Total chlorophyll and root nodules at various ages of soybean plants (*Glycine max* L.) in the wet-dry season

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Abstract Plants have a vulnerability to climate change. Soybean plants must be able to adapt to climate change stresses so that physiological processes and plant development run well and produce optimal soybean seed production. Planting soybean cultivars that can withstand climate change is recommended to maintain soybean production. The effect of soybean cultivars and plant age on the nodules' amount and leaf chlorophyll content planted during the wet-dry season needs to be studied. The study was designed with a factorial randomised block design and was repeated three times. The first factor is soybean cultivars, and the second factor is planting time. Parameters observed were weather data, chlorophyll content, nodules' amount, and nodules' mass. The results showed that the Gepak Hijau and Gepak Kuning cultivars planted at different times had significantly different root nodules' amount and dry mass of root nodules, while there was no significant difference in chlorophyll content. The nodules' amount positively correlated with the total chlorophyll. Relative humidity and rainfall reduce the nodules amount and the total chlorophyll. Solar radiation promotes growth in the nodules' amount and the total chlorophyll.

1. Introduction

Soybean is a type of legume plant belonging to the family Leguminosae. Soybean plants establish a mutually beneficial symbiosis with nitrogen-fixing bacteria. Bacteria get energy for growth and development from soybean plants, then bacteria provide N for soybean plant growth. Bacteria attack root hairs then enter the root tissue and form root nodules [1]. The symbiosis between soybean plants with N-fixing bacteria can be seen from the formation of root nodules. Root nodules as a morphological sign that soybean plants have a source of N from bacteria for their growth.

In leaves, chlorophyll biosynthesis increases along with the availability of nitrogen to be absorbed by plants and encourages better photosynthetic performance [2]. The availability of N in the soil will increase the chlorophyll content, but the chlorophyll content decreases when the availability of N is too high [3]. The average number of N fixed by biological nitrogen fixation (BNF) in legumes are in the range of 32 to 115 kg ha^{-1}. The amount of chlorophyll in soybean leaves was positively correlated with the root nodules' amount while the leaves' amount increased plant height [4].

Gepak Kuning and Gepak Hijau are Superior Varieties of Early Soybeans with maturity < 80 days after planting and productivity > 2.5 tons per ha. Many farmers in the Ponorogo region, East Java,

Indonesia, cultivate soybeans of the Gepak Kuning and Gepak Hijau varieties. The Gepak Kuning variety produces yellow soybean seeds with a larger size than the Gepak Hijau. The Gepak Hijau variety produces green soybean seeds and is smaller in size than the Gepak Kuning. Soybean cultivars affect the effectiveness of symbiotic N-fixing bacteria in fixing N and forming root nodules [5]. The amount and volume of root nodules affect the N content in soybean seed production. The formation and development of root nodules can be influenced by environmental conditions, such as land submerged in water or drought, soil type, tillage, N and phosphorus fertilisation, and weather conditions where soybean plants grow.

Research is needed to evaluate the effect of soybean cultivars and plant age on the leaf chlorophyll content and the nodules' amount planted during the wet-dry season. It is hoped that these findings can provide useful information in determining the timing of soybean plants for increasing the leaf chlorophyll content and the root nodules' amount.

2. Materials and Methods

The experimentation was carried out in the Research Field, Agrotechnology Department, Universitas Darussalam Gontor, Ponorogo, Indonesia from June 2022 to August 2022. The experimentation is arranged in a factorial complete randomised block design with three replicates as blocks. The first factor is soybean cultivars, including Gepak Kuning and Gepak Hijau. The second factor is planting time, including 70 days after planting (dap), 63 dap, 56 dap, 49 dap, 42 dap, 35 dap, and 21 dap. So that soybean plants were planted on June 5, June 12, June 19, June 26, July 3, July 10, and July 24. Soybean seeds are planted in rice fields after the rice harvest.

Observation parameters were weather data, chlorophyll content, nodules' amount, and dry mass of root nodules. The weather observations were made during the growth of soybean plants from planting until one day before the plant samples were taken, by observing the elements of daily mean daily temperature, mean daily humidity, total rainfall, a number of rainy days, and solar radiation. Weather data was obtained from Automatic Weather Station (AWS) UNIDA Gontor from June 2022 to August 2022. The leaves used to determine the chlorophyll content was the 3rd leaf from the top using the Winterman de Mots method with modification. Count the number of root nodules per plant. The way to observe the dry mass of the nodules is carried out by collecting the nodules then aerating them for 2 days at room temperature 27 ^{0}C and weighing them.

