

Correlation between Root Nodule Characteristic and Growth Component of Jack Bean Intercropped with Aloe Plant in Calcareous Soil

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Abstract—This research aims to find out the correlation between root nodules and the growth of jack bean intercropped with aloe plants in calcareous soil. The research was carried out in Karangmojo, Gunung Kidul, Yogyakarta, Indonesia. A Randomized Complete Block Design with two factors and three replications was used. The first factor was the application of four rates of cow manure (10, 15, 20, and 25 t ha⁻¹), and the second factor was the nitrogen fertilizer used, consisting of urea at a dose of 100 and 150 kg ha⁻¹ and ammonium sulfate at a dose of 200 and 300 kg ha⁻¹. Control treatment of jack bean monoculture was also made. The variables observed were the number of nodules, percentage of plant height, number of leaves, active nodules, leaf weight, root weight, and fresh weight of the plant. Analysis of variance was used for statistical analysis, and Duncan's Multiple Range Test at the 5% significance level was used for means comparison. The best growth of nodules was obtained at the combination of 20-ton manure with urea of 100 kg ha⁻¹, while the best growth of jack bean plants was gained at 15-ton manure combined with ammonium sulfate 200 kg ha⁻¹, and better than the control. There were correlations between nodule number with a number of active nodules and root weight, but no significant correlation with growth components of a jack bean plant.

Keywords— Cow manure; dry weight of the plant; jack bean; number of the active nodule.

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I. INTRODUCTION

Aloe barbadensis, *Aloe ferox*, *Aloe arborescens*, and *Aloe vera* are among the well-studied Aloe species; the plants of the Aloe genus, extensively spread in the old world, are well-known to be utilized as topical and oral therapeutic treatments for ages due to their medical, health, and skincare benefits. They are one of the most economically strategic medicinal herbs widely utilized in primary health care. In addition to being a rich source of essential phytochemicals, they play an important role in treating a variety of disorders by modulating biochemical and molecular processes. Aloe is a multifunctional crop used as an ornamental plant, healthy food industry, and in herbal medicine materials. Therefore, this plant is called the Miracle plant. Aloe leaves contain carbohydrates, proteins, fat, lignin, saponin, aloin, tannin, glucomannan, enzymes, vitamins A, B1, B2, C, E, and minerals, which are synergistically interacted [1]. Aloe vera L., according to Darini's [2] assessment, has a good chance of being developed because it offers a variety of purposes. This plant has not been widely cultivated in Yogyakarta because the quality and yield of the product are insufficient to entice

both the producer to cultivate the plant and the customer to consume it.

The objective of this research was to improve the leaf quality of seedlings grown on sandy soil by raising the content of aloin as the secondary metabolite of the seedlings grown on sandy soil. Aloe leaf contains secondary metabolites, such as aloin and vitamins A, B, C, E, and minerals. Therefore, it can be used as food containing antioxidants. Leaf length and seedling height did not significantly affect the increase in the fresh and dry weight of Aloe vera seedlings, but leaf number ($r = 0.738^*$) did. There was no difference in aloin concentrations in seedlings from a separate source grown on sandy soil. Aloin concentration was negatively correlated with proline concentration, with an r -value of -0.985 .

Meanwhile, the increase in aloin production of *Aloe vera* grown on sandy soil was affected by the growth of seedling and aloin concentration with r values of 0.957^* and 0.718^* , respectively [3]. The Aloe plant can endure adverse conditions, and Aloe can yield in eight months when intensively cultivated with relatively wide plant spacing. Based on their character, the land will be more efficiently used when aloe plants are intercropped with noncompetitive crops, such as legumes.

II. MATERIALS AND METHOD

A. Materials

Jack bean (*Canavalia ensiformis* L.) is one of the local legume crops in Indonesia, often called Jack Bean, and has the potential substitution for soybean and mung bean. The high protein content of 30% is close to the protein content of soybean and almost the same as that of mung bean. Besides, the seed also contains 60% carbohydrates and 2.6% fat. Jack bean has promising potential as a substitute for conventional foodstuff [4].

Jack bean is a diversified crop, and it could be included as a vegetable when harvested at a younger stage, grouped as a food source when harvesting the seeds, and withstands the biotic and abiotic environment. The productivity of jack bean is relatively high, up to 3-5 t ha⁻¹, while soybean yield is 1.2–2.0 t ha⁻¹. The seeds have many benefits and potential for agroindustry since they could be used directly for food, materials for the food and pharmaceutical industries, feed industry, and organic fertilizer [5]. Efforts for increasing the productivity of the jack bean on a large scale and its continuance need to be done by inoculants such as Rhizobium, one of the bacteria that can make nutrients available for plants. Through a symbiotic relationship with the legume crop, bacteria will infect the roots of the legume to form nodules.

