

A Comprehensive Study on Nitrogen Fixation and Growth and Development Regulation of Leguminous Plants

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Abstract Legumes are important nitrogen fixers that play significant roles in agricultural sustainability and ecosystem functioning. This review studied the relationship between nitrogen fixation and growth & development regulation in legumes. Firstly, it introduced the mechanism of legume nitrogen fixation, including root nodule formation with rhizobia, the nitrogenase system and nitrogen fixation process, as well as the interaction between nodule formation and the nitrogenase system. Secondly, it discussed the impacts of nodule formation on plant growth and development, signaling communication between rhizobia and plants, and rhizobia-induced plant metabolic regulation. Further, it summarized the advances in molecular mechanism research, agricultural applications of legume nitrogen fixation and growth & development regulation, as well as its ecological impacts. Finally, it proposed future research directions and challenges, including unknown mechanisms of legume nitrogen fixation, interactions with other growth and development regulatory pathways, and application prospects in nitrogen management and agricultural sustainability. This review provides important references for further studies in the field of legume nitrogen fixation and growth & development regulation.

Keywords Legumes; Nitrogen fixation; Growth and development regulation; Rhizobia; Agricultural sustainability

Leguminosae are a large family of plants, including many important crops and wild plants. The plants of this family have various uses, from food and feed crops to medicinal plants, all of which play extremely important roles on a global scale. Leguminous plants, such as soybeans, peas, and kidney beans, are also important sources of protein worldwide. The seeds of these plants are rich in high-quality protein, which is of great significance for the nutrition of humans and animals. In addition, many plants in the legume family have medicinal value and are widely used in traditional medicine and modern drug development. For example, yew is a commonly used Chinese medicinal herb that has anti-cancer and anti-inflammatory effects.

As early as 1888, German scientists discovered that leguminous plants coexisting with rhizobia can convert nitrogen into the nitrogen nutrients needed by plants. In the symbiosis between leguminous plants and rhizobia, leguminous plants provide a suitable nitrogen fixation environment and the necessary carbohydrates for the growth of rhizobia; In return, rhizobia convert nitrogen into nitrogen-containing compounds to meet the nitrogen requirements of leguminous plants. In addition, fixed nitrogen is also released into the soil and utilized by other plants. It is interesting that species that can symbiotically fix nitrogen with nitrogen fixing bacteria are only distributed in the order Fabales, Rosales, Cucurbitales, and Fagaceae, among which leguminous plants rhizobia symbiotic nitrogen fixation research is more extensive (Pagliai et al., 2020).

It was not until the 20th century that research on nitrogen fixation mechanisms in leguminous plants gradually began, as understanding of nitrogen cycling, plant microbial interactions, and other aspects deepened. Researchers have shown a strong interest in utilizing the nitrogen fixation capacity of leguminous plants to improve agricultural production efficiency and reduce environmental impacts, which has been driving research in related fields.

In recent decades, significant progress has been made in the research of leguminous plants in various aspects such as nitrogen fixation, growth and development regulation, and gene expression regulation. The in-depth study of the symbiotic relationship between rhizobia and plants has revealed the molecular mechanism of nodule formation and its role in nitrogen fixation. The application of molecular biology, genetics, and biochemistry methods has enabled us to better understand nitrogen fixation signaling pathways and the key molecules involved. At the same time, the technological development of genomics and transcriptomics provides powerful tools for the analysis of gene regulatory networks in leguminous plants.

The research process of leguminous plants has witnessed human attention and efforts towards agricultural production and ecological environment. From ancient times to the present, significant achievements have been made in the study of nitrogen fixation mechanisms and growth and development regulation. With the promotion of new technologies and interdisciplinary cooperation, the future prospects of leguminous plant research are still broad, which will further promote the sustainable development of agriculture and the healthy maintenance of ecosystems.

1 The Mechanism of Nitrogen Fixation in Leguminous Plants

1.1 Root nodule development mechanism

The symbiotic nitrogen fixation between leguminous plants and rhizobia is an efficient biological nitrogen fixation system and the most important green nitrogen source in natural soil. Analyzing the molecular regulatory mechanism of symbiotic nitrogen fixation between leguminous plants and rhizobia is not only of great scientific significance, but also an important guarantee for human beings to achieve sustainable agricultural development. Therefore, this research field has been one of the hot topics in plant biology for many years. The research team of Wang Ertao, a researcher at the Center for Excellence and Innovation in Molecular Plant Science of the Chinese Academy of Sciences, has made breakthrough research progress in this field, and discovered the molecular mechanism of SHR-SCR molecular regulation module regulating the initiation of root nodules of leguminous plants by determining the fate of cortical cells (Zhuang et al., 2021).

The cortex of the roots of leguminous plants undergoes rapid cell division and proliferation after invasion by rhizobia, leading to the formation of nodules. However, the cortex of non leguminous plants cannot respond to the invasion of rhizobia to produce nodules. Their research found that unlike the expression patterns of key genes SCR (SCARECROW, endothelial layer specific expression) and SHR (SHORTROOT, column specific expression) that regulate Arabidopsis root development, the homologous genes MtSHR and MtSCR in alfalfa appear in the root cortex. Further analysis revealed that the regular arrangement of key regulatory elements AT-1 box and enhancer sequences in the promoter region of MtSCR is conserved in leguminous plants, but there are significant differences in non leguminous plants. Genetic analysis shows that the expression of MtSCR and MtSHR in the cortex is extremely important for the occurrence of root nodules, and their mutant root nodule production ability is significantly reduced. Cytokinin has been shown to regulate the symbiotic relationship between plants and rhizobia, the division of cortical cells, and the initiation of root nodules. However, this promoting effect of cytokinin is significantly reduced in *Mtscr-1* and *MtSHR1-SRDX* (simulating SHR mutants by inhibiting the transcriptional activity of SHR), once again confirming the core role of MtSHR and MtSCR in root nodule initiation (Dong et al., 2021).