The data obtained from the observations of each parameter were analysed by ANOVA then a further test was carried out with LSD (Least Significance Different) test at a 95% confidence level. Weather data along with the root nodules' amount, dry mass of root nodules, and total chlorophyll were tested for correlation.

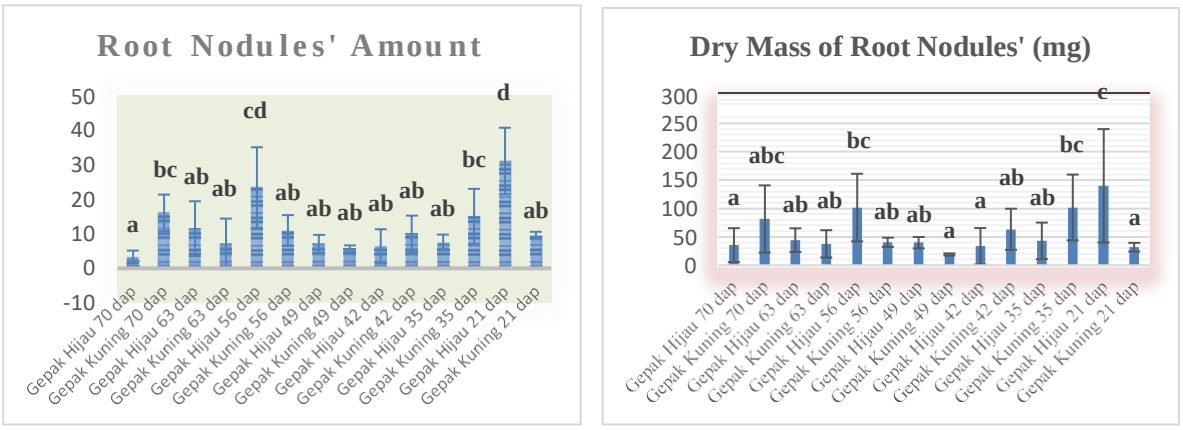
3. Results

The soybean planting season has changed because of the climate anomaly with the wet-dry season or should be the dry season, but there is still rainfall. In June there is still a high rainfall of 152.8 mm, in July and August there is still rain, although in small amounts (Table 1.). The presence of rain makes soybean planting experience changes in planting time. The average humidity is low even though there is rain because the intensity of solar radiation is quite high.

This study evaluated the effect of cultivars on different crops on the root nodules amount, dry mass of root nodules, and chlorophyll content of soybean leaves. According to Fig. 1, there was a significant difference in the root nodules' amount and dry mass of root nodules in the combination of cultivars and plant age. The highest amount of root nodules was in the Gepak Hijau cultivar at the age of 21 dap. The Gepak Hijau cultivar has the smallest amount of root nodules at 70 daps. The Gepak Hijau cultivar has the potential to have a greater number of root nodules than the Gepak Kuning.

Table 1. Weather data for June to August 2022 in the Ponorogo region, East Java, Indonesia

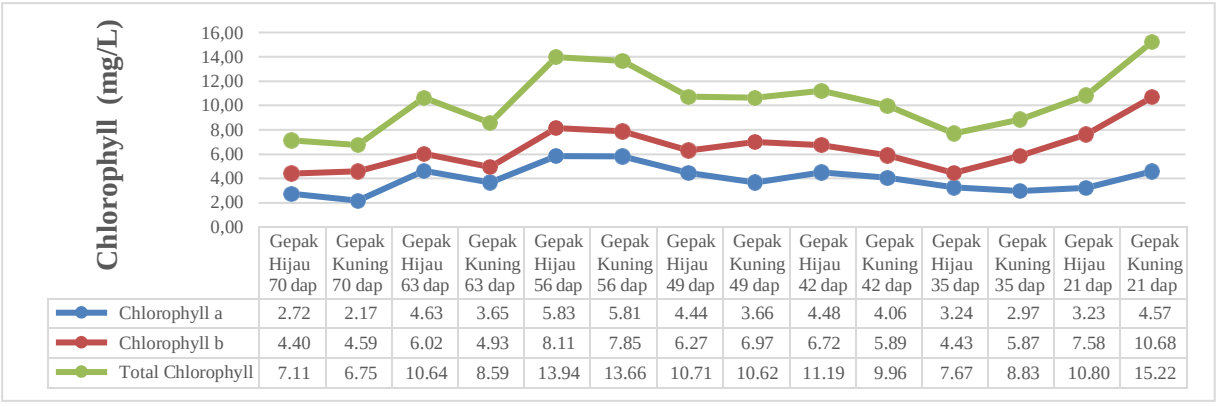
Month	Average temperature (°C)	Average air humidity (%)	Total rainfall (mm)	rainy days' amount	Solar radiation (Wm^{-2})
June	26.63	82.33	152.80	13.00	395.56
July	26.60	74.53	5.80	3.00	479.38
August	26.82	74.29	23.40	9.00	495.90



