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Symbiotic Relationships between Nitrogen-fixing Bacteria and Leguminous Plants Ecological and Evolutionary Perspectives: A Review

Sushma Raj Chellem [a*], Chiriki Vasantha Lakshmana Kishore [b], Gurrala Sai Vamsi Reddy [a], D. V. S. Akshay [c++], Kavuri Kalpana [a], Purimetla Lavanya [d#] and Kavya Harshita Choragudi [d#]

[a] Department of Genetics and Plant Breeding, KL College of Agriculture, Koneru Lakshmaiah Education Foundation, Guntur, Andhra Pradesh, India.
[b] Department of Agronomy, Himgiri zee university, Dehradun, India.
c College of Agriculture, ANGRAU, Bapatla, Andhra Pradesh, India.
[d] KL College of Agriculture, Koneru Lakshmaiah Education Foundation, Guntur, Andhra Pradesh, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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[++] PhD Scholar (Agronomy).
[#] UG Scholar;
**Corresponding author: sushmaraj@kluniversity.in;*

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ABSTRACT

The symbiotic relationships between nitrogen-fixing bacteria and leguminous plants play a Important role in enhancing soil fertility, boosting crop yields, and promoting sustainable agricultural practices. The molecular and biochemical mechanisms underpinning these interactions, including signal exchange, root nodule formation, and metabolic integration. The co-evolution of legumes and rhizobia, driven by reciprocal selective pressures, has resulted in highly specialized and efficient symbiotic relationships. Genetic adaptations in legumes, such as the evolution of receptor-like kinases and transcription factors, facilitate the establishment and maintenance of these symbioses. The evolutionary divergence among nitrogen-fixing bacteria, influenced by host specificity and environmental factors, has led to a diverse array of rhizobial strains with varying symbiotic capabilities. Horizontal gene transfer has further contributed to the spread of symbiotic traits, enhancing the adaptability and ecological success of rhizobia. In agricultural contexts, the benefits of symbiotic nitrogen fixation are substantial, reducing reliance on synthetic fertilizers and improving soil health. Sustainable practices such as crop rotation, intercropping, and cover cropping with legumes enhance soil nitrogen levels and overall farm productivity. Advances in genetic engineering and biofertilizer technology offer promising avenues to optimize symbiotic efficiency and extend nitrogen-fixing capabilities to non-leguminous crops. Challenges remain, including the need for effective delivery systems for biofertilizers and the variability of symbiotic performance under different environmental conditions. Future research should focus on understanding soil microbial interactions and developing resilient rhizobial strains. By leveraging these natural processes, we can enhance agricultural sustainability, ensuring food security and environmental health for future generations. This comprehensive review underscores the critical importance of nitrogen-fixing symbioses in agriculture and ecosystem management.

Keywords: Symbiosis; nitrogen-fixation; rhizobia; soil-fertility; biofertilizers.

1. INTRODUCTION

The symbiotic relationships between nitrogen-fixing bacteria and leguminous plants represent a cornerstone of ecological and agricultural systems. These interactions not only contribute significantly to the nitrogen cycle but also enhance soil fertility and plant productivity. This introduction aims to provide an in-depth overview of the symbiotic relationships, underscore the importance of nitrogen-fixing bacteria, highlight the significance of leguminous plants in agriculture and ecology, and outline the objectives and scope of this review. Symbiosis, a term derived from the Greek words "syn" (together) and "bios" (life), describes the close and often long-term interaction between different biological species. Among the various forms of symbiosis, mutualistic relationships, where both partners benefit, are particularly noteworthy in the context of nitrogen fixation. Nitrogen-fixing bacteria, primarily from the genera Rhizobium, *Bradyrhizobium*, *Sinorhizobium*, and *Azorhizobium*, establish mutualistic relationships with leguminous plants. In these associations, bacteria infect the roots of legumes, leading to the formation of specialized structures known as root nodules. Within these nodules, bacteria convert atmospheric nitrogen (N_2) into ammonia (NH_3), a form of nitrogen that plants can readily assimilate for their growth and development [1]. This mutualistic relationship is a classic example of co-evolution, where both the host plant and the symbiotic bacteria have evolved intricate signaling mechanisms to recognize and interact with each other. The legume-rhizobium symbiosis begins with the exchange of molecular signals; the plant roots exude flavonoids that attract the bacteria, which in turn produce Nod factors that trigger root nodule formation [2]. This tightly regulated process ensures that both partners derive maximum benefit from the association: the plant receives a crucial nutrient in the form of bioavailable nitrogen, while the bacteria obtain carbohydrates and a protected niche within the plant roots.

1.1 Importance of Nitrogen-Fixing Bacteria

Nitrogen-fixing bacteria play a pivotal role in the nitrogen cycle, a fundamental ecological process that sustains life on Earth. Atmospheric nitrogen, which constitutes approximately 78% of the Earth's atmosphere, is biologically inert and unavailable to most organisms. Nitrogen-fixing bacteria convert this inert nitrogen into ammonia through a process called biological nitrogen fixation, mediated by the enzyme nitrogenase [3,93]. This conversion is vital because nitrogen is a critical component of amino acids, proteins, nucleic acids, and other cellular constituents necessary for life. The ecological importance of nitrogen-fixing bacteria extends beyond their direct interaction with leguminous plants. By enriching the soil with bioavailable nitrogen, these bacteria enhance soil fertility and promote the growth of both legumes and non-leguminous plants in the vicinity. This process helps maintain ecosystem productivity and supports diverse plant communities. Furthermore, nitrogen-fixing bacteria contribute to reducing the dependency on synthetic nitrogen fertilizers, which are associated with environmental issues such as water pollution and greenhouse gas emissions [4].

