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ACCURATE AND PRACTICAL METHOD TO DETECT PHOTOTROPIC LEAF MOVEMENT OF COTTON: DIGITAL IMAGING

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Abstract

Cotton species have adapted to their originated environment by developing different physiological and morphological features. One of these features is heliotropic leaf movement. Gossypium hirsutum L. (GH) have diurnal leaf movement owing to pulvinus, which is formed by specialized cells on the contrary to Gossypium barbadense L. (GB). There are very few studies in literature about advantages and disadvantages of diaheliotropic leaf movement of cotton. To assess effects of diaheliotropic leaf movement of both cotton genotypes on their adaptation abilities, firstly need to quantify orientation of leaves towards light. Therefore, two different sequential experiments were carried out in this study. First one was angle-based leaf turn experiment that contained two different measurement method for detection of leaf movement which are midrib normal-incident angle (M_I) and midrib-petiole angle (M_P). Second was area-based leaf turn (T_A) experiment. In T_A experiment, digital images of plants from same direction of light source are analysed. Then, area of turning leaf lamina is calculated by pixel counting method using with an image processing software. Results of the study revealed that digital imaging method is easier and faster method for detecting diaheliotropic leaf movement. Furthermore, it is possible to quantify area based diaheliotropic leaf movement as centimetre square in T_A method unlike other two methods. According to our findings, T_A by using digital imaging is featured and accurate method to detect phototropic leaf movement of cotton.

***Key words**: cotton, phototropism, phototropic leaf movement, Gossypium hirsutum L., Gossypium barbadense L., digital imaging.*

INTRODUCTION

Some plants have capabilities that adapt to their environmental conditions in order to survive. This adaptation of plants can be possible by changing some physiological and morphological features. One of these plant's capabilities is leaf movement (Koller, 1990). It is possible to categorize leaf movement as circadian rhythm orientation and light-driven orientation. Some plants can adjust their leaves almost horizontally at day and positioning vertically at night. This type of leaf movement is known as nyctinasty and controlled by circadian rhythm of plants (Ueda and Nakamura, 2007). The other leaf movement is light-driven orientation and known as phototropism (Darwin, 1880). Growth-based phototropism most often observed in almost all plants. However, pulvinus-based phototropism which known as heliotropic leaf movement has developed in some plants by evolution to environment and it is more dynamic form of phototropism (Moore and Hines, 2017). There are two type of heliotropic leaf movements were reported (Darwin, 1880; Yao et al., 2018). One of them is paraheliotropic leaf movement which was developed by plants in order to avoid environmental stresses such as severe light and heat (Inamullah et al., 2006; Bressan, 2004; Kao and Forseth, 1992). The other one is diaheliotropic leaf movement which enables tracking to diurnal sun movement for enhance photosynthetic capacity (Zhang et al., 2009; Yao et al., 2018).

The heliotropic leaf movement commonly occurs in legumes and also in some other plants such as cotton species (Moore and Hines, 2017). *Gossypium hirsutum* L. (GH) have diaheliotropic leaf movement in contrast with *Gossypium barbadense* L. (GB) (Hejnak et al., 2015; Ehleringer and Hammond, 1987). GH leaves have a high sensitivity to different photosynthetic active radiation (PAR) (Yao et al., 2018; O'Carrigan et al., 2014; Arena et al., 2016) and can improve their photosynthetic capacity by the contribution of diaheliotropic leaf movement (Yao et al., 2018). Furthermore, some researchers reported that diaheliotropic leaf movement leads to exposure more incident light to GH leaves, thus these leaves can utilize more light energy (Yao et al., 2018), increase leaf N content (Yao et al., 2015; Werger and Hirose 1991) and leaf mass per area (Witkowski and Lamant, 1991; Niinemets, 1999; Yao et al., 2016) as well as improve leaf longevity (Kitajima et al., 1997). On the other hand, Fukai and Loomis (1976) suggested that diaheliotropic leaf movement of GH leaves lead to enhance total canopy yield in early stage, however canopy productivity could be reducing in late growing stage due to uneven light distribution. Especially in intensive agriculture, outermost leaves may limit penetration of light to inside of canopy in particular period of season due to diaheliotropic leaf movement (Ehleringer and Forseth, 1980; Thanisawanyongkura et al., 1997), thus it could be reducing total canopy photosynthesis (Ehleringer and Hammond, 1987).

