

Molecular Mechanisms of Nitrogen-Fixing Symbiosis in Fabaceae

Weiguo Lu, Qishan Chen ✉

Modern Agricultural Research Center, Cuixi Academy of Biotechnology, Zhuji, 311800, Zhejiang, China

✉ Corresponding email: qishan.chen@cuixi.org

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Abstract Nitrogen-fixing symbiosis in Fabaceae, facilitated by the interaction between legumes and rhizobia bacteria, plays a crucial role in sustainable agriculture by enhancing soil fertility and reducing the need for synthetic fertilizers. This study aims to provide a comprehensive overview of the molecular mechanisms underlying this symbiotic relationship. The study explores the genetic, biochemical, and physiological processes involved in the establishment and maintenance of nitrogen-fixing nodules. Key signaling pathways, gene expression profiles, and regulatory networks are discussed, highlighting the roles of nodulation factors, receptor kinases, and transcription factors. Furthermore, the study examines the latest advancements in molecular techniques and their applications in enhancing symbiotic efficiency and nitrogen fixation. Understanding these mechanisms is vital for developing strategies to improve crop productivity and environmental sustainability.

Keywords Nitrogen-fixing symbiosis; Fabaceae; Rhizobia; Nodulation factors; Gene expression profiles

1 Introduction

Nitrogen is a critical nutrient for plant growth, yet it is often a limiting factor in many ecosystems. Biological nitrogen fixation (BNF) is a process that converts atmospheric nitrogen (N₂) into ammonia, a form that plants can readily absorb and utilize. This process is primarily facilitated by nitrogenase enzymes found in certain bacteria, which can either live freely in the soil or form symbiotic relationships with plants (Dixon and Kahn, 2004). Among the plant families, Fabaceae (legumes) are particularly notable for their ability to engage in symbiotic nitrogen fixation with rhizobia bacteria. This symbiosis not only enhances the nitrogen content in the soil, promoting plant growth and soil fertility, but also reduces the need for synthetic nitrogen fertilizers, thereby contributing to sustainable agricultural practices (Liu et al., 2018; Aasfar et al., 2020).

The symbiotic relationship between legumes and rhizobia is a complex and highly regulated process. It begins with the infection of legume roots by rhizobia, leading to the formation of specialized structures called root nodules. Within these nodules, rhizobia differentiate into bacteroids, the form in which they fix nitrogen (Lodwig et al., 2003; Liu et al., 2018). The nitrogenase enzyme in bacteroids catalyzes the reduction of atmospheric nitrogen to ammonia, which is then assimilated by the plant. This process is energy-intensive and requires a continuous supply of carbon compounds from the plant to the bacteroids (Bellenger et al., 2020; Aasfar et al., 2021). Additionally, the symbiotic nitrogen fixation process is tightly regulated by both the host plant and the bacteria, involving intricate genetic and signaling interactions to ensure efficiency and responsiveness to environmental conditions (Dixon and Kahn, 2004; Berger et al., 2020).

This study aims to elucidate the molecular mechanisms underlying nitrogen-fixing symbiosis in Fabaceae. The specific objectives are to examine the genetic and biochemical pathways involved in the formation and functioning of root nodules, investigate the regulatory networks that control nitrogen fixation and their responses to environmental cues, and explore the evolutionary aspects of nitrogen-fixing symbiosis in Fabaceae, including the phylogenetic relationships and divergence times of key species. Additionally, the study will assess the potential applications of this knowledge in improving agricultural practices and promoting sustainable crop production. By synthesizing current research findings, this study seeks to provide a comprehensive understanding

of the molecular basis of nitrogen-fixing symbiosis in Fabaceae, highlighting its significance for both ecological and agricultural systems.

2 Historical Perspective and Evolution of Nitrogen-Fixing Symbiosis

2.1 Evolutionary origins of nitrogen fixation in Fabaceae

The evolutionary origins of nitrogen fixation in Fabaceae are deeply rooted in the plant's ability to form symbiotic relationships with nitrogen-fixing bacteria. This capability is believed to have evolved in response to nitrogen-limited environments that existed after the advent of phototrophy but before the widespread presence of oxygen in the atmosphere. The Fabaceae family, which includes legumes, has developed a highly specialized symbiotic relationship with rhizobia, a group of soil bacteria capable of fixing atmospheric nitrogen. This relationship is facilitated by the formation of root nodules, specialized structures that house the nitrogen-fixing bacteria (Liu et al., 2018). Phylogenomic studies have revealed that the ability to form these symbiotic relationships likely evolved from ancient gene duplication events that predate the origin of nodulation (Rutten et al., 2020). These events provided the genetic foundation necessary for the development of the complex signaling pathways and structural adaptations required for effective nitrogen fixation (Svistoonoff et al., 2013).

