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Symbiotic Nitrogen Fixation: The Role of Rhizobia in Enhancing Legume Growth and Soil Fertility

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Molecular Microbiology Research, 2024, Vol.14, No.2 doi: 10.5376/mmr.2024.14.0012
Received: 07 Mar., 2024
Accepted: 16 Apr., 2024
Published: 28 Apr., 2024

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Preferred citation for this article:
Chen Y.X., and Zhou X.H., 2024, Symbiotic nitrogen fixation: the role of rhizobia in enhancing legume growth and soil fertility, Molecular Microbiology Research, 14(2): 109-118 (doi: 10.5376/mmr.2024.14.0012)

Abstract Symbiotic nitrogen fixation, facilitated by rhizobia, plays a crucial role in enhancing legume growth and soil fertility. This symbiotic relationship has evolved to allow rhizobia to fix atmospheric nitrogen into a form usable by plants, significantly impacting agricultural productivity and ecological sustainability. This study summarizes the current understanding of the mechanisms, effectiveness, and ecological implications of rhizobia-legume symbiosis in nitrogen fixation. The study found that the effectiveness of nitrogen fixation varies significantly among different rhizobial strains, with some strains demonstrating high efficiency under specific conditions. The interaction between plant nitrogen demand and symbiotic efficiency is tightly regulated by systemic signaling pathways. Reactive oxygen and nitrogen species play a dual role in both promoting and regulating the symbiotic process. Environmental factors such as soil nitrate levels and herbivory also influence the allocation of fixed nitrogen in legumes. The rapid evolution of rhizobial strains through lateral gene transfer may lead to the emergence of competitive but less efficient nitrogen-fixing strains. Understanding the complexity and adaptability of the rhizobia-legume symbiosis can inform strategies to enhance nitrogen fixation efficiency, thereby improving legume growth and soil fertility. Future research should focus on unraveling the specific regulatory mechanisms and environmental interactions that optimize this symbiotic relationship.

Keywords Symbiotic nitrogen fixation; Rhizobia; Legumes; Soil fertility; Molecular mechanisms; Ecological impact

1 Introduction

Symbiotic nitrogen fixation is a critical biological process where atmospheric nitrogen (N_2) is converted into ammonia (NH_3) by symbiotic bacteria, primarily rhizobia, within the root nodules of leguminous plants (Sindhu et al., 2019). This process is essential for plant growth and soil fertility, as it provides a natural and sustainable source of nitrogen, a key nutrient for plant development (Liu et al., 2018a). The resulting symbiosis not only enhances the growth and productivity of legume crops but also contributes to improving soil fertility by increasing nitrogen content in the soil. Rhizobia have evolved a dual lifestyle, alternating between a free-living phase in the soil and an intracellular symbiotic phase within legume root nodules, which has allowed them to spread across diverse ecological niches and persist through evolutionary history (Masson-Boivin and Sachs, 2018). As global agriculture faces the challenges of increasing food demand, reducing environmental impact, and maintaining soil health, understanding and harnessing the benefits of symbiotic nitrogen fixation has become increasingly vital.

Rhizobia play a pivotal role in agriculture by enhancing the growth and productivity of leguminous crops, which are vital for food security and soil health. The symbiotic relationship between rhizobia and legumes not only boosts plant growth by providing essential nitrogen but also improves soil fertility, reducing the need for chemical fertilizers (Lindström and Mousavi, 2019). This mutualistic interaction is tightly regulated by the host plant, ensuring efficient nitrogen fixation and optimal plant health (Oldroyd et al., 2011). Additionally, rhizobia-mediated nitrogen fixation has been shown to enhance plant defense mechanisms against herbivores, further contributing to the overall fitness and resilience of leguminous plants (Thamer et al., 2011). The ability of rhizobia to adapt and evolve in response to environmental conditions and host plant requirements underscores their significance in sustainable agricultural practices (Nandasena et al., 2007).

This study will explore the mechanisms and efficiency of symbiotic nitrogen fixation in different legume-rhizobia associations and assess their impact on plant growth and soil fertility. The focus is on investigating the genetic, physiological, and environmental factors that influence this process and determining strategies to enhance the effectiveness of rhizobial inoculants in agricultural practices. A deeper understanding of the interactions between rhizobia and leguminous plants is expected to improve crop yields and reduce reliance on chemical fertilizers. This study aspires to develop innovative approaches to enhance the efficiency of biological nitrogen fixation, achieve more sustainable agricultural systems, and contribute to global food security and environmental protection.

2 Overview of Rhizobia

2.1 Definition and characteristics of rhizobia

Rhizobia are soil bacteria that form a close symbiotic relationship with leguminous plants, belonging to the family Rhizobiaceae or related taxa. These microorganisms infect the root tissues of host plants and induce the formation of structures known as nodules, which are the primary sites for nitrogen fixation. Inside the nodules, rhizobia convert atmospheric nitrogen (N_2) into ammonia (NH_3) using specialized enzyme systems such as nitrogenase (Masson-Boivin and Sachs, 2018; Lindström and Mousavi, 2019). This process is crucial for plants, as nitrogen is an essential element for the synthesis of amino acids, proteins, and nucleic acids. The ability of rhizobia to fix nitrogen not only improves the growth efficiency of leguminous plants but also enhances soil fertility, providing long-term agricultural benefits for crop rotation.

