Morpho-histological consistency on petiole length elongation of *Anthurium* under partial shades©

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Abstract

In *Anthurium*, the consistency of partial shading is of crucial importance in relation to the elongation of petiole length, but the anatomical basis for these responses is a question. In this study, we investigated the effects of partial shade on the anatomical aspect of elongation changes in petioles by determining the changes in cell size, cell number, and total cell area. From a histological perspective, three developmental processes, cell size, cell numbers, and total cell area, are responsible for the length of a given petiole. The experiment was conducted by utilizing three shading treatments, i.e., full sunlight, 40% reduced light, and 60% reduced light. Morphological traits (plant height and petiole length), histological traits (cell size, cell number, and total cell area), characterizing the petioles, as well as the physiological traits (SPAD value and leaf area) characteristics were measured. We found that plant height, leaf area, and SPAD value increased linearly with increasing partial shade. In this context, cell size, cell number, and total cell area also increased with increased petiole elongation.

INTRODUCTION

Light is an important environmental information source that plants use to modify their growth and development, and it regulates and optimizes the growth and development in biotic and abiotic condition (Begna et al., 2002; Kozuka et al., 2011). In general, light is sensed by photosensors that respond to different light wavelengths (Kozuka et al., 2011) and it has diversified physiological and phenotypical function by responding of photoreceptors (Briggs and Christie, 2002; Demarsy and Fankhauser, 2009). They regulate a wide range of responses in plants, including phototropism, chloroplast movement, stomatal opening, leaf flattening, and floral induction (Sakai et al., 2001; Sakamoto and Briggs, 2002); all of which influence photosynthetic efficiency. On the other hand, the quantity of light or light intensity influences photosynthesis in plant and that accumulates the biomass and dry matter (Devkota and Jha, 2010); in addition the partition of carbon is also mediated by the quantity and quality of light (Begna et al., 2002). Consequently, low light intensity is responsible for increasing intermodal elongation (Armitage, 1991). In addition, low and high light intensity also affects cell size and thus affects plant growth.

Under low light condition, cell wall modifying proteins increase cell wall extensibility and thereby facilitate cellular expansion during shade-induced extension growth (Keuskamp et al., 2010).

Anthurium, a beautiful cut flower, can be grown in low light; because it is a tropical shade plant, it does not thrive well under high light intensities and shade must be provided for its satisfactory growth and flowering (Hlatshwayo and Wahome, 2010). The quality and quantity of diffused light are the most important factors influencing foliage plant performances under shade conditions (Jeong et al., 2009; Vendrame et al., 2004). However, the growth performance of anthurium, including cell size determination is necessary for anthurium under shade condition. Because shade stimulates cellular expansion and rapid cell division this results in increased petiole length and plant height (Schoch, 1982). Shading treatment resulted in the tallest plants and, on the other hand, smallest plants are observed

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under shade free area (Miah et al., 2008). The quality and quantity of light are the main sources that plants use to regulate and optimize their phenotypical and physiological functions through the response of photoreceptors (Demarsy and Fankhauser, 2009). Even phenotypic response to light can vary within a species, suggesting that selection may allow for development of cultivars with enhanced shade tolerance (Siemann and Rogers, 2001). Therefore, this study was aimed at understanding the petiole length variation of anthurium under partial shades conditions.

MATERIALS AND METHODS

Plant materials and treatments

Anthurium andraeanum plants were obtained from a commercial nursery in July 2012 and then multiplied at Sher-e-Bangla Agricultural University Horticultural farm. New plantlets were produced from their suckers. The experiment was arranged in partially control environment at the Horticulture Farm, Sher-e-Bangla Agricultural University, Dhaka, during the period April 2013 to February 2014. This experiment was also carried out in a split-plot design with four replications which comprise 60 pots. Anthurium plants, more or less uniform size, were used with four leaves and a single plant was grown per pot. The size of each pot was 25 cm (10 in.) in diameter and 20 cm (8 in.) in height. Pulverized coconut husk was mixed with a small amount of soil (coco dust:soil, 10:1) as growing medium. Thereafter, three levels of light intensities were applied such as, L_0, control (100% light/full sunlight); L_1, 40% reduced light (60% of the full sunlight); and L_3, 60% reduced light (40% of the full sunlight). Plants were allowed to establish before the first sampling to end of the experiment with these treatments.