2.2 Historical advancements in the study of nitrogen fixation

The study of nitrogen fixation has seen significant advancements over the years, particularly with the advent of molecular and genomic technologies. Early research focused on the morphological and physiological aspects of nitrogen-fixing symbioses, but recent studies have delved into the genetic and molecular mechanisms underlying these interactions. For instance, the sequencing of plant genomes has provided insights into the genetic basis of nitrogen-fixing symbiosis and revealed the presence of conserved gene families involved in this process. Additionally, the discovery of multiple independent losses of nitrogen-fixing symbiosis in various plant lineages has highlighted the evolutionary fragility and complexity of this trait (Griesmann et al., 2018). Advances in model legume species, such as *Medicago truncatula*, have also uncovered the genetic components that allow rhizobia to establish chronic intracellular infections without causing harm to the host plant. These findings have paved the way for a deeper understanding of the co-evolutionary dynamics between plants and their symbiotic partners.

2.3 Comparative analysis of nitrogen-fixing and non-nitrogen-fixing Fabaceae

Comparative analyses between nitrogen-fixing and non-nitrogen-fixing Fabaceae have revealed significant differences in their genetic and physiological traits. Nitrogen-fixing species possess a suite of genes that are essential for the establishment and maintenance of symbiotic relationships with rhizobia. These genes are often conserved across different nitrogen-fixing species, indicating a common evolutionary origin (Battenberg et al., 2018). In contrast, non-nitrogen-fixing species lack these critical genes, and their genomes often show traces of lost pathways that could have supported nitrogen-fixing symbiosis. This suggests that the ability to fix nitrogen has been lost multiple times throughout the evolutionary history of Fabaceae, likely due to adverse selection pressures (Griesmann et al., 2018). Furthermore, the presence of specialized structures such as root nodules in nitrogen-fixing species contrasts with the absence of such structures in non-nitrogen-fixing species, highlighting the significant morphological adaptations associated with nitrogen fixation (Liu et al., 2018). These comparative studies underscore the evolutionary and ecological significance of nitrogen-fixing symbiosis in Fabaceae and provide valuable insights into the genetic and functional diversity within this plant family.

3 Molecular Mechanisms of Symbiotic Nitrogen Fixation

3.1 Signal exchange between host plants and rhizobium

The initiation of symbiotic nitrogen fixation in Fabaceae involves a sophisticated exchange of chemical signals between the host plant and rhizobium bacteria. Plant roots secrete flavonoids, which act as signaling molecules to activate the expression of nodulation (*nod*) genes in rhizobium. These genes are responsible for the synthesis of lipochitooligosaccharides known as Nod factors (NFs) (Lepetit and Brouquisse, 2023). Nod factors are crucial for inducing root hair deformation, cortical cell division, and the initiation of nodule organogenesis at very low concentrations. The structural specificity of Nod factors, determined by various substitutions on their

lipochitooligosaccharide core, is essential for the recognition and activity, indicating a co-evolutionary relationship between the symbiotic partners (Figure 1) (Zhao et al., 2021).

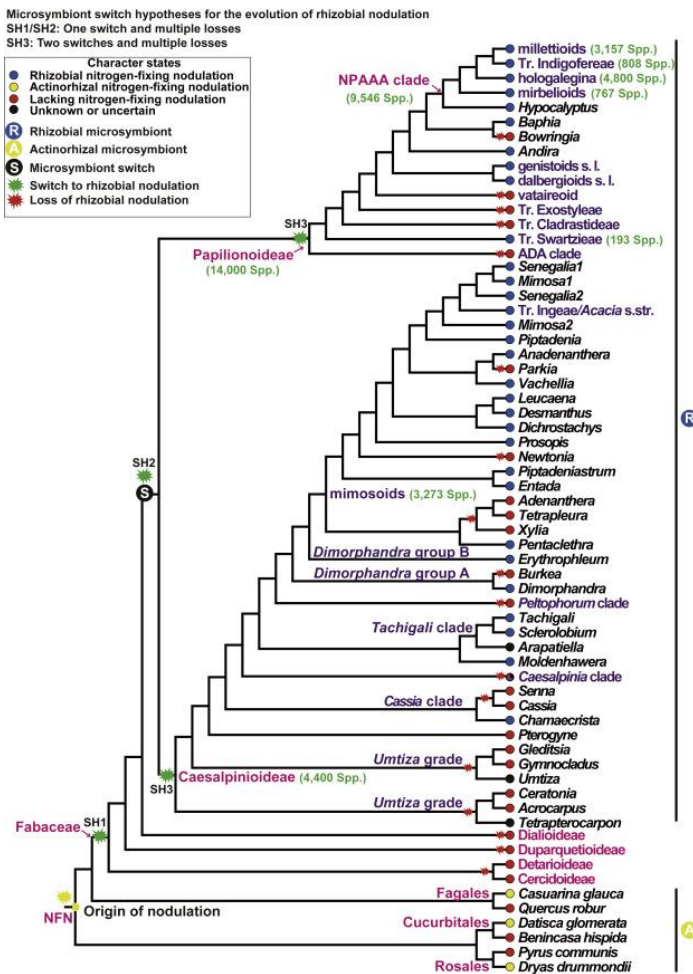


Figure 1 An overview of proposed evolutionary histories of the rhizobial nitrogen-fixing symbiosis in Fabaceae (Adopted from Zhao et al., 2021)

