



PHYSIOLOGICAL AND MORPHOLOGICAL STUDY OF *Croton tiglium* LEAVES EXPOSED TO DIFFERENT LIGHT CONDITIONS


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**ABSTRACT:** In this study we have taken *Croton tiglium* to study the effect of different light conditions on the physiological and morphological appearance of the plant. *Croton tiglium* is an ornamental plant used for the interior and garden decoration, belongs to the family Euphorbiaceae, is one of the most popular ornamental plants because of vivid foliage colours and varied leaf shapes. When it is allowed to grow under different light conditions and tested by various parameters like Chlorophyll and Carotenoid Content, Leaf Area and Specific Leaf Weight, Light Microscopy, statistical analysis etc the results shows Low light intensities (LLI) increases the concentration of *chl a* and *chl b* while high light intensities (HLI) on the otherhand reduces the level of *chl a* and *chl b* in the leaves of *Croton tiglium*.

**Key words:** *Croton tiglium*, LLI, HLI, *chl a*, *chl b*

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## INTRODUCTION

Generally it is found that the leaves of plants in shade are thinner and have numerous large chloroplasts than the leaves that are under the sunlight. Different species respond separately to different light intensities. Plant grown in low light condition have higher specific leaf area (SLA) and Leaf area ratio (LAR) and lower biomass and root shoot ration (R/S ratio) [1,2]. Light demanding species are more flexible in morphology and biomass allocation in response to light change than shade tolerant species. Hence, light plays an important role in the environment, controlling the process associated with dry matter accumulation and thus contributing to plant growth. The species adaptative plasticity to solar radiation depends on the adjustment of the photosynthetic apparatus, in order to render radiant energy conversion in carbohydrates highly efficient and, consequently, to promote higher growth [3-7].

The genus *croton*, established by Linnaeus in 1737, is extensive, 625 species being recognized in the Index Kewensis. We have a number of herbaceous species in this country, but none of any economic importance. The *croton* plant is a native of India and is grown all through the East Indies. It is a small tree fifteen to twenty feet high. The leaves are ovate, petiolate, acuminate, alternate, the margins faintly serrate. The flowers are borne in loose terminal spike-like racemes, and are monoicous, the male flowers being at the top of the the raceme, the females below. The male flowers have five sepals, each sepal bearing a yellow gland, five petals, and from ten to twenty stamens with slender filaments. The female flowers have floral envelopes similar to those of the male, and a large sessile, three-celled ovary, thickly covered with stellate hairs and bearing three slender styles, each style dividing into two linear stigmas. The fruit is a three-celled capsule, each cell having a single seed which yields the *croton* oil of commerce [8-11].

*Croton tiglium* is considered indigenous to Malabar, Ceylon, Amboina (of the Molucca islands), the Philippines and Java. Joannes Scott, in his dissertation on the medicinal plants of Ceylon (Edinburg, 1819), states that the seeds of *Croton tiglium* under the name of “gayapala,” are a most powerful purgative, and also that the leaves are very acrid, causing an intolerable burning in the mouth and throat [12]. This suggests that croton is a kind of plant that has good mechanisms for light acclimation. In this research programme the aim was to study the physiological, morphological and statistical analysis of adaptation of *Croton tiglium* leaves under different light conditions.

## MATERIALS AND METHODS

### Treatment plant materials with different light conditions

Stem-cutting croton *Croton tiglium* plants were soil grown in pots, with one plant per pot. Before starting the experiment, all the same-sized plants were maintained for three weeks in a glasshouse with medium light intensity. For the whole day (8 AM– 5 PM), the average intensity was about  $200 \mu\text{mol m}^{-2}\text{s}^{-1}$ . When the experiment was started, the croton plants were divided into two groups. A least 50 plants were used in each group. One group was exposed to full sunlight, of which the average light intensity for the whole day was  $640 \mu\text{mol m}^{-2}\text{s}^{-1}$ . This treatment is called the high light intensity (HLI) treatment. The other was grown under the shan, which reduced the average light intensity to  $25 \mu\text{mol m}^{-2}\text{s}^{-1}$ . This treatment is called the low light intensity (LLI) treatment. All plants were completely watered and nutritionally supplied for the whole experiment [13].