Beside all of these discussions, to clarify advantages and disadvantages of diaheliotropic leaf movement of GH in different growing stages has great importance and firstly need to have accurate, practical and quantitative measurement methods. Many studies were conducted in relation to diaheliotropic leaf movement of cotton by using different angle measurement methods such as cosine, azimuth, lamina and midrib angle (Ehleringer and Hammond, 1987; Zhang et al., 2009; Yao et al., 2018; Shell et al., 1974). Ehleringer and Hammond (1987) used cosine method in order to reveal leaf movement differences between GH and GB. Yao et al. (2018) suggested that midrib angle method is the main factor for detecting leaf movement in contrast to lamina and azimuth angle methods (Zhang et al., 2009). In these methods, generally inclinometer, protractor and compass were used on a couple of leaves for angle measurements. When taking account of all plant leaves, these angle measurement methods might have disadvantages especially in terms of practicality. Besides, estimation of area-based leaf turns (T_A) might be more accurate, quantitative and practical method to determine effective leaf movement.

Thus, objectives of this study were (*i*) verifying leaf movement differences between *G. hirsutum* L. and *G. barbadense* L. as revealed in previous studies and (ii) evaluating of angle measurement methods and T_A method in terms of accuracy and practicality by using digital imaging. When considering to diaheliotropic leaf movement as selection criteria, one of these methods is expected to be using in breeding programme for fast selection.

MATERIALS AND METHODS

This study was comprised of two different sequential experiments which have four replications. The experiments were consisted of two contrasting cotton genotypes in terms of heliotropic movement of their leaves: *G. hirsutum* L. (GH) and *G. barbadanse* L. (GB). The plants were grown in pots at fully controlled climate chamber (stable at 30˚C and 50% relative humidity).

Experiment 1th (Angle-based leaf movement)
Angle-based leaf movement experiment was carried out in 2018, in fully controlled climate chamber. Cotton plants were grown under daylight fluorescent lamps until second true leaf fully developed. Then, each plants were transferred to growth cabin which is isolated from light and contain own light source (50W daylight power LED). We have observed in preliminary experiments that GH leaves were showed remarkable leaf movement during first two hours (data not shown). Therefore, the leaves were exposed to one directional light (midrib of first true leaf is vertical to light source) during two hours in the growth cabin. Digital images collected from the leaves as vertical to linear of light source by using with high resolution camera with ten minute intervals during two hours. Then, the images were analysed by an image processing software for different angle measurement methods as described below.

Midrib - Petiole Angle (M_P) Method: M_P refers to angle between midrib and petiole. This measurement was performed by processing images as shown in Figure 1 a. This method could corroborate that leaf movement is pulvinus-based or not.

Midrib normal - Incident Angle (M_I) Method: M_I refers to angle between incident light and midrib normal. It was measured by processing images as shown in Figure 1b. Then, cosine of M_I was calculated to scale leaf movement between 0 to 1 according to Ehleringer and Hammond (1987).

