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# Photosynthetic pigment content and growth of chilli under low light intensity for agroforestry crop development

*Kandungan pigmen fotosintetik dan pertumbuhan cabai pada intensitas cahaya rendah untuk pengembangan cabai agroforestry*

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### ABSTRACT

#### Article history

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#### Keyword

Chilli; low light intensity; photosynthetic pigment

**Introduction:** Plants that grow and develop in a shaded environment are difficult to produce optimally. Therefore, the use of plant species that are able to produce optimally in a shaded environment is very important to be used as an agroforestry area. The research purposes was to observe morpho-physiological characters that can be used as characters to determine chilli plants that can produced in low light intensity area. **Methods:** A field experiment was conducted at farmer field in Pekuncen, Banyumas, Indonesia from May to October 2020. The research was arranged in randomized complete block design (RCBD) with three replications. The first plot was shading intensity (0% (control) and 50%) and the second plot consisted of nine chilli varieties, V1 (Segana), V2 (Lada Hijau), V3 (Bara), V4 (Catas), V5 (Kerinci), V6 (Raya), V7 (Genie), V8 (Sonar), and V9 (Rajo). **Results:** The results of this research showed that shade affected on leaves number and leaves area, but not affected on plant height and stem diameter. Shading net was affected on chlorophyll a and b, but not affected on chlorophyll content. Decreasing of total chlorophyll on 50% shade net occurring in shade sensitive varieties was significantly different than shade-tolerant varieties. Tolerant varieties based on the observation criteria were Bara (V3), Genie (V7), and Sonar (V8). **Conclusion:** Leaf area and leaf pigment character can be used as a reference for determining the resistance of varieties to low light.

### ABSTRAK

#### Riwayat artikel

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#### Kata kunci

Cabai; intensitas cahaya rendah; pigmen fotosintetik

**Pendahuluan:** Tanaman yang tumbuh dan berkembang pada lingkungan ternaungi sulit untuk berproduksi secara optimal. Oleh karena itu pemanfaatan jenis tanaman yang mampu berproduksi secara optimal pada lingkungan ternaungi menjadi sangat penting untuk dapat dimanfaatkan sebagai pengembangan kawasan agroforestri. Penelitian ini bertujuan mengevaluasi karakter morfo-fisiologi cabai sebagai karakter untuk menentukan tanaman cabai yang toleran naungan. **Metode:** Penelitian ini dilaksanakan di lahan petani desa Karang Kemiri Kecamatan Pekuncen Kabupaten Banyumas, Indonesia dari Mei hingga Oktober 2020. Rancangan percobaan dari penelitian ini adalah 1 Rancangan Acak Kelompok Lengkap (RAKL) dengan tiga kali ulangan. Perlakuan pertama adalah intensitas cahaya terdiri dari 0% (kontrol) dan 50% naungan, perlakuan ke dua adalah varietas cabai terdiri dari sembilan varietas, yaitu V1 (segana), V2 (lada hijau), V3 (bara), V4 (catas), V5 (kerinci), V6 (raya), V7 (Genie), V8 (Sonar), dan V9 (Rajo). **Hasil:** Naungan berpengaruh pada klorofil a dan klorofil b, namun tidak mempengaruhi kandungan klorofil total. Menurunnya kandungan klorofil total pada varietas peka di bawah naungan 50% sangat signifikan dibanding pada varietas yang toleran. Varietas toleran berdasarkan penelitian adalah Bara (V3), Genie (V7), dan Sonar (V8). **Kesimpulan:** Luas daun dan karakter pigmen daun dapat dijadikan acuan untuk menentukan ketahanan varietas terhadap cahaya rendah.

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### INTRODUCTION

Chilli is one of the commercial vegetable commodities in Indonesia. Based on data from the Ministry of Agriculture (2019), the total consumption of chilli at the household level in Indonesia during 2002-2018 fluctuated but increased by 3.29%. From 2019 to 2021, consumption of red chillies is predicted to increase to 1,905 kg/capita/year or an increase of 6.99% compared to the previous year. In 2018 there was a decrease in the harvested area for chilli plants

by 3.47% (BPS 2019). This decrease in the harvested area can lead to low chilli production in Indonesia. Therefore, it is necessary to increase chilli production to sufficient the chilli needs of the people.

