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SPECIAL ISSUE REVIEW

# Symbiotic Nodulation Enhances Legume Tolerance to Abiotic Stresses: Mechanisms and Perspectives

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## ABSTRACT

Abiotic stresses, such as drought, salinity, heavy metal contamination and cold, pose significant challenges to global agriculture, reducing crop productivity and threatening food security. Legume-rhizobium symbiosis not only facilitates biological nitrogen fixation but also improves plant tolerance to abiotic stresses. Nodulated leguminous plants exhibit better growth and improved productivity under abiotic stress conditions. In this review, we highlight recent advances in understanding how symbiotic nodulation mitigates abiotic stresses, focusing on physiological and biochemical responses, as well as molecular pathways. We then discuss future research directions to optimise rhizobial applications for stress-tolerant and climate-adaptive farming systems. Rhizobial inoculation is presented as a promising, sustainable and eco-friendly strategy for mitigating abiotic stresses, offering significant potential for stressed agricultural systems.

## 1 | Introduction

Global agriculture is facing increasing challenges due to adverse environmental conditions, particularly abiotic stresses such as drought, salinity and heavy metal contamination. These stresses have been further exacerbated by climate change, leading to increasingly severe growing environments and significant yield reductions (Datta et al. 2024). As the global population continues to grow, ensuring stable crop production under these unfavourable conditions has become a critical priority for sustainable agriculture.

Leguminous plants are vital in global food systems, serving as essential sources of protein, oil and forage for human nutrition and livestock feed. Beyond their economic and nutritional significance, legumes possess a unique ability to establish symbiotic relationships with a wide range of Gram-negative α- and β-Proteobacteria, collectively known as rhizobia, resulting in the formation of nitrogen-fixing root nodules. Symbiotic nitrogen fixation (SNF) through this relationship reduces the reliance on chemical fertilisers, contributes to nitrogen enrichment of the soil, and supports sustainable agricultural systems (Abd-Alla et al. 2023).

The formation of root nodules is a complex and highly regulated programme involving several developmental processes and multiple exchanges of signals between legumes and rhizobia. While nodulation primarily facilitates biological nitrogen fixation (BNF), recent research highlights its broader role in enhancing legume tolerance to abiotic stresses. Nodulated plants exhibit improved physiological responses under drought, salinity and heavy metal stress through enhanced water-use efficiency, better nutrient acquisition, reduced oxidative damage and altered gene expression. These adaptations not only

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ensure plant survival but also contribute to maintaining productivity under adverse conditions.

In this review, we focus on the effects of rhizobial nodulation on legume tolerance to abiotic stresses. We discuss the biochemical and physiological changes associated with nodulation, as well as the latest insights into the physiological and molecular mechanisms by which nodulation improves plant tolerance in stressful environments. By highlighting the potential of the legume-rhizobia symbiosis, we aim to provide insights into its application for developing stress-tolerant and sustainable agricultural systems.

## 2 | The Role of Symbiotic Nodulation in Abiotic Stress

Abiotic stress refers to adverse environmental factors, such as salinity, drought, extreme temperatures and metal stress, arising from natural phenomena or human-induced changes like climate change and unsustainable agricultural practices. These stresses pose significant challenges to global agriculture, as they not only reduce the amount of arable land available for cultivation but also diminish crop yields and threaten food security. In plants, abiotic stress disrupts vital physiological and metabolic processes, leading to stunted growth, reduced productivity, and even crop failure under severe conditions (Datta et al. 2024). Abiotic stresses are interconnected with one another and often influence each other, forming a chain of adverse effects. For instance, climatic variability such as changes in rainfall or temperature can lead to drought stress, which exacerbates salinity stress. Salinity stress contributes to soil alkalisation, reducing nutrient availability and causing nutrient stress (Sindhu et al. 2020).

For legumes, the impact of abiotic stress is particularly critical because they play a dual role in agriculture: providing essential sources of protein, oil and forage, while contributing to soil fertility through symbiotic nitrogen fixation. However, these stresses impair the growth and productivity of legumes, thereby limiting their ecological and economic contributions. Addressing these challenges requires a comprehensive understanding of how legumes respond and adapt to stress conditions. Nodulation, a key feature of legume-rhizobia symbiosis, has emerged as a vital process that enhances legume resilience to diverse abiotic stresses. In the following sections, we will review how nodulation specifically contributes to improving legume resilience to different abiotic stresses.

### 2.1 | Symbiotic Nodulation Enhances Salt Tolerance in Legumes

Salt stress, primarily caused by soil salinization, is a significant abiotic factor limiting global agricultural productivity. Soil salinization results from natural processes such as seawater intrusion and anthropogenic activities like improper irrigation and deforestation. Approximately 20% of the world's cultivated land are affected by salinity, with these numbers expected to rise due to climate change and unsustainable land management practices (Zhang et al. 2024a). Salt stress adversely impacts agricultural systems by reducing cultivated land and crop yields, thereby posing a serious threat to global food security. At the physiological level, high salt concentrations in soil create osmotic stress, hindering water uptake and causing cellular dehydration. Excessive sodium ($Na^+$) and chloride ($Cl^-$) ions accumulate in plant tissues, leading to ion toxicity, membrane instability and oxidative stress. These effects disrupt essential physiological processes such as photosynthesis, respiration and nutrient assimilation, ultimately stunting plant growth and significantly reducing productivity. To cope with salt stress, plants have evolved various mechanisms, including ion homoeostasis, osmotic adjustment and enhanced antioxidant defence systems to mitigate oxidative damage (Ma et al. 2023). Morphological and anatomical adaptations, such as changes in root structure and the development of salt-excreting glands, further contribute to salt tolerance. Additionally, leguminous plants can establish symbiotic relationships with rhizobial strains and develop root nodules to mitigate the adverse effects of salt stress (Figure 1).