The role of Rhizobium on plant growth is especially related to nitrogen availability for the host plant. Rhizobium bean inoculants soil and Rhizobium soybean inoculants do not affect the formation of nodules and do not increase either the growth or yield of the *Medicago sativa* in the sandy soil. Urea dosage 75 kg/ha can raise the growth and yield by 42.64% compared to the urea application dosage of 50 kg/ha [6]. According to Sutrisno and Yusnawan [7], organic fertilizer, combined with inorganic fertilizers, could not produce a significant increase in yield. However, plant secondary metabolites, particularly phenolic levels, total flavonoid, and antioxidant activity, increased by 28%, 37%, and 28%, respectively, when compared to those without fertilizer treatment. As a result, combining organic and inorganic fertilizers to improve the seed quality of mung bean could be advised.

Based on Ho et al. [8] stated that arable land has decreased and undergone conversion for the needs of industry areas, housing, offices, and others, affected in reducing food production. Spodosols have a limited nutrient retention capability due to their silica-rich sandy nature (low clay content). Therefore, supplying sufficient organic C is very important to maintain the fertility and productivity of sandy soils, especially after the removal of the litter layer covering the E horizon for cultivating pepper and oil palm.

To improve soil fertility, it is necessary to enhance the physical property, especially the structure and texture of the soil. Several local ameliorants are organic fertilizers, such as compost, humus, and manure. Manure is available, so it is easy to obtain, especially cow manure. However, to improve the soil's chemical fertility, it needs the addition of nitrogen fertilizer, such as urea and ammonium sulfate. Meanwhile, nitrogen-fixing bacteria that live inside the root nodules could supply the plants with nitrogen-fixed molecules. Therefore, it is necessary to detect a correlation between root nodule characteristics and the growth of jack bean intercropped with aloe plants in calcareous soil, combining combined organic fertilizer and nitrogen sources.

The materials in this research were taken from some areas or sources. Aloe seedlings were obtained from Karangnongko women farmer's group. Jack bean seedlings were taken from Faculty of Agriculture, Universitas Sarjanawiyata Tamansiswa. Jack bean Rhizobium was taken from Laboratory of Soil Science, Faculty of Agriculture Universitas Gadjah Mada. Cow manure was from a local location. Urea, ammonium sulfate, and triple superphosphate fertilizers were taken from the farmer shop.

B. Experimental Design

It was a two-factor Randomized Complete Block Design experiment consisting of three replications. The first factor was the application of cow manure at four rates (10, 15, 20, and 25 t ha⁻¹), and the second factor was nitrogen source, including urea at a dose of 100 and 150 t ha⁻¹ and ammonium sulfate (AS) at a dose of 200 and 300 kg ha⁻¹. Control treatment was jack bean monoculture, applied with manure and nitrogen fertilizer at minimum rates. Every plot with a size of (4 x 4) m was planted with row spacing of 80 x 80 cm, resulting in 25 aloe plants and 16 jack bean plants.

C. Land Preparation, Planting, Intercropping Operation, and Harvesting

Land preparation was started with soil tillage, then 51 plots were made for three blocks. Basal fertilizers of 16, 24, 32, and 40 kg plot⁻¹ cow manure were applied, and the aloe seedlings were planted one week later. Another week later, jack bean seeds were sowed at the mid-point of four aloe seedlings. Half of the urea and ammonium sulfate were applied, and the remaining half of the nitrogen fertilizers were applied one month afterward. The intercultural operation done, including watering, weeding, and pest management.

D. Data Collection

Growth components were collected on four sample plants. Variables measured were plant length, number of nodules, number of active nodules, percentage of active nodules, root weight, root length, number of leaves, leaf area, leaf weight, fresh plant weight, and plant dry weight.

E. Data Analysis

To determine significant ($P \leq 0.05$) treatment effect, analysis of variance was used, and post-hoc test of Duncan's Multiple Range Test was performed to determine differences between treatment means.

III. RESULTS AND DISCUSSION

Cow manure and nitrogen sources had a significant interaction effect on all variables measured except active nodule percentage and root weight. The cow manure and nitrogen source rate did not result in a different percentage of the active nodule. The combination of cow manure and nitrogen fertilizer application did not give a significantly different effect compared to the control (Table I). This result was not supported by Sutrisno and Yusnawan [7], reporting that applying organic fertilizer, inorganic fertilizer, and its combination could increase the nodule number of mung bean,

while the application of organic fertilizer combined with inorganic fertilizers did not considerably increase the yield. Nevertheless, the plant's secondary metabolites, particularly phenolic content, total flavonoid, and antioxidant activity, significantly increased by 28%, 37%, and 28%, respectively, when compared to those without fertilizer treatment. Therefore, combining organic and inorganic fertilizers to improve the seed quality of mung bean could be advised.

