JUATIKA

JURNAL AGRONOMI TANAMAN TROPIKA
VOL. 7 NO. 3 September 2025

DOI :https://doi.org/10.36378/juatika.v5i2.4853
eissn 2656-1727
pissn 2684-785X
pages : 763 – 769

RESEARCH ARTICLE Open Access

Optimization of Biological Nitrogen Fixation by Rhizobium Bacteria to Enhance Growth and Yield of Soybean (*Glycine max* L.)

Johannes Pardede[1], Noverina Chaniago[1,*], Murni Sari Rahayu[1]

Abstract

Rhizobium bacteria play a pivotal role in soybean (*Glycine max* L.) cultivation through their ability to perform biological nitrogen fixation. The effectiveness of Rhizobium in increasing crop yield is influenced by the soybean variety and the inoculation dose applied. Therefore, the application of Rhizobium inoculants appropriate to soybean varieties has the potential to increase the efficiency of nitrogen utilization in a sustainable and environmentally friendly manner. This study aims to determine the effectiveness of Rhizobium strain doses on the growth and production of two soybean varieties. The study was conducted using a factorial Randomized Block Design (RBD), consisting of two factors: soybean varieties (Anjasmoro and Dega 1) and Rhizobium doses (control/without Rhizobium, 10 g/kg seed, and 15 g/kg seed). Data were analyzed using analysis of variance (ANOVA) and continued with Duncan's Multiple Range Test (DMRT) at a significance level of 5%. The results showed that the interaction between soybean varieties and Rhizobium doses had a significant effect on leaf area. The best treatment combination was obtained with the Dega 1 variety and a Rhizobium dose of 15 g/kg of seeds. The Dega 1 variety had a significant effect on seed weight per plant and per plot. This variety demonstrates superior physiological capabilities and adaptability in utilizing growing resources for seed biomass accumulation, thereby contributing to increased national soybean yields. A Rhizobium dose of 15 g/kg seed had a significant effect on the number of root nodules and the number of soybean pods. Therefore, this dosage is recommended as the standard in soybean cultivation using inoculation technology.

Keywords: Biofertilizer, Inoculation, Legume, Sustainable Agriculture, Symbiosis

1. Introduction

Soybeans (*Glycine max* L.) are a strategic food commodity with high economic and nutritional value. According to the national food balance sheet projections, Indonesia's domestic soybean demand in 2024 is estimated to reach 2.64 million tons, while the estimated domestic production is only 291,401 tons, a figure that shows a downward trend in national production every year (Rachmawati 2024). One of the factors causing low domestic soybean productivity is low nutrient content, particularly nitrogen (N), which is an essential element for the vegetative growth of legumes. Nitrogen deficiency inhibits chlorophyll formation, reduces the rate of photosynthesis, and has a direct impact on pod formation and seed yield (Hungria and Mendes 2015). The availability of nitrogen in the soil is often limited, especially on marginal land, so farmers are highly dependent on inorganic fertilizers as a source of N.

However, efforts to increase soybean productivity through the application of synthetic nitrogen fertilizers face serious obstacles. The price of nitrogen fertilizers is becoming increasingly expensive due to rising prices of natural gas, the main raw material for urea production, and global market fluctuations (Nations 2022), 2022). This situation has increased farmers' production costs, while profit margins remain low. As a result, many farmers are reluctant to expand or intensify soybean cultivation, making it difficult for national production to keep up with demand. To reduce this gap, intensification efforts continue to be carried out, ranging from the use of superior varieties to the application of appropriate cultivation systems, one of which is utilizing Rhizobium bacteria to fix N2 in the

*Correspondence: noverinachaniago40515@gmail.com
1) Universitas Islam Sumatera Utara - Jl. Sisingamangaraja 191, Medan 20217, North Sumatra, Indonesia

atmosphere, with the aim of increasing the quantity and quality of soybean yields.