Growing and shade condition

Light intensity levels were maintained using black nylon (60 mesh) net (Black et al., 2003). A single and double layer net reduced light intensity by approximately 40% and approximately 60%, respectively. Light intensities were measured by a CEM DT-1301 Digital Lux and fc light meter (Shenzhen Everebest Manhinery Industry Co. Ltd., China). Hence, relative humidity and temperature were recorded in accordance with monthly weather report of Dhaka Weather Forecasting Department. Mitchell (1953) found that the speed and pattern of morphological development of the plant was determined by contemporary rather than previous light and temperature conditions.

Measurement and calculation

In morphological data, plant height, petiole length, and leaves number were recorded at the different days after transplanting (DAT). Plant height and petiole length were recorded in metric scale. A SPAD 502 meter (Minolta, Osaka, Japan) was used to determine relative leaf greenness (chlorophyll content), called SPAD value (Netto et al., 2005); three recently matured, fully expanded leaves were used from each plant during the experiment. Leaf area (LA) was determined by a non-destructive method using a Cl-202 leaf area meter (CID Bio-Science, Inc., USA). SPAD value and leaf area data were collected at three stages namely vegetative, reproductive, and harvesting, respectively.

Histological analysis

Five leaf petioles of anthurium grown under the three different light intensities were collected for histological studies. A 5 cm long and 4 mm diameter of petiole was taken from the midpoint from each petiole and fixed in neutral buffered formalin (NBF) for 24 h (Seago et al., 2000; Kraus and Arduin, 1997), then washed in abundant running water and dehydrated in a series of alcohol (10, 30, 50, 70, 90 and 100%) for 10 min at each concentration and embedded in a plastic petri dish. Then the petiole was cut in cross section by hand using steel blades. In order to make slides, sections were stained with 0.5% toluidine blue-color reagent for 1 minute. Then they were washed thoroughly in water. Finally, transverse sections were mounted between the slide and coverslip with 50%

glycerin, and sealed with clear nail polish. Then the slides were observed under microscope attached to the image capture and photography system, and the corresponding micrometric scales were displayed.

Image acquisition and processing

Anthurium petiole stained using DTZ were photographed at a 6 magnification using a stereoscopic zoom microscope (Nikon SMZ 1500, Japan) and a camera control unit (Nikon, SU-1). The 8-bit depth images were analyzed on a desktop or laptop computer using ImageJ software (Ferreira and Rasband, 2012). The intensity of staining was measured through the RGB color space (red, green and blue) defined by formula staining intensity values expressed as R þ G þ B/3. Intensity data represented the relative density of Zn in the grains, and was scored from 1 (less intense color), 2 (medium intense color), 3 (intense color) to 4 (very intense color) in accordance with the intensity of staining (RGB values, scale from 0 to 255).

Statistical analysis

The data was analysed by least significance difference (LSD) according to 5% level of significance and method was described by XLSTAT.

RESULTS

Plant height

The plant height was significantly tallest (29.4 cm) in 60% reduced light (L_2) at 100 DAT and the smallest (23.2 cm) was for control (L_0) at 100 DAT (Figure 1A).

Petiole length

The maximum petiole length (16.2 cm) was found in 60% reduced light at 100 DAT and the minimum (11.2 cm) was for direct sunlight at 100 DAT (Figure 1B).

