Influence of Light Intensity on Leaf Photosynthetic Traits and Alkaloid Content of Kiasahan (*Tetracera scandens* L.)

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Influence of Light Intensity on Leaf Photosynthetic Traits and Alkaloid Content of Kiasahan (*Tetracera scandens* L.)

T Setiawati[1], **A Ayalla**[1], **M Nurzaman**[1]**and A. Z Mutaqin**[1]

[1]Departement of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Jatinangor-Sumedang, 45363, Indonesia

E-mail:tia@unpad.ac.id

Abstract. Kiasahan (*Tetracera scandens* L.) is one of the plants that people use as atraditional medicine for various diseases. This study was conducted to determine the characteristics of anatomy, morphology, chlorophyll content, and alkaloids of Kiasahaan leaf (*T. scandens*) based on differences in light intensity in the Pananjung Pangandaran Nature Reserve. The survey method was used to determine the location of sampling.The location was determined based on the existence of *T. scandens* plant. The shaded and unshaded area had the light intensity of 5516 and 922,000 lux, respectively. Leaf samples were taken as many as three strands of three different individuals on the shaded and unshaded areas. Parameters measured were the thickness of leaf, leaf area, stomatal density, chlorophyll,and alkaloid content. Measurement of chlorophyll and alkaloids used chlorophyll meter and Mayer's reagent method, respectively. The results showed that the average leaf thickness, leaf area, stomatal density, chlorophyll content in theshaded area were 0.048 mm, 7.086 cm^2, 50.958 cells / mm^2, 15.38 CCI, respectively whereas in the unshaded area were 0.076mm, 6.496 cm^2, 62.678 cells / mm^2, 8.86 CCI, respectively. Alkaloid content of *T. scandens* leaves in the unshaded area was higher than in the shaded area.

1. Introduction

Plants produce secondary metabolites that are beneficial to humans such as food flavoring, insecticides, perfumes, and sources of medicinal materials [1]. As a medicinal substance, the secondary metabolites of plants have biological properties such as antioxidant activity, antimicrobial, detoxification enzyme modulation, immune system stimulation, decreased platelet aggregation and modulation of hormones metabolic, and anticancer [2]. One type of secondary metabolite that is widely used as a medicine is alkaloids. Role of alkaloids in plants is still unknown, but in the health aspect can be used as a medicine for neurosystem or as an additive compound [3].

There are several factors that affect the growth and development of plants, such as the intensity of light. According to Li et al. [5], light is an important environmental factor affecting growth, development, and secondary metabolism of plants. Spectrum, intensity,and quality of light have an important role in the process of plant development and metabolism [5]. The intensity of light that is too high or too low has an impact on morphology, physiology of photosynthesis, and the production of secondary metabolites [6]. As also stated by Coelho et al.[7] that the secondary metabolite synthesis and accumulation of plant is influenced by the light. Light affects the oxidative metabolism in the chloroplast further affecting light absorption and CO_2 diffusion which induce structural and physiological modifications to the leaf [8]. According to Vendramini et al. [9] leaf characteristics

usually reflect adaptive strategies plants to important environmental factors, including light, temperature, air, and nutrition. The research conducted by Kong et al.[10] showed that differences of light intensity affecting the external morphology of leaves, internal anatomy, and physiological characteristics; even induces changes in secondary metabolite production of *Mahonia bodinieri*.

Tetracera scandens commonly known to the local community as Kiasahan is one of the plants that people use for traditional medicine such as hypertension, rheumatism, inflammatory diseases, internal pains, urinary disorders, gout and hepatitis [11]. To the best of our knowledge, there has not been any publication about influence of light intensity on leaf photosynthetic traits and alkaloid content of kiasahan (*T. scandens* L.).

The purpose of this research was to know the traits of photosynthetic leaf and alkaloid content in Kiasahan plants (*T. scandens* L.) in areas with different light intensity.

2. Materials and Methods

The materials used in this study were leaves of *T. scandens* L. plant, distilled water, transparent nail polish, ammonia chloroform, sulfuric acid (H_2SO_4) 2N, and Mayer's reagent.

This study used a quantitative descriptive approach.The sampling technique used a survey method to determine the presence of *T. scandens* plants in two different locations i.e. the shaded area and the area exposed to full sunlight (unshaded area). A leaf that taken as a sample was the third, fourth, and fifth leaves from shoots that already fully open. Leaf samples were taken as many as three pieces of three different individuals in the shaded area and unshaded area. Leaf characteristics observed were the thickness of leaves, leaf area, stomatal density, chlorophyll,and alkaloids content. The data were analyzed descriptively.