Under salt stress conditions, nodulated Faba bean (*Vicia faba* L.), wild soybean (*Glycine soja*) and soybean (*Glycine max*) exhibited superior growth and productivity compared to non-nodulated plants (Nour El-Din et al. 2011; Yang et al. 2023). Symbiotic nodulation in alfalfa (*Medicago sativa* L.) and *Medicago truncatula* could effectively enhanced antioxidant system and concentrations of compatible solutes such as proline, soluble sugars and proteins, and consequently improve the salt tolerance (Wang et al. 2016; Irshad et al. 2021a). Faba bean (*Vicia faba* L.) with a higher number of nodules accumulated greater levels of soluble assimilates and nutrients, exhibited elevated enzymatic antioxidant activity, and maintained ionic homoeostasis by enhancing potassium ($K^+$) uptake while reducing sodium ($Na^+$) accumulation. These physiological changes alleviated the damage induced by salt stress and ultimately improved the yield attributes (Hafez et al. 2021). Moreover, inoculation with rhizobium improved the chlorophyll content, photosynthetic rate, water use efficiency, and phosphorus and protein concentration of mung bean (*Vigna radiata* L.) during salt stress. Additionally, it decreased $Na^+$ levels while increasing $K^+$ content, ensuring ionic balance under saline conditions (Ahmad et al. 2013).

Chakraborty et al. (2021) conducted transcriptome analysis of *Medicago truncatula* roots and found that symbiotic nodulation could downregulate one-third of salt-responsive gene expressions, restoring to control levels. Transcriptome sequencing of bacteroids in common bean nodules identified 1069 differentially expressed genes under salt stress, mainly involving in the transport of mineral ions, synthesis and transport of osmotolerance molecules, as well as the production of GSH and γ-aminobutyric acid (GABA). These findings suggest that bacteroids may facilitate mineral iron mobilisation, enhance osmotolerance, and supply GSH and GABA to host plants, thereby improving the salt tolerance of nodulated plants (Wekesa et al. 2022). Wang et al. (2022a; 2022b) conducted proteomic analysis on alfalfa shoots and roots, respectively. Plants with active nodules (NA) showed differential expression of proteins related to cell wall modification, antioxidant ability, protein sythesis and degradation, and stress responds compared to plants without nodules (NN) under NaCl treatment (Wang



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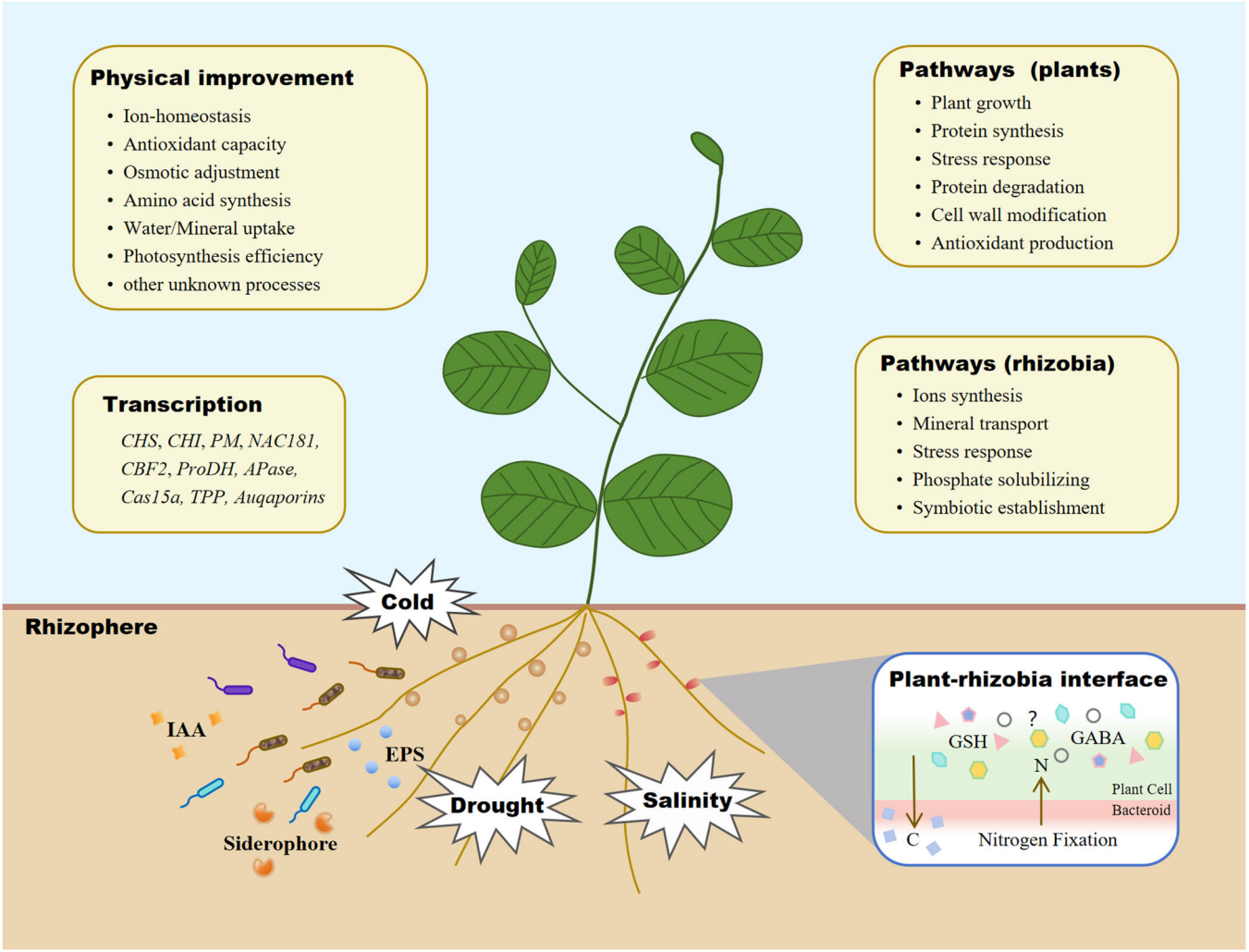



