EFFECT OF CANOPY CLOSURE ON CHLOROPHYLL CONTENT AND ANATOMY STRUCTURE OF *CASTANOPSIS BOISII* LEAVES IN THE REGENERATION STAGE IN BAC GIANG AND HAI DUONG, VIET NAM

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SUMMARY

The research was conducted in Bac Giang and Hai Duong in order to determine the relationship between canopy closure and chlorophyll a, b content and some anatomical features of *Castanopsis boisii* leaves in the regeneration stage. The survey result of 41 leaves samples on 20 transects shows that chlorophyll a content of the regeneration *Castanopsis boisii* leaves ranges from 1.12 to 1.75 (mg/g), chlorophyll b is from 0.47 to 1.0 (mg/g). The ratio of chlorophyll a/b is allocated according to the different canopy levels from 1.75 -2.4. Chlorophyll a content in all canopy levels is much higher than chlorophyll b content. Chlorophyll a accounts for 64-70% of total chlorophyll content in the research leaf samples while chlorophyll b only counts for 30-36% of total chlorophyll content. The first step is to assess the regenerated *Castanopsis boisii* trees in area with common height of less than 2.5 meters and belonging to shade tolerant stage. Quadratic equation and logarithmic equation are used to reflect the relationship between canopy closure and some anatomical features with a coefficient of determination (R^2) ranging from 0.31 to 0.59 indicating that the relationship is at the moderate level to the strong level. Through the regression equations, the study has identified that the light corresponding to canopy closure of 60% makes a difference on chlorophyll content and anatomical features of the regenerated *Castanopsis boisii* in the study area.

Keywords: Canopy closure, chlorophyll a, chlorophyll b, correlation, regenerated *Castanopsis boisii*.

I. INTRODUCTION

Plant life is always influenced by environmental factors, especially the plants at the young stage or the regeneration stage. The sunlight is essential to the existence, growth and development of plants. Besides affecting to the morphology, distribution of species, plants also have some changes in the anatomical features of the leaves when existing at different lighting conditions. *Han et al, 2013* researched and identified that chlorophyll affects to plants photosynthesis process. With light intensity smaller saturation point, photosynthetic rate will be reduced with the reduction of light intensity. Therefore light is considered to be a core factor affect to the efficiency of plant photochemical process (Han S. Cheng FL. 2013).

The light demand of plants is also indicated by the chlorophyll a and chlorophyll b content in the leaf. According to Viviane F. Favaretto (Viviane F. Favaretto et al., 2009), when comparing species under full light condition compared with partially shaded condition ones, the results showed that there is a significant difference in anatomical structure of the leaf, shaded species normally are sensitive to solar radiation and have chlorophyll a, chlorophyll b concentration higher than those in fully light conditions.

The most profound effect of light on plants is the change in the chlorophyll content, which will affect photosynthesis process. In the low light condition, the chlorophyll b content increases faster than that of the chlorophyll a. This leads to a decrease in the ratio of chlorophyll a and chlorophyll b (Bertamini et al., 2006; VF. Favaretto et al., 2011).

Akecop (2010) reported that the chlorophyll content at different leaf positions of the plant was significantly different. For examples, the 3 years old *A auriculiformis* in The Philippines

has the highest chlorophyll content in the low leaves (11.5g/mL); for *Pterocarpus macrocarpus*, the highest chlorophyll content was obtained in the middle leaves and was 18.3g/mL *(*Chanhsamone Phonguodume et al., 2012).

Castanopsis boisii has high economic and ecological value in the Northeast of Vietnam. However, *Castanopsis boisii* forest is currently being degraded both in quantity and quality.

Therefore, the research on "*Effect of canopy closure on chlorophyll content and some anatomical features of Castanopsis boisii leaf in the regeneration stage in Bac Giang and Hai Duong, Vietnam*" was conducted. The main objective of this research was to provide a basis for recommending solutions to adjust the sunlight needed by *Castanopsis boisii* trees at the regenerated stage for producing higher fruit yield at the mature stage.

II. MATERIALS AND METHODS

2.1. Study site

The study was conducted at the natural *Castanopsis boisii* forest in Hai Duong province (20°56′15″N 106°18′52″E) and Bac Giang province (21°16′29″N 106°12′06″ E) in the Northeast of Vietnam. The climate of this area is humid tropical having 4 seasons: spring, summer, autumn, winter, and it is strongly influenced by the northeast monsoon. The average rainfall is from 1500 to 1600mm/year. The average temperature is 23.3°C. The sunshine hours in a year are from 1,524 to 1,600 hours. The average relative humidity is 83% - 86%. Some areas of the high mountain area are very cold, dry, and have hoarfrost at winter, and less affected by the foehn wind in the summer. Some mountainous districts have widespread tornado phenomenon, hail, and floods in the rainy season *(Pham Ngoc Toan, Phan Tat Dac, 1993)*

2.2. Experimental design and sampling method

In the study area, 20 transects were selected so that the transects can go through the typical *Castanopsis boisii* population in different terrain conditions. The transects have a width of 10 meters, and the transects length is not fixed, average over 50m per one. On each transect, 2-3 regenerated trees were randomly selected for sampling the leaves. The selected plants are plants having good growing quality. The leaf samples were taken randomly in the height range of the regenerated trees in the area (<2.5 m), only good leaves on the branches having from 10 to 15 leaves are selected A tape ruler is used to identify the leaf sample's height comparing to the ground, at the accuracy level up to 0.5 cm. Considering to height of regenerated trees, it is divided into 4-5 levels *(Nguyen Toan Thang, 2015)*: Level I ($H < 0.5$ m; Level II ($0.5\ m \leq H < 1$ m); Level III ($1.0\ m \leq H < 1.5$ m); Level IV ($1.5\ m \leq H < 2$ m); Level V ($H \geq 2.0$ m). In the study area, most of the regenerated trees are less than 2.5 m in height, so in this research, the leaf samples were taken mainly at a height of 2 m or less.

The selected leaf samples are noted with symbol and information about height of sampling location to the ground, GPS coordinates, and canopy closure of the main tree layer.

Identify canopy closure of the main tree layer: The sampling tree is at the central position of a standard plot of 100 m^2 (10 m x 10 m). The canopy closure is measured by setting the survey grid systems; There were 50 investigation points in each standard plot The overall canopy closure will be calculated by total value of points multiply by 100/50.

2.3. The analysis method in the laboratory

The whole collected leaf samples were analyzed at the Institute of Forest Biotechnology, Vietnam National University of Forestry. The analysis procedures are as follows:

- Separate and measure chlorophyll a and b in the leaf according to the method by Benz et al. (1980).

- In the visible light region (λ = 400 - 700 nm), chlorophyll molecules's absorption is best in 2 zones: red zone (λ = 662 nm) and blue violet (λ = 430 nm). Therefore, based on the absorption of different wavelength of chlorophyll on the colorimetric machine, we can calculate their content.

