Field Crop 2024, Vol.7, No.2, 58-69
http://cropscipublisher.com/index.php/fc

Review Article Open Access

Nitrogen Fixation in Legumes: Genetic Mechanisms and Agricultural Applications

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Field Crop, 2024 Vol.7, No.2 doi: 10.5376/fc.2024.07.0007
Received: 13 Jan., 2024
Accepted: 24 Feb., 2024
Published: 16 Mar., 2024

Preferred citation for this article:
Huang W.Z., 2024, Nitrogen fixation in legumes: genetic mechanisms and agricultural applications, Field Crop, 7(2): 58-69 (doi: 10.5376/fc.2024.07.0007)

Abstract Biological nitrogen fixation (BNF) in legumes is a critical process that significantly contributes to sustainable agriculture by reducing the need for synthetic fertilizers. This study provides a comprehensive examination of the historical background, genetic mechanisms, symbiotic relationships, agricultural applications, and environmental impacts of nitrogen fixation in legumes. Key discoveries in legume nitrogen fixation and the evolution of symbiotic nitrogen fixation (SNF) research are highlighted, along with the identification of essential genes and genetic pathways involved in SNF. Advances in genetic modification techniques aimed at enhancing nitrogen fixation are discussed. This study also explores the role of rhizobia in nodule formation, plant-microbe signaling, and the benefits of incorporating legumes into cropping systems. Case studies demonstrating successful agricultural implementations and the environmental benefits of BNF are presented, emphasizing the reduction in synthetic fertilizer use and improvements in soil health. A detailed analysis of a case study on genetic modification in soybeans is included, providing insights into future agricultural practices. This study concludes by addressing the challenges and limitations of current genetic and agronomic approaches, proposing potential solutions, and highlighting future research directions. The integration of emerging technologies in genetic engineering and microbiome manipulation, along with the prospects for transferring nitrogen-fixing capabilities to non-legume crops, are discussed. This study underscores the importance of continued research and development in enhancing nitrogen fixation for sustainable agriculture.
Keywords Biological nitrogen fixation; Legumes; Symbiotic nitrogen fixation; Genetic modification; Sustainable agriculture

1 Introduction

Nitrogen fixation is a critical biological process that allows certain plants, particularly legumes, to convert atmospheric nitrogen (N_2) into a form that is usable by plants, such as ammonia (NH_3). This process is facilitated by symbiotic relationships between legumes and nitrogen-fixing bacteria, primarily rhizobia, which inhabit root nodules of the host plants. The symbiotic relationship between legumes and rhizobia is highly specialized and involves complex signaling pathways and genetic mechanisms that ensure successful colonization and nitrogen fixation (Mahmud et al., 2020; Kebede, 2021). The ability of legumes to fix atmospheric nitrogen not only benefits the legumes themselves but also enhances soil fertility, making nitrogen available to subsequent crops in a rotation system (Liu et al., 2011; Iannetta et al., 2016).

Biological nitrogen fixation (BNF) plays a pivotal role in sustainable agriculture by reducing the need for synthetic nitrogen fertilizers, which are associated with environmental issues such as nitrate pollution and greenhouse gas emissions (Mahmud et al., 2020; Ma et al., 2022). The integration of legumes into cropping systems can significantly improve soil health and productivity by increasing the availability of nitrogen and other nutrients, breaking pest cycles, and enhancing soil microbial activity (Olivares et al., 2013; Kebede, 2021). Moreover, BNF contributes to the overall nitrogen balance in agricultural ecosystems, supporting higher yields and reducing the dependency on chemical fertilizers (Iannetta et al., 2016; Batista and Dixon, 2019). The adoption of legume-based cropping systems and the development of biofertilizers that exploit BNF are essential strategies for achieving sustainable agricultural practices and mitigating the adverse effects of intensive fertilizer use (Olivares et al., 2013; Ma et al., 2022).

This study summarizes the current knowledge on the genetic and molecular basis of symbiotic nitrogen fixation in legumes, including the identification and functional characterization of key genes involved in the process. Additionally, it discusses the agronomic benefits of incorporating legumes into cropping systems, focusing on their role in enhancing soil fertility, reducing the need for synthetic fertilizers, and promoting sustainable agricultural practices. This study also highlights recent advancements in the manipulation of nitrogen-fixing mechanisms and the potential for transferring these capabilities to non-legume crops to further improve agricultural productivity and sustainability. By addressing these objectives, this study seeks to underscore the importance of BNF in legumes and its potential to revolutionize agricultural practices, contributing to global food security and environmental sustainability.

2 Historical Background

2.1 Early discoveries in legume nitrogen fixation

The phenomenon of nitrogen fixation in legumes has been recognized for over a century. Early studies identified the unique ability of legumes to enrich soil fertility through their symbiotic relationship with nitrogen-fixing bacteria, primarily rhizobia. This symbiosis was first observed in the late 19th century, leading to the understanding that legumes could convert atmospheric nitrogen into a form usable by plants, thus reducing the need for synthetic fertilizers (Kebede, 2021).

