

## Nodulation and Morphological Response of *Pueraria Javanica* Bent. to Sunlight Intensity

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### ABSTRACT

*Pueraria javanica* Bent. is a tropical leguminous ground cover crop commonly planted in oil palm plantations to reduce soil erosion, fix nitrogen, and suppress weed growth. This plant is known to be resistant to fluctuations in low and high light intensity. *Pueraria javanica* has the ability to induce nodule formation by growing root hairs on the main branched root through a symbiosis with the bacterium *Bradyrhizobium japonicum*, stimulating the root surface to encourage nodule entry. In addition, legumes have the ability to bind with soil rhizobial bacteria, which fix atmospheric N<sub>2</sub>. This is a form of N that is not available to plants, but can fix N<sub>2</sub> from the soil. The aim of this study was to analyze the effect of variations in sunlight intensity on nodule formation in *Pueraria javanica* and to determine the effect of sunlight intensity on vegetative growth and morphological development of *Pueraria javanica*. This research was conducted at the Experimental Garden of Asahan University campus, Asahan Regency, North Sumatra Province, with an altitude of  $\pm 25$  meters above sea level from January to March 2025. The research was conducted using a randomized block design method consisting of paranet and IAA auxin treatments. The use of paranet was divided into four levels, namely control without paranet, paranet with transparency of 50%, 75%, and 95%. Meanwhile, the application of IAA auxin consisted of three concentration levels, namely 100 ppm, 200 ppm, and 300 ppm. The observed variables were: plant height, number of leaves, number of leaflets, leaf area, fresh weight of plants, root length, number of root nodules, fresh weight of roots, dry weight of roots. The analysis of significant differences was made using the Tukey's HSD method. All data analysis used the SPSS application version 16.0 (SPSS, Chicago, United States). The results of the study showed that the treatment of various percentages of paranet on *Pueraria javanica* showed a significant effect on all observation parameters with the best growth in the control treatment. The application of auxin on *Pueraria javanica* showed a significant difference in the number of leaves, number of leaflets, leaf area, fresh weight of plants, root length, number of root nodules, fresh weight of roots, dry weight of roots. With the best growth in the control treatment. The two treatments above between the treatment of paranet percentage and the administration of auxin showed an interaction that had a significant effect on several parameters observed, including the number of root nodules, fresh weight of roots and dry weight of roots.

**Keywords:** *Pueraria javanica* Bent., nodulation, sunlight, legume cover crop, auxin.

## INTRODUCTION

*Pueraria javanica* Bent. is a tropical leguminous ground cover crop commonly planted in oil palm plantations to reduce soil erosion, fix nitrogen, and suppress weed growth. It is a low, evergreen herbaceous perennial plant found primarily in Southeast Asia (Halimshah et al., 2016). *P. javanica* has a higher soil-locking ability and produces a higher biomass (308.8 g) than *Calopogonium mucunoides* (Nursanti and Supriyanto, 2022). This plant is known to be resistant to fluctuations in low and high light intensity. Before large-scale planting, *Pueraria javanica* plants should be sown. This nursery system offers the advantage of producing high-quality seeds, strong durability and adaptability, and minimizing field mortality (Sukmawati and Kusnadi, 2022).

Nodulation (root nodule formation) is an abnormal tissue on plant roots resulting from the interaction of plant roots with rhizobia bacteria that help fix atmospheric nitrogen. Factors influencing nodule formation include soil pH, rhizobia type, temperature, and soil conditions. *Pueraria javanica* has the ability to induce nodule formation by growing root hairs on the branched main root through a symbiosis with the bacterium *Bradyrhizobia japonicum*, stimulating the root surface to encourage nodule entry. The bacteria enter the root tissue and multiply rapidly. This encourages nodule formation, and the number of nodules that can be produced increases due to more branched fibrous roots (Akbar et al., 2024).

