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THE ENDOGENOUS RESPONSES DURING THE FLOWERING STAGE OF *TORENIA FOURNIERI* L. UNDER LED LIGHT

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SUMMARY

The quality of light has played an important role not only in the vegetative growth, but also in the reproductive stage of the plant. Normally, the endogenous transformation has hardly been observed under the general condition. This study analyzed the endogenous changes, which were particularly influenced by the spectrum of light emitting diode (LED) with induction flowering time from 30^{th} to 40^{th} day. In the 40^{th} day of the flowering process, when the average number of flower buds get the highest, endogenous carbohydrate content was decreased. However, in this phase, plants need more energy; therefore, the net photosynthetic rate tends to increase in order to supply energy for flowering. Throughout 40 days of culture, the net photosynthetic rate had an increase in the concentration of CO_2. In particular, it reached the peak when being induced under the light combination ratio 7:3 of red and blue light (0.222 µmol mol^{-1} h^{-1}). The sugar content also followed the same trajectory; however, it dropped at the end of the period. Among all the experiments, the ratio of red and blue light 5:5 resulted in the highest content of endogenous carbohydrate source (722.30 µg g^{-1}). Moreover, the morphological anatomy of shoot apical meristem in flowering stage was also studied. The floral transition at meristem and floral architecture is as similar as that of *Arabidopsis*. A typical flower of *T. fournieri* also consists of a sequence: sepals – petals – stamens – carpels. The SAM is organized into three different zones such as the central zone (CZ), the peripheral zone (PZ) surrounding the CZ and the rib zone (RZ) underneath the CZ.

Keywords: *Endogenous carbohydrate content, in vitro flowering, LED, net photosynthetic rate, Torenia fournieri* L

INTRODUCTION

In the circle of a plant's life, reproduction is a basic strategy which is a cost to the plant both energy and nutrients. Photosynthesis is responsible for the production of energy and regulated by this reproductive stage depends mostly on the requirement of sink demand. Among environmental conditions in micropropagation, the importance of photosynthetic photon flux (PPF) that affects growth and photosynthesis of plantlets has already been demonstrated in many species (Kozai *et al.*, 1997; Nguyen *et al.*, 1999; Cui *et al.*, 2000). It is widely known that a certain spectrum has a significant influence on both vegetation and inflorescent developments. Light quality also plays an important role in morphogenesis, photosynthesis and the reproductive process (Hoenecke *et al.*, 1992; Saebo *et al.*, 1995; Dewir *et al.*, 2006; Zhou, 2006), influencing the way in which light is absorbed by the chlorophyll (Tennessen *et al.*, 1994; Tripathy, Brown, 1995). In addition to the energy, flowering is the process that demands the huge amount of carbohydrate for structuring and developing both floral and fruit organ by photo assimilation. The role of sugar and the effect of applying external sugar in medium on *in vitro* flowering have been reported in many species (Vu *et al.*, 2006; Taylor *et al*., 2007; Wang *et al*., 2009). However, there are not many studies observing the change of endogenous sugar according to the demand of reproduction and the difference between flowering or not.

The use of light sources that emit photons over a broad spectral range generally meets these two lighting requirements. Tissue culture and growth

rooms have long been using artificial light sources, including fluorescent lamps, high pressure sodium lamps, metal halide lamps, and incandescent lamps, *etc*. Among these, fluorescent lamps have been the most popular in tissue culture rooms (Gupta *et al*., 2012). However, a specific spectrum has a considerable effect on the growth of the plant. Apart from existing system, light emitting diode (LED) system hold numerous merits. Compared to conventional system, using LEDs can adjust the wavelength according to the framework of experiment; moreover, it has higher durability, small size, long operating lifetime, relatively cool emitting surface, and a photon output that is linear with the electrical input current, and the ability to control spectral composition (Hoenecke *et al.*, 1992; Brown *et al.*, 1995). This feature allows the implementation of LEDs with specific spectral ranges that are involved in plant responses, and also ensures the independent control of each spectral range and precise manipulation of spectral quality and light intensity (Folta *et al.*, 2005). Furthermore, the wavelength specificity of LEDs may be used to study the physiological qualities of plants grown in closed plant production systems (Schuerger *et al.*, 1997; Yeh, Chung, 2009; Nhut, Nam, 2010).

