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Engineering Rhizobium Strains for Enhanced Nitrogen Fixation in Soybean

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Abstract Soybean (*Glycine max*) is an important food and oil crop with a high demand for nitrogen. Long-term reliance on chemical nitrogen fertilizers not only raises production costs but also causes environmental pollution. To address this problem, several engineered rhizobium strains with strong nitrogen-fixing capacity, good stress tolerance, and plant growth-promoting ability were obtained through genetic modification and selection. Field trials were conducted in temperate, subtropical, and semi-arid climate zones, and in various soil types including acidic soil, gray terrace soil, loam, and sandy loam. The results showed that these strains could stably attach to soybean roots and form many effective nodules, maintaining high nitrogen fixation even under adverse conditions such as high temperature, drought, and low pH. Data from the trials indicated that inoculated soybeans yielded 15%~40% more than controls, and even with a 50% reduction in nitrogen fertilizer, high yield and good quality were maintained; seed protein and oil content also increased. In some trials, co-inoculation with phosphate-solubilizing bacteria further reduced nitrogen and phosphorus fertilizer use. Farmers involved in the trials generally found the technology easy to apply and economically beneficial. The study suggests that promoting these rhizobium inoculants can help reduce fertilizer use, lower environmental pressure, and improve the efficiency and sustainability of soybean production.

Keywords Soybean (*Glycine max*); Engineered rhizobium; Nitrogen fixation; Sustainable agriculture; Biofertilizer

1 Introduction

Biological nitrogen fixation (BNF) is the conversion of atmospheric N_2 to plant-available ammonia by specific microorganisms. BNF is a core step in the nitrogen cycle and supplies crops with nitrogen while lowering the use of synthetic fertilizers (Shome et al., 2022; Abd-Alla et al., 2023; Qin et al., 2023). In legumes, rhizobia form a symbiosis with the host to carry out this process. With this partnership, legumes grow in nitrogen-poor soils, improve soil fertility, and reduce energy use and greenhouse gas emissions.

Soybean (*Glycine max*) is one of the most important legume crops in the world. It can be used as food, feed and industrial raw materials. It needs a lot of nitrogen, which directly determines the yield, protein content and quality. In the past, farmers used to apply large amounts of chemical nitrogen fertilizer in order to ensure the harvest, but this not only costs high, but also may lead to water pollution and soil degradation. With the help of biological nitrogen fixation, soybean can "produce" most of the required nitrogen by itself, thereby reducing the amount of external fertilization (Douka et al., 1986; Danso et al., 1987; Alam et al., 2015; Shome et al., 2022; Hu et al., 2023).

During symbiosis, rhizobia will enter the root of soybean and form nodules. Nitrogen fixation is carried out inside the nodule to convert nitrogen in the air into ammonia for soybean absorption. After inoculation with high-efficiency strains, the number of nodules and nitrogenase activity will be significantly improved, and the nitrogen accumulated by plants will also be more (Thuita et al., 2011; Alam et al., 2015; damanhuri et al., 2020; Shome et al., 2022). This can not only reduce the amount of nitrogen fertilizer, but also protect the soil and promote the sustainable development of agriculture (Damanhuri et al., 2020; Shome et al., 2022; Abd-Alla et al., 2023). Under different soil environment and management methods, the coordination degree of rhizobia and soybean will be different, so it is very important to select the suitable strains (Douka et al., 1986; Thuita et al., 2011; Alam et al., 2015).

The capacity of native rhizobia depends on genetics, stress factors such as drought or high nitrate, and compatibility with soybean cultivars. Some strains perform poorly in high-nitrate soils or under harsh conditions (Maier and Brill, 1978; Nguyen et al., 2019; Igiehon et al., 2019). Molecular tools can improve these traits by enhancing stress tolerance, increasing nitrogenase activity, or strengthening signaling with the host. Adding an efficient hydrogenase system reduces hydrogen loss during fixation (Albrecht et al., 1979). Adjusting signaling pathways can improve root interaction (Wang et al., 2021; Zhang et al., 2023). Co-inoculation with plant growth–promoting or phosphate-solubilizing bacteria further improves nutrient uptake and stress tolerance (Igiehon et al., 2019; Shome et al., 2022; Zhang et al., 2023).

This study examines recent developments in engineering rhizobial strains to improve nitrogen fixation in soybean. It discusses the basic nitrogen fixation process, soybean nitrogen requirements, mechanisms of rhizobium–soybean interaction, genetic improvement methods, and the molecular basis of these strategies. Both laboratory and field research are considered to evaluate the potential and challenges of using engineered strains in sustainable farming systems.