2.2 Evolution of research in symbiotic nitrogen fixation (SNF)

Research into symbiotic nitrogen fixation (SNF) has evolved significantly over the past few decades. Since 1999, various genetic approaches have uncovered nearly 200 genes required for SNF in legumes. These discoveries have advanced our understanding of the evolution of SNF in plants and its relationship to other beneficial endosymbioses. Key areas of progress include signaling between plants and microbes, control of microbial infection of plant cells, nodule development, and the regulation of nodule senescence (Roy et al., 2020). Additionally, the integration of transcriptomic and metabolomic analyses has revealed that SNF enhances drought resistance in legumes, further highlighting the multifaceted benefits of this symbiotic relationship (López et al., 2023).

2.3 Milestones in BNF research

Several milestones have marked the progress of biological nitrogen fixation (BNF) research. The development of dynamic vegetation models, such as LPJ-GUESS, has enabled the global quantification of nitrogen fixation rates and crop yields, providing valuable insights into the role of BNF in agricultural sustainability (Ma et al., 2022). Furthermore, the identification of a symbiotic flowering pathway in legumes has elucidated how fixed nitrogen and symbiotic signals promote reproductive success, thereby enhancing legume growth and production in nitrogen-poor soils (Figure 1) (Yun et al., 2023). The ongoing efforts to transfer nitrogen-fixing mechanisms from legumes to non-legumes, such as rice, maize, and wheat, represent a significant frontier in BNF research, with the potential to revolutionize agricultural practices and reduce dependency on synthetic fertilizers (Pankievicz et al., 2019; Mahmud et al., 2020). In summary, the historical background of nitrogen fixation in legumes encompasses early discoveries, the evolution of SNF research, and key milestones that have shaped our current understanding and application of this critical biological process. The integration of genetic, molecular, and ecological studies continues to drive advancements in this field, promising sustainable agricultural solutions for the future.

3 Genetic Mechanisms of Nitrogen Fixation

3.1 Key genes involved in SNF

Symbiotic nitrogen fixation (SNF) in legumes is a complex process that involves numerous genes. Research has identified nearly 200 genes essential for SNF in model legumes such as *Medicago truncatula* and *Lotus japonicus*, as well as in crop species like soybean (*Glycine max*) and common bean (*Phaseolus vulgaris*) (Roy et al., 2020). These genes are involved in various stages of the symbiotic process, including signaling between plants and microbes, microbial infection of plant cells, nodule development, and the control of bacteroid differentiation and nodule senescence. Key genes such as those encoding for nodulation factors and receptors, transcription factors, and transporters play crucial roles in these processes (Roy et al., 2020).