The efforts to increase chilli production can be implemented through intensification and extensification. One of the efforts that can be done is the development of chilli as agroforestry vegetables through intercropping planting systems with other food or vegetable crops or with tree crops. This system can increase land productivity, reduce biotic stress and reduce the risk of crop failure. But, lack of light in chilli planted under the tree or as intercrops greatly influence plant growth, morphogenesis (Ritonga et al., 2018), production and photosynthetic devices (Fan et al., 2013). Low light intensity generally occurs in multiple cropping systems such as intercropping or agroforestry. The higher canopy of the main crop blocks reduces the level of light radiation received by the intercropping plants underneath (Susanto & Sundari, 2016). Plants that grow and develop in a shaded environment are challenging to produce optimally. Therefore, using plant species that can produce optimally in a shady environment is crucial to be used as an agroforestry area (Valladares et al., 2016).

The diversity of response to plant growth and production in low light-intensity is influenced, among others, by the physiological characteristics of plants, especially those related to photosynthetic activity (Soverda & Alia, 2013). Plants overcome the stress of low light intensity, among others, by increasing light capture efficiency. When conditions lack light, the plant will increase the light capture area, namely by increasing leaf number, leaf area, plant height, light-capturing pigment content, especially chlorophyll a and b, and decreasing the chlorophyll a / b ratio, leaf thickness, and the number of trichomes to capture light. become more efficient (Beneragama & Goto, 2011; Zhu et al., 2017).

In tomato plants, 25% shading increased relative yields across all tested genotypes, while 75% decreased relative yields by 10-97%. And the provision of 50% shading resulted in a high diversity between genotypes, namely 32%. (Baharuddin et al., 2014). Based on these considerations, in this study, 50% shade was used as the basis for determining the tolerance of chilli plants. Research by Soverda (2011) showed a higher increase in chlorophyll a and b in the tolerant soybean genotype. The higher chlorophyll content in tolerant plants will allow more light energy to be converted into chemical energy in the form of excited electrons. Increased levels of chlorophyll a and b are evidence of the ability of tomato plants to grow in the shade. Response of chlorophyll to shade is essential because chlorophyll a and chlorophyll b are components of the chloroplast peripheral antenna complex, whose response is determined by the light received. Light energy will be converted into chemical energy in the reaction centre, which can be used for photosynthesis resulting in high production.

It is hoped that the use of types or varieties that are resistant to low light intensity can be a more efficient way to prevent a decrease in chilli production in a shaded environment. Testing of several varieties at low light intensity and observing changes in growth and physiological characters are needed to obtain chilli varieties that are resistant to low light intensity.

## METHODS

A field experiment was conducted at a farmer's field in Pekuncen, Banyumas, Indonesia, from May to October 2020. Analysis of chlorophyll anthocyanin content was carried out in the Plant Breeding and Biotechnology Laboratory and UNSOED Research Laboratory. The experiment was arranged in Randomized Complete Block Design (RCBD) with two factors. i.e. variety and light intensity. The genotype consisted of 9 chilli genotypes: Segana, Lada Hijau, Bara, Kerinci, Raya, Genie, Sonar and Rajo, and shade consisted of no shade (0%) and 50% shade. Each treatment was repeated three times to obtain 54 experiments. Each experimental unit consists of 10 plants, so there are 540 plants.

The black paranet with a 50% reduction in light intensity was installed as high as 2 meters above the ground. Before planting, the seeds are sown in a seedbed and nurtured for about one month before planting in the field. Seedlings about 20 cm high are planted at a distance of 50 x 50 cm.

The parameters studied were leaf area, plant height, number of leaves, chlorophyll a, chlorophyll b, total chlorophyll, ratio of chlorophyll a/b, anthocyanin, production per plot and Stress Suspectibility Index. Leaf area, plant height, and number of leaves were measured 20 week after planting. Plant height was measured from the soil surface to the point of growth. Chilli was harvested based on the fruit characteristics that was really old which was indicated by the formation of dense seeds, when pressed the fruit was hard, and the fruit was dark green or reddish green.

Chlorophyll contents were measured using the spectrophotometric method following the procedure of (Kurniawan et al., 2010). Chilli leaves were taken from three parts as replicates. Each individual from various variations was taken 2 leaves and crushed with a mortar and then weighed with a weight of 0,15 gram. The leaf samples were then extracted with 15 ml of 80% acetone, stirring until the color was removed from the tissue. The extract was then filtered using filter paper. The obtained filtrate is placed in a cuvet and then analyzed using a spectrophotometer at a wavelength of 480 nm, 645 nm and 663 nm. Total chlorophyll content and carotenoids were calculated using the formula (Lee et al., 1993):

$$\begin{aligned} \text{Total Chlorophyll (mg/l)} &= (8,02 \times A663) + (20,2 \times A645) \\ \text{Chlorophyll a (mg/l)} &= (12,7 \times A663) - (2,69 \times A645) \\ \text{Chlorophyll b (mg/l)} &= (22,9 \times A645) - (4,68 \times A663) \end{aligned}$$