TABLE I
EFFECTS OF THE DOSES OF COW MANURE AND NITROGEN SOURCES ON THE ACTIVE NODULE AND ROOT WEIGHT

Rate of Manure (ton ha ⁻¹) /Nitrogen Source (Urea/AS, kg ha ⁻¹)		Means of Traits	
		Active Root Nodule Percentage (%)	Root Fresh Weight (g)
Cow Manure's Rate (ton ha ⁻¹)	10	55.70 a	1.76 c
	15	51.79 b	1.84 c
	20	52.92 a	2.20 b
	25	58.67 a	2.93 a
Nitrogen Fertilizers' Source	Urea 100	55.70 a	2.18 a
	Ammonium 150	57.63 a	2.25 a
	Sulphate 200	56.66 a	2.18 a
Interaction		51.24 b	2.12 a
Means		P _≥ 0.05	P _≥ 0.05
Control		55.55 x	2.18 x
		55.36 x	1.73 x

Remarks: Values followed by different letters within the same column signify differences based on Duncan's Multiple Range Test at P_≤0.05.

Table I shows that a higher rate of cow manure increased the fresh weight of root, in which the highest value was obtained at the rate of 25-ton ha⁻¹ manure. However, nitrogen source had no significant effect on the root fresh weight. The treatment combination of organic and inorganic fertilizer resulted in the same root fresh weight as control, which was not under the statement of Mukhtar et al. [9], revealed that due to its properties in releasing nutrients and improving other soil qualities, organic fertilizer is capable of reducing the use of synthetic fertilizer. The combination of organic fertilizer (compost/manure) with nitrogen increased the fresh weight of Chinese cabbage. The fresh weight and number of leaves in green mustard increased when cow manure combined with litter compost was applied with an extra nitrogen fertilizer at a rate of 25 Mg ha⁻¹. However, the application of 25 Mg ha⁻¹ organic fertilizers without additional nitrogen fertilizer had no significant effect on most observed parameters, implying that organic fertilizer on green mustard is effective in reducing nitrogen fertilizer.

Table II shows that the combination of 20-ton ha⁻¹ manure and 100 kg urea produced the highest number of nodules, while the lowest nodule number was found in 20-ton ha⁻¹ manure combined with 150 kg urea. Treatment combinations or control resulted in no differences in active nodule number. Prasanti et al. [10] reported that Organic manures had been shown to improve soil fertility and structure and affect microbial enzyme activity and soil biodiversity. The investigation at GKVK, Bangalore, during 2013-2014 revealed that 20 tons of FYMha⁻¹ showed a higher abundance of soil mesofauna (18.53) when compared to inorganic fertilizer and the recommended treatment, showing an abundance of soil mesofauna of only 10.30 and 12.35, respectively. Meanwhile, a single application of organic

fertilizer up to 20-ton ha⁻¹ raised the number of soybean nodules by 64.93 per plant.

TABLE II
EFFECTS OF THE DOSES OF COW MANURE AND NITROGEN SOURCE ON ACTIVE NODULE NUMBER, LEAF NUMBER, LEAF AREA, AND LEAF FRESH WEIGHT OF JACK BEAN

Treatment Combination Manure Rate (t ha ⁻¹)/Nitrogen Source (kg ha ⁻¹)	Means of Traits			
	Active Nodule Number	Leaf Number	Leaf Area (cm ²)	Leaf Fresh Weight (g)
10 ton/100 kg urea	4.0 c	10.31 h	478.99 c	9.86 b
10 ton/150 kg urea	4.3 c	8.30 j	384.57 f	7.74 d
10 ton/200 kg AS	5.3 a	8.00 j	176.14 k	4.35 h
10 ton/300 kg AS	4.3 c	24.00 a	375.82 f	7.73 d
15 ton/100 kg urea	4.0 c	6.00 k	126.16 l	4.57 h
15 ton/150 kg urea	3.7 d	7.70 j	534.84 b	10.35 b
15 ton/200 kg AS	3.3 e	20.30 b	573.73 a	11.12 a
15 ton/300 kg AS	3.3 e	12.70 f	374.48 f	8.25 c
20 ton/100 kg urea	5.0 a	11.30 g	227.41 j	5.60 g
20 ton/150 kg urea	3.3 e	14.70 d	345.74 h	6.93 f
20 ton/200 kg AS	3.3 e	13.70 e	438.46 d	8.35 c
20 ton/300 kg AS	3.0 f	12.70 f	419.50 e	8.50 c
25 ton/100 kg urea	3.7 d	15.70 d	361.95 g	7.35 e
25 ton/150 kg urea	4.0 c	8.70 i	253.55 i	5.66 g
25 ton/200 kg AS	4.7 b	18.30 c	419.59 e	8.53 c
25 ton/300 kg AS	3.7 d	11.30 g	130.64 l	3.56 i
Interaction	P _≤ 0.05	P _≤ 0.05	P _≤ 0.05	P _≤ 0.05
Means	3.7 x	11.06 x	438.47 x	8.43 x
Control	4.1 x	10.22 y	351.35 y	7.40 y

Remarks: Values followed by different letters within the same column signify differences based on Duncan's Multiple Range Test at P_≤0.05.

