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Morphological and Physiological Responses of *Clinacanthus Nutans* Under Different Light Conditions

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Abstract: Abiotic stress such as light stress can cause injuries or damage to the plant cells or biochemical processes in the plants. Difference of light conditions resulted in different morphological and physiological responses of plants. This study is done to determine the response of *Clinacanthus nutans* under different light condition as the results of plant defense mechanism. *Clinacanthus nutans* is one of the plant species that contains higher medicinal value and currently being commercialised as alternative treatment to treat various ailments. Four weeks of treatment was done on five samples of *Clinacanthus nutans* in each condition. Observation done morphologically shows that samples under condition A (exposed fully under sunlight) appear bigger in stem diameter, longer in root length, higher in plant height and pale in colour compared to samples in condition B (shaded under polyethylene netting). However, for leaf size parameter, samples in condition B has bigger leaves compared to condition A. Meanwhile, for physiological analysis, shows that there is difference in chlorophyll content based on different condition (0.83 ± 0.463 *Ca*, 1.46 ± 0.740 *Cb* and 0.03 ± 0.015 carotenoids for condition A, and 0.50 ± 0.321 *Ca*, 1.47 ± 0.539 *Cb* and 0.03 ± 0.105 carotenoids for condition B) and samples under condition A contain higher photosynthetic rate as a result of higher dry matter content (0.27 ± 0.017) in plants. It is suggested that to ensure the results is more accurate, the period of treatment should be longer and analysis on primary and secondary metabolites should be conducted.

Keywords: Light conditions, morphology and physiology responses, photosynthetic rates, chlorophyll content and, primary and secondary metabolites

1. Introduction

Abiotic stress for a plant is a condition where the plants face non-living environmental stress such as extreme levels of light, radiation, temperature, water and any chemical factors [1] that harm or negatively impacts the plants. Light stress is one of the stresses that can cause injuries to the plant. Over exposure of plants under light can lead to photo-oxidative damage [2], while under low light stress, the plant cells may damage and inhibit the biosynthesis process and activity [3]. According to [1], these impacts of abiotic stress have forced the plants to adapt and respond to the altering condition of climates in order for them to keep on surviving and maintaining their genetics. The adaptation or response of plants due to any changes in the environment can be seen in their defense mechanism. A plant defense system is a mechanism used by all plant species to fight or combat the biotic or abiotic stress pressure on them. Under unfavorable environmental conditions, the plant development or growth may be affected which will inhibit their productivity and thus prevent them from performing their full potential of biochemical processes [4]. Plant defense mechanisms work by regulating the hormone and enzyme in the plants to increase the physiological and biochemical response to survive threats such as abiotic stress [5].

Light plays a significant role in plant growth and development regulations, including seed germination and establishment [6]. According to the study conducted by [7], different light conditions are believed to affect on the growth

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of plant development. This study suggested that optimal light intensity of 10 µmol m^{-2} s^{-1} is enough to grow young plants for the regeneration system of succulent plants. Besides, light quality and intensity also affect the leaves size, density, thickness and colour [6]. For example, under the low light intensity plants leaves are smaller and thinner compared to plant leaves under high light intensity [8].

The chlorophyll content in a leaf usually is influenced by the light intensity factor as the higher the light intensity, the lower the chlorophyll content can be found [9]. This is to avoid the process of photoinhibition which is the process of the inhibition of photosynthesis by receiving excessive light resulting in stunted plant growth which will cause damage to the plants [10].

A study conducted by [9], also stated that the higher the light intensity, the greater the photosynthetic rate that can be found in the leaf area. An increase in light intensity will provide more energy to undergo photosynthesis. However, reductions in light intensity may affect the balance of carbons in plants due to the increasing demand for carbohydrate while the production of them decreases, thus increasing the physiological process while decreasing the yield of photosynthetic [8].

Clinacanthus nutans comes from the family of Acanthacea is a tall perennial herb that can grow up to 1 m tall [11]. The leaves of *Clinacanthus nutans* are simple and narrowly elliptic-oblong or lanceolate in shape where they are located opposite each other [12]. The petioles hold the leaf range between 0.3 – 2.0 cm, while the stem is in a cylindrical shape and has a bamboo-like segmented part. The flowers of this species are sordidly yellow or greenish-yellow in colour, covered with 5-alpha cymules and are very dense at the top of the branches. Two ovules are present in each ovary cell that is compressed into two cells. The oblong capsule is wrapped into four seeded short stalks [12].