Leaf samples to be analyzed were previously sorted. Then drained and dried in an oven at 50°C for 24 hours. The dry sample was crushed using a mortar and weighed 2 grams. Maceration of the sample by soaking 2 grams of leaf powder with 200 ml of 70% ethanol solvent at a temperature of 25 °C for 24 hours, then filtered and the filtrate was taken. 10 ml of each macerated extract was dissolved in 10 ml of ethanol as solvent. Then the absorbance was measured at 510 and 700 nm wavelengths. To determine the total amount, you can use the following equation:

$$\text{Total anthocyanins (mg/L)} = \text{sumber: Maulid \& Laily (2015)}$$

The grouping of chili varieties based on their tolerance to low light intensity stress is carried out based on the value of the Stress Susceptibility Index (SSI) (Fischer and Maurer, 1978) with the formula:

$$S = \frac{1 - \left(\frac{Y}{Y_p}\right)}{1 - \left(\frac{X}{X_p}\right)}$$

- Y = observed value for one genotype in stressed condition  
 Yp = observed value for one genotype under control conditions  
 X = the average value of observations for all genotypes under stress conditions  
 Xp = mean value of observations for all genotypes under control conditions.

Chili genotypes were grouped into tolerant (T) if SSI <0.5, moderate (M) if 0.5<SSI<1, and sensitive (S) to salinity stress if SSI>1. The data obtained were analyzed by the F test. If there was a significant difference between the tested factors, then proceed with the DMRT test.

## RESULT AND DISCUSSION

### Micro climate condition

Observations of the microclimate conditions in the experimental land consisting of the average daily light intensity, temperature and air humidity were presented in Table 1. In 50% shade conditions, the average daily light intensity data is 17.63 \* 1000 lx, lower and significantly different compared to the shadeless condition, which is 40.25 \* 1000 lx. Daily humidity in 50% shade was 83.73%, while the daily humidity in the shade without shade was 76.86%. The average daily temperature in the 50% shade was 27.17 ° C, while the average daily temperature in control was 28.17 ° C.

Table 1. Light intensity, humidity and average daily temperature of chilli genotype grown under control (0%) and 50% shading

Variable	Level of shade	
	0%	50%
Light intensity	40.25*1000 lux	17.63*1000 lux
Humidity	76.86%	83,73%
Temperature	28.17 °C	27.17 °C

The vegetative growth variables observed were plant height, leaf area, and number of leaves. Plant height is affected by shade and variety. Shade reduces leaf area in chilli plants, possibly because the chilli varieties observed are mostly included in the 50% shade sensitive group, so chilli plants are unable to form an adaptation mechanism by expanding leaves, this is following the research of Chairudin et al. (2015), stated that shaded soybean plants decreased in total leaf area. Plant height and number of leaves were affected by the variety. The stem height of the tested varieties decreased, but not significantly. Raya variety has the highest plant height compared to other varieties, 31.85 cm. The Catas and Sonar varieties had the lowest plant height ranging from 28.27 cm - 29.88 cm.

The number of leaves in shade conditions decreased, but not significantly. The number of leaves is affected by the variety. The highest number of leaves was the Sonar variety, 52.17, and the least number was the Rajo variety, 19.17 cm. The growing diversity of the chilli plants tested was due to the nature of the plant (genetic), and differences in genetic composition were the factors causing the diversity of plant growth. Sitompul and Guritno (1995) stated that the genetic composition is expressed in one or all of the different growth phases and can be expressed in various plant traits, Resulting in a diversity of plant growth and yields.

Leaf area was significantly influenced by shading and variety. In the shade condition, it is known that the average height of the chillies is 26.86 cm. This value is lower but not different from the conditions without shade, which have an average plant height of 28.23 cm. While the leaf area of chilli plants in shade conditions reached 27.32 cm<sup>2</sup>, bigger and significantly different when compared to the treatment without shade. Fiorucci & Fankhauser (2017) and Yusof et al., (2021) states that changes in plant morphology due to shade, especially in leaf organs, are a form of plant adaptation through avoidance mechanisms so that the photosynthesis process is more efficient (Baharuddin et al.,

2014). Increasing leaf area allows plants to be able to use all of the limited amounts of light that hit them (Sulistiyowati et al., 2019)

Table 2. Leaf area, plant height, number of leaves of chilli varieties under control (0%) and 50% shading

