Physical Education, Health and Social Sciences

https://journal-of-social-education.org

E-ISSN: 2958-5996

P-ISSN: 2958-5988

# Nitrogen Fixation Efficiency and Rhizobial Diversity in Soybean

**Hasham Feroz Ghuman[1], Naveed Murad[2], Muhammad Taimur Maqbool[3]**

[1] Centre of Agricultural Biochemistry and Biotechnology, University of Agriculture Faisalabad. Hashigh2@gmail.com

[2] Institute of Soil and Environmental Sciences, Muhammad Nawaz Shareef University of Agriculture, Multan. naveedbugti1188@gmail.com

[3] Department of Agronomy, University of Agriculture Faisalabad. taimuragronomist@gmail.com

**DOI: https://doi.org/10.63163/jpehss.v4i1.1206**

## Abstract

Soybean (*Glycine max* [L.] Merr.), the world's leading grain legume with annual production exceeding 353 million tons, relies heavily on biological nitrogen fixation (BNF) to meet its high nitrogen demands (50–80% or more of total N), offering a sustainable alternative to inefficient synthetic fertilizers (30–60% utilization) that contribute to environmental degradation. This review synthesizes current knowledge on BNF efficiency, effectiveness, and specificity in soybean, emphasizing the role of rhizobial diversity primarily *Bradyrhizobium japonicum*, B. *diazoefficiens*, and *Sinorhizobium fredii* in nodule formation, nitrogenase activity, and symbiotic performance. Genetic factors, including host genes (GmNFR1, GmNFR5, GmNIN) and bacterial nodulation factors (Nod factors), govern specificity and efficiency, while environmental stressors (drought, salinity, acidity) and agronomic practices (inoculation, co-inoculation with PGPR/mycorrhizae) modulate outcomes. Advances in microbiome engineering, horizontal gene transfer of symbiosis islands, and selection for elite strains promise enhancements in BNF rates (up to 300 kg N/ha), reducing fertilizer dependency and emissions. The analysis highlights integrated strategies for optimizing soybean productivity in diverse agroecosystems, supporting global food security and sustainability.

**Keywords:** Soybean, Biological Nitrogen Fixation, Rhizobial Diversity, Bradyrhizobium, Sinorhizobium, BNF Efficiency, Nod Factors, Symbiotic Specificity, Microbiome Engineering, Nitrogenase Activity, Horizontal Gene Transfer, PGPR Co-inoculation

## 1. Introduction

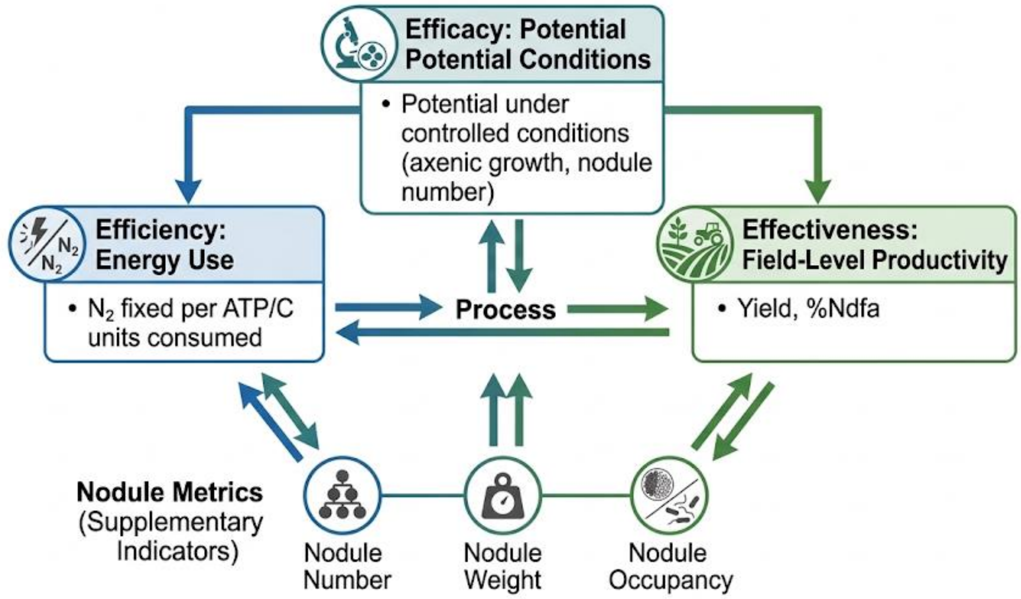
The global agricultural sector faces the formidable challenge of securing food for an expanding population while addressing environmental degradation. Soybean (*Glycine max* [L.] Merr.) Represents a pivotal crop in this endeavor, serving as the world's primary source of vegetable protein and oil (Alves et al., 2003). With global annual production reaching approximately 353 million tons, soybean is the most economically significant grain legume. The high nutritional value of soybean, characterized by seed protein (35–45%) and oil (17–25%) content, imposes a substantial nitrogen demand during its growth cycle (Wang et al., 2024). Historically, this demand was met via synthetic fertilizers; however, these exhibit low efficiency (30–60%) and contribute to soil acidification and greenhouse gas emissions (Zhang et al., 2023). Consequently, biological nitrogen fixation (BNF) has emerged as a critical sustainable alternative, capable of providing 50–80%, and in some cases over 90%, of total nitrogen needs (Mastrodomenico & Purcell, 2012).