IAA is a naturally occurring auxin hormone found in plants. This hormone can influence rapid plant growth, increasing cell elongation, cell

differentiation, and stem elongation (Ardiana and Advinda, 2022). Plants respond to sunlight with horizontal growth. Sunlight also affects plant hormones, particularly auxin. Hormonal responses to light levels in plants can influence the production and distribution of plant hormones, such as auxin, which plays a key role in regulating cell growth and differentiation. Plants grown in low-light areas may produce more auxin in response to the low-light environment, which can stimulate further stem growth as an adaptation. Thus, the ability of plants to adapt to lighting conditions is key to their survival and growth (Aprimonika et al., 2024).

Increased light can improve the growth parameters of shoots, leaves, and roots of leguminous ground cover plants, and conversely, the root-to-shoot ratio decreases with decreasing light intensity, indicating that low light intensity is particularly detrimental to shoot growth. Low light exposure causes plants to grow larger, thinner leaves with large stomata, a thin epidermal cell layer, more leaves, and wider intercellular spaces. To adapt to low-light environments, plants change their molecular, biochemical, anatomical, morphological, physiological, and agronomic structures (Bayu et al., 2024).

Based on the above review, This study aimed to analyze the effect of variations in sunlight intensity on nodule formation in *Pueraria javanica*. Furthermore, to determine the effect of sunlight intensity on vegetative growth and morphological development of *Pueraria javanica*.

## Materials and Tools

The tools used in this research include: 10 x 15 cm polybags, hygrometers, digital scales, paranet (50%, 75%, 95%), stationery, research plot boards, meters, machetes, watering cans, and bamboo. The materials used in this study were *Pueraria javanica* Bent. seeds and IAA auxin as a plant growth hormone.

## Methods

The study was conducted using a

## RESEARCH METHODS

### Research Location

The research was conducted in the campus of Asahan University, precisely in the experimental garden field of the Faculty of Agriculture, Asahan University, Asahan Regency, North Sumatra Province with an altitude of  $\pm 25$  meters above sea level. The implementation of this research began in January to March 2025.

randomized block design consisting of shade net treatment and IAA auxin administration. The shade net use was divided into four levels: control (without shade net), shade net with 50% transparency, 75% transparency, and 95% transparency. Meanwhile, IAA auxin administration consisted of four concentration levels: 0 ppm (control), 100 ppm, 200 ppm, and 300 ppm. For this study, polybags measuring 15 cm long and 10 cm wide were used.

A soil sample analysis was conducted before the experiment. The type of sample taken was soil sent by the Faculty of Agriculture of Asahan University, located at Jl. Jend. Ahmad Yani, Kisaran 21224, with letter number 001/025/FP-UNA/2024 in 1 sample wrapped in plastic. After being analyzed by the Palm Oil Research Center, PPKS Laboratory, PT. RPN Medan on January 29–March 7, 2024, order number 21–24 was known to have results with certificate number 427/0.1/Sert/III/2024.

Fill the polybag with soil media from the land, namely up to two-thirds full, and ensure the media in the polybag is sufficiently moist by watering it. Planting seeds in polybags is best done in the morning or evening to give the seedlings sufficient time to adapt. The prepared seeds are then placed in the growing medium to a depth of 2 cm to ensure optimal growth and strong root development. One seedling should be planted in each planting hole.

Maintenance includes watering, replanting, fertilizing, and controlling plant pests. Watering is done in the morning and evening using a sprinkler. Weed control is carried out using a hoe to remove weeds between plants. This step is crucial to prevent competition with the cultivated crops. Pest and disease control is carried out mechanically.

The treatment of sunlight intensity in this study was carried out by comparing plants grown without shade netting and those protected by shade netting with transparency levels of 50%, 75%, and 95%. That is, with 4 levels. Plants without shade netting will receive direct sunlight, while plants under shade netting will receive varying protection, with 50% shade netting providing more light than 75% and 95% shade netting, which further limits the intensity of light received.

Field temperature and humidity measurements are conducted twice a week, at 6:00 a.m. and 1:00 p.m. WIB, using a hygrometer.

