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Growth and Flowering of Cut Chrysanthemum as Affected by Source and Time of Light-Emitting Diodes

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The study examined the effects of light sources and light time on the growth and flowering responses of cut 'Baekma' and 'Jinba' chrysanthemum [*Dendranthema grandiflorum* (Ramat.) Kitamura] under in vitro and greenhouse conditions in South Korea. In vitro shoot explants were treated with light-emitting diodes (LEDs) sources with white, red (660 nm), blue (450 nm), and red-blue for 5 wk. In a greenhouse experiment, 4-h supplemental lighting was provided with incandescent bulb, red LEDs, or white LEDs staining at 20:00, 22:00, 00:00, or 02:00 h, with 12 h of day length, for 8 wk in 'Baekma' and for 7 wk in 'Jinba', depending on their typical weeks to flowering. In vitro red LED treatments extended stems of 'Baekma' (2.9 cm) and 'Jinba' (3.7 cm) adventitious shoots. In the greenhouse, growth and flowering of 'Jinba' were little influenced by source and time of light. Total fresh weight (FW) and chlorophyll content of 'Baekma' flowers were high under white LEDs at 22:00 h in the greenhouse, resulting in successfully delayed flowering and enlarged flower size.

Key Words: chrysanthemum, flowering, growth, light sources, light time

Abbreviations: EC – electrical conductivity, FW – fresh weight, LED – light-emitting diode

INTRODUCTION

Cut chrysanthemum ranked second in production and cultivation area in recent years among the floricultural crops in South Korea that were mostly exported to Japan. Approximately 81% of the cultivation area and 80% of the total sales of cut chrysanthemum were standard type chrysanthemum in 2011 (MIFAFF 2012), and the main cultivar was 'Baekma', followed by 'Baeksun' and 'Jinba' developed in Japan. 'Baekma' was released by the National Institute of Horticultural and Herbal Science in South Korea in 2004. It is a hybrid between 'Baeksun' and 'Jinba', which has many petals (300–350) on each flower and the natural flowering occurs at the end of July. 'Jinba', a white cultivar, has large flowers that bloom at the end of November. 'Jinba' is very popular in Japan, and the cultivation area in South Korea has increased to meet the demand for export of the flowers. Year-round cultivation of 'Baekma', 'Baeksun', and 'Jinba' was attainable through control of day length and temperature, using incandescent bulb or fluorescent lamp in many floricultural farms (Shin et al. 2005).

Incandescent bulbs and fluorescent lamps have a short life span and low power efficiency, and the frequent replacement of old lamps may cause substantial environmental pollution as well (Choi et al. 2012; Hong et al. 2013; Kwon et al. 2013). Light-emitting diodes (LEDs) concentrated on red (660 nm) light of the spectrum improved light use efficiency (Okamoto et al. 1996), and blue (450 nm) light was necessary for vegetative growth and floral induction (Hoenecke et al. 1992), which had

significantly varied responses among floricultural crops (Massa et al. 2008).

Little research was conducted on the effects of various wave lengths of LEDs on growth of 'Baekma' and 'Jinba' cut chrysanthemum grown under in vitro and greenhouse conditions. Flowering of chrysanthemum could be manipulated by changing day length due to its sensitivity to photoperiod, and the reduction of day length induced flower bud development (Jeong et al. 2012). The use of red LED lighting from 22:00 to 2:00 was not effective in delaying flower bud initiation and flowering of 'Baekma' and 'Jinba' compared with the use of fluorescent lamps (Choi et al. 2012). White LEDs improved vegetative growth of 'Baekma' and retarded flowering development under indoor conditions (Jeong et al. 2012).

Prolonged light duration for 3–5 h, called night breaks, extended flower stem length, and delayed days to flower budding and flowering of 'Baekma' and 'Jinba' grown under red LEDs and fluorescent lamp (Kwon et al. 2013). Night break lighting typically begins at 22:00 h in many 'Baekma' and 'Jinba' flower farms during all seasons. The start of break time depends on different day length and temperature in each season, and little research has been devoted to explore how light time influences the physiological responses of 'Baekma' and 'Jinba'. LEDs should be evaluated under in vitro conditions to understand their effects on plant performance under controlled conditions.

Growers of cut chrysanthemum have to distribute production to increase prices competitively (MIFAFF 2012). LED study has been mostly conducted on seedling, biological activity, and growth of vegetable crops, with little research on delay of flowering time for cut chrysanthemum to the LED sources staining at different times (Choi et al. 2012). This study was conducted to evaluate the most effective LED spectra and light time for optimum growth and delay of flowering time of cut chrysanthemum under in vitro and greenhouse conditions.

MATERIALS AND METHODS

In Vitro Experiment

The study was conducted in 2012 with cut chrysanthemum 'Baekma' and 'Jinba' grown in vitro at the Chonnam National University in Gwangju, South Korea (35°N, 127°E) and under greenhouse condition (300-m long single house) using different sources and hours of light treatments, with 12 h of day length in the Jeollanam-do Agricultural Research and Extension Services, Naju, South Korea (35°N, 127°E). The in vitro study was designed to understand the effects of treatment on vegetative and reproductive responses under controlled conditions as a pre-experiment.