Optimization of BNF efficiency and characterization of rhizobial diversity are essential for advancing agroecological sustainability (Herridge et al., 2022).

## 2. Conceptual Framework: Efficiency, Effectiveness, and Efficacy in Symbiosis

A rigorous understanding of symbiotic nitrogen fixation (SNF) requires clear distinctions between efficiency, effectiveness, and efficacy. These parameters reflect different physiological and agronomic benchmarks (Karges et al., 2022). Optimizing soybean BNF requires understanding the distinction between efficiency, effectiveness, and efficacy, summarized in Figure 1.

**Figure 1: Conceptual Framework: Efficiency, Effectiveness, and Efficacy in Soybean BNF**



### 2.1 Thermodynamic and Energy Efficiency

Efficiency in SNF is strictly defined from an energy perspective, focusing on the relationship between energy input and the resulting dinitrogen (N2) fixed (Phillips, 1980). This includes the function of the nitrogenase enzyme complex and the energy provided by the host plant to support the biochemical reaction. Nitrogen fixation is metabolically expensive, requiring significant adenosine triphosphate (ATP) and reducing power (Lyu et al., 2022). Efficiency measures how effectively the bacterial symbiont utilizes carbon skeletons primarily malate to fuel the nitrogenase complex (Yang et al., 2021).

### 2.2 Agronomic Effectiveness and System Productivity

Effectiveness describes the utility of the symbiotic system for practical purposes, such as dry matter and protein production. It integrates energy efficiency with biological and environmental factors. For instance, a rhizobial strain might be highly efficient in a laboratory but ineffective in the field if it fails to compete with indigenous soil populations (Zilli et al., 2025). Effectiveness is largely determined by the delivery of photosynthates from the shoots to the nodules and the synchronization of nitrogen supply with peak crop demand (Santachiara et al., 2019).

### 2.3 Controlled Efficacy and Potential

Efficacy refers to the performance of the system under ideal, axenic conditions (Phillips, 1980). While seldom used in field studies, efficacy measures the potential of a specific rhizobium-legume pairing in controlled environments like growth pouches, allowing researchers to isolate genetic potential from environmental variables (Tominaga et al., 2012).

**Table 1. Distinction between Efficiency, Effectiveness, and Efficacy in Symbiosis**

| Term | Domain | Primary Focus | Measurement Variable |
|---|---|---|---|
| Efficiency | Biochemical | Energy utilization | N2 fixed per unit of ATP or Carbon |
| Effectiveness | Agronomic | System productivity | Dry matter, protein yield, %Ndfa |
| Efficacy | Laboratory | Ideal performance | Nitrogen fixed in axenic culture |
| Nodulation | Developmental | Spatial establishment | Nodule number, weight, occupancy |

## 3. Rhizobial Diversity and Taxonomic Evolution

The taxonomy of soybean-nodulating bacteria has undergone significant revision as genomic sequencing reveals high genetic diversity (Salvagiotti et al., 2008).