SHR-SCR is a key module in the stem cell program of plant development, expressed in the stem cell region and endothelial layer of plants. This study found that during the evolution of leguminous plants, the key stem cell gene SCR in leguminous plants is expressed in cortical cells, and another key stem cell transcription factor SHR moves to cortical cells after vascular bundle expression. As a result, the cortical cells of leguminous plants obtain the SHR-SCR stem cell submodule. This stem cell molecular module endows the cortex cells of leguminous plants with the ability to divide, making the cortex of leguminous plants different from non leguminous plants. At the same time, the stem cell molecular module can be activated by signals from rhizobia, inducing cortical division and forming nodules in leguminous plants such as alfalfa (Dong et al., 2021) (Figure 1).

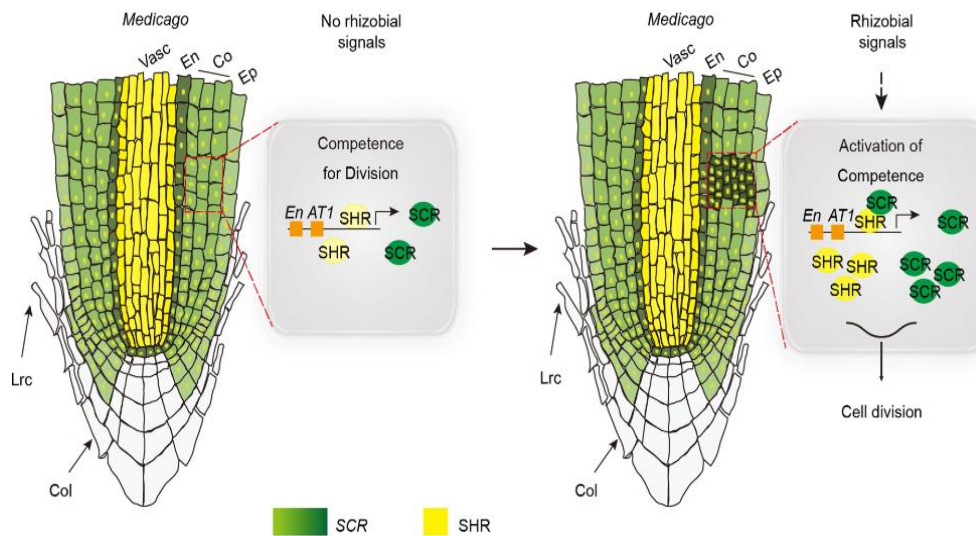


Figure 1 Root nodule development mechanism of leguminous plants (Dong et al., 2021)

When the SHR-SCR molecular module is overexpressed in the roots of leguminous plants such as alfalfa, it can induce cortical cell division to form a nodule like structure. Ectopic overexpression of SHR-SCR molecular modules in non leguminous plant *Arabidopsis* and rice roots can also induce root cortex cell division. Therefore, the SHR-SCR molecular module is a sufficient and necessary condition for plant cortex cell division, indicating that the acquisition of SHR-SCR stem cell program modules by leguminous plant cortex cells may be a prerequisite event for symbiotic nodulation and nitrogen fixation in leguminous plants.

1.2 Nitrogen enzyme system and nitrogen fixation process

Nitrogen is a large amount of essential nutrients for plant growth and development, and is an important component of molecules such as proteins and nucleic acids in living organisms. Although the Earth's atmosphere contains 78.1% nitrogen, it cannot be directly utilized by the vast majority of plants, making agricultural production highly dependent on industrial nitrogen fertilizers. However, the production of nitrogen fertilizer requires a large amount of fossil fuels, and excessive application of nitrogen fertilizer can cause soil compaction degradation and water pollution, affecting the sustainable development of agriculture. Biological nitrogen fixation is the largest natural source of nitrogen available to organisms in nature. Leguminous plants and rhizobia can interact to form a unique organ, known as symbiotic nodules. Symbiotic nitrogen fixation in root nodules is an important pathway for nitrogen reduction to ammonia that can be utilized by plants in the Earth's ecosystem. It contributes over 60% of terrestrial biological nitrogen fixation annually, affecting primary production and carbon sinks in agriculture and natural ecosystems. To reduce reliance on industrial nitrogen fertilizers and develop green agriculture, it is of great significance (Ke et al., 2022).

Symbiotic nitrogen fixation is a high energy consuming enzyme catalytic process, and the carbohydrates fixed by plant photosynthesis are the main carbon and energy sources for symbiotic nitrogen fixation (Figure 2). Therefore, the nitrogen fixation ability of symbiotic nodules needs to be coordinated with the carbon source and energy level of leguminous plants, in order to balance the carbon consumption of symbiotic nitrogen fixation and other life processes and ensure the normal growth of leguminous plants in different environments. However, it is not clear how leguminous plants respond to carbon sources and energy levels to regulate high energy consuming biological nitrogen fixation processes (Ke et al., 2022).

The nitrogen fixation ability of leguminous plant nodules is affected by environmental changes. When the external oxygen and phosphorus supply changes, the nitrogen fixation ability of the nodules will be rapidly adjusted, and the energy state of the nodules will also change accordingly, indicating that the change in the energy state of the nodules may be an important inducement for the change in nitrogen fixation ability.