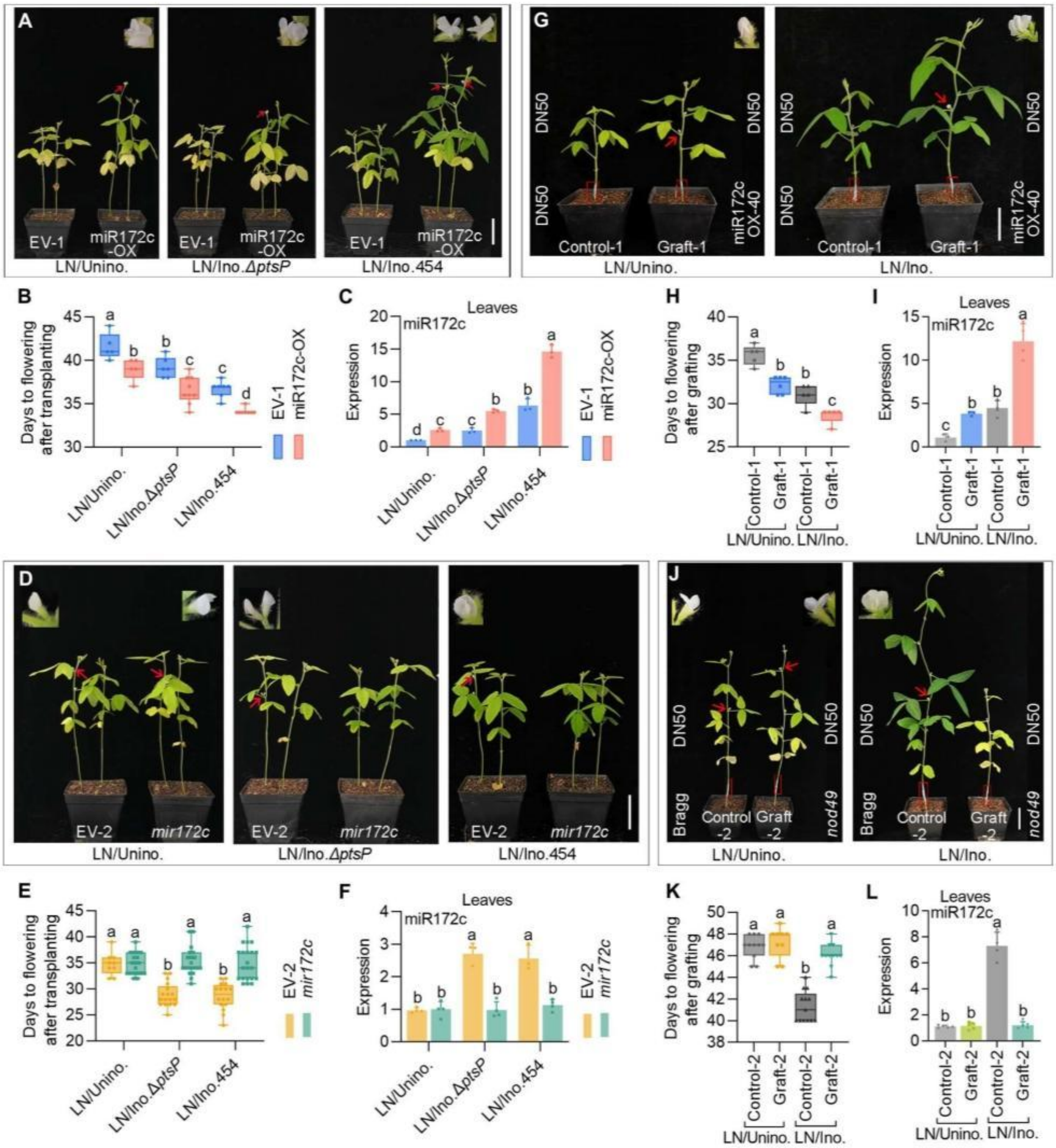

Figure 1 Long-distance transmission of symbiotic *miR172c* accelerates flowering (Adopted from Yun et al., 2023)

Image caption: (A) Phenotypes of composite plants expressing EV-1 and 35S: miR172c (miR172c-OX) inoculated without or with *S. fredii* CCBAU 45436 or the mutantstrain *ΔptsP* under LN conditions. (B) Daysfrom transplanting to flowering of EV-1 and miR172c-OX under the above treatments. Data are means±SDs (n≥5). (C) Quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis of miR172c expression in leaves of different EV-1 and miR172c-OX treatments. Data are means ± SDs (n=3). (D) Phenotype of composite plants expressing EV-2 or CRISPR-Cas9 knockout *mir172c* roots inoculated without or with *S. fredii* CCBAU 45436 or mutant strain *ΔptsP* under LN conditions. (E) Days from transplanting to flowering of composite plants described in (D). Data are means±SDs (n≥10). (F) qRT-PCR analysis of miR172c abundance in leaves of the EV-2 and mir172c composite plantsin (D). Data are means±SDs (n≥3). (G and H) Phenotypes and flowering time of self-grafted wild-type DN50 (left) and intraspecific grafts between a wild-type scion and stale miR172cOX-40 rootstock (right) inoculated without and with *B. diazoefficiens* USDA110 under LN conditions. Data are means±SDs (n≥5). (I) miR172c abundance in leaves of grafted plants in (G). Data are means±SDs (n≥3). (J and K) Phenotypes and flowering time of grafted plants with non-nodulating mutant nod49 root stock inoculated without or with *B. diazoefficiens* USDA110 under LN conditions. Data are means±SDs (n≥10). (L) miR172c abundance in leaves of grafted plants shown in (J). Data are means±SDs (n≥4). Photos were taken at appearance of the first flowering. For each phenotype photo, red arrows indicate the first flowering buds, and red boxes indicate grafted sites. Scale bars, 5 cm. One-way ANOVA with Tukey's test was used for statistical analysis ($P≤0.05$) (Adopted from Yun et al., 2023)

3.2 Genetic pathways and regulatory mechanisms

The genetic pathways and regulatory mechanisms governing SNF are intricate and tightly controlled. The interaction between legumes and rhizobia involves a sophisticated signaling network that ensures successful symbiosis. This includes the perception of rhizobial signals by plant receptors, activation of downstream signaling cascades, and regulation of gene expression to facilitate nodule formation and function (Roy et al., 2020). For instance, the symbiotic microRNA miR172c has been shown to play a role in coupling symbiotic and nutritional signals to promote flowering in soybeans, highlighting the integration of SNF with other physiological processes (Yun et al., 2023). Additionally, the interplay between nitrogen fixation and phosphorus nutrition is regulated through specific signaling crosstalk, which is essential for efficient SNF (Figure 2) (Zhong et al., 2022).

