Research article

Evaluation of Growth, Chlorophyll Content, and Photosynthesis Rate of *Curcuma xanthorrhiza* With Different Shade Levels

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Abstract

Keywords

plant growth;

Curcuma xanthorrhiza;

chlorophyll;

photosynthesis rate;

shade

Curcuma xanthorrhiza RoxB. is a medicinal plant found in Indonesia and is often cultivated for therapeutic purposes. *Curcuma xanthorrhiza* typically thrives in shaded, low-light environments. In this study, plant growth, photosynthetic rate, and chlorophyll content of *C. xanthorrhiza* in the same growing environment with different shade treatments were evaluated. The level of shade was adjusted using paranet at 0%, 25%, 50%, and 75%. Growth observations of *C. xanthorrhiza* were carried out from 1 to 6 months after planting (MAP). Chlorophyll content was measured using a UV-Vis spectrophotometer at 649 and 665 nm. The photosynthetic rate was measured using Li-Cor 6400 at 5 MAP. The results revealed that 50% shade gave the best response for plant height at 2 MAP. However, the highest number of leaves was found at 25% shade at 4 and 5 MAP. The shade level of 25% produced a significant response for *C. xanthorrhiza* rhizome biomass. The highest photosynthetic rate was found at 0% shade (26.69 µmol CO_2 $m^{-2}s^{-1}$) and the lowest at 75% shade (15.12 µmol CO_2 $m^{-2}s^{-1}$). Meanwhile, the chlorophyll content and number of tillers of *C. xanthorrhiza* leaves were unaffected by shade treatment. This study shows that shade treatments at 25%-50% levels gave the best growth responses in the *C. xanthorrhiza* plants.

1. Introduction

Curcuma xanthorrhiza, also known as temulawak in Indonesia, is a native Indonesian rhizomatous medicinal plant commonly cultivated in the countries of Southeast Asia including Indonesia, Malaysia, Thailand, Vietnam, and the Philippines [1]. In addition, cultivars can be found in India, China, Korea, and Japan [2]. This plant, which belongs to the *Zingiberaceae* family, has been

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utilized as a medicinal herb by the Indonesian people for generations. For instance, the rhizomes of *C. xanthorrhiza* can be used to treat infections, loss of appetite, fatigue, common fever, liver complaints, and gastrointestinal disorders [3]. The rhizomes of *C. xanthorrhiza* possess anti-inflammatory, antibacterial, antihyperlipidemic, and antioxidant activities [4, 5]. *Curcuma xanthorrhiza* production in Indonesia reached 32.28 thousand tons in 2021, having steadily increased since 2019 [6]. To increase the yield of a plant, environmental management is required [7]. Therefore, environmental engineering is needed to increase *C. xanthorrhiza* rhizome production.

The amount of light that reaches the leaf surfaces of a plant significantly affects its growth, and in particular the photosynthetic characteristics of its leaves. This is because light plays a significant role in the opening and closing of stomata and chlorophyll synthesis [8]. A previous study showed that reducing the relative light intensity increased the turmeric rhizome curcumin content (%) [9]. In addition, light is also considered a stress factor that significantly affects plant growth [10]. Plants respond to light stress by modifying cellular, molecular, and biochemical processes across the plant's hierarchy [11]. Unpredictable and quick climate shifts frequently expose plants to varying light levels. In conjunction with daily oscillations, these shifts modify the photosynthetic process, which determines how the plant adapts to these fluctuations [12]. As with shade-adapted plants, a quick increase in light intensity induced by light spots (sunspots) can increase photosynthesis or photoinhibition and generate a rapid stomatal reaction [13].

Moreover, plant acclimation to different light intensities is species-specific, depending on ambient factors and plant genotype [14]. *Curcuma xanthorrhiza* is typically grown under bamboo and teak trees in shady settings, which are similar to its native habitat [15]. Bhuiyan *et al.* [16] stated that turmeric's maximum growth parameters were observed under 25-30% shade, whereas yield characteristics and yield were most significant at 70-80%. Shade treatment also greatly influenced leaf shape. The largest leaves were found on plants cultivated in 50% and 70% shade, whereas the shortest leaves were found on plants planted in direct sunlight [17]. Shade-tolerant plants can grow, survive, and flourish in low-light conditions. In contrast, shade-intolerant plants require full sunlight and low competition [18]. Shade-tolerant plants can modify their morphophysiology to maximise light acquisition, including biomass allocation pattern, chlorophyll concentration, and leaf area and thickness [19].