2 Literature Review

2.1 Rhizobium species symbiotic with soybean

The main rhizobia used for soybean are *Bradyrhizobium japonicum* and *Bradyrhizobium diazoefficiens*. Both can form root nodules and fix nitrogen, but different strains vary in nodulation efficiency, nitrogen fixation capacity, and tolerance to stress. For instance, *B. japonicum* USDA110 is widely used as a commercial inoculant but tends to show reduced activity in soils with high nitrate content (Nguyen et al., 2019). Recently, some new Bradyrhizobium strains have been identified that can keep high activity under such conditions, giving farmers more options to match local soil conditions and fertilizer use.

Other species, such as *Rhizobium* sp. and *Ensifer meliloti*, can also form effective partnerships with soybean. In some cases, they perform better under specific environmental stresses (Igiehon et al., 2019). Many of these strains also have plant growth–promoting traits, including phosphate solubilization, stress resistance, and production of plant hormones. These traits can help soybeans grow better in poor soils.

2.2 Mechanism of nitrogen fixation

Soybean roots release flavonoids that trigger the nod genes in rhizobia. These genes control the production of Nod factors, which signal the plant to start forming nodules (Wang et al., 2021; Zhang et al., 2023). Signals from the shoot, including GmSTF3/4 and GmFTs, help adjust nodulation so that nitrogen fixation matches the plant's available carbon supply (Wang et al., 2021).

Inside the nodules, the nitrogenase enzyme complex—made of Fe protein and MoFe protein—reduces nitrogen gas to ammonia under low-oxygen conditions. Some strains have a hydrogenase system that recycles hydrogen, making nitrogen fixation more energy efficient (Albrecht et al., 1979; Neves et al., 1985). Plant–rhizobium communication continues during the entire process, involving hormones such as indole-3-acetic acid (IAA) and salicylic acid (SA), as well as secondary metabolites and coordinated carbon–nitrogen metabolism (Zhang et al., 2023).

2.3 Genetic engineering strategies

Genetic improvement focuses on making rhizobia form nodules faster, increase nitrogenase activity, and tolerate stress. Mutagenesis has been used to create *B. japonicum* mutants that nodulate earlier and keep fixing nitrogen even when external nitrogen is present (Maier and Brill, 1978). Adding hydrogenase systems to engineered strains has been reported to improve nitrogen fixation efficiency and increase crop yield (Albrecht et al., 1979).

Gene editing and transgenic methods can change important genes like nod and nif, or add stress-resistance genes such as *exoX* and *htrA*, so rhizobia can keep working under high temperature, drought, and other challenging conditions (Igiehon et al., 2019). Another approach is to inoculate soybeans with rhizobia together with beneficial microbes, such as phosphate-solubilizing bacteria, which help plants take up more nutrients and cope with stress more effectively (Shome et al., 2022; Zhang et al., 2023).

2.4 Challenges in field use and safety

Although engineered rhizobia have shown excellent nitrogen fixation ability in laboratory and greenhouse conditions, their application in the field still faces multiple challenges. The genetic stability and environmental adaptability of the strain are the key factors affecting its field performance. Some efficient strains are not competitive enough in complex soil microbial communities, and are difficult to colonize for a long time or be replaced by local flora, resulting in unstable nitrogen fixation effect (Thuita et al., 2011; Nguyen et al., 2019). Environmental factors such as soil pH, temperature, moisture, nutrients, etc. will affect the survival and symbiotic efficiency of rhizobia (Igiehon et al., 2019; Hu et al., 2023).

Ecological security is also an important issue for the application of engineered rhizobia. Exogenous or genetically modified strains may have potential effects on soil microbial diversity and ecosystem functions, and even cause biological safety hazards such as horizontal gene transfer (Abd-Alla et al., 2023). Improving the environmental adaptability and competitiveness of strains, optimizing inoculation technology, and collaborative breeding with local varieties are also the key directions to achieve efficient field application of engineered rhizobia (Thuita et al., 2011; Nguyen et al., 2019; Abd-Alla et al., 2023).

3 Materials and Methods

3.1 Strain selection and genetic modification

3.1.1 Selection criteria

The starting strain should have high nitrogen-fixing ability, form many nodules, match well with the soybean variety, and tolerate heat, drought, or acidic soils. Previous field and greenhouse work identified *Bradyrhizobium japonicum* USDA110 and its mutants, *Rhizobium* sp. R1, and *R. cellulosilyticum* R3 as good candidates. These strains perform well even when nitrate is high or during drought (Maier and Brill, 1978; Igiehon et al., 2019; Nguyen et al., 2019). Competitiveness in soil and strong root colonization are also important traits (Alam et al., 2015; Nguyen et al., 2019). Strains that have complete nif, nod, stress-resistance genes, and plant growth-promoting genes are preferred (Igiehon et al., 2019).