Rhizobium bacteria play an important role in the soybean cultivation system, forming a mutualistic symbiotic relationship with soybeans by infecting soybean roots to form root nodules as centers for the biological fixation of nitrogen (N2) in the atmosphere, converting it into a form of nitrogen that is available to soybean plants, such as ammonium ions (NH_4^+) (Kiptiyah, Soeparjono, and Sa'diyah 2025; Nakei, Venkataramana, and Ndakidemi 2022). In addition, the integration of Rhizobium bacteria in soybean cultivation practices offers an environmentally friendly approach to reducing dependence on synthetic nitrogen fertilizers. The application of Rhizobium also has the potential to support increased plant growth in both the vegetative and generative phases, thereby having a positive impact on productivity (Abd-alla, Al-amri, and El-enany 2023). According to Novriani (2011), under optimal symbiotic conditions, nitrogen fixation by Rhizobium can reach more than 80 kg N_2 per hectare per year.

The success of nitrogen fixation by Rhizobium is influenced by various variables such as soybean variety, microbial strain compatibility, the amount of inoculant applied, and environmental factors where the plants are cultivated.

The soybean root system follows an allorhizic pattern, in which the primary root develops from the hypocotyl and forms a strong taproot. Lateral roots grow transversely from the main root, and functional root hairs are found around the tips of both taproots and lateral roots. In addition, a special structure called a nodule forms on the lateral roots as a result of interaction with Rhizobium bacteria, where the nitrogen fixation process takes place (Fatima, Kataria, and Jain 2024). The formation of root nodules in soybean plants begins with infection by Rhizobium bacteria that enter the root hair cells through infection threads. These threads develop in the cytoplasm of the root cells, then penetrate the root cortex layer and initiate the formation of nodule primordium structures. The primordium cells, together with uninfected cortex cells, then undergo division and differentiation into mature root nodules capable of biological nitrogen fixation. This process generally occurs within 12–18 days after infection. Auxin is key in nodule formation, as nodule growth is stimulated by the presence of the compound tryptophan, which is a precursor to the hormone auxin (IAA). Both plants and bacteria produce IAA, and the local accumulation of this hormone at the site of nodule formation is essential for nodule initiation and development (Jaiswal et al. 2021). Although nodules stop developing after reaching 29–37 days after planting (DAP), nitrogen fixation activity continues until the nodules begin to senesce around 50 to 60 days. Visually, active nodules appear pink when cut open, while dead or inactive nodules turn blackish brown.

The novelty of this study lies in its applied approach, which tests the effects of three levels of Rhizobium inoculant dosage (0, 10, and 15 g/kg of seeds) on the growth and production of two soybean varieties (Dega 1 and Anjasmoro). This study not only observed the formation of nodules as a physiological response, but also evaluated the agronomic implications in terms of the number of nodules, number of pods, seed weight per plant, and seed weight per plot. Thus, this study bridges theory and practice: while previous studies focused more on hormonal mechanisms (IAA) in nodule formation, this study adds a new dimension in the form of soybean variety response and Rhizobium inoculant dose effectiveness, thereby producing practical recommendations for farmers.This study aims to determine the effectiveness of Rhizobium strain doses on the growth and production of two soybean varieties.

2. Material and Methods

This research was carried out in the greenhouse of the Faculty of Agriculture, Islamic University of North Sumatra, Jln. Karya Wisata, Gedung Johor, Medan Johor District, Medan City, North Sumatra Provincewith coordinates 3°33'03.8"N 98°40'13.1"E and an altitude of 25 m above sea level. The duration of the research is from March to September 2024.

The materials used in this study were Anjasmoro and Dega soybean seeds, Rhizobium japonicum bacteria, and polybags. The tools used in this study were hoes, watering cans, measuring tapes, knives, labels, calculators, analytical scales, paper, and leaf stalk applications.