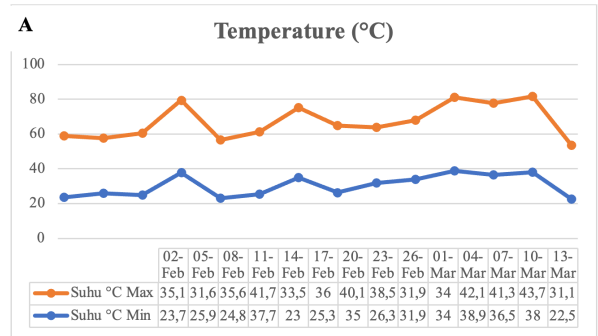


Figure 1. Minimum and maximum temperature (°C) (January-March 2025) in the research area.

### Research Parameters

#### Plant Height (cm)

Plant height was measured from the soil surface to the tip of the tallest leaf. Plant height was measured when 50% of the leaves began to turn yellow after treatment.

#### Number of Leaves and Leaflets

The number of leaves counted is the number of fully opened leaves. This is done by counting the total number of leaves on the *Pueraria javanica* plant. The number of leaves counted are leaflets that are fully opened and is done by counting the total number of leaflets of the *Pueraria javanica*.



Figure 2. Leaf and leaflets on the *Pueraria javanica*

#### Leaf Area (cm<sup>2</sup>)

The leaf area measured is the area of a fully opened leaf, which is then measured using a ruler or tape measure. Leaf area measurements utilize the

ImageJ application. Calculations are performed after the plant has been harvested.

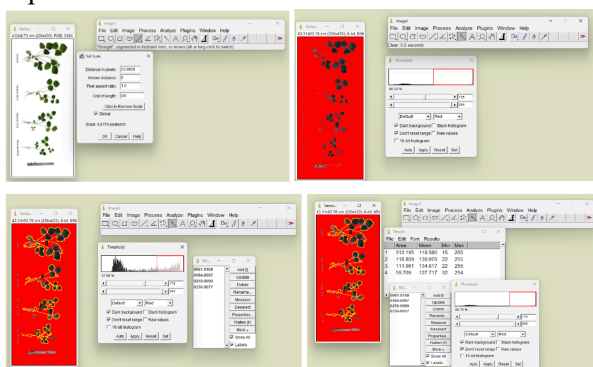


Figure 3. Leaf Area Analysis Using Image J Application

### Weight of Fresh Plant (g)

Fresh plant weight is measured after harvest. Before weighing the fresh plant weight, the lower part of the plant (roots) must be separated. Plants are weighed using a digital scale while the plants are still fresh.

### Root Length

Root length observations were conducted at the end of the study, using a measuring tool in cm.

### Number of Effective Root Nodules

The number of effective root nodules can be influenced by rhizobium inoculation of seeds or soil. The number of effective root nodules is monitored after harvest by counting the number of nodules.

### Fresh Root Weight (g)

This observation was carried out by weighing the roots, namely the root neck to the tip of the root which was still fresh in g units.

### Root Dry Weight (g)

Observations were made by weighing the plant roots after harvesting and drying them until the weight was constant in g units.

### Data analysis

The data obtained from the observations were then analyzed using analysis of variance (ANOVA) and post-hoc tests. Diagrams were created using the Microsoft Excel application, using the insert menu, namely PivotChart. Analysis of the significant

difference test used the Tukey's HSD (Honestly-Significant Difference) method. All data analysis used the SPSS application version 16.0 (SPSS, Chicago, United States).

## RESULTS AND DISCUSSION

### Plant Height (cm)

From the observation results, it can be seen that the paranet treatment showed a significant difference in the variable of plant height at 6 weeks after planting. The provision of IAA auxin showed no significant effect at 6 weeks after planting. There was no interaction between IAA and paranet treatments. From the histogram image, it can be seen that the control treatment had the best plant height, namely 21.9 cm, which was significantly different from the treatments of 50%, 75%, and 95%. This shows that plants that did not receive paranet treatment were able to grow more optimally than those under paranet, which was caused by better light and ventilation factors. In the paranet treatments of 50% and 75%, plant height did not show a significant difference. In the paranet treatment of 50% with an average plant height of 20.03 cm and paranet 75% with an average of 19.76 cm, which indicates that both levels of paranet may provide similar conditions for plant growth.