Identifying the chlorophyll of the leaf:

- Take a leaf sample of interest to analyze, weigh it accurately up to 0.5g.

- Crush the leaf sample with 2ml acetone solution 80% in a ceramic bowl.

Filter the sample and collect extracts through the glass funnel, then wash several times with acetone 80% until extracts are colorless.

- Transfer the extract into 50 ml flask and use acetone 80% to increase the extract volume up to the quota line.

- Measure the optical density of the extract at the wavelength of 646.6 nm to 663.6 nm on the colorimetric machine.

Then, the content of photosynthetic pigments is calculated by the formula:

$$X_{Chla} = 12.7. D_{663} - 2.69. D_{645} \text{ (mg/l)}$$

$$Y_{Chlb} = 22.9. D_{645} - 4.68 . D_{663} \text{ (mg/l)}$$

Where D_{663} and D_{645} is the optical density measured at the wavelength of 663nm and 645nm separately.

The amount of the pigment in 1g fresh leaves is calculated using the formula:

$$A = \frac{C.V}{P.1000}$$

Where: A is the chlorophyll content (mg/g fresh leaf);

V is the extract volume (ml); P is sample weight (g);

C is the pigment concentration (mg/l); 1000 is conversion coefficient.

Identifying the anatomical feature of the leaf

The sampled leaves are kept freshly, the shape of the leaf is intact. If the samples are dried, they need to be boiled or soaked in the boiling water before cutting, the soak or boil time depends on the solid level of the sample. Cut the leaf sample then put it immediately into a petri dish filled with distilled water.

The sample is bleached with Javen detergent water. Some other samples were dyed. The purposes of bleaching and dyeing are to get clearer, more beautiful and easier to see pictures. Then put the samples on the glass with distilled water or glycerin, and place the glass on microscope to observe composition of the cells.

Using the microscope named Optika M-699 connected to a computer, using the relevant lens size 40 to take photos and measure the size of the cells. Conduct 10 time measurements, and then take the average value to be recorded.

2.4. Data analysis method

The correlation analysis was conducted to establish the relationship between canopy closure and chlorophyll content of the leaves and the leaf anatomical features (using the P value and coefficient of determination R^2). The data were analyzed by SPSS software (*Statistical Package for the Social Sciences 15.0*).