Image caption: This summary tree is a simplified version with tips collapsed as noted in the tip names. Three slightly different hypotheses for switch(es) from actinorhizal to rhizobial nodulation with subsequent multiple losses are marked as SH1, SH2, and SH3 next to the affected nodes. NFN indicates the nitrogen-fixing clade. The origin of rhizobial nodulation for the nitrogen-fixing clade and multiple losses of rhizobial nodulation are marked with green and red asterisks, respectively (Adopted from Zhao et al., 2021)

Upon receiving flavonoid signals from the plant, rhizobium bacteria respond by producing Nod factors, which are recognized by specific receptors on the plant root cells. This recognition triggers a cascade of events, including polarized root-hair tip growth and invagination associated with bacterial infection. The plant enforces strict control over the infection process, ensuring that only compatible rhizobium strains can successfully initiate symbiosis. This control extends to the enforcement of terminal differentiation of bacteria within nodules, which is crucial for effective nitrogen fixation (Bellés-Sancho et al., 2022).

3.2 Nodule formation and development

The early stages of nodule formation involve significant architectural changes in the plant root, such as root hair morphogenesis, cortical cell enlargement, and vascular patterning. These changes are driven by the recognition of Nod factors, leading to the formation of infection threads that guide the bacteria into the root cortex. The specificity and timing of these early events are critical for successful symbiosis, with the plant exerting control over the process to ensure compatibility and efficiency (Figure 2) (Bellés-Sancho et al., 2022; Lepetit and Brouquisse, 2023).

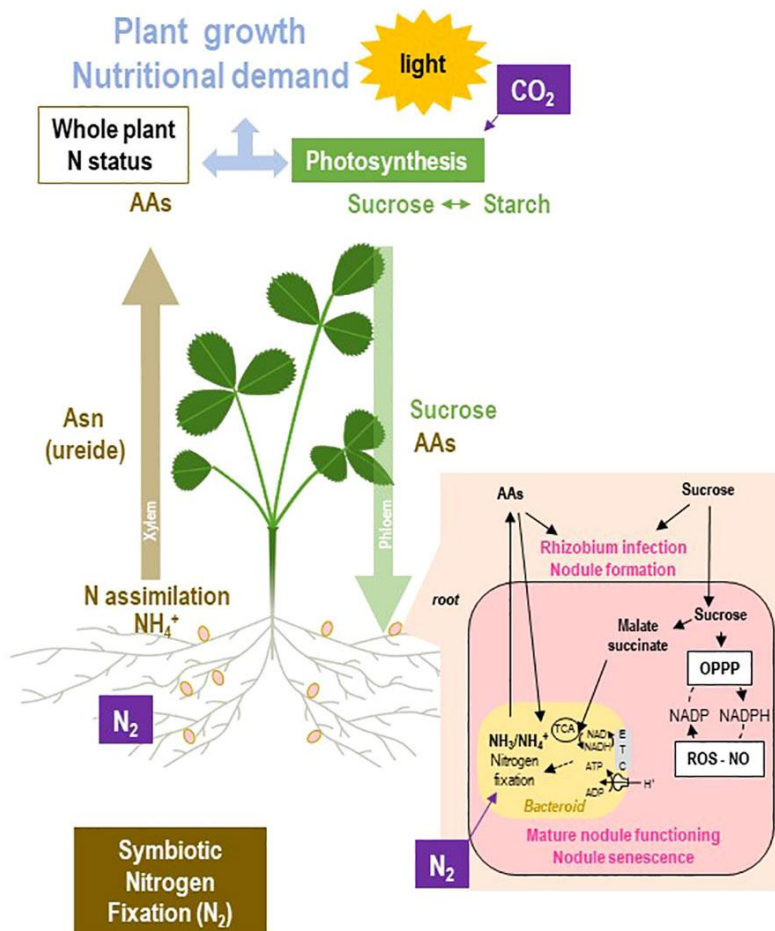


Figure 2 Overview of the N and C fluxes within the rhizobium-legume holobiont using symbiotic nitrogen fixation as the sole source of nitrogen (Adopted from Lepetit and Brouquisse, 2023)

Image caption: Atmospheric N_2 is fixed into NH_3/NH_4^+ by nitrogenase in the bacteroids of the root nodule. The resulting NH_4^+ is delivered to the plant cells where it is assimilated into amino acids (AAs). In temperate legumes, asparagine (Asn) is the main form of transport of nitrogen from the root to the shoot through the xylem flux, whereas in some tropical legumes, this transport involves ureides (Adapted from Lepetit and Brouquisse, 2023)

Nodule organogenesis is a complex process that involves the differentiation of root cortical cells into a new organ capable of housing nitrogen-fixing bacteria. This process is tightly regulated by both plant and bacterial signals, ensuring the formation of a functional nodule. The plant's systemic signaling mechanisms, which integrate nutritional demands and environmental conditions, play a crucial role in regulating nodule formation and development. These mechanisms ensure that nodule formation is synchronized with the plant's overall nutritional status and photosynthetic capacity (Oldroyd et al., 2011).