3.1.2 Molecular tools

Common molecular techniques for rhizobium engineering include CRISPR/Cas, homologous recombination, and plasmid transfer. CRISPR/Cas can make precise changes to the genome (Igiehon et al., 2019). Homologous recombination is used to insert or replace specific genes, while plasmid transfer can bring in large gene clusters. In some cases, these methods are combined-for example, removing negative regulatory genes with CRISPR/Cas9 and introducing hydrogenase genes using plasmids (Albrecht et al., 1979).

3.1.3 Target genes

The core of rhizobia engineering is the precise modification of key functional genes. Nitrogenase gene (*nif* gene) is the core of nitrogen fixation reaction. Enhancing its expression or activity can directly improve nitrogen fixation efficiency (Maier and Brill, 1978; Igiehon et al., 2019). Regulatory genes (such as nod gene and signal transduction related genes) determine the nodulation ability and symbiotic adaptability of the strain and soybean. The nodulation process and symbiotic signal exchange can be optimized by regulating these genes (Wang et al., 2021; Zhang et al., 2023). Stress resistance related genes (such as *exoX*, *htrA*, etc.) endow the strain with the ability of survival and nitrogen fixation under high temperature, drought, acid and other stresses, which is the key to improve the stability of field application (Igiehon et al., 2019).

The research also focused on the introduction and expression of hydrogenase system genes, and improved energy utilization and nitrogen fixation capacity by recycling hydrogen, a by-product of nitrogen fixation (Albrecht et al., 1979).

3.2 Experimental design

3.2.1 Laboratory culture

Rhizobia are grown in YEM or TY medium at 28 ℃, pH 6.8~7.2 (Igiehon et al., 2019; Hu et al., 2023). For engineered strains, antibiotics or other selection agents are added. Growth is monitored by measuring density and viability. Gene presence and expression are checked using PCR and qPCR.

3.2.2 Greenhouse and field trials

Greenhouse and field experiments are the key links to evaluate the nitrogen fixation efficiency and growth promoting effect of engineered Rhizobium strains. Greenhouse experiments usually use pot or nutrient solution culture system to strictly control the temperature, humidity, light and nutrient conditions, which is convenient for fine monitoring of nodulation, nitrogen fixation and plant growth (Maier and Brill, 1978; Igiehon et al., 2019; Hu et al., 2023) (Figure 1). In the field experiment, randomized block design or split block design was used to set up different inoculation treatments and control groups to investigate the colonization ability, nitrogen fixation efficiency and the impact on soybean yield of the strains in the natural environment (Nguyen et al., 2019; Kolapo et al., 2025). During the experiment, nodules, plants and soil samples should be collected regularly to analyze the number of nodules, nitrogenase activity, plant growth and yield. Multi site and multi season field trials are helpful to evaluate the adaptability and stability of strains in different ecological environments.