III. RESULTS

Figure 01. Morphology of the regenerated *Castanopsis boisii* leaf

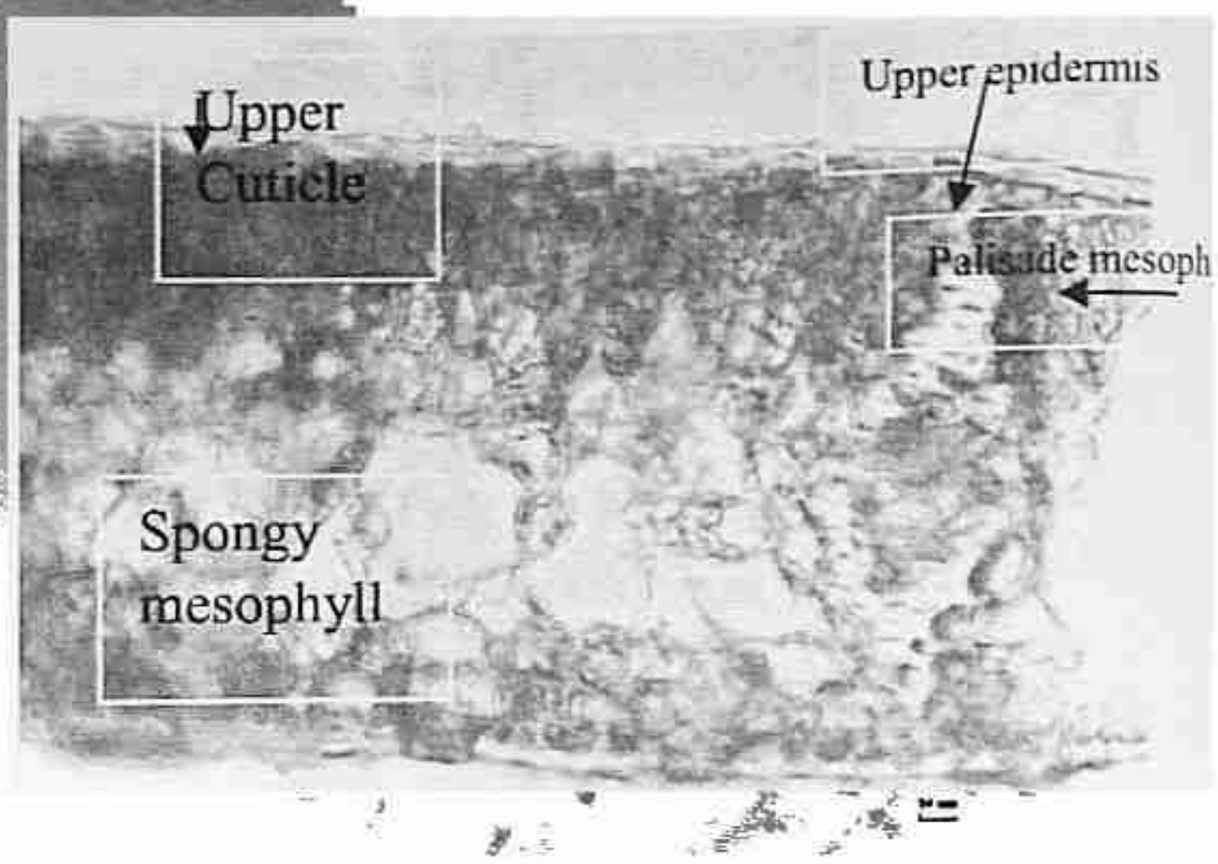

Figure 02. Cross section of *Castanopsis boisii* leaf

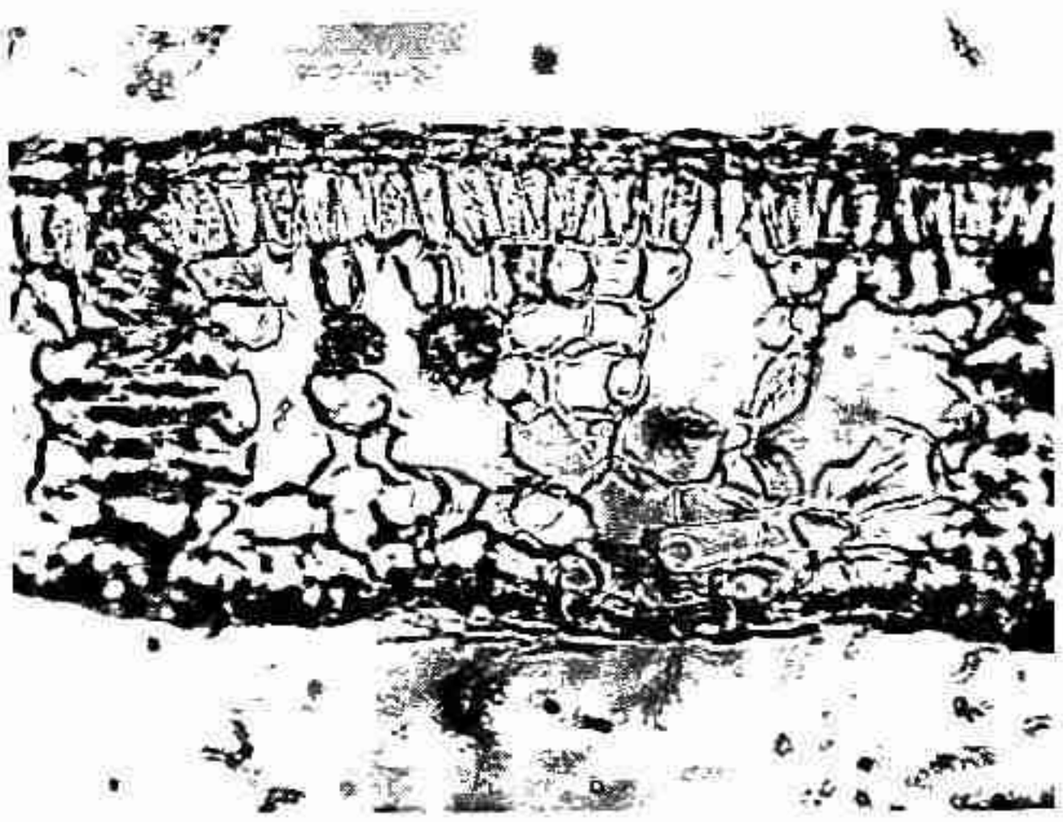

Figure 03. Cross section of the regenerated *Castanopsis boisii* leaf

3.1. The chlorophyll (Chl) a, b content

Based on the data of 41 leaf samples, the chlorophyll content is summarized according to the canopy closure as following:

Table 01. Chlorophyll content of *Castanopsis boisii* leaf according to the canopy closure

	Criteria	Cc < 20%	20%≤ Cc ≤ 40(%)	40% < Cc ≤ 60(%)	60% < Cc ≤ 80(%
Chl a	Average (mg/g)	1.12	1.47	1.75	1.71
	Std.	0.11	0.2	0.15	0.3
	CV (%)	9.83	13.92	8.54	17.4
Chl b	Average (mg/g)	0.47	0.72	1	0.94
	Std.	0.04	0.17	0.12	0.22
	CV (%)	8.57	23.43	11.84	23.18
Chl a + b	Average (mg/g)	1.59	2.19	2.75	2.66
	Std.	0.14	0.35	0.25	0.5
	CV (%)	8.74	15 99	9 1	18.74
Chl a/b	Average	2.4	2.07	1.75	1.84
	Std.	0.2	0 29	0.14	0.23
	CV (%)	8.22	14.17	7.74	12.51

(Where Cc is the canopy closure (%); Std. is Standard deviation; CV is Coefficient of variation)

The Chlorophyll a of the regenerated *Castanopsis boisii* leaf is from 1.12 to 1.75 (mg/g). Chlorophyll b reaches from 0.47 to 1.0 (mg/g). Total chlorophyll a and b is from 1.59 - 2.75 (mg/g), the ratio of chlorophyll a/b is from 1.75 - 2.4. Chlorophyll a content in all canopy closure levels is much higher than that of chlorophyll b. Chlorophyll a accounts for 64

70% of total chlorophyll content in the analysed leaves. While chlorophyll b only accounts for 30% to 36% of the total chlorophyll content. In general, variation in chlorophyll content are quite small, all are less than 25%. The coefficient of variation of chlorophyll a, chlorophyll a +b and chlorophyll a/b ratio are all less than 20%.

For chlorophyll b, the coefficient of variation is higher, ranging from 8.57% to 23.43% at different canopy closure levels. The data shows that when the canopy closure increases from 0 to 60%, the content of chlorophyll a, b tends to increase, and then reduce despite that the canopy closure still continues increasing. This means that the less the amount of the sunlight the plants received, the greater the chlorophyll a and b contents. Up to a certain threshold corresponding to the canopy closure of 60% or more, when sunlight is reduced to a certain threshold, the chlorophyll content does not increase any more and tend to decrease.

Table 02. Chlorophyll content of *Castanopsis boisii* leaf based on the height level of the sampled leaves

	Criteria	H ≤ 0.5 m	0.5m < H ≤ 1.0m	1.0m < H ≤ 1.5m	1.5m < H ≤ 2.0m
Chl a	Average (mg/g)	1.61	1.54	1.47	1.66
	Std.	0.36	0.3	0.29	0.26
	CV (%)	22.7	19.11	19.8	15.58
Chl b	Average (mg/g)	0.87	0.81	0.72	0.94
	Std.	0.22	0.25	0.21	0.22
	CV (%)	25.49	30.98	28.52	23.81
Chl a + b	Average (mg/g)	2.47	2.35	2.19	2.6
	Std.	0.59	0.53	0.48	0.47
	CV (%)	23.68	22.61	22.01	17.94
Chl a/b	Average	1.86	1.99	2.1	1.83
	Std.	0.05	0.31	0.31	0.33
	CV (%)	2.88	15.42	14.93	17.84

(Where H is the height level from the ground to the selected leaf sample, meter (m); Std. is Standard deviation; CV is Coefficient of variation)

The higher, the more sunlight the leaves receive, chlorophyll a and b content decrease more. Up to a height of 1.5 meters or higher, the trend is opposite, it means the content of chlorophyll a, b increases. Chlorophyll a content is from 1.47 - 1.66 mg/g, chlorophyll b content is from 0.72 - 0.94 mg/g, the ratio of chlorophyll a/b is from 1.83 2.1. The coefficient of variation of these criteria is quite high, ranging from 15% to 31%, especially for chlorophyll b content; the coefficient of variation is highest with about 31%.

Therefore, it can be seen that although based on the sampling height level or canopy closure level, the chlorophyll b content always has higher coefficient of variation comparing to that of the chlorophyll a content. Chlorophyll a content is always higher than

that of chlorophyll b, accounting for over 60% of the total chlorophyll content. The ratio of chlorophyll a/b varies significantly from 1.75 - 2.4.

3.2. Anatomical features of Castanopsis boisii leaf

Our microscopic observations showed that the leaves of regenerated *Castanopsis boisii* trees have anatomic structure including full parts as seen in the leaves of the common dicotyledonous plants.

When approaching closer to the midrib, we can observe the thickness of palisade mesophyll is increasing, while the thickness of spongy mesophyll is decreasing gradually. Similarly, the closer the palisade mesophyll to the midrib, the more the layers of palisade mesophyll, being seen in thestreamlined and long shapes. This is true for the common characteristics of the leaves of the dicotyledonous plants. According to Phan Nguyen Hong (1991), the leaf near the stem and on the lower braches is thicker than the leaf on the higher branches due to the development of the palisade mesophyll and spongy mesophyll layers.

