Application of Rhizobium Inoculum and Plant Population on Nodulation and Productivity of Edamame (*Glycine max* (L.) Merr.)

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Abstract. Root nodulation is a crucial process for legumes, including edamame (*Glycine max* (L.) Merr.), as it enables biological nitrogen fixation, enhancing nitrogen availability for plant growth. The implementation of *Rhizobium* inoculum has been demonstrated to reduce the necessity for synthetic fertilizers, thereby enhancing the health and yield of the plants in question. Furthermore, optimal plant population management ensures adequate access to light, water, and nutrients, maximizing productivity. The combination of *Rhizobium* inoculation and population management represents a crucial strategy for enhancing the productivity and sustainability of legume crops, such as edamame. This study examines the impact of *Rhizobium* inoculation and plant population management on the nodulation and productivity of edamame. The research was conducted using a randomized block design, with two factors: the application of *Rhizobium* (*Bradyrhizobium japonicum*) at two levels (without inoculation and with inoculation) and plant spacing (20 x 15 cm and 20 x 30 cm). Each treatment was replicated five times. The observed parameters included number of root nodulations, plant height, leaf area, leaf greenness, number of filled and empty pods, weight of filled and empty pods, wet weight, and 100 seed weight. The data were subjected to analysis of variance, followed by Duncan's Multiple Range Test. The findings indicated that *Rhizobium* inoculation markedly enhanced root nodulation, with the 20 x 15 cm spacing in conjunction with inoculation yielding the highest total nodulation, number and wight filled pods, wet weight, and 100 seed weight.

Keywords: Edamame, Rhizobium inoculum, Root nodulation, Plant population, Sustainable agriculture.

INTRODUCTION

Edamame is a specialty soybean (*Glycine max* (L.) Merr.) that is harvested as a vegetable when the seeds are immature (R6 stage) and has expanded to fill 80 to 90 percent of the pod width. Like field-dried soybeans, edamame variety seeds are rich in protein and highly nutritious [1]. To fulfill the demand for edamame domestically and for export, it is necessary to support the increase in production. Edamame soybean production since 2017 has been targeted at 9 tonnes/ha of wet pods to increase export. Edamame production can be increased by expanding the planting area and increasing the plant population per hectare, which still needs information about regional and seasonal differences in Indonesia [2].

Cultivation techniques such as nitrogen mineral fertilizer have been widely adopted to increase edamame production. Fertiliser application resulted in taller plants with greater above-ground biomass and dry matter yield than plants grown without Mineral Nitrogen [3]. In addition, fertilizer application resulted in a linear increase in the seed yield of legume crops up to a maximum rate of 180 kg N ha-1 [4]. Although these fertilizers are used repeatedly, N is easily lost through leaching, evaporation, and denitrification [5]. Hence, there are high potential economic and environmental losses associated with nitrogen fertilization [6].

Sustainable agriculture is increasingly recognized as a method to address the abovementioned challenges. Sustainable agriculture uses technologies that aim to increase N availability and productivity of legumes, emphasizing the production system's economic and ecological aspects. An example is biological N fixation, which can be an alternative to N supply, reducing the use of N fertilizers and, consequently, minimizing the environmental impact of N leaching in rivers and lakes [7].

One of the Nitrogen fixing technologies is through legin. Legin is a biofertiliser made from pure cultures of *Rhizobium* bacteria. *Rhizobium* is a symbiotic bacterium capable of fixing Nitrogen by forming root nodules in legume plants. In legume crops, Rhizobium contributes to nitrogen availability of 24-584 N/ha/year compared to nonsymbiotic bacteria, which is only 15 kg/ha/year [8]. Plant population is also an approach to sustainably increasing productivity. Increasing plant population by adjusting plant spacing can affect leaf area, plant dry weight, and plant root system. Appropriate plant population settings can increase plant leaf area and help individual plants get more nutrients, water, and sunlight. However, population settings that are too narrow can cause competition for nutrients among plants, one of which is the element Nitrogen. Population settings with plant spacing significantly affect plant height and number of pods on plants. The results of further tests that have been carried out on plant spacing settings show that the components of growth and yield of edamame increase together with an increase in the spacing applied [9].