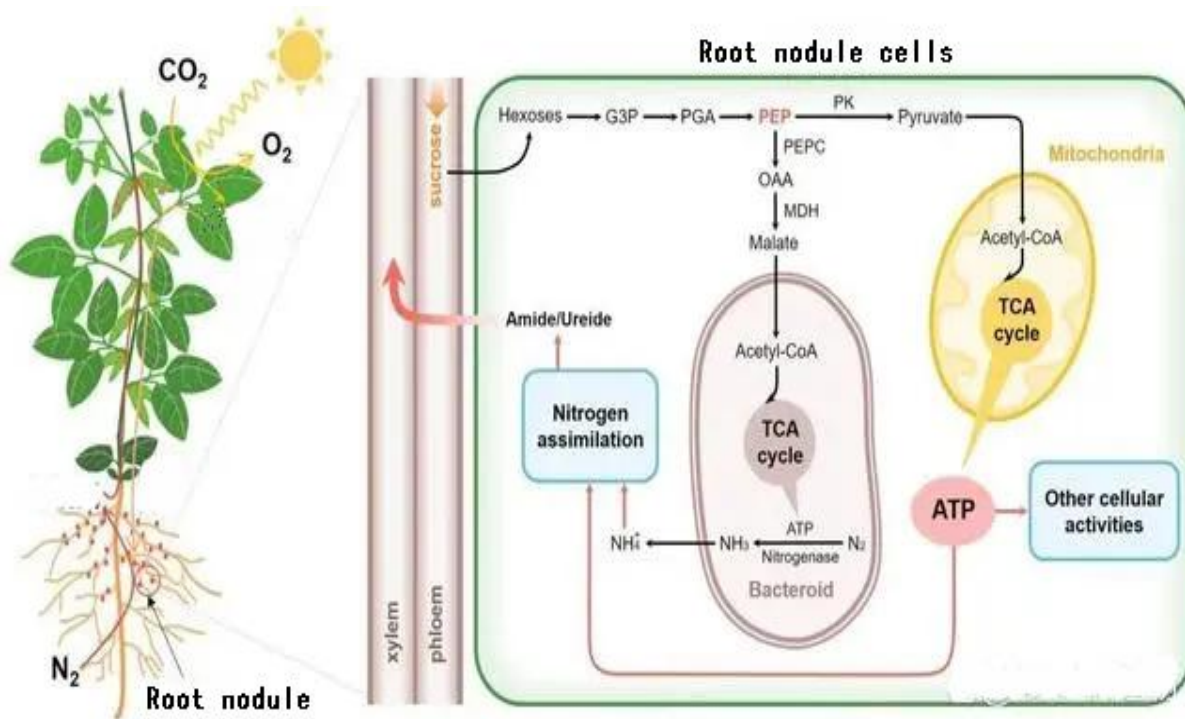


Figure 2 Source and distribution of carbon and energy in symbiotic nitrogen fixation (Ke et al., 2022)

1.3 Interaction between nodule formation and nitrogenase system

The process of root nodule formation is a series of ordered molecular events. Early studies have found that Nod factor can activate gene expression related to cell division and differentiation in the nucleus of plant roots, induce the inclusion and division of root hair cells, and form early nodule root structures. Through DNA microarray technology, it was found that NifA can directly control the expression of Nod genes and promote the secretion of Nod factors at this stage. The Nod factor regulates root fertilizer differentiation and growth through the recognition of plant adjacent membrane receptor complexes, obtaining more nutrient supply for root nodule development. The development process of rhizobia also regulates nitrogen fixation. By constructing different mutant strains of rhizobia, it was found that the ability of mutants lacking NifA related genes to form nodules was affected. This means that the nitrogen fixing gene promoter NifA can directly promote the expression of Nod gene and maintain the process of root nodule formation (Prithiviraj et al., 2003).

Mature nodules have an appropriate structure that can provide a low oxygen atmosphere for rhizobia to isolate from the outside world. In addition, the abundant vacuoles in nodule cells can also provide abundant ammonia transport and storage space for nitrogen fixation. Research has found that under different oxygen concentration conditions, rhizobia can regulate NifA activity through an oxygen suffocation system, optimize nitrogenase levels and structures, and adapt to the needs of the root nodule microenvironment. This reveals the important role of root nodule morphology regulation in providing optimized conditions for nitrogen fixation.

The above discusses the deep regulation of nitrogen fixation at different stages of nodule formation, as well as how the mature structure of nodules can in turn optimize the nitrogen fixation function of rhizobia, forming a complete interdependent regulatory network to jointly promote the efficient operation of both. It lays the foundation for in-depth research on this important symbiotic relationship mechanism.

2 The Relationship between Nitrogen Fixation and Growth and Development Regulation in Leguminous Plants

2.1 The impact of root nodule formation on plant growth and development

The formation of root nodules in leguminous plants has a significant impact on their growth and development.

The formation of root nodules provides additional nitrogen sources for plants, and through the formation of symbiotic mycorrhizal fungi, it provides the ability to absorb other nutrients, thereby promoting plant growth and development.

The formation of root nodules enables leguminous plants to absorb nitrogen from the atmosphere through symbiotic relationships with rhizobia and convert it into ammonia nitrogen that can be utilized by plants. This provides additional nitrogen sources for plants to meet their nitrogen needs for growth and development. Due to nitrogen being a key nutrient element in plant growth and development, the formation of root nodules can increase the biomass accumulation and yield of plants.