The highest number of active nodules was gained at the combined 10-ton manure with 200 kg ammonium sulfate or 20-ton manure with 100 kg urea, whereas the lowest number of active nodules was found at the application of 20-ton manure with 300 kg ammonium sulfate. A field study by Khan et al. [11] evaluated the efficacy of organic amendments (biochar, phosphorous, and potassium combination) in reducing the detrimental impact of low water quality on soil properties and the growth of chickpeas (*Cicer arietinum*). The collection of soil samples was carried out before organic amendments were applied, and after crop harvesting, the application of biochar, phosphorous, and potassium increased the nodule number of chickpeas. The application of 10-ton manure combined with 300 kg ammonium sulfate resulted in the highest number of leaves. Meanwhile, the application of 15-ton manure with 100 and 150 kg urea per hectare produced the lowest number of leaves. The combined application of manure and two nitrogen sources increased leaf number compared to the control. It can be inferred that low-quality underground water can be used efficiently for sustainable agriculture production in the region where the study was conducted. However, further fieldwork is required to assess the long-term impact of such water on the properties of soil containing organic materials so that a sustainable plan for the region can be proposed. In accordance with Donatus's [12] statement, almost all soil nutrient properties improved significantly (P 0.05) when poultry manure was applied. The pot treated with 2-ton ha⁻¹ poultry manure and 1-ton ha⁻¹ urea showed the best improvements in soil fertility, including soil pH, total nitrogen, organic carbon, effective cation exchange Capacity (ECEC), available phosphorus, and total

exchangeable acidity (TEB) when compared to the control treatment.

Likewise, adding urea to poultry manure significantly increased the number of leaves and pods (P0.05). When urea was applied without poultry manure, it raised the number of leaves more than when poultry manure was applied alone. However, poultry manure in combination with urea increased the number of pods significantly more than when urea was applied alone. When 2 tons ha⁻¹ of poultry manure was combined with 1 ton of urea, the highest increase in the number of leaves and pods was observed. It was also shown that poultry manure increased the number of nodules while urea decreased the value. It is therefore recommended that urea be combined with poultry manure to boost soil fertility and improve groundnut yield. Groundnut production should not use a significant amount of urea to promote nodulation and nitrogen availability in the soils of Unwana, Southeastern Nigeria; instead, chicken manure can be applied. The application of poultry manure with urea increased the leaf number of groundnuts.

The highest leaf area of jack bean was gained at 15-ton manure combined with 200 kg ha⁻¹ ammonium sulfate, and the lowest was found at 25-ton manure combined with 300 kg ammonium sulfate. This finding was not in line with Situmeang et al. [13], who stated that fertilizer types had no significant effect on the observed variables. However, the phonska, compost, and biochar rates significantly affected all variables except stem diameter. Maize plants grew the fastest when treated with 10-20 tonnes/ha compost, 5-10 tonnes/ha biochar, and 150-300 kg/ha phonska. Based on the regression analysis, with the combined application of compost, bamboo biochar, and Phonska, the optimum rates of bamboo biochar of 10.93 tonnes/ha raised the total leaf area of maize.

The application of 15-ton manure with 200 kg ammonium sulfate gave the highest leaf fresh weight, while the lowest leaf fresh weight was obtained at 25-ton manure combined with 300 kg ammonium sulfate. The application of manure and nitrogen resulted in higher leaf fresh weight than control. According to Mukhtar et al. [9], organic fertilizers can reduce synthetic fertilizers' usage by releasing nutrients and acting as soil ameliorants. Combining organic fertilizer (compost/manure) with nitrogen increased the fresh weight of *Chinese cabbage*. The number of leaves and fresh weight of green mustard increased when litter compost and cattle manure was applied at 25 Mg ha⁻¹ combined with nitrogen fertilizer. However, organic fertilizers applied at a rate of 25 Mg ha⁻¹ without combining with nitrogen fertilizer did not significantly affect most variables, implying that using organic fertilizer on green mustard can reduce the use of nitrogen fertilizer use.

In contrast to Kamtchoum et al. [14], there were no significant differences in the nodule's yield and dry matter content. However, poultry manure was more effective in improving shoot length, collared diameter, and number of leaves than the chemical fertilizer used (NPK 14 – 24 – 14). This study demonstrates that chemical fertilizers can be used to grow beans.

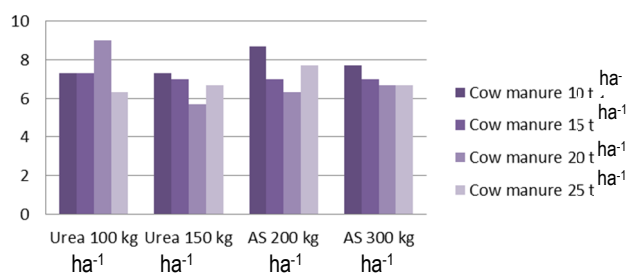


Fig. 1 The number of root nodules at various doses of cow manure and nitrogen sources

Table III shows that the highest root length was found in a combination of 15-ton cow manure with 150 kg urea, 20-ton manure with 200-300 kg ammonium sulfate, and 25-ton manure with 100 kg urea. Application of manure with nitrogen fertilizer made jack bean root longer than the control.