The nitrogen-fixing capability of rhizobia plays a significant role in sustainable agriculture by substantially reducing the reliance on synthetic nitrogen fertilizers. The extensive use of synthetic nitrogen fertilizers can lead to environmental issues such as water eutrophication and greenhouse gas emissions, whereas rhizobia offer a natural and environmentally friendly alternative. By forming symbiotic relationships with leguminous plants, rhizobia not only meet the nitrogen needs of the plants but also enhance the nitrogen cycling in ecosystems, contributing to soil health and productivity. This natural nitrogen source shows great potential in organic and low-input agricultural systems, promising to advance the sustainability of agricultural practices (Pankievicz et al., 2019).

2.2 Types of rhizobia

Rhizobia are diverse and can be classified into several genera, including *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, and *Mesorhizobium*, among others. Each genus contains various species, which differ in their ability to form nodules with different leguminous host plants and their nitrogen-fixing efficiency. For example, *Bradyrhizobium diazoefficiens*, from the genus *Bradyrhizobium*, is well-known for its symbiotic relationship with soybeans, while *Rhizobium leguminosarum* from the genus *Rhizobium* commonly forms symbioses with peas and lentils (Nguyen et al., 2019; Dupin et al., 2020). *Sinorhizobium* typically forms nodules with leguminous plants like alfalfa, similar to those formed by *Rhizobium*. *Mesorhizobium* is associated with plants like chickpeas and lentils, with nodules that exhibit characteristics intermediate between determinate and indeterminate types.

Each type of rhizobia has evolved to adapt to specific environmental conditions and form symbiotic relationships with particular legume populations. Recent research has revealed the adaptability of rhizobia under different environmental conditions, especially their nodulation and nitrogen-fixing abilities in high nitrate environments. These newly discovered rhizobial strains demonstrate the potential to maintain efficient nitrogen fixation even in conditions traditionally considered unfavorable for nodule formation and nitrogen fixation. The emergence of these strains offers new hope for improving the nitrogen-fixing efficiency of leguminous plants under adverse soil conditions, particularly in the high nitrate environments commonly found in agricultural production, and they may have significant application prospects (Nguyen et al., 2019).

2.3 Rhizobia-host specificity

Rhizobia exhibit a high degree of host specificity, meaning that specific rhizobial strains can only be compatible with particular leguminous plants. This specificity is primarily determined by the molecular signaling exchanges between the plant and the rhizobia. Flavonoids secreted by the plant roots trigger the production of nodulation factors (Nod factors) in the rhizobia, which are then recognized by the plant, leading to nodule formation (Masson-Boivin and Sachs, 2018; Lepetit and Brouquisse, 2023). This specificity of interaction ensures that rhizobia can only successfully infect and form nodules on compatible leguminous plants.

For example, *Rhizobium leguminosarum* biovar viciae forms nodules on peas and field peas, while *Bradyrhizobium japonicum* specifically symbiotically associates with soybeans (Kimeklis et al., 2019). The genetic basis of this specificity lies in the symbiotic genes carried by the rhizobia, which determine their ability to interact with specific plant hosts. Understanding the host specificity of rhizobia is crucial for developing effective inoculants to enhance crop production in various agricultural environments.

Rhizobia are essential for legume growth and soil fertility due to their unique ability to fix atmospheric nitrogen. The diversity among rhizobial species and their specific interactions with legume hosts highlight the complexity and importance of this symbiotic relationship in agricultural ecosystems.

3 Mechanisms of Symbiotic Nitrogen Fixation

3.1 Infection and nodule formation

Root hair curling is the initial step in the infection process where rhizobia attach to the root hairs of legumes. This attachment triggers the root hairs to curl around the bacteria, forming an infection pocket. This process is tightly regulated by the exchange of signaling molecules between the plant and the rhizobia. The plant releases flavonoids that induce the production of Nod factors by rhizobia, which in turn trigger root hair curling (Masson-Boivin and Sachs, 2018; Chakraborty et al., 2022). Following root hair curling, rhizobia enter the plant root through infection threads, which are tubular structures formed by the invagination of the root hair cell wall. The development of infection threads is a critical step that allows rhizobia to travel from the root hair to the root cortex. This process is regulated by various plant hormones and signaling pathways, including cytokinin and auxin, which promote infection thread formation and progression (Liu et al., 2018b; Teulet et al., 2019).

Nodule organogenesis involves the differentiation of root cortical cells into a new organ, the nodule, where nitrogen fixation occurs. This process is initiated by the perception of Nod factors and involves complex signaling pathways that coordinate cell division and differentiation. Key transcription factors and signaling molecules, such as NODULE INCEPTION (NIN) and cytokinin, play crucial roles in nodule organogenesis (Velzen et al., 2018; Chakraborty et al., 2022). Additionally, certain rhizobial effectors, like ErnA, can directly trigger nodule formation by modulating host plant signaling pathways (Teulet et al., 2019).

3.2 Nitrogen fixation process

Once the nodules form, the rhizobia inside the nodules convert atmospheric nitrogen (N_2) into ammonia (NH_3) through nitrogenase. The plant then assimilates this ammonia into amino acids, providing an essential nitrogen source for plant growth. The nitrogen fixation process is highly energy-intensive, relying on carbohydrates provided by the plant from photosynthesis to support the activity of nitrogenase (Figure 1) (Lindström and Mousavi, 2019; Lepetit and Brouquisse, 2023).