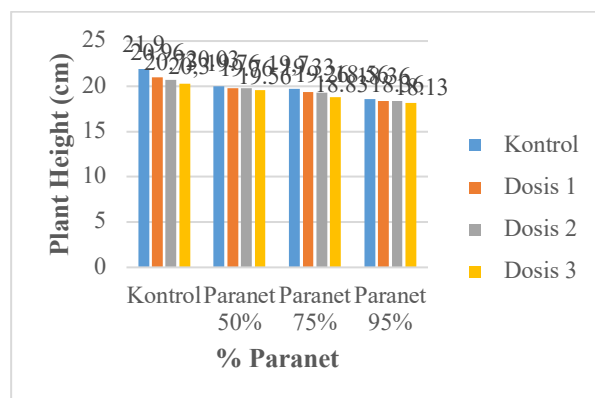


Figure 4. Diagram of Plant Height Observations 6 Weeks After Planting

Lack of sunlight can disrupt photosynthesis and plant growth, although light requirements vary depending on the plant species. Plants deprived of sunlight during their growth period tend to grow tall, slender, and pale. Besides affecting growth, light is also essential for photosynthesis. Plants deprived of

light cannot produce chlorophyll, resulting in pale leaves. However, too much light can damage chlorophyll (Zannah et al., 2023).



Figure 5. Height of *Pueraria javanica* Plants Bent. Without Paranet, 50% Paranet, 75% Paranet, 95% Paranet (dose 300 ppm).

Number of Leaves and Leaflets

The results of the data on the variety of leaf numbers in jack bean plants aged 4 weeks after planting which received drought stress treatment were not much different because at the age of 4 weeks the jack bean plants had only produced 2 leaves. Based on the observation results, the paranet treatment showed a significantly different effect on the number of leaves at 6 weeks of auxin administration; IAA showed a significant difference in the number of leaves. The interaction of paranet and auxin treatments showed no significant effect on the number of leaves. The control treatment showed the results of the number of leaves with an average of 7.33 strands, significantly different from the 95% paranet treatment of 6 strands, while the 50% and 75% paranet treatments did not show any significant difference from each other. This is because plants that grow in bright areas generally have higher levels of photosynthesis compared to plants in dark areas. After all, the presence of sufficient light supports the process of energy absorption to take place more efficiently. On the other hand, in a dark environment, the process of photosynthesis is hampered or does not even take place, because there is no energy that can be used to carry out photochemical reactions. A decrease in light received can cause physiological stress in plants, negatively impacting their ability to produce new leaves (Zega et al., 2024).