In addition to providing additional sources of nitrogen, the formation of root nodules also promotes the absorption of other nutrients by plants through the formation of symbiotic mycorrhizae. Symbiotic mycorrhizal fungi can increase the absorption surface area of plant roots and provide other nutrients such as phosphorus and potassium required by plants through symbiosis with rhizobia. This symbiotic relationship enables leguminous plants to more effectively absorb and utilize nutrients in the soil, thereby improving plant growth and development (Dixon and Kahn, 2004).

In addition, the formation of root nodules also affects the root structure and morphological development of plants. The formation of root nodules can lead to changes in the morphology of plant roots, such as thickening and thickening of the root system. Such changes help to increase the contact area between the rhizosphere and soil of plants, enhance the absorption capacity of roots for water and nutrients, and promote plant growth and development.

Overall, the formation of root nodules has a significant impact on the growth and development of leguminous plants. The formation of nodules provides an additional source of nitrogen to meet the nitrogen requirements for plant growth and development. Meanwhile, the formation of root nodules promotes the absorption of other nutrients by plants through the formation of symbiotic mycorrhizae. In addition, the formation of root nodules also affects the root structure and morphological development of plants. The in-depth study of the relationship between root nodule formation and plant growth and development will help us better understand the growth and development regulation mechanisms of leguminous plants, and provide scientific basis for the optimization cultivation and variety improvement of leguminous crops.

2.2 Regulatory mechanisms of signal exchange between rhizobia and plants

The signal exchange between rhizobia and plants is a crucial step in the formation of nodules, which is regulated through a series of signaling molecules and pathways. During the formation of nodules, rhizobia induce the formation of nodules in plant roots by releasing a special type of signaling molecule called Nod factors. Nod factors are a diverse class of signaling molecules with highly diverse structures and biological activities. The receptors in plant roots can recognize and respond to specific Nod factors, thereby initiating signaling pathways for root nodule formation.

The perception and response process of Nod factor is achieved through a series of receptors and signaling molecules. In leguminous plants, receptor proteins NFR1 and NFR5 play a crucial role in the formation of root nodules. These receptors are able to recognize and bind Nod factors, thereby activating downstream signaling pathways. Once receptors bind to Nod factors, they activate a series of signaling molecules, such as kinases and phosphatidylinositol kinases. These signaling molecules further regulate a series of gene expression, thereby initiating the process of root nodule formation (People et al., 1994).

In addition to the signal transduction of Nod factors, rhizobia also regulate the formation of nodules by releasing other signaling molecules. For example, some rhizobia can release a class of small molecule compounds called symbiotic effectors. These symbiotic effectors can regulate the morphology and physiological status of plant roots, further promoting the formation of root nodules. The regulatory mechanism of symbiotic effectors is not fully

understood, but it has been found that they can be achieved by activating plant hormone signaling pathways and regulating plant gene expression.

Overall, signal exchange between rhizobia and plants is a key regulatory mechanism in the process of nodule formation. The Nod factor and other symbiotic effectors activate a series of signaling pathways by binding to plant receptors, thereby regulating the formation of root nodules. The in-depth study of the signal exchange regulation mechanism between rhizobia and plants will help us better understand the molecular mechanism of nodule formation, and provide scientific basis for nodule formation and variety improvement of leguminous crops (Han et al., 2020).

2.3 Plant metabolic regulation induced by rhizobia

The symbiotic relationship between rhizobia and plants not only affects nodule formation, but also affects plant growth and development by regulating plant metabolic processes. Rhizobia induce the formation of nodules in plant roots, providing additional nitrogen sources for plants. The increase of this nitrogen source can cause changes in plant metabolism. Firstly, the nodules induced by rhizobia contain a large number of enzymes related to protein synthesis and nitrogen fixation, which increases the protein synthesis and nitrogen metabolism activity of plants; Secondly, rhizobia provide the ability to absorb phosphorus and other nutrients through the formation of symbiotic mycorrhizae, which can alter plant phosphorus metabolism and the utilization of other nutrients. These metabolic changes will affect the growth and development of plants.

In addition, rhizobia regulate plant metabolism by activating plant hormone signaling pathways. Rhizobia can induce plant synthesis of the hormone gibberellin and activate the gibberellin signaling pathway, thereby promoting plant growth and root nodule formation. The synthesis and signaling pathways of gibberellin regulate the activity of auxin in plants, affecting the growth and development of plant roots. In addition, rhizobia can also regulate the synthesis and signaling pathways of plant hormones jasmonic acid and ethylene, further affecting plant metabolism and growth and development (Dixon and Kahn, 2004).

Rhizobia can initiate the formation of nodules by secreting Nod factors, and can also induce the activation and expression of a series of secondary metabolic pathways in plant root cells. Early studies have shown that the recognition of Nod factors can lead to the accumulation of secondary messengers such as calcium and cGMP in plant cells. These secondary messengers affect the transcription levels of plant hormone related genes such as flavonoid biosynthesis and steroids through transcription factors and their regulatory groups. Meanwhile, studies have also found that Nod factor can upregulate the expression of a unique solanine metabolism pathway enzyme in leguminous plants. This provides an important intracellular regulatory mechanism for promoting nodule formation.

In combination with Nod factor, polysaccharides secreted by rhizobia can also have a profound impact on plant metabolism. Polysaccharides, as edible carbon sources, can directly participate in the synthesis and regulation of plant central gluconeogenesis pathways and photosynthetic products after absorption. However, in addition, polysaccharides from rhizobia can also indirectly regulate plant cell division and differentiation through hormone pathways. Research has found that polysaccharides can upregulate the expression levels of cytokinin and auxin in plant cells. These two plant hormones act as secondary messengers and can promote root and branch expansion and thickening, thereby increasing nutrient absorption area.

