLE peptides of soybean control NARK-dependent nodule formation. *Mol Plant-Microbe Interact*. 2011;**24**:606–18
28. Ferguson BJ, Li D, Hastwell AH. *et al.* The soybean (*Glycine max*) nodulation-suppressive CLE peptide, GmRIC1, functions interspecifically in common white bean (*Phaseolus vulgaris*), but not in a supermodulating line mutated in the receptor PvNARK. *Plant Biotechnol J*. 2014;**12**:1085–97
29. Krusell L, Madsen LH, Sato S. *et al.* Shoot control of root development and nodulation is mediated by a receptor-like kinase. *Nature*. 2002;**420**:422–6
30. Schnabel E, Journet EP, de Carvalho-Niebel F. *et al.* The *Medicago truncatula* SUNN gene encodes a CLV1-like leucine-rich repeat receptor kinase that regulates nodule number and root length. *Plant Mol Biol*. 2005;**58**:809–22
31. Searle IR, Men AE, Laniya TS. *et al.* Long-distance signaling in nodulation directed by a CLAVATA1-like receptor kinase. *Science*. 2003;**299**:109–12
32. Gautrat P, Mortier V, Laffont C. *et al.* Unraveling new molecular players involved in the autoregulation of nodulation in *Medicago truncatula*. *J Exp Bot*. 2019;**70**:1407–17
33. Takahara M, Magori S, Soyano T. *et al.* Too much love, a novel Kelch repeat-containing F-box protein, functions in the long-distance regulation of the legume-rhizobium symbiosis. *Plant Cell Physiol*. 2013;**54**:433–47
34. Sasaki T, Suzaki T, Soyano T. *et al.* Shoot-derived cytokinins systemically regulate root nodulation. *Nat Commun*. 2014;**5**:4983
35. Imin N, Mohd-Radzman NA, Ogilvie HA. *et al.* The peptide-encoding CEP1 gene modulates lateral root and nodule numbers in *Medicago truncatula*. *J Exp Bot*. 2013;**64**:5395–409
36. Laffont C, Huault E, Gautrat P. *et al.* Independent regulation of symbiotic nodulation by the SUNN negative and CRA2 positive systemic pathways. *Plant Physiol*. 2019;**180**:559–70
37. Mohd-Radzman NA, Laffont C, Ivanovici A. *et al.* Different pathways act downstream of the CEP peptide receptor CRA2 to regulate lateral root and nodule development. *Plant Physiol*. 2016;**171**:2536–48
38. Luo Z, Moreau C, Wang J. *et al.* NLP1 binds the CEP1 signalling peptide promoter to repress its expression in response to nitrate. *New Phytol*. 2022;**234**:1547–52
39. Fu M, Yao X, Li X. *et al.* GmNLP1 and GmNLP4 activate nitrate-induced CLE peptides NIC1a/b to mediate nitrate-regulated root nodulation. *Plant J*. 2024;**119**:783–95
40. Zhou S, Zhang C, Huang Y. *et al.* Characteristics and research progress of legume nodule senescence. *Plan Theory*. 2021;**10**:1103
41. Van de Velde W, Guerra JCP, De Keyser A. *et al.* Aging in legume symbiosis. A molecular view on nodule senescence in *Medicago truncatula*. *Plant Physiol*. 2006;**141**:711–20
42. Escuredo PR, Minchin FR, Gogorcena Y. *et al.* Involvement of activated oxygen in nitrate-induced senescence of pea root nodules. *Plant Physiol*. 1996;**110**:1187–95
43. Du M, Gao Z, Li X. *et al.* Excess nitrate induces nodule greening and reduces transcript and protein expression levels of soybean leghaemoglobins. *Ann Bot*. 2020;**126**:61–72
44. Puppo A, Groten K, Bastian F. *et al.* Legume nodule senescence: roles for redox and hormone signalling in the orchestration of the natural aging process. *New Phytol*. 2005;**165**:683–701
45. Yang Z, Zheng J, Zhou H. *et al.* The soybean beta-expansin gene GmINS1 contributes to nodule development in response to phosphate starvation. *Physiol Plant*. 2021;**172**:2034–47