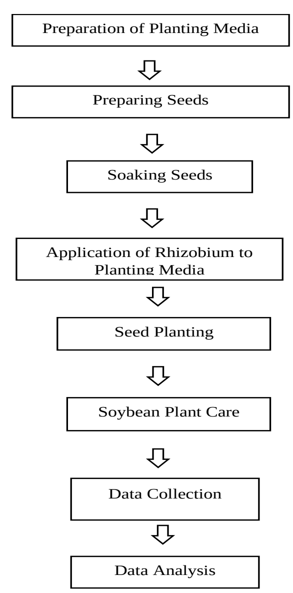

Figure 1. Research Flow Diagram

The design model used was a factorial randomized block design (RAK), consisting of two factors, namely soybean varieties (Anjasmoro and Dega I) and Rhizobium strain doses (control/without Rhizobium, 10 g/kg of seeds and 15 g/kg of seeds). The variables observed in this study included analysis of soil nutrient content before planting and after the end of the study, namely: organic C (%), total N (%), P -Bray I (ppm P) and K-dd (me/100 mg), as well as measurements of agronomic components, including plant height, leaf area, number of root nodules, number of pods, seed weight per plant, and seed weight per plot.

The agronomic component data were analyzed using analysis of variance and further tested with Duncan's Multiple Range Test (DMRT) at a significance level of 5%.The analysis was performed using the BM SPSS Statistics program in 2025.

3. Results and Discussion

The results of the analysis of initial soil nutrient content (before Rhizobium inoculation) and final soil nutrient content (after harvesting) for several nutrients, namely: organic C (%), total N (%), P-Bray I (ppm P), and K-dd (me/100 mg), are presented in Table 1.

Table 1. Results of initial soil nutrient content analysis (before rhizobium inoculation) and final analysis (after Rhizobium inoculation)

No	Type of Analysis	Soil samples before (initial) and after Rhizobium inoculation with various doses (final)			
		Initial	Final		
			0 g	10 g	15 g
1	C-Organik (%)	0,22	0,15	0,28	0,32
2	N-total (%),	0,24	0,14	0,25	0,30
3	P-Bray I (ppm P)	5,14	4,14	4,76	4,07
4	K-dd (me/100 mg)	1,99	1,72	2,35	2,55

Source: Soil Laboratory, North Sumatra Agricultural Instrument Standardization Agency

The results of the analysis of initial soil nutrient content after Rhizobium inoculation showed an increase in organic C%, total N (4.2%), and K-dd (18.1%), but a decrease in P-Bray I of 7.4%. This was because the phosphorus contained in the soil could be absorbed by soybean roots for soybean growth and production

A comparison of initial and final soil analysis results shows that the higher the Rhizobium dose applied, the greater the increase in organic carbon (27.3% - 45.5%), nitrogen (4.2% - 25%), and potassium (18.1% - 28.1%) content. However, phosphorus content decreased (7.4%–20.8%).

The application of Rhizobium to soybean fields has been proven to have a positive impact on increasing the content of organic C, total N, and K-dd in the soil after harvest. This phenomenon is not only related to the main function of Rhizobium as a nitrogen-fixing agent, but also involves complex biological and biogeochemical mechanisms in the soil, which contribute to improving soil quality and fertility.

The increase in organic C (27.3%–45.5%) was mainly due to the greater contribution of plant biomass in the Rhizobium-inoculated treatment. The symbiosis formed between Rhizobium and soybean roots increased the plant's ability to obtain sufficient nitrogen to support vegetative and generative growth. More extensive root growth also produces root exudates in the form of simple organic compounds, such as organic acids, sugars, and amino acids, which act as a carbon source for soil microorganisms. After harvesting, plant residues in the form of roots, stems, and leaves that remain will undergo decomposition, thereby increasing the soil's organic matter reserves. Thus, there is an increase in organic C content, which is directly related to biological activity and plant biomass accumulation (Hungria and Mendes 2015). This is in line with the statement by Ladha et al. (2016), that an increase in organic C can be attributed to the contribution of root biomass, root exudates, and decomposed plant residues during growth.