1.2 Significance of Leguminous Plants in Agriculture and Ecology

Leguminous plants, encompassing a wide variety of species including peas, beans, lentils, and clovers, are integral to both natural ecosystems and agricultural systems. They are unique in their ability to establish symbiotic relationships with nitrogen-fixing bacteria, thereby significantly enhancing soil nitrogen levels. This capability allows legumes to thrive in nitrogen-deficient soils where other plants might struggle, making them valuable for improving soil fertility and sustaining agricultural productivity [5]. In agriculture, legumes are often used in crop rotations and intercropping systems to replenish soil nitrogen levels naturally. This practice not only reduces the need for chemical fertilizers but also enhances soil structure, reduces pest and disease pressures, and improves overall crop yields. Additionally, legumes are a vital source of protein for human and animal consumption, making them essential for food security and nutrition [6]. Ecologically, leguminous plants play a crucial role in maintaining biodiversity and ecosystem stability. Their ability to fix nitrogen supports diverse plant and microbial communities, contributing to the overall health and resilience of ecosystems. Legumes also provide habitat and food for various organisms, from insects to mammals, further highlighting their ecological significance [7].

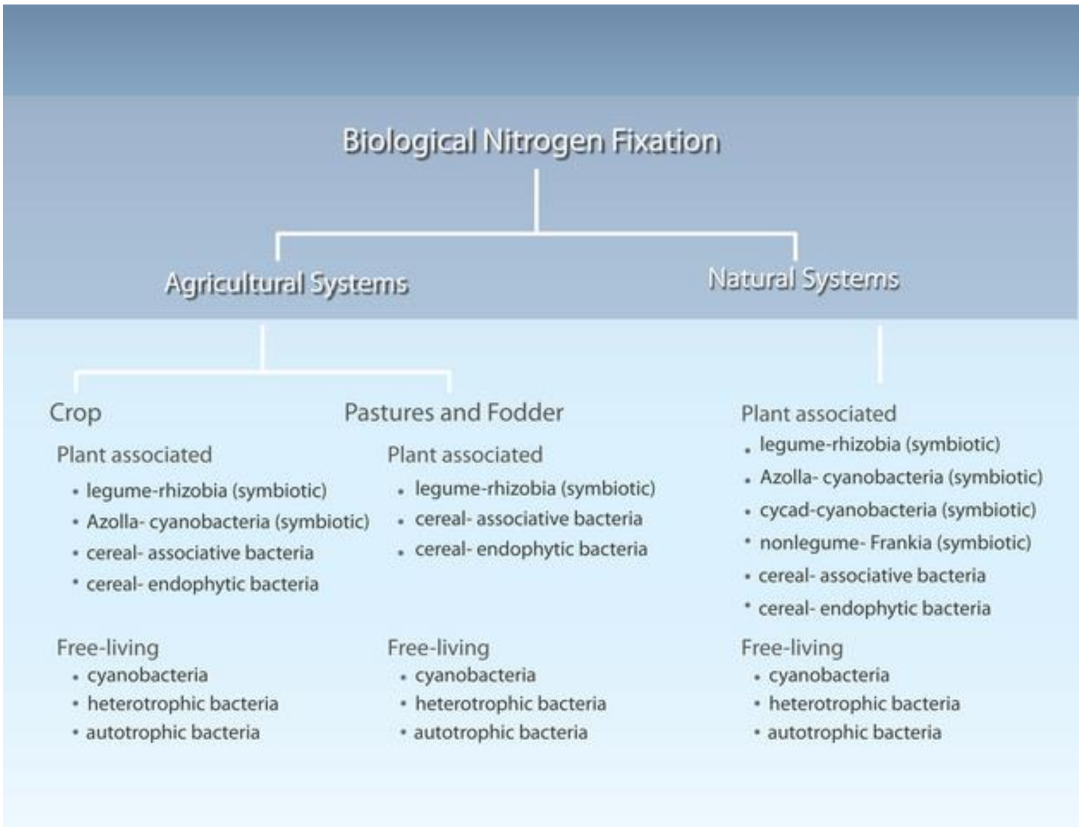

Fig. 1. Nitrogen-fixing organisms found in agricultural and natural systems (Source: Nature Journal)

1.3 Objectives and Scope of the Review

The primary objective of this review is to provide a comprehensive analysis of the symbiotic relationships between nitrogen-fixing bacteria and leguminous plants, with a focus on ecological and evolutionary perspectives. This review aims to synthesize current knowledge on the molecular and biochemical mechanisms underlying these symbioses, explore their ecological roles and implications, and examine the evolutionary dynamics that have shaped these interactions over time. By integrating insights from various fields, including microbiology, plant biology, ecology, and evolutionary biology, this review seeks to enhance our understanding of these complex and essential relationships. The scope of this review encompasses both fundamental and applied aspects of legume-rhizobium symbiosis. It will cover the historical development of research in this area, the molecular dialogue between plants and bacteria, the formation and functioning of root nodules, and the ecological impacts of nitrogen-fixing symbioses. Additionally, this review will explore the evolutionary processes that have driven the diversification and adaptation of both legumes and their symbiotic partners. By addressing these topics, the review aims to provide a holistic perspective on the significance of nitrogen-fixing symbioses in both natural and managed ecosystems.

2. HISTORY

The study of nitrogen fixation and the symbiotic relationships between nitrogen-fixing bacteria and leguminous plants has a rich historical background.