However, there is a lack of data on the effects of different shade levels on *C. xanthorrhiza* growth, photosynthetic rate, and leaf chlorophyll content. Therefore, this study was conducted to observe the growth response, photosynthetic rate, and leaf chlorophyll content of *C. xanthorrhiza* at different shade levels. This study is expected to offer knowledge about optimal environmental engineering conditions for the growth of *C. xanthorrhiza* plants, which will help enhance the production of high-quality *C. xanthorrhiza.*

2. Materials and Methods

2.1 Location and experimental design

Curcuma xanthorrhiza seeds weighing 50-70 g and of the same age were obtained from the LPPM IPB Conservation Center for Tropical Biopharmaceutical Cultivation. From January to August 2022, *C. xanthorrhiza* seeds were planted on an open field in the Tropical Biopharmaceutical Conservation Unit Park of LPPM IPB, Cikabayan Gardens Block C Bogor, at the elevation of 240 m asl. All plants were given the same dose of fertilizer: urea at 250 kg/ha, SP-36 at 200 kg/ha, KCl at 200 kg/ha and cow manure at 20 tons/ha. This study used paranet shading made of polyethene-containing materials in the form of woven plastic nets installed to form shade houses. The four treatments included 0% (no shading), 25%, 50% and 75% shade (Figure 1). Each treatment was carried out in three replications, so that there were 12 experimental units. Paranet density, marked with a percentage, indicated the ability of the paranet to withstand the intensity of sunlight hitting the plants. The greater the percentage, the tighter the paranet is woven, so the less light intensity hits the plants. Meanwhile, the chlorophyll content and photosynthetic rate of *C. xanthorrhiza* leaves were tested five months after planting (MAP), and the third leaf of each plant was selected for these tests.

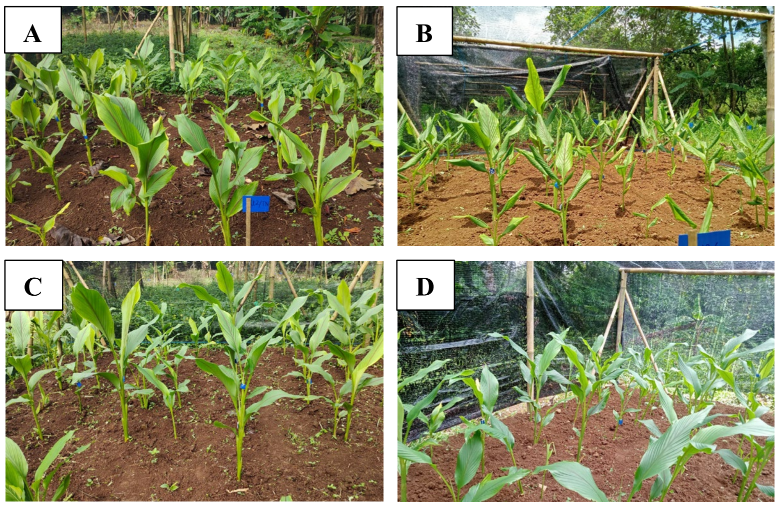

Figure 1. *Curcuma xanthorrhiza* planting conditions at different shade levels at 1 MAP (A) 0% shade; (B) 25% shade; (C) 50% shade; (D) 75% shade

2.2 Agro-morphological character

The observations of the agro-morphological character of *C. xanthorrhiza* are based on Lestari *et al.* [20] with modification. From each experimental unit, five plants were taken for observation. The factors observed in this investigation were number of leaves, plant height, and number of tillers of *C. xanthorrhiza*. Observations were made up to 6 MAP (month after planting), monthly. The plant height of *C. xanthorrhiza* was measured from the base of the stem to the tip of the highest leaf. The total number of leaves was counted, excluding leaf buds. And the fresh weight of the plant was measured after harvest at 7 MAP by weighing the cleaned *C. xanthorrhiza* rhizomes.

2.3 Chlorophyll content analysis

Analysis of the chlorophyll content of *C. xanthorrhiza* leaves was conducted with a modified method of Parry *et al.* [21]. The third leaf of each *C. xanthorrhiza* plants were taken for samples and cut into small pieces and 0.1 g lots were weighed and used for each parameter. Leaf samples were immersed in 7 mL DMSO solution in a test tube and heated in a bath at 65°C for 25 min until the disc faded. Chlorophyll concentration was measured using a UV-Vis spectrophotometer at the wavelengths of 649 and 665 nm. The blank used was a DMSO solution. The calculation of chlorophyll content was performed using Arnon's equation [22]:

$$\text{Chlorophyll a (mg/g)} = [(12.47 \times A665) - (3.62 \times A649)].\ V/1000.\ W$$
$$\text{Chlorophyll b (mg/g)} = [(25.06 \times A649) - (6.5 \times A665)].\ V/1000.\ W$$
$$\text{Total Chlorophyll} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