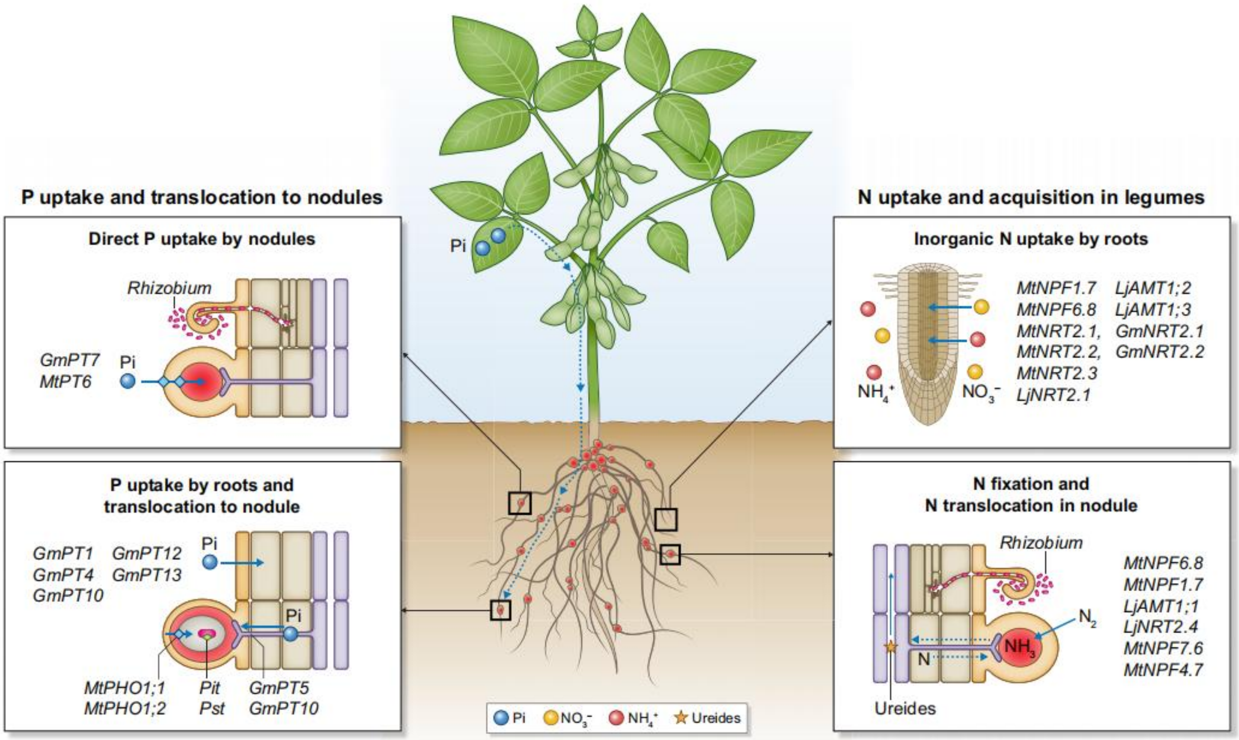

Figure 2 Uptake and translocation of phosphorus (P) and nitrogen (N) to legume nodules (Adopted from Zhong et al., 2022)

Image caption: Transporters involved in direct and indirect uptake of P to nodules and the acquisition of N in legumes are shown. Nodules have two ways for P acquisition: direct and indirect uptake. Direct uptake is mediated by transporters expressed in the nodules, such as GmPT7 and MtPT6, while indirect uptake includes uptake of P by host plant roots and translocation from roots to the nodules through transporters such as GmPT4, GmPT5, and GmPT10, followed by loading to the fixation zone byMtPHO1;1 and MtPHO1;2. On the contrary, legumes could take up inorganic N in the form of NO_3 and NH_4^+ by nitrate transporters (MtNPF1.7, MtNPF6.8, MtNRT2.1, MtNRT2.1, MtNRT2.3, LjNTR2.1, GmNRT2.1, and GmNRT2.2) and/or ammonium transporters (LjAMT1;2 and LjAMT1;3), respectively. Also, legumes could obtain N from the atmosphere through symbiotic nitrogen fixation with rhizobia in nodules. Pit, inorganic phosphate transporter; Pst, phosphate specific transporter (Adopted from Zhong et al., 2022)

3.3 Advances in genetic modification to enhance nitrogen fixation

Recent advances in genetic modification have opened new avenues for enhancing nitrogen fixation in legumes. Techniques such as genome editing and mutagenesis have been employed to improve SNF traits. For example, breeding programs have focused on enhancing symbiotic tolerance to nitrate, inducing supernodulation, and promoting selective nodulation (Herridge and Rose, 2000). Moreover, efforts are underway to transfer nitrogen-fixing capabilities to non-legume crops, which could revolutionize agricultural practices by reducing the reliance on synthetic nitrogen fertilizers (Mahmud et al., 2020). The integration of SNF genetics into mainstream breeding programs and the use of dynamic simulation models to predict and optimize nitrogen fixation under various environmental conditions are also promising strategies for future improvements (Herridge and Rose, 2000; Liu et al., 2011).

4 Symbiotic Relationships

4.1 Role of rhizobia in nitrogen fixation

Rhizobia are soil bacteria that form symbiotic relationships with leguminous plants, leading to the formation of root nodules where nitrogen fixation occurs. This process is crucial for converting atmospheric nitrogen (N_2) into ammonia (NH_3), which plants can use for growth. Rhizobia's ability to fix nitrogen has been a subject of extensive research due to its ecological and agricultural importance. The symbiotic relationship between rhizobia and legumes has evolved to be highly efficient, allowing these plants to thrive in nitrogen-poor soils (Masson-Boivin and Sachs, 2018; Lindström and Mousavi, 2019).

4.2 Mechanisms of nodule formation and function

Nodule formation begins with the recognition of rhizobial Nod factors by the plant, which triggers a series of events including root hair curling, infection thread formation, and cortical cell division. These processes lead to the development of nodules, specialized structures where nitrogen fixation takes place. Within the nodules, rhizobia differentiate into bacteroids, which are capable of fixing nitrogen. The plant tightly regulates this process to ensure efficient nitrogen fixation while maintaining control over the bacterial population (Oldroyd et al., 2011; Roy et al., 2020; Lepetit and Brouquisse, 2023).