At the same time, some small molecule substances secreted by rhizobia, such as amino inorganic acids and fatty acids, also participate in regulating plant metabolic processes. Amino acids can achieve balanced transformation of inorganic nitrogen and organic nitrogen in plants, which is conducive to the dynamic allocation and utilization of nitrogen. Fatty acids can promote the improvement of photosynthetic efficiency in plant leaves by affecting key enzymes involved in the biosynthesis pathway of biological pigments. In addition, rhizobia can also enter plant cells through RNA molecules packaged outside cells, participate in the coupling of post transcriptional regulatory networks, and affect the rate of key reactions.

It can be seen that rhizobia secrete various types of signaling molecules to trigger and bind multiple cascade regulatory mechanisms within plant cells, forming a macroscopic regulatory network. This multi-level interaction helps rhizobia finely manipulate various physiological functions of the host plant and optimize their steady-state symbiosis within the root nodules. The revelation of this deep regulatory mechanism will lay a solid foundation for our understanding of rhizobial biology.

3 Research Progress and Application

3.1 Progress in molecular mechanism research

Significant progress has been made in the study of molecular mechanisms in the symbiotic relationship between rhizobia and plants. Through in-depth research on the signal exchange and metabolic regulation mechanisms between rhizobia and plants, we have gained a deeper understanding of the molecular mechanisms underlying nodule formation. We have been able to decipher the relationship between different Nod factor structures and biological activities in the study of the signal molecule Nod factor produced by rhizobia. This provides a theoretical basis for designing and synthesizing more effective Nod factors. In addition, we also conducted in-depth research on the receptors and signaling pathways of plants towards Nod factors. By analyzing the structure and function of receptor proteins, we can better understand how plants recognize and respond to Nod factors, and initiate signaling pathways for nodule formation.

Significant progress has also been made in the study of other signaling molecules released by rhizobia, and we have found that some rhizobia can release symbiotic effectors to regulate plant growth and development. By studying the structure and biological activity of symbiotic effectors, we can understand how they regulate plant metabolism and growth and development. In addition, we also discovered some symbiotic effectors that regulate plants by activating plant hormone signaling pathways and regulating plant gene expression (Christina and Simona, 2021).

Our research on the molecular mechanisms of the symbiotic relationship between rhizobia and plants also provides some new ideas for agricultural applications. By deeply understanding the signal exchange and metabolic regulation mechanisms between rhizobia and plants, we can develop new agricultural technologies and variety improvement strategies. Researchers can use gene editing techniques to alter the structure of plant receptors for Nod factors, thereby enhancing the plant's ability to infect rhizobia. Symbiotic effectors can also be used to regulate plant growth and development, improving crop yield and quality.

3.2 Agricultural applications of nitrogen fixation and growth and development regulation in leguminous plants

Leguminous plants have the ability to coexist with rhizobia and provide the necessary nitrogen source for plants through nitrogen fixation by rhizobia. This characteristic has important application value in agriculture, which can improve soil fertility, reduce the use of fertilizers, and increase crop yield and quality. A typical example is the application of leguminous plant soybean in agriculture. Soybean, as an important economic crop, has a high nitrogen demand. Through symbiosis with rhizobia, soybean can form nitrogen nodules in the nodules and convert atmospheric nitrogen into usable forms for plants through the nitrogen fixation ability of rhizobia. This enables soybeans to reduce their dependence on fertilizer nitrogen and lower the cost of agricultural production. Meanwhile, rhizobial symbiosis can also provide other nutrients such as phosphorus and potassium, further promoting the growth and development of soybeans.

In many agricultural systems, corn and soybean are common crop rotation plants because soybean's nitrogen fixation ability can provide nitrogen for non nitrogen fixing crops such as corn. Soybeans can fix a large amount of nitrogen through symbiosis with rhizobia. In maize soybean rotation, nitrogen fixation of soybeans provides a nitrogen source for the next season of maize crops (Sanjay et al., 2021).

In addition to soybeans, other leguminous crops such as peas, lentils, and peanuts can also benefit from symbiotic relationships with rhizobia. These crops obtain additional nitrogen sources by co growing with rhizobia,

improving crop yield and quality. Under certain poor soil conditions, symbiosis of rhizobia can significantly improve soil nitrogen supply, reduce dependence on fertilizers, and reduce environmental pollution.

In addition, introducing the symbiotic ability of rhizobia into non leguminous plants also has certain agricultural application potential. By using transgenic technology to introduce the symbiotic genes of rhizobia into other crops, such as wheat and corn, they can coexist with rhizobia, thereby improving crop nitrogen utilization efficiency, reducing the demand for fertilizers, and achieving sustainable agricultural production (Jeanine et al., 1998).

Overall, the symbiotic relationship between leguminous plants and rhizobia has important application value in agriculture. By utilizing this symbiotic relationship, the nitrogen utilization efficiency of crops can be improved, the use of chemical fertilizers can be reduced, soil fertility can be improved, and sustainable agricultural production can be achieved.

3.3 Ecological impacts of nitrogen fixation and growth and development regulation in leguminous plants

Leguminous plants have a significant impact on the nitrogen cycling and biodiversity of ecosystems through their symbiotic nitrogen fixation ability with rhizobia. Leguminous plants can convert atmospheric nitrogen into usable forms for plants through rhizobia symbiosis, allowing them to input nitrogen into the soil through nitrogen fixation and increase soil nitrogen content. This is particularly important for poor soil, as it can improve soil fertility, promote the growth of other plants, and affect the material cycle of the entire ecosystem.