### Analysis of statistical data

All experiments were done with Completely Randomized Design (CRD) with 3 replicates. Means were compared by one-way analysis of variance and Least Significant Difference (LSD).

### Measurement of level of Carotenoid and Chlorophyll content

Total photosynthetic pigments were extracted from 0.5 g of leaf tissues with 10 ml of 80% acetone. The supernatant was then used for the determination of the chlorophyll *a* (chl *a*), chlorophyll *b* (chl *b*), and carotenoid contents [13].

### Measurement of specific leaf weight and area

Leaf area was determined by using leaf area meter (Systronics digital portable leaf area meter Model WDY500A). To determine the specific leaf weight, fifteen leaf discs from each sample were randomly chosen to measure leaf fresh weight and dry weight. Then, the specific leaf weight was calculated from the equation:

Specific leaf weight = leaf dry weight / leaf area. [13]

### Light Microscope

At least 15 slice of leaf tissues were sampled from one leaf. For each treatment, 3-5 replicates for each leaf position were obtained. Forty-micrometer thick leaf cross-sections were viewed using a Nikon microscope. Photomicrographs were taken using Kodak Gold 100 film with automatic exposure setting.

## RESULTS AND DISCUSSION

### Adaptation of Photosynthetic Pigment to Different lighting condition

Three replicates were used for the measurements to determine the photosynthetic pigment contents changes, chl *a* and chl *b*, and carotenoid content in the leaves, these were mature before starting the light treatment (the fifth leaf from the top). Data collection was performed every two weeks for seven weeks. The pigment content determination was also done in the mature leaves, developing under different light intensities. Three replicates were used for each experiment. Each treatment was done by using 20, 30 and 40 day-old leaves. Chl *a* and chl *b* contents in the fifth leaves when the plants were moved to the light treatment condition (time = week 0) were approximately  $580 \mu\text{g/g}$  FW, and  $190 \mu\text{g/g}$  FW, respectively (Fig. 1). After three weeks in high light intensity, chl *a* and chl *b* contents in the LLI leaves were significantly different from those in the HLI leaves. Compared with the chlorophyll content in the leaves at time 0, both chl *a* and chl *b* contents in LLI leaves increased, while chl *a* and chl *b* contents in HLI leaves decreased. The increase in chlorophyll content in LLI leaves and the decrease in chlorophyll content in HLI leaves were also detected in the fifth week after the light treatment. In the seventh week, both chl *a* and chl *b* levels were similar to the levels previously detected in week 5 (Fig. 1). No light intensity effect could be detected for the carotenoid content of the mature croton leaves (Fig. 2). After seven weeks of light treatment, the leaf colours of the LLI leaves and HLI leaves could be easily distinguished. HLI leaves were more yellowish than the LLI leaves. Therefore, this characteristic was in agreement with the photosynthetic pigment content data, indicating that the chlorophyll content increased in LLI leaves and decreased in HLI leaves. Interestingly, a decrease in chlorophyll content was observed in the yellowish leaves, while throughout the period of the light treatment the carotenoid content was maintained at the same level (Fig. 2).

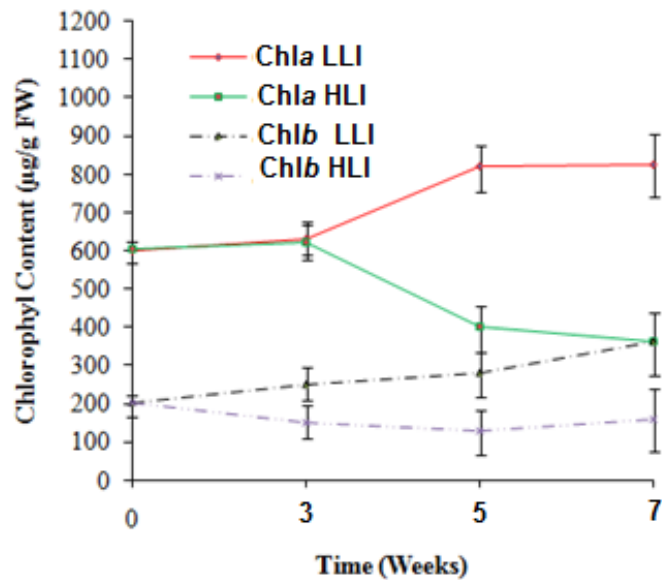


Figure 1. Statistical analysis data of level of Chl *a* and *b* contents in the fifth leaves from the top. Error bars represent  $\pm 1$  SD