TABLE III
RATE OF COW MANURE AND NITROGEN SOURCE EFFECT ON ROOT LENGTH, LEAF LENGTH, LENGTH OF THE PLANT, AND PLANT FRESH WEIGHT

Treatment Combination Manure Rate (t ha ⁻¹) / Nitrogen Source (kg ha ⁻¹)	Means of Traits			
	Root Length (cm)	Stem Length (cm)	Plant Length (cm)	Plant Fresh Weight (g)
10 ton/100 kg urea	6.93 e	27.67 h	34.60 c	19.26 c
10 ton/150 kg urea	6.15 f	26.57 i	32.72 c	17.93 e
10 ton/200 kg AS	7.04 e	36.11 b	43.15 b	13.15 h
10 ton/300 kg AS	7.66 c	34.05 c	41.71 b	18.50 d
15 ton/100 kg urea	6.12 g	29.12 g	35.24 c	9.55 j
15 ton/150 kg urea	8.52 a	25.93 i	34.45 c	20.50 b
15 ton/200 kg AS	4.60 i	34.76 b	39.36 b	25.72 a
15 ton/300 kg AS	5.45 h	18.51 j	23.96 e	16.04 f
20 ton/100 kg urea	7.23 d	22.11 j	29.34 d	13.84 h
20 ton/150 kg urea	7.64 c	29.85 f	37.49 c	15.87 f
20 ton/200 kg AS	9.47 a	26.07 i	35.54 c	13.21 h
20 ton/300 kg AS	9.47 a	31.22 e	40.69 b	18.69 c
25 ton/100 kg urea	8.89 a	26.59 i	35.48 c	16.27 f
25 ton/150 kg urea	7.75 c	44.59 a	52.34 a	15.49 g
25 ton/200 kg AS	8.11 b	32.46 d	40.57 b	20.75 b
25 ton/300 kg AS	4.55 i	28.65 g	32.20 c	10.79 i
Interaction	P≤0.05	P≤0.05	P≤0.05	P≤0.05
Means	7.04 x	32.97 x	34.60 x	16.60 x
Control	4.53 y	29.67 y	30.34 y	12.84 y

Remarks: Values followed by different letters within the same column signify differences based on Duncan's Multiple Range Test at P≤0.05.

The lengthier main stem was obtained at 25-ton manure with 150 kg urea, while 15-ton manure with 300 kg ammonium sulfate or 20-ton manure with 100 kg urea resulted in the shortest one. The same result was obtained in plant length as well. The highest plant fresh weight and dry weight were gained at the application 15-ton manure with 200 kg ammonium sulfate, while the lowest ones were found with 15-ton manure combined with 100 kg urea. Dautas et al. [15] also reported fixed doses (29, 30, 43, and 250 kg ha⁻¹) identified by legume biomass.

The nitrogen fixation by intercropped jack beans supplies more nitrogen than that distributed to the fruit, thereby resulting in a positive balance higher than 100 kg ha⁻¹. Sutrisno and Yusnawan [7] also found that inorganic fertilizer, organic fertilizer, and their combination might increase the number of nodules in mung bean, while organic fertilizer combined with inorganic fertilizer did not effectively improve yield. However, there was a rise in

secondary metabolites in plants, particularly phenolic content, total flavonoid, and antioxidant activity. The crops treated with manure and NPK fertilizers produced seeds with an increase in phenolic content, total flavonoid, and antioxidant activity by 37%, 28%, and 28%, respectively, when compared to those without fertilizer treatment.

Therefore, combining inorganic and organic fertilizers may be proposed to improve seed mung bean quality. Applying organic and inorganic fertilizers enhanced the mung bean's fresh weight, whereas according to Otieno et al. [16], manure, inorganic fertilizers, and lime treatments had substantial effects on all variables. Over the control treatment, lime, manure, and manure + lime raised soil pH by 2.19, 1.33, and 2.28, respectively. Control (1.71-1.81 percent), NPK (1.85-1.98 percent), and manure (2.00-2.11 percent) produced lower shoot N than all other treatments. The number of nodules and dry weights per plant was the highest in the lime + manure treatment. NPK uptake was positively correlated with soil pH ($R^2 = 0.30-0.77$) and biomass ($R^2 =$ from 0.68-0.99) (R^2 ranged between 0.68 and 0.99). Based on the findings of this study, manure, and lime are able to enhance soil pH, allowing greater nutrient uptake, nodule development, and soybean productivity in Western Kenya.

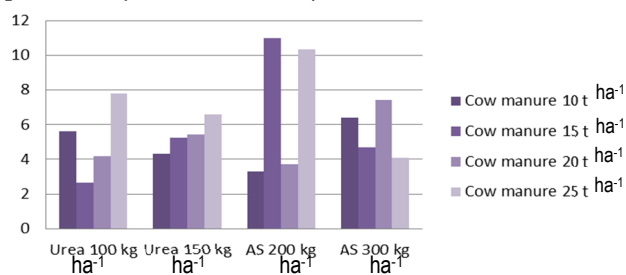


Fig. 2 Plant dry weight (g) at various doses of cow manure and nitrogen sources

The efficacy of organic amendments (farmyard manure, compost, and poultry manure) alone and in combination to counteract the negative impact of poor quality water on soil properties and the growth of chickpea (*Cicer arietinum*) was evaluated in a field study by Malik et al. [11].