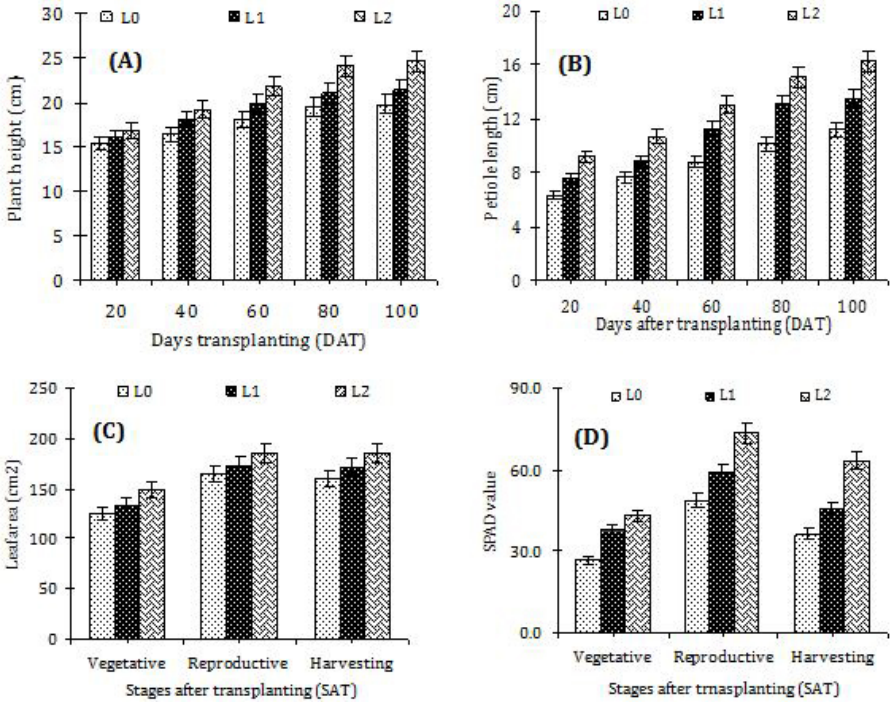

Figure 1. Treatment effects (mean ± SE) on (A) plant height, (B) petiole length, (C) leaf area and (D) SPAD value. All characters were significantly affected by treatments, i.e. L_0, full sunlight; L_1, 40% reduced light and L_2 60% reduced light.

Leaf area

Leaf area was significantly influenced by shade treatments at different SAT and the maximum leaf area (185.6 cm^2) occurred in 60% reduced light, while the minimum (164.9 cm^2) was for control at the reproductive stage.

SPADE value

The 60% reduced light (T_2) had the highest value (73.6) in the partial shade condition at reproductive stage, while the 40% reduced light (T_0) had the lowest (49.0).

Cell length

The 60% reduced light had largest cells (79.2±2.3 µm) in the petiole-length direction which was also larger than the 40% reduced light. On the other hand, the direct sunlight had smaller cells (41.4±2.3 µm) in petiole length directions (Figures 2A and 3).

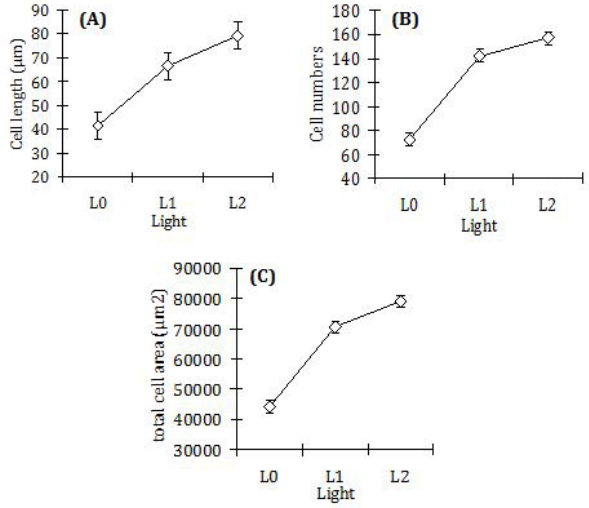

Figure 2. Treatment effects (mean±SE) on (A) cell length, (B) cell numbers, and (C) total cell area. All characters were significantly affected by treatments, i.e. L_0, full sunlight; L_1, 40% reduced light and L_2 60% reduced light.

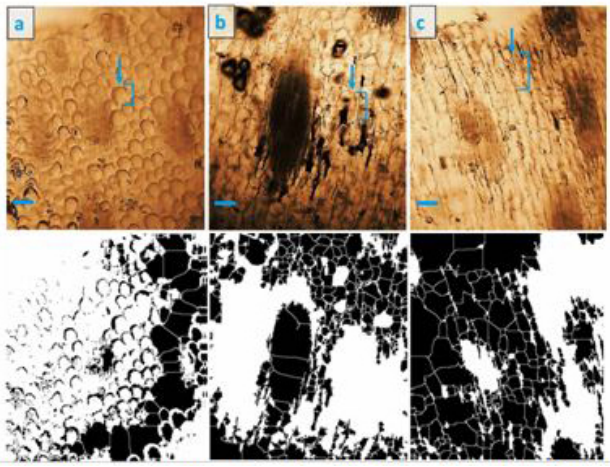

Figure 3. Photograph showing cross sectional view (histological analysis) of petioles from pink anthurium grown under (a) full sunlight, (b) 40% reduced light, and (c) 60% reduced light. Photographs were taken under a microscope with photographic outfit. Bar = 20 µm in (a) and (c) and 25 µm in (b).