Treatment	Leaf area	Plant height	Number of leaves
<b>Shade</b>			
0%	27.31 a	28.23	32.89
50%	18.19 b	26.86	31.76
<b>Varieties</b>			
Segana	32.53 a	29.23 bc	25.83 f
Lada Hijau	23.73 b	26.60 d	23.83 f
Bara	19.20 b	30.3 b	46.00 b
Catas	14.93 e	23.88 e	32.50 d
Kerinci	22.93 b	28.27 c	24.50 f
Raya	23.73 b	31.85 a	30.00 e
Genie	16.80 d	28.03 c	42.00 c
Sonar	17.33 d	29.88 e	52.17 a
Rajo	33.60 a	19.95 f	19.17 g

Note: Data are means; different letters represent statistically significant differences ( $P < 0.05$ )

### Physiological response

The content of photosynthetic pigments in leaves, especially chlorophyll a and b, can be used as an indicator of plant tolerance to low light intensity conditions (Croft & Chen, 2017; Kunderlikova et al., 2016). The results of variance analysis was clearly shown that the level of shade had a significant effect on chlorophyll a and b content (Table 3). In the shaded condition, the chlorophyll a (10.59 mg g<sup>-1</sup>) and chlorophyll b (5.58 mg g<sup>-1</sup>) content was higher and significantly different than the non-shaded conditions (Table 3). Shade caused an increase in chlorophyll a by 23.86% while chlorophyll b increased by 36.10%. This is in line with several previous studies which reported that low light intensity increased chlorophyll a and chlorophyll b content (Latifa et al., 2019). The increase of chlorophyll a and chlorophyll b content on chilli plants in shade treatment is evidence that chilli plants carry out a tolerance mechanism for low light intensity (Sulistiyowati et al., 2016). Under conditions of low light intensity, plants will increase the chlorophyll content and reduce the ratio of chlorophyll a to b, by improving the amount of chlorophyll b (Taiz and Zeiger 2002). This is caused by changes in the size and shape of the antenna. Plants at low light intensity have PS II, containing more Light-Harvesting Complex (LHC) II antenna. So, the chlorophyll a/b ratio is low, and the LHC II/PS II ratio is high (Khumaida 2002). An increase in plant chlorophyll at the low light intensity is closely related to the increase in chlorophyll a/b protein in LHC II. This was also shown by the tolerant genotype of upland rice, which had higher chlorophyll a and b and a lower a/b ratio than sensitive (Soverda 2002). The same research result was also found in soybeans, and tolerant taro also found the same research result (Sopandie et al. 2003).

Table 3. Chlorophyll a, chlorophyll b, total chlorophyll, and anthocyanin of chilli varieties under control (0%) and 50% shading

Treatment	Chlorophyll a	Chlorophyll b	Total chlorophyll	Ratio of chlorophyll a/b	Anthocyanin
<b>Shade</b>					
0%	8.55 b	4.10 b	12.65	2.08 a	0.043
50%	10.59 a	5.58 a	16.17	1.89 b	0.037
<b>Varieties</b>					
Segana	8.71 d	3.82 d	12.59 e	2.28	0.043
Lada Hijau	9.48 c	4.90 c	14.45 c	1.93	0.042
Bara	9.85 c	4.61 c	14.54 c	2.13	0.039
Catas	10.69 b	5.27 b	16.04 b	2.03	0.033
Lada Hijau	9.53 c	4.84 c	14.44 c	1.97	0.045
Raya	8.86 d	4.57 c	13.49 d	1.94	0.034
Genie	10.89 b	5.35 b	16.33 b	2.03	0.040
Sonar	11.72 a	6.55 a	18.36 a	1.79	0.046
Rajo	6.41 e	3.63 d	10.08 f	1.77	0.034

Note: Data are means; different letters represent statistically significant differences ( $P < 0.05$ )

Chlorophyll b is part of the photosynthetic antenna complex that tasked for capturing and collecting and transferring light to the reaction center. The reaction center is composed of chlorophyll a. It is in this reaction center that light energy changes into chemical energy which is then used for the reduction process in photosynthesis (Read et al., 2008). The percentage increase in chlorophyll b content that is higher than chlorophyll a at low light intensity

conditions is related to an increase in chlorophyll protein so that it will increase the efficiency of the photosynthetic antenna function in Light Harvesting Complex II (LHCII) (Djukri & Purwoko, 2003). Increasing the number of antennas in photosystem II (PSII) also will increase the efficiency of light harvesting (Belgio et al., 2012).