46. Fujikake H, Yamazaki A, Ohtake N. *et al.* Quick and reversible inhibition of soybean root nodule growth by nitrate involves a decrease in sucrose supply to nodules. *J Exp Bot*. 2003;**54**:1379–88
47. Wang L, Tian T, Liang J. *et al.* A transcription factor of the NAC family regulates nitrate-induced legume nodule senescence. *New Phytol*. 2023;**238**:2113–29
48. Wang X, Qiu Z, Zhu W. *et al.* The NAC transcription factors SNAP1/2/3/4 are central regulators mediating high nitrogen responses in mature nodules of soybean. *Nat Commun*. 2023;**14**:4711
49. Wang YY, Cheng YH, Chen KE. *et al.* Nitrate transport, signaling, and use efficiency. *Annu Rev Plant Biol*. 2018;**69**:85–122
50. Vidal EA, Alvarez JM, Araus V. *et al.* Nitrate in 2020: thirty years from transport to signaling networks. *Plant Cell*. 2020;**32**:2094–119
51. Dechorgnat J, Nguyen CT, Armengaud P. *et al.* From the soil to the seeds: the long journey of nitrate in plants. *J Exp Bot*. 2011;**62**:1349–59
52. Huang NC, Liu KH, Lo HJ. *et al.* Cloning and functional characterization of an Arabidopsis nitrate transporter gene that encodes a constitutive component of low-affinity uptake. *Plant Cell*. 1999;**11**:1381–92
53. Morere-Le Paven MC, Viau L, Hamon A. *et al.* Characterization of a dual-affinity nitrate transporter MtNRT1.3 in the model legume *Medicago truncatula*. *J Exp Bot*. 2011;**62**:5595–605
54. Liu KH, Huang CY, Tsay YF. CHL1 is a dual-affinity nitrate transporter of Arabidopsis involved in multiple phases of nitrate uptake. *Plant Cell*. 1999;**11**:865–74
55. Tsay YF, Schroeder JI, Feldmann KA. *et al.* The herbicide sensitivity gene CHL1 of Arabidopsis encodes a nitrate-inducible nitrate transporter. *Cell*. 1993;**72**:705–13
56. Parker JL, Newstead S. Molecular basis of nitrate uptake by the plant nitrate transporter NRT1.1. *Nature*. 2014;**507**:68–72

57. Sun J, Bankston JR, Payandeh J. et al. Crystal structure of the plant dual-affinity nitrate transporter NRT1.1. *Nature*. 2014;**507**:73–7
58. Teillet A, Garcia J, de Billy F. et al. Api, a novel *Medicago truncatula* symbiotic mutant impaired in nodule primordium invasion. *Mol Plant-Microbe Interact*. 2008;**21**:535–46
59. Yendrek CR, Lee YC, Morris V. et al. A putative transporter is essential for integrating nutrient and hormone signaling with lateral root growth and nodule development in *Medicago truncatula*. *Plant J*. 2010;**62**:100–12
60. Bagchi R, Salehin M, Adeyemo OS. et al. Functional assessment of the *Medicago truncatula* NIP/LATD protein demonstrates that it is a high-affinity nitrate transporter. *Plant Physiol*. 2012;**160**:906–16
61. Valkov VT, Rogato A, Alves LM. et al. The nitrate transporter family protein LjNPF8.6 controls the N-fixing nodule activity. *Plant Physiol*. 2017;**175**:1269–82
62. Xiao Q, Chen Y, Liu C-W. et al. MtNPF6.5 mediates chloride uptake and nitrate preference in *Medicago* roots. *EMBO J*. 2021;**40**:e106847
63. Wang Q, Huang Y, Ren Z. et al. Transfer cells mediate nitrate uptake to control root nodule symbiosis. *Nat Plants*. 2020;**6**:800–8
64. Vittozzi Y, Nadzieja M, Rogato A. et al. The *Lotus japonicus* NPF3.1 is a nodule-induced gene that plays a positive role in nodule functioning. *Front. Plant Sci*. 2021;**12**:688187
65. Misawa F, Ito M, Nosaki S. et al. Nitrate transport via NRT2.1 mediates NIN-LIKE PROTEIN-dependent suppression of root nodulation in *Lotus japonicus*. *Plant Cell*. 2022;**34**:1844–62