Where V = volume of the extract (ml); W = Weight of fresh leaves (g)

2.4 Photosynthesis rate

The rate of photosynthesis of *C. xanthorrhiza* leaves was measured at 09.00-10.00 am following the method of Suwanmontri *et al.* [23]. The absorption rate of carbon dioxide (CO_2) at that time was higher than measured in the afternoon or evening. Photosynthesis rate measurement was carried out on the third leaf using the LI-COR 6400 Portable Photosynthesis System (LI-COR Inc., Lincoln, Nebraska, USA). Leaves of *C. xanthorrhiza* in each shade treatment were put into the chamber for 3 min, and the photosynthetic rate was measured in three replications. The observed data of photosynthesis rate measurement were: internal CO_2 concentration, photosynthesis rate, transpiration rate, and stomatal conductance.

2.5 Data analysis

The acquired data were analyzed using variance (F test) at a significance level of 5%. To determine the actual effects, IBM SPSS Statistics 22 was used to conduct Duncan's Multiple Range Test at a 5% significance level. For data interpretation of each parameter (Agromorphology, photosynthesis rate, chlorophyll content, and correlation), GraphPad Prism version 9.4.1 (California USA) was used. MetaboAnalyst 5.0 was used for the overall correlation analysis parameters.

3. Results and Discussion

3.1 Plant agro-morphological character

3.1.1 Plant height

The plant height of *C. xanthorrhiza* was significantly affected by shade at 2 MAP but not up to 6 MAP (P-*value* 0.024 < 0.05). During the 2 MAP period, there was no significant difference between the 75% and 50% shade levels (Figure 2). However, the 75% shade level was much more significant than other treatments. As shown in Figure 3, the highest *C. xanthorrhiza* plants were found at 50% shade. Meanwhile, the shortest *C. xanthorrhiza* plants were found in 25% shade, followed by 0%. Different light conditions cause significant differences in plant morphology, metabolism, and interactions with other organisms [24]. The light intensity hitting plants was adjusted using various shading techniques. *Curcuma xanthorrhiza* plants adapted well to the shade level, as demonstrated by the optimal plant height occurring at 50% shade.

In addition, Alam *et al.* [25] also stated that plant height, leaf length, shoot biomass, and fresh and dry weight of rhizomes increased significantly between 33 and 50% shade levels. The etiolation symptoms of *C. xanthorrhiza* plants that occur at 50% shade were caused by the activity of the auxin hormone, which is triggered by a lack of light during plant development. The parts of the plant canopy that are exposed to light grow slowly because light inhibits the action of auxin, while the parts that are not exposed to light overgrow. This condition causes the apex of the plant to experience the most substantial development, allowing it to seek light and carry out photosynthesis optimally. Reducing light intensity via the use of shading results in etiolation [26].

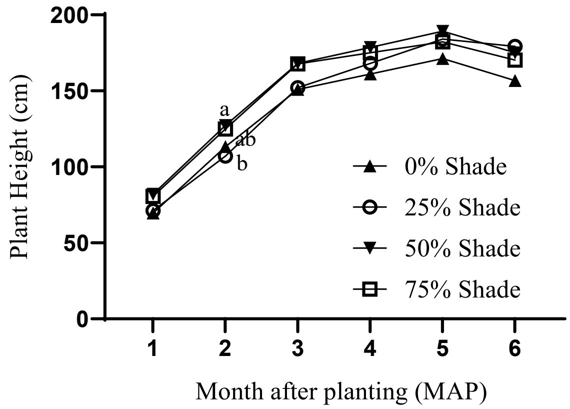

Figure 2. Plant height of *C. xanthorrhiza* under different shade levels. Plant height was significantly affected by shade at 2 MAP in 50% shade level. The data obtained were analyzed at the 5% level, as determined by Duncan's multiple range test.

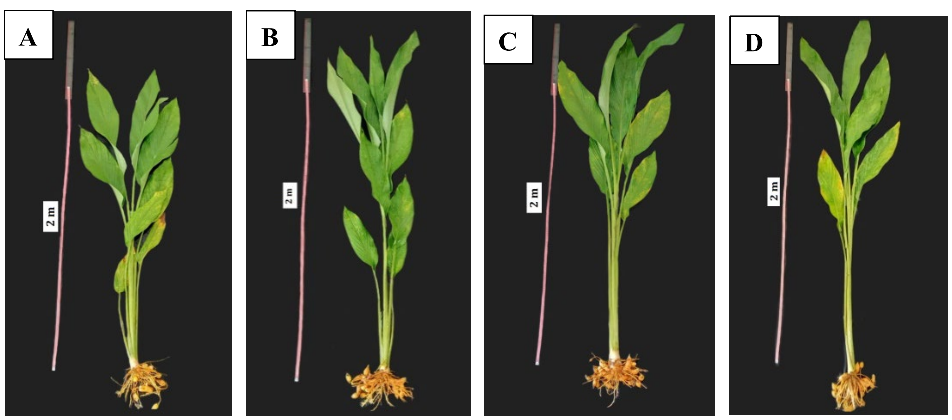

Figure 3. *Curcuma xanthorrhiza* plant height differences between shades at 5 MAP (A) 0% shade; (B) 25% shade; (C) 50% shade; (D) 75% shade