The nitrogen fixation ability of leguminous plants gives them a competitive advantage in plant communities. Due to their ability to independently obtain nitrogen sources, leguminous plants can grow better in nitrogen limited environments. This may lead to leguminous plants dominating certain ecosystems, affecting the structure and species composition of plant communities.

In traditional agricultural production, fertilizers are used to meet the nitrogen needs of crops, but excessive use of fertilizers can lead to nitrogen loss in the soil, thereby causing pollution to water bodies and ecosystems. However, through the nitrogen fixation symbiotic ability of leguminous plants, dependence on fertilizers can be reduced, thereby reducing the risk of nitrogen loss and minimizing negative impacts on ecosystems.

The symbiotic relationship between leguminous plants and rhizobia provides important support for the maintenance of biodiversity in many ecosystems. The nitrogen fixation ability of leguminous plants can provide additional nitrogen sources for other plants, thereby promoting their growth and development. This is crucial for maintaining plant diversity and ecosystem stability. It can be seen that the nitrogen fixation ability and growth and development regulation of leguminous plants have important impacts on ecosystems. They can improve soil fertility, affect plant community structure, reduce nitrogen loss, and maintain biodiversity. These ecological impacts are of great significance for the health and sustainability of ecosystems.

4 Future Research Directions and Challenges

4.1 Unknown nitrogen fixation mechanisms in leguminous plants

Although the nitrogen fixation mechanism of leguminous plants has been largely studied and understood, there are still some unknown aspects and challenges. Currently, we know that the symbiotic relationship between leguminous plants and rhizobia is established through the interaction of signaling molecules. However, the specific mechanisms of these signaling molecules are still limited. Future research can focus on revealing the molecular mechanisms of signal transmission between rhizobia and leguminous plants, as well as the establishment and maintenance mechanisms of symbiotic relationships.

Although the nitrogen fixation ability of leguminous plants has been widely recognized, there are still some unknowns on how nitrogen fixation affects plant growth and development. Future research can explore the regulatory mechanisms of nitrogen fixation on plant root development, leaf growth, flower bud differentiation, and other aspects. In addition to symbiotic relationships with rhizobia, leguminous plants also have symbiotic relationships with other symbiotic bacterial groups, such as cocoon silkworms. Although these symbiotic

relationships have been studied, there is still limited understanding of the specific roles and interaction mechanisms of these symbiotic microbial communities in nitrogen fixation in leguminous plants. Future research can explore the interaction between these symbiotic microbial communities and leguminous plants, as well as their functions and regulatory mechanisms in nitrogen fixation processes.

The nitrogen fixation capacity of leguminous plants is influenced by environmental factors such as soil pH, temperature, humidity, etc. However, our understanding of the specific effects and regulatory mechanisms of these environmental factors on nitrogen fixation is still limited. Future research can explore the interaction between nitrogen fixation and environmental factors, as well as the impact of these environmental factors on the nitrogen fixation capacity of leguminous plants (Zhang et al., 2021).

Overall, there are still some unknown areas and challenges in the nitrogen fixation mechanism of leguminous plants. Future research can focus on revealing the symbiotic mechanism of rhizobia, the regulation of nitrogen fixation on plant growth and development, the interaction between leguminous plants and other symbiotic bacterial communities, and the interaction between nitrogen fixation and environmental factors. These studies will contribute to a deeper understanding of the mechanisms of nitrogen fixation in leguminous plants and provide a better foundation for future agricultural and ecological applications.

4.2 Interaction between nitrogen fixation and other growth and development regulatory pathways in leguminous plants

There is a complex interaction between the nitrogen fixation ability of leguminous plants and other growth and development regulatory pathways. The nitrogen fixation process of leguminous plants involves multiple signaling pathways and regulatory factors, which interact and regulate with other growth and development regulatory pathways.

Multiple hormones are involved in the nitrogen fixation process of leguminous plants and interact with other growth and development regulatory pathways. It is well known that the plant hormone gibberellin can promote the formation and development of root nodules, thereby increasing nitrogen fixation capacity. Meanwhile, other hormones such as ethylene and abscisic acid also participate in the nitrogen fixation process of leguminous plants and interact with the growth and development regulation pathways of plants.

Photosynthesis is an important process for plant growth, development, and nitrogen fixation. Leguminous plants synthesize organic compounds through photosynthesis and use them for energy supply during nitrogen fixation. The photosynthetic products produced by photosynthesis, such as carbohydrates, have a regulatory effect on the nitrogen fixation ability of leguminous plants. In addition, photosynthesis also interacts with other growth and development regulatory pathways such as the plant auxin signaling pathway, jointly regulating the growth and nitrogen fixation of leguminous plants (Liu et al., 2023).

The root system architecture of leguminous plants has a significant impact on their nitrogen fixation ability. The morphological structure and distribution of the root system play a crucial role in the formation of nodules and the efficiency of rhizobial symbiosis. At the same time, the development of the root system also interacts with other growth and development regulatory pathways, such as plant hormones and root tip activity, to jointly regulate the growth and nitrogen fixation of leguminous plants.

In addition, the nitrogen fixation capacity of leguminous plants is regulated by environmental factors, such as soil nitrogen content, soil moisture, temperature, etc., which can affect the nitrogen fixation capacity of leguminous plants. Meanwhile, these environmental factors also interact with other growth and development regulatory pathways, jointly regulating the growth and nitrogen fixation of leguminous plants.