### 3.1 The Genus Bradyrhizobium and Global Distribution

The genus Bradyrhizobium remains the primary symbiont for soybean. Modern phylogenomic analyses identify seven major clades, with the B. japonicum and B. elkanii superclades hosting the majority of commercial inoculants (Ormeño-Orrillo et al., 2019). Distribution is strongly influenced by latitude and soil temperature. In Japan and the United States, B. japonicum strains dominate northern latitudes, while B. elkanii clusters are more prevalent in warmer southern regions (Saeki et al., 2000).

### 3.2 Diversification Beyond the Primary Clades

Recent isolation efforts have expanded the list of effective microsymbionts. In addition to B. japonicum, species such as B. diazoefficiens, B. brasilense, B. vignae, and B. yuanmingense have been documented (He et al., 2020). Furthermore, some soybean cultivars form symbioses with members of Sinorhizobium (Ensifer), Mesorhizobium, and Rhizobium, particularly in alkaline soils (Han et al., 2020).

### 3.3 Genetic Plasticity and Symbiosis Islands

The ability of diverse bacteria to nodulate soybean is facilitated by horizontal gene transfer (HGT) of symbiotic genetic elements known as symbiosis islands. These islands contain essential nod, nif, and fix genes (Liu et al., 2023). While housekeeping genes (e.g., gyrB, recA) display high diversity, island genes are often highly conserved, suggesting they move as discrete units across taxonomic backgrounds (Andrews et al., 2018).

## 4. Molecular Mechanisms of Interaction and Nodule Formation

The establishment of symbiosis is a multi-stage process governed by a reciprocal molecular dialogue (Tian et al., 2019).

### 4.1 Signal Exchange and Recognition

The process begins with the exudation of flavonoids by soybean roots, recognized by the bacterial NodD protein, which activates nodulation genes. The resulting Nod factors are perceived by receptors in root hairs, triggering curling and infection thread initiation (Zeffa et al., 2020).

### 4.2 The Role of Kinases and Transcription Factors

Internal signaling regulates the infection process. The enzyme GmSK2-8, a kinase of the GSK3 family, modulates nodulation under stress. In high-salinity environments, GmSK2-8 phosphorylates transcription factors GmNSP1a and GmNSP1b, weakening their ability to bind to gene promoters and inhibiting nodule formation (Wang et al., 2022).

### 4.3 Genomic Determinants: Enrei type SEN1 and GmNMHC5

Specific genes enhance nitrogen fixation capacity. The SEN1 gene, identified in the cultivar Enrei, is a crucial nitrogen fixation enhancing gene that reduces the need for fertilizer. Similarly, the GmNMHC5 gene regulates carbon and nitrogen allocation; knockout mutations elevate gibberellin levels, leading to increased protein yield per plant (Yang et al., 2022).

## 5. Biochemical Regulation of the Nodule Environment

Once nodules are formed, the interior environment must be managed to protect the nitrogenase enzyme (Seefeldt et al., 2009).

### 5.1 Oxygen Management and Leghemoglobin

Nitrogenase is sensitive to oxygen (O2), which can inactivate its Fe-Mo cofactor. The plant produces leghemoglobin to maintain a hypoxic microenvironment while facilitating O2 transport to bacterial respiratory chains (Alves et al., 2003).

### 5.2 Trace Element Synergies: Iron, Molybdenum, and Nickel

BNF relies on critical micronutrients. Iron (Fe) is essential for leghemoglobin and nitrogenase Fe-S clusters. Molybdenum (Mo) is a key component of the nitrogenase cofactor, while Nickel (Ni) serves as a cofactor for hydrogenase, which recycles H2 gas produced during nitrogenase reactions to reclaim energy (Siqueira et al., 2014).

**Table 2. Role of Trace Elements in Biological Nitrogen Fixation**

| Element | Role in BNF | Consequence of Deficiency |
|---|---|---|
| Iron (Fe) | Leghemoglobin and Nitrogenase Fe-S cluster | Reduced nodule density and O2 transport |
| Molybdenum (Mo) | Nitrogenase MoFe-cofactor component | Loss of nitrogenase catalytic activity |
| Nickel (Ni) | Hydrogenase and Urease cofactor | Energy loss via H2 emission; urea toxicity |
| Cobalt (Co) | Vitamin B12 precursor; bacteroid development | Poor nodule maturation |

## 6. Environmental and Management Influences on BNF

Soybean BNF is sensitive to abiotic stressors and soil management practices (Ferguson et al., 2019).