2.1 Discovery of Nitrogen Fixation

The discovery of nitrogen fixation was a pivotal moment in the field of plant science and soil microbiology. The journey began in the late 19th century when researchers sought to understand the sources of nitrogen available to plants. Early agronomists and chemists recognized that nitrogen was an essential nutrient for plant growth, but the mechanisms by which plants acquired nitrogen were not well understood. A French chemist, conducted pioneering experiments demonstrating that legumes could grow in nitrogen-poor soils and still accumulate nitrogen [8]. This observation suggested that legumes had a unique ability to obtain nitrogen from an unknown source [9]. However, the exact mechanism remained elusive until later studies provided more concrete evidence. The breakthrough came when German agronomist and Dutch microbiologist independently demonstrated that certain soil bacteria were responsible for nitrogen fixation in legumes [10]. An experiment showing that the presence of nodules on legume roots was essential for nitrogen fixation [11]. They proposed that these nodules housed bacteria that converted atmospheric nitrogen into a form usable by the plant. Symbiotic bacteria found on the roots of legume and responsible for nitrogen fixation [12]. The symbiotic relationship between legumes and rhizobia, marking the beginning of modern research in biological nitrogen fixation [13].

2.2 Early Studies on Legume-Rhizobia Symbiosis

Following the discovery of nitrogen fixation, early studies on legume-rhizobia symbiosis focused on understanding the nature of the interaction between plants and bacteria. These studies were crucial in elucidating the biochemical and physiological processes involved in nodule formation and nitrogen fixation. The term "symbiosis" to describe the mutually beneficial relationship between legumes and rhizobia [14]. He emphasized the importance of this symbiotic relationship in enhancing soil fertility and agricultural productivity [15]. During this period, researchers began to investigate the specific conditions under which nitrogen fixation occurred. Studies in the late 19th and early 20th centuries explored the environmental factors affecting nitrogen fixation, such as soil pH, temperature, and nutrient availability [16]. These investigations provided valuable insights into optimizing conditions for effective nitrogen fixation in agricultural systems. The work of American agronomist contributed significantly to the understanding of legume-rhizobia symbiosis [17]. He promoted the use of crop rotations with legumes to improve soil fertility and reduce reliance on chemical fertilizers. His advocacy for sustainable agricultural practices highlighted the practical applications of legume-rhizobia symbiosis in enhancing soil health and crop yields [18].

2.3 Evolution of Research Techniques in Symbiotic Studies

The evolution of research techniques in symbiotic studies has been instrumental in advancing our understanding of the molecular and genetic mechanisms underlying legume-rhizobia interactions. The development of new technologies and methodologies has allowed researchers to delve deeper into the intricacies of symbiosis and uncover the complex signaling pathways involved. In the mid-20th century, the advent of molecular biology techniques revolutionized the study of symbiotic relationships. The discovery of the structure of DNA provided a framework for investigating the genetic basis of nitrogen fixation [19]. Subsequently, researchers developed techniques for isolating and characterizing the genes involved in nodule formation and nitrogen fixation. The identification and sequencing of nodulation (nod) genes in rhizobia were landmark achievements in symbiotic research. These genes encode proteins responsible for the synthesis and recognition of Nod factors, signaling molecules that initiate the nodule formation process. Studies by researchers elucidated the roles of Nod factors in mediating the early stages of legume-rhizobia symbiosis [20]. Advances in microscopy techniques, such as electron microscopy and confocal microscopy, have provided detailed visualizations of nodule structures and the interactions between plant cells and bacteria. These imaging techniques have enabled researchers to observe the dynamic processes of nodule development and nitrogen fixation at the cellular and subcellular levels [21]. More recently, the advent of genomics and high-throughput sequencing technologies has opened new avenues for exploring the genetic diversity and evolutionary dynamics of nitrogen-fixing bacteria and leguminous plants. Comparative genomics studies have revealed the evolutionary history of symbiotic genes and the horizontal gene transfer events that have shaped the diversity of rhizobia [22].

3. MECHANISMS OF NITROGEN FIXATION

Understanding the mechanisms of nitrogen fixation is crucial for appreciating how certain organisms convert atmospheric nitrogen into a form usable by plants.

3.1 Biological Nitrogen Fixation Process

Biological nitrogen fixation is a process by which certain prokaryotes, including free-living bacteria, cyanobacteria, and symbiotic bacteria such as rhizobia, convert atmospheric nitrogen (N_2) into ammonia (NH_3). This process is essential because nitrogen is a critical nutrient for all living organisms, yet most cannot utilize atmospheric nitrogen directly due to its strong triple bond, which makes it chemically inert [23]. The biological nitrogen fixation process begins with the binding of atmospheric nitrogen to the nitrogenase enzyme complex within the nitrogen-fixing organism. This process occurs under anaerobic or microaerophilic conditions since nitrogenase is highly sensitive to oxygen. The fixation of nitrogen involves a series of reduction reactions, where nitrogen (N_2) is progressively reduced to ammonia (NH_3) through the transfer of electrons and protons. The overall reaction can be summarized as follows [24]:

N2+8H++8e−+16ATP→2NH3+H2+16ADP+16PiN2+8H++8e−+16ATP→2NH3+H2 +16ADP+16Pi

This reaction Shows the high energy requirement of the nitrogen fixation process, necessitating significant ATP consumption. The energy is provided by the host plant in the case of symbiotic nitrogen fixers like rhizobia, which inhabit root nodules of leguminous plants. The fixed nitrogen is then assimilated into organic compounds such as amino acids and nucleotides, which are vital for plant growth and development [25].