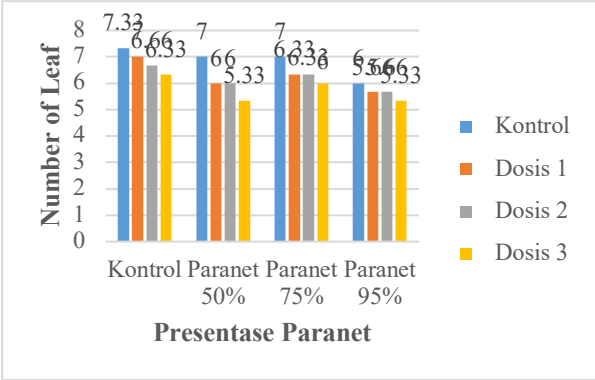


Figure 16. Diagram of Observation of Number of Leaves 6 Weeks After Planting

From the results of observations and Tukey's significant difference test, it can be seen that the treatment of various percentages of paranet significantly differed on the number of leaflets. Auxin treatment with different concentrations showed a significantly different effect on root length at 6 weeks after planting. The interaction between paranet treatment and auxin treatment showed no significant effect on the variable number of leaflets. The results of the study showed that the shade net treatment had a significantly different effect on the number of leaflets in *Pueraria javanica* plants. The interaction between the shade net and auxin treatments did not show any significant effect. Based on the histogram analysis, the 95% shade net treatment showed a significant difference with the lowest average number of leaflets of 14, compared to the control treatment with an average of 17.66 leaves, as well as the 50% shade net with an average of 18.33 leaves and the 75% shade net with an average number of leaflets of 18.33 leaves. The treatment of auxin administration with a concentration of 300 ppm was not significantly different from the auxin concentrations of 100 ppm and 200 ppm in the variable number of leaflets, but was significantly different from the control treatment. According to Gibson and Smirnov (2017), plants grown in dark places are more likely to experience decreased growth because they cannot produce sufficient energy. In the long term, these plants will experience symptoms such as yellowing leaves and stunted growth. The number of tillers is one indicator of plant growth and development during the vegetative phase. The number of tillers can be used to estimate the resulting plant weight.

Light intensity specifically influences the growth of tillers (Nopriani et al., 2014).

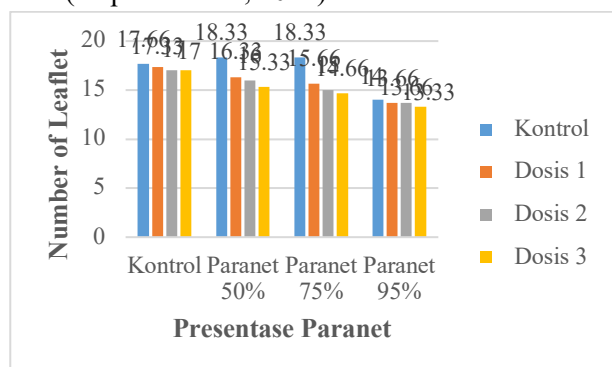


Figure 7. Leaflet Observation Diagram 6 Weeks After Planting

### Leaf Area (cm<sup>2</sup>)

Observations showed that the shade net treatment significantly affected the number of leaflets in *Pueraria javanica* plants. Furthermore, the auxin treatment also showed a significant difference. However, the interaction between the shade net and auxin treatments did not show any significant effect. Based on the results of the Tukey test, the control treatment showed a significant difference in having the highest leaf area of 55.17 cm<sup>2</sup> compared to the 50%, 75%, and 95% shade net treatments. On the other hand, the 50% shade net treatment had an average of 42.41 cm<sup>2</sup>, significantly different from the 75% shade net treatment, while the 75% and 95% shade net treatments did not show any significant difference from each other. The administration of auxin with a concentration of 300 ppm showed a significant difference compared to no treatment. However, the control treatment did not show any significant difference from the administration of auxin at concentrations of 100 ppm and 200 ppm. In addition, the administration of auxin at concentrations of 100 ppm, 200 ppm, and 300 ppm also did not show any significant difference between them. Although auxin can have a positive impact, lower concentrations do not significantly affect growth compared to no treatment. The number of leaves does not always affect leaf area, because shaded leaves tend to widen to obtain the necessary sunlight. The leaf area index is influenced by the amount of sunlight received by the plant; the wider the leaf area, the higher the leaf area index that can be achieved. This indicates that leaf morphological

development is crucial for plant adaptation to the environment, particularly in efforts to increase photosynthetic efficiency. Solar radiation intensity plays a crucial role in increasing leaf area; the higher the leaf area index, the more light the plant absorbs. This study underscores the importance of proper lighting management in plant cultivation, as optimal lighting can increase leaf growth and overall plant yield (Masyitho et al., 2022).