Leaf thickness was measured using a micrometer screw. The measurement was done five times the repetition and then averaged.Measurement of leaf area was conducted using the gravimetric method [12] i.e. by comparing the weight between leaf patterns made on paper with other paper patterns of extension known [13].Observations of stomata density used a replica method. Transparent nail polish applied to the leaf surface, allowed to dry, and then spread transparent nail polish peeled away slowly. Replica of stomata placed on a glass slide and examined under a light microscope then density of stomata was calculated. Chlorophyll content was measured with chlorophyllmeter Opti-Science CCM-20. Measurement on each leaf sample was taken as many as fiverepetitions, thereafter were averaged.

Alkaloid content was determined using Mayer's reagent. The leaf sample was weighed 2 grams and mashed, then put in a test tube, added chloroform and an ammonia solution of 5 mL each. The mixture was heated, shaken, and filtered. H_2SO_4 2 N solution of 5 drops was added to the filtrate and shaken. The top of the filtrate was taken and tested with Mayer's reagent. The positive test results contain alkaloids when there was white precipitate [14].

Environmental parameters measured include light intensity, temperature, humidity, and pH. Lux meter was used to measure light intensity, Thermo hygrometer was used to measure temperature and humidity, and soil tester was used to measure soil pH. Environmental parameters measurements were performed at two locations (shaded and unshaded areas).

3. Results and Discussion

3.1. Environmental parameter measurement

Environmental parameters showed that the intensity of light in the shaded area was 5,516 lux, while in the unshaded area was 922.000 lux. Under these conditions, the temperature of the shaded and unshaded area indicated 28˚C and 26˚C, respectively.The result ofhumidity measurement showed thatin the shaded area of 55% while the unshaded area of 50%. This condition indicates that in the higher the shade level, the air humidity also increases. Soil pH measurements showed that the shaded area was 4, while in the unshaded area was 4.8. The acidity (pH) of the soil was classified as acid (<7).

3.2. The thickness of leaf and leaf area

The average leaf thickness and leaf area of *T. scandens* in the shaded and unshaded areas can be seen in **Figure 1** and **2**.

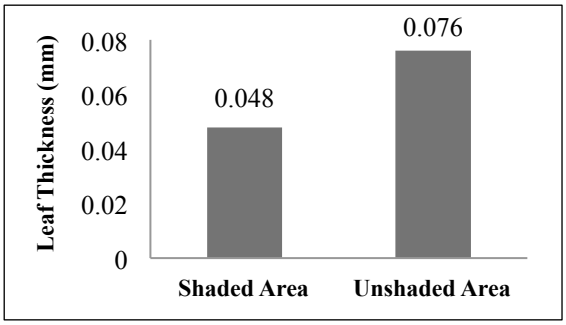

Figure 1. Average leaf thickness of *T.scandens*in shaded and unshaded areas

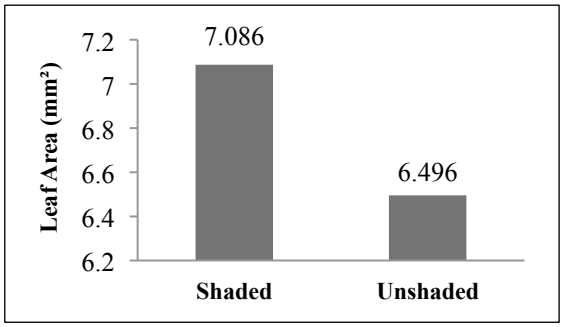

Figure 2. Average leaf area of *T. scandens* in shaded and unshaded areas

Figure 1showed that the average thickness of *T. scandens* leaf was 0.048 mm while in the unshaded area of 0.076 mm. Average leaf thickness in the shaded area was lower than in the unshaded area. In accordance with a recent report [15] that leaves that grow in unshaded areas or commonly called the sun leaves are thicker than the leaves in shade areas or shade leaves. Tucić et al. [16] reported thatmany plants growing in exposed areas of full sunlight produce thicker leaves to minimize excess light interception. Leaf thickness is affected by the thickness of the tissue layer on the leaves, such as mesophyll tissue.Likewise, according to [17] that characteristic changethe leaves on the shaded condition becomes wide and thin. Leaf thinning is caused by a decrease in the palisade layer in leaf mesophyll cells [18].