3.2 Role of Nitrogenase Enzyme

The nitrogenase enzyme complex is central to the nitrogen fixation process. It is composed of two main protein components: the dinitrogenase reductase (Fe protein) and the dinitrogenase (MoFe protein). The Fe protein provides the reducing power needed for nitrogen fixation by transferring electrons to the MoFe protein in an ATP-dependent manner [26]. The MoFe protein, which contains a molybdenum-iron cofactor (FeMo-co), is the site where atmospheric nitrogen is reduced to ammonia. The structure of the FeMo-co is complex, consisting of iron, molybdenum, sulfur, carbon, and homocitrate. This cofactor is crucial for the catalytic activity of nitrogenase, facilitating the binding and reduction of nitrogen [27]. The mechanism of nitrogenase action involves multiple steps of electron transfer and protonation. Electrons from the Fe protein are transferred to the MoFe protein, where they reduce the nitrogen molecule bound at the active site. The process is highly coordinated, with each electron transfer coupled with ATP hydrolysis to provide the necessary energy for the reduction reactions [28]. Given its sensitivity to oxygen, nitrogenase operates in an environment protected from oxygen exposure. In symbiotic systems, legume root nodules create a low-

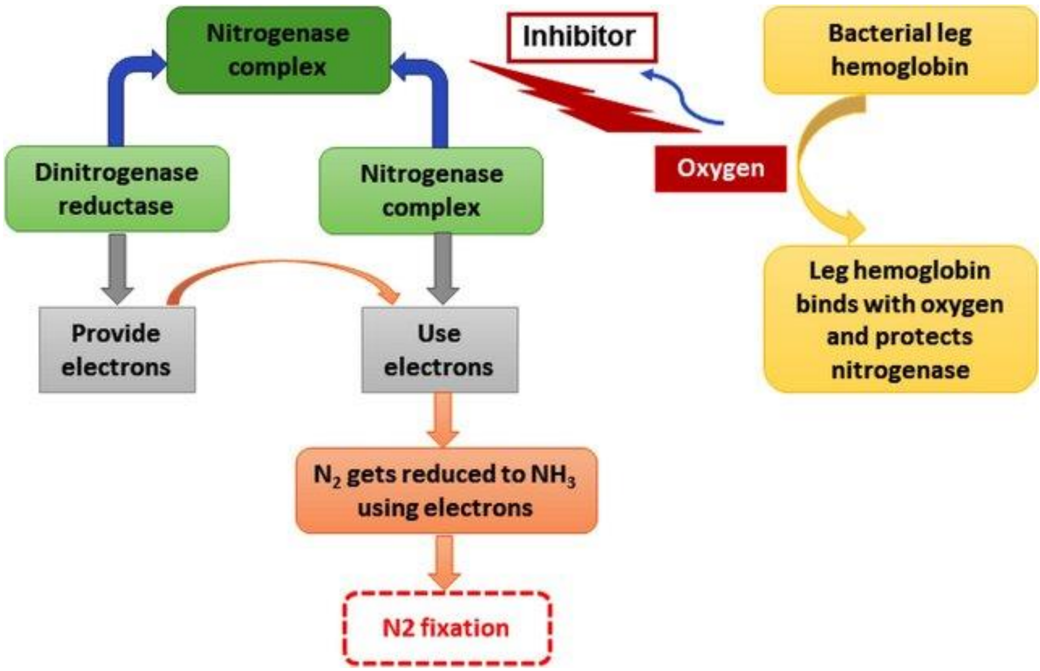

Fig. 2. Nitrogen fixation process

oxygen environment through the production of leghemoglobin, which binds and sequesters oxygen, maintaining the necessary conditions for nitrogenase activity [29].

3.3 Genetic Basis of Nitrogen Fixation

The genetic basis of nitrogen fixation is encoded by a cluster of genes known as the nif (nitrogen fixation) genes. These genes are responsible for the synthesis, assembly, and regulation of the nitrogenase enzyme complex, as well as other proteins involved in the nitrogen fixation process [30]. The nif gene cluster typically includes the following key genes: nifH: Encodes the dinitrogenase reductase (Fe protein). nifD and nifK: Encode the α and β subunits of the dinitrogenase (MoFe protein). nifE and nifN: Involved in the biosynthesis of the FeMo-cofactor. nifB: Plays a role in the early steps of FeMo-co synthesis. nifV: Encodes homocitrate synthase, essential for FeMo-co synthesis. nifA and nifL: Regulatory genes that control the expression of other nif genes in response to environmental conditions [31]. In addition to these core genes, several accessory genes assist in the maturation and function of the nitrogenase complex. These include genes involved in the synthesis of iron-sulfur clusters, electron transport proteins, and those ensuring the protection of nitrogenase from oxygen damage [32]. In symbiotic nitrogen-fixing bacteria like rhizobia, the nif genes are often located on symbiotic plasmids or within symbiosis islands in the genome. These genetic elements can be transferred horizontally between bacteria, facilitating the spread of nitrogen-fixation capabilities among different species and strains [33]. Regulation of nif gene expression is tightly controlled by environmental factors, primarily oxygen and fixed nitrogen levels. Under low-oxygen conditions, the nifA gene activates the transcription of nif operons, while nifL inhibits nifA under high-oxygen conditions, ensuring that nitrogenase is produced only when conditions are favorable for nitrogen fixation [34].

4. SYMBIOTIC RELATIONSHIPS: MOLECULAR AND BIOCHEMICAL RELATION

The symbiotic relationship between nitrogen-fixing bacteria and leguminous plants is a complex and finely tuned interaction that involves multiple molecular and biochemical processes.