(a) (b)

Figure 1. Root nodules' amount (a) and Dry mass of root nodules' (b)

The chlorophyll content was not significantly different at all ages of observation and cultivars. The content of chlorophyll b is more than the content of chlorophyll a (fig. 2). Gepak Hijau and Gepak Kuning still have chlorophyll until the age of 70 days after planting (R6), so they are still photosynthesizing so that there is still enough energy for pod filling.



	Gepak Hijau 70 dap	Gepak Kuning 70 dap	Gepak Hijau 63 dap	Gepak Kuning 63 dap	Gepak Hijau 56 dap	Gepak Kuning 56 dap	Gepak Hijau 49 dap	Gepak Kuning 49 dap	Gepak Hijau 42 dap	Gepak Kuning 42 dap	Gepak Hijau 35 dap	Gepak Kuning 35 dap	Gepak Hijau 21 dap	Gepak Kuning 21 dap
Chlorophyll a	2.72	2.17	4.63	3.65	5.83	5.81	4.44	3.66	4.48	4.06	3.24	2.97	3.23	4.57
Chlorophyll b	4.40	4.59	6.02	4.93	8.11	7.85	6.27	6.97	6.72	5.89	4.43	5.87	7.58	10.68
Total Chlorophyll	7.11	6.75	10.64	8.59	13.94	13.66	10.71	10.62	11.19	9.96	7.67	8.83	10.80	15.22

Figure 2. Leaf' chlorophyll content at various ages of soybean plants

The results of the correlation analysis between the root nodules' amount and the dry mass of root nodules have very strong support. The total chlorophyll with root nodules' amount is weakly correlated. The root nodules' amount is negatively correlated with temperature, humidity, and rainfall, but positively correlated with solar radiation. Chlorophyll is positively correlated with average air

temperature and solar radiation but negatively correlated with humidity, rainfall, and rainy days' amount (Table 2).

Table 2. Correlation between root nodules' amount, dry mass of nodules, and total chlorophyll to the weather elements

	Nodules' Amount	Dry mass of nodules (mg)	Total Chlorophyll (mg/L)	Average temperature (ºC)	Average air humidity (%)	Total rainfall (mm)	Rainy days' amount	Solar radiation (Wm^{-2})
Nodules' Amount	1.00							
Dry mass of nodules (mg)	0.93	1.00						
Total Chlorophyll (mg/L)	0.22	-0.03	1.00					
Average temperature (ºC)	-0.22	-0.29	0.13	1.00				
Average air humidity (%)	-0.23	-0.13	-0.58	0.10	1.00			
Total rainfall (mm)	-0.17	-0.08	-0.61	0.00	0.86	1.00		
Rainy days amount	-0.21	-0.18	-0.46	0.27	0.94	0.87	1.00	
Solar radiation (Wm^{-2})	0.31	0.27	0.41	-0.58	-0.85	-0.74	-0.93	1.00

4. Discussion

Observing how the development of the root nodules' amount, dry mass of root nodules, and chlorophyll in soybeans planted during the wet-dry season provides an important illustration for improving soybean cultivation technology when climate anomalies occur. The Gepak Hijau cultivar was significantly different and had more nodules than the Gepak Kuning cultivar (fig. 1). Different cultivars have different responses in nodule formation and nodule size [5]. The availability of N in the soil from N fertilisation can reduce the number of root nodules and their size. Several cultivars were responsive to N fertilisation so that the number of root nodules decreased by 23.27% and some decreased by 4.72%.

The dry mass of root nodules was strongly correlated with the root nodules' amount (table 2.). The increase in the root nodules' amount makes the dry mass of root nodules bigger. Between the ages of soybean plants there is a difference in the root nodules' amount but did not form a pattern of increasing the root nodules' amount with increasing plant age (fig. 1). This is different from the results of studies [6] that along with the growth of soybean plants, there is an increase in the root nodules' amount. The root nodules' amount has a weak negative correlation with rainfall, temperature, and humidity, and a weak positive correlation with solar radiation (table 2). Temperature and rainfall affect the number of nodules [7]. The increase in solar radiation slowly makes the number of nodules increase. If the amount of rainfall is greater, then the number of root nodules decreases. Rain-containing lightning will raise the nitrate content in the soil. Nitrates affect the formation of nodules [1] [5]. Rain makes the soil high in nitrate and the soil is saturated with water or excessively wet. So that the amount of rainfall inhibits the activity of bacteria in forming nodules.