Similarly, close to the midrib, the epidermis has the additional dermis layer. The dermis layer is located between the palisade mesophyll and epidermis. This layer is to enhance photosynthesis, and limit the harmful effects caused by the direct and strong sunlight. In overall, the cell has thick wall.

Table 03. Anatomical features (average) based on the canopy closure

Criteria	Cc < 20%			20%≤ Cc ≤ 40 (%)			40% < Cc ≤ 60 (%)			60% < Cc ≤ 80(%)		
	Average	Std.	CV (%)	Average	Std.	CV (%)	Average	Std.	CV (%)	Average	Std.	CV (%)
Leaf thickness (µm)	174 73	12.54	7.18	156 17	22 62	14.48	147.62	17 60	11.92	144.99	23 53	16 23
Cuticle (µm)	2 33	0 68	29 22	2 37	0 42	17 93	2 87	0 36	12 64	2 22	0 52	23.42
Upper epidermis (µm)	8 02	1 38	17 21	8 11	1 55	19 06	8.43	2 45	29.02	7 36	1 70	23 13
Palisade mesophyll (µm)	55 22	2 64	4 78	48 81	7 14	14 64	37 22	8 86	23 81	41 51	7 93	19 11
Spongy mesophyll (µm)	90.08	12.96	14 38	91 21	17 97	19 70	89 70	14 39	16.05	84 99	16 39	19 29
Lower epidermis (µm)	6 09	0 67	11 06	6 19	1 45	23.49	6 86	1 75	25 48	6 18	1 40	22.66
Lower cuticle (µm)	1 73	0 33	19 36	1 84	0 34	18 37	1 93	0 21	10 73	1 72	0 30	17 68
Palisade spongy mesophyll	0 62	0 10	15 78	0 56	0 14	25 21	0 43	0 15	33 77	0 52	0 18	34 00

(Where Std is Standard deviation; CV is Coefficient of variation; Cc is the canopy closure (%))

Based on ourcroscopic observations, the cross section of the leaves at the upper side has thick cuticle layer and there are 1 - 3 layers with the thickness of 2 22 2.87µm The thickness of the upper epidermis and upper cuticle accounts for 5 9% to 7 6% of the leaf thickness; the lower cuticle includes one cell layer with the thickness of 1 72 1.93µm, representing 1.0%-1 3% of the leaf thickness The total thickness of epidermis and cuticle layers account for 10 4% to 13.6% of the leaf thickness. Between two epidermis layers is the

virtue mesophyll layer, the cells of this layer have a clear separation between palisade mesophyll and spongy mesophyll. The thickness of palisade mesophyll layer is from 37.22 - 55.22μm, while the thickness of the spongy mesophyll layer is from 84.99 91.21μm, the total thickness of these layers accounts for 83% - 86% of the leaf thickness.

The ratio of the palisade mesophyll thickness to that of the spongy mesophyll is corresponding to the canopy closure levels < 20%, 20 - 40%, > 40 - 60%, >60 - 80% are 0.62; 0.56; 0.43 and 0.52 separately. The ratio reduces from the canopy closure level less than 20% up to 60%, and then it increases a little bit. The ratio of the palisade mesophyll thickness to that of the spongy mesophyll of the regenerated *Castanopsis boisii* leaf in the study area is from 0.43 - 0.62.

Other indices such as the thicknesses of the upper and lower cuticle, upper and lower epidermis are all increasing accordingly to the increase of the canopy closure up to the threshold of 60%. Over 60% canopy closure, the values have a decreasing trend.

Table 04. Anatomical feature (average) according to the height of the sampled leaves

Criteria	H ≤ 0.5 m			0.5m < H ≤ 1.0 m			1.0m < H ≤ 1.5 m			1.5m < H ≤ 2.0 m		
	Average	Std.	CV (%)	Average	Std.	CV (%)	Average	Std.	CV (%)	Average	Std.	CV (%)
Leaf thickness (μm)	133.35	14.34	10.75	151.85	16.21	10.68	158.12	24.54	15.52	159.27	33.12	20 79
Cuticle (μm)	2.42	1.06	43.93	2.47	0 45	18.40	2.49	0.60	24.03	2.36	0.52	22.10
Upper epidermis (μm)	4.59	2.01	43.69	7.99	1.26	15.73	8.92	1.53	17.18	7.66	2.47	32.20
Palisade mesophyll (μm)	43.51	11 59	26 64	43.82	10.92	24.92	49.57	7.80	15.73	42.72	4 26	9.98
Spongy mesophyll (μm)	75.08	4 91	6 54	88.28	12.44	14.09	91.69	16.37	17.85	92.91	24 86	26.76
Lower epidermis (μm)	5.45	1.14	20.86	6.16	1.13	18.38	7.01	1.67	23.81	6 07	1 82	29 99
Lower cuticle (μm)	1.70	0.20	11.88	1.83	0 34	18.51	1 75	0.28	16.20	1.94	0.24	12.30
Palisade/spongy mesophyll	0.58	0.12	20 28	0 51	0 17	33.42	0.56	0 13	23 78	0 49	0.15	30.86

The results showed that the anatomical structural features of the leaves increases as the height of selected leaf samples increases. However, the thickness of cuticle, upper epidermis and palisade mesophyll only increase until reaching to the height of 1.5m then tended to decrease. The change of the ratio of palisade mesophyll to spongy mesophyll doesn't follow any certain trend.

3.3. Relationship between canopy closure and some anatomical features of the regenerated Castanopsis boisii leaf

The analysis results of the relationship between the main tree layer's canopy closure and some anatomical features of the leaves are showed by the following charts:

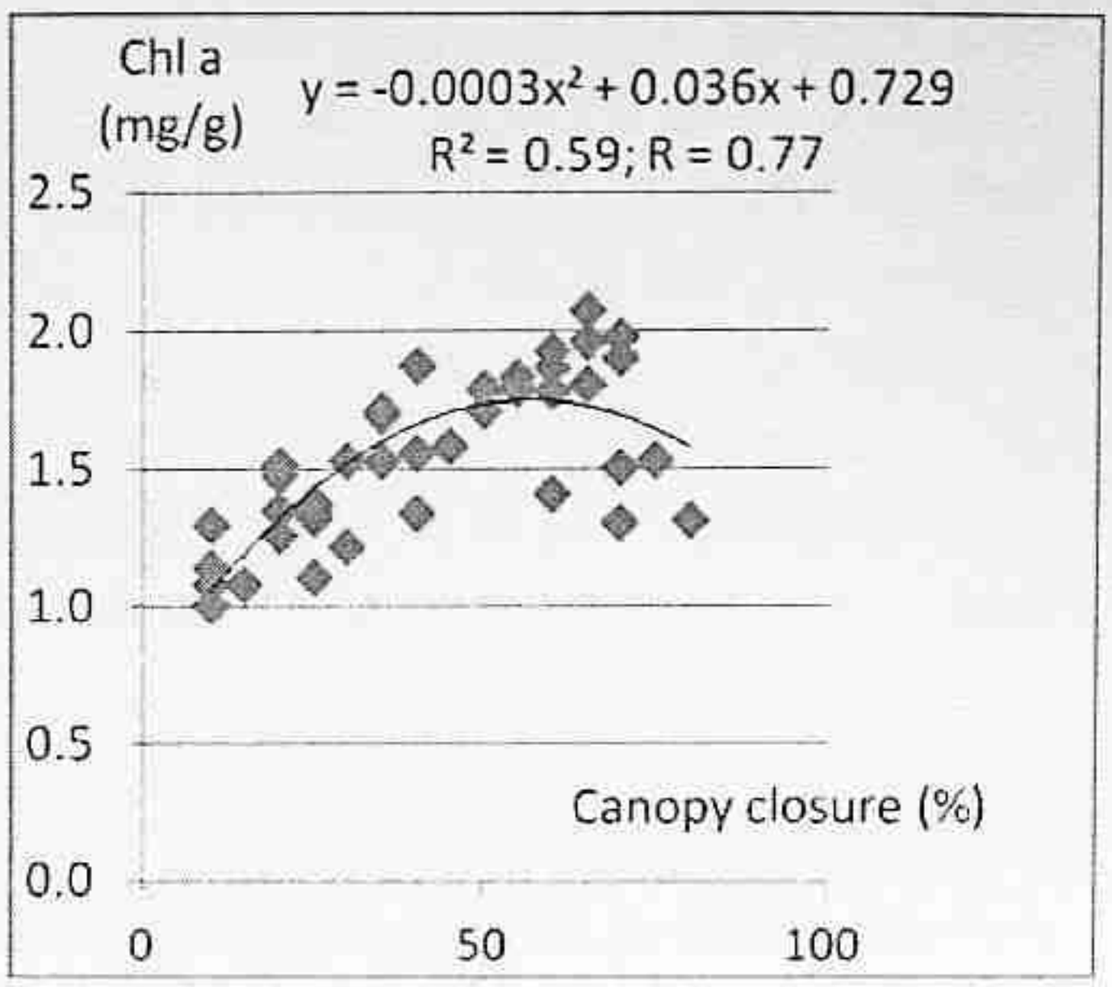

Figure 04. Relationship between canopy closure and chlorophyll a content

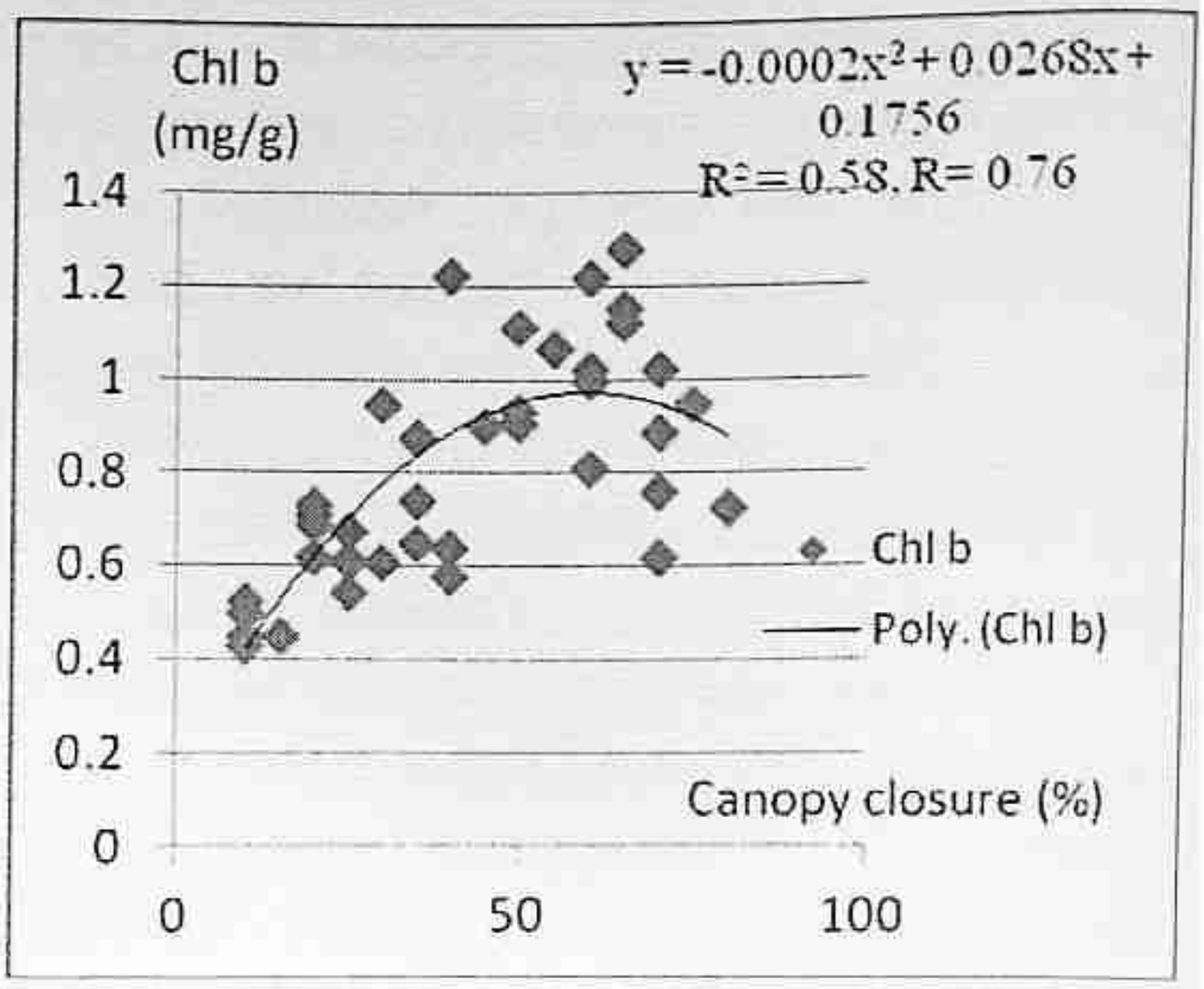

Figure 05. Relationship between canopy closure and chlorophyll b content