66. Luo Z, Wang J, Li F. et al. The small peptide CEP1 and the NIN-like protein NLP1 regulate NRT2.1 to mediate root nodule formation across nitrate concentrations. *Plant Cell*. 2023;**35**:776–94
67. Rogato A, D'Apuzzo E, Barbulova A. et al. Tissue-specific down-regulation of LjAMT1.1 compromises nodule function and enhances nodulation in *Lotus japonicus*. *Plant Mol Biol*. 2008;**68**:585–95
68. Rogato A, D'Apuzzo E, Barbulova A. et al. Characterization of a developmental root response caused by external ammonium supply in *Lotus japonicus*. *Plant Physiol*. 2010;**154**:784–95
69. D'Apuzzo E, Rogato A, Simon-Rosin U. et al. Characterization of three functional high-affinity ammonium transporters in *Lotus japonicus* with differential transcriptional regulation and spatial expression. *Plant Physiol*. 2004;**134**:1763–74
70. Qin L, Zhao J, Tian J. et al. The high-affinity phosphate transporter GmPT5 regulates phosphate transport to nodules and nodulation in soybean. *Plant Physiol*. 2012;**159**:1634–43
71. Chen L, Qin L, Zhou L. et al. A nodule-localized phosphate transporter GmPT7 plays an important role in enhancing symbiotic N₂ fixation and yield in soybean. *New Phytol*. 2019;**221**:2013–25
72. Liu J, Yang R, Yan J. et al. VPT-like genes modulate rhizobium-legume symbiosis and phosphorus adaptation. *Plant J*. 2023;**116**:112–27
73. Nguyen NNT, Clua J, Vetal PV. et al. PHO1 family members transport phosphate from infected nodule cells to bacteroids in *Medicago truncatula*. *Plant Physiol*. 2021;**185**:196–209
74. Liu JJ, Liu J, Cui M. et al. Investigate the effect of potassium on nodule Symbiosis and uncover an HAK/KUP/KT member, GmHAK5, strongly responsive to root nodulation in soybean. *J Plant Biol*. 2022;**65**:459–71
75. Desbrosses G, Kopka C, Ott T. et al. *Lotus japonicus* LjKUP is induced late during nodule development and encodes a potassium transporter of the plasma membrane. *Mol Plant-Microbe Interact*. 2004;**17**:789–97
76. Cao HR, Peng W-T, Nie M-M. et al. Carbon-nitrogen trading in symbiotic nodules depends on magnesium import. *Curr Biol*. 2022;**32**:4337–4349 e5
77. Krusell L, Krause K, Ott T. et al. The sulfate transporter SST1 is crucial for symbiotic nitrogen fixation in *Lotus japonicus* root nodules. *Plant Cell*. 2005;**17**:1625–36
78. Ma Y, Zhu W, Zhao W. et al. MtESN2 is a subgroup II sulphate transporter required for symbiotic nitrogen fixation and prevention of nodule early senescence in *Medicago truncatula*. *Plant Cell Environ*. 2023;**46**:3558–74
79. Brear EM, Bedon F, Gavrín A. et al. GmVTL1a is an iron transporter on the symbiosome membrane of soybean with an important role in nitrogen fixation. *New Phytol*. 2020;**228**:667–81
80. Liu S, Liao LL, Nie MM. et al. A VIT-like transporter facilitates iron transport into nodule symbiosomes for nitrogen fixation in soybean. *New Phytol*. 2020;**226**:1413–28
81. Escudero V, Abreu I, Tejada-Jiménez M. et al. *Medicago truncatula* Ferroportin2 mediates iron import into nodule symbiosomes. *New Phytol*. 2020;**228**:194–209
82. Suganuma N, Nakamura Y, Yamamoto M. et al. The *Lotus japonicus* Sen1 gene controls rhizobial differentiation into nitrogen-fixing bacteroids in nodules. *Mol Gen Genomics*. 2003;**269**:312–20
83. Walton JH, Kontra-Kováts G, Green RT. et al. The *Medicago truncatula* vacuolar iron transporter-like proteins VTL4 and VTL8 deliver iron to symbiotic bacteria at different stages of the infection process. *New Phytol*. 2020;**228**:651–66