Figure 2showed that the average leaf area of *T. scandens*in the shaded area washigher than in the unshaded area. Average leaf area in the shaded and unshaded area was7,086 mm² and6,496 mm², respectively.Under low light, plants often get features to capture themore optical energy, such as leaf area enlargement [19].As wellKong et al.[10] reported that the leaves in sun area are thicker and smaller with low stomatal density on both leaf surfaces. Similar to the results of research conducted by[6] which indicates that *Epimedium pseudowushanense*seedlings grow under relatively low light intensity have larger leaves than those grown under high light intensity. According to Fitter and Hay[8], plants under the shading adapt to low light intensity conditions by increasing the leaf area to obtain a larger surface for light absorption. Moreover, adecrease in leaf size in full sunlight area will reduce leaf temperature, potential water loss and damage to leaf photosystems [20].

3.3. Density of stomata

The average stomatal density of *T.scandens* leaf in the shaded and unshaded areas can be seen in **Figure 3**, which was calculated based on the distribution of stomata number on the abaxial longitudinal incision of *T. scandens* leaf (**Figure 4** and **5**).

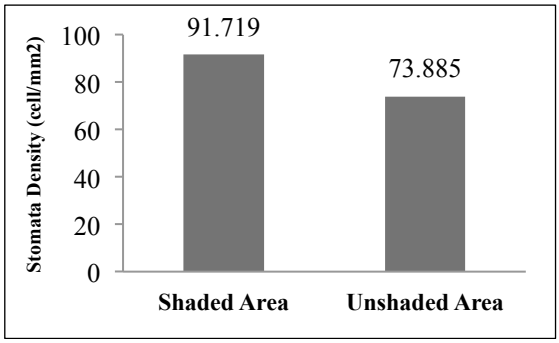

Figure 3. Average stomatal density of *T. scandens*in shaded and unshaded area

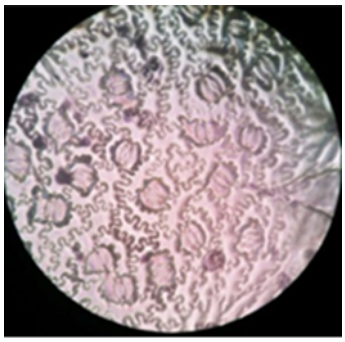

Figure 4. Distribution of stomata number of *T. S scandens*in the unshaded area

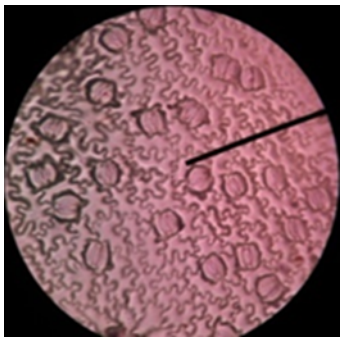

Figure 5. Distribution of stomata number of *T. scandens* in the shaded area

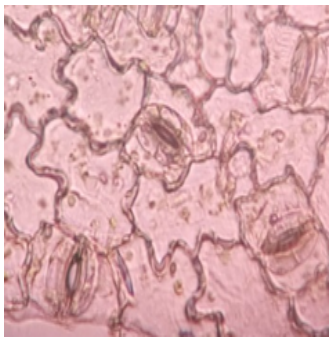

Figure 6. Paracytic stomata of *T. scandens* leaf

Figure 3 indicated that the average stomata density of *T. scandens* shaded leaves of 50.958 cells /mm^2, whereas in the unshaded area of 62.678 cells/mm^2. These results indicated that the average density of stomata in the shaded area was lower than the unshaded area. Stomata control gas exchange, water loss, and temperature of leaves. Stomata will be found more in leaves less exposed to sunlight to reduce evaporation or water loss. Species with higher stomatal density tend to be more responsive to the increase in CO_2, so the rate of photosynthesis is greater [21]. In general, stomatal density decreases with increased CO_2 concentration and compared with leaf developed under low light intensity, the sun leaves have ahigher density of stomata [22].

Observation of stomata on *T. scandens* leaf indicated the type of parasitic (**Figure 6**). According to [23], Kiasahan (*T. scandens*) plants including the Dilleniaceae family, usually have anomocytic stomata type in which each guard cellis surrounded by a number of cells that are not different in size and shape from other epidermal cells; but exceptions to the species of *Tetracera*, the type of parasitic stomata [24]. Type of paracytic / Rubiaceous stomata where each guard cell is accompanied by one or more neighboring cells with the long axis of the neighboring cell is parallel to the guard cell and pore [25].

3.4. Chlorophyll content

The result of chlorophyll content analysis of *T. scandens* leaf on the shaded and unshaded areas can be seen in **Figure 7**.