Furthermore, the increase in soil total N is the main effect of the biological nitrogen fixation process by Rhizobium, through the activity of the nitrogenase enzyme. Rhizobium is able to convert atmospheric nitrogen (N_2) into ammonia (NH_3), which is then utilized by soybean plants to form organic nitrogen compounds such as amino acids, nucleotides, and proteins. Not all of the fixed nitrogen is absorbed into plant tissues; some returns to the soil through leaf fall, root exudates, and the decomposition of plant residues after harvest. This process ultimately increases the total Nitrogen content of the soil, which is not only beneficial for soybean cultivation but can also be a source of nitrogen for subsequent crops in the rotation system (Namkeleja, Mtei, and Ndakidemi 2016).

Meanwhile, the increase in K-dd (exchangeable potassium) in soil with Rhizobium application occurs indirectly. Better plant growth due to nitrogen availability encourages more intensive root activity, thereby increasing the release of root exudates, including organic acids. These compounds play a role in dissolving soil minerals, which then release K^+ ions from clay fractions and primary minerals. In addition, soybean plant residues are also an important source of potassium. When plant residues undergo decomposition, the potassium contained in plant tissues is released back into the soil in a form that is available and exchangeable (Meena et al. 2017). Rhizobium indirectly promotes the accumulation of organic matter through increased plant growth and the addition of organic matter to the soil. This organic matter also improves soil physical properties and provides energy for soil microorganisms, thereby enhancing nutrient mineralization, including K-dd (Lal 2015).

The 18.1%-28.1% increase in K-dd was due to improved soil structure and microbial activity that released potassium from soil minerals through biological weathering (Basak et al. 2017). Therefore, soil K-dd content increased after soybean harvest in treatments with Rhizobium inoculation.

Overall, the results of this study indicate that Rhizobium not only acts as a biofertilizer to increase nitrogen availability, but also contributes significantly to improving soil quality

through the accumulation of organic matter, total nitrogen, and exchangeable potassium. The improvement in these three indicators suggests that the use of Rhizobium in soybean cultivation systems has the potential to be an environmentally friendly, sustainable nutrient management approach that supports long-term increases in land productivity.

The decrease in phosphorus content (7.4%-20.8%) is caused by increased phosphorus uptake by soybean plants during vegetative and generative growth. In addition, phosphorus is also used in energy (ATP) formation in the nitrogen fixation process, so that some of the available phosphorus is taken up for the metabolic needs of plants and symbiotic microbes (Vance et al. 2003). On the other hand, phosphorus availability in the soil is greatly influenced by soil pH and interactions with other ions, so even though total phosphorus may not change, the available form (P-Bray I) may decrease.

Overall, Rhizobium application not only contributes to an increase in total N, but also improves organic C and K-dd content, although additional strategies such as the use of phosphate fertilizers or phosphate-solubilizing biofertilizers are needed to compensate for the decrease in available P in the soil.

The results of agronomic component measurements during the vegetative and reproductive growth phases are presented in Table 2. The results of the analysis of variance and Duncan's 5% mean difference test showed that the two varieties studied differed significantly in terms of seed weight per plant and seed weight per plot. The Dega 1 variety produced higher seed weight per plant and seed weight per plot than Anjasmoro.This is in accordance with the description issued by Balitkabi (2014), where Dega 1 is a short-maturing superior variety, a cross between Grobogan and Malabar, which can be harvested at 69-73 days after planting. Its main characteristics include large seeds, bright in color and oval in shape, with a weight of 100 seeds around 22.98 g, approximately 29 pods per plant, and high productivity with a potential yield of 3.82 tons/ha. Anjasmoro is a nationally recognized superior variety known for its large seeds, with a weight of 100 seeds around 14.8-15.3 g, a potential yield ranging from 2.03-2.25 tons/ha, and can be harvested at 82-92 days after planting.