84. Wu X, Wang Y, Ni Q. et al. GmYSL7 controls iron uptake, allocation, and cellular response of nodules in soybean. *J Integr Plant Biol*. 2023;**65**:167–87
85. Castro-Rodríguez R, Escudero V, Reguera M. et al. *Medicago truncatula* yellow stripe-Like7 encodes a peptide transporter participating in symbiotic nitrogen fixation. *Plant Cell Environ*. 2021;**44**:1908–20
86. Gavrín A, Loughlin PC, Brear E. et al. Soybean yellow stripe-like 7 is a symbiosome membrane peptide transporter important for nitrogen fixation. *Plant Physiol*. 2021;**186**:581–98
87. Castro-Rodríguez R, Abreu I, Reguera M. et al. The *Medicago truncatula* yellow Stripe1-Like3 gene is involved in vascular delivery of transition metals to root nodules. *J Exp Bot*. 2020;**71**:7257–69
88. Kaiser BN, Moreau S, Castelli J. et al. The soybean NRAMP homologue, GmDMT1, is a symbiotic divalent metal transporter capable of ferrous iron transport. *Plant J*. 2003;**35**:295–304
89. Tejada-Jimenez M, Castro-Rodríguez R, Kryvoruchko I. et al. *Medicago truncatula* natural resistance-associated macrophage protein1 is required for iron uptake by rhizobia-infected nodule cells. *Plant Physiol*. 2015;**168**:258–72
90. Gil-Diez P, Tejada-Jiménez M, León-Mediavilla J. et al. MtMOT1.2 is responsible for molybdate supply to *Medicago truncatula* nodules. *Plant Cell Environ*. 2019;**42**:310–20
91. Tejada-Jimenez M, Gil-Diez P, León-Mediavilla J. et al. *Medicago truncatula* molybdate transporter type 1 (MtMOT1.3) is a plasma membrane molybdenum transporter required for nitrogenase activity in root nodules under molybdenum deficiency. *New Phytol*. 2017;**216**:1223–35
92. Moreau S, Thomson RM, Kaiser BN. et al. GmZIP1 encodes a symbiosis-specific zinc transporter in soybean. *J Biol Chem*. 2002;**277**:4738–46
93. Abreu I, Saéz Á, Castro-Rodríguez R. et al. *Medicago truncatula* zinc-iron Permease6 provides zinc to rhizobia-infected nodule cells. *Plant Cell Environ*. 2017;**40**:2706–19

94. Leon-Mediavilla J, Senovilla M, Montiel J. et al. MtMTP2-facilitated zinc transport into intracellular compartments is essential for nodule development in *Medicago truncatula*. *Front Plant Sci.* 2018;**9**:990
95. Senovilla M, Castro-Rodríguez R, Abreu I. et al. *Medicago truncatula* copper transporter 1 (MtCOPT1) delivers copper for symbiotic nitrogen fixation. *New Phytol.* 2018;**218**:696–709
96. Takanashi K, Yokosho K, Saeki K. et al. LjMATE1: a citrate transporter responsible for iron supply to the nodule infection zone of *Lotus japonicus*. *Plant Cell Physiol.* 2013;**54**:585–94
97. Kryvoruchko IS, Routray P, Sinharoy S. et al. An iron-activated citrate transporter, MtMATE67, is required for symbiotic nitrogen fixation. *Plant Physiol.* 2018;**176**:2315–29
98. Frungillo L. Getting to the root of nodulation: how legumes and rhizobia use nitrate uptake to control symbiosis. *Plant Cell.* 2022;**34**:1443–4
99. Temple SJ, Vance CP, Gantt JS. Glutamate synthase and nitrogen assimilation. *Trends Plant Sci.* 1998;**3**:0–56
100. Smith PM, Atkins CA. Purine biosynthesis. Big in cell division, even bigger in nitrogen assimilation. *Plant Physiol.* 2002;**128**:793–802
101. Cordoba E, Shishkova S, Vance CP. et al. Antisense inhibition of NADH glutamate synthase impairs carbon/nitrogen assimilation in nodules of alfalfa (*Medicago sativa* L.). *Plant J.* 2003;**33**:1037–49
102. Coletto I, Trenas AT, Erban A. et al. Functional specialization of one copy of glutamine phosphoribosyl pyrophosphate amidotransferase in ureide production from symbiotically fixed nitrogen in *Phaseolus vulgaris*. *Plant Cell Environ.* 2016;**39**:1767–79