4.1 Signal Exchange Between Host Plants and Bacteria

The symbiotic relationship between legumes and rhizobia begins with a sophisticated signal exchange process, which ensures the precise recognition and initiation of symbiosis. This communication is crucial for the establishment of a successful symbiotic interaction. The first step in this molecular dialogue involves the secretion of flavonoids by the legume roots into the rhizosphere. These flavonoids serve as chemoattractants for rhizobia, which contain specific receptors to detect these compounds. Upon sensing the flavonoids, rhizobia activate the expression of nodulation (nod) genes, leading to the synthesis of Nod factors [35]. Nod factors are lipochitooligosaccharides (LCOs) that play a pivotal role in the symbiotic signaling process. Nod factors bind to receptor-like kinases (RLKs) located on the surface of legume root hairs. This binding triggers a cascade of intracellular signaling events within the plant cells, including calcium spiking and the activation of specific transcription factors [36]. These signals ultimately lead to root hair curling, trapping the rhizobia, and initiating the infection process. The recognition and response to Nod factors are highly specific, with different legume species recognizing distinct sets of Nod factors produced by compatible rhizobial strains. This specificity is mediated by the structural variations in the Nod factors, such as the length and saturation of the fatty acid chain, as well as the presence of additional chemical modifications like acetylation or sulfation [37].

4.2 Formation of Root Nodules

Following the successful signal exchange, the formation of root nodules begins. This process involves coordinated plant and bacterial responses that result in the development of specialized structures capable of nitrogen fixation. The infection process starts with the entry of rhizobia into the root hair cells through infection threads, which are tubular structures formed by the invagination of the plant cell wall. The rhizobia proliferate within these threads, which extend into the root cortex [38]. Meanwhile, cortical cells undergo dedifferentiation and proliferation, leading to the formation of the nodule primordium. As the infection threads reach the nodule primordium, rhizobia are released into the plant cells within symbiosomes, which are membrane-bound compartments derived from the plant plasma membrane. Each symbiosome contains one or more rhizobia, now referred to as bacteroids, which will carry out nitrogen fixation [39]. The differentiation of plant cells and rhizobia within the nodule is tightly regulated by a network of plant hormones, including auxins, cytokinins, and ethylene. Cytokinins, in particular, play a crucial role in nodule organogenesis by promoting cell division and differentiation in the root cortex [40].

4.3 Metabolic Integration in the Symbiotic Relationship

Metabolic integration between the host plant and rhizobia is essential for the symbiotic relationship to function effectively. This integration ensures the exchange of nutrients and metabolic intermediates necessary for nitrogen fixation and plant growth. Within the nodule, the host plant supplies the bacteroids with carbon sources, primarily in the form of dicarboxylates such as malate and succinate, which are derived from photosynthetically fixed carbon [41]. These carbon compounds are transported into the bacteroids via specific transporters and are utilized in the tricarboxylic acid (TCA) cycle to generate ATP and reducing power required for nitrogen fixation. The fixed nitrogen, in the form of ammonia, is assimilated into amino acids and transported back to the plant cells. This assimilation process involves the enzymes glutamine synthetase and glutamate synthase, which convert ammonia into glutamine and glutamate, respectively [42]. These amino acids are then used by the plant for protein synthesis and other metabolic processes. Oxygen regulation within the nodule is another critical aspect of metabolic integration. Nitrogenase, the enzyme complex responsible for nitrogen fixation, is highly sensitive to oxygen. To protect nitrogenase while ensuring an adequate oxygen supply for respiration, leguminous plants produce leghemoglobin, a heme-containing protein that binds and buffers oxygen levels within the nodule [43].

4.4 Host Specificity and Compatibility Factors

Host specificity and compatibility between legumes and rhizobia are determined by a combination of genetic, molecular, and biochemical factors. These factors ensure that only compatible partners establish a successful symbiotic relationship. The specificity of the initial signal exchange, mediated by flavonoids and Nod factors, is one of the primary determinants of host compatibility. The structural diversity of Nod factors and the specificity of their recognition by plant receptors are crucial for ensuring the correct pairing of host and symbiont [44]. Genetic determinants in both the host plant and the rhizobia also play a significant role in compatibility. In rhizobia, the nod, nif, and fix genes are essential for nodulation, nitrogen fixation, and the maintenance of the symbiotic relationship. Mutations in these genes can disrupt the symbiosis, leading to ineffective or non-functional nodules [45]. In the host plant, several genes have been identified that are involved in nodule formation and function. These include genes encoding receptor-like kinases, transcription factors, and proteins involved in hormone signaling and transport. Mutations in these genes can affect nodule development and the symbiotic efficiency [46]. Compatibility factors also include the presence of specific microbial partners in the soil microbiome. The interactions between rhizobia and other soil microorganisms can influence the establishment and effectiveness of the symbiotic relationship. For example, the presence of certain mycorrhizal fungi can enhance the colonization of roots by rhizobia and improve nodule function [47].

5. ECOLOGICAL PERSPECTIVES

The symbiotic relationships between nitrogen-fixing bacteria and leguminous plants play a crucial role in shaping ecological systems.

5.1 Role of Nitrogen-Fixing Bacteria in Soil Fertility

Nitrogen-fixing bacteria, particularly those in symbiotic relationships with legumes, are fundamental contributors to soil fertility. These bacteria convert atmospheric nitrogen (N_2) into ammonia (NH_3), a form of nitrogen that plants can readily assimilate. This process, known as biological nitrogen fixation, enriches the soil with bioavailable nitrogen, which is a limiting nutrient in many ecosystems [48]. The role of nitrogen-fixing bacteria in soil fertility is multifaceted. They not only increase the nitrogen content of the soil but also improve soil structure and microbial activity. The organic matter produced by decomposing legume residues enhances soil organic carbon levels, which in turn improves soil texture, water retention, and aeration [49]. Additionally, the fixed nitrogen supports the growth of non-leguminous plants in the surrounding area, thereby promoting plant diversity and overall ecosystem productivity [50]. In agricultural systems, the incorporation of leguminous crops in crop rotations or as cover crops is a common practice to enhance soil fertility naturally. This reduces the need for synthetic nitrogen fertilizers, which are associated with environmental issues such as groundwater contamination and greenhouse gas emissions. Moreover, legumes can improve the availability of other essential nutrients, such as phosphorus, by altering the soil pH and enhancing microbial activity that facilitates nutrient cycling [51].