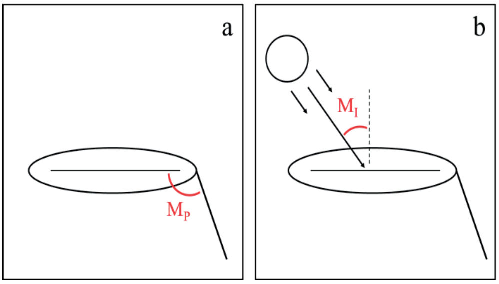

Figure 1. A visual of how measured M_P (a) and M_I (b) in Experiment 1th.
M_P: Midrib - Petiole Angle
M_I: Midrib normal - Incident Angle

Experiment 2nd (Area-based leaf movement)
Area-based leaf movement (T_A) experiment was carried out in 2019 under fully controlled climate chamber conditions. Cotton plants were grown under daylight fluorescent lamps until they had four fully developed leaves. Then, the plants were exposed to light treatment by using lighting equipment which is designed as parallel to ground and consist of four pieces of daylight power LED (50W). This one directional light treatment was implemented during two hours (09:00-11:00) according to the previous experiment during six days. The light treatment wasn't conducted in fully dark ambiance in order to simulate sunrise effect.
Then digital images of plants were collected from 09.00am to 11.00am during the light treatment from the same direction with light source in order to estimation of area-based leaf movement. Adaxial leaf areas were calculated using with an image processing software as centimetre square by using pixel counting method as shown in Figure 2. The leaf area differences between 09:00am and 11:00am indicated leaf movement capability and quantity for each plant.

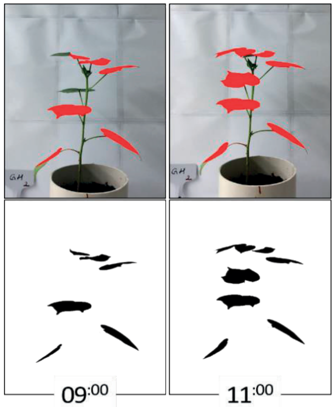

Figure 2. A visual of how measured T_A in Experiment 2nd.
T_A: Area - based leaf movement

RESULTS AND DISCUSSIONS

In order to quantify phototropic movement of plants, all leaves including cotyledons have to be analysed to examine changes in full-plant position. Furthermore, need to non-destructive and zero-touch method while measuring leaf movement for conducting reliable studies. Some digital methods were used to detect heliotropic response in many previous studies (Bawhey et al., 2003; Rakocevic et al., 2010). In our study, we observed that digital imaging method is appropriate method for measuring leaf movement.
Two types of angle measurement (M_I and M_P) were performed during ten minutes interval in the first experiment. Leaf movements of both cotton species were clearly detected at minutes of 0, 50, 80 and 110. M_I of GH had decreasing trend during two hours of light treatment. However, M_I of GB was stable and only decreased in 50 min (Figure 3 a). It is possible to suggest that GB leaves had no diaheliotropic feature, only showed growth-based phototropism in 50 min; however, GH had linear increase in diaheliotropic leaf movement during light treatment. Earlier, this method to measure diaheliotropic leaf movement was examined by Ehleringer and Hammond (1987). Shell et al. (1974) estimating light interception capability of plants.

Joint-like pulvinus is located between leaf lamina and petiole (Moore and Hines, 2017). These specialized cells enable diaheliotropic feature to plants by help of turgor pressure (Moore and Hines, 2017). Therefore, M_P of both GH and GB were measured in order to support previous suggestion that is related to diaheliotropic leaf movement. M_P of GH had decreasing trend similar to M_I of GH, but GB had almost same M_P during two hours (Figure 3 b). It is proving that GH had diaheliotropic leaf movement however, GB only showed growth-based phototropic leaf orientation.

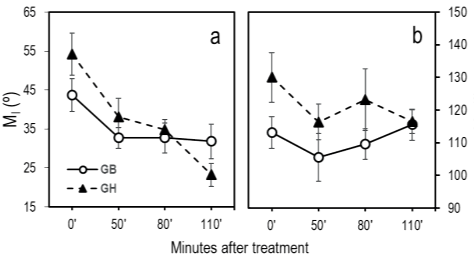

Figure 3. Effects of light treatment on M_I (a) and M_P (b) value of GB and GH during minutes after treatment.
M_P: Midrib - Petiole Angle
M_I: Midrib normal - Incident Angle
GB: *Gossypium barbadense* L.
GH: *Gossypium hirsutum* L.