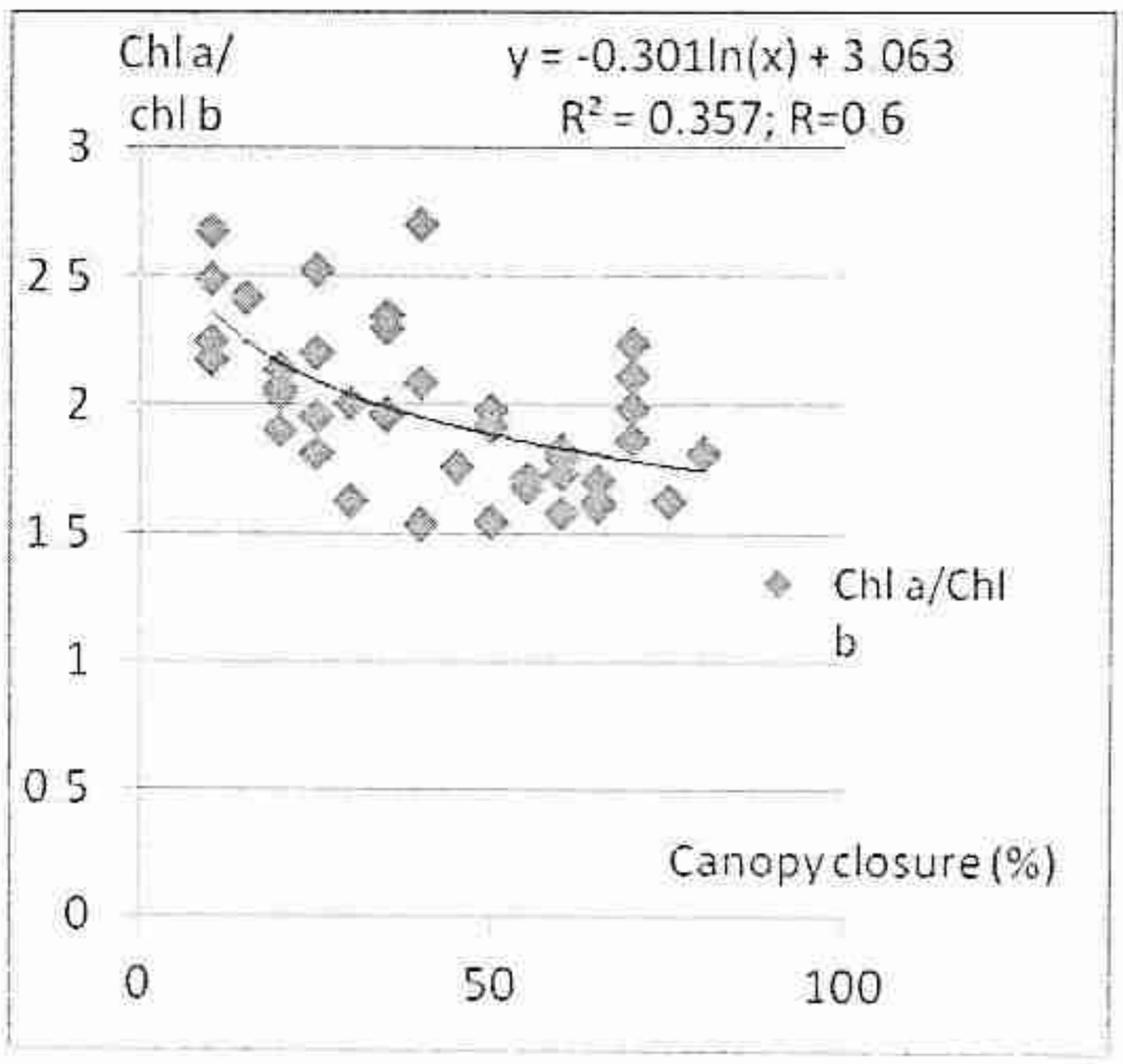

Figure 06. Relationship between canopy closure and the ratio of chl a and chlb

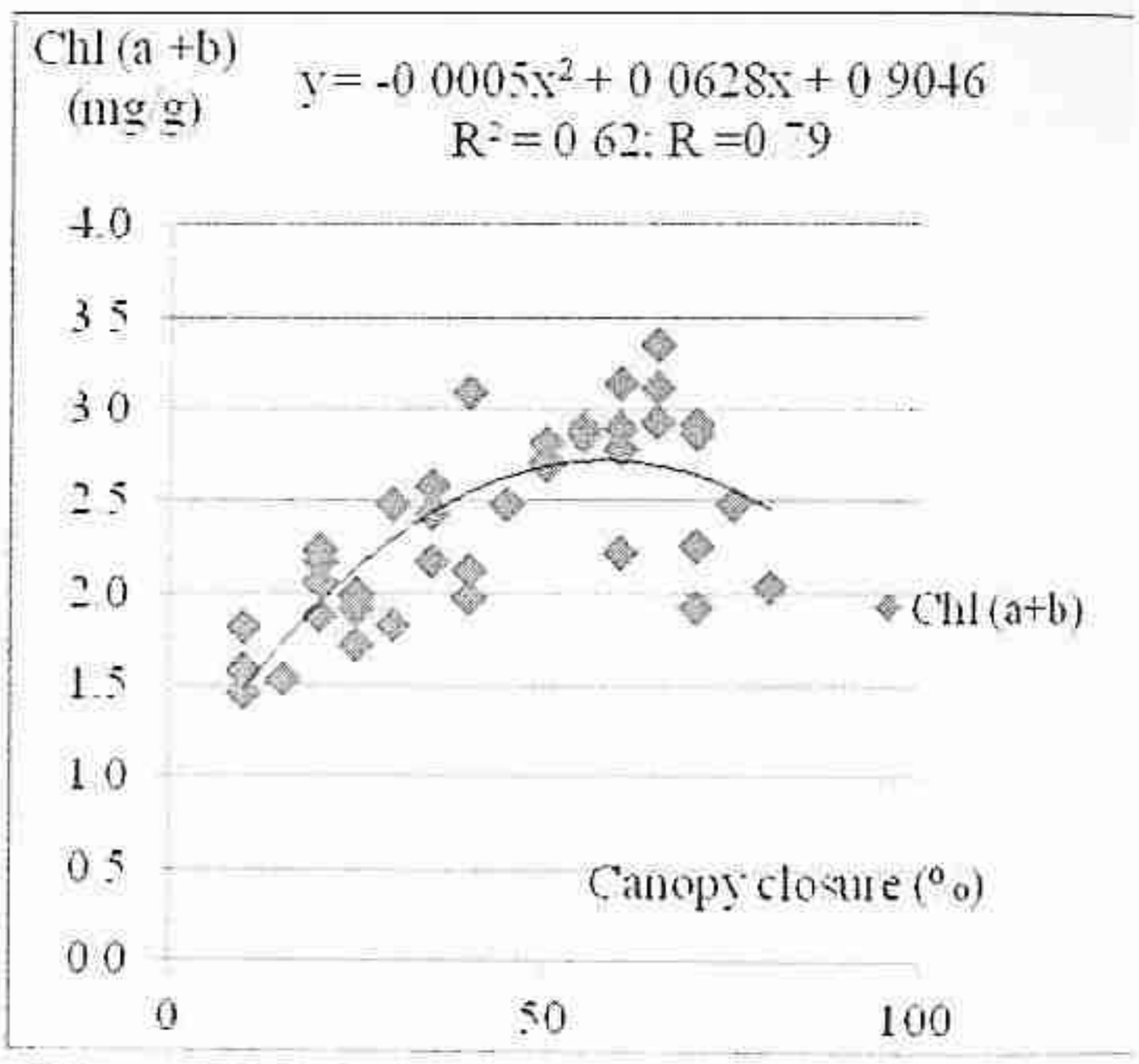

Figure 07. Relationship between canopy closure and total chlorophyll(a+b)