5.2 Impact on Plant Community Dynamics

Nitrogen-fixing bacteria significantly influence plant community dynamics by altering the availability of nitrogen in the soil. This can affect species composition, competition, and successional patterns within plant communities. The presence of nitrogen-fixing legumes in an ecosystem can lead to increased nitrogen levels, benefiting both the legumes and neighboring plants that can utilize the additional nitrogen [52]. In nitrogen-poor environments, legumes and their symbiotic bacteria can establish themselves as keystone species, playing a critical role in the establishment and maintenance of plant communities. By improving soil nitrogen levels, legumes facilitate the growth of other plant species, promoting greater biodiversity and stabilizing plant communities [53]. This positive feedback loop enhances ecosystem resilience and the capacity of plant communities to withstand disturbances such as herbivory, disease, and climatic variations. Conversely, the introduction of nitrogen-fixing species into ecosystems where they were previously absent can lead to shifts in plant community structure. In some cases, this can result in the displacement of native species and the proliferation of invasive species that can outcompete the native flora due to the increased nitrogen availability [54]. Therefore, understanding the ecological context is crucial when considering the introduction or management of nitrogen-fixing plants in natural and agricultural ecosystems.

5.3 Influence on Ecosystem Productivity and Stability

The influence of nitrogen-fixing bacteria on ecosystem productivity and stability is profound. By enhancing soil nitrogen levels, these bacteria contribute to the primary productivity of ecosystems, which is the foundation for the energy flow and nutrient cycling within ecological networks. Increased nitrogen availability typically leads to higher rates of photosynthesis and biomass production, supporting more robust and productive ecosystems [55]. In natural ecosystems, nitrogen-fixing plants often play a pivotal role in primary succession, particularly in environments where soil nitrogen is initially low, such as newly formed volcanic landscapes or post-glacial terrains. These pioneering species establish themselves, improve soil fertility, and create conditions conducive to the establishment of other plant species, thereby driving the successional process [56]. The stability of ecosystems is also closely linked to the presence of nitrogen-fixing plants. These plants contribute to nutrient cycling and soil fertility maintenance, which are essential for sustaining long-term ecosystem productivity. In ecosystems subject to periodic disturbances, such as fire or grazing, nitrogen-fixing plants can facilitate recovery and enhance ecosystem resilience [57]. Their ability to rapidly colonize disturbed areas and improve soil nitrogen levels supports the regrowth of plant communities and the restoration of ecosystem functions.

5.4 Symbiosis and Environmental Stress Adaptation

Symbiotic relationships between nitrogen-fixing bacteria and legumes enhance the ability of plants to adapt to environmental stresses, such as nutrient deficiency, drought, and salinity. These symbiotic partnerships provide several benefits that improve the stress tolerance of both the host plant and the associated microbial community. In nutrient-poor soils, the ability to fix atmospheric nitrogen gives leguminous plants a competitive advantage over non-leguminous plants. This is particularly important in marginal environments where nitrogen is a limiting factor for plant growth. The symbiosis with nitrogen-fixing bacteria enables legumes to thrive in such conditions, contributing to the stabilization and sustainability of these ecosystems [58]. Drought is another significant stress factor that affects plant growth and productivity. Symbiotic nitrogen fixation can enhance the drought tolerance of legumes by improving their water-use efficiency and maintaining nitrogen supply under water-limited conditions. Studies have shown that legumes with effective symbiotic relationships exhibit better growth and higher yield under drought stress compared to non-symbiotic plants [59]. The production of osmoprotectants and antioxidants by both the plant and the bacteria during drought stress further aids in maintaining cellular integrity and function [60]. Salinity stress, which affects large areas of agricultural land worldwide, can also be mitigated through symbiotic nitrogen fixation. Legumes associated with salt-tolerant rhizobial strains can grow in saline soils, where other crops might fail. The symbiotic bacteria help in reducing the negative effects of salinity by improving nitrogen availability and promoting plant growth, even under high salt concentrations [61].

6. EVOLUTIONARY PERSPECTIVES

The evolutionary dynamics of the symbiotic relationships between legumes and nitrogen-fixing bacteria, primarily rhizobia, have shaped their interactions and adaptations over millions of years.

6.1 Co-evolution of Legumes and Rhizobia

Co-evolution, the reciprocal evolutionary influence between interacting species, has played a significant role in the development of the legume-rhizobia symbiosis. This mutualistic relationship, where both partners benefit, has driven the co-evolutionary arms race, resulting in highly specialized interactions. The co-evolution of legumes and rhizobia can be traced back to the early Cretaceous period, approximately 100 million years ago, when the first leguminous plants appeared [62]. Phylogenetic studies suggest that the ability to form symbiotic relationships with nitrogen-fixing bacteria has evolved multiple times independently within the legume family (Fabaceae), highlighting the adaptive significance of this trait [63]. The evolution of symbiotic signaling mechanisms is a prime example of co-evolution. Legumes secrete specific flavonoids that are recognized by compatible rhizobia, which then produce Nod factors that trigger nodule formation in the host plant. This precise molecular dialogue ensures that only compatible partners engage in symbiosis, minimizing the risk of exploitation by non-beneficial microbes [64]. Over evolutionary time, both legumes and rhizobia have refined these signaling mechanisms, leading to the diversity and specificity observed in modern legume-rhizobia interactions.