3.3 Nitrogenase enzyme complex

The nitrogenase enzyme complex is responsible for the reduction of atmospheric nitrogen (N_2) to ammonia (NH_3), a form that can be assimilated by the plant. This complex consists of two main components: the dinitrogenase reductase and the dinitrogenase, which work together to catalyze the reduction process under low oxygen conditions (diCenzo et al., 2020). The activity of nitrogenase is highly sensitive to oxygen, necessitating a microaerobic environment within the nodule for optimal function.

The expression and activity of nitrogenase are tightly regulated by both bacterial and plant genes. In rhizobium, the key regulator NifA controls the expression of nitrogenase genes, ensuring that the enzyme is produced only under appropriate conditions. Additionally, the plant exerts control over nitrogenase activity through systemic signaling pathways that respond to the plant's nitrogen and carbon status, further fine-tuning the symbiotic relationship (Oldroyd et al., 2011; Lindström and Mousavi, 2019).

3.4 Metabolic integration between host and symbiont

The symbiotic relationship between legumes and rhizobium involves a complex exchange of metabolites, with the plant providing carbon sources in the form of dicarboxylates to the bacteria, and the bacteria supplying fixed nitrogen in the form of ammonia to the plant. This exchange is crucial for maintaining the energy balance and supporting the high metabolic demands of nitrogen fixation. Transport proteins play a vital role in facilitating the exchange of metabolites between the host plant and the symbiotic bacteria. These proteins ensure the efficient transfer of dicarboxylates from the plant to the bacteria and the export of ammonia from the bacteria to the plant. The regulation of these transport processes is critical for the overall efficiency of the symbiotic relationship, with both partners contributing to the control mechanisms (diCenzo et al., 2020).

4 Genetic Insights into Symbiosis

4.1 Genomic analysis of Fabaceae species

The identification of symbiosis-related genes in Fabaceae species has significantly advanced our understanding of nitrogen-fixing symbiosis. Through high-throughput sequencing technologies, researchers have pinpointed key genes involved in the establishment and maintenance of symbiotic relationships between Fabaceae plants and nitrogen-fixing bacteria. These genes are typically associated with nodule formation, signaling pathways, and nutrient exchange mechanisms. For instance, the genes *NOD* factor receptor 1 (*NFR1*) and *NOD* factor receptor 5 (*NFR5*) are crucial for the recognition of rhizobial signals, leading to nodule formation. Additionally, genes like *ENOD40*, which is involved in nodule organogenesis, and NCR (Nodule Cysteine-Rich) peptides, which play a role in the differentiation of rhizobia, have been extensively studied (Van de Velde et al., 2010).

Comparative genomics between symbiotic and non-symbiotic Fabaceae species provides insights into the evolutionary adaptations associated with nitrogen-fixing symbiosis. Symbiotic species possess a suite of genes that facilitate the intricate interaction with rhizobia, which are absent or significantly different in non-symbiotic species. Genomic comparisons have revealed that symbiotic Fabaceae species often exhibit expansions in gene families related to signal transduction and immune response modulation, highlighting the evolutionary pressures to accommodate beneficial symbionts while managing potential pathogens (Roux et al., 2014). Moreover, the presence of symbiosis islands, which are distinct genomic regions enriched with symbiosis-related genes, underscores the genetic basis for symbiotic capability in these plants (Gonzalez et al., 2010).

4.2 Role of transcription factors in regulating symbiosis

Transcription factors (TFs) play a pivotal role in regulating the gene expression necessary for nodule development in Fabaceae. Among the key TFs, NIN (NODULE INCEPTION) is paramount, as it directly activates genes involved in early nodule formation and development. Another significant TF is ERN (ERF Required for Nodulation), which is involved in the downstream signaling pathways initiated by rhizobial infection. Additionally, TFs such as NSP1 (NODULATION SIGNALING PATHWAY 1) and NSP2 form a complex that regulates the expression of early nodulation genes, further illustrating the complexity of the transcriptional regulation involved in nodule development (Hirsch et al., 2009).

The regulatory networks controlling gene expression during symbiosis involve a complex interplay of TFs, signaling molecules, and feedback mechanisms. These networks ensure the precise activation and repression of genes at various stages of nodule formation and function. For instance, the activation of NIN leads to the induction of early nodulation genes, while the TFs NF-YA1 and NF-YA2 (Nuclear Factor Y) regulate the expression of genes necessary for nodule maturation and function (Comber et al., 2006). Additionally, microRNAs (miRNAs) have emerged as critical regulators within these networks, fine-tuning gene expression to optimize symbiotic efficiency and nodule development (Wang et al., 2015).