The efficiency of nitrogen fixation can be affected by various factors, including the energy supply for the nitrogenase, interactions with the host plant, and external environmental conditions (Lindström and Mousavi, 2019; Sauviac et al., 2022). Research by Lindström and Mousavi (2019) indicates that the high energy consumption of nitrogenase is a major limiting factor for nitrogen fixation efficiency, and adjusting the oxygen levels and carbon metabolism within the nodules can enhance fixation efficiency. Additionally, differences in nitrogen fixation effectiveness exhibited by rhizobia in specific host plants highlight the need to optimize nitrogen fixation in different plant-microbe symbiotic systems.

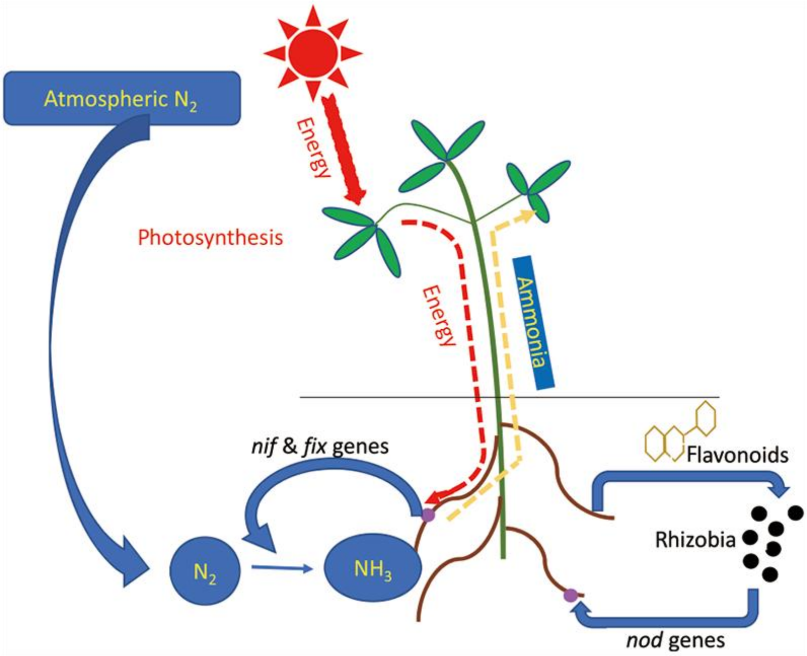

Figure 1 Summarized model for symbiotic nitrogen fixation in legumes by rhizobia (Adopted from Lindström and Mousavi, 2019)
Image caption: The figure illustrates how rhizobia interact with the roots of leguminous plants to form nodules and carry out nitrogen fixation. Rhizobia fix atmospheric nitrogen and convert it into ammonia, which plants can utilize, thereby enhancing plant growth and improving soil fertility (Adapted from Lindström and Mousavi, 2019)

3.3 Molecular signaling between rhizobia and legumes

The symbiotic relationship between rhizobia and legumes is orchestrated by a complex network of molecular signals. The initial recognition involves the exchange of flavonoids and Nod factors, which activate downstream signaling pathways in both the plant and the bacteria. In legumes, microRNAs such as miR2111 play a crucial role in maintaining the susceptibility of roots to rhizobial infection by regulating key suppressors of symbiosis (Tsikou et al., 2018). Additionally, systemic signaling mechanisms, including the allocation of sucrose and the oxidative pentose phosphate pathway, adjust nodule formation and function based on the plant's nutritional status (Lepetit and Brouquisse, 2023). Transcription factors also integrate environmental signals with symbiotic signaling to modulate nodule development and function (Chakraborty et al., 2022).

4 Role of Rhizobia in Enhancing Legume Growth

4.1 Nutrient uptake and utilization

Rhizobia play a crucial role in enhancing nitrogen uptake in legumes through the process of symbiotic nitrogen fixation. This process involves the conversion of atmospheric nitrogen (N2) into ammonia (NH_3), which is then assimilated into amino acids by the plant. Studies have shown that inoculation with effective rhizobium strains significantly increases nitrogen fixation and uptake in legumes. For instance, *Vicia faba* inoculated with strains NSFBR-12 and NSFBR-15 fixed 87.7% and 85.5% of the total nitrogen uptake, respectively, demonstrating the substantial contribution of rhizobia to nitrogen nutrition in legumes (Allito et al., 2020). Additionally, the effectiveness of nitrogen fixation in rhizobia has been a focal point of research, aiming to improve the efficiency of this process for sustainable food production (Lindström and Mousavi, 2019). Rhizobia not only enhance nitrogen uptake but also improve the utilization of other essential nutrients such as phosphorus and potassium. The presence of rhizobia can lead to better phosphorus uptake, as observed in *Vicia faba*, where soil available phosphorus and pH significantly influenced phosphorus uptake in inoculated plants (Allito et al., 2020). Moreover, rhizobia can solubilize precipitated phosphorus, making it more available to the plant, and produce siderophores that help in the uptake of iron, which is crucial for various plant metabolic processes (Fahde et al., 2023).

Rhizobia also play a role in enhancing the availability of micronutrients to legumes. They produce siderophores that chelate iron, making it more accessible to the plant. Additionally, rhizobia can produce phytohormones and other growth-promoting substances that improve the overall nutrient uptake and utilization efficiency of the plant (Fahde et al., 2023). This multifaceted role of rhizobia in nutrient uptake underscores their importance in legume growth and soil fertility.