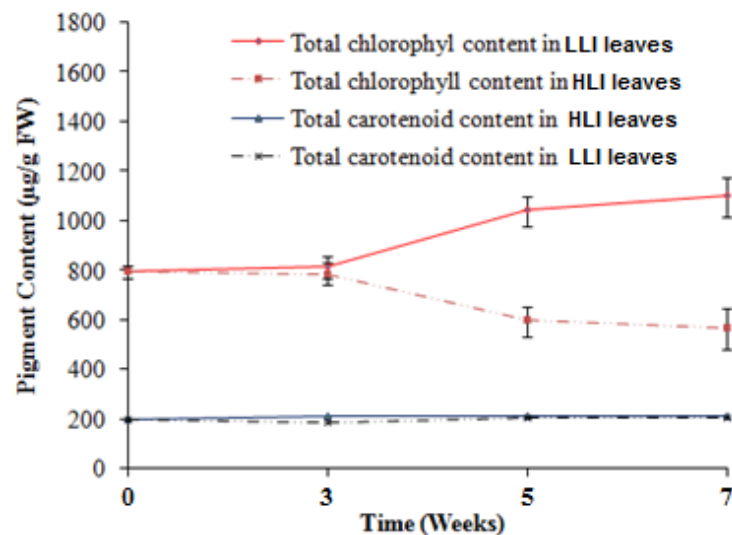
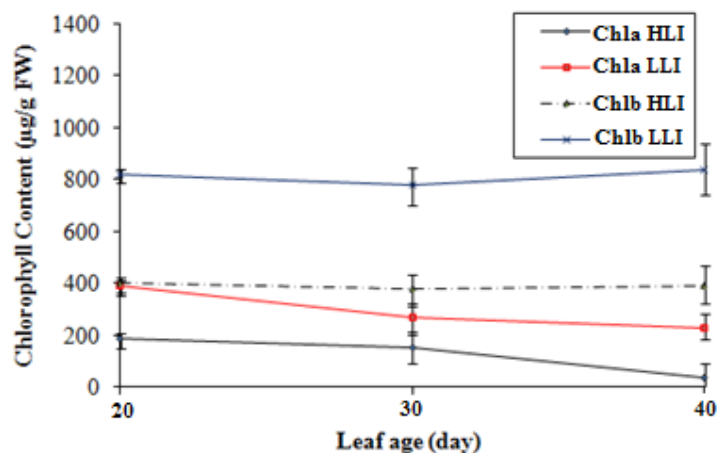


Figure 2. Total chlorophyll and total carotenoid contents in the fifth leaves from the top, starting the experiment (week 0). Results are the means of five replicates and error bars represent  $\pm 1$  SD.

At the ages of 20, 30, and 40 days old the photosynthetic pigment level of leaves were detected developing under the LLI and HLI conditions. Leaves at different ages under the same conditions showed similar levels of chlorophyll content, but both chl *a* and chl *b* levels in the HLI leaves were significantly lower than the ones in LLI leaves (Fig. 3). The carotenoid level is independent on leaf age. A slightly higher level of carotenoid content was detected in the LLI leaves when compared with the HLI leaves (Fig. 4). This suggests that in case of the LLI leaves there is an increase in the antenna complex size. The chl *a/b* ratios of 20, 30, and 40 day-old leaves developing in the same light conditions, were similar. The average chl *a/b* ratio of the LLI leaves was 2.5, which was significantly different from that of HLI leaves, 3.4. These data indicated that when the croton leaves developed in the low light intensity condition, the antenna complex was increased in order to maximize the light absorption for photosynthetic processes in the low light intensity environment. However, a difference in chl *a/b* ratio in the LLI leaves and HLI leaves that were fully developed before the light treatment was not clearly seen from this experiment. The adaptation in chlorophyll content composition in croton leaves to the different light regimes is similar to that found in other species. [2,13].