**FIGURE 1** | The Mechanisms of symbiotic nodulation improving legume tolerance to abiotic stress. Symbiotic nodulation enhances plant tolerance to abiotic stress through a variety of mechanisms, which includes the modulation of antioxidant defence systems, promotion of osmotic adjustment, and regulation of ion homoeostasis. Additionally, nodulation improves photosynthetic efficiency and optimises water and mineral uptake. The expression of genes/proteins involved in cell wall modification, protein synthesis and degradation, as well as the regulation of stress-responsive and plant growth-related genes/proteins, is significantly altered during nodulation-mediated stress tolerance. The secretion of rhizobial-derived compounds such as IAA, EPS and siderophores into the rhizosphere, along with increased phosphate solubility driven by rhizobial activity, further contribute to the enhancement of plant stress resilience.

et al. 2022a; Wang et al. 2022b). Additionally, Soybean (*Glycine max*) plants inoculated with rhizobium showed increased shoot biomass and grain yield during salt stress, and the proteomic analysis revealed that proteins involved in plant growth and stress response were upregulated (Ilangumaran et al. 2021; Ilangumaran et al. 2022).

Flavonoid compounds are essential to the communication between symbiotic partners and plant stress response. *Sinorhizobium meliloti* inoculation in soybean seedlings could significantly increase salt tolerance by upregulating the key genes related to flavonoids metabolism, including *chalcone synthase* (*CHS*), *chalcone isomerase* (*CHI*) and *cytochrome P450 monooxygenase* (*CPM*) (Qu et al. 2016). The process of symbiotic nodulation between legumes and rhizobial strains is quite sensitive to salt stress. Wang et al. (2022c) found that the overexpression of salt responsive gene *GmNAC181* significantly increased soybean root nodule numbers under salt stress by activating expression of *GmNINa*, a central gene fuction in nodulation, suggesting that *GmNAC181* could mitigate the negative effects of salt stress on nodulation. Acid phosphatase (APase) and Trehalose 6-phosphate phosphatase (TPP) play an essential role in plant salt tolerance by sustaining inorganic phosphate levels and stabilising dehydrated enzymes, membranes and biological structures. Rhizobium inoculation in common bean enhanced salt tolerance through modulating nodule APase and TPP activities. Interestingly, common bean plants could acquire diffenrent levels of salt tolerance when inoculated with diffenrent rhizobium strains (Faghire et al. 2013). Nodulated *Phaseolus vulgaris* plants showed improved water status compared to non-nodulated plants under salt stress conditions. The rhizobial symbiosis increased osmotic root water flow and the expression levels of PIP (plasma membrane intrinsic protein) aquaporins, while decreasing sodium ($Na^+$) content in the roots (Franzini et al. 2019).



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## 2.2 | Symbiotic Nodulation Enhances Drought Tolerance in Legumes

Drought stress, caused by insufficient water availability and exacerbated by climate change and unsustainable agricultural practices, is a major threat to global food security. Drought affects 45% of the world's agricultural land, with increasing severity projected due to the growing frequency and intensity of extreme weather events (Abdelraheem et al. 2019). In agriculture, drought stress reduces crop yields, deteriorates soil quality, and accelerates land degradation, posing significant challenges to farming systems. Drought stress disrupts key physiological processes in plants, such as photosynthesis, respiration and nutrient uptake. It causes stomatal closure, which reduces water loss but limits $CO_2$ assimilation, thereby lowering photosynthetic efficiency. Prolonged drought leads to osmotic stress, resulting in cellular dehydration, loss of turgor pressure and impaired growth. Moreover, drought stress induces the accumulation of reactive oxygen species (ROS), which cause oxidative damage to lipids, proteins and nucleic acids, further undermining cellular integrity. Plants employ several strategies to cope with drought stress, including stomatal closure to minimise water loss and root elongation to access deeper water reserves (Zhang et al. 2024b). Additionally, they accumulate compatible solutes to stabilise cellular structures and maintain turgor pressure, and activate antioxidant defence systems to mitigate oxidative damage. Phytohormones like abscisic acid (ABA) also play a crucial role in drought adaptation. In addition to these adaptive mechanisms, the formation of symbiotic nodules with rhizobia provides legumes with an added advantage in coping with drought stress (Figure 1).