4.2 Hormonal regulation

Rhizobia influence the hormonal regulation in legumes, which is essential for various growth and developmental processes. They produce phytohormones such as auxins, cytokinins, and gibberellins, which can enhance root growth and development, leading to better nutrient and water uptake. The production of these hormones by rhizobia can also modulate the plant's hormonal balance, promoting overall plant health and growth (Fahde et al., 2023). Furthermore, systemic signaling mechanisms involving the plant's nitrogen demand and photosynthetic capacities are crucial for the regulation of nodule organogenesis and functioning, highlighting the complex interplay between rhizobia and plant hormonal regulation (Lepetit and Brouquisse, 2023).

4.3 Stress tolerance

Rhizobia contribute to the stress tolerance of legumes by enhancing their ability to cope with various abiotic and biotic stresses. For example, rhizobia can produce ACC deaminase, an enzyme that lowers ethylene levels in plants, thereby reducing the negative effects of stress conditions such as drought (Fahde et al., 2023). Additionally, rhizobia can induce systemic resistance in the host plant, providing protection against pathogens and pests. This enhanced stress tolerance is particularly important in agricultural systems where legumes are exposed to fluctuating environmental conditions. The ability of rhizobia to improve stress tolerance in legumes underscores their role in promoting sustainable agriculture and enhancing crop resilience.

5 Impact of Rhizobia on Soil Fertility

5.1 Soil nitrogen content

Rhizobia play a crucial role in enhancing soil nitrogen content through the process of symbiotic nitrogen fixation. This process involves the conversion of atmospheric nitrogen (N_2) into ammonia (NH_3), which plants can readily assimilate. The symbiotic relationship between rhizobia and legumes results in the formation of root nodules where nitrogen fixation occurs, significantly enriching the soil with nitrogen. This biological nitrogen fixation is particularly important for sustainable agriculture as it reduces the need for chemical nitrogen fertilizers, which are associated with greenhouse gas emissions and environmental pollution (Mabrouk et al., 2018; Lindström and Mousavi, 2019; Goyal et al., 2021). Additionally, the efficiency of nitrogen fixation can be influenced by various factors, including the genetic compatibility between the rhizobia and the host plant, as well as environmental conditions such as soil nitrate levels and herbivory (Wang et al., 2018; Thompson and Lamp, 2021).

5.2 Soil structure and health

Beyond nitrogen enrichment, rhizobia contribute to soil structure and health. The presence of rhizobia and their symbiotic interactions with legumes can lead to improved soil aggregation and porosity. This is partly due to the organic matter added to the soil from decaying root nodules and plant residues, which enhances soil organic carbon content and microbial activity. Improved soil structure facilitates better water infiltration and retention, reducing soil erosion and promoting root growth (Mabrouk et al., 2018; Costa et al., 2021). Furthermore, rhizobia can help in solubilizing phosphates and producing phytohormones, which further support plant growth and soil health (Mabrouk et al., 2018).

5.3 Interaction with soil microbiome

Rhizobia interact with a diverse soil microbiome, influencing the overall microbial community structure and function. These interactions can have synergistic effects, where the presence of rhizobia promotes the growth of other beneficial microorganisms, such as plant growth-promoting rhizobacteria (PGPR). This can enhance plant resistance to biotic and abiotic stresses, including pathogen attacks and heavy metal contamination (Clúa et al., 2018; Bellabarba et al., 2019). The complex interplay between rhizobia and other soil microorganisms underscores the importance of a holistic approach to understanding soil health and fertility. The ability of rhizobia to coexist and cooperate with other microbes highlights their role in maintaining a balanced and productive soil ecosystem (Masson-Boivin and Sachs, 2018; Sachs et al., 2018).

6 Agricultural Applications and Practices

6.1 Inoculant development and use

The development and use of rhizobial inoculants have shown significant promise in enhancing legume growth and soil fertility. Rhizobia, as plant growth-promoting bacteria, form symbiotic relationships with legumes, facilitating nitrogen fixation, which is crucial for plant nutrition and soil health. The effectiveness of rhizobial inoculants has been demonstrated in various studies. For instance, specific rhizobium strains have been shown to significantly enhance nodulation, nitrogen fixation, and nutrient uptake in *Vicia faba*, leading to improved soil nitrogen balance (Allito et al., 2020). Additionally, novel rhizobial strains have been identified that exhibit superior nodulation and nitrogen fixation even under high nitrate concentrations, making them promising candidates for use in nitrate-applied soils (Nguyen et al., 2019). The use of rhizobial inoculants not only boosts legume productivity but also contributes to sustainable agricultural practices by reducing the need for chemical fertilizers (Masson-Boivin and Sachs, 2018; Mabrouk et al., 2018).

Additionally, several novel rhizobial strains have been identified that maintain excellent nodulation and nitrogen-fixing capabilities even under high nitrate concentrations, making them promising candidates for use in soils with nitrate application (Nguyen et al., 2019). Research has shown that the traditional rhizobial strain *Bradyrhizobium diazoefficiens* USDA110 exhibits significantly reduced nodulation ability at higher nitrate concentrations, whereas several newly isolated rhizobial strains (NKS4, NKM2, and NKTG2) can still effectively form nitrogen-fixing nodules at a nitrate concentration of 20 mM (Figure 2). Comparisons of nodulation efficiency, nitrogen-fixing activity, and competitiveness reveal that these new strains exhibit superior nodulation capabilities and growth characteristics compared to USDA110. The study also found that the new strains can enhance the expression of symbiotic signaling genes in soybeans and reduce the expression of nitrate-dependent nodulation repression genes. The discovery of these new strains offers potential microbial inoculant options for improving soybean production under high nitrogen fertilizer conditions. The use of rhizobial inoculants not only increases the productivity of leguminous crops but also promotes sustainable agricultural practices by reducing the need for chemical fertilizers (Masson-Boivin and Sachs, 2018; Mabrouk et al., 2018).