### 6.1 Impact of Soil Nitrogen and "Nitrate Inhibition"

High soil nitrate concentrations inhibit root nodulation. Plants prioritize direct root absorption of nitrogen over energy-intensive BNF when soil N is abundant. Excessive N disrupts the GS/GOGAT (glutamine synthetase/glutamate synthase) pathway and reduces the translocation of ureides the products of SNF from nodules to stems (Zhang et al., 2023).

### 6.2 Abiotic Stressors: pH, Salinity, and Temperature

Soil pH and salinity significantly limit productivity. Low pH (acidity) increases the solubility of toxic metals like aluminum (Al3+), which damage roots. High salinity decreases microbial populations and prevents infection thread formation. Extreme temperatures disrupt the secretion of flavonoids and the transcription of symbiotic genes (He et al., 2020).

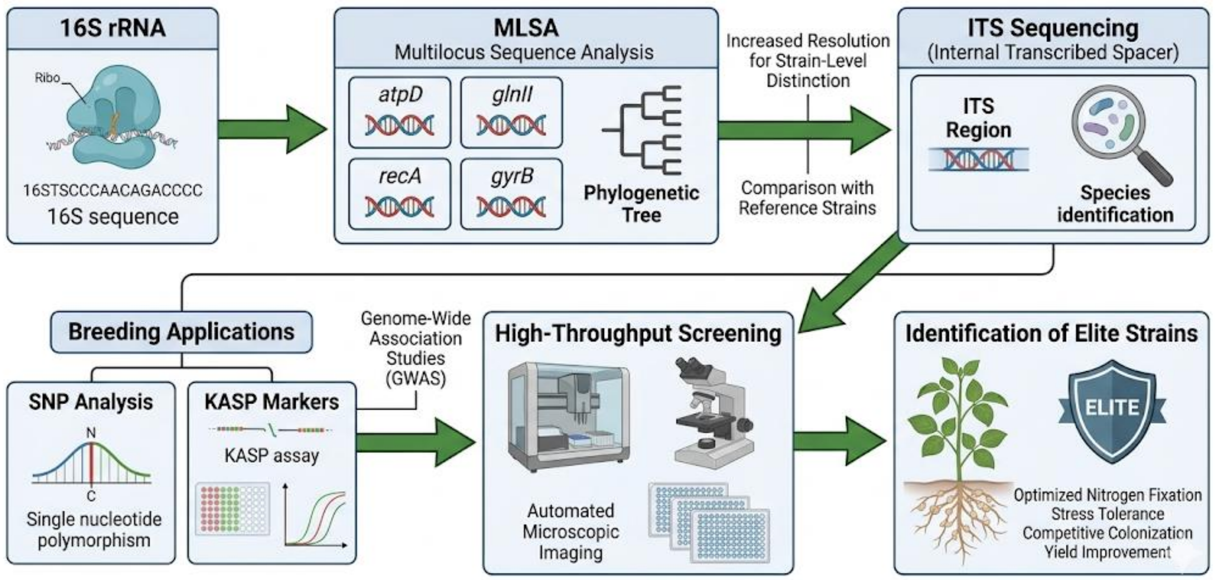
**Table 3. Impact of Abiotic Stress on the Symbiotic System**

| Abiotic Stress | Primary Target | Mechanism |
|---|---|---|
| Acidity (Low pH) | Signaling | Reduced flavonoid secretion; Al3+ toxicity |
| Salinity | Population | Osmotic shock; reduced leghemoglobin |
| Drought | Carbon Flow | Reduced malate supply; O2 limitation |
| High Heat | Survival | Decreased Nod factor induction; cell death |

## 7. Advanced Methodologies for Characterizing Diversity

The shift to genotypic identification has revolutionized the study of rhizobial diversity (Zilli et al., 2025). Characterizing rhizobial diversity has evolved from basic 16S rRNA sequencing to high-resolution MLSA and SNP analysis, illustrated in Figure 2.

Figure 2: Advanced Methodologies for Characterizing Rhizobial Diversity



### 7.1 Multi-Locus Sequence Analysis (MLSA) and ITS Sequencing

Traditional 16S rRNA sequencing often lacks the resolution to distinguish closely related species. Researchers increasingly rely on MLSA targeting genes such as atpD, glnII, recA, and gyrB. The intergenic transcribed spacer (ITS) region is also an effective marker for delineating lineages (Biol. Life Sci. Forum, 2025).