Zhang et al. (2013) demonstrated that nodulated alfalfa plants exhibited higher superoxide dismutase (SOD) and peroxidase (POD) activities, along with increased accumulation of glutathione (GSH) and osmotic solutes such as proline and soluble sugars during drought stress, enhancing both reactive oxygen defence capability and osmotic adjustment ability. Nodulated pea (*Pisum sativum* L.) and faba bean plants showed improved drought tolerance, associated with greater chlorophyll content, increased photosynthetic rate and antioxidant enzyme activities compared to non-nodulated plants (Frechilla et al. 2000; Li et al. 2023). Nodulation in black locust (*Robinia pseudoacacia* L.) improved photosynthesis and reduce the content of malondialdehyde (MDA) and hydrogen peroxide, and thus increased drought tolerance (Yuan et al. 2022). In *Medicago truncatula*, nodulated plants exhibited a significant delay in drought-induced leaf senescence, maintaining higher potassium ($K^+$) concentrations, and osmolytes, such as proline (Staudinger et al. 2016). Liu et al. (2022a) found that symbiotic nodulation could significantly increase the synthesis of drought responsive amino acids, including proline, arginine, alanine and glutamate, and consequently increase survival rate of Chinese milk vetch (*Astragalus sinicus* L.) under drought stress. Additionally, the level of drought tolerance is also related to the efficiency of symbiotic nodulation. The study in common bean demonstrated that plants with a greater number of nodules accumulated more plant height and shoot dry matter when subjected to drought stress conditions, indicating stronger drought tolerance (Figueiredo et al. 2008).

Nodulation in pea and vetch (*Vicia sativa*) plants signifficantly increased survival rates and seed productions under drought stress by improving plant water status and osmotic regulation, and reducing the damage of membrane lipid peroxidation. Subsequently, they conducted transcriptomic analysis on both pea and vetch leaves and found that differentially expressed genes were mainly involved in nitrogen metabolism and drought stress response, including abscisic acid (ABA) signalling, stomatal closure and redox balance mechanisms (Álvarez-Aragón et al. 2023). Proteomic analysis on *Medicago truncatula* revealed the upregulation of proteins involved in photosynthesis, hormonal signalling and translational regulation, which help maintain leaf function and delay senescence during drought stress (Staudinger et al. 2016; Staudinger et al. 2012). Meanwhile, metabolomic profiling indicated the increased accumulation of osmolytes, such as soluble sugars and proline, contributing to osmotic adjustment and improved drought stress tolerance (Staudinger et al. 2012). Igiehon et al. (2019) explored genomic insights into rhizobial strains that enhanced soybean seed germination under drought stress and identified genes involved in symbiotic establishment, exopolysaccharide (EPS) production, indole-acetic-acid (IAA) production, siderophore production and stress response.

## 2.3 | Symbiotic Nodulation Enhances Cold Tolerance in Legumes

Cold stress, which includes chilling stress (between 0°C and 15°C) and freezing stress (below 0°C), has a significant negative impact on crop yield and geographical distribution (Larran et al. 2023). This is particularly problematic in tropical and subtropical regions, where plants are more sensitive to low temperatures. The frequency and severity of cold stress have increased due to the rising occurrence of extreme weather events caused by global climate change. Chilling stress reduces cell membrane fluidity and impairs enzyme activity, while freezing stress causes ice crystal formation in intercellular spaces, leading to cell dehydration and structural damage. Cold stress disrupts essential plant processes such as photosynthesis, respiration and the uptake of water and mineral nutrient. It often triggers oxidative stress through excessive production of ROS, resulting in damages to lipids, proteins and DNA. Plants have evolved several adaptive mechanisms to cope with cold stress. They accumulate osmoprotectants to maintain cellular osmotic balance and stabilise proteins. Additionally, the activation of antioxidant defence systems plays a crucial role in mitigating ROS-induced damage (Vicente et al. 2016). Beyond these intrinsic mechanisms, plants can also benefit from interactions with rhizobia through symbiotic nodulation. While much of the research to date has focused on drought and salt stress, emerging evidence suggests that symbiotic nodulation also plays a vital role in enhancing host plant tolerance to cold stress (Figure 1).

Studies on nodulated alfalfa revealed significantly higher activities of antioxidant enzymes, such as SOD, CAT and POD, which mitigate oxidative damage caused by ROS. Nodulated plants also accumulated osmoprotectants, including proline, soluble proteins and soluble sugars, which play crucial roles in maintaining osmotic balance and stabilising cellular structures



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under cold stress (Li et al. 2016; Liu et al. 2019a). In alfalfa and *Medicago truncatula*, inoculation with rhizobia improved photosynthesis, antioxidant capacity, osmotic regulation ability and nutrient uptake (N, P, K), which collectively contributed to higher tolerance to cold stress (Liu et al. 2018; Irshad et al. 2021b). Lentil (*Lens culinaris* L.) plants with more active nodules showed stronger cold tolerance, as evidenced by greater dry weight accumulation. This was associated with increased chlorophyll content, improved uptake of nutrients (N, P), and higher levels of physiologically available iron (Mishra et al. 2011).

Megu et al. (2024) isolated low-temperature tolerant rhizobial strains from wild legumes and found that these strains possess the ability of IAA production and phosphate solubilizing, indicating their potential roles to improve cold stress tolerance of the host plants. In nodulated alfalfa plants, the expression of regulatory and metabolism associated genes, such as *CBF2*, *Cas15a* and *ProDH*, was altered, leading to the accumulation of soluble proteins and sugars. These molecular changes provide a robust foundation for nodulated alfalfa to cope with low-temperature environments (Liu et al. 2019a).