The soil samples were collected before applying organic amendments and after crop harvesting. The number of nodules positively correlated with the stem length of the chickpea. Active root nodules correlated with root weight, stem length, and percentage of active root nodules. Yang et al. [17] used correlation networks to compare correlations found for hyper nodulating N nutrition features and C nutrition traits. Biological Nitrogen Fixation can improve crop productivity, reducing N fertilizer application in Western Canada. The findings show that decreased N₂ fixation in hyper nodulating mutants is due to restricted shoot growth. There was a correlation between the number of active root nodules and the number of root nodules, reducing the plant's ability to accumulate N₂. Soybeans can utilize atmospheric nitrogen through biological fixation by forming a symbiotic connection with Rhizobium bacteria, as reported by Zi et al. [18]. Nitrogen (N) is a very mobile element in the continuum of the soil-plant-atmosphere because it undergoes intensive chemical and microbiological transformations. There was a correlation between active root nodule with nodule number, leaf number, and soybean plant height. Meanwhile, Fenta et

al. [19] noted that it is critical to provide a suggestion for inoculating common bean seeds with the correct species of Rhizobium bacteria before sowing for the first time common bean cultivation.

Another suggestion would be to decrease nitrogen fertilizer, as common bean plants grew well, even with few root nodules. Most were dormant, implying that soils had sufficient plant-available nitrogen to support plant development without nitrogen fixation. Nevertheless, further research is required before solid recommendations on reduced nitrogen fertilization of common beans can be made. Many root nodules and active root nodules were not correlated with common bean biomass (*Phaseolus vulgaris*L.). There was a correlation between root weight with the number of root nodules, root length, stem length, leaf number, leaf weight, leaf area, and plant fresh weight. According to Kamtchoum et al. [14], poultry manure was more effective in improving shoot length, collared diameter, and number of leaves of common beans, compared to the chemical fertilizer used (NPK 14 – 24 – 14). This investigation, therefore, indicates the possibility of growing beans using chemical fertilizers. There was a correlation between nodule number with stem length and fresh weight of bean (*Phaseolus vulgaris* L.) plant. Many systems, according to Schwember et al. [20], interact with each other in the symbiotic relationship between nodules and plants, including nitrogen (N) metabolism, carbon (C) metabolisms, oxidative stress, oxygen transport through nodules, and phosphorous (P) levels. This research extensively reviewed these mechanisms, which are known to influence the fixation of N₂ and are highly adjusted on a whole-plant basis. A critical pathway inside nodules implicated in this mechanism is the carbonic anhydrase (CA)-phosphoenolpyruvate carboxylase (PEPC)-malate dehydrogenase (MDH), and malate appears to play an important role in controlling the symbiotic N₂ fixation. Understanding N₂ fixation control on a whole-plant level requires understanding legumes' mechanism in sensing N-status and activating the regulatory mechanisms. Because there is currently no cohesive theory explaining all of the variables involved in N₂ fixation, this will need to be thoroughly investigated in the future.

Advances in functional genomics and molecular techniques, like as miRNAs, have made it possible to discover a large number of regulatory components for extensive research of mechanisms in specific N₂ fixation linked to physiological responses under abiotic stress. Combined with existing information, these plentiful genetic molecular techniques can be used in identifying mechanisms in specific N₂ fixation. Nodule correlated with plant dry weight, the growth of legume. Stem length correlated with number of root nodules, active root nodules, root weight, root length, number of leaves, plant fresh and dry weight, and percentage of active root nodule. Linstrom and Mousavi [21] stated the relationship between rhizobia and their legume hosts had been explored at the physiological, morphological, agronomic, and molecular levels, producing a wealth of information about the processes involved, but identifying fundamental bottlenecks in nitrogen fixation efficiency has been proven to be complex. Otieno et al. [16] also reported a relationship between number of root nodules and plant dry weight.

Positive correlation was found between leaf number and root weight, root length, stem length, leaf area, leaf weight, plant fresh and dry weight, and active root nodule percentage. There was a positive correlation between leaf area with root weight, root length, leaf number, leaf weight, plant fresh and dry weight. A positive correlation was also found between plant fresh weight with root weight, root length, number of leaves, leaf weight, leaf area, and plant dry weight. A correlation was also found between the dry weight of plants with root length, stem length, number of leaves, leaf weight, leaf area, and percentage of active root nodule. Meanwhile, Kuntuyastuti et al. [22] reported the results showed that in the lowlands Vertisol in Ngawi, which is rich in P elements, the addition of 5 tons.ha⁻¹ of cow dung, 3 tons.ha⁻¹ of chicken manure, 1.5 – 2.5 tons.ha⁻¹ of SANTAP-NM1 and SANTAP-NM2 fertilizers, and 300 kg.ha⁻¹ Phonska was not necessary. Local Results Central Java / Sinabung-1036 soybean strains reached 1.95 t ha⁻¹. The increase in soybean yield by 21-27 percent in Madiun's lowland vertisols with poor P elements required the application of 5 tons.ha⁻¹ of cow dung, 1.5-2.5 tons.ha⁻¹ of SANTAP-NM2 fertilizer, and 150 kg.ha⁻¹ of

Phonska. The use of organic fertilizer combined with inorganic fertilizer can be an alternative to help preserve the soil properties, soil productivity, and crop cultivation in sustainable agriculture.