103. Weaver CD, Shomer NH, Louis CF. et al. Nodulin 26, a nodule-specific symbiosome membrane protein from soybean, is an ion channel. *J Biol Chem.* 1994;**269**:17858–62
104. Miao GH, Verma DP. Soybean nodulin-26 gene encoding a channel protein is expressed only in the infected cells of nodules and is regulated differently in roots of homologous and heterologous plants. *Plant Cell.* 1993;**5**:781–94
105. Fortin M, Morrison N, Verma D. Nodulin-26, a peribacteroid membrane nodulin is expressed independently of the development of the peribacteroid compartment. *Nucleic Acids Res.* 1987;**15**:813–24
106. Masalkar P, Wallace IS, Hwang JH. et al. Interaction of cytosolic glutamine synthetase of soybean root nodules with the C-terminal domain of the symbiosome membrane nodulin 26 aquaglyceroporin. *J Biol Chem.* 2010;**285**:23880–8
107. Collier R, Tegeder M. Soybean ureide transporters play a critical role in nodule development, function and nitrogen export. *Plant J.* 2012;**72**:355–67
108. Carter AM, Tegeder M. Increasing nitrogen fixation and seed development in soybean requires complex adjustments of nodule nitrogen metabolism and partitioning processes. *Curr Biol.* 2016;**26**:2044–51
109. Yang SY, Lin WY, Hsiao YM. et al. Milestones in understanding transport, sensing, and signaling of the plant nutrient phosphorus. *Plant Cell.* 2024;**36**:1504–23
110. Raghothama KG. Phosphate acquisition. *Annu Rev Plant Physiol Plant Mol Biol.* 1999;**50**:665–93
111. Sa TM, Israel DW. Energy status and functioning of phosphorus-deficient soybean nodules. *Plant Physiol.* 1991;**97**:928–35
112. Dokwal D, Romsdahl TB, Kunz DA. et al. Phosphorus deprivation affects composition and spatial distribution of membrane lipids in legume nodules. *Plant Physiol.* 2021;**185**:1847–59
113. Tang C, Hinsinger P, Drevon JJ. et al. Phosphorus deficiency impairs early nodule functioning and enhances proton release in roots of *medicago truncatula* L. *Ann Bot.* 2001;**88**:131–8
114. Isidra-Arellano MC, Pozas-Rodríguez EA, del Rocío Reyero-Saavedra M. et al. Inhibition of legume nodulation by pi deficiency is dependent on the autoregulation of nodulation (AON) pathway. *Plant J.* 2020;**103**:1125–39
115. Chmelikova L, Hejcman M. Effect of nitrogen, phosphorus and potassium availability on emergence, nodulation and growth of *Trifolium medium* L. in alkaline soil. *Plant Biol.* 2014;**16**:717–25
116. Sulieman S, Tran LS. Phosphorus homeostasis in legume nodules as an adaptive strategy to phosphorus deficiency. *Plant Sci.* 2015;**239**:36–43
117. Gu M, Chen A, Sun S. et al. Complex regulation of plant phosphate transporters and the gap between molecular mechanisms and practical application: what is missing? *Mol Plant.* 2015;**9**:396–416
118. Nussaume L, Kanno S, Javot H. et al. Phosphate import in plants: focus on the PHT1 transporters. *Front Plant Sci.* 2011;**2**:83
119. Zhu WL, Yang LF, Yang SP. et al. Overexpression of rice phosphate transporter gene *OsPT2* enhances nitrogen fixation and ammonium assimilation in transgenic soybean under phosphorus deficiency. *J Plant Biol.* 2016;**59**:172–81
120. Rubio V, Linhares F, Solano R. et al. A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae. *Genes Dev.* 2001;**15**:2122–33
121. Gonzalez E, Solano R, Rubio V. et al. Phosphate transporter traffic Facilitator1 is a plant-specific SEC12-related protein that enables the endoplasmic reticulum exit of a high-affinity phosphate transporter in *Arabidopsis*. *Plant Cell.* 2005;**17**:3500–12