The objective of this research was analyzing the impact of LED system on the endogenous change, in particular the net photosynthetic, the endogenous concentration of carbohydrate and its morphology, during the flowering time of *Torenia fournieri* L., a typical plant.

MATERIALS AND METHODS

Materials

Explants that were used for all treatments will be *T. fournieri* shoot culture *in vitro* (taken from Plant Cell Technology Department – Institute of Tropical Biology). These shoot were 2.0 cm in height.

Methods

Experiment design

The *in vitro* shoot of *T. fournieri* having 2.0 cm in height was cultured on MS medium supplemented with 30 g L^{-1} sucrose, 8 g L^{-1} agar. Light condition: Fluorescent lamp (HQ) (36W, Phillip), 100% LED red light, 100% LED blue light, LED with various combined ratio of red light and blue light: 5:5, 6:4, 7:3, 8:2 and 9:1.

After 10, 15, 20, 25, 30, and 40 days of culture, the collected data were based on these criteria: the net photosynthetic rate (µmol mol^{-1} h^{-1}) and the endogenous carbohydrate content (µg g^{-1}).

Experiment condition

The sample was exposed under the light 10 hours per day, with 45 ± 2 µmol m^{-2} s^{-1} photosynthetic photon flux density, temperature was maintained around 25 ± 2°C, average moisture: 60 ± 5%. Every experiment was repeated three times and is completely randomized design. The collected data was analyzed by using Statgraphic software (P = 0.05).

The net photosynthetic rate measurement by GC (Walter, 2000)

Set up the CO_2 standard:

Using a syringe to take out 250 µL standard CO_2, which is commensurate with these concentrations: 100 µL, 300 µL and 1000 µL. Then pumping the gas to GC-2000 for identifying the content of CO_2. The standard line of CO_2 will illustrate the correlation between the standard concentration of CO_2 and the content of CO_2.

The correlation between the concentration of CO_2 and the value from GC-2000 is illustrated by a formulation:

$$y = 0.0031x - 22.503$$

Note: x: the concentration of CO_2

y: the value of gas chromatograph of GC-2000 (A_{GC})

Therefore, the content of CO_2 is identified basing on this formulation:

$$x = \frac{(A_{GC} + 22.503)}{0.0031} (\mu L)$$

The net photosynthetic rate measurement bases on formulation of Fujiwara (Fujiwara *et al*., 1987).

$$P_n = \frac{k \; x \; N \; x \; V \; x \; (C_{out} - C_{in})}{n}$$

Note: P_n: net photosynthetic rate (µmol mol^{-1} h^{-1} $plant^{-1}$)

k: the constant exchanging CO_2 from volume to molecular mass, k = 0.0000409 ɣ mol cm^{-3} at 25°C.

N: the number of gas exchanging times in 1 hour. The number of gas exchanging times of Magneta without air filter is 0.2 times h^{-1}, with 2 air filters is 3.96 times h^{-1} (Kozai *et al*., 1986).

C_{out}: using a syringe to take out 250 µl air of culture room. Then use GC - 2000 to identify the content of CO_2

C_{in}: pick out one culture box per repeated treatment then use a syringe to take out 250 µl of the air inside the culture box then use GC - 2000 to identify the content of CO_2.

Sugar content measurement by UV-VIS

The total sugar content is extracted and estimated by the method of Coombs *et al*. (1987). The prepared solution of sucrose and glucose were based on the array of concentration: 10, 20, 30, 40, 50, 60, 70 µg L^{-1}. Dyeing the solution by phenol 5% and condensed H_2SO_4. The volume ratio of sugar solution: phenol: condensed H_2SO_4 = 1:1:5.

After dyeing the sugar solution, shake it carefully and let it rest in 15 minutes. Then, the optical density of these solutions was calculated at the 490 nm wavelength. After collecting the data, establish the standard line of sucrose. The standard line of sucrose will illustrate the correlation between the standard concentration of sucrose and the absorbance of optical density at 490 nm.