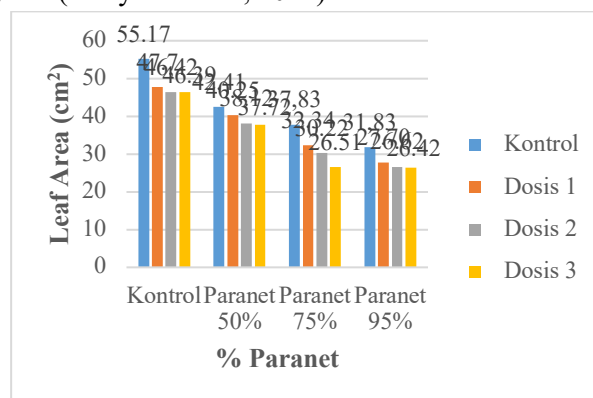


Figure 8. Leaf Area Observation Diagram 6 Weeks After Planting

### Fresh Weight (g)

From the results of observations and analysis of the variety of paranet treatments, it was found that the effect of shade netting on plant growth was significantly different, while the auxin treatment also showed a significant difference. However, the interaction analysis between the shade netting and auxin treatments showed that there was no significant interaction between the two. In the control treatment, the highest average was 7 grams in the fresh weight variable of the plant, there was a significant difference compared to the 50% shade netting 3.74 grams, the 75% shade netting 2.14 grams, and the 95% shade netting 0.95 grams, which showed that the light intensity from the shade netting significantly affected plant growth. In addition, the 50%, 75%, and 95% shade netting treatments also showed significant differences from each other, indicating that the higher the intensity of the shade netting, the greater the effect on plant development. According to (Primadani et al., 2018), which states that the photosynthesis process is higher when the light rays given are longer. A high rate of photosynthesis will produce a lot of photosynthesis which is then accumulated throughout the plant

organs so that the fresh weight of the plant will increase. The control treatment showed a significant difference with auxin at a concentration of 300 ppm, indicating that auxin can increase plant growth at that concentration. However, the control treatment did not show a significant difference with auxin at concentrations of 100 ppm and 200 ppm, or between 200 ppm and 300 ppm.

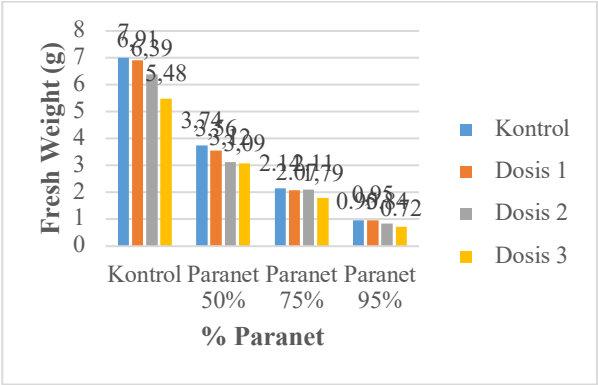


Figure 9. Fresh Weight Observation Diagram of Plants 6 Weeks After Planting

### Root Length (cm)

From the results of observations and Tukey's significant difference test, it can be seen that the treatment of various percentages of paranet significantly differed in root length. Auxin treatment with different concentrations showed a significantly different effect on root length at 6 weeks of age. The interaction of paranet treatment and auxin treatment showed no significant effect on root length at the observation age of 6 weeks. The control treatment produced the highest root length, with an average value reaching 13.57 cm. This shows that without paranet treatment, plants can grow well, utilizing resources optimally. In the 50% paranet treatment, the root length was recorded at 12.73 cm, which still shows good growth, although there was a reduction compared to the control. The 75% paranet treatment showed an average root length of 10.9 cm, which indicates a decrease in the quality of root growth along with increasing paranet intensity. The 95% paranet treatment showed the lowest results, with a root length reaching 9.16 cm. This decrease indicates that too high light intensity can have a negative impact on root development, which may be caused by stress due to excessive lighting. According to Kendek et al. (2024), root length increase is a root response to soil nutrient availability. Observing root

length aims to provide information on a plant's root capacity to absorb nutrients available in the soil. Root growth involves root elongation and widening, which are influenced by both media and environmental factors. Plant nutrients are the organs responsible for absorbing water and nutrients and are closely related to the ability to support root growth.