IV. CONCLUSION

The interaction was observed between the doses of cow manure and nitrogen sources on the variables measured, except on the root weight and percentage of active nodules. Rates of manure did not significantly affect root nodules and jack bean plant growth, while sourcing and dosages of nitrogen affected root nodules and jack bean plant growth. A manure rate of 20 tons combined with urea of 100 kg ha⁻¹ gave the best growth of root nodules, whereas the best growth of the jack bean plant was obtained at the combination of 15-ton manure with 200 kg ammonium sulfate. The number of root nodules was correlated with the number of active nodules and root weight, but a significant correlation with the growth of the jack bean plant was not observed.

TABLE IV
PEARSON CORRELATION COEFFICIENT

	RNN	ActRN	RW	RL	SL	LN	LW	LA	PFW	PDW	%PActN
RNN	1.000	0.723**	0.290*	-0.128	0.021	-0.093	-0.222	-0.255	-0.020	-0.177	-0.001
ActRN		1.000	0.207	-0.045	0.194	-0.045	-0.323*	-0.317*	-0.093	-0.105	0.679**
RW			1.000	0.087	0.094	0.084	0.277*	0.218	0.332*	-0.051	-0.003
RL				1.000	0.570**	0.430**	0.308*	0.339*	0.506**	0.598**	0.053
SL					1.000	0.164	-0.179	-0.166	0.226	0.320*	0.261
LN						1.000	0.364**	0.389**	0.520**	0.651**	0.025
LW							1.000	0.969**	0.801**	0.571**	-0.246
LA								1.000	0.801**	0.580**	-0.209
PFW									1.000	0.778**	-0.125
PDW										1.000	0.031
%PActN											1.000

Notes: * Significant ($\alpha=5\%$), ** Very Significant ($\alpha=1\%$), Root Nodule Number (RNN), Active Root Nodule (ActRN), Root Weight (RW), Root Length (RL), Stem Length (SL), Leaf Number (LN), Leaf Weight (LW), Leaf Area (LA), Plant Fresh Weight (PFW), Plant Dry Weight (PDW), Percentage of Active Nodule (%PActN)

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REFERENCES

[1] B. Salehi et al., "Aloe genus plants: From farm to food applications and phytopharmacotherapy," *Int. J. Mol. Sci.*, vol. 19, no. 9, 2018, doi: 10.3390/ijms19092843.

[2] M. Theresia, "The responses and correlation of growth and aloin production of *Aloe vera* L. Seedlings from various planlets in sandy soil," *Int.J.Curr. Agric. Res.* vol. 5, no. 12, pp. 229–232, 2017.

[3] M. T. Darini and F. Amalia, "Growth response and antioxidant content of *Aloe vera* L. plant under different types and dosage of green mulch in sandy soil," *Int. J. of Advances in Sci. Eng. and Tech.* vol 5 no. 2, pp. 59–63, 2017.

[4] S. G. Solomon, V. T. Okomoda, and O. Oguche, "Nutritional value of raw *Canavalia ensiformis* and its utilization as partial replacement for soybean meal in the diet of *Clarias gariepinus* (Burchell, 1822)

fingerlings," *Food Sci. Nutr.*, vol. 6, no. 1, pp. 207–213, 2018, doi: 10.1002/fsn3.548.

[5] N. Nurmuliana and M. A. Akib, "Plant growth analysis of jack been (*Canavalia ensiformis* L.) at different spacing to determine the application time of cutback technology," *Agrotech J.*, vol. 4, no. 1, pp. 1–7, 2019, doi: 10.31327/atj.v4i1.907.

[6] G. Shen, W. Ju, Y. Liu, X. Guo, W. Zhao, and L. Fang, "Impact of urea addition and rhizobium inoculation on plant resistance in metal contaminated soil," *Int. J. Environ. Res. Public Health*, vol. 16, no. 11, 2019, doi: 10.3390/ijerph16111955.

[7] S. Sutrisno and E. Yusnawan, "Effect of manure and inorganic fertilizers on vegetative, generative characteristics, nutrient, and secondary metabolite contents of mungbean," *Biosaintifika J. Biol. Biol. Educ.*, vol. 10, no. 1, pp. 56–65, 2018, doi: 10.15294/biosaintifika.v10i1.12716.

[8] S. Y. Ho, M. E. Bin Wasli, and M. Perumal, "Evaluation of physicochemical properties of sandy-textured soils under smallholder agricultural land use practices in Sarawak, East Malaysia," *Appl. Environ. Soil Sci.*, vol. 2019, 2019, doi: 10.1155/2019/7685451.