Soybean plants planted anywhere will form root nodules. Soybean plants planted on land that has never been planted with soybeans will produce a small number of root nodules compared to soybeans planted on land that is often planted with legumes. The root nodules' amount can be increased by inoculation of N-fixing bacteria [8]. The soil for research every year in the dry season is always planted with soybeans and the results of the study show that the number of nodules is relatively small (fig. 1), so to increase the root nodules' amount, it is necessary to inoculate N-fixing bacteria when planting soybeans. The inoculation of soil N-fixing bacteria was 10^4 bacteria per g of soil. If the soil already has N 10^3 bacteria fixing bacteria per g of soil, then the number of bacteria inoculated must be more than 10^4 bacteria per g of soil [9]. Overcrowding of soybean plant populations reduces the root nodule' amount [12]. Phosphorus fertilisation and compost increased the number of nodules. The location of

the nodules on the roots affects the productivity of N-fixing bacteria and the supply of N to plants. Nodules located on taproots are more productive than secondary roots [10].

The content of chlorophyll was not significantly different at all ages of observation and cultivars (fig. 2). Chlorophyll content was positively correlated with the root nodules' amount (table 2). This supports the results of the study [4]. Leaf chlorophyll content correlates with N content because nitrogen increases chlorophyll biosynthesis in leaves. Nitrogen as a limiting factor for the formation of chlorophyll. If there is a deficiency of N, the chlorophyll content decreases and the plant will turn yellow, especially on the leaf blade and photosynthetic activity is hampered.

When plants are in symbiosis with N-fixing bacteria, they will form root nodules [1]. Not all of the root nodules formed were effective in fixing N. Root nodules that were small in size and spread over the roots of branches were relatively ineffective in fixing N. More nodules will increase N content and leaf chlorophyll. Root nodule' amount and N content have a strong correlation [8]. But too high N content reduces chlorophyll content [3]. There are things to note about the dry weight of the nodules which has a very weak negative correlation with chlorophyll content (table 2). According to [11] N-fixing bacteria in nodules consume most of the energy produced by the photosynthesis of soybean plants, and the greater the root nodules' amount inhibiting the growth of soybean plants. Hyper nodulation soybean plants growth stunted due to inhibition of shoot growth.

Solar radiation is positively correlated with chlorophyll content (table 1). High light intensity without obstructions in the atmosphere increases the total chlorophyll of plant leaves [12]. The presence of obstruction of solar radiation in the form of rain and humidity can reduce the chlorophyll content. The content of chlorophyll in soybean leaves has a negative correlation with air humidity and rain (table 2). Soybean plants require direct sunlight, shade can reduce the content of chlorophyll a and b, and reducethe rate of photosynthesis of soybean plants [13].

Chlorophyll content has a very weak positive correlation with temperature (table 1). Warm temperatures encourage N fixation to increase and are inhibited when temperatures are too (<15 ^{0}C) andtoo hot (>35 ^{0}C) [1] [14]. So that the temperature supports an increase in the chlorophyll content but when the temperature is extreme, the chlorophyll content can decrease. In addition to weather elements that affect the chlorophyll content, there are elements of cultivation technology that can increase leaf chlorophyll content. Tillage increases the chlorophyll content [15]. The ploughed soil becomes loose and has enough air voids so that it contains a lot of oxygen and nutrients to stimulate the formation of chlorophyll. Without tillage, it can overcome the loss and lack of groundwater in the dry season so that the formation of plant chlorophyll is not disturbed during the dry season. The chlorophyll content decreases when there is a shortage and excess of groundwater. Chlorophyll content decreases when there is a shortage of groundwater. The most sensitive chlorophyll to lack of water is chlorophyll a than chlorophyll b [16].

5. Conclusion

In conclusion, the Gepak Hijau and Gepak Kuning cultivars planted at different times had significantly different root nodules' amounts and dry mass of root nodules, while there was no significant difference in chlorophyll content. The nodules' amounts are positively correlated with the total chlorophyll. Relative humidity and rainfall reduce the nodules' amount and the total chlorophyll. Solar radiation promotes growth in the nodules' amount and the total chlorophyll.

Acknowledgments

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