122. Lu M, Cheng Z, Zhang XM. et al. Spatial divergence of *PHR-PHT1* modules maintains phosphorus homeostasis in soybean nodules. *Plant Physiol.* 2020;**184**:236–50
123. Xing X, Du H, Yang Z. et al. *GmSPX8*, a nodule-localized regulator confers nodule development and nitrogen fixation under phosphorus starvation in soybean. *BMC Plant Biol.* 2022;**22**:161
124. Zhuang Q, Xue Y, Yao Z. et al. Phosphate starvation responsive *GmSPX5* mediates nodule growth through interaction with *GmNF-YC4* in soybean (*Glycine max*). *Plant J.* 2021;**108**:1422–38
125. Kanai S, Ohkura K, Adu-Gyamfi JJ. et al. Depression of sink activity precedes the inhibition of biomass production in tomato plants subjected to potassium deficiency stress. *J Exp Bot.* 2007;**58**:2917–28
126. Karnik R, Waghmare S, Zhang B. et al. Commandeering channel voltage sensors for secretion, cell turgor, and volume control. *Trends Plant Sci.* 2017;**22**:81–95
127. Ragel P, Raddatz N, Leidi EO. et al. Regulation of K⁺ nutrition in plants. *Front Plant Sci.* 2019;**10**:281
128. Fedorova EE, Coba de la Peña T, Lara-Dampier V. et al. Potassium content diminishes in infected cells of *Medicago truncatula* nodules due to the mislocation of channels MtAKT1 and MtSKOR/GORK. *J Exp Bot.* 2021;**72**:1336–48
129. Mengel K, Haghparast M, Koch K. The effect of potassium on the fixation of molecular nitrogen by root nodules of *Vicia faba*. *Plant Physiol.* 1974;**54**:535–8
130. Cakmak I, Hengeler C, Marschner H. Partitioning of shoot and root dry-matter and carbohydrates in bean-plants suffering from phosphorus, potassium and magnesium-deficiency. *J Exp Bot.* 1994;**45**:1245–50
131. White PJ, Broadley MR. Calcium in plants. *Ann Bot.* 2003;**92**:487–511

132. Capoen W, Sun J, Wysham D. et al. Nuclear membranes control symbiotic calcium signaling of legumes. *Proc Natl Acad Sci*. 2011;**108**:14348–53
133. Ehrhardt DW, Wais R, Long SR. Calcium spiking in plant root hairs responding to rhizobium nodulation signals. *Cell*. 1996;**85**:673–81
134. Sieberer BJ, Chabaud M, Timmers AC. et al. A nuclear-targetedameleon demonstrates intranuclear Ca²⁺ spiking in *Medicago truncatula* root hairs in response to rhizobial nodulation factors. *Plant Physiol*. 2009;**151**:1197–206
135. Watkin ELJ, O'Hara GW, Glenn AR. Calcium and acid stress interact to affect the growth of rhizobium *leguminosarum biovar trifolii*. *Soil Biol Biochem*. 1997;**29**:1427–232
136. Reeve WG, Tiwari RP, Dilworth MJ. et al. Calcium affects the growth and survival of rhizobium-meliloti. *Soil Biol Biochem*. 1993;**25**:581–6
137. Redondo-nieto M, Wilmot AR, El-Hamdaoui A. et al. Relationship between boron and calcium in the N₂-fixing legume-rhizobia symbiosis. *Plant Cell Environ*. 2003;**26**:1905–15
138. D'haeze W, Holsters M. Nod factor structures, responses, and perception during initiation of nodule development. *Glycobiology*. 2002;**12**:79R–105R
139. Kanamori N, Madsen LH, Radutoiu S. et al. A nucleoporin is required for induction of Ca²⁺ spiking in legume nodule development and essential for rhizobial and fungal symbiosis. *Proc Natl Acad Sci*. 2006;**103**:359–64
140. Andreev IM, Dubrovo PN, Krylova VV. et al. Functional identification of ATP-driven Ca²⁺ pump in the peribacteroid membrane of broad bean root nodules. *FEBS Lett*. 1999;**447**:49–52