It can be seen that the nitrogen fixation ability of leguminous plants has complex interactions with other growth and development regulation pathways, including hormone regulation, photosynthesis and nitrogen metabolism, root architecture and nitrogen fixation, as well as environmental factors and nitrogen fixation, jointly regulating

the growth and nitrogen fixation ability of leguminous plants. Further research can explore the mechanisms of these interactions and gain a deeper understanding of the overall regulatory network of nitrogen fixation and growth and development regulation in leguminous plants.

4.3 Application prospects of nitrogen fixation by leguminous plants in nitrogen management and sustainable agricultural development

The nitrogen fixation of leguminous plants has important application prospects in nitrogen management and sustainable agricultural development. Leguminous plants can directly fix nitrogen from the atmosphere and convert it into organic nitrogen to supply plants through symbiosis with rhizobia. This nitrogen fixation ability enables leguminous plants to partially replace chemically synthesized nitrogen fertilizers. By utilizing leguminous plants for rotation or intercropping in a reasonable manner, the dependence of farmland on chemical nitrogen fertilizer can be reduced, nitrogen input in agricultural production can be reduced, thereby reducing the negative impact on the environment.

The nitrogen fixation process of leguminous plants releases organic acids and other compounds, which have a positive effect on soil improvement. Organic acids can lower soil pH, improve soil structure and permeability, and promote the decomposition and mineralization of organic matter in the soil. In addition, nitrogen fixation can increase the number and diversity of nitrogen fixing bacteria in soil, improve soil biological activity, and help maintain soil health and ecosystem balance (Li et al., 2008).

The nitrogen fixation ability of leguminous plants can provide available nitrogen sources for crops, thereby improving their growth, development, and yield. Leguminous plants can also improve the nitrogen supply in the soil, providing organic nitrogen sources for the growth of other crops. In addition, the nitrogen fixation process of leguminous plants also releases some growth regulating substances, such as gibberellins, which promote the growth and development of other crops. Therefore, reasonable use of leguminous plants for rotation or intercropping can improve the yield and quality of the entire farmland.

The nitrogen fixation ability of leguminous plants can reduce the dependence of agricultural production on chemical nitrogen fertilizers, thereby reducing the amount of nitrogen fertilizer used and the risk of loss. Excessive application of chemical nitrogen fertilizer can lead to nitrogen overload in soil and water, causing environmental problems such as eutrophication and soil acidification. Using leguminous plants as nitrogen fertilizer substitutes can reduce the probability of these environmental problems and minimize negative impacts on the environment. In addition, the nitrogen fixation process of leguminous plants can also contribute to mitigating climate change by reducing greenhouse gas emissions and carbon sequestration.

It can be seen that the nitrogen fixation of leguminous plants has broad application prospects in nitrogen management and sustainable agricultural development. By reasonably utilizing the nitrogen fixation ability of leguminous plants, nitrogen fertilizer substitution, soil improvement, improving crop yield and quality, and environmental protection can be achieved, promoting sustainable agricultural development. This will help improve the productivity of farmland, reduce negative impacts on the environment, and contribute to achieving sustainable agricultural development. However, it should be noted that in practical applications, it is also necessary to consider factors such as the suitable matching planting of leguminous plants with other crops, soil suitability, and management techniques, in order to fully tap into the potential of leguminous plants for nitrogen fixation.

5 Outlook

In the past few decades, significant progress has been made in the study of nitrogen fixation and growth and development regulation in leguminous plants, with in-depth exploration of the mechanisms, regulatory factors, and application prospects of this key physiological process. The research on nitrogen fixation and growth and development regulation of leguminous plants is increasingly integrating knowledge from different disciplines, forming interdisciplinary research cooperation. Experts in fields such as ecology, molecular biology, genetics, and

agriculture actively collaborate to explore the interrelationship between nitrogen fixation and growth and development, providing a more comprehensive perspective for the study of leguminous plants.

Researchers have gradually elucidated the molecular mechanism of nitrogen fixation through in-depth exploration of the symbiotic relationship between rhizobia and leguminous plants. Rhizobia infect plant roots, forming nodules and establishing symbiotic relationships. Nitrogen enzymes catalyze the conversion of nitrogen in the atmosphere into ammonia, providing plants with available nitrogen and promoting the improvement of soil fertility.

Researchers have found that nitrogen fixation not only affects nitrogen supply, but is also closely related to plant growth and development. Nitrogen fixation has a significant impact on the overall growth of plants by affecting root development, leaf photosynthesis, and plant nutrient allocation. The mechanism of regulating nitrogen fixation and balancing growth and development has gradually become a focus of research.

The development of genomics and molecular biology has enabled researchers to reveal the molecular mechanisms underlying nitrogen fixation and growth and development regulation. The hormone signaling pathway and gene expression regulatory network are gradually being revealed, and the key role of transcription factors in regulating nitrogen fixation and growth and development processes is gradually becoming apparent.

Research has found that regulating nitrogen fixation and balancing growth and development can bring enormous potential to agricultural production and the ecological environment. By utilizing nitrogen fixation capacity, scientists can improve the nitrogen utilization efficiency of crops, reduce the use of chemical nitrogen fertilizers, and thus reduce the impact on the environment. In addition, the introduction of gene editing technology is expected to further optimize the efficiency of nitrogen fixation, providing more possibilities for sustainable agricultural development.

With the advocacy of global sustainable development, research on nitrogen fixation of leguminous plants is of great significance in promoting agricultural sustainability. By improving nitrogen fixation efficiency and reducing reliance on chemical nitrogen fertilizers, the negative impact of agriculture on the environment can be reduced, soil and water pollution can be reduced, and a more environmentally friendly agricultural production model can be achieved.