### 2.4 | Symbiotic Nodulation Enhances Heavy Metal Tolerance in Legumes

Heavy metal stress, caused by toxic elements such as cadmium (Cd), arsenic (As) and copper (Cu), poses significant challenges to plant growth and agriculture systems. These metals enter ecosystems through natural processes, such as volcanic activity and rock weathering, as well as human activities, including mining, industrial emissions and excessive fertiliser use. Unlike essential micronutrients, many heavy metals have no biological function and are toxic even at low concentrations, posing long-term risks as they accumulate in soils. Heavy metal-contaminated soils limit plant growth and introduce toxic elements into the food chain, thereby endangering human and environmental health. Heavy metal stress interferes with key physiological and metabolic processes in plants, such as photosynthesis, respiration and nutrient uptake, often resulting in stunted growth, leaf chlorosis, and reduced reproductive capacity. Heavy metals also induce excessive ROS production, leading to oxidative damage to membranes, proteins, and DNA (Mansoor et al. 2023). To mitigate heavy metal stress, plants employ various strategies, such as chelation and sequestration of metals in vacuoles to minimise cytoplasmic toxicity. Antioxidant systems play a crucial role in scanving ROS and preventing oxidative damage. Additionally, osmotic solutes stabilise cellular structures and maintain osmotic balance under stress conditions (Ghori et al. 2019). Symbiotic nodulation has also emerged as a promising strategy to enhance the tolerance of leguminous plants to heavy metal stress (Figure 2).

Fan et al. (2018) isolated a Cu-tolerant *Mesorhizobium loti* strain HZ76 from a mining area, which exhibited plant growth-promoting (PGP) traits, including IAA production, siderophore production and 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity. HZ76 inoculation on *Robinia pseudoacacia* seedlings significantly improved plant tolerance to Cu stress. When exposed to Cu stress, nodulated *Medicago lupulina* plants showed improved growth, associated with the enhanced activities of antioxidant enzymes, such as SOD, ascorbate peroxidase (APX) and glutathione reductase (GR) (Kong et al. 2015). Similar results were observed in alfalfa plants, where rhizobial inoculation alleviated Cu-induced damage by increasing antioxidant enzyme activities and the content of chlorophyll and soluble protein, as well as decreasing MDA content (Jian et al. 2019; Duan et al. 2022). *Sinorhizobium meliloti* CCNWSX0020, a Cu-tolerant strain isolated from *Medicago lupulina* root nodules, could effectively improve *Medicago lupulina* growth under Cu stress (Fan et al. 2011). Li et al. (2014) further identified three genes from this *S. meliloti* strain, including *lpxXL*, *merR* and *omp*, which were responsible for Cu stress tolerance by creating a random insertion library. Moreover, inoculation with the same strain, *S. meliloti* CCNWSX0020, significantly enhanced Cu tolerance of alfalfa plants by regulating the expression of genes related to antioxidant responses, phytochelatin (PC) biosynthesis and metallothionein biosynthesis (Chen et al. 2018).

Symbiotic nodulation also positively affects plant tolerance to As stress. It is reported that rhizobial inoculation increased biomass in soybean plants by 38% under As stress compared to non-nodulated controls (Reichman 2007). Another study further revealed that the growth promotion of the nodulated soybean plants under As stress was attributed to the increased concentrations of siderophores and auxins in the rhizosphere (Seraj et al. 2020). Ye et al. (2021) further investigated the molecular mechanisms by which Rhizobium-*Medicago truncatula* symbiosis reduces As accumulation under As stress conditions. The symbiosis converts atmospheric $N_2$ into ammonium, which enhances nitrogen levels in shoots to mitigate As accumulation. Additionally, ammonium inhibits the biosynthesis of ABA and linalool in roots, leading to reduced expression of *NRT3.1-like* genes, which in turn limit the uptake and accumulation of As in plants.

The study in *Medicago sativa* demonstrated that compared with non-inoculated plants, *Sinorhizobium meliloti* nodulation dramatically mitigated the chlorosis and growth reduction effect caused by Cd. The improved nodulated plant performance under Cd stress is likely attributed to the promoted accumulation of nutrients, like Mg, Ca and K in the shoot (Ghnaya et al. 2015). Transcriptomics and transcriptome-wide $m^6A$ methylation analysis results on soybean indicated that RNA methylation in root nodules plays a crucial role in Cd and Pb resistance mediated by nodulation. The overexpression of ammonium transporter gene *GmAMT1;1*, which was identified as differentially methylated genes (DMG) and differentially expressed genes (DEG) in the analysis could significantly enhance soybean nodule tolerance to Pb and Cd stress (Ma et al. 2024). Dual-transcriptomics analysis conducted in *Medicago truncatula* revealed that plants might detoxify mercury (Hg) via targeted detoxification mechanisms, while Cd responses involve diverse genotype-specific regulatory mechanisms. The retention and compartmentalisation of toxic metals in nodules of legumes facilitated by genetically adapted heavy metal tolerant rhizobia strains might need to be systemically investigated in the future (Sharma et al. 2025).

## 3 | Conclusions and Perspectives

The legume-rhizobium symbiosis not only facilitates nitrogen fixation but also plays a critical role in enhancing plant