Ehleringer and Hammond (1987) reported that when cos (M_I) value close to 1, there is a strong diaheliotropic feature in leaves. According to this suggestion, in the previous study, GH had remarkable increase in leaf movement and higher orientation than GB at the end of the light treatment (Figure 4).

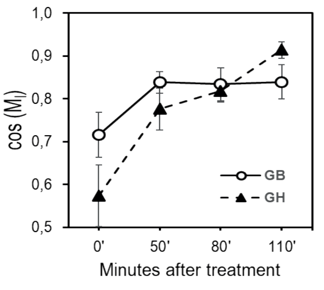

Figure 4. Effects of light treatment on cos (M_I) value of GB and GH during minutes after treatment.
cos (M_I): Cosine of Midrib normal - Incident Angle
GB: *Gossypium barbadense* L.
GH: *Gossypium hirsutum* L.

The second experiment which considered area-based full leaves movement (T_A) was more reliable method in principle since it covered changes in whole plant position. In the present study, GB and GH leaves were tending to turn towards light as shown in Figure 5. GH generally had more orientation to light during six days, but T_A of GB and GH were almost same in 3th, 4th and 5th days. This orientation similarity between GB and GH probably was resulted from growth-based phototropism of GB as mentioned before. This finding is also supported by increasing of differences again between T_A of GH and GB at 6th day. The growth-based phototropism of GB is also explained by growing of cotton plants in lower light conditions on contrary to sunlight.

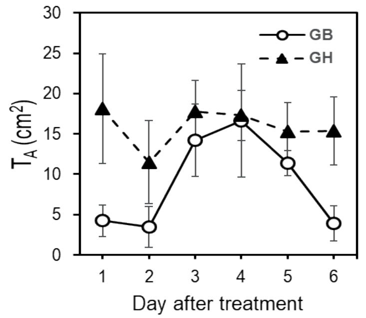

Figure 5. Effects of two hours light treatment on T_A of GB and GH during day after treatment.
T_A: Area-based leaf turn (cm^2)
GB: *Gossypium barbadense* L.
GH: *Gossypium hirsutum* L.

It is possible to estimate and quantify total leaf movement by using T_A method. We found that total T_A of GH is significantly higher than T_A of GB (Figure 6).

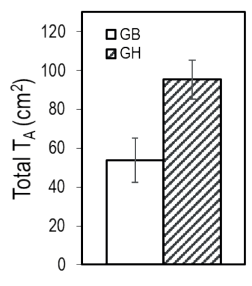

Figure 6. Total T_A of GB and GH through six days of light treatment.
T_A: Area-based leaf turn (cm^2)
GB: *Gossypium barbadense* L.
GH: *Gossypium hirsutum* L.

Although GH has relatively smaller leaf area than GB (Hejnak et al., 2015; Wise et al., 2000), leaf turn of GH were found significantly higher than GB. It is mean that diaheliotropic feature of GH leaves lead to turn more leaves towards light source than growth-based phototropic feature of GB leaves.

CONCLUSIONS

The main objective of study was determining to accurate and practical method for quantifying leaf movement of cotton. All measurement methods of this study were based on digital imaging. It was possible to measuring of phototropic leaf movement via easier and non-destructive way by digital imaging.

Furthermore, estimation of area-based leaf movement (T_A) using with pixel counting method could be suggested as featured method to detecting and quantifying of phototropic leaf movement in terms of accuracy and reliability. According to our results, GH showed strong phototropic leaf movement on the contrary of GB. T_A is expected to use in physiological and breeding studies as accurate and practical method.

However, growth-based phototropic leaf movement of GB were detected in all measurement methods. Therefore, measuring of diaheliotropic leaf movement on same cotton species without lead to growth-based phototropic leaf movement could be more effective to determine accurate method.

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