The correlation between the concentration of sucrose and the value of absorbance of optical density is illustrated by this formulation:

$$y = 0.011x - 0.00201$$

Note: x : the concentration of sucrose
y : absorbance at 490 nm (A_{490})

Therefore, the formulation of the sugar content is:

$$x = \frac{(A_{490} + 0.00201)}{0.011} (\mu g)$$

Morphological anatomy

The morphological method of the shoot apical meristem, inflorescence meristem and flower meristem based on Nguyen Tien Ban *et al.,* (1979). Firstly, the sample of observed meristem was cut into pieces and put in Javel liquid (Viso, Vietnam) in 10 minutes. Then the explants were washed by distilled water in order to remove Javel completely. And put them into acetic acid 10% solution in 10 minutes; wash them again with distilled water. Make them dry then drop 1 drop of carmine-iodine onto them. After 15 minutes, they were washed again by distilled water, and observe under the microscope at X4 and X10.

RESULTS

As it has been known, the process of flowering is a complicated transformation with numerous changes in not only the morphology but also the physiology. Given are two tables below illustrating the difference in sugar content and photosynthetic efficiency within 40 days when the sample of *T. fournieri* was exposed under various light conditions.

In general, the net photosynthetic rate continuously increased until the last day of observation; meanwhile, the sugar content reached its peak at 30 days then decreased. Regarding the net photosynthetic rate, in the first 20 days after culture, the ability of photosynthesis was still low. However, the net photosynthetic rate of the treatment, which combined 70% red light with 30% blue light, was second to none in the last 20 days, when the plant started the reproduction process. In terms of the sugar content, there was a significant change after 30 days of culture compare to others. In particular, both ratios of red light : blue light = 5:5 or 6:4 had the greatest content then decreased after 40 days.

Table 1. The effect of LED light on the net photosynthetic rate of *T. fournieri.*

Experiment	Net photosynthetic rate (µmol mol^{-1} h^{-1})					
	10 days	**15 days**	**20 days**	**25 days**	**30 days**	**40 days**
Blue	0.003^{d}	0.010^{c}	**0.051**^{a}	0.021^{b}	0.027^{c}	0.025^{d}
Red	0.001^{d}	**0.064**^{a}	0.017^{b}	0.025^{b}	0.035^{bc}	0.041^{d}
HQ	0.018^{c}	0.013^{c}	0.020^{b}	0.023^{b}	0.032^{bc}	0.165^{abc}
L 5:5	0.046^{b}	0.040^{abc}	0.022^{b}	0.042^{b}	0.050^{bc}	0.088^{cd}
L 6:4	**0.069**^{a}	0.033^{bc}	**0.055**^{a}	0.050^{b}	0.090^{b}	0.173^{ab}
L 7:3	0.022^{c}	0.062^{ab}	**0.065**^{a}	**0.122**^{a}	**0.222**^{a}	**0.247**^{a}
L 8:2	0.041b	0.048^{ab}	0.014^{b}	0.039^{b}	0.045^{bc}	0.056^{d}
L 9:1	0.022^{b*}	0.038^{abc}	0.008^{b}	0.020^{b}	0.048^{bc}	0.093^{bcd}

*Note: *Value in the same column followed by different letters are significantly different (p=0.05) by using LSD Multiple Range Test.*

Table 2. The effect of LED light on the endogenous sugar content of *T. fournier*i.