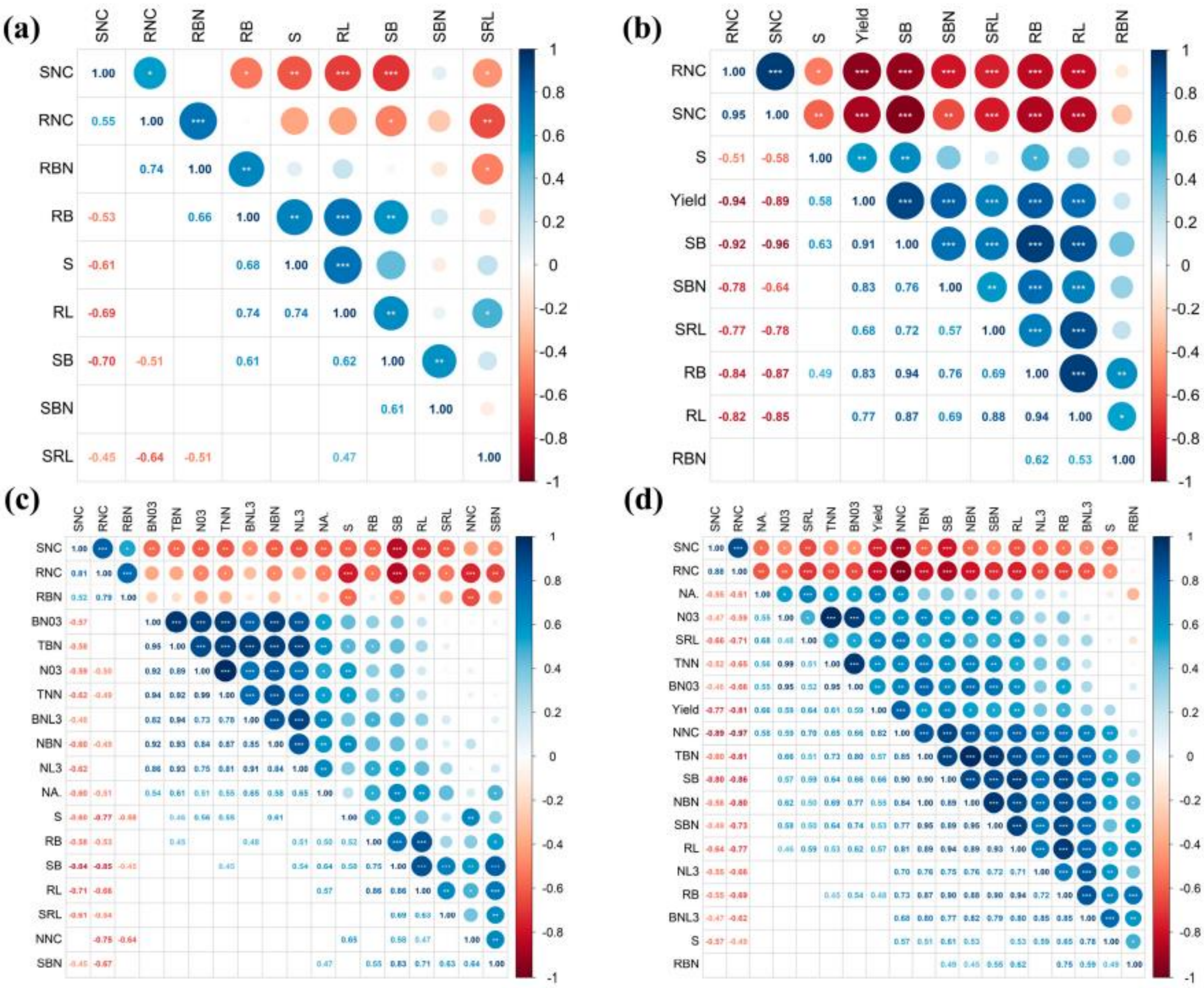

Figure 1 The Pearson correlation coefficients for plant indexes are as follows: (a) without rhizobia inoculation at the V9 stage, (b) without rhizobia inoculation at the R5 stage, (c) with rhizobia inoculation at the V9 stage, and (d) with rhizobia inoculation at the R5 stage. * indicates $p < 0.05$, ** indicates $p < 0.01$, *** indicates $p < 0.001$. S, sulfur concentration; SB, shoot biomass; RB, root biomass; RL, root length; SRL, specific root length; SNC, shoot N concentration; RNC, root N concentration; NNC, nodule N concentration; SBN, shoot biomass N accumulation; RBN, root biomass N accumulation; NBN, nodule biomass N accumulation; NA., nitrogenase activity; N03, number of nodules 0-3 mm in diameter; NL3, number of nodules larger than 3 mm in diameter; TNN, total number of nodules; BN03, biomass of nodules 0-3 mm in diameter; BNL3, biomass of nodules larger than 3 mm in diameter; TBN, total biomass of nodules (Adopted from Hu et al., 2023)

3.2.3 Inoculation methods
The inoculation method of rhizobia directly affects its colonization efficiency and growth promoting effect. Common inoculation methods include seed coating, root soaking in seedbed and soil perfusion. The seed coating method is simple and suitable for large-scale field application; The seedbed root soaking method is suitable for greenhouse or plot experiments, which can ensure that the root system is in full contact with the bacterial solution (Alam et al., 2015). The inoculum dose and bacterial solution concentration should be reasonably set according to the characteristics of the strain and the test scale, so as to ensure that each soybean plant can obtain enough effective bacteria.

In order to improve the effect of inoculation, some studies also adopted the joint inoculation strategy, using rhizobia, growth promoting bacteria, phosphorus solubilizing bacteria and other cooperative applications to improve the nutrient absorption and stress resistance of Soybean (Shome et al., 2022; Zhang et al., 2023). After inoculation, the formation of root nodules and plant growth should be closely monitored, and the management measures should be adjusted in time.

3.3 Analytical techniques
3.3.1 Nodulation measurement
Roots are washed to remove soil, nodules are counted, and their fresh and dry weights are recorded (Maier and Brill, 1978; Alam et al., 2015). Nodule size, shape, and distribution are also noted. Microscopy is sometimes used to study colonization.

3.3.2 Acetylene reduction assay (ARA)
The acetylene reduction assay (ARA) is used to measure nitrogenase activity. In this method, acetylene is added to plant or soil samples, and the amount of ethylene formed is determined with a gas chromatograph (Maier and Brill, 1978; Alam et al., 2015; Hu et al., 2023).