4.3 Genetic engineering for enhanced symbiotic efficiency

Genetic engineering offers promising strategies to enhance symbiotic efficiency in Fabaceae species. Techniques such as CRISPR/Cas9 and RNA interference (RNAi) allow for precise manipulation of symbiosis-related genes to improve nodule formation, nitrogen fixation, and overall plant health. For example, overexpression of NIN and other key TFs has been shown to enhance nodule number and functionality, leading to increased nitrogen fixation

rates (Soyano et al., 2013). Additionally, engineering plants to express more efficient or novel nodulation genes from other species can potentially broaden the range of rhizobia that can establish symbiosis, thus improving nitrogen fixation under diverse environmental conditions (Rogers and Oldroyd, 2014).

Several case studies highlight the success of genetic enhancements in improving symbiotic efficiency. In one study, the overexpression of a modified version of the TF NIN in *Lotus japonicus* resulted in a significant increase in nodule number and enhanced nitrogen fixation, demonstrating the potential of targeted gene overexpression (Soyano et al., 2013). Another example involves the transfer of the symbiosis island from a highly efficient nitrogen-fixing bacterium to a less efficient strain, resulting in improved symbiotic performance and plant growth (Gonzalez et al., 2010). These case studies underscore the potential of genetic engineering to optimize symbiotic interactions and enhance agricultural productivity.

5 Case Study: Rhizobium-Legume Symbiosis in Soybean (*Glycine max*)

5.1 Overview of soybean's agricultural importance

Soybean (*Glycine max*) is a critical crop globally, valued for its high protein and oil content, which makes it a staple in both human and animal diets. The agricultural significance of soybean is further enhanced by its ability to engage in symbiotic nitrogen fixation with rhizobium bacteria, reducing the need for nitrogenous fertilizers and promoting sustainable farming practices. Soybean's role in agriculture is multifaceted. It is a major source of protein and oil, with applications ranging from food products to industrial uses. The crop's ability to fix atmospheric nitrogen through symbiosis with rhizobium bacteria is particularly important in regions with nitrogen-deficient soils. This symbiotic relationship not only improves soil fertility but also enhances crop yields and reduces the environmental impact of synthetic fertilizers (Dall'Agnol et al., 2014; Zhao et al., 2021; Veličković et al., 2022).

Research has shown that inoculation with *Rhizobium japonicum* can significantly increase soybean yield, particularly in nutrient-poor soils. For instance, an experiment conducted in the southern savanna region of Ghana demonstrated that rhizobium inoculation, along with nitrogen and phosphorus supplementation, significantly improved various growth parameters and yield components of soybean (Dall'Agnol et al., 2014). This highlights the potential of rhizobium inoculation to enhance soybean productivity in challenging agricultural environments. The interaction between soybean and rhizobium is influenced by various factors, including the presence of other symbiotic organisms such as mycorrhizal fungi. Studies have indicated that the tripartite symbiosis involving soybean, rhizobium, and mycorrhizal fungi can lead to improved photosynthetic efficiency and nutrient uptake, further boosting plant growth and yield (Veličković et al., 2022).

5.2 Molecular mechanisms specific to soybean-rhizobium symbiosis

The molecular mechanisms underlying the soybean-rhizobium symbiosis are complex and involve a series of genetic and biochemical interactions that facilitate the establishment and maintenance of this mutualistic relationship. Several key molecular players and pathways have been identified that are specific to the soybean (*Glycine max*) and its interaction with rhizobium. One critical aspect of this symbiosis is the role of the Glycogen Synthase Kinase 3 (GSK3)-like kinase, GmSK2-8, which has been shown to inhibit symbiotic signaling and nodule formation under salt stress conditions. GmSK2-8 interacts with *Glycine max* Nodulation Signaling Pathway 1 (GmNSP1) transcription factors, phosphorylating them and thereby inhibiting their ability to bind to the promoters of symbiotic genes. This phosphorylation reduces nodule formation under stress conditions, highlighting a regulatory mechanism that modulates symbiosis in response to environmental stress (He et al., 2020).

Another significant molecular component is the Yellow Stripe-like 7 (GmYSL7) transporter, which is localized on the symbiosome membrane in soybean nodules. GmYSL7 is responsible for transporting oligopeptides, which are crucial for proper nodule development and nitrogenase activity. Silencing GmYSL7 results in reduced nitrogenase activity and impaired nodule development, indicating its essential role in the symbiotic process (Figure 3) (Gavrin et al., 2021). Hydrogen sulfide (H₂S) has also been identified as a positive signaling molecule in the soybean-rhizobium symbiotic system. Exogenous application of H₂S has been shown to promote soybean growth,

nodulation, and nitrogenase activity. This is achieved through the upregulation of symbiosis-related genes in both the rhizobium and soybean, suggesting that H₂S enhances the nitrogen fixation ability of the symbiotic system (Fan et al., 2022). Iron transport across the symbiosome membrane is another critical factor for effective nitrogen fixation. The iron transporter GmVTL1a, localized on the symbiosome membrane, is responsible for supplying iron to the bacteroids, which is essential for the activity of the nitrogenase enzyme. Mutations in GmVTL1a that impair iron transport result in reduced nitrogen fixation, underscoring the importance of iron homeostasis in the symbiotic relationship (Bender et al., 2022).