Experiment	The endogenous sugar content (µg/g)					
	10 days	**15 days**	**20 days**	**25 days**	**30 days**	**40 days**
Blue	302.41^{e}	337.33^{b}	**365.30**^{a}	336.66^{c}	482.85^{c}	257.63^{e}
Red	308.93^{de}	323.60^{c}	**361.63**^{a}	**363.34**^{a}	440.23^{d}	241.46^{f}
HQ - control	324.56^{cd}	336.91^{b}	348.60^{abc}	**365.45**^{a}	546.22^{b}	273.89^{d}
L 5:5	318.97^{cde}	337.34^{b}	361.93^{a}	361.89^{ab}	**722.30**^{a}	**580.75**^{a}
L 6:4	**353.92**^{a}	340.13^{b}	332.59^{c}	344.40^{bc}	**716.83**^{a}	292.57^{c}
L 7:3	**363.30**^{a}	**362.38**^{a}	**360.77**^{a}	299.52^{d}	410.57^{e}	310.45^{b}
L 8:2	347.67^{ab}	321.66^{c}	340.16^{bc}	356.69^{ab}	419.05^{e}	249.47^{f}
L 9:1	329.70^{bc}	328.88^{bc}	353.20^{ab}	310.69^{d}	319.71^{f}	191.85^{g}

*Note: *Value in the same column followed by different letters are significantly different (p=0.05) by using LSD Multiple Range Test.*

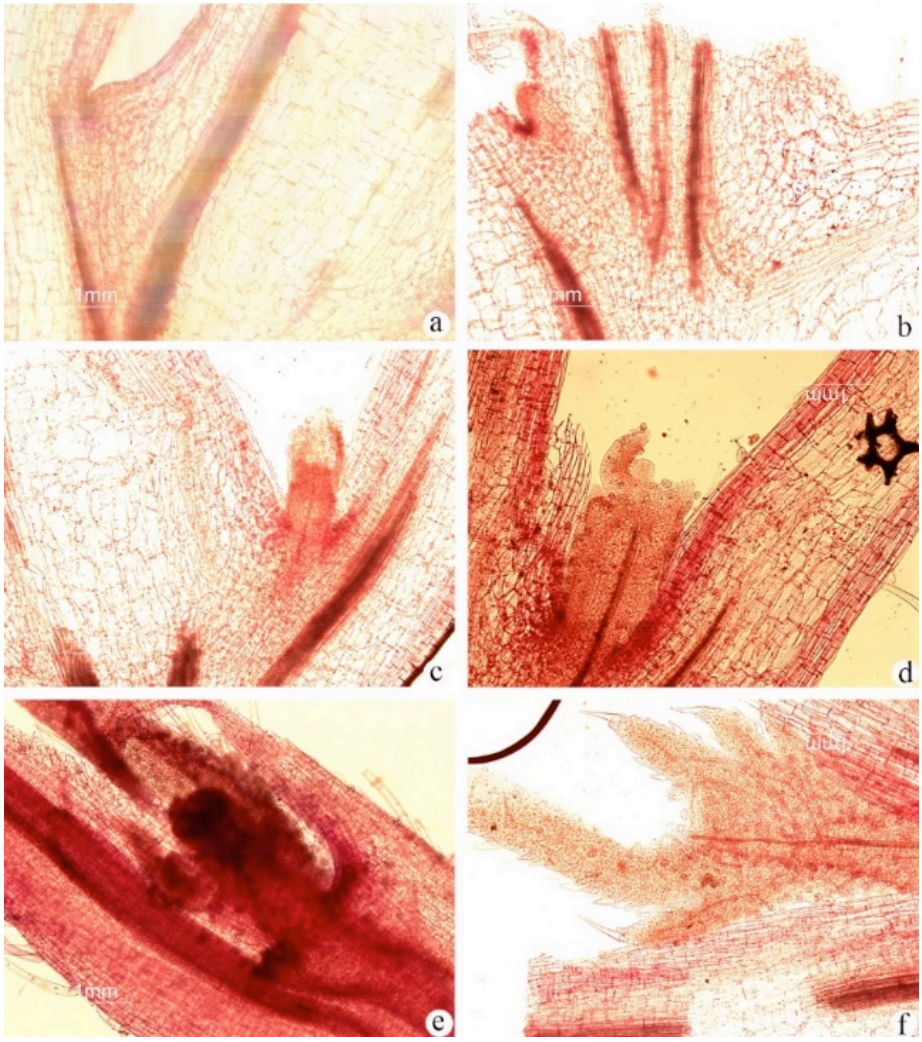

Figure 1. Axillary shoot apical meristem of *Torenia fournieri* L. a), b): The cell division of an axillary bud in vegetative development stage; c) The formation of vegetative shoot apical meristem; d) The reproductive transition of vegetative shoot apical meristem; e), f): Floral meristem.