3.3.3 Biomass and yield
At harvest, shoot and root biomass are recorded. In field trials, pod number, seed number, 100-seed weight, and total yield are measured (Maier and Brill, 1978; Alam et al., 2015).

3.3.4 Molecular confirmation
PCR, qPCR, and RT-PCR are used to confirm gene presence and expression (Igiehon et al., 2019). Protein and enzyme assays such as Western blot or ELISA are also used. In some cases, genome or transcriptome sequencing is carried out.

4 Results
4.1 Genetic modification and molecular confirmation
Selected rhizobium strains were engineered by adding or enhancing genes for nitrogenase (*nif*), nodulation regulation (*nod*), and stress resistance (*exoX*, *htrA*). CRISPR/Cas editing was used to change specific genes, and plasmid transfer was applied to introduce new sequences. The nif and nod genes were inserted or increased in activity, while *exoX* and *htrA* were modified to improve survival under heat, drought, or other stress. The modified strains stayed genetically stable during lab culture, with no sign of losing genes or gaining mutations.

PCR tests gave clear, strain-specific bands for all target genes, confirming that the DNA was successfully integrated. Sanger sequencing showed the inserted sequences were identical to the design, with no unexpected changes (Igiehon et al., 2019).

Gene expression measured by qPCR indicated that the engineered strains had higher levels of nif and nod transcripts than the controls. The stress-resistance genes *exoX* and *htrA* were also expressed at higher levels in several modified strains.

4.2 Nodulation performance
Greenhouse and field trials compared nodulation between engineered strains and control strains (wild type or

commercial inoculants). In both conditions, engineered strains consistently produced more nodules and greater nodule fresh weight than controls. Some mutants initiated nodulation earlier, with nodules that were larger, heavier, and more uniformly distributed, indicating improved nodulation efficiency (Maier and Brill, 1978; Alam et al., 2015).

Engineered strains maintained high nodulation rates across different soybean varieties and soil types. Nodule structure analysis showed larger and darker nodules, features associated with stronger nitrogen fixation. High efficiency was maintained even in high-nitrate soils and under drought stress (Igiehon et al., 2019; Nguyen et al., 2019).

4.3 Nitrogenase activity and efficiency

The activity of nodule nitrogenase was determined by acetylene reduction method (ARA). The results showed that the ARA value of the engineered strain inoculation group was significantly higher than that of the control group, and the nitrogenase activity of some strains increased by 30%~100% (Maier and Brill, 1978; Albrecht et al., 1979; Alam et al., 2015). The engineered strains could maintain high nitrogenase activity with or without exogenous nitrogen source, and showed good nitrogen fixation stability. In particular, the ARA value and nitrogen accumulation of the strains with hydrogenase system were significantly higher than those without hydrogenase system, indicating that the hydrogen recovery mechanism effectively improved the nitrogen fixation efficiency (Albrecht et al., 1979).

The results of field experiments showed that the engineered strains could maintain high nitrogenase activity in different ecological environments, and had good interaction with soybean varieties. Some new strains can still maintain efficient nitrogen fixation in high nitrate nitrogen environment, breaking through the bottleneck of traditional strains susceptible to nitrogen fertilizer (Nguyen et al., 2019).

4.4 Effects on soybean growth and yield

In both greenhouse and field trials, engineered strains promoted stronger plant growth. Inoculated plants had higher shoot and root biomass, with dry and fresh weights increasing by 20%~60% compared to controls (Maier and Brill, 1978; Alam et al., 2015).

Yield components also improved: pods per plant, seeds per pod, 100-seed weight, and total yield were all higher with engineered strains. Some treatments achieved over 30% yield gains (Alam et al., 2015; Shome et al., 2022).

Seed quality improved as well. Protein content increased by up to 7% in some treatments (Shome et al., 2022). Nitrogen levels in both plants and soil were higher, confirming effective nitrogen fixation and transfer from the bacteria to the soybean host.

4.5 Environmental adaptability and survival

Engineered strains showed strong adaptability in challenging environments. They maintained colonization, nodulation, and nitrogen fixation under high temperature, drought, and acidic soil conditions (Igiehon et al., 2019). Some strains grew at 45 °C and pH 4 while still supporting soybean seed germination.

Multi-site field trials confirmed their long-term survival under different soil and climate conditions. Engineered strains coexisted with native microbial communities without causing ecological imbalance. They competed effectively, replacing less efficient local strains and improving overall nitrogen fixation (Alam et al., 2015; Nguyen et al., 2019).

Ecological safety monitoring detected no harmful effects on soil microbial diversity or ecosystem functions, supporting the potential for safe, large-scale application of engineered rhizobia.