6.2 Genetic Adaptations in Leguminous Plants

Leguminous plants have developed a range of genetic adaptations that facilitate and optimize their symbiotic relationships with nitrogen-fixing bacteria. These adaptations include the evolution of specific genes and regulatory networks that control nodule development, function, and maintenance. One of the key genetic adaptations in legumes is the evolution of receptor-like kinases (RLKs) that recognize Nod factors produced by rhizobia. The LysM domain-containing RLKs, such as NFR1 and NFR5 in Lotus japonicus, are essential for Nod factor perception and signaling [65]. These receptors initiate a signaling cascade that leads to nodule formation, highlighting the importance of precise molecular recognition in symbiotic interactions. Legumes have also evolved complex regulatory networks to control nodule organogenesis and function. The transcription factor NIN (NODULE INCEPTION) is a central regulator of nodule development, coordinating the expression of genes involved in cell division, differentiation, and infection thread formation [66]. Additionally, leguminous plants have adapted mechanisms to control the number and location of nodules, ensuring efficient use of resources and preventing excessive nodule formation [67]. Genetic adaptations in legumes also include the evolution of mechanisms to protect the nitrogenase enzyme from oxygen damage. The production of leghemoglobin, which buffers oxygen levels within nodules, is a crucial adaptation that allows nitrogenase to function in an oxygen-limited environment [68]. These genetic innovations have enabled legumes to establish and maintain effective symbiotic relationships with rhizobia, contributing to their ecological success.

6.3 Evolutionary Divergence Among Nitrogen-Fixing Bacteria

The diversity of nitrogen-fixing bacteria, particularly rhizobia, reflects their evolutionary divergence and adaptation to different leguminous hosts and environmental conditions. Phylogenetic analyses indicate that rhizobia belong to several distinct lineages within the Alphaproteobacteria and Betaproteobacteria classes, including the genera Rhizobium, Bradyrhizobium, Sinorhizobium, and Azorhizobium [69]. The evolutionary divergence among rhizobia has been driven by their interactions with different leguminous hosts. Host specificity, mediated by the production of specific Nod factors and the recognition of these signals by legume receptors, has played a key role in shaping rhizobial diversity [70]. This specialization has resulted in the co-evolution of distinct rhizobial strains adapted to particular legume species or groups. Environmental factors have also contributed to the evolutionary divergence of rhizobia. Soil pH, temperature, and nutrient availability influence the distribution and competitiveness of rhizobial strains. For instance, Bradyrhizobium species are often found in acidic soils and have evolved strategies to thrive in such environments, while Sinorhizobium species are more common in neutral to alkaline soils [71]. The genomic plasticity of rhizobia, characterized by the presence of large plasmids and symbiosis islands, has facilitated their adaptation to diverse ecological niches. These genetic elements harbor key symbiotic genes, such as those involved in Nod factor production and nitrogen fixation, and can be horizontally transferred between strains, contributing to the rapid evolution of symbiotic capabilities [72].

6.4 Horizontal Gene Transfer and Symbiotic Evolution

Horizontal gene transfer (HGT) has played a crucial role in the evolution of symbiotic nitrogen fixation. The transfer of symbiotic genes between different bacterial species and strains has facilitated the spread of nitrogen-fixing capabilities and the emergence of new symbiotic associations. Evidence for HGT in rhizobia includes the presence of symbiotic genes on plasmids and symbiosis islands that can be transferred between bacteria. For example, the symbiotic plasmid pSymA in Sinorhizobiummeliloti carries genes for Nod factor production and nitrogen fixation, and its transfer can confer symbiotic capabilities to non-symbiotic strains [73]. This genetic mobility allows for the rapid acquisition and dissemination of symbiotic traits, promoting adaptation to new hosts and environments. HGT has also contributed to the genetic diversity and evolution of rhizobial populations. By acquiring new symbiotic genes, rhizobia can expand their host range and improve their competitive abilities in the rhizosphere. This process has led to the emergence of rhizobial strains with enhanced symbiotic efficiency and ecological versatility [74]. The role of HGT in symbiotic evolution is not limited to rhizobia. Other nitrogen-fixing bacteria, such as Frankia and cyanobacteria, have also acquired symbiotic genes through HGT, highlighting the widespread impact of this evolutionary mechanism across different lineages of nitrogen-fixing organisms [75].

7. AGRICULTURAL RELATION

The symbiotic relationship between nitrogen-fixing bacteria and leguminous plants has significant implications for agriculture, providing sustainable solutions to enhance crop yields and reduce dependency on chemical fertilizers.

7.1 Enhancing Crop Yield through Symbiotic Nitrogen Fixation

Symbiotic nitrogen fixation plays a critical role in enhancing crop yields, particularly in leguminous crops such as soybeans, peanuts, lentils, and peas. These crops have the unique ability to form symbiotic relationships with nitrogen-fixing bacteria, primarily from the genera Rhizobium and Bradyrhizobium, which convert atmospheric nitrogen into ammonia, a form of nitrogen that plants can utilize for growth [76]. The enhanced availability of nitrogen through biological fixation improves the nutritional status of the soil, leading to increased plant growth, higher biomass, and ultimately greater yields. Studies have shown that legumes inoculated with efficient strains of rhizobia produce significantly higher yields compared to non-inoculated plants or those grown in nitrogen-deficient soils [77]. This process not only boosts the productivity of the leguminous crops themselves but also benefits subsequent crops in rotation by enriching the soil with residual nitrogen [78]. In addition to direct nitrogen fixation, legumes also contribute organic matter to the soil through root exudates and decaying plant residues. This organic matter enhances soil structure, water-holding capacity, and microbial activity, creating a more favorable environment for plant growth. The cumulative effect of these benefits can lead to substantial increases in overall farm productivity [79].