6.2 Crop rotation and intercropping

Crop rotation and intercropping are traditional agricultural practices that can be optimized through the use of rhizobia to enhance soil fertility and crop yields. Intercropping, in particular, has been shown to maximize the use of resources and improve nitrogen utilization. A meta-analysis of grain legume-cereal intercropping revealed that intercropping increases the proportion of nitrogen derived from nitrogen fixation in legumes and enhances soil nitrogen acquisition in cereals (Rodriguez et al., 2020). This practice not only improves the overall nitrogen balance in the soil but also reduces the need for external nitrogen inputs, thereby promoting sustainable agriculture. Furthermore, intercropping systems involving rhizobial inoculation, such as maize/faba bean intercropping, have demonstrated increased productivity and nitrogen fixation, along with reduced nitrogen losses, especially in challenging environments like reclaimed desert soils (Mei et al., 2021).

6.3 Sustainable agriculture practices

Sustainable agriculture practices aim to maintain high productivity while minimizing environmental impact. The use of rhizobia in sustainable agriculture is a key strategy to achieve this balance. Rhizobia enhance plant growth and resilience by fixing atmospheric nitrogen, solubilizing phosphates, and producing phytohormones, which collectively improve plant nutrition and defense mechanisms (Mabrouk et al., 2018). Rhizobia can tolerate various abiotic stresses, such as extreme temperatures, pH, salinity, and drought, making them valuable in diverse agroecosystems. The integration of rhizobia into sustainable agricultural practices, such as the use of biofertilizers and biopesticides, can lead to healthier and more productive farming systems.

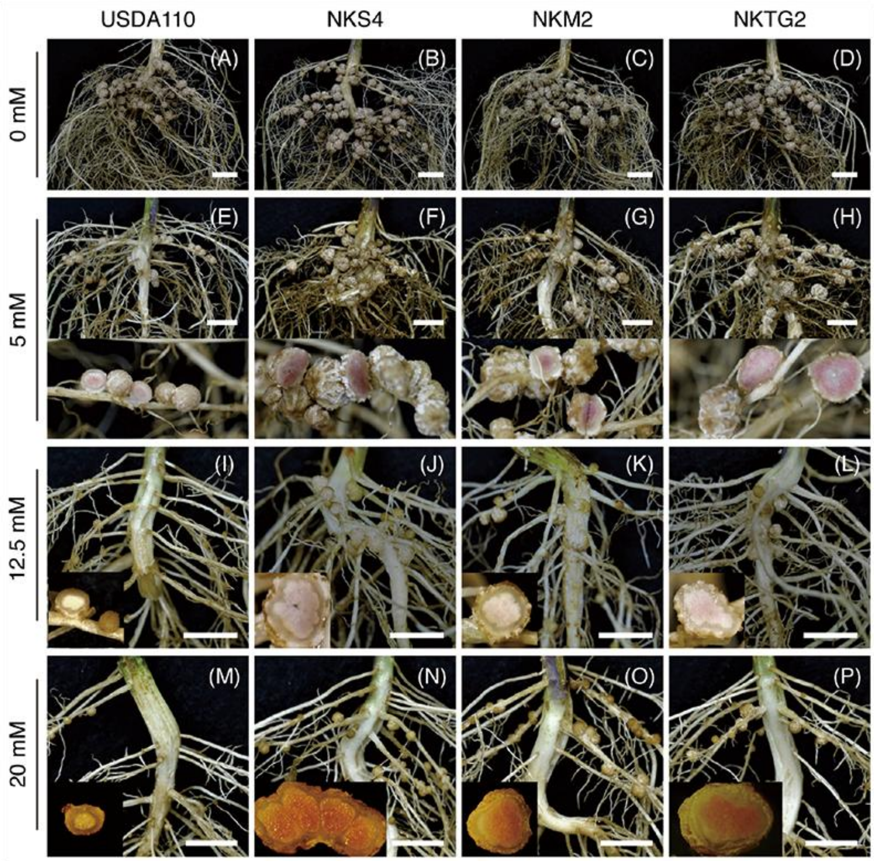

Figure 2 Roots of *G. max* cv. Enrei inoculated with the rhizobial strains. The soybeans were grown in the presence of 0 (A–D), 5 (E–H), 12.5 (I–L) and 20 mM (M–P) of potassium nitrate, respectively. The Enrei roots and nodule sections were photographed at 30 dpi. Scale bars: 1 cm (Adopted from Nguyen et al., 2019)

Image caption: The figure compares the nodulation effects of the traditional rhizobial strain USDA110 with three novel rhizobial strains (NKS4, NKM2, NKTG2). The results indicate that USDA110 is almost unable to form effective nodules under high nitrate conditions (especially at 12.5 mM and 20 mM), whereas the novel strains can still produce larger and heme-rich pink nodules under these conditions, demonstrating their superior nodulation capability in high nitrate environments (Adapted from Nguyen et al., 2019)

Moreover, the application of rhizobia in metal-contaminated and high saline lands has been shown to improve crop yield and soil health, further underscoring their role in sustainable agriculture (Bellabarba et al., 2019). By leveraging the symbiotic relationship between rhizobia and legumes, farmers can enhance soil fertility, reduce dependency on chemical fertilizers, and promote environmentally friendly farming practices (Lindström and Mousavi, 2019; Thompson and Lamp, 2021).