In term of tissue aspect, during the floral transition, figure 1 and figure 2 illustrated numerous changes. In term of morphology, the critical conversion from vegetative shoot apical meristem to inflorescent meristem or floral meristem is hardly noticed under the ordinary condition. Shoot apical meristem (SAM) is responsible for forming leaf, lateral shoot to create branch and elongate during the growth and development of plant. However, when it comes to flowering time, the cell differentiation occurs vigorously at this place, which can be observed under the microscope. The first difference between these meristems is the general morphology, in particular the changing from flat to dome shape. The basic floral architecture is mostly conserved among the so-called core eudicots that make up over 73% of extant flowering plants (Drinnan *et al.*, 1994) including *Torenia*. Flowers within this group generally have four concentric whorls of organs that are specified, from the outside to the center of the flower, in the sequence: sepals, petals, stamens, and carpels (Elena *et al.*, 2010). *Torenia* has this type of floral architecture.

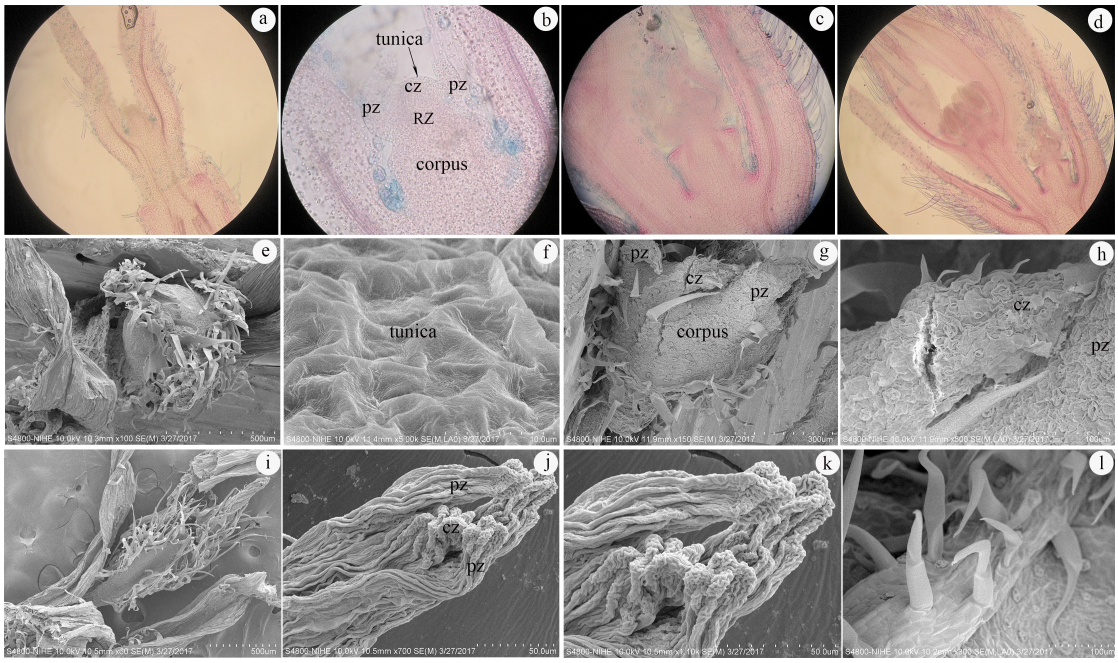

Figure 2. Shoot apical meristem of *Torenia fournieri* L. a), b): The formation of vegetative shoot apical meristem c); d) The reproductive transition of vegetative shoot apical meristem; e), f), g), h), i), j), k), l): Floral meristem is observed by SEM; CZ: central zone, PZ: peripheral zone.