141. Krylova V, Andreev IM, Zartdinova R. et al. Biochemical characteristics of the Ca²⁺ pumping ATPase in the peribacteroid membrane from broad bean root nodules. *Protoplasma*. 2013;**250**:531–8
142. Kopriva S, Malagoli M, Takahashi H. Sulfur nutrition: impacts on plant development, metabolism, and stress responses. *J Exp Bot*. 2019;**70**:4069–73
143. Li Q, Gao Y, Yang A. Sulfur homeostasis in plants. *Int J Mol Sci*. 2020;**21**:8926
144. Anderson AJ, Spencer D. Sulphur in nitrogen metabolism of legumes and non-legumes. *Aust J Sci Res B*. 1950;**3**:431–49
145. Varin S, Cliquet JB, Personeni E. et al. How does Sulphur availability modify N acquisition of white clover (*Trifolium repens* L.)? *J Exp Bot*. 2010;**61**:225–34
146. Ohki Y, Munakata K, Matsuoka Y. et al. Nitrogen reduction by the Fe sites of synthetic [Mo₃S₄Fe] cubes. *Nature*. 2022;**607**:86–90
147. Rodriguez-Haas B, Finney L, Vogt S. et al. Iron distribution through the developmental stages of *Medicago truncatula* nodules. *Metallomics*. 2013;**5**:1247–53
148. O'Hara GW, Dilworth MJ, Boonkerd N. et al. Iron-deficiency specifically limits nodule development in peanut inoculated with *Bradyrhizobium* sp. *New Phytol*. 1988;**108**:51–7
149. Brear EM, Day DA, Smith PM. Iron: an essential micronutrient for the legume-rhizobium symbiosis. *Front Plant Sci*. 2013;**4**:359
150. Gonzalez-Guerrero M, Matthiadis A, Saez A. et al. Fixating on metals: new insights into the role of metals in nodulation and symbiotic nitrogen fixation. *Front Plant Sci*. 2014;**5**:45
151. Tang C, Robson AD, Dilworth MJ. The role of iron in nodulation and nitrogen fixation in *Lupinus angustifolius* L. *New Phytol*. 2006;**114**:173–82
152. Slatni T, Krouma A, Aydi S. et al. Growth, nitrogen fixation and ammonium assimilation in common bean (*Phaseolus vulgaris* L) subjected to iron deficiency. *Plant Soil*. 2007;**312**:49–57
153. Buren S, Jimenez-Vicente E, Echavarri-Erasun C. et al. Biosynthesis of nitrogenase cofactors. *Chem Rev*. 2020;**120**:4921–68
154. Terry RE, Soerensen KU, Jolley V. et al. The role of active *Bradyrhizobium japonicum* in iron stress response of soybeans. *Plant Soil*. 1991;**130**:225–30
155. Day DA, Smith PMC. Iron transport across symbiotic membranes of nitrogen-fixing legumes. *Int J Mol Sci*. 2021;**22**:432
156. Chen ZC, Peng WT, Li J. et al. Functional dissection and transport mechanism of magnesium in plants. *Semin Cell Dev Biol*. 2018;**74**:142–52
157. Li J, Yokosho K, Liu S. et al. Diel magnesium fluctuations in chloroplasts contribute to photosynthesis in rice. *Nat Plants*. 2020;**6**:848–59
158. Seefeldt LC, Hoffman BM, Dean DR. Mechanism of Mo-dependent nitrogenase. *Annu Rev Biochem*. 2009;**78**:701–22
159. Peng WT, Zhang LD, Zhou Z. et al. Magnesium promotes root nodulation through facilitation of carbohydrate allocation in soybean. *Physiol Plant*. 2018;**163**:372–85
160. Kaiser BN, Gridley KL, Ngaire Brady J. et al. The role of molybdenum in agricultural plant production. *Ann Bot*. 2005;**96**:745–54
161. Hille R, Nishino T, Bittner F. Molybdenum enzymes in higher organisms. *Coord Chem Rev*. 2011;**255**:1179–205
162. Mendel RR, Hansch R. Molybdoenzymes and molybdenum cofactor in plants. *J Exp Bot*. 2002;**53**:1689–98
163. Delgado MJ, Tresierra-Ayala A, Talbi C. et al. Functional characterization of the *Bradyrhizobium japonicum modA* and *modB* genes involved in molybdenum transport. *Microbiology*. 2006;**152**:199–207
164. Tejada-Jimenez M, Chamizo-Ampudia A, Galvan A. et al. Molybdenum metabolism in plants. *Metallomics*. 2013;**5**:1191–203