7 Technological and Environmental Considerations

7.1 Genetic engineering of rhizobia

Genetic engineering of rhizobia has emerged as a promising approach to enhance the efficiency of symbiotic nitrogen fixation (SNF) and improve legume growth. Advances in molecular biology have enabled the modification of rhizobial strains to increase their tolerance to abiotic stresses such as extreme temperatures, pH, salinity, and drought, which are critical for maintaining effective nitrogen fixation under varying environmental conditions (Mabrouk et al., 2018; Owaresat et al., 2023). Additionally, genetic modifications have been aimed at improving the nodulation process and nitrogen fixation efficiency even in the presence of high nitrate concentrations, which typically inhibit these processes (Nguyen et al., 2019). The development of SNF-efficient rhizobial species tailored to the genetic makeup of specific legume hosts has shown potential in enhancing the overall productivity and sustainability of legume crops (Goyal et al., 2021).

7.2 Ecological impact and environmental safety

The ecological impact and environmental safety of using genetically engineered rhizobia must be carefully considered. While the introduction of genetically modified organisms (GMOs) into the environment raises concerns, the benefits of enhanced nitrogen fixation and reduced reliance on chemical fertilizers are significant. Rhizobia play a crucial role in improving soil fertility and promoting sustainable agriculture by reducing the need for synthetic nitrogen fertilizers, which are associated with greenhouse gas emissions and nitrogen pollution (Goyal et al., 2021). However, it is essential to ensure that genetically engineered rhizobia do not disrupt existing microbial communities or lead to unintended ecological consequences. Studies have shown that rhizobia can adapt to various environmental conditions and maintain their symbiotic efficiency, which is promising for their safe integration into agricultural systems (Masson-Boivin and Sachs, 2018; Thompson and Lamp, 2021).

7.3 Integration with modern agricultural practices

Integrating genetically engineered rhizobia with modern agricultural practices involves developing effective inoculant formulations and application methods. The use of peat carrier-based inoculants has been shown to enhance nodulation, nitrogen fixation, and nutrient uptake in legumes such as *Vicia faba* (Allito et al., 2020). Additionally, novel rhizobial strains that exhibit superior nodulation and nitrogen fixation under high nitrate conditions can be particularly beneficial for agroecosystems where chemical fertilizers are commonly used (Nguyen et al., 2019). The compatibility between legumes and rhizobia is crucial for establishing successful nitrogen-fixing symbioses, and understanding the molecular mechanisms underlying this interaction can inform the development of more effective inoculants (Clúa et al., 2018). By integrating these advanced rhizobial strains into modern agricultural practices, it is possible to achieve higher crop yields, improved soil health, and reduced environmental impact (Mabrouk et al., 2018; Lindström and Mousavi, 2019; Schulte et al., 2021).

8 Concluding Remarks

The symbiotic relationship between rhizobia and legumes plays a crucial role in biological nitrogen fixation (BNF), which is essential for enhancing legume growth and soil fertility. Rhizobia infect legume roots, forming nodules where they convert atmospheric nitrogen into ammonia, a form usable by plants. This process not only benefits the host plants but also improves soil nitrogen levels, making it available for subsequent crops. The effectiveness of this symbiosis is influenced by various factors, including soil nitrate levels, herbivory, and the compatibility between specific rhizobia strains and legume genotypes. Additionally, the interaction between rhizobia and other soil microorganisms, such as arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR), further enhances nutrient acquisition and plant growth.

Rhizobia are pivotal in sustainable agriculture due to their ability to naturally fix nitrogen, reducing the need for chemical fertilizers. This not only lowers production costs but also minimizes environmental pollution associated with synthetic fertilizers. The integration of legumes into cropping systems through practices like crop rotation, intercropping, and green manuring can significantly improve soil fertility and crop yields. Moreover, the symbiotic relationship between rhizobia and legumes enhances the resilience of agricultural systems to biotic and abiotic stresses, promoting overall ecosystem health. The dual symbiosis with AMF further supports plant growth by improving nutrient uptake and soil structure.

Future research should focus on several key areas to further enhance the benefits of rhizobia in agriculture. There is a need to identify and develop more rhizobial strains that can perform well under various environmental stresses, such as high nitrate levels, extreme temperatures, and drought conditions. Understanding the molecular mechanisms and systemic signaling pathways that regulate nodule formation and function can provide insights into optimizing nitrogen fixation. In addition, exploring the interactions between rhizobia and other soil microorganisms, including both beneficial and parasitic species, can help develop integrated pest and nutrient management strategies. Field studies should also be conducted to evaluate the long-term impacts of rhizobial inoculation on soil health and crop productivity across different agroecosystems. By addressing these research areas, we can further harness the potential of rhizobia in promoting sustainable agriculture and enhancing global food security.

Acknowledgments

The MicroSci Publisher extends its sincere gratitude to the two anonymous peer reviewers for their contributions during the evaluation of this manuscript.