4.3 Plant-microbe signaling and interaction

The interaction between legumes and rhizobia involves complex signaling pathways. The plant releases flavonoids that attract rhizobia, which in turn produce Nod factors to initiate nodule formation. This signaling is highly specific and ensures that only compatible rhizobia infect the plant. Additionally, systemic signaling mechanisms within the plant adjust nodule formation and function based on the plant's nitrogen status. For instance, if the plant has sufficient nitrogen, nodule formation is inhibited, and existing nodules may senesce. Conversely, nitrogen deficiency can stimulate nodule formation and enhance nitrogen fixation (Figure 3) (Wang et al., 2018; Lepetit and Brouquisse, 2023).

5 Agricultural Applications

5.1 Benefits of incorporating legumes in cropping systems

Incorporating legumes into cropping systems offers numerous benefits, primarily due to their ability to fix atmospheric nitrogen through symbiosis with rhizobia. This process, known as biological nitrogen fixation (BNF), enhances soil fertility and reduces the need for synthetic nitrogen fertilizers, thereby promoting sustainable agriculture (Peoples et al., 2009; Iannetta et al., 2016; Kebede, 2021). Legumes also contribute to the diversification of cropping systems, which can improve resilience against pests and diseases and enhance overall ecosystem services (Rodriguez et al., 2020; Kebede, 2021). Additionally, the inclusion of legumes in crop rotations or intercropping systems can lead to improved yields of subsequent crops due to the residual nitrogen left in the soil (Peoples et al., 2009; Iannetta et al., 2016).

5.2 Techniques for measuring and enhancing nitrogen fixation in the field

Several techniques are employed to measure and enhance nitrogen fixation in the field. These include the use of isotopic methods, such as the 15N natural abundance method, and non-isotopic methods, like the acetylene reduction assay (Herridge and Rose, 2000; Liu et al., 2011; Anglade et al., 2015). Enhancing nitrogen fixation can be achieved through the selection and breeding of legume varieties with high BNF potential, inoculation with effective rhizobial strains, and optimizing agronomic practices such as proper planting density and intercropping arrangements (Herridge and Rose, 2000; Liu et al., 2011; Kebede, 2021). Additionally, the use of nitrate-tolerant legumes and the integration of legumes into low-input cropping systems can further enhance nitrogen fixation and overall system productivity (Liu et al., 2011).

5.3 Case studies of successful agricultural implementations

Several case studies highlight the successful implementation of legumes in agricultural systems. For instance, intercropping grain legumes with cereals has been shown to increase the use of soil-derived and biologically fixed nitrogen, thereby enhancing yields and reducing the need for external nitrogen inputs (Rodriguez et al., 2020). In Europe, historical data from experimental cropping systems have demonstrated that legume-derived nitrogen can maintain productivity and reduce the reliance on mineral fertilizers (Iannetta et al., 2016). Another example is the use of legume-based cropping systems in temperate agroecosystems, which has been shown to improve nitrogen use efficiency and overall sustainability (Anglade et al., 2015; Rodriguez et al., 2020). These case studies underscore the potential of legumes to contribute significantly to sustainable agricultural practices through enhanced nitrogen fixation and improved soil fertility.

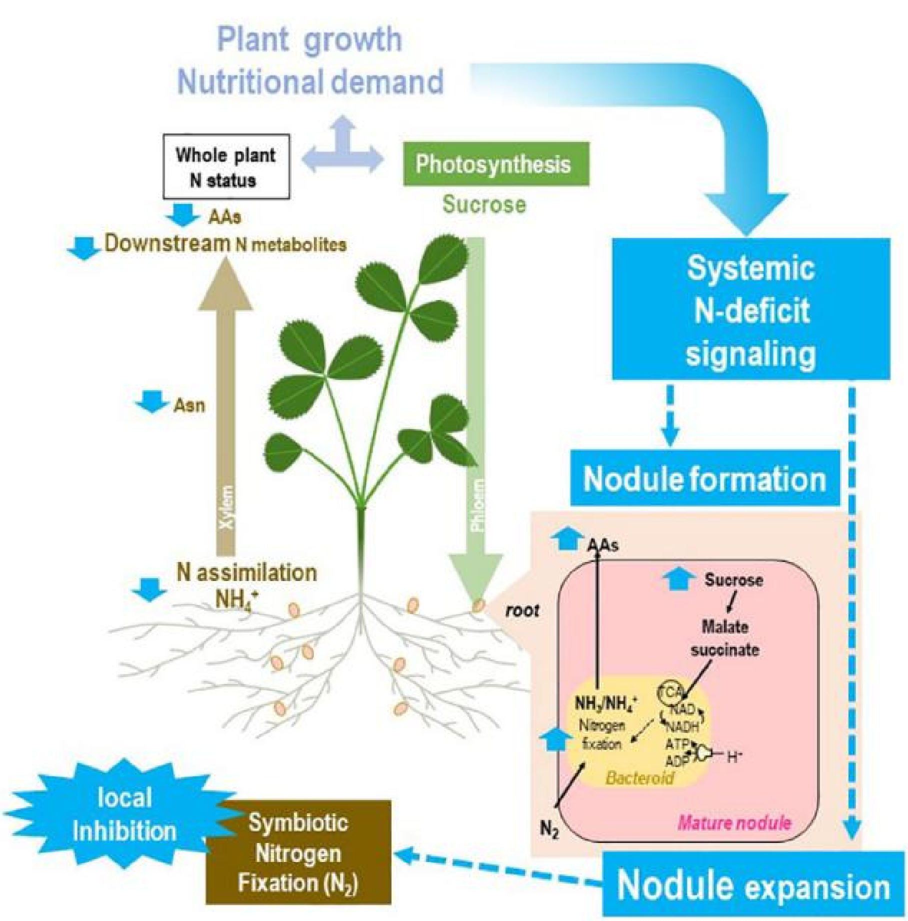

Figure 3 Systemic responses of the rhizobium-legume holobiont to plant N deficit (Adopted from Lepetit and Brouquisse, 2023)