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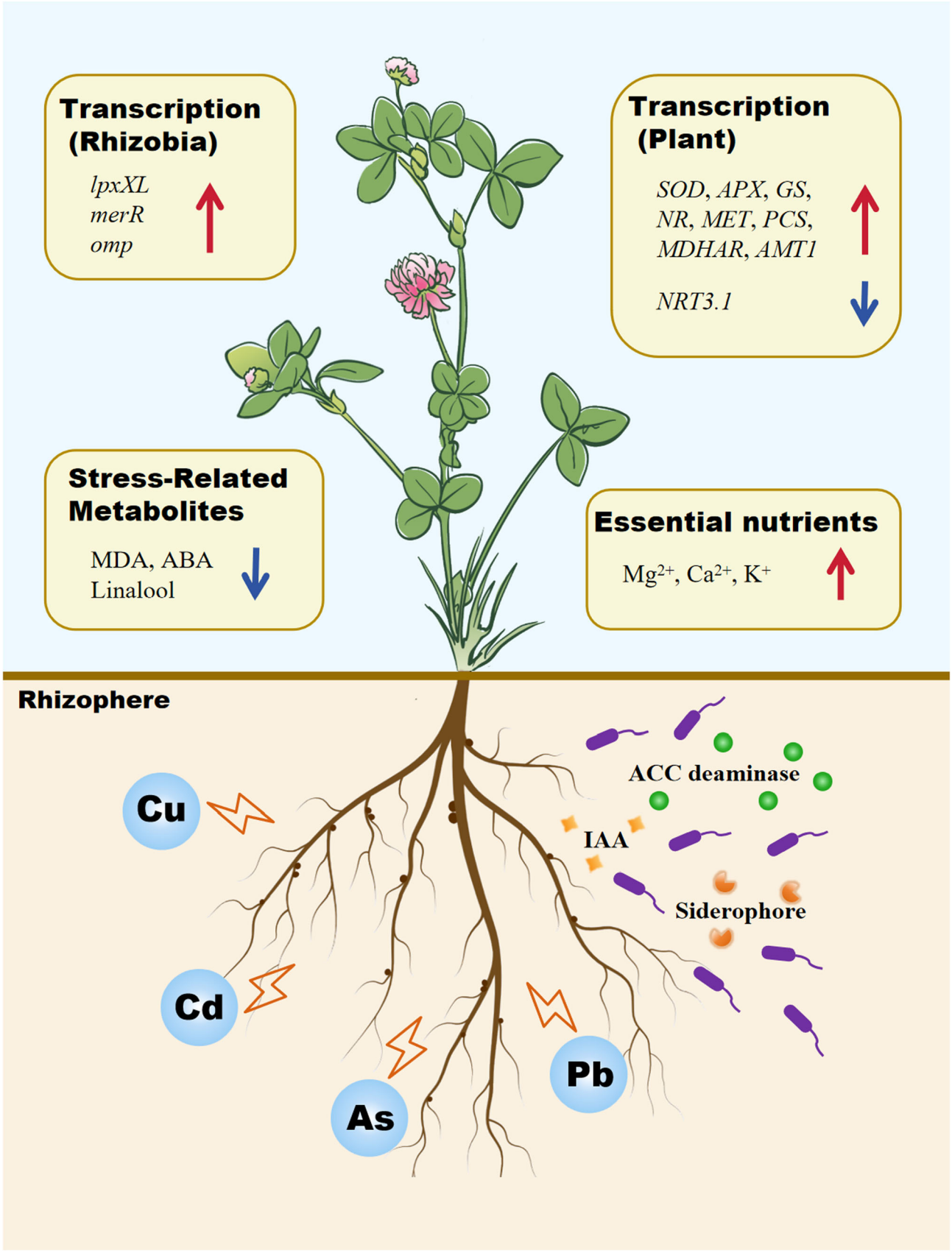



**FIGURE 2** | Legend on next page.



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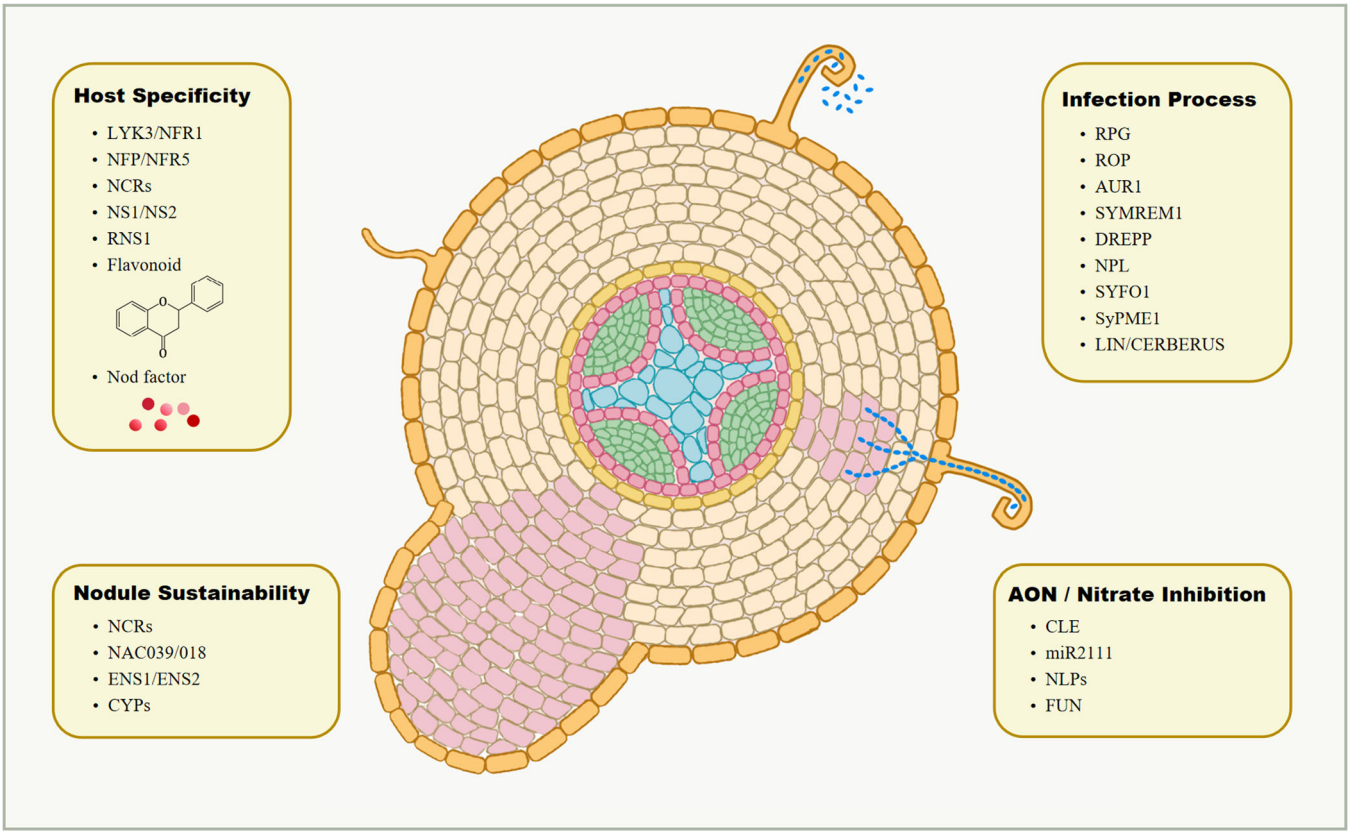