### 7.2 KASP Markers and SNP Analysis in Breeding

To improve BNF efficiency, scientists use high-throughput tools like Kompetitive Allele Specific PCR (KASP) markers. By analyzing single nucleotide polymorphisms (SNPs) across accessions, researchers identify regions that control traits like restricted nodulation of inefficient strains (USDA Annual Report, 2024).

## 8. Bioinoculants and the Potential of Co-Inoculation

Rhizobial inoculants ensure adequate nodulation where indigenous populations are lacking (Karges et al., 2022).

### 8.1 Seed Inoculation with Bradyrhizobium japonicum

Inoculation with B. japonicum has been demonstrated to enhance the symbiotic apparatus even in soils with existing native populations, increasing fixed nitrogen by 33.9–36.1 kg/ha (Meena et al., 2018).

### 8.2 Synergistic Effects of PGPR Co-Inoculation

A promising tool is the co-inoculation of Bradyrhizobium with PGPR such as Azospirillum, Bacillus, and Pseudomonas. Meta-analyses indicate significant increases in nodule number (11.40%), nodule biomass (6.47%), and root biomass (12.84%) through these consortia (Zeffa et al., 2020).

**Table 4. Synergistic Effects of Co-Inoculant Genera on Soybean Growth**

| Co-Inoculant Genus | Primary Mechanism | Observed Impact |
|---|---|---|
| Azospirillum | N-fixation; auxin production | Enhanced root development |
| Bacillus | P-solubilization; biocontrol | Increased pod numbers |
| Pseudomonas | ACC deaminase; mineral uptake | Improved stress tolerance |

## 9. Regional Variations in Fixation Rates and N-Balance

The contribution of BNF varies by region, reflecting soil health and management intensity (Qiu et al., 2019).

### 9.1 Global Comparison of %Ndfa

The percentage of nitrogen derived from the atmosphere (%Ndfa) shows three distinct regional groupings:

- **Brazil:** 78% average %Ndfa, the highest globally due to long-term inoculation programs
- **North America and Argentina:** 61% average %Ndfa, influenced by higher soil nitrogen pools (Tamagno et al., 2018).
- **Europe:** 44% average %Ndfa, potentially due to cooler climates and recent crop introduction (Alves et al., 2003).

### 9.2 Apparent Nitrogen Balance and Yield Gaps

The nitrogen balance the difference between N fixed and N removed in seeds is often negative in commercial systems. Fields with low yield gaps (0–5%) tend to exhibit higher %Ndfa and more favorable N balances than high-gap fields (Herridge et al., 2022).

## 10. Seasonal Dynamics and Environmental Predictors

The maximum rate of N fixation typically occurs around the beginning of pod formation (R3 stage), while the maximum %Ndfa is reached after full pod formation (R4). Advanced predictive models have identified atmospheric vapor pressure deficit (VPD) and precipitation during early reproductive growth as key predictors of fixation cumulative rates (Santachiara et al., 2019).

## 11. Future Outlook: Breeding and Microbiome Engineering

The future of soybean BNF lies in integrating genomics and soil microbiology. Breeding varieties that restrict inefficient indigenous strains while preferring elite inoculants offers a major opportunity (USDA Annual Report, 2024). Furthermore, engineering the root microbiome through composite bacterial inoculants can improve nutrient accessibility and enhance overall plant resilience (Arif et al., 2020).

## 12. Conclusion

Biological nitrogen fixation in soybean exemplifies a sustainable pathway to meet escalating global protein demands while mitigating environmental impacts from synthetic fertilizers. The interplay of rhizobial diversity, host genetics, and agronomic interventions underscores that optimizing BNF efficiency requires a multifaceted approach: selecting promiscuous yet effective strains, leveraging horizontal gene transfer for enhanced symbiosis, and integrating PGPR/mycorrhizal consortia to bolster resilience against abiotic stresses. Achieving BNF contributions exceeding 90% of plant N needs is feasible through targeted breeding and microbiome engineering, potentially reducing greenhouse emissions and soil degradation. Future research should prioritize field-scale validation of engineered symbionts, climate-adapted inoculants, and integrated crop management systems to fully harness soybean's potential in low-input, high-output agriculture, ensuring long-term agroecological stability and nutritional security.