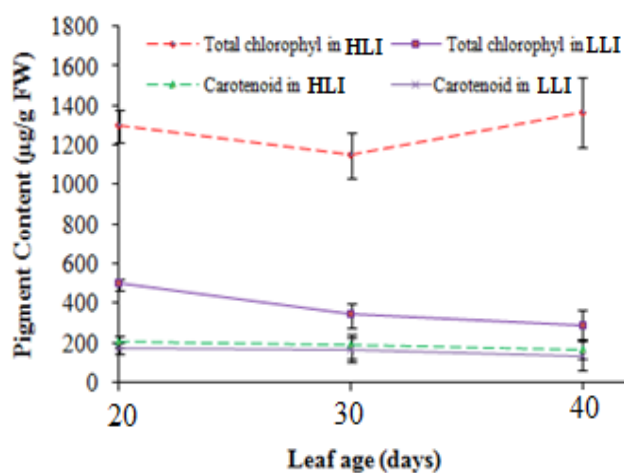


**Figure 3. Chl *a* and *b* contents in the mature leaves that developed in the high light intensity (HLI) or low light intensity (LLI) condition.**

There was quite stable carotenoid content observed under both conditions. Because of the photoprotective role of carotenoids, their content was expected to increase under the high light condition [11]. The stable carotenoid content in croton leaves in high light intensity conditions suggested that the conditions used in this experiment were not so stressful that the plants had to adjust themselves by increasing the carotenoid content. Only the reduction of the antenna complex size by decreasing the chlorophyll content was enough for plant survival in such conditions.

#### The Effects of Light Intensity on the Leaf Area

The fully developed fifth leaf from the top of the plants was chosen to measure the leaf area. The fifth leaf was developed before the treatment started. The leaf sizes were varied (Fig. 5), but the varying light intensity did not lead to a significant difference in leaf area. On the contrary, the leaf areas of new mature leaves developing in the different light conditions were significantly different. The 20 day-old LLI leaves were approximately 4 times larger than HLI leaves at the same age (Fig. 6). In the high light intensity condition, the leaves at the different ages did not show significant differences in leaf size, but in the low light treatment, the newer leaves tended to be significantly larger than the previous ones that developed in the same condition (Fig. 6). These data indicated the adaptation of the leaf size in the low light condition in order to maximize the photosynthetic capacity by enlarging the leaf size. The smaller leaf area in the HLI leaves was advantageous for the HLI grown plants. The higher plant growth rate can be achieved as the smaller leaves on the top part of the plants will allow the sun light to get through to the lower ones, maximizing the capacity for the photosynthesis of the whole plant.



**Figure 4. Total chlorophyll and total carotenoid contents in the mature leaves that developed in the high intensity light (HLI) or low intensity light (LLI) environment.**

### The Effects of Light Intensity on Leaf Thickness

Thickness of leaf was indicated by specific leaf weight data. Light intensity did not affect the thickness of the leaves that were mature before the light treatment was started, according to the specific leaf weight data (Fig. 7). A consistent result was also found from the leaf anatomy study. There is no significant difference between observed between LLI and HLI leaf structure during seven weeks of the light treatment (data not shown). In contrast, the light intensity effects on leaf thickness could be clearly seen in the leaves that developed in the different light conditions (Fig. 7). The leaves developing to maturation in the LLI condition were thinner than the same-aged leaves developing in the high light condition, resulting in lower specific leaf weight (Fig. 7).