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.

References

Allito B., Ewusi-Mensah N., and Logah V., 2020, Legume-rhizobium strain specificity enhances nutrition and nitrogen fixation in faba bean (*Vicia faba* L.), Agronomy, 10(6): 826.
https://doi.org/10.3390/agronomy10060826

Bellabarba A., Fagorzi C., diCenzo G., Pini F., Viti C., and Checcucci A., 2019, Deciphering the symbiotic plant microbiome: translating the most recent discoveries on rhizobia for the improvement of agricultural practices in metal-contaminated and high saline lands, Agronomy, 9(9): 529.
https://doi.org/10.3390/agronomy9090529

Chakraborty S., Valdés-López O., Stonoha-Arther C., and Ané J., 2022, Transcription factors controlling the rhizobium-legume symbiosis: integrating infection, organogenesis, and the abiotic environment, Plant & Cell Physiology, 63(10): 1326-1343.
https://doi.org/10.1093/pcp/pcac063

Clúa J., Roda C., Zanetti M., and Blanco F., 2018, Compatibility between legumes and rhizobia for the establishment of a successful nitrogen-fixing symbiosis, Genes, 9(3): 125.
https://doi.org/10.3390/genes9030125

Costa S., Ng J., and Mathesius U., 2021, Interaction of symbiotic rhizobia and parasitic root-knot nematodes in legume roots-from molecular regulation to field application, Molecular Plant-Microbe Interactions : MPMI, 34(5): 470-490.
https://doi.org/10.1094/MPMI-12-20-0350-FI

Dupin S., Dupin S., Geurts R., and Kiers E., 2020, The non-legume *Parasponia andersonii* mediates the fitness of nitrogen-fixing rhizobial symbionts under high nitrogen conditions, Frontiers in Plant Science, 10: 1779.
https://doi.org/10.3389/fpls.2019.01779

Fahde S., Boughribil S., Sijilmassi B., and Amri A., 2023, Rhizobia: a promising source of plant growth-promoting molecules and their non-legume interactions: examining applications and mechanisms, Agriculture, 13(7): 1279.
https://doi.org/10.3390/agriculture13071279

Goyal R., Mattoo A., and Schmidt, M., 2021, Rhizobial-host interactions and symbiotic nitrogen fixation in legume crops toward agriculture sustainability, Frontiers in Microbiology, 12: 669404.
https://doi.org/10.3389/fmicb.2021.669404

Kimeklis A., Chirak E., Kuznetsova I., Sazanova A., Safronova V., Belimov A., Onishchuk O., Kurchak O., Aksenova T., Pinaev A., Andronov E., and Provorov N., 2019, Rhizobia isolated from the relict legume *Vavilovia formosa* represent a genetically specific group within *Rhizobium leguminosarum* biovar viciae, Genes, 10(12): 991.
https://doi.org/10.3390/genes10120991

Lepetit M., and Brouquisse R., 2023, Control of the rhizobium-legume symbiosis by the plant nitrogen demand is tightly integrated at the whole plant level and requires inter-organ systemic signaling, Frontiers in Plant Science, 14: 1114840.
https://doi.org/10.3389/fpls.2023.1114840

Lindström K., and Mousavi S., 2019, Effectiveness of nitrogen fixation in rhizobia, Microbial Biotechnology, 13: 1314-1335.
https://doi.org/10.1111/1751-7915.13517

Liu A., Contador C., Fan K., and Lam H., 2018a, Interaction and regulation of carbon, nitrogen, and phosphorus metabolisms in root nodules of legumes, Frontiers in Plant Science, 9: 1860.
https://doi.org/10.3389/fpls.2018.01860

Liu H., Zhang C., Yang J., Yu N., and Wang E., 2018b, Hormone modulation of legume-rhizobial symbiosis, Journal of Integrative Plant Biology, 60(8): 632-648.
https://doi.org/10.1111/jipb.12653

Mabrouk Y., Hemissi I., Salem I., Mejri S., Saidi M., and Belhadj O., 2018, Potential of rhizobia in improving nitrogen fixation and yields of legumes, Symbiosis, 13(4): 1-20.
https://doi.org/10.5772/INTECHOPEN.73495

Masson-Boivin C., and Sachs J., 2018, Symbiotic nitrogen fixation by rhizobia-the roots of a success story, Current Opinion in Plant Biology, 44: 7-15.
https://doi.org/10.1016/j.pbi.2017.12.001

Mei P., Wang P., Yang H., Gui L., Christie P., and Li L., 2021, Maize/faba bean intercropping with rhizobial inoculation in a reclaimed desert soil enhances productivity and symbiotic N_2 fixation and reduces apparent N losses, Soil & Tillage Research, 213: 105154.
https://doi.org/10.1016/J.STILL.2021.105154

Nandasena K., O'Hara G., Tiwari R., Sezmiş E., and Howieson J., 2007, *In situ* lateral transfer of symbiosis islands results in rapid evolution of diverse competitive strains of mesorhizobia suboptimal in symbiotic nitrogen fixation on the pasture legume *Biserrula pelecinus* L., Environmental Microbiology, 9(10): 2496-2511.
https://doi.org/10.1111/J.1462-2920.2007.01368.X