The light has played a crucial role in the growth and development of living creatures in general and plant in particular. Not only it regulates every biological activity, but also be one of the factors, which trigger the flowering. Importantly, these movements are also influenced by the spectrum of light and *T. fournieri* is not an exception. According to the previous research (Trần Trọng Tuấn *et al.*, 2015), the ratio of blue and red light at 5:5 is specifically essential for this plant in the reproductive as well as vegetative stage, which can significantly enhance the sustainability of the next generation, with 19.59% of flowering. This study sets an aim of deepening the knowledge of endogenous transformation of this special event.

It is well-known that flowering is a complicated phenomenon with several events occurring simultaneously. To flower, plant must experience many endogenous physiological changes, including net photosynthetic rate and the sugar content as a well preparation of energy source for a special event. In general, as can be seen from figure 3, the net photosynthetic rate gradually increased over time; meanwhile the sugar content reached a peak at 30 days then dropped.

Regarding monochromatic light conditions, which did not have any flower buds, the net photosynthetic rate and the sugar content were considerably low, which acquired approximately 30

µmol mol^{-1} h^{-1} and around 500 µg g^{-1} (blue light, red light and fluorescent lamp) at 30 days, when floral signal was recorded. Interestingly, in terms of the combined light conditions, the optimal net photosynthetic rate and the optimal endogenous sugar content were in reverse. In particular, the LED 7:3 experiment has the highest net photosynthetic rate, 0.222 µmol mol^{-1} h^{-1} and the sugar content (410.57 µg g^{-1}) was considerably low. Whereas, the equal combination of red light and blue light, resulting in the highest flowering response had only 0.050 µmol mol^{-1} h^{-1}, much lower than the optimal result, but the remained experiment did not obtain the sugar content as immense as LED 5:5 (722.30 µg g^{-1}).

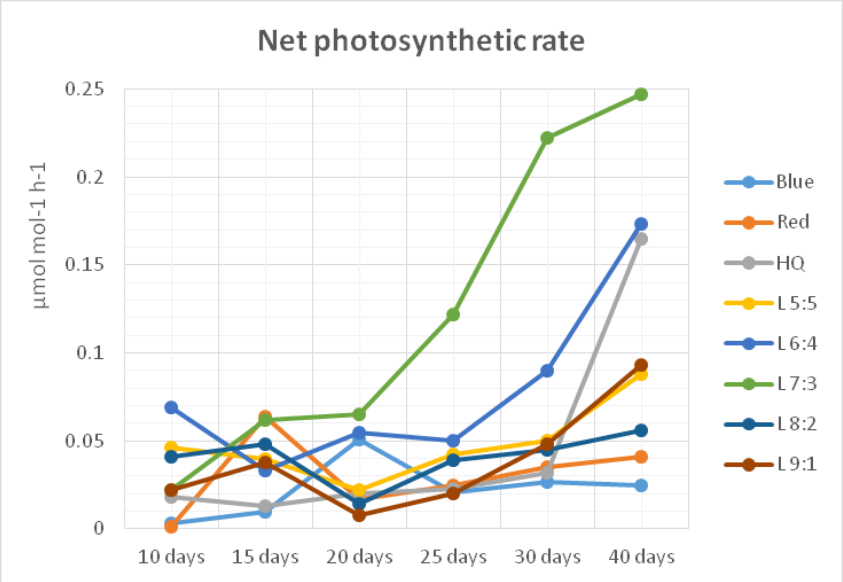

Figure 3. The net photosynthetic rate of *T. fournieri*.

After 40 days of culture, although the sugar content of all experiments considerably drops, that of LED 5:5 was still remarkably high (Table 2). Hence, the result demonstrated that the difference between 30 days and 40 days could possibly use for the floral formation and blooming process. The remains could be used for other purposes, such as the reproductive structure or the formation and ripening of fruit and seed.