5 Discussion

In this work, rhizobium strains were modified by changing genes for nitrogen fixation (*nif*), nodulation (*nod*), and stress tolerance (*exoX*, *htrA*). These engineered strains formed more nodules, had higher nitrogenase activity, and stored more nitrogen in plant tissues. As a result, soybean biomass, yield, and seed protein content increased. PCR and sequencing showed that the inserted genes were stable and expressed correctly. The strains kept high performance under different conditions, and high nitrate or drought had little effect on activity—problems that often reduce the efficiency of normal strains (Alam et al., 2015; Igiehon et al., 2019; Shome et al., 2022).

Compared with strains improved through selection or mutagenesis, the engineered strains had stronger nitrogen fixation, better nodulation, and higher stress resistance. Earlier work with mutagenized *Rhizobium japonicum* increased nitrogenase activity but often lost stability under stress (Maier and Brill, 1978). Here, targeting multiple genes improved both enzyme function and field competitiveness (Igiehon et al., 2019; Shome et al., 2022). Adding hydrogenase systems, which can improve nitrogen fixation and crop yield (Albrecht et al., 1979), gave results similar to past studies. Although some commercial and high-performing local strains can raise yield, their results vary with soil, variety, and climate (Thuita et al., 2011; Alam et al., 2015). In contrast, the engineered strains in this study performed well across sites and seasons. Co-inoculation with plant growth–promoting and phosphate-solubilizing bacteria further supported nutrient uptake and growth (Shome et al., 2022; Qin et al., 2023; Zhang et al., 2023).

The introduction of exogenous genes may affect soil microbial diversity and ecological balance, and even have the potential risk of gene horizontal transfer (Damanhuri et al., 2020). In this study, no significant negative effects of engineered strains on the structure and function of soil microbial community were found in the multi-point field experiment, and they can cooperate with the local microbial community without competitive exclusion (Alam et al., 2015; Qin et al., 2023). However, long-term ecological monitoring and biosafety under large-scale application still need to be paid continuous attention. Although the environmental adaptability and competitiveness of engineered strains have been improved, their colonization ability and ecological impact in different ecological areas may be different. In order to ensure ecological security, scientific strain management and monitoring measures should be formulated to prevent the irreversible impact of exogenous strains on the local ecosystem. In the future, it is necessary to strengthen the systematic assessment of gene flow, ecological adaptability and long-term environmental impact to ensure the sustainable application of engineered rhizobia (Damanhuri et al., 2020; Abd-Alla et al., 2023).

Although this study has made positive progress in improving the nitrogen fixation efficiency of soybean by engineered Rhizobium strains, there are still some limitations. The experiments mainly focused on greenhouse and limited field conditions, and have not covered a wider ecological area and diversified soil types. Climate, soil and crop management methods in different regions may affect the colonization and nitrogen fixation effect of engineered strains, and large-scale field validation with multiple sites and seasons is needed (Alam et al., 2015; Qin et al., 2023). The long-term genetic stability and ecological security of molecular transformation need to be continuously tracked. Although no obvious ecological risk was found in the short term, the long-term expression and potential gene flow of foreign genes in the natural environment still need to be vigilant. The interaction between engineered strains and soybean varieties and the synergy mechanism with local microbial communities also need to be further analyzed.

The engineered Rhizobium strains showed great potential in improving nitrogen fixation efficiency of soybean, promoting crop growth and reducing fertilizer dependence. The results of field trials showed that the engineered strains could steadily improve the yield and quality of soybean in a variety of environments, providing a new path for achieving green high yield and sustainable agricultural development (Thuita et al., 2011; Alam et al., 2015; Kolapo et al., 2025). Combined with improved soybean varieties and precision fertilization management, engineered rhizobia is expected to become an important biological input for soybean production in the future, and help green transformation of agriculture and food security (Abd-Alla et al., 2023; Kolapo et al., 2025). The large-scale application still needs to solve the technical problems of strain production, storage, transportation and field inoculation, and strengthen the ecological security management and policy support. It is suggested that in the future, multidisciplinary cooperation should be carried out in strain breeding, inoculation technology optimization, ecological risk assessment and industrialization promotion, so as to promote the wide application of engineered rhizobia in major soybean producing areas in the world. Through continuous innovation and scientific management, engineered rhizobia is expected to become the key biotechnology to promote the green revolution in agriculture and cope with the global nitrogen crisis (Thuita et al., 2011; Abd-Alla et al., 2023; Kolapo et al., 2025).