**FIGURE 3** | Potential modification targets to enhance nodulation. Host specificity of rhizobia is determinated by a fine-tuned exchange of molecular signals and can affect nodulation efficiency and performance dramatically. Genetic engineering targeting the genes responsible for recognition and infection may substantially enhance nodulation. Small peptides that involove in AON pathway are proved to be effective in nodulation optimisation. NLPs and transcription factor FUN can improve nodulation efficiency by mitigating the inhibitory effects of rhizosphere nitrate on root nodule development and function. The sustainability of nodulation may be further improved through the action of NCR peptides and genes associated with nodule senescence. Metal nutrients, such as zinc, iron and molybdenum also play important roles in nodulation enhancement. The figure was created with Biorender (https://www.biorender.com/). [Color figure can be viewed at wileyonlinelibrary.com]

tolerance under adverse environmental conditions. Nodulated plants exhibited better growth and increased productivity under abiotic stresses through physiological, biochemical and molecular responses. Rhizobial inoculation represents a promising and eco-friendly solution for mitigating abiotic stresses in agriculture. Unlike chemical treatments, which often have negative environmental and economic consequences, rhizobial inoculation is cost-effective and fits well with sustainable farming principles. In regions where soils are heavily affected by salinity, drought, or heavy metal contamination, rhizobia-based biofertilizers have the potential to significantly improve crop productivity while remaining friendly to the environment.

Despite these promising applications, symbiotic nodulation is the unique capacity of leguminous plants, which limits the use of rhizobia biofertilizer in improving stress tolerance in non-leguminous plants. Additionally, the efficiency of nodulation and nitrogen fixation varies significantly among different rhizobium-plant pairs, leading to considerable differences in levels of abiotic stress tolerance. Therefore, besides exploring the molecular mechanisms underlying how nodulation improves plant tolerance to abiotic stresses, future research should also focus on enhancing the efficiency of nodulation or nitrogen fixation. To do so, there are four potential paths: via the selection of rhizobia strains/species that hold broader compatibility with legume plants; via the increase of the infection efficiency; via the enhancement of the nodulation capacity; via the improvement of the sustainability of symbiosis (Figure 3).

The compatibility between symbiotic partners is determined by both sides. A complex recognition mechanism has evolved to aid the formation of optimal match between rhizobia and the

**FIGURE 2** | Enhancement of heavy metal tolerance in legume plants through nodulation. Genes associated with antioxidant responses, as well as the biosynthesis of phytochelatins and metallothioneins, play a role in nodulation-mediated tolerance to heavy metals. Ammonium transporter (AMT1) acts as positive regulator of heavy metal tolerance, whereas the downregulation of nitrate transporters can reduce heavy metal accumulation. Nodulation also promotes the uptake and transport of essential nutrients to the shoot, while suppressing the accumulation of MDA, ABA and linalool, thereby improving plant performance under heavy metal stress. Additionally, the secretion of IAA, siderophores, and the increased activity of ACC deaminase in the rhizosphere further contribute to the enhancement of plant tolerance to heavy metals. [Color figure can be viewed at wileyonlinelibrary.com]



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host legume. This mechanism helps achieve an efficient symbiosis and reduce the risk of collateral damage caused by incompatible infection. Selection or creation of suitable rhizobial partner might enhance nitrogen fixation and resistance to stress of the host plant at the same time. Host range of rhizobia could be manipulated via the engineering of the flavonoid sensor NodD and the nodulation (Nod) factor biosynthesis enzymes from the rhizobia side and Nod factor receptor on the legume host side. Over-expression of the NodD2 gene from *Rhizobium tropici* CIAT 899 was proved to be able to promote symbiosis and extend rhizobial nodulation range through constitutive stimulation of Nod factor production (Ayala-García et al. 2022). Besides Nod Factors, rhizobial surface polysaccharides and secreted proteins/type I/III secretion system (T1SS/T3SS) also contribute to the determination of symbiotic specificity (Walker et al. 2020; Liu et al. 2022b). So far, our understanding of how host legume control the range of symbiotic partnership remains poorly understood. Nodule specific Cysteine-Rich (NCR) peptides secreted by the IRLC clade legumes were demonstrated to play a role in symbiont discrimination (Wang et al. 2017; Yang et al. 2017). High-throughput functional genomics and large scale genome manipulation facilitated by the development of leading-edge techniques might help us understand better the nodulation host-range specificity (diCenzo et al. 2019). Comparative genomic and transcriptomic analyses have shed lights on expanding the host range of rhizobia to legume and even non-legume crops in nitrogen-fixing and stress resistance enhancement (Yuan et al. 2024).