Image caption: Various steps of the regulatory loop are indicated in blue. A local suppression of SNF may be obtained artificially (by replacing locally air by a mixture Ar/O_2 80/20 v/v) or as the result of abiotic stresses. The local inhibition of symbiosis in the roots exposed to these conditions results in a partial decrease of the whole plant SNF. As the whole plant N demand is not fully satisfied, the systemic signaling promoting symbiosis is activated, resulting in the formation of new nodules on the other roots not exposed to the constraint. In mature nodules of these roots, the Ndeficit systemic signaling results in a strong increase in nodule sucrose and organic acid levels associated with nodule expansion. This increase in nodule biomass is associated with higher levels of SNF in roots not exposed to the local constraint that may compensate the plant N deficit (Adopted from Lepetit and Brouquisse, 2023)

6 Environmental Impact

6.1 Reduction of synthetic fertilizer use through BNF

Biological nitrogen fixation (BNF) in legumes significantly reduces the need for synthetic nitrogen fertilizers in agricultural systems. Legumes, through their symbiotic relationship with rhizobia, can fix atmospheric nitrogen, making it available to the plant and subsequently to the soil. This process not only enhances soil fertility but also reduces the dependency on synthetic fertilizers, which are often costly and environmentally damaging (Iannetta et al., 2016; Kessel and Hartley, 2000; Kebede, 2021). Intercropping legumes with cereals has been shown to increase the efficiency of nitrogen use, further reducing the need for synthetic fertilizers (Rodriguez et al., 2020; Jensen et al., 2020). This practice can lead to a reduction in the global requirement for synthetic nitrogen fertilizers by approximately 26% (Jensen et al., 2020).

6.2 Environmental benefits and challenges

The environmental benefits of BNF are substantial. By reducing the need for synthetic fertilizers, BNF helps to lower greenhouse gas emissions associated with fertilizer production and application (Rodriguez et al., 2020; Kebede, 2021). Additionally, BNF contributes to the reduction of nitrate pollution in water bodies, which is a common issue with synthetic fertilizers (Iannetta et al., 2016; Mahmud et al., 2020). However, there are challenges associated with maximizing the benefits of BNF. The efficiency of nitrogen fixation can be influenced by various factors, including soil properties, legume species, and environmental conditions (Kessel and Hartley, 2000). Moreover, the persistence and productivity of legumes in mixed cropping systems can be affected by competition with other plants and soil nutrient status.

6.3 Impact on soil health and ecosystem sustainability

BNF plays a crucial role in improving soil health and promoting ecosystem sustainability. The incorporation of legumes into cropping systems enhances soil structure, increases organic matter content, and promotes microbial activity (Iannetta et al., 2016; Kebede, 2021). These improvements in soil health can lead to better water retention, reduced soil erosion, and increased resilience of the agroecosystem (Rodriguez et al., 2020; Kessel and Hartley, 2000). Furthermore, the use of legumes in crop rotations and intercropping systems can break pest and disease cycles, reducing the need for chemical pesticides and promoting biodiversity (Jensen et al., 2020; Kebede, 2021). However, the long-term sustainability of BNF depends on the careful management of cropping systems to maintain the balance between nitrogen fixation and soil nitrogen levels (Kessel and Hartley, 2000).

7 Case Study

7.1 Detailed analysis of a specific case where genetic modification improved nitrogen fixation in soybeans

A notable case of genetic modification enhancing nitrogen fixation in soybeans involves the identification and manipulation of specific genes responsible for symbiotic nitrogen fixation (SNF). Research has uncovered nearly 200 genes required for SNF in legumes, including soybeans (*Glycine max*) (Roy et al., 2020). One significant advancement was the development of soybean varieties with enhanced symbiotic tolerance to nitrate, which traditionally inhibits nitrogen fixation. This was achieved through mutagenesis-induced supernodulation, leading to increased nodule formation and nitrogen fixation even in the presence of nitrate (Herridge and Rose, 2000).

7.2 Results and implications for future agricultural practices

The genetic modifications in soybeans have shown promising results. Enhanced nitrogen fixation has led to improved plant growth and yield, particularly in nitrogen-poor soils. For instance, the introduction of supernodulation traits resulted in a significant increase in the amount of nitrogen fixed by the plants, thereby reducing the need for synthetic nitrogen fertilizers (Herridge and Rose, 2000). This not only lowers production costs for farmers but also mitigates environmental issues associated with excessive fertilizer use, such as nitrate pollution (Santi et al., 2013; Mahmud et al., 2020). The implications for future agricultural practices are profound. By integrating genetically modified soybeans with enhanced nitrogen fixation capabilities into mainstream breeding programs, it is possible to develop cultivars that are more resilient to varying soil nitrogen levels. This can lead to more sustainable agricultural systems that rely less on chemical inputs and more on natural biological processes (Herridge and Rose, 2000; Yun et al., 2023).