3.1.2 Number of leaves

Leaves are plant-specific organs for photosynthetic activity. Leaf development is complex and influenced by light [27]. Analysis of the variance of *C. xanthorrhiza* leaves that had been carried out indicated that shading significantly affected the number of *C. xanthorrhiza* leaves at 4 MAP (P-*value* 0.030 < 0.05) (Figure 4). Based on this study, the highest number of leaves was found in 25% shade with 75% light intensity. Conversely, the least number of leaves was found at 75% shade, with the intensity of light hitting the plants at 25%. In addition, shade also significantly affected the number of *C. xanthorrhiza* leaves at 5 MAP (P-*value* 0.002 < 0.05). Likewise, during the planting period of 4 MAP, the highest number of *C. xanthorrhiza* leaves was found in 25% shade at 5 MAP. Meanwhile, at the shade level of 75%, the least number of leaves was found.

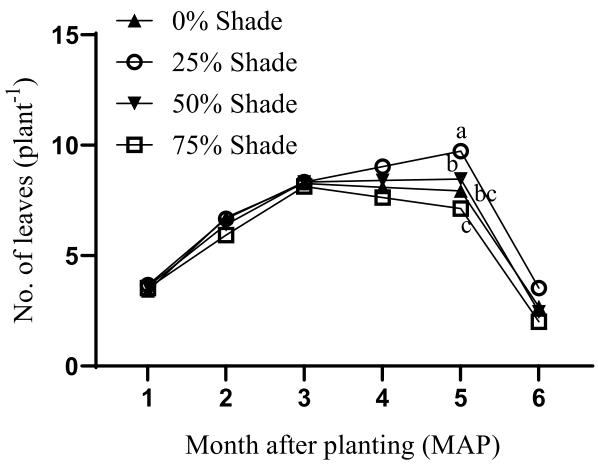

Figure 4. The number of leaves of *C. xanthorrhiza* under different shade levels. The number of leaves is significantly affected by shade at 4 MAP and very significantly affected at 5 MAP in 25% shade level. The data obtained were analyze at the 5% level, as determined by Duncan's multiple range test.

As shown in Figure 4, the highest number of leaves of *C. xanthorrhiza* was found at 25% shade. It also stated that *Curcuma* plants could grow under shade at relatively low sunlight intensity [28]. Plants at a low light intensity grow more slowly than those at a high light intensity. Hence plants on broad terrain grow faster. Meanwhile, 75% shade conditions produced the fewest number of *C. xanthorrhiza* leaves. Because the 75% shade level had the highest paranet density compared to the other treatments, the plant leaves captured the least sunlight [29]. According to previous studies, plants undergo morphological changes such as longer internodes, fewer leaves, and thinner leaves in order to adapt to low light and maximize the light they receive [30].

3.1.3 Number of tillers and fresh biomass

The number of tillers of *C. xanthorrhiza* plants was observed from 2 to 5 MAP. *Curcuma xanthorrhiza* plants observed at 1 MAP had no tillers. At 6 MAP, the *C. xanthorrhiza* plants had turned yellow, and no tillers were found. This was because the *C. xanthorrhiza* plant had entered the generative phase at 6 MAP. In this phase, plant growth tends to be focused on forming secondary metabolites such as curcuminoids and xanthorrhizol [31]. Typically, at the approach to the harvesting stage, the leaves of the plants usually dry and turn yellow followed by the withering of the pseudo stems of the plants [32]. Shade treatment did not significantly affect the quantity of *C. xanthorrhiza* plant tillers (P-*value* > 0.05). In addition, the fresh weight of *C. xanthorrhiza* rhizome was calculated to see the level of shade treatment that produced the highest biomass.

Curcuma xanthorrhiza rhizomes were harvested at 7 MAP (Figure 5). The fresh weight at 9 months was lower than the fresh weight at 7 and 8 months. This pattern emerged because the water content of the rhizomes was maximum at 7 months [33]. According to Faiza *et al.* [31], *C. xanthorrhiza* or Javanese turmeric grown with manure and LMO produced larger rhizomes and higher yield at 7 MAP than the control turmeric at 9 MAP. Furthermore, the maximum amount of *xanthorrhizol* was also discovered at 7 MAP [34]. Data on *C. xanthorrhiza* rhizome weight after harvest is shown in Figure 6.