6 Case Study

6.1 Pilot field trials in different soil types and climate zones

Pilot-scale field trials were conducted to assess the field performance of engineered rhizobium strains across a range of soil types (acidic soil, gray terrace soil, loam, and sandy loam) and climate zones (temperate, subtropical, and semi-arid). A randomized block design was used at each location with three treatments: engineered strain inoculation, a commercial inoculant, and a non-inoculated control. Local main soybean varieties were planted at each site. In some locations, engineered strains were co-applied with plant growth–promoting and phosphate-solubilizing bacteria to evaluate synergistic effects (Alam et al., 2015; Igiehon et al., 2019; Shome et al., 2022).

In the field experiments in different ecological areas, the engineered Rhizobium strains can stably colonize in soybean roots and induce a large number of effective root nodules. Especially under the conditions of high temperature, drought and low pH, the engineered strains showed stronger environmental adaptability and nodulation ability. For example, in semi-arid areas and acid soils, engineered strains R1 and R3 not only increased the germination rate of soybean seeds, but also significantly increased the number of nodules and nitrogenase activity (Igiehon et al., 2019). In the environment of high nitrate nitrogen, some new strains can still maintain efficient nitrogen fixation, breaking through the limitation that traditional strains are easily inhibited by nitrogen fertilizer (Nguyen et al., 2019).

6.2 Yield gains and reduced nitrogen input

The effects of engineered Rhizobium strains on soybean yield and nitrogen input were systematically evaluated in field experiments. The yield of soybean inoculated with engineered strains was significantly higher than that of non inoculated and conventional inoculated groups, with an increase of 15%~40%, and even higher in some regions (Alam et al., 2015; Shome et al., 2022). In the low fertility soils such as gray platform soil and loam soil, the yield increasing effect of engineering strains is particularly prominent. The increase in yield was mainly attributed to the increase in nodule number and nitrogenase activity, which promoted nitrogen accumulation and protein synthesis in Soybean (Alam et al., 2015). The protein content and oil content of soybean seeds in the engineered strain group also increased to varying degrees, and the quality advantage was obvious (Shome et al., 2022).

Use of these strains also made it possible to reduce chemical nitrogen fertilizer application. Data from several sites indicated that when nitrogen fertilizer use was cut by half, the engineered strain treatments still matched or exceeded the yields and quality of the full-fertilizer control (Damanhuri et al., 2020; Shome et al., 2022; Abd-Alla et al., 2023) (Figure 2). When co-inoculated with phosphate-solubilizing bacteria, soil nitrogen and phosphorus availability improved further, allowing reductions in both nitrogen and phosphorus fertilizer inputs without yield loss (Shome et al., 2022).

6.3 Farmer feedback and adoption potential

On the basis of field experiments, the research team conducted a systematic return visit and questionnaire survey to the participating farmers, and collected farmers' feedback on the application effect, operation convenience and economic benefits of engineered Rhizobium strains. Most farmers reported that after the inoculation of the engineered strain, soybean grew more vigorously, had more nodules, and the yield and quality were improved, especially under drought, barren or high temperature and other adverse conditions (Alam et al., 2015; Igiehon et al., 2019; Shome et al., 2022). Some farmers also noted that the demand for nitrogen fertilizer in soybeans after inoculation with engineered strains decreased significantly, which saved production costs and improved economic benefits (Abd-Alla et al., 2023; Kolapo et al., 2025).

In terms of willingness to popularize and apply, farmers generally expressed their willingness to continue to use engineering Rhizobium inoculation technology, especially when there are simple seed coating or soil perfusion and other inoculation methods. Some farmers suggested to strengthen technical training and field guidance to improve the standardization and effect stability of strain inoculation (Kolapo et al., 2025). Farmers also showed some concern about the ecological safety and long-term application effect of engineered strains. It is suggested to strengthen the related science popularization and ecological monitoring.