7.3 Future prospects and recommendations

Looking ahead, the future prospects for genetically modified soybeans with improved nitrogen fixation are promising. Continued research into the genetic mechanisms underlying symbiotic nitrogen fixation (SNF) can lead to the discovery of new genes and pathways that can be targeted for further improvements. The use of advanced genome editing technologies, such as CRISPR/Cas9, holds potential for precise modifications that can enhance nitrogen fixation efficiency even further (Roy et al., 2020). Recommendations for future research and agricultural practices include the integration of nitrogen fixation traits into broader legume breeding programs to develop high-yielding, nitrogen-efficient cultivars (Herridge and Rose, 2000). Conducting extensive field trials to validate the performance of genetically modified soybeans under diverse environmental conditions and management practices is crucial (Rodriguez et al., 2020; Ma et al., 2022). Promoting the use of these genetically modified soybeans in sustainable agricultural practices, such as crop rotation and intercropping, can maximize the benefits of biological nitrogen fixation. Additionally, developing policies and regulatory frameworks that support the adoption of genetically modified crops while ensuring environmental safety and public acceptance is essential (Herridge et al., 2008). By following these recommendations, the agricultural sector can harness the full potential of genetic modifications to improve nitrogen fixation in soybeans, leading to more sustainable and productive farming systems.

8 Challenges and Limitations

8.1 Current limitations in genetic and agronomic approaches

Despite significant advancements in understanding the genetic mechanisms underlying nitrogen fixation in legumes, several limitations persist. One major challenge is the variability in nitrogen fixation efficiency among different legume species and even among cultivars within a species. This variability complicates breeding programs aimed at enhancing nitrogen fixation traits (Herridge and Rose, 2000; Kebede, 2021). Additionally, the effectiveness of the rhizobia-host plant symbiosis is influenced by environmental factors such as soil nitrogen levels and other abiotic stresses, which can limit the overall nitrogen fixation capacity (Kessel and Hartley, 2000). Furthermore, the integration of nitrogen-fixing traits into non-legume crops through genetic engineering remains a long-term goal, with current efforts facing significant technical and regulatory hurdles (Pankievicz et al., 2019; Mahmud et al., 2020).

8.2 Barriers to widespread adoption of nitrogen-fixing crops

The adoption of nitrogen-fixing crops in agricultural systems is hindered by several factors. Firstly, the economic and political landscape often favors the use of synthetic nitrogen fertilizers due to their immediate and predictable effects on crop yields (Olivares et al., 2013). Secondly, there is a lack of awareness and knowledge among farmers regarding the benefits and management practices required for optimizing biological nitrogen fixation (BNF) (Kebede, 2021)). Additionally, the initial costs associated with adopting new cropping systems, such as purchasing legume seeds and inoculants, can be prohibitive for small-scale farmers (Rodriguez et al., 2020). The variability in nitrogen fixation efficiency and the potential for lower yields compared to non-legume crops also contribute to the reluctance in adopting these systems (Iannetta et al., 2016).

8.3 Potential solutions and future research directions

To overcome these challenges, several strategies can be employed. Enhancing communication and collaboration between researchers, farmers, and policymakers is crucial for the successful implementation of nitrogen-fixing crops. Breeding programs should focus on integrating nitrogen fixation traits into mainstream legume breeding efforts and evaluating these traits under low nitrogen conditions to ensure their effectiveness (Herridge and Rose, 2000). Additionally, interdisciplinary research efforts involving synthetic biologists, microbiologists, and agronomists are needed to engineer nitrogen-fixing capabilities in non-legume crops (Pankievicz et al., 2019; Mahmud et al., 2020).

Improving the availability and effectiveness of biofertilizers and rhizobial inoculants can also enhance nitrogen fixation in agricultural systems (Olivares et al., 2013). Developing sustainable agronomic practices, such as intercropping and crop rotation with legumes, can maximize the benefits of BNF and reduce the reliance on synthetic fertilizers (Rodriguez et al., 2020; Kebede, 2021). Finally, increasing farmer education and providing financial incentives for adopting nitrogen-fixing crops can facilitate their widespread adoption and contribute to more sustainable agricultural practices (Kessel and Hartley, 2000; Kebede, 2021).

By addressing these challenges through coordinated research and policy efforts, the potential of nitrogen-fixing crops to enhance agricultural sustainability and productivity can be fully realized.

9 Future Directions

9.1 Emerging technologies in genetic engineering and microbiome manipulation

The future of nitrogen fixation in legumes is poised to benefit significantly from advancements in genetic engineering and microbiome manipulation. Recent research has identified nearly 200 genes involved in symbiotic nitrogen fixation (SNF) in legumes, providing a robust foundation for genetic modifications aimed at enhancing this process (Roy et al., 2020). Techniques such as CRISPR/Cas9 and other genome editing tools offer the potential to fine-tune these genes, thereby improving the efficiency and effectiveness of nitrogen fixation.