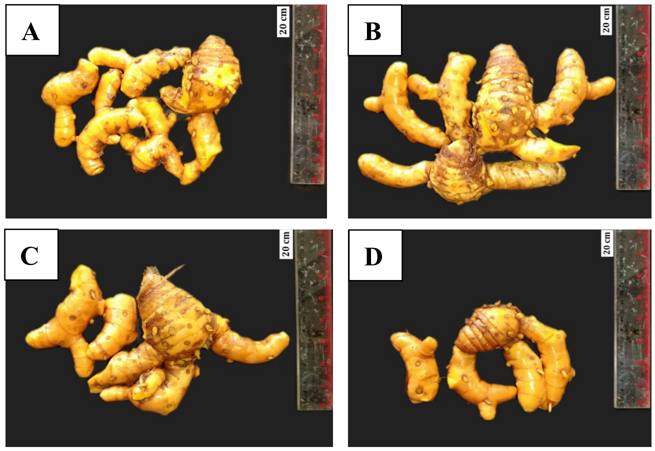

Figure 5. *Curcuma xanthorrhiza* rhizome after harvest with different shade levels (a) 0% shade; (b) 25% shade; (c) 50% shade; (d) 75% shade

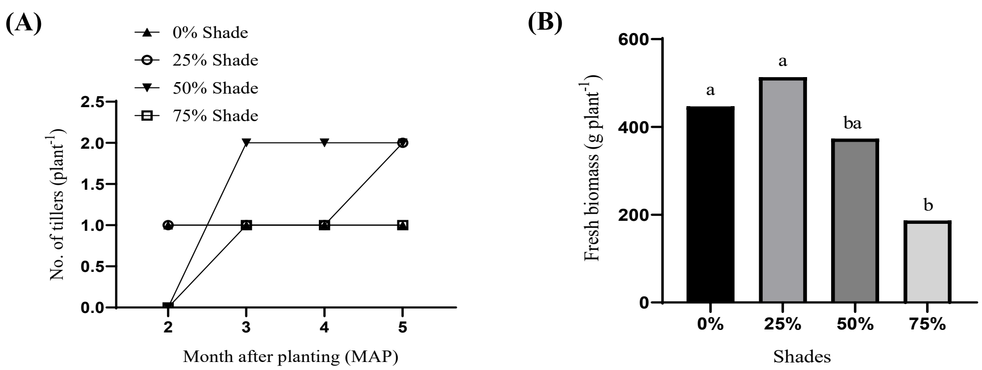

Figure 6. The number of tillers (A) and rhizome biomass (B) of *C. xanthorrhiza* under different shade levels. Number of tillers is not significantly affected by shade. Meanwhile fresh biomass is significantly affected by shade at the 5% level, as determined by Duncan's multiple range test at the 5% level.

Shading generally lowered the tiller number and slowed tiller emergence and growth [35]. However, the data obtained in this study showed that shade did not affect the number of tillers of *C. xanthorrhiza.* The reason for this was probably that the formation, growth, and production of tillers are affected by both internal (genetic variables, plant growth period, and cultivars) and external (environmental, horticultural, and management) elements [34]. The rhizome's fresh weight varied greatly depending on the shade the plants were exposed to, with the largest fresh biomass occurring at 25% shade. Moreover, direct and indirect light contribute to *C. xanthorrhiza* biomass synthesis. Variations in environmental light levels and harvest timing are known to increase or reduce productivity. Plant physiological, metabolic, anatomical, and growth properties may respond differently depending on age and environmental changes. These factors can alter both chemical and biological processes [36].

3.2. Photosynthetic rate

The rate of photosynthesis of *C. xanthorrhiza* leaves was measured using a Li-Cor 6400 Portable Photosynthesis System at 5 MAP. The results of measurements of photosynthesis rate, stomatal conductance and water use efficiency are shown in Figure 7. Shade significantly affected the photosynthesis rate of *C. xanthorrhiza* leaves, with a P-*value* (0.0001<0.05). In addition, there was a difference in the rate of photosynthesis between the shades; the highest rate of photosynthesis found at 0% shade was 26.69 µmol CO_2 $m^{-2}s^{-1}$. Meanwhile, the lowest photosynthetic rate found at 75% shade was 15.12 µmol CO_2 $m^{-2}s^{-1}$. Moreover, the photosynthetic rates from the largest to the smallest were found at the 0%, 25%, 50%, and 75% levels of shade, respectively.