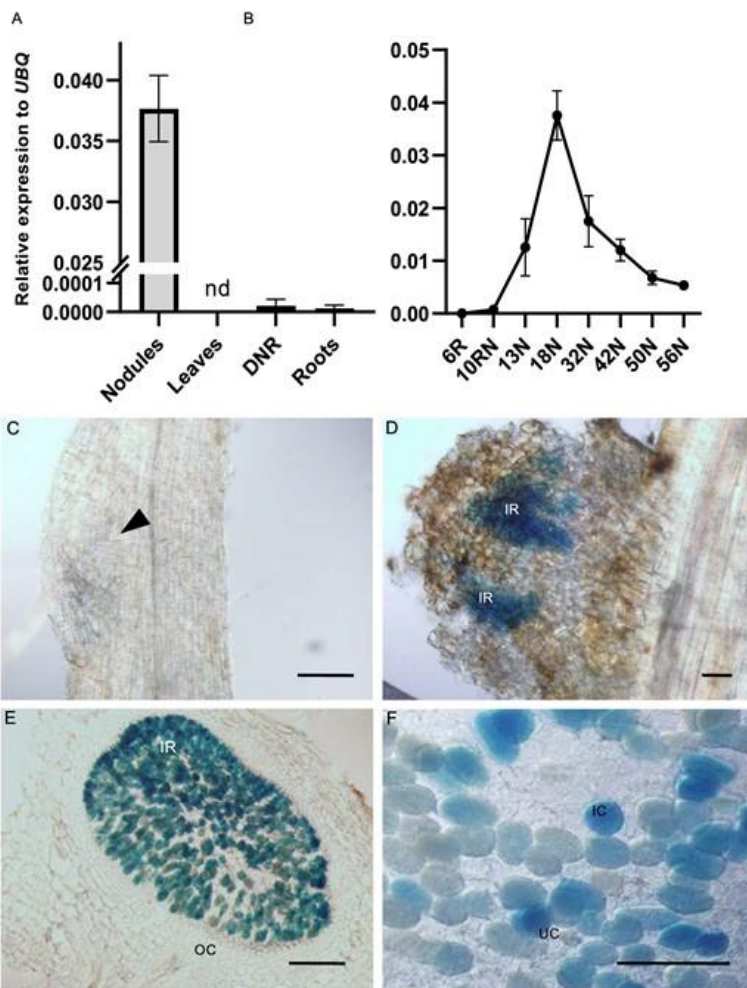


Figure 3 GmYSL7 is expressed in infected cells of soybean root nodules (Adopted from Gavrin et al., 2021)
 Image caption: A, Transcript level of *GmYSL7* in tissue samples from different organs. DNR, denodulated roots; nd, not detected. B, Transcript level of *GmYSL7* during nodule development. 6R, roots 6 d after inoculation (DAI); 10RN, roots and nodules 10 DAI; 13N-26N, nodules the indicated DAI. Data shown are from three independent time courses. Error bars are SE ($n = 3$). Nitrogenase activity was first detected at 18 DAI. The relative expression was quantified by RT-qPCR and normalized to a soybean ubiquitin gene (GmUBI3, Glyma20g27950.1). C, Transgenic root expressing pYSL7: GFP-GUS. GUS staining was not detectable in the very early stages of nodule development. Arrowhead indicates a nodule initiation. D, Transgenic pYSL7: GFP-GUS 10-d-old nodule primordia. IR, infected region. E, Transgenic pYSL7:GFP-GUS mature nodule. GUS staining is restricted to infected cells. OC, outer cortex. F, Magnification of E. Scale bars, 150 μ m (Adopted from Gavrin et al., 2021)

5.3 Genetic basis of soybean's nodulation efficiency

The genetic basis of nodulation efficiency in soybean (*Glycine max*) is a complex interplay of various genes and molecular pathways that regulate the formation and function of nodules, which are essential for symbiotic nitrogen fixation. This section delves into the key genetic components and mechanisms that contribute to the nodulation efficiency in soybean. GmACP plays a crucial role in nodulation by participating in fatty acid biosynthesis. Silencing of GmACP results in a significant reduction in nodule numbers, indicating its importance in the nodulation process (Li et al., 2022).