During the flowering stage, the distinction between the shoot apical meristem and floral meristem was discovered only when observing under the microscope. Apart from normal cells, which was in the normal stage of development, shoot apical meristem consisting of stem cells required more cell divisions, which Figure 1a showed that several small cells appeared at the axillary bud contrasting to the nearby large cells. When vegetative shoot apical meristem (SAM) transformed into inflorescence meristem (IM), the major contrast was the dome-shaped of the meristem instead of being flat (Figure 2). The SAM of the Arabidopsis inflorescence consisted of a small dome of cells organized into different regions. Cell divisions within these meristem layers were exclusively anticlinal and the new cell walls were formed perpendicular to the surface of the meristem (Alvarez-Buylla *et al.*, 2010). As being classified as a eudicot, the floral transition at meristem and floral architecture is as similar as that of *Arabidopsis*. A typical flower of *Torenia* also consists of a sequence: sepals – petals – stamens – carpels (Figure 4). Because sepals are also a foliar type, it is designed to resemble a normal leaf, which means it has stomata on the surface for respiration. The SAM is organized into three different cytohistological zones, each with characteristic cytoplasmic densities and cell division rates: the central zone (CZ), the peripheral zone (PZ) surrounding the CZ and the rib zone (RZ) underneath the CZ (Bowman, 1994; Bowman, Eshed, 2000). This arrangement is illustrated by the figure 4.

Figure 4. Torenia flower buds are cut longitudinally.

CONCLUSION

In general, flowering is the major change in the life cycle of a plant, which requires a huge energy accumulation because they need to postpone the vegetative process for the concentration on the sustainability of their next generation. In this research, induction flowering time is from 30th to 40th day. In the 40th of the flowering process, when the average number of flower buds get the highest (Tran Trong Tuan *et al.*, 2016), endogenous carbohydrate content was decreased. However, in this phase, plants need more energy. Therefore, the net photosynthetic rate tended to increase in order to supply energy for flowering. The net photosynthetic rate reached the peak when being induced under the light combination ratio 7:3 of red and blue light. Among all the experiments, the ratio of red and blue light 5:5 resulted in the highest content of endogenous carbohydrate source.

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SỰ BIẾN ĐỔI NỘI SINH TRONG QUÁ TRÌNH RA HOA CỦA CÂY HOA MÕM CHÓ (*TORENIA FOURNIERI* L.) DƯỚI ÁNH SÁNG ĐÈN LED

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TÓM TẮT

Chất lượng ánh sáng đóng một vai trò quan trọng không chỉ trong sự tăng trưởng, mà còn trong giai đoạn sinh sản của thực vật. Thông thường, sự biến đổi nội sinh hầu như không được quan sát thấy trong điều kiện chung. Nghiên cứu này phân tích những thay đổi nội sinh, đặc biệt những thay đổi dưới sự ảnh hưởng của đèn LED. Trong nghiên cứu này, thời gian cảm ứng ra hoa là từ 30 đến 40 ngày. Vào ngày thứ 40 của quá trình ra hoa, khi số lượng trung bình các chồi hoa cao thì hàm lượng carbohydrate nội sinh giảm. Tuy nhiên, trong giai đoạn này, thực vật cần thêm năng lượng, do đó, tỷ lệ quang hợp thuần có xu hướng gia tăng để cung cấp năng

lượng cho quá trình ra hoa. Trong suốt 40 ngày nuôi cấy, tỷ lệ quang hợp thuần có sự gia tăng theo nồng độ CO_2. Đặc biệt, nó đạt đến đỉnh khi được nuôi dưới điều kiện ánh sáng có tỷ lệ 70% ánh sáng đỏ : 30% ánh sáng xanh. Hàm lượng đường cũng theo cùng một quỹ đạo; tuy nhiên, nó đã giảm vào giai đoạn cuối. Trong tất cả các thí nghiệm, tỷ lệ ánh sáng đỏ và xanh 5:5 đạt hàm lượng đường nội sinh cao nhất. Hơn nữa, nghiên cứu này cũng đã khảo sát sự thay đổi cấu trúc hình thái trong các giai đoạn hình thành hoa. Sự chuyển tiếp ra hoa và cấu trúc hoa cũng tương tự như cây *Arabidopsis*. Hoa Torenia cũng bao gồm: lá đài, cánh hoa, nhị hoa, lá noãn.

Từ khóa: *Hàm lượng đường nội sinh, hiệu suất quang hợp thuần, LED, ra hoa in vitro, Torenia fournieri* L