Fig. 1 - The leaves and stem of *Clinacanthus nutans* [13]

Clinacanthus nutans poses a lots of medicinal properties that can helps to treat various diseases. A study conducted stated that his species contains anti-viral properties which can be used to treat Herpes simplex virus (HSV) because of the bioactive constituents found in *Clinacanthus nutans* such as polyphenolics, glycosides and terpenes that have being identified as anti-HSV agents [12]. Other than that, *Clinacanthus nutans* also contains antioxidant properties due to the high radical scavenging activity found. The high activity of radical scavenging found may be due to the presence of alkaloids, flavonoids and flavones in the plants [12].

As one species that possessed medicinal properties, *Clinacanthus nutans* were commercialized into various types of products including herbal tea, and supplements in the form of pills and tablets. However, to ensure this species contains the best quality content of medicinal properties to best serve for humans, the growth and development of *Clinacanthus nutans* must be in a suitable condition where the environmental factor such as abiotic stress does not being a threat for this species to grow.

Based on the study done to review *Clinacanthus nutans*, the main problem is how this species responds to different light conditions. Light is a significant factor that helps plant development and growth [7]. Changes in light conditions may affect the plant development as the different rate of light being absorbed may bring different responses morphologically and physiologically to the plant. Hence, this project was done to determine the response of *Clinacanthus nutans* under the different light conditions as the results of plant defense mechanisms.

This paper also is meant to observe the morphological responses of *Clinacanthus nutans* under different light conditions such as plant height, stem diameter, number of leaves, root length leaf size and leaf colour. Furthermore, analysis on physiological responses of *Clinacanthus nutans* under different light conditions such as chlorophyll nad carotenoids content has also been determined in this paper. Furthermore, this paper may help people to understand on how important it is *Clinacanthus nutans* as alternative treatment to modern medicine which also highlighting the knowledge from ethnomedicinal field. Other than that, this paper also is significant in optimizing leaf growth for future references for farmers and company that commercialised products based on *Clinacanthus nutans*.

2. Methodology

2.1 Sample Preparation

Firstly, the plant sample of *Clinacanthus nutans* was bought from the nursery of Nasuha Herbs and Spices Farm located at Muar, Johor in the condition of budding stage growth level. The samples were then relocated in the college area of Universiti Tun Hussein Onn Malaysia, Pagoh campus, to be treat with different light conditions which were condition A and B.

Five plant samples of *Clinacanthus nutans* were grown in two different conditions of light each for 4 weeks. The first five samples were grown under direct exposure of sunlight (A), and another five plant samples were shaded under 50% of polyethylene netting (B) to minimize the sunlight exposure on the plant. The samples were watered once a day in the evening and all other environmental factors remain constant except for the light factor that were controlled using polyethylene netting. The control of the light exposure was to differentiate the response of *Clinacanthus nutans* under different light conditions and to observe its morphological and physiological response.

2.1 Morphological Observation

Morphological observations were done to observe six different parameters changes that occur on the 10 plant samples from the two different light conditions which are plant height, stem diameter, number of leaves, leaves size, leaves colour and root length along with the growth of the sample of *Clinacanthus nutans* for four weeks. The observations were made every week along the four weeks to observe its morphology changes after being exposed to two different conditions. The parameters for plant height, stem diameter, leaves size and root length were measured using measuring tape while the parameters such as the number of leaves being counted manually and the leaves colour were determined using naked eyes.

2.2 Physiological Analysis

2.2.1 The Rate of Photosynthesis

The rate of photosynthesis was calculated by measuring the plant increase in dry matter content (DMC). The higher the dry matter content of the plant sample will indicate the active photosynthesis reaction process undergo by the plant. To undergo this method, the plant sample must be completely dry by using an oven-drying method for 48 hours under 40 ºC. After completely dry, the samples were then weighed using an analytical balance and were calculated using the equation 1 [14].

$$DMC = \frac{dry\ weight}{fresh\ weight} \quad (1)$$

2.2.2 Chlorophyll Content

The chlorophyll content in *Clinacanthus nutans* sample followed the procedures of pigment quantification based on a study conducted by Mizuno & Amaki (2011) [16]. A 10 mm^2 square of leaves sample will be prepared from the top leaves part and soaked overnight in 1 mL of 80% acetone to extract chlorophyll *a*, *b* and carotenoids. The solutions then were centrifuged and the absorbance (*A*) was measured using a spectrophotometer at 663.2 nm, 646.8 nm, and 470 nm wavelength for chlorophyll *a* (*Ca*), chlorophyll *b* (*Cb*) and carotenoids respectively. Meanwhile for the total content of Ca, Cb and carotenoids was determined by using the formula as equation 2, 3 [15] and [16] respectively.