This gene is involved in the shoot-to-root signaling pathway that regulates nodulation and nitrogen nutrition. Variations in the promoter region of GmNN1/FT2a can lead to decreased transcription levels and nitrogen deficiency phenotypes. Manipulating this gene enhances nodulation, plant growth, and nitrogen nutrition (Bender et al., 2022). GmPT7 is a nodule-localized phosphate transporter that plays a significant role in enhancing symbiotic nitrogen fixation and yield. Overexpression of GmPT7 promotes nodulation and increases plant biomass, shoot nitrogen, and phosphorus content, thereby improving soybean yield (Wang et al., 2018). GmHSP17.9 is specifically expressed in the infected regions of nodules and is crucial for nodule development and nitrogen fixation. Overexpression or silencing of this gene significantly affects nodule number, nitrogenase activity, and overall plant growth and seed yield (Yang et al., 2021).

5.4 Practical applications and implications for soybean breeding

The molecular mechanisms underlying nitrogen-fixing symbiosis in Fabaceae, particularly in soybean (*Glycine max*), offer significant potential for practical applications in soybean breeding. Understanding these mechanisms can lead to the development of soybean varieties with enhanced nitrogen fixation capabilities, improved growth, and higher yields, especially in nitrogen-poor soils. The discovery of the symbiotic flowering pathway involving microRNA172c (miR172c) and fixed nitrogen signals provides a crucial insight into how nitrogen fixation can be leveraged to improve soybean flowering and reproductive success. By promoting the expression of florigen-encoding FLOWERING LOCUS T (FT) homologs (GmFT2a/5a), breeders can develop soybean varieties that flower more efficiently under low-nitrogen conditions, thereby enhancing their reproductive success and yield (Yun et al., 2023). The differential performance of symbiotic nitrogen fixation between soybean and common bean highlights the importance of the root and nodule microbiomes. Soybean's efficient symbiosis with *Bradyrhizobium*, as opposed to the less effective symbiosis in common bean, suggests that selecting for specific rhizobial strains and optimizing co-inoculation strategies could significantly improve nitrogen fixation and plant growth. This approach can reduce the reliance on chemical fertilizers, promoting more sustainable agricultural practices (Bender et al., 2022).

Iron and phosphate are critical for effective nitrogen fixation. The identification of iron transporters such as GmVTL1a and phosphate transporters like GmPT7, which are localized to the symbiosome membrane and nodule cortex respectively, underscores their roles in enhancing nitrogen fixation and overall plant health. Breeding strategies that enhance the expression of these transporters can lead to soybean varieties with improved nutrient uptake, better nodulation, and higher yields (Brear et al., 2020). The metabolic modeling of *Sinorhizobium fredii* and its interaction with soybean provides a framework for identifying key genes that control nitrogen fixation. By targeting these genes through genetic engineering or selective breeding, it is possible to enhance the nitrogen-fixing efficiency of symbionts, thereby improving the nitrogen availability to the host plant and reducing the need for external nitrogen inputs (Contador et al., 2020).

The role of GmNN1/FT2a in shoot-to-root signaling and its impact on nodulation and nitrogen nutrition highlights a critical regulatory pathway that can be manipulated to improve soybean growth. By selecting for alleles that enhance the expression of GmNN1/FT2a, breeders can develop soybean varieties with better nodulation and nitrogen uptake, leading to improved plant health and yield. The phosphorylation of RIN4, a key immune regulator, upon rhizobial inoculation, and its essential role in nodulation, suggests that integrating immune and symbiotic signaling pathways can enhance the establishment of effective symbiosis. Breeding strategies that focus on optimizing RIN4 function could improve the compatibility and efficiency of nitrogen-fixing symbiosis in soybean.

6 Environmental and Agricultural Implications

6.1 Impact of nitrogen-fixing symbiosis on soil fertility

Nitrogen-fixing symbiosis in Fabaceae plays a crucial role in sustainable agriculture by enhancing soil fertility through the biological nitrogen fixation (BNF) process. This symbiotic relationship between legumes and rhizobia bacteria allows the conversion of atmospheric nitrogen into a form that plants can utilize, reducing the need for chemical fertilizers. This process not only improves soil health but also supports the growth of subsequent crops

by enriching the soil with essential nutrients (Liu et al., 2018; Raza et al., 2020; Cui et al., 2021). The ability of legumes to fix nitrogen biologically makes them an integral part of sustainable agricultural practices, promoting long-term soil fertility and reducing the environmental impact of synthetic fertilizers.

Incorporating nitrogen-fixing legumes into crop rotation and intercropping systems can significantly enhance soil fertility and crop yields. Legumes, when used in rotation with non-leguminous crops, replenish soil nitrogen levels, thereby reducing the dependency on chemical fertilizers. This practice not only improves soil structure and fertility but also helps in breaking pest and disease cycles, leading to healthier crops (Liu et al., 2018; Raza et al., 2020). Intercropping legumes with other crops can also provide mutual benefits, such as improved nutrient availability and better pest management, contributing to more resilient and productive agricultural systems (Cui et al., 2021).