Furthermore, to the rate of photosynthesis, shade also significantly affected the stomata conductance of *C. xanthorrhiza* leaves, with P-*value* (0.0341<0.05). As shown in Figure 7, the most significant stomata conductance was found in the 0% shade, which was 2.83 mol H_2O $m^{-2}s^{-1}$. Moreover, the smallest stomatal conductance was found at 75% shade with 1.39 mol H_2O $m^{-2}s^{-1}$. The stomatal conductance of the leaves of *C. xanthorrhiza*, from the largest to the smallest, was found at 0%, 50%, 25% and 75% shade, respectively. At the same time, the shade had no significant effect on the internal CO_2 concentration and transpiration rate of *C. xanthorrhiza* leaves.

Compared to leaves grown in the shade, leaves grown under the sun had higher photosynthetic net CO_2 assimilation (PN) rates probably due to their sun-type chloroplasts. Sun grown leaves had a much higher stomatal conductance (gs), a result that was consistent with the increased photosynthetic rate of sun leaves. Sun-loving plants have higher gs values. The increase in stomatal conductance observed in leaves exposed to direct sunlight is a reaction to an increase in radiation. As a result, leaves growing in the shade do not acquire the same gs as leaves growing in the sun. The effect of shadowing on gs can be linked to the leaves' abscisic acid (ABA) concentration, as ABA content is thought to decrease with increasing mesophyll radiation, resulting in increased stomata opening [37].

3.3. Analysis of chlorophyll content

The *C. xanthorrhiza* chlorophyll content measurements were carried out at wavelengths of 649 and 665 nm, as shown in Figure 8. Our results suggested that shade treatment did not significantly affect chlorophyll contents. However, an increase in chlorophyll content of *C. xanthorrhiza* leaves is directly proportional to increase in shade intensity. Plants increase the interception of low light by increasing chlorophyll content to ensure maximum photosynthetic carbon accumulation as light intensity decreases, indicating that plant adapted to the decrease in light duration and photosynthetically active radiation was beneficial to *C. xanthorrhiza* photosynthesis in a low light environment [38].

Moreover, *C. xanthorrhiza*, as previously stated, is a plant that thrives in shade [15]. Shade tolerance varies between shade-tolerant plant species, resulting in the reconstruction of morphology, growth, and behaviors. In general, the chlorophyll content of the leaves of shade-tolerant plants decreases as light intensity increases. Instead, the chlorophyll a/b content increased. This happens because under shaded conditions, plants express chlorophyl biosyntetic genes to maintain photosynthesis rate [39]. Plants respond to decreasing light levels by creating more chlorophyll. The higher the shade level, the greater the rise in chlorophyll concentration is expected [40]. Increase in the chlorophyll content is an adaptive mechanism for shade-tolerant species that can promote the maximization of light capture.