## References

Alves, B. J. R., Boddey, R. M., & Urquiaga, S. (2003). The success of BNF in soybean in Brazil. *Plant and Soil*, *252*(1), 1-9. https://doi.org/10.1023/A:1024143825010

Andrews, M., De Meyer, S., James, E. K., Stępkowski, T., Hodge, S., Simon, M. F., & Young, J. P. W. (2018). Horizontal transfer of symbiosis genes within and between rhizobial genera: occurrence and importance. Genes, 9(7), 321.

Arif, I., Batool, M., & Schenk, P. M. (2020). Plant microbiome engineering: expected benefits for improved crop growth and resilience. Trends in Biotechnology, 38(12), 1385-1396.

Biol. Life Sci. Forum. (2025). Comparative evaluation of 16S rRNA and housekeeping gene-specific primer pairs for rhizobia and agrobacteria metagenomics. *Biology and Life Sciences Forum*, *46*(1), 1-9. https://doi.org/10.3390/blsf2025046001

Ferguson, B. J., Mens, C., Hastwell, A. H., Zhang, M., Su, H., Jones, C. H., Chu, X., & Gresshoff, P. M. (2019). Legume nodulation: The host controls the party. *Plant, Cell & Environment*, *42*(1), 41-51. https://doi.org/10.1111/pce.13348

Han, Q., Ma, Q., Chen, Y., Tian, B., Xu, L., Bai, Y., ... & Li, X. (2020). Variation in rhizosphere microbial communities and its association with the symbiotic efficiency of rhizobia in soybean. The ISME journal, 14(8), 1915-1928.

Haroon, M., Shehu, U. A., Ali, S., Haq, A., Essa, Y., Junaid, M., & Jan, A. (2025). Red Rot Disease of Sugarcane: Current Status, Pathogen Biology, and Integrated Management Strategies. Global Research Journal of Natural Science and Technology.

He, X., Zhang, Y., & Li, S. (2020). Improving nitrogen fixation in soybean: Insights into rhizobium. *Molecular Microbiology Research*, *10*(1), 3898.

Herridge, D. F., Peoples, M. B., & Boddey, R. M. (2022). Global estimates of biological nitrogen fixation by agricultural legumes. *Plant and Soil*, *311*(1-2), 1-18. https://doi.org/10.1007/s11104-008-9632-z

Jan, A., Adil, S., Ali, T., Ahmed, B., Ahmed, Z., & Hussain, Z. (2025). Desert and medicinal plants as novel sources of antimicrobial agents for crop protection. Planta Animalia, 4(3), 197-218.

Jan, A., Adil, S., Ali, T., Ahmed, B., Ahmed, Z., & Hussain, Z. (2025). Desert and medicinal plants as novel sources of antimicrobial agents for crop protection. Planta Animalia, 4(3), 197-218.

Jan, A., Ali, T., Chirag, S., Ahmed, S., Ali, M., Wali, S., ... & Ullah, K. (2025). Eco-Friendly Management of Insect Pests and Plant Diseases Using Botanical Extracts. Global Research Journal of Natural Science and Technology.

Jan, A., Ali, T., Chirag, S., Ahmed, S., Ali, M., Wali, S., ... & Ullah, K. (2025). Eco-Friendly Management of Insect Pests and Plant Diseases Using Botanical Extracts. Global Research Journal of Natural Science and Technology.

Jan, A., Hussain, Z., Ullah, A., Ahmed, Z., Bakhsh, B. P., Latif, A., ... & Ahmed, M. (2025). Sugarcane Whip Smut: A Comprehensive Review of Pathogen Biology, Epidemiology, and Control Measures. Annual Methodological Archive Research Review, 3(5), 211-232.

Jan, A., Hussain, Z., Ullah, A., Ahmed, Z., Bakhsh, B. P., Latif, A., ... & Ahmed, M. (2025). Sugarcane Whip Smut: A Comprehensive Review of Pathogen Biology, Epidemiology, and Control Measures. Annual Methodological Archive Research Review, 3(5), 211-232.

Jan, A., Razzaq, F., Umair, M., Ullah, I., Shamsullah, S., Uzair, M., Ikram, M., Ayyaz, M., & Ali, T. (2025). Cotton Leaf Curl Disease: Pathogen Diversity, Whitefly Ecology, and Integrated Management Approaches. Planta Animalia, 4(4), 363-371.