There may be a correlation between population arrangements and plant spacing, soil microbial activity, and the availability of nutrients, such as nitrogen, which can increase the productivity of edamame soybean plants. Dense plant spacing can increase competition for light uptake in soils colonized by *Rhizobium* bacteria, impacting nutrient and water uptake [10]. In soil colonized by *Rhizobium* bacteria, high availability of nutrients such as nitrogen can increase soil microbial activity, which affects plant productivity [11].

Considering the increasing interest in and drive towards using *Rhizobium* bacteria inoculation to mitigate the harmful effects of chemical fertilization on the environment and its activity on plant population as an effort to increase edamame production, we analyzed the effect of *Rhizobium* inoculum and plant population on nodulation and edamame production.

METHODS

The research was conducted from May to October 2024 at the seasonal food crop garden, Politeknik Negeri Lampung, Bandar Lampung, Indonesia. The research was conducted using a Randomized Group Design (RGD) with 4 treatments and 5 replicates, including j_1r_0 (20x15 cm without *rhizobium* inoculum), j_1r_1 (20x15 cm with *rhizobium* inoculum), j_2r_0 (20x30 cm without *Rhizobium* inoculum), j_2r_1 (20x30 cm with *Rhizobium* inoculum). Data were analyzed using variance analysis (ANOVA) at a 95% confidence interval, and treatments with significant effects were further tested using Duncan's Multiple Range Test (DMRT) at a 5% level. The *Rhizobium* inoculum used in this study contains *Bradyrhizobium japonicum* in ± 10^8 CFU/g.

The research was conducted on a 15 m × 22 m flat dryland. Tillage was carried out using a rotary or hoe at a depth of 20 cm, accompanied by applying 1 tonne/ha of manure. After processing, the land was levelled and fermented for 2 weeks. The research land was divided into 20 experimental plots, each measuring 3 m × 4 m and a height of 30 cm. The distance between plots was 50 cm. The research plots were divided into two planting distance treatment groups: 10 plots with a spacing of 20 cm × 15 cm (± 300,000-350,000 plants/ha) and 10 plots with a spacing of 20 cm × 30 cm (± 150,000-175,000 plants/ha). One week before planting, liming was carried out using dolomite lime to increase the availability of Ca and Mg, which play a role in plant growth and include plant height, number of leaves, number of branches, and number of pods [12].

Seed preparation was done by wetting edamame seeds using sufficient water. Next, the seeds were inoculated with legin (Rhizobium inoculum) at 10 grams/kg seed [13]. Mixing was done in the shade to avoid direct sunlight exposure that could affect the viability of Rhizobium inoculum. The inoculation process aims to ensure the colonization of Rhizobium bacteria on the roots of edamame plants. After the inoculation process, the seeds are directly planted on the prepared plots.

Observations were made on 10 sample plants by measuring root nodulation, edamame growth, and production parameters. Root nodulation parameters include the average number of total root nodules. The total number of root nodules was observed at 26 days after planting (DAP) by uprooting the sample plants and counting all root nodules formed after cleaning.

The growth and production parameters of edamame included several aspects of observation. Plant height was measured at 2, 3, and 4 weeks after planting (WAP) by measuring the distance from the soil surface to the last stem growth point. Leaf area was measured on the top trifoliate leaf that had optimal development. Chlorophyll content was observed using a SPAD-502 plus chlorophyll meter.

In production, yield components were counted and weighed, including the number and weight of filled and empty pods on each sample plant. Filled pods are filled, while empty pods are not filled. Wet weight was weighed at harvest. To measure 100 seed weight, pods from each plot were threshed, and 100 seeds were counted and weighed to determine their weight.

RESULTS AND DISCUSSION

EDAMAME ROOT NODULATION DUE TO SPACING AND RHIZOBIUM INOCULUM APPLICATION

The effect of plant spacing and Rhizobium inoculation on edamame root nodulation showed varying responses across treatments (**Table 1**). The results demonstrate that Rhizobium inoculation significantly influenced nodule formation, particularly in closer plant spacing arrangements.