Table 2. Average plant height, leaf area, number of root nodules, number of pods, seed weight per plant, and seed weight per plot for soybean varieties and rhizobium dosages

Treatment	Plant Height (cm)	Leaf Area (cm^2)	Number of Root Nodules	Number of Pods	Seed Weight per Plant (g)	Seed weight per area (g)
Soybean Varieties (V)						
Anjasmoro	54.1±1.24	48.6±0.9	124.0±14.03	49.3±0.87	85.7±1.56 b	257.0± 4.69 b
Dega 1	54.8±1.24	48.7±0.9	145.9±14.03	50.8±0.87	96.7±1.56 a	290.2± 4.49 a
Rhizobium Dosage (R)						
0 g/kg	55.4 ±1.52	48.0±0.11	84.9 ±17.19c	48.6±1.06 b	90.0±1.91	269.9±5.75
10 g/kg	52.4±2.14	47.0±0.15	138.4 ±24.30b	48.1±1.50 b	88.4±2.70	265.1±8.13
15 g/kg	55.5±1.52	50.0±0.10	181.6 ±17.19a	53.4±1.06 a	95.3±1.91	285.8±5.75
V x R Interaction						
V1 R0	56.0±3.26	50.0±0.22ab	78.0±20.57	50.1±3.67	85.8±9.33	257.5±28.06
V1 R1	52.5±3.61	49.0±0.45ab	129.0±3.53	49.1±2.32	85.6±11.07	256.8±33.29
V1 R2	53.8±4.12	47.0±0.29 b	165.0±28.31	48.6±2.78	85.6±6.57	256.8±19.80
V2 R0	54.8±0.56	46.0±0.43 b	91.8±12.33	47.0±5.35	94.1±9.63	282.3±28.89
V2 R1	52.3±3.61	45.0±0.46 b	147.8±28.89	47.1±6.92	91.2±8,76	273.5±26.28
V2 R2	57.2±3.30	53.0±0.21 a	198.3±26.23	58.3±2.11	104.9±3.38	314.8±10.14

Remarks: The mean values within the same column followed by similar small letter are not significantly different at 5% DMRT.

However, these two varieties did not have a significant effect on plant height, leaf area, number of root nodules, and number of pods. Although descriptively, the Anjasmoro variety had a greater average plant height (64–68 cm) than the Dega variety (±53 cm) (Balitkabi, 2014), this genetic expression was highly dependent on growing conditions. Factors such as water availability, light, and soil fertility can suppress the expression of genetic potential, so that both varieties show relatively similar plant height growth (Haque and Haque 2016). Vegetative growth parameters such as plant height, leaf area, and root nodule formation are generally greatly influenced by agroecological conditions. The environmental conditions at the time of the study were relatively homogeneous. The plants were grown in polybags with soil of the same fertility level, placed in a greenhouse, and managed uniformly. Therefore, there were no significant differences in growth response between varieties (Taiz et al. 2015). The number of pods is the result of the interaction between genetic factors and the physiological condition of the plant. However, in many cases, environmental factors such as water availability during the reproductive phase are more decisive than variety factors. Therefore, although the Anjasmoro and Dega varieties have different agronomic descriptions, relatively uniform environmental conditions mean that the number of pods between varieties does not differ significantly (Board and Kahlon 2011).

The results showed that Rhizobium application at various doses had a significant effect on the number of root nodules and soybean pods. An increase in the Rhizobium dose was shown to stimulate the formation of more root nodules, especially at the highest dose (15 g/kg of seeds) (Figure 2). The increase in the number of root nodules due to higher doses of Rhizobium can be seen from the linear regression graph in Figure 3, with the equation $\hat{Y} = 6.29x + 82.55$, and a correlation coefficient of 0.983 (Figure 3).

This can be explained by the fact that the higher the amount of inoculant given, the greater the chance for Rhizobium bacteria to infect soybean root hairs. The infection then forms infection threads that develop into the root cortex, and subsequently stimulate the formation of nodules as centers of nitrogen fixation activity. In other words, increasing the inoculation dose increases the population of effective bacteria that symbiotically interact with the roots, thereby significantly increasing the number of root nodules (Hungria and Mendes 2015).