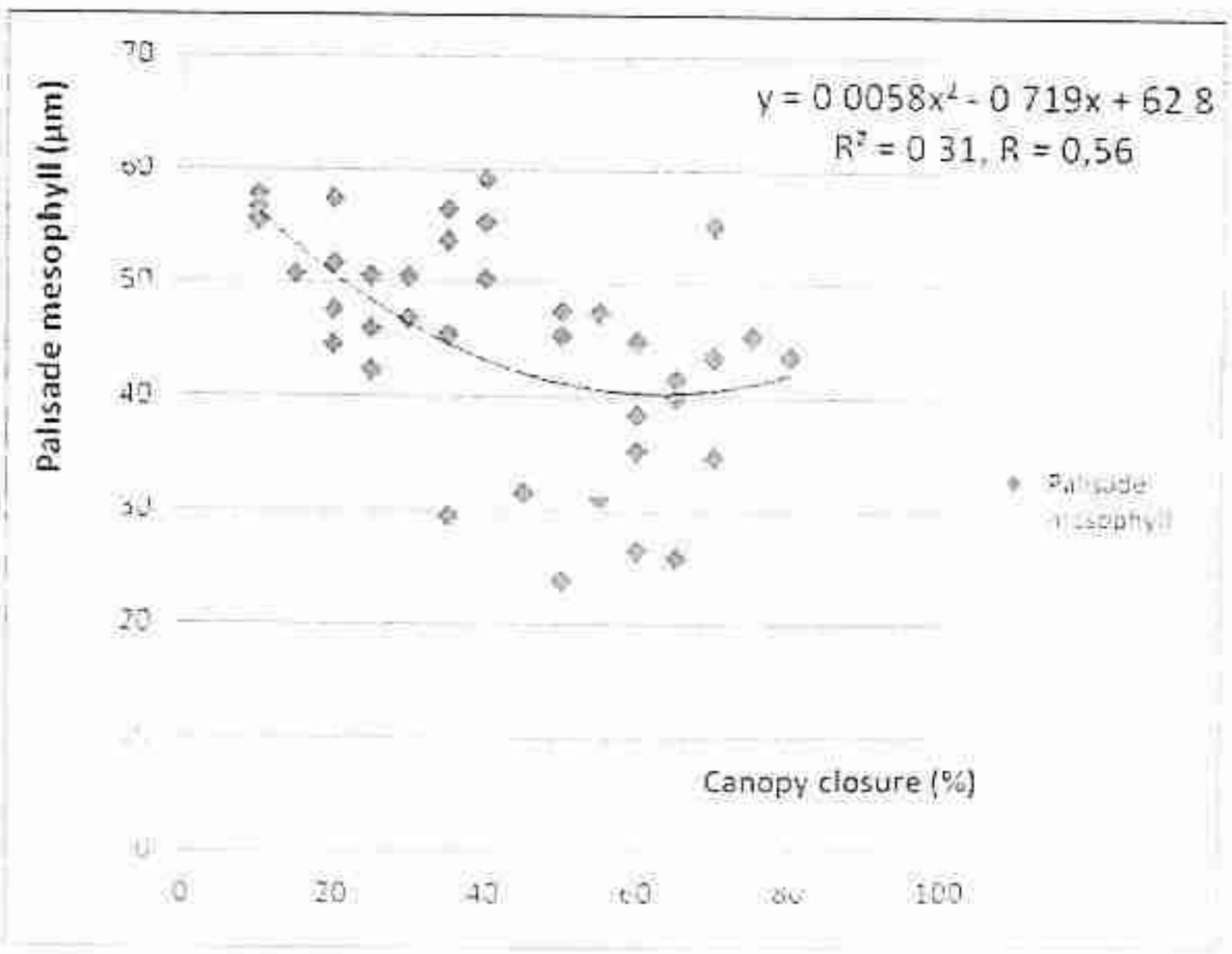

Figure 08. Relationship between canopy closure and the thickness of palisade mesophyll

The chlorophyll a, and b content, the total chlorophyll (a+b) have a relatively strong relationship with the canopy closure with the coefficient of determination (R^2) from 0.59 – 0.62 and it is represented by the quadratic and logarithm equations. Based on these results, we can identify the best canopy closure for the regenerated *Castanopsis boisii* tree is at the inflection point of the quadratic equation curve. Accordingly, the chlorophyll content will increase positively with the canopy closure until reaching to the threshold of 60%, and then it will gradually decrease.

IV. DISCUSSION

4.1. Chlorophyll content

The result of chlorophyll content at the Table 1 and Table 2 shows that the total content of chlorophyll a and b are from 2.59 - 2.75 mg/g. This content is quite small when comparing to the total chlorophyll content of *Tilia cordata*, *Populus nigra* in Salicaceae familia and *Platanus acerifolia* in Platanaceae familia in the shaded conditions (the total chlorophyll content of three trees are from 3.71 – 4.59 mg/g) (H.K. Lichtenthaler, 2009). The ratio of chlorophyll a and b of the *Castanopsis boisii* trees is from 1.75 - 2.4. Based on these results, we can conclude that the *Castanopsis boisii* trees in the regeneration stage belong to the shade tolerant plant group (with the ratio of chlorophyll a and b is less than 2.3). These values are not higher than the those of the common green trees (2.6 3.3) (H.K. Lichtenthaler, 2009). When compared to the ratio of chlorophyll a and b of the *Schyzolobium parahybum* in the strong and low lighting conditions of 3.25 ± 0.4 and 3.27 ± 0.2, the ratio of the 2 chlorophylls (a and b) of the *Castanopsis boisii* trees is not high. And similarly when comparing to the *Hymenaea stilbocarpa* trees with the ratio of chlorophyll a/b of 3.16 ± 0.3 (strong lighting mode) and 2:20 ± 0.1 (weak lighting mode), the ratio of chlorophyll a/b of *Catanopsis boisii* trees are mostly lower (U. C. Malavasi and M. M. Malavasi, 2001).

The data analysis figures shows that the less the sunlight coming down to the lower canopy layers due to the canopy closure of the main tree canopy layer, the chlorophyll a and b content and the total chlorophyll a + b all increases. In most of the samples, the change in chlorophyll a content is greater than the change in chlorophyll b content. The ratio of chlorophyll a/b decreases when canopy closure increases. These rules are presented in the canopy closure of 0 up to 60%. When the canopy closure is over 60%, the trend of chlorophyll content is opposite. It means that chlorophyll content will not increase continuously but decrease, the ratio of chlorophyll a/b will increase. Similarly, the higher the level of selected leaf samples, and the more the sunlight the trees received, the more the decreasing of the chlorophyll content. This rule is valid when the height level of the sampled leaves is less than or equals to 1.5m. When the height is over 1.5m, the chlorophyll content increases significantly.