[9] Z. Mukhtar, D. Putri, and N. Setyowati, "Reduction of synthetic fertilizer for sustainable agriculture: Influence of organic and nitrogen fertilizer combination on growth and yield of green mustard," *Int. J. Adv. Sci. Eng. Inf. Technol.*, vol. 6, no. 3, pp. 361–364, 2016, doi: 10.18517/ijaseit.6.3.802.

[10] G. Prasanthi, N. G. Kumar, S. Raghu, N. Srinivasa, and H. Gurumurthy, "Study on the effect of different levels of organic and

- inorganic fertilizers on microbial enzymes and soil mesofauna in soybean ecosystem,” *Legum. Res.*, vol. 42, no. 2, pp. 233–237, 2019, doi: 10.18805/LR-3850.
- [11] S. Khan *et al.*, “Soil fertility, n₂ fixation and yield of chickpea as influenced by long-term biochar application under mung–chickpea cropping system,” *Sustain.*, vol. 12, no. 21, pp. 1–14, 2020, doi: 10.3390/su12219008.
- [12] E. Donatus, “Effect of poultry manure and urea on soil chemical properties, nodulation and yield of groundnut (*Arachis hypogaea*) in Akanu Ibiam Federal Polytechnic, Unwana Afikpo Ebonyi State,” *Asian J. Adv. Agric. Res.*, vol. 3, no. 3, pp. 1–8, 2017, doi: 10.9734/ajaar/2017/37677.
- [13] Y. P. Situmeang, I. M. Adnyana, I. N. N. Subadiyasa, and I. N. Merit, “Effectiveness of bamboo biochar combined with compost and npk fertilizer to improved soil quality and corn yield,” *Int. J. Adv. Sci. Eng. Inf. Technol.*, vol. 8, no. 5, pp. 2241–2248, 2018, doi: 10.18517/ijaseit.8.5.2179.
- [14] S. M. Kamtchoum *et al.*, “Production of bean (*Phaseolus vulgaris* l.) under organo-mineral fertilization in humid forest agro-ecological zone with bimodal rainfall pattern in cameroon,” *Annu. Res. Rev. Biol.*, vol. 29, no. 4, pp. 1–11, 2018, doi: 10.9734/arrb/2018/44607.
- [15] E. F. Dantas *et al.*, “Biological fixation, transfer and balance of nitrogen in passion fruit (*Passiflora edulis* Sims) orchard intercropped with different green manure crops,” *Aust. J. Crop Sci.*, vol. 13, no. 3, pp. 465–471, 2019, doi: 10.21475/ajcs.19.13.03.p1559.
- [16] H. M. O. Otieno, G. N. Chemining’wa, and S. Zingore, “Effect of farmyard manure, lime and inorganic fertilizer applications on soil ph, nutrients uptake, growth and nodulation of soybean in acid soils of Western Kenya,” *J. Agric. Sci.*, vol. 10, no. 4, p. 199, 2018, doi: 10.5539/jas.v10n4p199.
- [17] C. Yang, R. Bueckert, J. Schoenau, A. Diederichsen, H. Zakeri, and T. D. Warkentin, “Evaluation of growth and nitrogen fixation of pea nodulation mutants in western Canada,” *Can. J. Plant Sci.*, vol. 97, no. 6, pp. 1121–1129, 2017, doi: 10.1139/cjps-2016-0383.
- [18] J. C. N’Zi, A. P. Koua, J. Kahia, K. D. Kouassi, A. S. P. N’Guetta, and C. Kouamé, “Evaluating nodulation and its effects on some agromorphological parameters of soybean varieties (*Glycine max* L.),” *Asian J. Plant Sci.*, vol. 15, no. 1–2, pp. 26–34, 2016, doi: 10.3923/ajps.2016.26.34.
- [19] B. A. Fenta, S. E. Beebe, and K. J. Kunert, “Role of fixing nitrogen in common bean growth under water deficit conditions,” *Food Energy Secur.*, vol. 9, no. 1, pp. 1–14, 2020, doi: 10.1002/fes3.183.
- [20] R. Schwember, J. Schulze, A. Pozo, and R. A. Cabeza, “Regulation of symbiotic nitrogen fixation in legum root nodules,” *Plant*, vol. 8, no. 333, pp 1-18, 2019.
- [21] K. Lindström and S. A. Mousavi, “Effectiveness of nitrogen fixation in rhizobia,” *Microb. Biotechnol.*, vol. 13, no. 5, pp. 1314–1335, 2020, doi: 10.1111/1751-7915.13517.
- [22] H. Kuntuyastuti, Sutrisno, and A.D. Lestari. "Effect of application of organic and inorganic fertilizers on soybean yield on lowland vertisols," *J. Degrad. Min. L. Manag.*, vol. 8, no. 1, pp. 2439–2450, 2020, doi: 10.15243/jdmlm.2020.081.2439.