The highest number of root nodules was observed in treatment j_1r_1, which was significantly higher than both non-inoculated treatments. This finding aligns with previous research [14], Inoculation with specific Rhizobium strains significantly increases the number of nodules compared to non-inoculated controls. For example, inoculating faba bean varieties with strains like NSFBR-15 resulted in a notable increase in nodule number, with the variety Dosha producing up to 88.5 nodules per plant when inoculated with this strain.

TABLE 1. Edamame root nodulation due to spacing and Rhizobium inoculum application

Treatment	Number of root nodulation
j_1r_0 (20x15 cm without *rhizobium* inoculum)	33,67 a
j_1r_1 (20x15 cm with *rhizobium* inoculum)	63,99 b
J_2r_0 (20x30 cm without *Rhizobium* inoculum)	29,20 a
J_2r_1 (20x30 cm with *Rhizobium* inoculum)	45,47 ab

Note: Based on Duncan Multiple Range Test (DMRT) test at 5% level.

Interestingly, the effect of Rhizobium inoculation appeared to be more pronounced in closer plant spacing (20x15 cm) compared to wider spacing (20x30 cm). In the wider spacing treatment, j_2r_1 showed a moderate increase in nodulation (45.47) compared to its non-inoculated counterpart j_2r_0 (29.20), though this difference was not statistically significant. The reduced effectiveness of Rhizobium inoculation in wider spacing contradicts the general understanding that wider spacing should promote better root colonization by reducing competition for resources [15]. However, this unexpected result might be explained by the complex interactions between plant density and root system development. While closer spacing might increase competition for resources, it may also create a more favorable microenvironment for Rhizobium colonization, possibly through increased root contact and enhanced signaling between plants and bacteria. For successful colonization, rhizobia must compete effectively with other soil microorganisms. The ability to utilize carbon sources from legume root exudates is essential for competitive nodulation, indicating that a rich microbial community can influence the success of Rhizobium establishment [16].

EDAMAME GROWTH DUE TO SPACING AND RHIZOBIUM INOCULUM APPLICATION

The analysis of edamame growth response reveals that plant spacing and rhizobium inoculation treatments had relatively limited effects on plant growth parameters. Most parameters showed no significant differences between treatments. For plant height (**Figure 1.A**), all treatments showed the same from 2 to 4 WAP, indicating that combinations of plant spacing and rhizobium inoculation did not significantly affect plant height growth. Similarly, for leaf number (**Figure 1.B**), no significant differences were found between treatments during the observation period. Treatment effects were only observed in a few specific parameters. Leaf area (**Figure 1.C**) showed different responses at 2 WAP, where j_1r_0 treatment had higher values compared to other treatments. Differences were also observed in chlorophyll content (**Figure 1.D**), particularly at 4 WAP observation, where j_2r_1 treatment showed higher values compared to j_1r_1.

These results indicate that although plant spacing and rhizobium inoculation combinations were applied, their effects on edamame growth tended to be minimal. Significant differences were only observed in leaf area and chlorophyll content parameters, while main growth parameters such as plant height and leaf number showed no significantly different responses. This suggests that edamame plants have a high level of plasticity in responding to different growth conditions, where plants can maintain relatively uniform growth patterns regardless of treatment variations. A comprehensive study by [17] revealed that while plant spacing influenced certain growth parameters, legumes exhibited a degree of plasticity that allowed them to maintain overall growth patterns. This indicates that edamame may similarly adapt to spacing variations without significant impacts on key growth metrics. Further evidence comes from a detailed study conducted in Ethiopia examined the impact of *Rhizobium* inoculation and inter-row spacing on faba bean yield. It found that while significant effects were noted for leaf area index and grain yield, main growth parameters like plant height and number of leaves per plant did not show significant differences among treatments. This suggests a similar trend may occur in edamame, where specific physiological traits are influenced without affecting overall growth metrics [18].