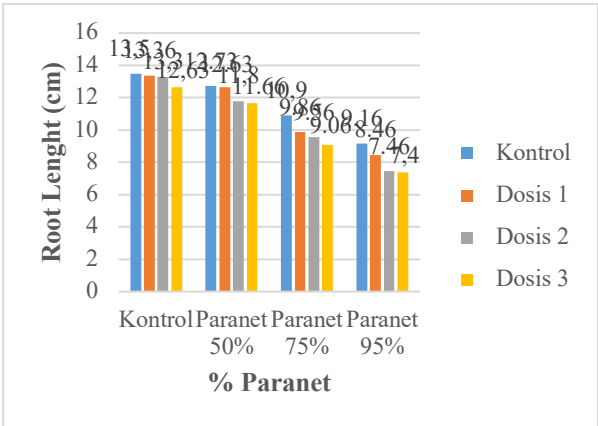


Figure 10. Root Length Observation Diagram 6 Weeks After Planting

### Number of Effective Root Nodules

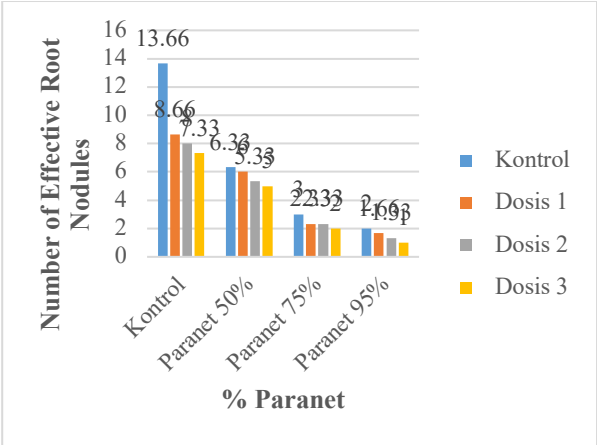


Figure 11. Diagram of Observation of the Number of Effective Root Nodules 6 Weeks After Planting

The dose variations (Dose 1, Dose 2, and Dose 3) applied at each shade level showed a relatively similar trend, with the number of root nodules tending to decrease with increasing shade net density. Although there was a slight fluctuation between doses at each shade level, the main effect clearly visible was the negative impact of increased shade on root nodule formation. According to Izlal et al. (2025), the higher the shade level, the lower the activity of root nodules in supplying nitrogen to plants, which results in reduced nutrient availability

in the soil. Overall, these results indicate that LCC grows optimally under conditions without shade. Higher shade levels cause a decrease in vegetative growth and root nodule activity, which impacts nitrogen fixation efficiency. Therefore, in plantation systems, the selection of LCC types and the regulation of shade levels need to be considered so that plants can function optimally in increasing soil fertility and supporting the growth of the main crop.

Root Fresh Weight (g)