7.2 Sustainable Agricultural Practices

Incorporating legumes and their symbiotic partners into agricultural systems is a cornerstone of sustainable farming practices. This approach helps to reduce the reliance on synthetic nitrogen fertilizers, which are energy-intensive to produce and can cause environmental problems such as soil acidification, water pollution, and greenhouse gas emissions [80]. Crop rotation and intercropping with legumes are effective strategies for maintaining soil fertility and reducing pest and disease pressure. By rotating legumes with non-leguminous crops, farmers can naturally replenish soil nitrogen levels, breaking the cycle of dependency on chemical fertilizers. Intercropping, where legumes are grown alongside other crops, can lead to synergistic interactions that enhance nutrient availability and improve overall crop performance [81]. Cover cropping with legumes is another sustainable practice that protects the soil during fallow periods, preventing erosion and maintaining soil health. Cover crops such as clover and vetch can fix nitrogen, suppress weeds, and improve soil organic matter, providing multiple ecosystem services that contribute to sustainable agriculture [82]. Agroforestry systems, which integrate trees and shrubs with crops and livestock, can also benefit from the inclusion of nitrogen-fixing legumes. These systems enhance biodiversity, improve soil fertility, and provide additional sources of income through the production of timber, fruits, and other products [83]. The deep root systems of some leguminous trees can access nutrients from deeper soil layers, bringing them to the surface and making them available to other plants.

7.3 Genetic Engineering and Biofertilizers

Advances in genetic engineering offer promising opportunities to enhance the efficiency of nitrogen fixation and expand its benefits to a wider range of crops. Genetic modification of both rhizobia and leguminous plants can improve symbiotic performance, increase nitrogen fixation rates, and enhance stress tolerance. One approach involves the genetic engineering of rhizobia to improve their competitiveness, nitrogen fixation efficiency, and ability to thrive in diverse environmental conditions. For example, overexpression of nitrogenase genes or the introduction of genes for better nodulation and stress resistance can lead to more effective and resilient bacterial strains [84]. Genetic engineering of leguminous plants focuses on enhancing their ability to establish and maintain symbiotic relationships with rhizobia. This includes the modification of genes involved in nodulation signaling, nodule development, and nitrogen assimilation. For instance, overexpression of the transcription factor NIN (NODULE INCEPTION) has been shown to enhance nodule formation and increase nitrogen fixation efficiency [85]. Biofertilizers, which are formulations containing beneficial microorganisms such as rhizobia, offer an environmentally friendly alternative to chemical fertilizers. These biofertilizers can be applied to seeds, soil, or plants, promoting the establishment of effective symbiosis and improving crop yields. Inoculants containing elite strains of rhizobia have been successfully used in many regions to boost legume productivity and soil health [86]. The development of biofertilizers that combine rhizobia with other beneficial microbes, such as mycorrhizal fungi and plant growth-promoting rhizobacteria (PGPR), is an area of active research. These multi-strain formulations can provide synergistic benefits, enhancing nutrient uptake, disease resistance, and overall plant health [87].

7.4 Challenges and Future in Agricultural Applications

While the agricultural benefits of symbiotic nitrogen fixation are well recognized, several challenges must be addressed to maximize its potential and ensure widespread adoption. One of the primary challenges is the variability in the effectiveness of rhizobia-legume symbiosis under different environmental conditions. Factors such as soil pH, temperature, moisture, and nutrient availability can influence the establishment and efficiency of the symbiotic relationship. Developing rhizobial strains that are highly effective and resilient under a range of conditions is crucial for consistent performance [88]. Another challenge is the need for effective delivery systems for biofertilizers. Ensuring that inoculants remain viable and effective from production to application requires advancements in formulation, storage, and application techniques. Developing cost-effective and user-friendly biofertilizer products will be key to their adoption by farmers, particularly in resource-limited settings [89]. The integration of genetic engineering with traditional breeding programs offers promising avenues for improving legume performance and expanding the benefits of nitrogen fixation to non-leguminous crops. Research into transferring nitrogen fixation traits to cereals, such as rice and wheat, through genetic engineering or synthetic biology approaches is ongoing and holds great potential for transforming global agriculture [90]. Future research should also focus on understanding the complex interactions within the soil microbiome and how these interactions influence symbiotic nitrogen fixation. Advances in metagenomics and systems biology can provide insights into the microbial communities that support or hinder symbiosis, leading to more targeted and effective management practices [91]. Fostering collaborations between researchers, policymakers, and farmers is essential to translating scientific advancements into practical solutions. Extension services and farmer education programs can play a vital role in promoting the benefits of symbiotic nitrogen fixation and supporting the adoption of sustainable agricultural practices [92].

8. CONCLUSION

The symbiotic relationship between nitrogen-fixing bacteria and leguminous plants offers immense benefits for agriculture and ecosystem stability, enhancing crop yields and soil fertility while reducing the need for synthetic fertilizers. Advances in genetic engineering and biofertilizer technology hold promise for optimizing these interactions and extending their benefits to a broader range of crops. However, challenges such as environmental variability, effective delivery systems, and integration with traditional breeding programs must be addressed. Future research should focus on understanding microbial interactions within the soil and fostering collaborations to translate scientific advancements into practical applications. By leveraging these natural processes, we can move towards more sustainable and resilient agricultural systems, ensuring food security and environmental health for future generations.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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