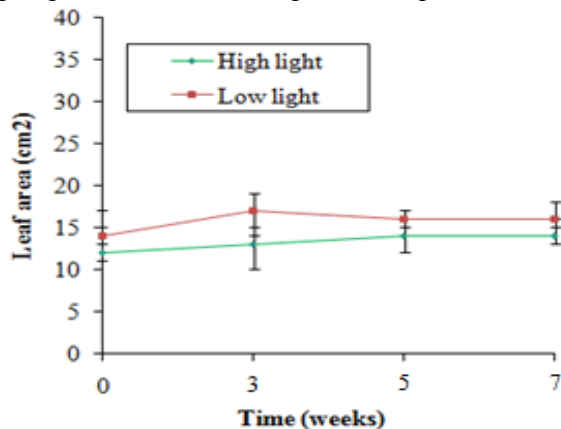


Figure 5. Leaf area of the fifth leaf from the top of the plant starting the experiment (week 0).

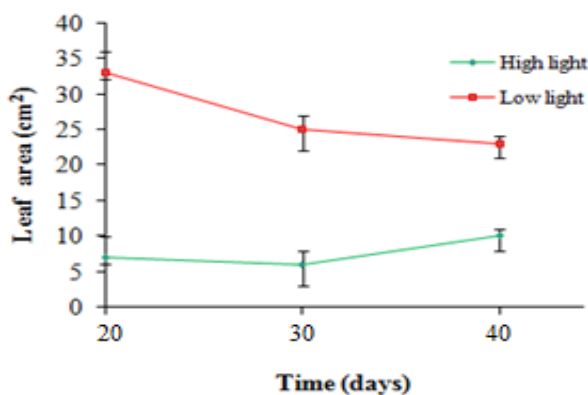


Figure 6. Leaf area of the mature leaves (20, 30, and 40 day-old leaves) developing in the provided light treatments.

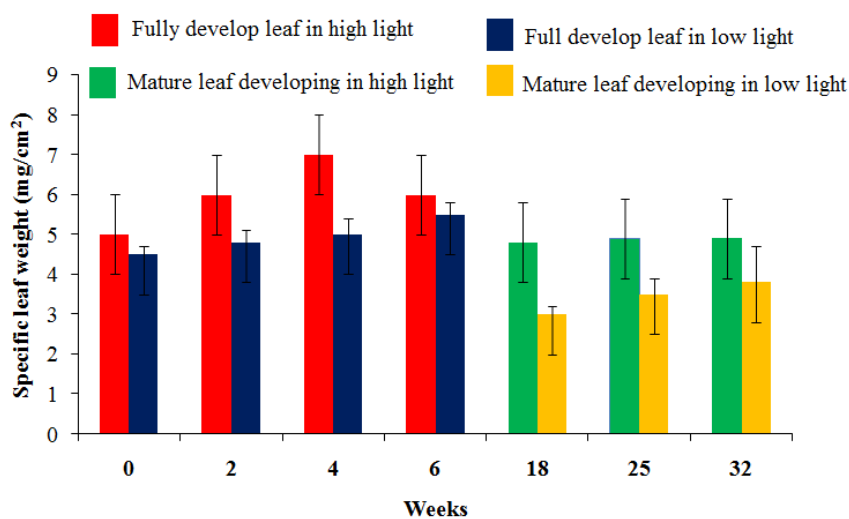


Figure 7. Specific leaf weight of the leaves that fully developed before and during the light treatment. The results obtained from fully developed leaves are the means of five replicates, and the results obtained from the developing leaves are means of three replicates.

Because of the sieve effect and light channeling, the light penetrates the first layer of palisade cells, [13] In the high intensity light condition, many cell layers benefit from the light absorption, but in the low light condition, the irregular shaped cells increase the interfaces between air and water which reflects and refracts the light, thereby randomizing its direction of travel. This process of light scattering increases the probability for light absorption [13]. The second layer of irregular-shaped palisade cells in the LLI leaves may help the light scattering process to increase the light absorption.

## CONCLUSION

The physiological, morphological and anatomical adaptations of croton leaves were strongly affected by the intensities of lights. The chlorophyll content has found to be changed as compared after the treatment of leaves with different intensities of lights. LLI increases the concentration of chl *a* and chl *b* while HLI on the other hand reduces the level of chl *a* and chl *b* in the leaves of *Croton tiglium*. Mature leaves remained unaffected and shows no morphological and anatomical changes.

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