Figure 2. Root nodule growth with various combinations of variety and rhizobium dosage treatments (Source: Research Collection)

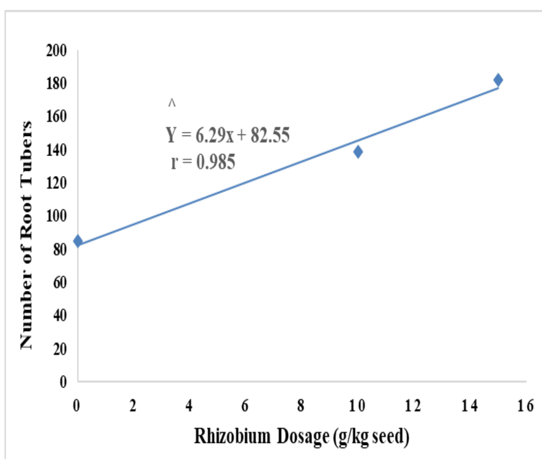

Figure 3. Linear regression graph of Rhizobium dose against number of root nodules

Meanwhile, in terms of pod number, higher doses of Rhizobium also showed a positive effect, although not all treatments were significantly different. A dose of 10 g/kg of seeds showed no significant difference from the control, while a dose of 15 g/kg of seeds produced higher pod yields. This indicates that pod formation is not only influenced by the number of nodules formed, but also by nodule efficiency, the availability of other nutrients such as phosphorus and potassium, and environmental conditions (water, light, and temperature) during the generative phase of the plant (Kaschuk et al. 2010; Board and Kahlon 2011). Thus, an increase in the number of root nodules due to high Rhizobium doses is not always directly proportional to an increase in the number of pods. This is because not all nodules are active in fixing nitrogen, and plant reproductive outcomes are more complexly influenced by the sink–source balance, which is the relationship between the availability of assimilates from photosynthesis (source) and the needs of the generative organs (sink) (Voisin et al. 2003).

Research by Pattipeilohy and Sopacua (2014) shows that Rhizobium inoculation can increase the number of root nodules. Some of the advantages of using Rhizobium as a biological fertilizer include: increasing the availability of nutrients, especially nitrogen, without causing negative impacts; its efficiency can be improved so that the risk of environmental pollution can be minimized; the cost of application is relatively low; and the application technology is quite simple and easy.

The results of research by Alam et al. (2015) reported that the application of rhizobia to soybean plants resulted in a higher number of pods compared to plants without rhizobia. This increase in the number of pods due to rhizobia was caused by better plant growth as a result of high nitrogen absorption in the plants. The application of rhizobia to leguminous plants such as soybeans causes an association between rhizobia and plants to fix nitrogen (N_2) from the air, which can then be utilized by plants to enhance plant growth (Prakamhang et al., 2015).

The interaction between soybean varieties and Rhizobium doses only had a significant effect on leaf area, while it had no significant effect on plant height, root nodule count, or production variables. The 5% DMRT results showed that the interaction between the Dega 1 variety and a Rhizobium dose of 15 g/kg of soybean seeds (V2R2) produced the highest leaf area (53.0 cm2). The leaf area is the morpho-physiological component that responds most rapidly to increased N availability due to the effects of biological nitrogen fixation by Rhizobium. This response is often influenced by genotype x environment x Rhizobium inoculant specific factors, so that interactions are easily detected in leaf area characteristics, compared to other characteristics such as plant height.

The results of a cross-location study by Tamagno et al. (2018), show that leaf area index (LAI) and canopy components are more sensitive to N/inoculation treatments and interactions with other factors than height or final yield. The dose of 15 g/kg is within the effective range for field practice. The general recommendation for legume inoculants is ~10–50 g/kg of seeds (with a concentration range of 10^8 CFU g^{-1}); within this range, differences in dose often modulate the rate of initial colonization without always translating into differences in nodulation/yield at the end of the season. The most consistent effect is seen in early leaf expansion due to faster N supply.