165. Liu Y, Khan AR, Gan Y. C2H2 zinc finger proteins response to abiotic stress in plants. *Int J Mol Sci*. 2022;**23**:2730
166. Klug A. The discovery of zinc fingers and their applications in gene regulation and genome manipulation. *Annu Rev Biochem*. 2010;**79**:213–31
167. Hakoyama T, Niimi K, Watanabe H. et al. Host plant genome overcomes the lack of a bacterial gene for symbiotic nitrogen fixation. *Nature*. 2009;**462**:514–7
168. Rubio MC, James EK, Clemente MR. et al. Localization of superoxide dismutases and hydrogen peroxide in legume root nodules. *Mol Plant-Microbe Interact*. 2004;**17**:1294–305
169. Lin J, Bjørk PK, Kolte MV. et al. Zinc mediates control of nitrogen fixation via transcription factor filamentation. *Nature*. 2024;**631**:164–9
170. Andresen E, Peiter E, Kupper H. Trace metal metabolism in plants. *J Exp Bot*. 2018;**69**:909–54
171. Soupene E, Foussard M, Boistard P. et al. Oxygen as a key developmental regulator of *rhizobium meliloti* N₂-fixation gene expression within the alfalfa root nodule. *Proc Natl Acad Sci*. 1995;**92**:3759–63
172. Preisig O, Zufferey R, Hennecke H. The *Bradyrhizobium japonicum fixGHIS* genes are required for the formation of the high-affinity *cbb3*-type cytochrome oxidase. *Arch Microbiol*. 1996;**165**:297–305
173. Hawkins FKL, Kennedy C, Johnston AWB. A *rhizobium leguminosarum* gene required for symbiotic nitrogen fixation, melanin synthesis and normal growth on certain growth media. *Microbiology*. 1991;**137**:1721–8
174. Navarro-Gomez C, León-Mediavilla J, Küpper H. et al. Nodule-specific Cu⁺-chaperone NCC1 is required for symbiotic nitrogen fixation in *Medicago truncatula* root nodules. *New Phytol*. 2024;**241**:793–810

175. Schulze J, Temple G, Temple SJ. *et al.* Nitrogen fixation by white lupin under phosphorus deficiency. *Ann Bot.* 2006;**98**:731–40
176. Ferguson BJ, Lin MH, Gresshoff PM. Regulation of legume nodulation by acidic growth conditions. *Plant Signal Behav.* 2013;**8**:e23426
177. Glenn AR, Dilworth MJ. The life of root nodule bacteria in the acidic underground. *FEMS Microbiol Lett.* 1994;**123**:1–9
178. Graham PH. Stress tolerance In *rhizobium* and *Bradyrhizobium*, and nodulation under adverse soil-conditions. *Can J Microbiol.* 1992;**38**:475–84
179. Vassileva V, Milanov G, Ignatov G. *et al.* Effect of low pH on nitrogen fixation of common bean grown at various calcium and nitrate levels. *J Plant Nutr.* 1997;**20**:279–94
180. Unkovich MJ, Sanford P, Pate JS. Nodulation and nitrogen fixation by subterranean clover in acid soils as influenced by lime application, toxic aluminium, soil mineral N, and competition from annual ryegrass. *Soil Biol Biochem.* 1996;**28**:639–48
181. Hernandez JA, George SJ, Rubio LM. Molybdenum trafficking for nitrogen fixation. *Biochemistry.* 2009;**48**:9711–21
182. O'Hara GW. Nutritional constraints on root nodule bacteria affecting symbiotic nitrogen fixation: a review. *Aust J Exp Agr.* 2001;**41**:417–33
183. O'Hara GW, Boonkerd N, Dilworth MJ. Mineral constraints to nitrogen fixation. *Plant Soil.* 1988;**108**:93–110
184. Nishida H, Suzuki T. Nitrate-mediated control of root nodule symbiosis. *Curr Opin Plant Biol.* 2018;**44**:129–36
185. Morère-Le Paven MC, Clochard T, Limami AM. NPF and NRT2 from *pisum sativum* potentially involved in nodule functioning: lessons from *medicago truncatula* and *lotus japonicus*. *Plants (Basel).* 2024;**13**:322