Despite significant research progress, there are still some challenges that need to be overcome. The nitrogen fixation mechanisms of leguminous plants may vary under different environmental and ecological conditions, therefore further research is needed to reveal their diversity. In addition, the application of gene editing technology also requires careful consideration of potential ecological and environmental risks.

The research on nitrogen fixation and growth and development regulation of leguminous plants has shown enormous potential, providing us with new perspectives on plant physiology and molecular biology, as well as new ideas for sustainable agricultural development and ecological environment protection. In the future, interdisciplinary cooperation, technological innovation, and in-depth research on ecological impacts will continue to drive the development of this field, making greater contributions to the sustainability of agriculture and global food safety. The understanding of this process has also brought new directions for the development of agriculture, ecological environment, and biotechnology. In the future, we can look forward to more interdisciplinary research collaborations to further reveal the growth and development mechanisms of leguminous plants, promote sustainable agricultural development, and maintain the health of ecosystems.

References

- Christina K., and Simona R., 2021, Understanding Nod factor signalling paves the way for targeted engineering in legumes and non-legumes, *Current Opinion in Plant Biology*, 62: 102026.
<https://doi.org/10.1016/j.pbi.2021.102026>

- Dong W.T., Zhu Y.Y., Chang H.C., Wang C.H., Yang J., Shi J.C., Gao J.P., Yang W.B., Lan L.Y., Wang Y.R., Zhang X.W., Dai H.L., Miao Y.C., Xu L., He Z.H., Song C.P., Wu S., Wang D., Yu N., and Wang E.T., 2021, An SHR-SCR module specifies legume cortical cell fate to enable nodulation, *Nature*, 589: 586-590.
<https://doi.org/10.1038/s41586-020-3016-z>
- Dixon R., and Kahn D., 2004, Genetic regulation of biological nitrogen fixation, *Nature Reviews Microbiology*, 2: 621-631.
<https://doi.org/10.1038/nrmicro954>
- Han Q., Ma Q., Chen Y., Tian B., Xu L.X., Bai Y., Chen W.F., and Li X., 2020, Variation in rhizosphere microbial communities and its association with the symbiotic efficiency of rhizobia in soybean, *The ISME Journal*, 14: 1915-1928.
<https://doi.org/10.1038/s41396-020-0648-9>
- Jeanine L., Kay-Shoemake A., Mary E., Watwood a, Rodrick D. Lentz B., Robert E., and Sojka B., 1998, Polyacrylamide as an organic nitrogen source for soil microorganisms with potential effects on inorganic soil nitrogen in agricultural soil, 30: 1045-1052.
[https://doi.org/10.1016/S0038-0717\(97\)00250-2](https://doi.org/10.1016/S0038-0717(97)00250-2)
- Ke X.L., Xiao H., Peng Y.Q., Wang J., Lv Q., and Wang X.L. Phosphoenolpyruvate reallocation links nitrogen fixation rates to root nodule energy state, 378(6623): 971-977.
<https://doi.org/10.1126/science.abq8591>
- Li Z.A., Zou B., Xia H.P., Ding Y.Z., Tan W.N., and Fu S.L., 2008, Role of Low-Molecule-Weight Organic Acids and Their Salts in Regulating Soil pH, *Pedosphere*, 18(2): 137-148
[https://doi.org/10.1016/S1002-0160\(08\)60001-6](https://doi.org/10.1016/S1002-0160(08)60001-6)
- Liu Z.J., Kong X.Y., Long Y.P., Liu S.R., Zhang H., Jia J.B., Cui W.H., Zhang Z.M., Song X.W., Qiu L.J., Zhai J.X., and Zhe Y., 2023, Integrated single-nucleus and spatial transcriptomics captures transitional states in soybean nodule maturation, *Nature Plants*, 9: 515-524.
<https://doi.org/10.1038/s41477-023-01387-z>
- Pagliai M., Vignozzi N., and Pellegrini S., 2020, Soil structure and the effect of management practices, *Soil and Tillage Research*, 79(2): 131-143.
<https://doi.org/10.1016/j.still.2004.07.002>
- Peoples M.B., Herridge D.F., and Ladha J.K., 1994, Biological nitrogen fixation: An efficient source of nitrogen for sustainable agricultural production? *Management of Biological Nitrogen Fixation for the Development of More Productive and Sustainable Agricultural Systems*, pp.3-28.
https://doi.org/10.1007/978-94-011-0055-7_1
- Prithiviraj B., Zhou X., Souleimanov A., Kahn W., and Smith D., 2003, A host-specific bacteria-to-plant signal molecule (Nod factor) enhances germination and early growth of diverse crop plants, *Planta* 216: 437-445.
<https://doi.org/10.1007/s00425-002-0928-9>
- Sanjay K., Jaiswall M.M., Fadimata Y.I., and Ibny F.D., 2021, Rhizobia as a Source of Plant Growth-Promoting Molecules: Potential Applications and Possible Operational Mechanisms, *Frontiers in Sustainable Food Systems*, 4: 12-31.
<https://doi.org/10.3389/fsufs.2020.619676>
- Zhuang Q.L., Xu Y.B., Yao Z.F., Zhu S.N., Liang C.Y., Hong L., Jiang T., 2021, Phosphate starvation responsive GmSPX5 mediates nodule growth through interaction with GmNF-YC4 in soybean (*Glycine max*), 108(5): 1422-1438.
<https://doi.org/10.1111/tpi.15520>