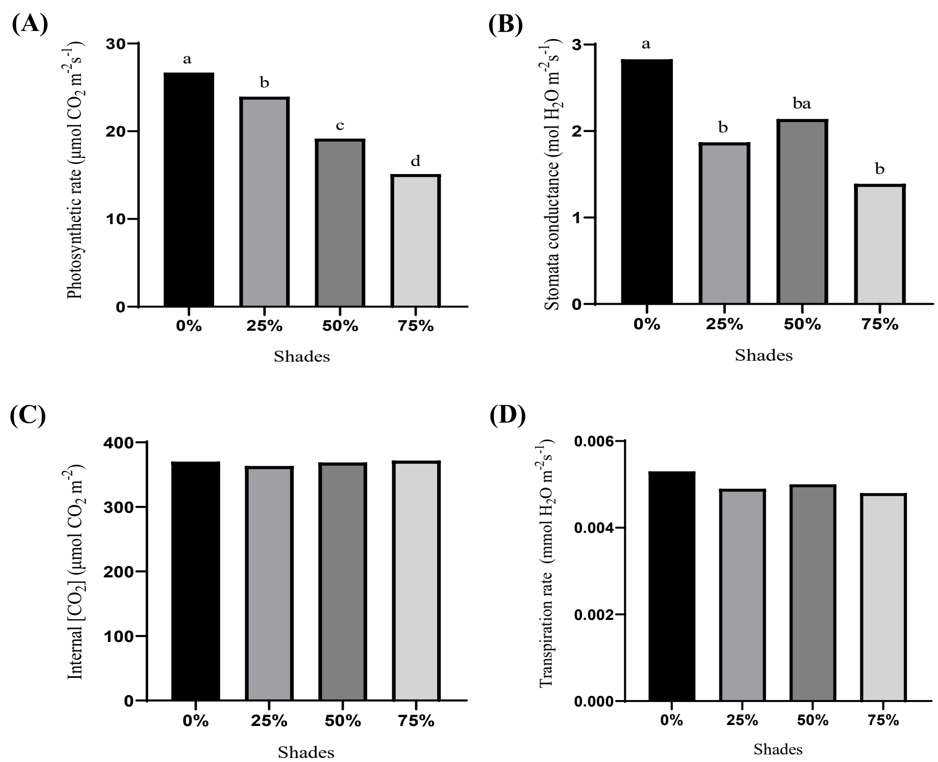

Figure 7. Response of photosynthetic rate (A), stomatal conductance (B), internal [CO_2] (C), and transpiration rate (D) to shade. Photosynthesis rate is very significantly affected by shade and significantly affects the stomatal conductance at the 5% level, as determined by Duncan's multiple spacing test.

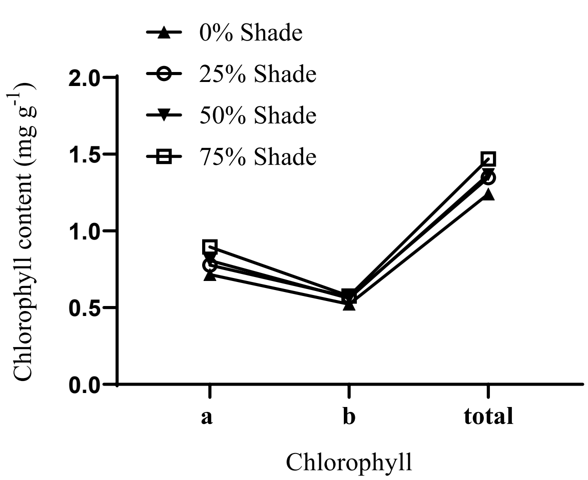

Figure 8. Chlorophyll content of *C. xanthorrhiza* leaves at the 5% level, as determined by Duncan's multiple spacing test.

Furthermore, the increase in chlorophyll content caused by shade is assumed to be related to an increase in the expression of a gene involved in chlorophyll synthesis, notably the CsPOR gene. By decreasing the light, the gene's expression can be raised. Light can block the expression of the CsHY5 gene, which has consequences for enhancing the transcription of the CsPORL-2 gene and increasing plant chlorophyll levels [41].

3.4. Correlation between photosynthetic rate and chlorophyll content

The correlation between photosynthetic rate and total chlorophyll is shown in Figure 9. Pearson analysis using Graphpad Prism 9.4.1 was performed. The Pearson correlation value between photosynthesis rate and total chlorophyll content showed a strong correlation, r (-0.3841 < -0.5). Figure 9 shows that the total leaf chlorophyll of *C. xanthorrhiza* decreased as photosynthesis increased. As shown in Figure 7 previously, the rate of photosynthesis decreased with increasing shade intensity, while the total chlorophyll content increased due to higher shade intensity (Figure 8). As stated by Ren *et al*. [42] it can be explained that the chloroplast, where photosynthesis takes place, is very sensitive to the external environment, and its structure can directly affect the rate of plant photosynthesis plants.

Due to the influence of shade, low light intensity that hits plants can damage the chloroplast ultrastructure and reduce chlorophyll synthesis, carbon dioxide fixation, and photosynthetic capacity. The low light intensity hitting the plant, as occurs at 75% shade, causes the plant to induce more superoxide, H_2O_2, and hydroxyl radicals. *Curcuma xanthorrhiza* plants deficient in light at 75% shade will change their morphology and nutrient distribution by carrying out environmental adaptation to accommodate the lack of light. Plants adapt to a dim light environment by synthesizing more chlorophyll as part of the light-gathering complex. This is due to plants having complex physiological and biochemical responses to environmental stressors [43].

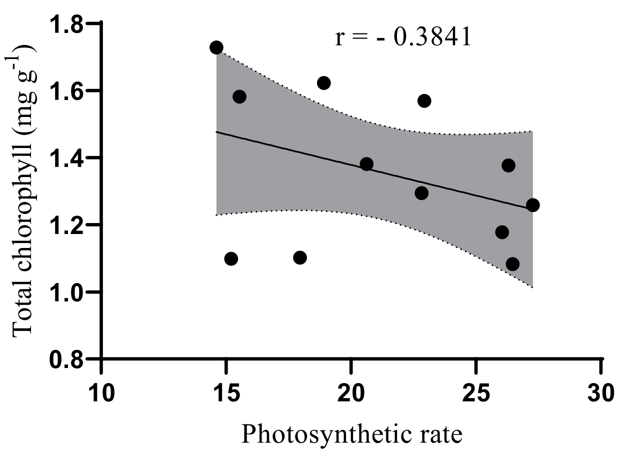

Figure 9. Correlation between photosynthetic rate and chlorophyll content *C. xanthorrhiza* leaves