Nodulation is established on successful infection and delivery of rhizobia to the inner layers of cortical cells on host plant roots. Thanks to the breakthroughs made mainly in the past 10 years in forward genetics, genes including *symbiotic remorin 1* (*SYMREM1*), *symbiotic formin 1* (*SYFO1*), *developmentally regulated plasma membrane polypeptide* (*DREPP*), *symbiosis-specific pectin methyl esterases 1* (*SyPME1*), *nodulation pectate lyase* (*NPL*), *rhizobium-directed polar growth* (*RPG*), *lumpy infections* (*LIN*, also known as *CERBERUS*), *Rho of plants* (*ROP*), *Aurora kinase 1* (*AUR1*) were reported to govern the attachment of rhizobia to root hair, endocytotic uptake of rhizobia into root hair cell, delivery and release of rhizobia to the cortical cells (Liang et al. 2018; Liang et al. 2021; Su et al. 2020; Su et al. 2023; Liu et al. 2019b; Lace et al. 2023; Gao et al. 2021; Gao et al. 2022). Manipulation of these genes holds the ability to increase rhizobia infection efficiency and the formation of more nodule primordia on legume plants.

Nodulation is controlled by a complex internal control circuit, called the autoregulation of nodulation (AON). In brief, perception of rhizobial lipochitooligosaccharides (Nod factors) by legume Nod factor receptor triggers the cortical and pericycle cell divisions to form nodule primordia, leading to the travel of CLAVATA3-like (CLE) peptides to the shoot via xylem; host plants could coordinate the demand of nitrogen and the serviceability of photosynthesis product through the production of systemic nodulation suppressing signals in responding to CLE; thus, a balanced nodule development is achieved. It was demonstrated that signalling transduction disorder during this process could lead to non-nodulation, or supernodulation, meaning nodulation capacity manipulation is theoretically achievable. Nodulation optimisation targeting to the CLE peptide genes in soybean has been shown to be able to increase yield and protein content (Zhong et al. 2024), demonstrating that genetically coordination of nodulation is a practical strategy in increasing yield, quality and stress-tolerance of leguminous crops. Nodulation can be also enhanced by releasing the suppression effect of rhizosphere nitrate on root nodule development and function. NIN-like protein (NLP) transcription factors play central role in regulating legume response to soil nitrate and bZIP protein fixation under nitrate (FUN) has been demonstrated to participate in the adaptation of legume to high nitrate condition through a zinc-dependent filamentation mechanism (Lin et al. 2018; Lin et al. 2024). In addition to zinc, iron delivery and concentration can also affect the response of host legume nodulation to inorganic nitrogen in the soil (Zhou et al. 2024).

Due to the lack of suitable mutant materials and high complexity, study on the mechanism of how symbiotic nitrogen fixation process is sustained and prolonged remains limited. NCR peptides were reported to govern the terminal differentiation of the nitrogen-fixing bacteroids inside nodule cells (Van de Velde et al. 2010; Wang et al. 2010). Recent study demonstrated that NCR peptides also play essential roles in the maintenance of viability of terminally differentiated bacteroids. Abolishments of *NCR086*, *NCR169*, *NCR211*, *NCR314*, *NCR343* and *NCR-new35* all lead to early senescence of root nodule, and loss of function in the *NCR* gene transcription factor AT-Hook Motif Nuclear Localised 1 (AHL1) can significantly impair nodule development (Saifi et al. 2024; Horváth et al. 2015; Kim et al. 2015; Horváth et al. 2023; Zhang et al. 2023). AP2 family transcription factor Ethylene-responsive transcription factors required for Nodule Senescence 1 (ENS1) and ENS2 can be induced by ethylene and trigger root nodule senescence by interacting with NAC039/018 to stimulate the expression of cysteine protease genes, such as *CYP35/37/39/45* (Xiao et al. 2024; Yu et al. 2023). Suppression of ethylene induced nodule senescence and taking advantage of NCR peptides may sustain root nodule function and might contribute to the enhancement of symbiotic nitrogen fixation and host plant resistance to stress conditions conferred by root nodules.

Furthermore, recent studies suggest that the legume-rhizobia symbiotic signalling pathway also contributes to the mitigation of abiotic stresses. Key signalling molecules such as NLPs, nodulation signalling pathway 1 (NSP1) and NSP2, have been demonstrated to modulate plant tolerance to drought, salinity, cold and vanadium (V) toxicity (Ma et al. 2025; Yi et al. 2022; Ding et al. 2022; Liu et al. 2024). However, the roles of other molecules within this pathway in regulating plant tolerance to abiotic stresses remain to be elucidated. Exploring their multiple functions represents a promising direction for future research and uncovers novel mechanisms for improving crop resilience under adverse environmental conditions.

In conclusion, the legume-rhizobium symbiosis offers a sustainable and environmentally friendly approach to improve plant tolerance to abiotic stresses. In addition to exploring the mechanisms that regulate these processes, future research should also focus on improving nodulation and nitrogen fixation efficiency, as well as exploring the potential function of the symbiotic signalling pathway in the mitigation of abiotic stresses, all of which are crucial for addressing the challenges of



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climate change and resource limitations in global agriculture. Rhizobial inoculation, therefore, represents not only a tool for improving agricultural productivity, but a cornerstone of sustainable, climate-resilient farming systems.


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### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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