Jan, A., Razzaq, F., Umair, M., Ullah, I., Shamsullah, S., Uzair, M., Ikram, M., Ayyaz, M., & Ali, T. (2025). Cotton Leaf Curl Disease: Pathogen Diversity, Whitefly Ecology, and Integrated Management Approaches. Planta Animalia, 4(4), 363-371.

Jan, A., Shaikh, G. Y., Ullah, S., Saddam, S., Ali, T., u Rehman, A., ... & Ahmed, M. (2025). In-vitro antifungal activity of medicinal plant extracts against Fusarium oxysporum causing wilt in okra. Indus Journal of Bioscience Research, 3(8), 406-414.

Jan, A., Shaikh, G. Y., Ullah, S., Saddam, S., Ali, T., u Rehman, A., ... & Ahmed, M. (2025). In-vitro antifungal activity of medicinal plant extracts against Fusarium oxysporum causing wilt in okra. Indus Journal of Bioscience Research, 3(8), 406-414.

Karges, K., Gatzke, B., & He, J. (2022). Biological nitrogen fixation by soybean (Glycine max [L.] Merr.) requires inoculation with non-native bradyrhizobia in new regions. *Frontiers in Agronomy*, *5*, 1196873. https://doi.org/10.3389/fagro.2023.1196873

Liu, S., Jiao, J., & Tian, C. F. (2023). Adaptive evolution of rhizobial symbiosis beyond horizontal gene transfer: From genome innovation to regulation reconstruction. Genes, 14(2), 274.

Lyu, X., Ke, D., & Yang, S. (2022). Proteomics analysis of the soybean-rhizobium interaction and nitrogen fixation efficiency. *Molecular Soil Biology*, *16*(5), 230-240.

Mastrodomenico, A. T., & Purcell, L. C. (2012). Soybean nitrogen fixation and initialization under drought stress. *Symbiosis*, *58*(1), 1-12.

Meena, R. S., Vijayakumar, V., Yadav, G. S., & Mitran, T. (2018). Response and interaction of Bradyrhizobium japonicum and arbuscular mycorrhizal fungi in the soybean rhizosphere. Plant Growth Regulation, 84(2), 207-223.

Noor, R. N., Atiq, M., Usman, M., Jan, A., Nawaz, A., Iqbal, S., ... & Rajput, N. A. (2025). Physiology, Epidemiology and Fungicidal Subdual Strategies for whip smut of sugarcane Caused by Sporisoriums Scitamineum.

Noor, R. N., Atiq, M., Usman, M., Jan, A., Nawaz, A., Iqbal, S., ... & Rajput, N. A. (2025). Physiology, Epidemiology and Fungicidal Subdual Strategies for whip smut of sugarcane Caused by Sporisoriums Scitamineum.

Noor, S., Nawaz, A., Ahmed, M., Akhtar, H., Ahmed, K., Irshad, M. S., ... & Jan, A. (2025). Beneficial Fungi and Bacteria as Biocontrol Agents against Fungal and Bacterial Plant Pathogens. Global Research Journal of Natural Science and Technology, 3(1).

Noor, S., Nawaz, A., Ahmed, M., Akhtar, H., Ahmed, K., Irshad, M. S., ... & Jan, A. (2025). Beneficial Fungi and Bacteria as Biocontrol Agents against Fungal and Bacterial Plant Pathogens. Global Research Journal of Natural Science and Technology, 3(1).

Ormeño-Orrillo, E., & Martínez-Romero, E. (2019). A genomotaxonomy view of the Bradyrhizobium genus. Frontiers in microbiology, 10, 1334.

Phillips, D. A. (1980). Efficiency of symbiotic nitrogen fixation in legumes. *Annual Review of Plant Physiology*, *31*, 29-49.

Qiu, Z., Egidi, E., Liu, H., Kaur, S., & Singh, B. K. (2019). New frontiers in agriculture productivity: optimised microbial inoculants and in situ microbiome engineering. Biotechnology advances, 37(6), 107371.

Roonjha, M. A., Roonjho, R., Ali, M., Anas, M., Khalid, H., & Jan, A. (2025). Aphid-Transmitted Plant Viruses: Epidemiology and Integrated Vector Management. International Journal of Agriculture Innovations and Cutting-Edge Research (HEC Recognised), 3(3), 109-126.