The characteristics of the Dega 1 variety support rapid canopy response. Dega 1 is an early maturing Indonesian variety (±70–73 days after sowing) known for its sturdy stature and high yield potential. early maturing varieties generally allocate vegetative resources quickly in the early phase, so that an increase in early N supply (through effective inoculation) is more easily reflected as a larger leaf area, without having to change the plant height (Herawati et al. 2020; Fattah et al. 2024).

The competition of native rhizobia often suppresses the effect of inoculant dose/strain, so that the number of nodules does not always increase with dose or differ between soybean varieties, especially in soils with a history of soybean cultivation. What is more relevant is the occupation of nodules by effective strains, not the total number. This explains the minimal interaction in “number of nodules” despite the response in leaf area.

4. Conclusion

The interaction between soybean varieties and Rhizobium doses had a significant effect on leaf area. The best treatment combination was obtained with the Dega 1 variety and a Rhizobium dose of 15 g/kg of seeds. The Dega 1 variety had a significant effect on seed weight per plant and per plot. A Rhizobium dose of 15 g/kg of seeds had a significant effect on the number of root nodules and the number of soybean pods.

Acknowledgment

For this research, we would like to thank the Faculty of Agriculture, Islamic University of Sumatra, for providing the facilities for this research.

References

Abd-Alla, M. H., Al-Amri, S. M., & El-Enany, A. E. E. (2023). Enhancing *Rhizobium-legume* symbiosis and reducing nitrogen fertilizer use are potential options for mitigating climate change. *Agriculture*, 13(11), 2092. https://doi.org/10.3390/agriculture13112092

Basak, A. K., Chatterjee, T., Ghosh, S. K., & Chakravarty, A. (2017). Impacts of dietary exposure to sodium or potassium salts of nitrate and nitrite on the development of *Drosophila melanogaster*. *Interdisciplinary Toxicology*, 10(2), 70–78. https://doi.org/10.1515/intox-2017-0012

Board, J. E., & Kahlon, C. S. (2011). Soybean yield formation: What controls it and how it can be improved. In H. A. El-Shemy (Ed.), *Soybean physiology and biochemistry*. InTech. https://doi.org/10.5772/17596

Fatima, A., Kataria, S., & Jain, M. (2024). Synchrotron tomography of magnetoprimed soybean plant root system architecture grown in arsenic-polluted soil. *Frontiers in Plant Science*, 15, 1391846. https://doi.org/10.3389/fpls.2024.1391846

Fattah, A., Idaryani, Herniwati, Yasin, M., Suriani, S., Salim, Nappu, M. B., et al. (2024). Performance and morphology of several soybean varieties and responses to pests and diseases in South Sulawesi. *Heliyon*, 10(5), e25507. https://doi.org/10.1016/j.heliyon.2024.e25507

Haque, M. A., & Haque, M. M. (2016). Growth, yield and nitrogen use efficiency of new rice variety under variable nitrogen rates. *American Journal of Plant Sciences*, 7(3), 612–622. https://doi.org/10.4236/ajps.2016.73054

Herawati, N., Erawati, B. T. R., Aisah, A. R., Sugianti, T., & Hidayah, B. N. (2020). Adaptation of nine new Indonesian improved soybean varieties in paddy fields of Central Lombok, Indonesia. *Russian Journal of Agricultural and Socio-Economic Sciences*, 107(11), 150–159. https://doi.org/10.18551/rjoas.2020-11.18

Hungria, M., & Mendes, I. C. (2015). Nitrogen fixation with soybean:

The perfect symbiosis? In F. De Bruijn (Ed.), *Biological nitrogen fixation* (Vol. 2, pp. 1005–1019). John Wiley & Sons. https://doi.org/10.1002/9781119053095.ch99

Jaiswal, S. K., Mohammed, M., Ibny, F. Y. I., & Dakora, F. D. (2021). Rhizobia as a source of plant growth-promoting molecules: Potential applications and possible operational mechanisms. *Frontiers in Sustainable Food Systems*, 4, 619676. https://doi.org/10.3389/fsufs.2020.619676