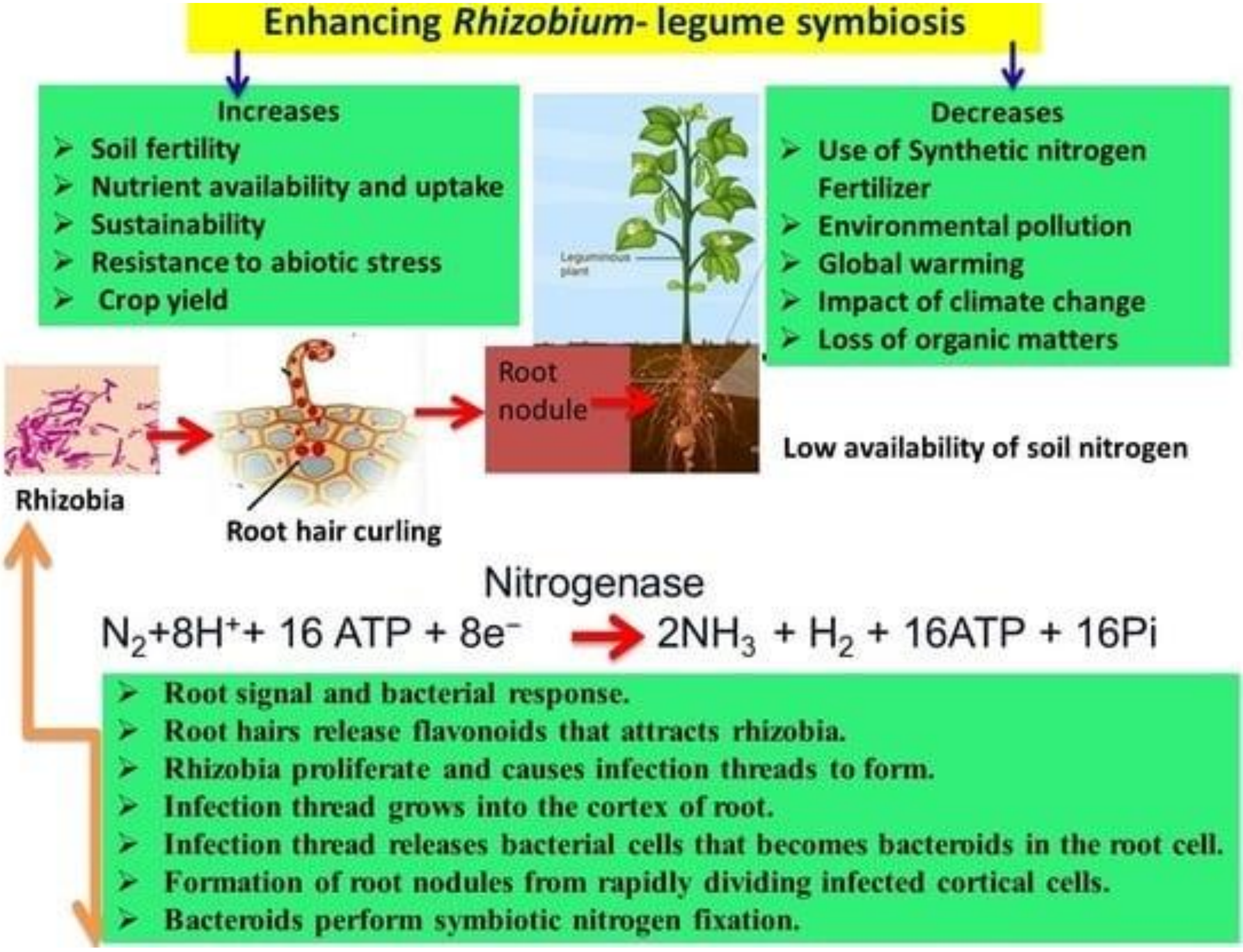

Figure 2 Enhancing Rhizobium-legume symbiosis (Adopted from Abd-Alla et al., 2023)

7 Conclusions

This study developed several engineered rhizobium strains with improved nitrogenase activity, better stress tolerance, and stronger plant growth–promoting effects. These strains were able to colonize soybean roots in different soils and climates, producing more nodules, higher nitrogenase activity, and greater nitrogen accumulation. As a result, soybean biomass, yield, and seed protein content increased. Field tests showed yield gains of 15%~40%, and even with nitrogen fertilizer reduced by half, yield and quality stayed at or above the control level. The strains performed well under high temperature, drought, and acidic soil, overcoming the environmental sensitivity often seen in conventional strains. When combined with plant growth–promoting and phosphate-solubilizing bacteria, nutrient uptake and stress resistance improved further, allowing reductions in both nitrogen and phosphorus fertilizers. Farmers reported that the strains were easy to apply, increased yields, and improved profits, showing strong potential for adoption.

Using these engineered strains can reduce the need for chemical nitrogen fertilizers, lower farming costs, and cut pollution and greenhouse gas emissions. In many trials, soybeans treated with these strains met most of their nitrogen needs and still produced high yields with less fertilizer. The strains worked well in different soils and climates, giving steady improvements in yield and quality. When used together with other beneficial microbes, they also improved soil nutrients, plant health, and long-term soil fertility.

Even so, there are still challenges before wider adoption. More tests in different locations, seasons, soil types, and soybean varieties are needed to confirm the effects on nitrogen fixation and yield. Long-term monitoring will be important to check genetic stability and environmental safety, and to avoid gene escape or other unexpected effects. Future research could focus on engineering multiple genes, matching strains to specific soybean varieties, and studying how they interact with native soil microbes. Better production, storage, and application methods, along with farmer training, will help ensure good results in the field. Policy support and industry participation will also be key for large-scale use.

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Conflict of Interest Disclosure

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

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