4.2. Anatomic structure

The leaf thickness decreases gradually from 174.74 147.62μm according to the canopy closure from < 20% to 60%, then the leaf thickness has an upward trend.

The thickness of the upper and lower cuticle layers both have a rising trend of the range of the canopy closure from less than 20% to 60%, then they have a gradual decreasing trend. The

thickness of the lower cuticle layer is equal to 67.2 - 77.6% of the thickness of the upper cuticle layer. The upper epidermis and lower epidermis change is in accordance with the similar rules of the leaf thickness and the cuticle layer. The upper epidermis is greater than the lower epidermis, and its thickness is equal 1.19 - 1.32 times of lower epidermis. Although the thickness of palisade mesophyll is smaller than that of spongy mesophyll (on average only equals to half), the total of these two mesophylls accounts for over 80% of the leaf thickness. The change rules of palisade mesophyll and spongy mesophyll are also opposite. While palisade mesophyll decreases according to the canopy closure level from < 20% to 60%, spongy mesophyll increases. And from 60% threshold, the rule is reversed.

Considering the height level of the sampled leaves, when the height increases, the trees receive more sunlight, and almost indices of anatomic structure increase to the height level of 1.5m, and then decrease.

The ratio of palisade mesophyll and spongy mesophyll changes is not according to any certain rule. However, there are many ratios of palisade mesophyll and spongy mesophyll having the value of over 1.0. If this ratio is used to confirm that the *Castanopsis boisii* trees in the regeneration stage belongs to the shade tolerant plant group, this result is completely matched with the above result based on the effects of the canopy closure levels.

4.3. Correlation between canopy closure and chlorophyll content and palisade mesophyll

Table 05. Summary of correlation between canopy closure and chlorophyll content and palisade mesophyll

No	Equation	The correlation factor	R^2	Sig
1	$Y = -0.0003 X^2 + 0.036 X + 0.729$	Canopy closure and chlorophyll a	0.59	0.000
2	$Y = -0.0002 X^2 + 0.0268 X + 0.1756$	Canopy closure and chlorophyll b	0.58	0.000
3	$Y = -0.0005 X^2 + 0.0628 X + 0.9046$	Canopy closure and chlorophyll a +b	0.62	0.000
4	$Y = -0.301 * Ln.(X) + 3.063$	Canopy closure and chlorophyll a/b	0.36	0.000
5	$Y = -0.0058 X^2 - 0.719X + 62.8$	Canopy closure and thickness of palisade mesophyll.	0.31	0.001

Based on the above equations, the canopy closure has the strongest relationship with total chlorophyll a + b, chlorophyll a, and chlorophyll b.

In regard of the ratio of chlorophyll a/b and the thickness of palisade mesophyll, with the coefficient of determination (R^2) is from 0.31-0.35 corresponding to the correlation coefficient R from 0.56 - 0.59, the relationship is at moderate level. If this result is compared to the chlorophyll content of the mother plant, in complete open sunlight conditions, we can determine the canopy closure, the regenerated tree's height so the regenerated *Castanopsis boisii* trees will move from the shade tolerant ones to the photophilic group. This is a very useful basis to determine the operational measures for providing the sunlight needed by the regenerated *Castanopsis boisii* trees in different growing stages.

V. CONCLUSION

This study has identified the chlorophyll a and chlorophyll b content, the ratio of chlorophyll a and chlorophyll b, the anatomical features of the regenerated *Castanopsis boisii* leaves of the tree height of less than 2.5m. Accordingly, the ratio of chlorophyll a and chlorophyll b on different canopy levels ranged from 1.75 to 2.4, therefore, the *Castanopsis boisii* trees at this stage belong to the shade tolerant plant group. The chlorophyll a content is always greater than that of chlorophyll b, accounts for 64 - 70% of the total chlorophyll in the analyzed leaves. Our quadratic equations and logarithmic equations were used to reflect the relationship between canopy closure and chlorophyll contents; between canopy closure and some anatomical features of the leaves. The coefficient of determination (R^2) of those relationships ranges from 0.31 to 0.59, indicating that there existed from moderate level to strong level of the relationships. Through the regression analyses, our research initially identifies that the sunlight corresponding with the canopy closure of 60% has significant effects on the chlorophyll contents and the anatomical features of the regenerated *Castanopsis boisii* leaves of the tree height less than 2.5 m in the study areas.

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ẢNH HƯỞNG CỦA ĐỘ TÀN CHE ĐẾN HÀM LƯỢNG DIỆP LỤC VÀ CẤU TRÚC GIẢI PHẪU LÁ DẺ ĂN QUẢ (*CASTANOPSIS BOISII* HICKEL ET A . CAMUS) Ở GIAI ĐOẠN TÁI SINH TẠI BẮC GIANG VÀ HẢI ĐƯƠNG, VIỆT NAM

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TÓM TẮT

Nghiên cứu được thực hiện ở Bắc Giang và Hải Dương nhằm xác định mối quan hệ giữa độ tàn che với hàm lượng diệp lục a, b và một số đăc điểm giải phẫu của lá Dẻ ăn quả ở giai đoạn tái sinh. Kết quả điều tra 41 mẫu lá trên 20 tuyến điều tra cho thấy: Hàm lượng diệp lục a của lá Dẻ tái sinh dao động từ 1,12 - 1,75 (mg/g), diệp lục b đạt từ 0,47 - 1,0 (mg/g). Tỷ lệ diệp lục a/b phân theo các cấp tàn che khác nhau từ 1,75 - 2,4. Hàm lượng diệp lục a trong tất cả các cấp tàn che đều cao hơn nhiều so với diệp lục b. Diệp luc a chiếm từ 64 - 70% tổng hàm lương diệp lục trong lá cây nghiên cứu. Trong khi đó diệp lục b chỉ chiếm từ 30 - 36% tổng lượng diệp luc. Bước đầu đánh giá cây Dẻ tái sinh trong khu vực với độ cao thường gặp nhỏ hơn 2,5m thuộc nhóm cây chịu bóng. Phương trình bậc hai và phương trình dạng logarit được sử dụng để phản ánh mối quan hệ giữa đô tàn che với môt số đặc điểm giải phẫu lá với hê số xác đinh R^2 từ 0,31 - 0,59, quan hệ ở mức từ vừa đến chăt. Qua các phương trình, nghiên cứu đã xác đinh ánh sáng ứng với độ tàn che 60% tạo sư khác biệt về hàm lương diệp luc và đặc điểm giải phẫu của Dẻ tái sinh tại khu vực nghiên cứu

Từ khóa: Dẻ ăn quả tái sinh, diệp lục a, diệp lục b, độ tàn che, tương quan.

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