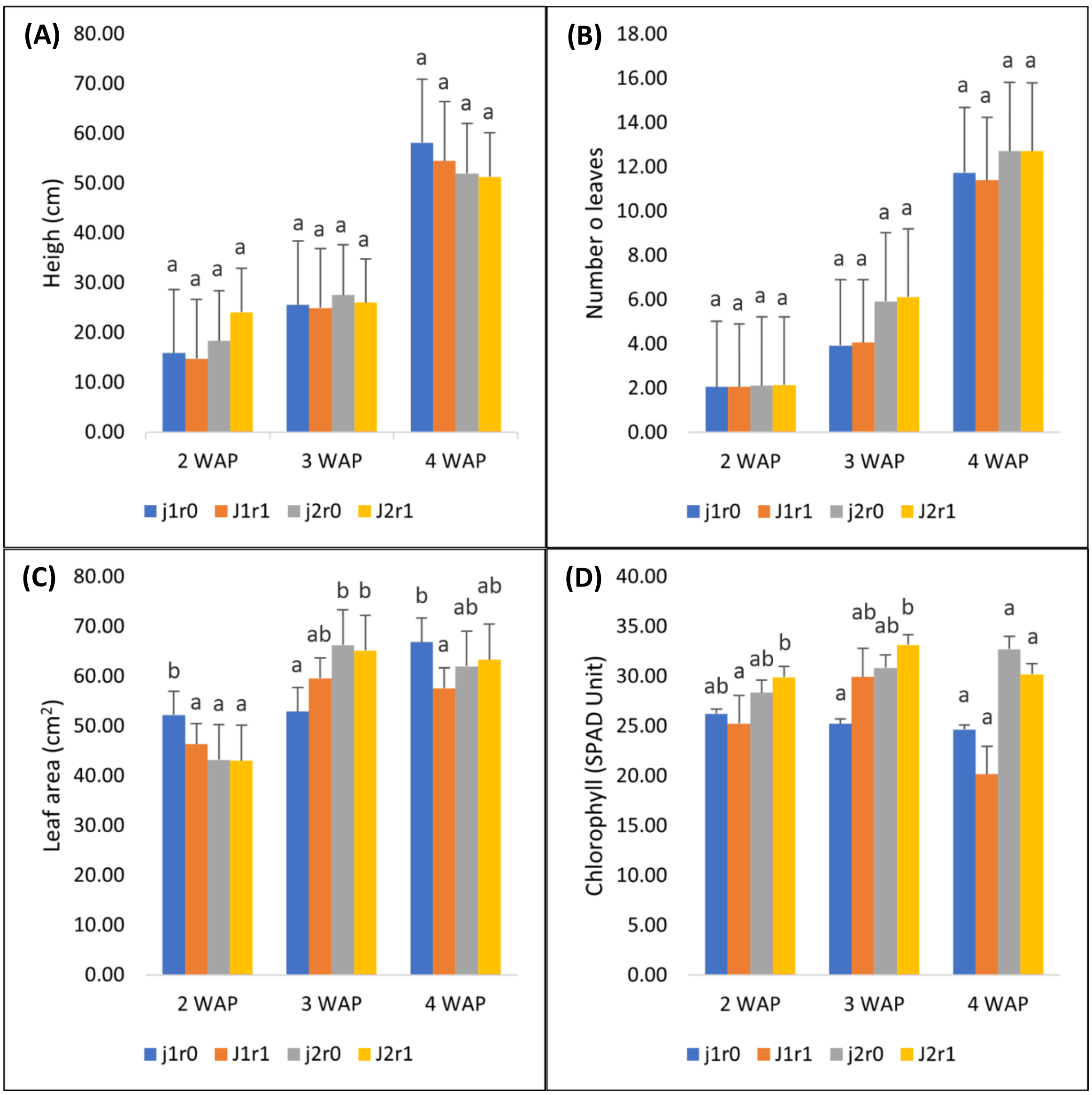

FIGURE 1. Heigh **(A)**, Number of leaves **(B)**, Leaf area **(C)**, and Chlorophyll content **(D)** of edamame due to spacing and *Rhizobium* inoculum application. Note: Based on Duncan Multiple Range Test (DMRT) test at 5% level.