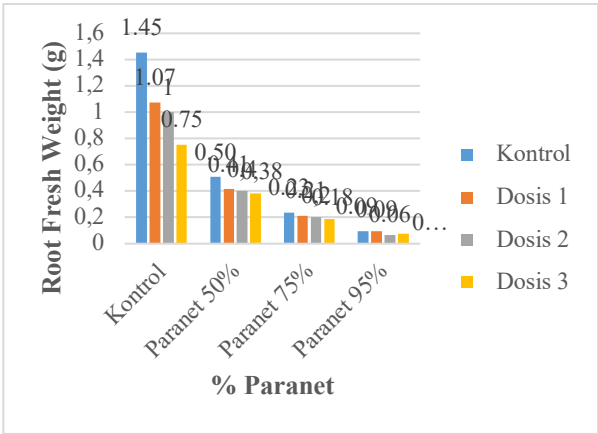


Figure 12. Diagram of Fresh Root Weight Observations 6 Weeks After Planting

In this study, root fresh weight measurements were conducted after 6 weeks of planting under different treatments, namely control (without shade netting), 50% shade netting, 75% shade netting, and 95% shade netting. The control showed the highest average root fresh weight value, reaching 1.45 grams. This indicates that without the restriction of shade netting, plants are able to grow optimally and absorb enough light and nutrients from the soil to produce healthy roots. 50% shade netting showed a decrease in root fresh weight to 0.50 grams. Although still showing a relatively good weight, this treatment indicates a negative impact of reducing the intensity of light received by the plants. 75% shade netting showed a further decrease in weight, namely 0.23 grams. At this level of shade, plants experience a reduction in their growth capacity, possibly due to limited light, which plays an important role in the process of photosynthesis. 95% shade netting showed the lowest result, namely 0.09 grams. The treatment of fresh root weight at the highest shade level reflects conditions where light received is very

minimal, resulting in stunted root growth and indicating that the plant is significantly affected by light deficiency. Available nutrients are absorbed and utilized by plants for the formation of shoots and roots in a relatively equal ratio, even though the supply of light intensity is different. As stated by Sitompul and Guritno (1995), the concept of morphological balance means that the growth of one part of the plant is followed by the growth of other parts. The increase in shoot weight linearly follows the increase in root weight.

Root Dry Weight (g)

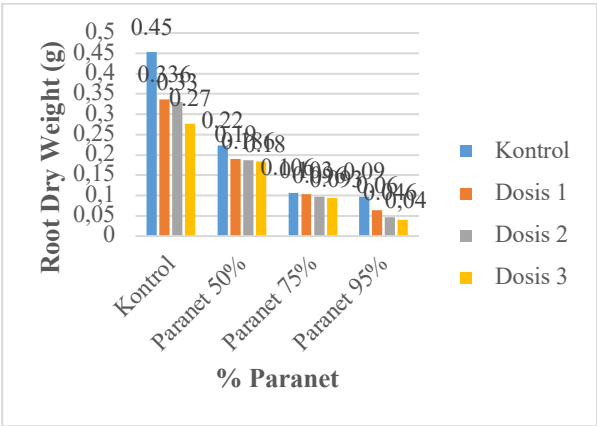


Figure 13. Diagram of Root Dry Weight Observations 6 Weeks After Planting

The control treatment (without shade netting) showed the highest root dry weight, averaging 0.45 grams. This result indicates that with full access to sunlight, plants are able to optimally photosynthesize, increasing energy accumulation and ultimately producing higher root biomass. The 50% shade netting treatment showed a significant decrease to approximately 0.22 grams. While still within the healthy growth range, this treatment indicates that the significant reduction in light is beginning to impact the plants' ability to develop roots optimally. The 75% shade netting treatment recorded a lower root dry weight of 0.1 grams. This decrease reflects the greater negative impact of light blockage. As a result, plants struggle to produce the carbohydrates necessary for healthy root growth, resulting in reduced biomass. The 95% shade netting treatment showed the lowest results, with a root dry weight of only 0.09 grams. This reflects that at very high levels of shade, plants experience significant stress, with photosynthesis barely occurring, leaving

very little energy available for root growth. This is in accordance with Dewi et al. (2021), who stated that the root system is a crucial component in supporting plant growth and development. It is a plant's ability to absorb water. Water availability in the soil affects root dry weight. Decreased soil water availability results in low root dry weight. Plants with high root dry weight have larger roots and a higher tolerance to drought.

## CONCLUSION

The treatment of various percentages of shade nets for *Pueraria javanica* showed a significant effect on all observation parameters, with the best growth in the control treatment, or without shade. The administration of IAA auxin to *Pueraria javanica* showed a significant difference in the number of leaves, number of leaflets, leaf area, fresh weight of plants, root length, number of root nodules, fresh weight of roots, and dry weight of roots. With the best growth in the control treatment. The two treatments above, between the percentage of shade nets and the administration of auxin, showed an interaction that had a significant effect on several observed parameters, including the number of root nodules, fresh weight of roots, and dry weight of roots.

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