Catas is variety that has decreased the chlorophyll a and b content and the chlorophyll total, while the Rajo variety has the lowest chlorophyll content. The chlorophyll content is thought to be one of the causes of the inability to adapt in low light intensity conditions. Meanwhile, the varieties of Sonar, genie, and Bara which contain higher photosynthetic pigments are better able to adapt by maintaining the rate of photosynthesis. The use of plant species that are able to grow, develop, and produce well in low light intensity environments is very important to be able to take advantage of the land between the stands. A photosystem (PS) consists of an antenna which is a collection of pigments and accessory pigments that capture and transfer photon energy to the reaction center, and a reaction center (composed of chlorophyll a) which transfers electrons out of the reaction center (Belgio et al., 2012). That is, chlorophyll a is not the only photosynthetically important pigment in chloroplasts but there are other pigments in the thylakoid membrane that can absorb light and transfer its energy to chlorophyll a (Sulistiyowati et al., 2016; Guidi et al., 2017).

The results showed a change in the ratio of chlorophyll a/b in the shade treatment. This indicates that the increase in chlorophyll b is higher than the increase in chlorophyll a in the chilli genotypes tested. The value of the ratio of chlorophyll a/b in the shaded chilli genotype was 2.08; after being given shade treatment, it decreased to 1.89. This is in line with Anggraeni (2010) research that under shaded conditions, the tolerant genotype has a higher chlorophyll b content and a lower chlorophyll a/b ratio than the sensitive genotype. According to Khumaida (2002), the decreased chlorophyll a/b ratio in shaded plants was caused by an increase in chlorophyll b, which was associated with an increase in chlorophyll a/b protein would increase the efficiency of photosynthetic antenna function in light-harvesting complex II (LHC II).

The shade treatment did not significantly affect the anthocyanin content, but it did not significantly decrease. According to Mazandarani et al., (2011) anthocyanins are part of the flavonoid component that has an antioxidant effect, namely cardioprotective. The results of this study are in line with those reported by on lisianthus (*Eustoma grandiflorum*) that low light intensity can reduce the total anthocyanin content. Zhu et al., (2017) explained that reducing the anthocyanin content in light deficit conditions is one form of avoidance response by plants.

#### Yield and stress susceptibility index

Chili yield data per plot was taken from 5 chili plants per experimental plot. The results of the analysis showed that there was an interaction effect of variety and shade on yields per plot. There was an increase in chili production under shade conditions in the Sonar variety with an SSI value of -1.45 so that this variety was included in the category of shade-loving varieties. The Bara, Raya varieties are categorized as moderate varieties because they have an SSI value of 0.55-0.60 and the Genie varieties are categorized as tolerant varieties because they have an SSI value of 0.16, while the Segana, Lada Hijau, Catas, Kerinci and Rajo varieties are sensitive varieties because they have high values. SSI 1.06 – 2.62.

Table 4. Production per plot and stress susceptibility index of several chili varieties under 50% shade conditions

Varietas	Production per plot (kg)		SSI	Criteria
	0%	50%		
Segana	3.58 a	0.97 d	2.62	Sensitive
Lada hijau	1.86 cd	0.79 d	2.08	Sensitive
Bara	1.54 cd	1.28 cd	0.60	Moderate
Catas	1.91 cd	1.16 d	1.43	Sensitive
Kerinci	1.44 cd	1.02 d	1.06	Sensitive
Raya	1.56 cd	1.32 cd	0.55	Moderate
Genie	1.65 cd	1.57 cd	0.16	Tolerant
Sonar	2.48 bc	3.47 ab	-1.45	Shade-loving
Rajo	0.52 e	0.32 e	1.38	Sensitive

Note: Data are means; different letters represent statistically significant differences ( $P < 0.05$ )

According to Sulistiyowati et al., (2019), shade-loving and shade-tolerant tomatoes have photosynthesis and respiration efficiency mechanisms so that they can produce sufficient photosynthetic results for plant growth and development. The decrease in respiration rate can suppress the process of carbohydrates assimilation in plants so the photosynthate can be accumulated in the fruit. In sensitive shade plants, according to (Purwoko, 2003), shade can result in a decrease in photosynthetic activity, resulting in a decrease in photosynthate stored in the fruit.

## CONCLUSION

Based on the data obtained, it can be concluded that leaf area and leaf pigment character can be used as a reference for determining the resistance of varieties to low light. Varieties that have the ability to grow optimally at low light intensity are Sonar and Genie varieties. This variety can be used for agroforestry under three-year-old rubber stands, which are equivalent to 50% light intensity.

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