6.2 Environmental benefits of reduced nitrogen fertilizer use

The use of nitrogen-fixing legumes in agriculture can lead to a significant reduction in greenhouse gas emissions. Chemical nitrogen fertilizers are a major source of nitrous oxide, a potent greenhouse gas. By reducing the need for these fertilizers, biological nitrogen fixation helps mitigate the release of nitrous oxide into the atmosphere, thereby contributing to climate change mitigation (Ibañez et al., 2016; Raza et al., 2020). The adoption of legume-based cropping systems can thus play a vital role in reducing the carbon footprint of agricultural practices (Jach et al., 2022).

Excessive use of chemical fertilizers often leads to nutrient runoff, causing water pollution and eutrophication in aquatic ecosystems. Nitrogen-fixing legumes help mitigate this issue by naturally enriching the soil with nitrogen, reducing the need for synthetic fertilizers and consequently decreasing the risk of nutrient leaching into water bodies (Raza et al., 2020; Jach et al., 2022). This environmentally friendly approach not only protects water quality but also supports the health of aquatic ecosystems and biodiversity (Ibañez et al., 2016).

6.3 Challenges and future directions

Climate change poses significant challenges to the effectiveness of nitrogen-fixing symbiosis in Fabaceae. Changes in temperature, precipitation patterns, and soil conditions can affect the symbiotic relationship between legumes and rhizobia, potentially reducing the efficiency of nitrogen fixation. Future research should focus on understanding the impacts of climate change on this symbiosis and developing strategies to enhance the resilience of legume-rhizobia interactions under changing environmental conditions (Lipa and Janczarek, 2020; Cui et al., 2021). This may include breeding climate-resilient legume varieties and exploring the use of stress-tolerant rhizobial strains (Ficano et al., 2021).

Advancements in molecular biology and genomics offer new opportunities to enhance the efficiency of nitrogen-fixing symbiosis in Fabaceae. Future research should aim to identify and manipulate key genetic and regulatory pathways involved in nitrogen fixation to improve symbiotic efficiency and compatibility between legumes and rhizobia (Zhao et al., 2021). Additionally, exploring the potential of engineering nitrogen-fixing capabilities into non-leguminous crops, such as cereals, could revolutionize agricultural practices and reduce the reliance on chemical fertilizers (Delaux et al., 2015). Continued research and technological innovations in this field are essential for developing sustainable and resilient agricultural systems that can meet the growing global food demand while minimizing environmental impacts (Cui et al., 2021).

7 Concluding Remarks

The study on the molecular mechanisms of nitrogen-fixing symbiosis in Fabaceae has elucidated several critical insights. Firstly, the phylogenetic analyses have revealed a highly resolved phylogeny of Fabaceae, supporting numerous polyploidization events and providing a foundation for understanding the evolutionary history of rhizobial nitrogen-fixing symbiosis. Additionally, the study of genomic traces has highlighted the fragility and evolutionary susceptibility of nitrogen-fixing symbiosis, with multiple independent losses observed across different species. The diversity and phylogenetic patterns of symbiotic genes in *Paraburkholderia* and *Bradyrhizobium* species have been mapped, revealing horizontal gene transfer events and the existence of distinct

symbiovars. Furthermore, the comparative genomics of rhizobia strains has underscored the complexity and variability of symbiotic genes, suggesting the absence of a simple core symbiome. The genetic mechanisms underlying the symbiosis, including the role of antimicrobial peptides and the regulation of nitrogen fixation, have also been explored, providing insights into the intricate interactions between host plants and symbiotic bacteria.

The findings from this study have significant implications for future research and agricultural practices. Understanding the phylogenetic relationships and evolutionary history of nitrogen-fixing symbiosis can guide the development of more efficient and resilient legume crops. The identification of key symbiotic genes and their regulatory mechanisms opens avenues for genetic engineering to enhance nitrogen fixation capabilities in non-leguminous crops, potentially reducing the reliance on chemical fertilizers and promoting sustainable agriculture. Additionally, the insights into the genetic diversity and horizontal gene transfer events among symbiotic bacteria can inform the selection and breeding of more effective and adaptable rhizobial strains for use in different environmental conditions. Future research should focus on unraveling the molecular pathways and regulatory networks involved in symbiosis, as well as exploring the potential for transferring these traits to other plant species.

Understanding the molecular mechanisms of nitrogen-fixing symbiosis in Fabaceae is of paramount importance for both scientific and practical reasons. This knowledge not only advances our comprehension of plant-microbe interactions and evolutionary biology but also holds the key to addressing global challenges related to food security and environmental sustainability. By harnessing the natural ability of legumes to fix atmospheric nitrogen, we can develop agricultural systems that are less dependent on synthetic fertilizers, thereby reducing environmental pollution and enhancing soil health. Moreover, the insights gained from studying these mechanisms can be applied to improve crop yields and resilience, contributing to more sustainable and productive agricultural practices. Ultimately, the continued exploration of nitrogen-fixing symbiosis at the molecular level will pave the way for innovative solutions to some of the most pressing issues facing humanity today.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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