Moreover, manipulating the soil microbiome to favor beneficial nitrogen-fixing bacteria can further enhance biological nitrogen fixation (BNF). Studies have shown that associative, endosymbiotic, and endophytic nitrogen fixation in non-legumes can be optimized through microbiome manipulation, suggesting similar potential in legumes (Mahmud et al., 2020). The integration of these emerging technologies could lead to more sustainable agricultural practices by reducing the reliance on synthetic nitrogen fertilizers and improving soil health.

9.2 Prospects for transferring nitrogen-fixing capabilities to non-legume crops

One of the most exciting future directions in the field of nitrogen fixation is the potential to transfer nitrogen-fixing capabilities to non-legume crops. This would revolutionize agriculture by enabling major crops like rice, maize, and wheat to fix atmospheric nitrogen, thereby reducing the need for synthetic fertilizers. Current research efforts are focused on understanding the molecular mechanisms of BNF in non-legume plants and exploring ways to transfer these mechanisms to economically important crops (Santi et al., 2013; Mahmud et al., 2020; Soumare et al., 2020).

The successful transfer of nitrogen-fixing capabilities to non-legume crops would require a deep understanding of the symbiotic relationships between plants and nitrogen-fixing bacteria. Advances in genetic engineering and synthetic biology are making this goal increasingly attainable. For instance, the identification and manipulation of key genes involved in nodule formation and nitrogen fixation in legumes could provide a blueprint for engineering similar capabilities in non-legume crops (Roy et al., 2020).

9.3 Integration of BNF into sustainable agricultural practices

Integrating biological nitrogen fixation into sustainable agricultural practices is essential for meeting the growing global food demand while minimizing environmental impact. Legumes play a crucial role in this integration by improving soil fertility and reducing the need for synthetic nitrogen fertilizers. Practices such as crop rotation, intercropping, and green manuring can maximize the benefits of BNF in agricultural systems (Kebede, 2021).

To fully realize the potential of BNF, it is important to select appropriate legume genotypes, inoculate them with effective rhizobia, and employ suitable agronomic practices. Research has shown that optimizing the balance between legumes and non-legume crops can enhance productivity and soil health (Iannetta et al., 2016). Additionally, simulation models that quantify legume BNF can provide better guidance for farmers, helping them to manage nitrogen more effectively and sustainably (Liu et al., 2011).

In conclusion, the future of nitrogen fixation in legumes and its application in agriculture holds great promise. By leveraging emerging technologies in genetic engineering and microbiome manipulation, exploring the transfer of nitrogen-fixing capabilities to non-legume crops, and integrating BNF into sustainable agricultural practices, we can create more resilient and productive agricultural systems.

10 Concluding Remarks

The research on nitrogen fixation in legumes has revealed several critical insights. Legumes play a significant role in enhancing soil fertility through biological nitrogen fixation (BNF), which is facilitated by their symbiotic relationship with rhizobia. This process not only benefits the legumes themselves but also improves the yield and nutrient availability for subsequent crops in the rotation. Genetic studies have identified nearly 200 genes involved in symbiotic nitrogen fixation, advancing our understanding of the molecular mechanisms underlying this process. Additionally, breeding programs and genetic modifications have shown potential in increasing the efficiency of nitrogen fixation, although practical application in cultivars remains challenging. The integration of legumes into cropping systems through practices such as crop rotation, intercropping, and green manuring has been shown to enhance soil properties and break pest cycles, further promoting agricultural productivity.

Continued research and development in the field of nitrogen fixation in legumes are crucial for several reasons. First, the growing global population necessitates increased food production, and enhancing nitrogen fixation in legumes can contribute to this goal by reducing the reliance on synthetic fertilizers, which are costly and environmentally damaging. Second, understanding the genetic and molecular mechanisms of nitrogen fixation can lead to the development of more efficient legume varieties, which can thrive in low-nitrogen soils and improve overall crop yields. Third, integrating legumes into sustainable agricultural practices can mitigate environmental issues such as nitrate contamination and greenhouse gas emissions, promoting a more sustainable and resilient agricultural system. Therefore, prioritizing research in this area is essential for achieving long-term food security and environmental sustainability.

The future of nitrogen fixation in legumes holds great promise for transforming agricultural practices. Advances in genetic engineering and breeding programs are expected to produce legume varieties with enhanced nitrogen-fixing capabilities, which can significantly reduce the need for synthetic fertilizers and lower agricultural costs. Moreover, the integration of legumes into diverse cropping systems will not only improve soil health and crop productivity but also contribute to sustainable farming practices that are resilient to climate change. As research continues to uncover the complex interactions between legumes and their symbiotic partners, new strategies and technologies will emerge to optimize nitrogen fixation and maximize its benefits for agriculture. Ultimately, the successful application of these findings will depend on collaborative efforts between scientists, farmers, and policymakers to implement innovative solutions that address the challenges of modern agriculture.

Acknowledgements

Author thanks the two anonymous peer reviewers for their thorough review of this study and for their valuable suggestions for improvement.

Conflict of Interest Disclosure

Author affirms 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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