$$Total\ content\ of\ Chlorophyll\ a\ (g/m^2) = 12.25A_{663.2} - 2.79A_{646.8} \quad (2)$$

$$Total\ content\ of\ Chlorophyll\ b\ (g/m^2) = 21.50A_{646.8} - 5.10A_{663.2} \quad (3)$$

$$Total\ content\ of\ carotenoids = \frac{A\ x\ V(mL)\ x\ 10^4}{2592\ x\ P(g)} \quad (4)$$

2.2.3 Statistical Analysis

Statistical analysis was done to analyse the variance to ensure accurate results. By using Statistical Product and Service Solution (SPSS), analysis of variance (ANOVA) was calculated by using the One-Way ANOVA test to compare the means of the data. All the samples are presented in mean ± standard deviation for five replicates.

3. Results and Discussion

3.1 Morphological Observation

Morphological observation was done for a period of four weeks and the observation data been recorded every week to observe any changes occur in different light conditions where condition A means fully exposed under sunlight and condition B means shaded under 50% of polyethylene netting. The average data for every week then were tabulated as below:

Table 1 - Comparison of morphological changes on *Clinacanthus nutans* sample in different condition after four weeks of treatment

Parameters	Condition A	Condition B
Plant height (cm)	56.32 ± 1.010	49.38 ± 1.709
Stem diameter (cm)	1.48 ± 0.083	1.39 ± 0.050
Number of leaves	120 ± 10.330	106 ± 8.468
Root length (cm)	23.62 ± 0.949	18.63 ± 4.647
Largest leaf size (*w x l*) (cm)	2.7 x 12.3	5 x 12
Leaves colour	Lighter green	Darker green

Based on table 1, for parameters plant height, stem diameter, number of leaves and root length in condition A, the samples show a higher value than condition B which indicates that samples that received more sunlight (condition A) will appear bigger and longer than samples in the shade. Plant height in condition A (56.32 ± 1.010) is higher than in condition B (49.38 ± 1.709), as the samples of *Clinacanthus nutans* used in this research is in saplings stage where it required more light and grew faster in high light condition [17]. The number of leaves in condition A (120 ± 10.330) also higher than condition B (106 ± 8.468) as the higher the plant height indicates more leaves and shoots growth [17]. Meanwhile for stem diameter parameter, condition A has higher value than condition B. This is because, under low light condition, the non-structural carbohydrate content in stem is reduced [18]. For root length parameter, root length in condition A is longer than condition B. This is because samples in condition A are more likely to be dry due to more exposure to sunlight which will cause the root to grow longer to find and absorb more water in the soil [19]. However, for leaf size, the biggest size found in condition B is 5 x 12 cm compared to condition A, 2.7 x 12.3cm. This is due to the plant adapting to a bigger surface area in the shade condition to absorb more sunlight for photosynthesis [18]. This result can be supported from a study conducted, where the leaves in the high light condition were observed much smaller than the leaves in low light condition [18]. For statistical analysis result in morphological observation, there is no significance difference between all parameters in both condition. This is due to the p value that higher than 0.05 ($p > 0.05$).

a b

Fig. 2 - Morphological comparison based on colour of *Clinacanthus nutans* samples. (a) Samples in condition A; (b) Samples in condition B

There is also a distinct colour between the samples in each condition. Along four weeks of treatment, leaves samples in condition A slowly turned to lighter green while leaves sample in condition B slowly turned to darker green. Based on fig 2, samples in condition A appeared paler or light green in colour, while samples in condition B appeared in dark green, which indicates more chlorophyll content in the plant [9]. This shows that the higher the light received by the plant, the lower chlorophyll content can be found in the plant [9].