Kaschuk, G., Hungria, M., Leffelaar, P. A., Giller, K. E., & Kuyper, T. W. (2010). Differences in photosynthetic behaviour and leaf senescence of soybean (*Glycine max* [L.] Merrill) dependent on N_2 fixation or nitrate supply. *Plant Biology*, 12(1), 60–69. https://doi.org/10.1111/j.1438-8677.2009.00211.x

Kiptiyah, M., Soeparjono, S., & Sa'diyah, H. (2025). Application of *Rhizobium* dosage and cytokinin concentration on growth, yield, and quality of edamame soybean (*Glycine max* (L.) Merrill). *Agroteknika*, 8(2), 409–428. https://doi.org/10.55043/agroteknika.v8i2.525

Ladha, J. K., Reddy, C. K., Cassman, K., Verma, S., Powlson, D. S., Van Kessel, C., Richter, D. D. B., Chakraborty, D., & Pathak, H. (2016). Global nitrogen budgets in cereals: A 50-year assessment for maize, rice, and wheat production systems. *Scientific Reports*, 6, 19355. https://doi.org/10.1038/srep19355

Lal, R. (2015). Restoring soil quality to mitigate soil degradation. *Sustainability*, 7(5), 5875–5895. https://doi.org/10.3390/su7055875

Meena, V. S., Meena, S. K., Verma, J. P., Kumar, A., Aeron, A., Mishra, P. K., Bisht, J. K., Pattanayak, A., Naveed, M., & Dotaniya, M. L. (2017). Plant beneficial rhizospheric microorganism (PBRM) strategies to improve nutrients use efficiency: A review. *Ecological Engineering*, 107, 8–32. https://doi.org/10.1016/j.ecoleng.2017.06.058

Nakei, M. D., Venkataramana, P. B., & Ndakidemi, P. A. (2022). Soybean-nodulating rhizobia: Ecology, characterization, diversity, and growth promoting functions. *Frontiers in Sustainable Food Systems*, 6, 824444. https://doi.org/10.3389/fsufs.2022.824444

Namkeleja, Y., Mtei, K., & Ndakidemi, P. A. (2016). Isolation and molecular characterization of elite indigenous rhizobia nodulating *Phaseolus* bean (*Phaseolus vulgaris* L.). *American Journal of Plant Sciences*, 7(14), 1755–1765. https://doi.org/10.4236/ajps.2016.714175

Food and Agriculture Organization of the United Nations. (2022). *World fertilizer trends and outlook to 2022: Summary report* (Vol. 5). FAO.

Rachmawati, D. (2024, June 28). Begini nasib harga kedelai impor imbas rupiah melemah. *Bisnis.com*. https://ekonomi.bisnis.com/read/20240628/12/1777841/begini-nasib-harga-kedelai-impor-imbas-rupiah-melemah

Taiz, L., Zeiger, E., Moller, I. M., & Murphy, A. (2015). *Plant physiology and development* (6th ed.). Sinauer Associates.

Tamagno, S., Sadras, V. O., Haegele, J. W., Armstrong, P. R., & Ciampitti, I. A. (2018). Interplay between nitrogen fertilizer and biological nitrogen fixation in soybean: Implications on seed yield and biomass allocation. *Scientific Reports*, 8, 35672. https://doi.org/10.1038/s41598-018-35672-1

Vance, C. P., Uhde-Stone, C., & Allan, D. L. (2003). Phosphorus acquisition and use: Critical adaptations by plants for securing a nonrenewable resource. *New Phytologist*, 157(3), 423–447. https://doi.org/10.1046/j.1469-8137.2003.00695.x

Voisin, A. S., Salon, C., Jeudy, C., & Warembourg, F. R. (2003). Symbiotic N_2 fixation activity in relation to C economy of *Pisum sativum* L. as a function of plant phenology. *Journal of Experimental Botany*, 54(393), 2733–2744. https://doi.org/10.1093/jxb/erg290