EDAMAME PRODUCTION DUE TO SPACING AND RHIZOBIUM INOCULUM APPLICATION

The results in **Table 2.** show the effect of *Rhizobium* inoculum and spacing on various edamame production parameters, including number of filled pods (NFP), number of empty pods (NEP), weight of filled pods (WFP), weight of empty pods (WEP), total wet weight (WW), and weight of 100 seeds (W100). Treatments with *Rhizobium* inoculum (J_1r_1 and J_2r_1) generally produced higher NFP compared to treatments without *Rhizobium* inoculum (J_1r_0 and J_2r_0). The J_1r_1 treatment had the highest NFP, at 39.73, significantly different from J_1r_0. This indicates that applying *Rhizobium* inoculum can increase the formation of filled pods. NEP and WEP parameters showed no significant differences among all treatments. This indicated that *Rhizobium* inoculum and spacing did not significantly affect the number of empty pods. These results were identified by [14], who focused on the effectiveness of different Rhizobium strains and their impact on legume growth. It found that while effective strains enhanced nodulation and biomass, they did not significantly change the number of empty pods, suggesting that other factors may play a more critical role in determining pod fill. While the study in [7] indicated that mineral nitrogen fertilization (NF) was more effective than *Rhizobium* inoculation alone, it also highlighted scenarios where RI could be more beneficial, particularly under specific soil conditions. This suggests that optimizing Rhizobium use can lead to significant improvements in filled pod weight

TABLE 2. Effect of Plant Spacing and Rhizobium Inoculum Application on Edamame Yield Components

Treatment	NFP	NEP	WFP (g)	WEP (g)	WW (g)	W100 (g)
j_1r_0 (20x15 cm without *rhizobium* inoculum)	20.73 a	8.20 a	38.67 a	15.20 a	113.00 b	43.44 a
j_1r_1 (20x15 cm with *rhizobium* inoculum)	39.73 b	6.80 a	66.40 b	13.80 a	115.67 b	59.75 b
J_2r_0 (20x30 cm without *Rhizobium* inoculum)	22.20 a	7.40 a	37.33 a	14.53 a	76.33 a	46.47 ab
J_2r_1 (20x30 cm with *Rhizobium* inoculum)	25.33 ab	8.80 a	62.73 a	13.73 a	84.00 a	49.75 ab

Note: Based on Duncan Multiple Range Test (DMRT) test at 5% level. Note: **NFP** (Number of filled pods), **NEP** (Number of empty pods), **WFP** (Weight of filled pods), **WEP** (Number of empty pods), **WW** (Wet weight), and **W100** (100 seed weight).

The highest filled pod weight was found in the J_1r_1 treatment with a value of 66.40 grams, significantly higher than the treatment without *Rhizobium*. This supports the finding that *Rhizobium* inoculum increased the productivity of edamame plants in terms of filled pod weight. The results from [19] suggest that Rhizobium inoculant application positively affects nitrogen-fixing activity during critical growth stages, such as pod formation. This increased nitrogen availability supports better growth and filled pod weight. The j_1r_1 treatment also showed the highest wet weight of 115.67 grams, which was higher than j_1r_0, only 113 grams. This indicates that *Rhizobium* inoculation at a tighter planting distance resulted in greater plant wet weight, Rhizobium inoculation significantly increased biomass production in legumes, including peanuts. The study indicated that tighter planting distances, when combined with effective Rhizobium strains, resulted in greater plant wet weight due to improved nodulation and nutrient uptake [20]. In the 100 seed weight parameter, the J_1r_1 treatment had the highest weight of 59.75 grams, significantly higher than the J_1r_0 treatment, which was only 43.44 grams. This indicates that *Rhizobium* inoculation also positively impacted edamame seed enlargement. The application of Rhizobium has been linked to improved growth metrics such as biomass accumulation and nutrient uptake, which indirectly supports higher pod production. Effective nitrogen fixation by Rhizobium enhances leaf growth and overall plant vigor, contributing to increased pod yield [21].

CONCLUSIONS

Rhizobium inoculation combined with closer plant spacing significantly improved key production parameters of edamame. The treatment positively affected root nodulation, number of filled pods, weight of filled pods, wet weight, and 100-seed weight. The number and weight of empty pods showed no significant response to the treatments. These findings indicate that the combination of Rhizobium inoculation and appropriate plant spacing can effectively enhance edamame productivity through improved pod filling and seed development.

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