3.2 The Rate of Photosynthesis

The dry matter content was measured on different light conditions to determine the photosynthetic rates of *Clinacanthus nutans* sample under sun and shade condition. The results were tabulated as below:

Table 2 - Comparison of dry matter content (DMC) on plant sample in different condition

	Condition A	Condition B	Significance
Fresh weight (g)	8.56 ± 3.989	8.73 ± 3.083	0.943
Dry weight (g)	2.29 ± 0.927	1.57 ± 0.535	0.168
DMC	0.27 ± 0.017	0.18 ± 0.011	<0.001

Based on table 2, there is significance difference between DMC in condition A and Condition B. The DMC calculated from condition A is higher than condition B. The DMC was calculated from the ratio of dry weight and fresh weight in grams of the *Clinacnathus nutans* samples. In condition A, the DMC calculated is (0.27 ± 0.017) while in condition B the DMC calculated is (0.18 ± 0.011). Higher DMC indicates a higher photosynthetic rate that occurs in plants which resulted from the condition that was fully exposed under sunlight. Photosynthetic rate is important in measuring the gross of light energy being captured and fixed into organic compounds [21]. Higher photosynthetic rates show the activity of light energy being captured during photosynthesis is increased due to the high light condition in condition A which contributes to the higher DMC [21]. From 2, there is no significance difference between parameters in the measurement of rate of photosynthesis except for DMC in both conditions. The result shows that there is significance difference of DMC as the p value is lower than 0.05 ($p < 0.01$).

3.3 Total Chlorophyll and Carotenoids Content

Chlorophyll and carotenoids plays significant role in plant photosynthesis and biochemical processes. Chlorophyll *a* (*Ca*) is the major pigment involved in photosynthesis while chlorophyll *b* (*Cb*) is the accessory pigments that helps in absorbing light energy and pass it into *Ca* [22]. Under different light conditions, the total content of chlorophyll and carotenoids may be different. The results achieved by the calculation of total chlorophyll and carotenoids content in the sample of *Clinacanthus nutans* were tabulated as below:

Table 3 - Comparison of chlorophyll and carotenoids content on plant sample in different condition

Total chlorophyll content	Condition A	Condition B	Significance
Chlorophyll *a* (g/m^2)	0.83 ± 0.463	0.50 ± 0.321	0.224
Chlorophyll *b* (g/m^2)	1.46 ± 0.740	1.47 ± 0.539	0.992
Carotenoids (μg/g)	0.03 ± 0.015	0.03 ± 0.105	0.742

Based on table 3, there is no significance difference between the total chlorophyll and carotenoids content in the samples of both condition. The p value for each parameter are higher than 0.05 ($p > 0.05$). In condition B the total content of *Cb* (1.47 ± 0.539) is greater than the total content of *Ca* (0.50 ± 0.321). This is due to condition B which is shade under netting and receives less sunlight indicating a higher amount of chlorophyll found in leaves. The abundance and increases of *Cb* shows an adaptation to the shade, as it allows the plants to absorb a broader range of wavelengths of light and indicates prevention of chlorophyll from damage [23]. Meanwhile, in condition A, the total content of *Ca* (0.83 ± 0.463) is higher than condition B (0.50 ± 0.321). Lower *Ca* contents observe in condition B explained the lower photosynthetic rates [23] in the condition as table 2. In both condition, the total *Cb* content are higher than *Ca*. This is due to the plant develop strategies to adapt in changing environment where in condition A, *Cb* is used to help absorbing light after the decreases of *Ca* due to excessive of light [24], while in condition B, *Cb* is used to help absorbing broader range of wavelength of light after being shade[23].For the total content of carotenoids, both condition has equal average of content which are 0.03 ± 0.015 and 0.03 ± 0.105 respectively for condition A and condition B. Carotenoids are also involved in the photosynthetic process by transferring some of light energy to chlorophylls in plants under shade and prevent photooxidative damage on plants that are overexposed to sunlight [25]. Under low light condition, carotenoids also play it role in absorbing the light and transfer it to chlorophyll [24].

4. Conclusion

This study showed that *Clinacanthus nutans* in condition A appeared bigger on average and contained higher photosynthetic rates and total content of *Ca*. Meanwhile, the total content of *Cb* and carotenoids of *Clinacanthus nutan*s sample seems equal in both conditions. In conclusion, for morphological observations parameters such as plant height, stem diameter, number of leaves and root length is best to observe under condition A, while leaf size and leaf colour parameters is best to observe under condition B. For physiological observation, photosynthetic rates and total chlorophyll *a* content is best to measure under condition A, while total chlorophyll *b* content is best to measure under condition B. However, it is suggested that the period of treatment should be longer to ensure more accurate results and additional studies on primary and secondary metabolites should be conducted to determine which condition of sun and shade is preferred for the best quality of *Clinacanthus nutans* sample.

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