Roonjha, M. A., Roonjho, R., Ali, M., Anas, M., Khalid, H., & Jan, A. (2025). Aphid-Transmitted Plant Viruses: Epidemiology and Integrated Vector Management. International Journal of Agriculture Innovations and Cutting-Edge Research (HEC Recognised), 3(3), 109-126.

Saeki, Y., Akagi, I., Takaki, H., & Nagatomo, Y. (2000). Diversity of indigenous Bradyrhizobium strains isolated from three different Rj-soybean cultivars. *Soil Science and Plant Nutrition*, *46*(4), 917-926.

Salvagiotti, F., Cassman, K. G., Specht, J. E., Walters, D. T., Weiss, A., & Dobermann, A. (2008). Nitrogen uptake, fixation and response to fertilizer N in soybeans: A review. *Field Crops Research*, *108*(1), 1-13. https://doi.org/10.1016/j.fcr.2008.03.001

Santachiara, G., Borrás, L., Rotundo, J. L., Reyes, A., Salvagiotti, F., & Ciampitti, I. A. (2019). Relative importance of environmental factors driving soybean nitrogen fixation. *Field Crops Research*, *239*, 51-59. https://doi.org/10.1016/j.fcr.2019.05.013

Seefeldt, S. S., Shi, Y., & Subbarao, G. V. (2009). Introduction of Enrei type SEN1 as a nitrogen fixation enhancing gene. *Plant Science*, *176*, 23-30.

Siqueira, A. F., Schmidt, S., & Delamuta, J. R. M. (2014). Bradyrhizobium species used in commercial inoculants. *Agricultural Reviews*, *35*(2), 110-125.

Tamagno, S., Córdova, S. C., & Ciampitti, I. A. (2018). N fixation dynamics throughout the soybean growing season. *Frontiers in Plant Science*, *9*, 1668.

Tian, C. F., & Young, J. P. W. (2019). Evolution of Symbiosis Genes: Vertical and Horizontal Gene Transfer. In Ecology and Evolution of Rhizobia: Principles and Applications (pp. 145-152). Singapore: Springer Singapore.

Tominaga, T., Klein, R., & Hasibuan, R. (2012). QTL analysis of symbiotic nitrogen fixation in experimental lines. *Nature Communications*, *3*, 1112.

U.S. Department of Agriculture (USDA). (2024). *Annual report on soybean germplasm and nitrogen fixation efficiency*. Agricultural Research Service.

Wang, G., Yue, L., & Guo, J. (2024). Soybeans as a crucial crop for global food security: Protein and oil metabolism. *Chemical Reviews*, *124*(3), 1453-1470.

Wang, X., Chen, K., Zhou, M., Gao, Y., Huang, H., Liu, C., ... & Li, X. (2022). GmNAC181 promotes symbiotic nodulation and salt tolerance of nodulation by directly regulating GmNINa expression in soybean. New Phytologist, 236(2), 656-670.

Yang, S., Ke, D., & Lyu, X. (2021). Efficient nitrogen fixation in root nodules: Energy metabolism and antioxidant defense. *Plant, Cell & Environment*, *44*, 3110-3125.

Yang, S., Wang, J., & Chen, L. (2022). Targeted knockout of GmNMHC5 enhances protein yield in soybean by modulating GA levels. *Plant Physiology*, *188*(2), 1232-1245. https://doi.org/10.1093/plphys/kiab567

Zeffa, D. M., Fantin, L. H., Santos, O. J. A. P., Canteri, M. G., & Gonela, A. (2020). The influence of plant growth-promoting rhizobacteria co-inoculation on soybean yield: A meta-analysis. *Frontiers in Plant Science*, *11*, 523561. https://doi.org/10.3389/fpls.2020.523561

Zhang, W., Li, X., & Zhang, Y. (2023). Molecular and physiological response mechanisms of soybean root nodules to high nitrogen levels. *Frontiers in Plant Science*, *14*, 1604251. https://doi.org/10.3389/fpls.2023.1604251

Zilli, J. E., Alves, B. J. R., & Rouws, L. F. M. (2025). Genetic diversity and symbiotic potential of Bradyrhizobium strains in cowpea and soybean. *Frontiers in Microbiology*, *15*, 12737117. https://doi.org/10.3389/fmicb.2024.12737117