Nguyen H., Miwa H., Obirih-Opareh J., Suzaki T., Yasuda M., and Okazaki S., 2019, Novel rhizobia exhibit superior nodulation and biological nitrogen fixation even under high nitrate concentrations, FEMS Microbiology Ecology, 96(2): fiz184.
https://doi.org/10.1093/femsec/fiz184

Oldroyd G., Murray J., Poole P., and Downie J., 2011, The rules of engagement in the legume-rhizobial symbiosis, Annual Review of Genetics, 45: 119-144.
https://doi.org/10.1146/annurev-genet-110410-132549

Owaresat J., Siam M., Dey D., Jabed S., Badsha F., Islam M., and Kabir M., 2023, Factors impacting rhizobium-legume symbiotic nitrogen fixation with the physiological and genetic responses to overcome the adverse conditions: a review, Agricultural Reviews, 44(1): 22-30.
https://doi.org/10.18805/ag.rf-257

Pankievicz V., Irving T., Maia L., and Ané J., 2019, Are we there yet? The long walk towards the development of efficient symbiotic associations between nitrogen-fixing bacteria and non-leguminous crops, BMC Biology, 17: 99.
https://doi.org/10.1186/s12915-019-0710-0

Rodriguez C., Carlsson G., Englund J., Flöhr A., Pelzer E., Jeuffroy M., Makowski D., and Jensen E., 2020, Grain legume-cereal intercropping enhances the use of soil-derived and biologically fixed nitrogen in temperate agroecosystems, a meta-analysis, European Journal of Agronomy, 118: 126077.
https://doi.org/10.1016/j.eja.2020.126077

Sachs J., Quides K., and Wendlandt C., 2018, Legumes versus rhizobia: a model for ongoing conflict in symbiosis, The New Phytologist, 219(4): 1199-1206.
https://doi.org/10.1111/nph.15222

Sauviac L., Rémy A., Huault E., Dalmasso M., Kazmierczak T., Jardinaud M., Legrand L., Moreau C., Ruiz B., Cazalé A., Valière S., Gourion B., Dupont L., Gruber V., Boncompagni E., Meilhoc E., Frendo P., Frugier F., and Bruand C., 2022, A dual legume-rhizobium transcriptome of symbiotic nodule senescence reveals coordinated plant and bacterial responses, Plant, Cell & Environment, 45(10): 3100-3121.
https://doi.org/10.1111/pce.14389

Schulte C., Borah K., Wheatley R., Terpolilli J., Saalbach G., Crang N., Groot D., Ratcliffe R., Kruger N., Papachristodoulou A., and Poole P., 2021, Metabolic control of nitrogen fixation in rhizobium-legume symbioses, Science Advances, 7(31): eabh2433.
https://doi.org/10.1126/sciadv.abh2433

Sindhu S., Sharma R., Sindhu S., and Sehrawat A., 2019, Soil fertility improvement by symbiotic rhizobia for sustainable agriculture, Soil Fertility Management for Sustainable Development, 7: 101-166.
https://doi.org/10.1007/978-981-13-5904-0_7

Teulet A., Busset N., Fardoux J., Gully D., Chaintreuil C., Cartieaux F., Jauneau A., Comorge V., Okazaki S., Kaneko T., Gressent F., Nouwen N., Arrighi J., Koebnik R., Mergaert P., Deslandes L., and Giraud E., 2019, The rhizobial type III effector ErnA confers the ability to form nodules in legumes, Proceedings of the National Academy of Sciences of the United States of America, 116: 21758-21768.
https://doi.org/10.1073/pnas.1904456116

Thamer S., Schädler M., Bonte D., and Ballhorn D., 2011, Dual benefit from a belowground symbiosis: nitrogen fixing rhizobia promote growth and defense against a specialist herbivore in a cyanogenic plant, Plant and Soil, 341: 209-219.
https://doi.org/10.1007/s11104-010-0635-4

Thompson M., and Lamp W., 2021, Herbivory enhances legume-rhizobia symbioses function, increasing aboveground allocation of biologically fixed nitrogen, but only in soils without additional nitrate, Plant and Soil, 465: 301-316.
https://doi.org/10.1007/s11104-021-04999-6

Tsikou D., Yan Z., Holt D., Abel N., Reid D., Madsen L., Bhasin H., Sexauer M., Stougaard J., and Markmann K., 2018, Systemic control of legume susceptibility to rhizobial infection by a mobile microRNA, Science, 362: 233-236.
https://doi.org/10.1126/science.aat6907

Velzen R., Holmer R., Bu F., Rutten L., Zeijl A., Liu W., Santuari L., Cao Q., Sharma T., Shen D., Roswanjaya Y., Wardhani T., Kalhor M., Jansen J., Hoogen J., Güngör B., Hartog M., Hontelez J., Verver J., Yang W., Schijlen E., Repin R., Schilthuizen, M., Schranz M., Heidstra R., Miyata K., Fedorova E., Kohlen W., Bisseling T., Smit S., and Geurts R., 2018, Comparative genomics of the nonlegume *Parasponia* reveals insights into evolution of nitrogen-fixing rhizobium symbioses, Proceedings of the National Academy of Sciences of the United States of America, 115(20): E4700-E4709.
https://doi.org/10.1073/pnas.1721395115

Wang Q., Liu J., and Zhu H., 2018, Genetic and molecular mechanisms underlying symbiotic specificity in legume-rhizobium interactions, Frontiers in Plant Science, 9: 313.
https://doi.org/10.3389/fpls.2018.00313

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