Cell number

The response of cell numbers (157.3±2.1) under light treatments was increased in 60% reduced light compare with direct sunlight with the number of cells the lowest (72.6±2.1µm) in L_0 treatment (Figures 2B and 3).

Cell area

Under the shade treatments, the cell area (79108.667±772.3 μm^2) was significantly higher for 60% reduced light and the lowest (44083.6±772.3 μm^2) was found in direct sunlight (Figures 2C and 3).

DISCUSSIONS

Light is undoubtedly the most important environmental variable for plant growth and development; plants not only use radiant energy in photosynthesis, they also respond to the quantity, quality, direction, and timing of incident radiation through photomorphogenic responses that can have huge effects on the rate of growth and the pattern of development (Smith, 1994). At the end of the experimental period, anthurium had a greater height and petiole length under shade than full light treatment (Figures 1A and B). From the results, we can say that light is the fundamental aspect of plant growth, which operate as an energy source for photosynthesis and an environmental signal with regard to its intensity, wavelength, and direction. Due to the variation of treatments in Figure 1A, it is clarified that plant height was slowly increasing in trend lines at 100 DAT. Partial shading enhances the plant height versus full sunlight, which compared the plant growth under shade and it also enhances the microclimate (Medany et al., 2009). In this context, 60% reduce light contributes to make the results in plant height of anthurium. Hence, the reduction of red/far-red photon flux ratio (600-700/700-800 nm) (R/FR) of daylight present in low light promotes plant height and petiole length (Murakami et al., 1997). Germana et al. (2001) also supported these results. Khawlhring et al. (2012) similarly showed in *A. andraearum* that taller plant heights were obtained from those grown under a shade house with 75% shade. Consequently, transverse sections of same-sized petiole were also observed for identifying cell size, cell numbers, and areas in relation to different light intensity of anthurium petiole length during elongation (Figure 3). In histological analysis, longer cell size was found in 60% reduced light than control and 40% reduced light (Figure 2A). However, 40% reduced light also increased cell size. In case of diffused light, low red and far red light radiation is mediated by phytochrome (PhyB) (Smith, 1994) which stimulates indoleacetic acid and gibberellin (Kurepin et al., 2007), and increases cell size (Maddonni et al., 2002). In addition, Cookson and Granier (2006) observed that changes in leaf expansion dynamics were accompanied by a decrease in epidermal cell number which was partly compensated for by an increase in epidermal cell area. Our study investigated that the elongation of cells is controlled by partial shade in petioles (Figures 2A and 3). However, it was also apparent that the partial shade affected not only the enlargement of cells but also the number of cells in the petiole (Figure 2B). Weijschede et al. (2008) found that cell number was the main trait explaining petiole length differences among genotypes grown under high light, while both cell number and length changed in response to shading. In contrast to these results we found that only the size of cells are responsible, not the number of cells as in *Polygonum* species (Griffith and Sultan, 2006). Plants receive solar radiation and capture more energy during the growth period under shade for photosynthesis (Chella and Bakker, 1998), which increases leaf area (Reich et al., 1998). Particularly, shade induces leaf plasticity in leaf cells for expansion of leaf area (Cookson and Granier, 2006). Even more, plant cell expansion and division stimulate to increase individual leaf area in shading plants (Schoch, 1982). Stanton et al. (2010) showed that partial shade increased individual leaf area and higher specific leaf area. These results were also supported by Li et al. (2014). However, Srikrishnah et al. (2012) found in *Dracaena sanderiana* that plants grown at 50 and 70% shade levels produced the higher leaf area and biomass than plants subjected to 80% shade. During the reduced light regimes under shade, chlorophyll content increased with increase ratio of chlorophyll a/b in seagrass (Dennison and Alberte, 1982), though it does not change unit

character of photosynthesis in low light. The decrease in chloroplast density is found in reduce light, which responses to UV light blocking and increase chlorophyll in 50% reduced light (Abal et al., 1994). This chloroplast is not only characterized higher number of thaylakoids per granum and higher stacking degree of thylakoids, but also broader granum in low light which promotes to increase chlorophyll flouroscence (Lichtenthaler et al., 1981).

CONCLUSION

In short, this study showed that anthurium petiole length elongation was maximum in 60% reduced light (L_2) due to the increase of cell length and cell number.

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