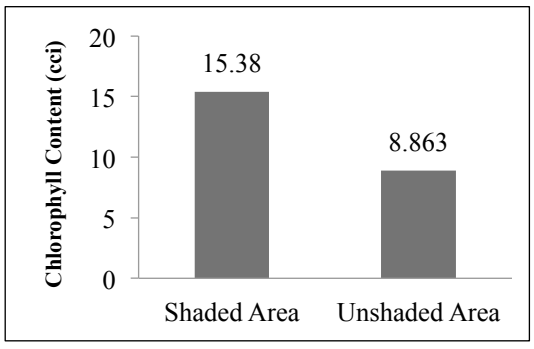

Figure 7.Average chlorophyll content of *T. scandens* in the shaded and unshaded area

Figure 8. The color of *T. scandens* leaves in the shaded(left) and unshaded area (right)

In **Figure 7** appeared that the average chlorophyll content of *T. scandens* leaves in the shaded area of 15.38 CCI higher than in the unshaded area of 8.863 CCI.A higher amount of chlorophyll in plants

under shade serves to maximize light absorption under low light conditions. Chlorophyll in shaded plants is arranged in photo-taxis state [17]. According to Watson & Dallwitz [26] one of the characteristics of the adjustment to low irradiation due to shade is an increase in leaf chlorophyll content. This increase is related to the increase of light harvesting complex (Light Harvesting Complex II) and the enlargement of the antenna in photosystem II which resulted in increased light capture efficiency. Light provides an energy source for photosynthesis but excess light can damage photosynthetic apparatus and cause photo-oxidation of chlorophyll. Photo-damage (photo-oxidation of chlorophyll and cell death) occurs only when the plant is exposed to extreme or prolonged pressure. Similar research reported that decreased light intensity followed by an increase in chlorophyll a (Chl. a), chlorophyll b (Chl. b) and total chlorophyll (Chl. a + b) on *Lithocarpus litseifolius*[5] and on *Mahonia bodinieri*[10]. The high light intensity causes chloroplast damage on *E. pseudowushanense* plant [6] resulting in a decrease of chlorophyll content.

Figure 8 indicated differences in leaf color of *T. Scandens* which grow in the shaded and unshaded areas. The leaves of *T. Scandens* which grow on the shaded area have more green color than in the unshaded area. This may be due to the leaves on the shaded have higher levels of chlorophyll than the unshaded area. The study conducted by Stanton [27] revealed that shading of 40% treatment on *Spiraea alba* plant resulted in darker green leaf.

3.5. Alkaloid content

The results of the analysis on the presence of alkaloids on the leaves of *T. scandens* showed that alkaloid found in the leaves of *T. scandens* both in the shaded and unshaded areas. In this study, alkaloid analysis is qualitative using Mayer's precipitation method, however alkaloid content is quantified based on the amount of precipitate formed. In accordance with the statement by Verma et al. [28] that the hydrochloride salt deposits from the leaf extract show the number of alkaloids formed.

In the leaves of *T. scandens* at the unshaded area are found more sediment than in the shaded area, so it can be assumed that the level of alkaloid in the unshaded area is higher than in the shaded area. The intensity of light is a major factor affecting the synthesis and accumulation of secondary metabolites [7]. According to Pompelli et al. [29] in the *Coffee arabica* plant, high-intensity stress triggers increased alkaloid production, such as caffeine, allantoin,and theophylline. Stress in the form of high intensity of light triggers the increase in the presence of free nitrogen. Nitrogen is the source for the passage of metabolism. Plants that live at low concentrations of nitrogen will experience caffeine degradation (xanthine alkaloids), so the resulting caffeine will be lower than at high concentrations of nitrogen. The similar research in *Iris pumila* plants showed that secondary metabolite levels of anthocyanin and phenol were detected higher in areas exposed to full sunlight [30].

4. Conclusion

Based on the results of this study can be concluded that the intensity of the light affects the thickness ofleaves, leaf area, stomatal density, chlorophyll, and alkaloid content of *T.scandens*. There were different characteristics of leaf photosynthetic of *T. scandens* in the shaded and unshaded areas. In the shaded area, average the leaf thickness (0.048 mm), stomatal density (50.958 cells / mm^2) and alkaloid content of *T. scandens* leaves were lower than in the unshaded area. While the average leaf area (7.086 cm^2) and chlorophyll content (15.38 CCI) of *T. scandens*leaves were higher in the shaded area than in unshaded ones.

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