3.5. Statistical analysis heatmap correlation

The correlation between the growth parameters of *C. xanthorrhiza* were expressed as a heatmap analysis using the Metaboanalyst software. The columns and rows listed on the heatmap display represent each test parameter. Red indicates a positive correlation of each parameter, while a negative correlation is illustrated in blue. The result of the heatmap analysis of the present study is illustrated in Figure 10. In this study, the heatmap correlation analysis showed no relationship between the number of tillers and the total chlorophyll content of *C. xanthorrhiza*. It indicates that other parameters affected the total chlorophyll content of *C. xanthorrhiza* plants. Influential parameters such as chlorophyll a and b contents positively correlated with total chlorophyll. Meanwhile, this heat map illustration shows a negative correlation between total chlorophyll content and photosynthetic rate.

Based on Thapa *et al*. [44] stated that the external plants on both sides, which received more sunlight, water, and nutrients, generally had more tillers per plant than the inside plants, so there was no correlation between the number of tillers and total chlorophyll because the production of the number of tillers was affected by the intensity of light into the plant. The development of a plant's tiller is controlled by genetic and environmental variables working in tandem, making it a feature of great importance. Various genetic, physiological, and environmental factors work together to control tiller growth.

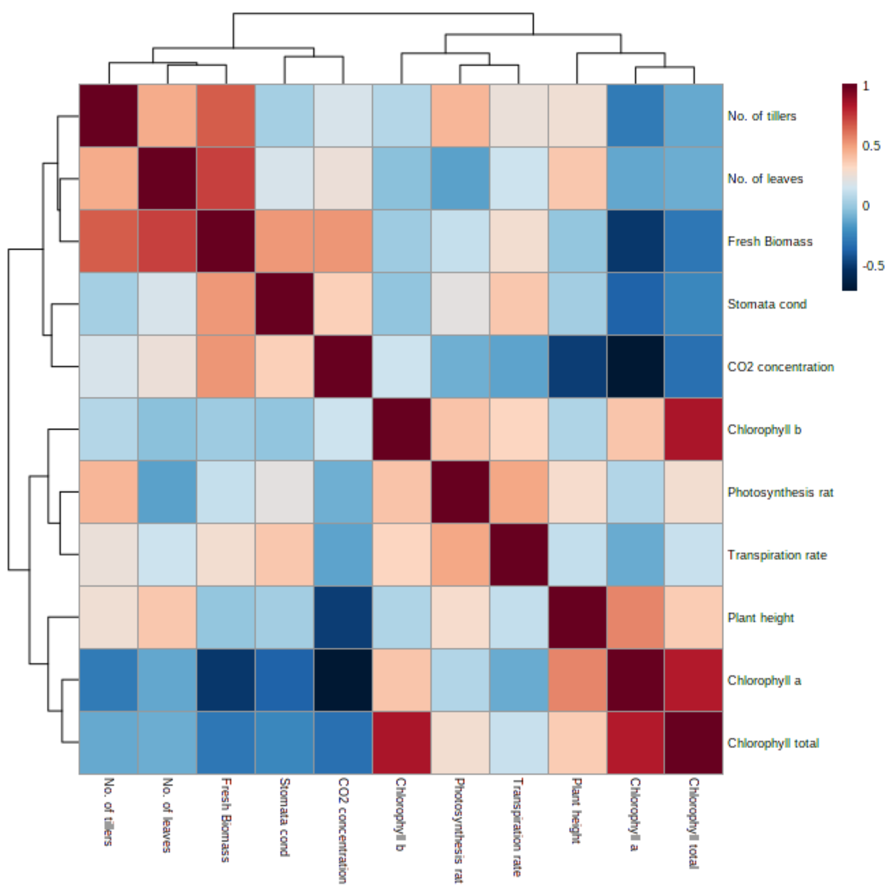

Figure 10. Heatmap showing correlation of the plant height, number of leaves, number of tillers, fresh biomass, photosynthesis rate, and chlorophyll content. The color in the heatmap scales ranges from -0.5 (blue) to 1 (red) and reflected the correlation of individual pair of types.

4. Conclusions

This study obtained information regarding the growth response, photosynthetic rate, and chlorophyll content of *C. xanthorrhiza* leaves. Shade treatment influenced plant height, number of leaves, fresh biomass, photosynthetic rate, and leaf stomatal conductance in *C. xanthorrhiza* plants. The best agro-morphological characteristics for the number of leaves and fresh biomass of *C. xanthorrhiza* plants were found at a shade level of 25%, implying that the optimal environmental engineering conditions for *C. xanthorrhiza* plant growth were found to be a shade level of 25%.

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