Shoots of 'Baekma' and 'Jinba' were collected, disinfected with 70% ethanol for 1 min and 2% NaOCl for 20 min, and then washed using sterile water. The developed leaves on the shoots were removed, and a 0.5-cm long adventitious shoot tip was preliminarily detached from the mother shoot. The explants were vertically cultured in tubes containing MS (Murashige and Skoog 1962) plant medium including 3% sucrose, 0.8% agar, B5 vitamins, and 2 mg L-1 2,4-D for 5 wk. They were treated with LEDs, provided white, red (660 nm), blue (450 nm), and red-blue (1:1) for 5 wk at 21 ± 1 °C. LED arrays (TI-24L, T&I Co., Gwangju, Korea) were placed at 30 cm above the top of the explants. Photosynthetic Photon Flux Density (PPFD) of the white LED, red LED, and blue LED were approximately 8, 4, and 4 μmol m^{-2} s^{-1}, respectively, based on measurements using a quantum sensor (LI-250, LI-COR, Lincoln, USA). Six repetitions were used per treatment and each of them contained two shoot tips.

Rooting percentage, number and length of roots, leaf number, shoot length, number of nodes, internode length, stem length, total fresh weight (FW, g), and stem:root (S:R) ratio were measured at harvest.

Greenhouse Experiment

The stock plants of 'Baekma' and 'Jinba' were obtained from an outdoor ornamental farm (Namsan farm, Youngam, Korea) at 10 d before planting. Basal parts of approximately 7-cm long cuttings of 'Baekma' and 'Jinba' were treated with indole-3-butyric acid (IBA)-water solution at 200 mg L^{-1} for 18 h. The cuttings were placed in a cold room at 0 ± 1 °C for 2 d for the cut surface to fully heal. Then the cuttings were planted directly with 10 cm × 10 cm density in commercial potting substrate (Baroker, Seoul Bio Co., Eumseong, Korea) on 13 April for 'Baekma' and on 25 August for 'Jinba'. Relative humidity (RH) in the greenhouse was maintained at approximately 90% using sprinkler irrigation systems initially at 3 d after planting. Artificial shade was also arranged on the roof of the greenhouse initially for 3 d to prevent wilting of flowers. Ten cuttings (replicates) were used for 'Baekma' and 'Jinba' treated with lights for 8 wk and for 7 wk from planting dates, respectively, with maintaining minimum temperature at 16 °C during the night. Flowers were not pinched.

Two-factor design (sources and time of light) was applied on the greenhouse experiment. Incandescent bulb, red LEDs, and white LEDs at a distance of 1.0 m above the ground surface were included as light sources.

Lighting which started at 20:00, 22:00, 00:00, and 02:00 h for 4 h was used for the light time treatments. Lighting which started at 22:00 h, the control treatment of the experiment, is typically applied in many 'Baekma' and 'Jinba' flower farms to control flowering. The 128 h of light treatments lasted from 13 April to 12 June for 'Baekma', with 112 h of light treatments from 25 August to 14 October for 'Jinba'. Metal Halide Lamps (250 W) were illuminated from 7 am to 7 pm for all treatment flowers with maintaining 500 μmol m^{-2} s^{-1} of PPFD. White LEDs were combined with 50% white light intensity and 50% blue light intensity. Irradiation was approximately 7 μmol m^{-2} s^{-1} for incandescent bulb, 4 μmol m^{-2} s^{-1} for red LED, and 8 μmol m^{-2} s^{-1} for white LED (PGL-PFL B07-7Watt, Parus Co., Cheonan, South Korea). The wavelength of red LED was 660 nm, and for blue LED, 450 nm.

The Netherlands PBG (Proefstation voor Bloemisterij en Glasgroente) solution was used as a nutrition solution for both cultivars, at approximately 1.5 dS m^{-1} of electrical conductivity (EC) from the 8th to the 20th day after planting. PBG solution was set at approximately 1.8 dS m^{-1} of EC from the 21st day after planting to flower bud initiation, and then at 2.0 dS m^{-1} of EC during flower bud formation. PBG solution was reset at approximately 1.8 dS m^{-1} of EC after flower bud formation. PBG solution has three nutrient solutions in separate containers to prevent precipitation. Solution A consisted of 630.0 mg L^{-1} $Ca(NO_3)_2 \cdot 2H_2O$, 87.2 mg L^{-1} KNO_3, 35.0 mg L^{-1} NH_4NO_3, 19.1 mg L^{-1} HNO_3, and 11.2 mg L^{-1} FeEDTA (12.5%); Solution B consisted of 312.8 mg L^{-1} KNO_3, 300.0 mg L^{-1} $MgSO_4 \cdot 7H_2O$, 140.0 mg L^{-1} KH_2PO_4, and 29.6 mg L^{-1} H_3PO_4. Micro-mineral nutrients contained 1.26 mg L^{-1} H_3PO_3, 1.01 mg L^{-1} $ZnSO_4 \cdot 7H2_O$, 0.83 mg L^{-1} $MnSO_4 \cdot H_2O$, 0.20 mg L^{-1} $CuSO_4 \cdot 5H_2O$, and 0.13 mg L^{-1} $N_2MoO_4 \cdot 2H_2O$.

Number of leaves, and length, width and fresh weight (FW) of leaf were recorded for each cultivar. Leaf chlorophyll contents were measured colorimetrically on the fourth fully developed leaves from the shoot tips using a non-destructive soil and plant analyzer development (SPAD) 502 meter (Minolta Co., Tokyo, Japan) at harvest. The thickest section of stem was cut transversally, and the diameter for both cultivar and cavity only observed for 'Baekma' were measured using a Vernier caliper. Length and FW of stem were also measured. Floral differentiation was visually determined 23 d after planting when the floral buds began to develop, and the flowering was visually observed. The number of petals, peduncle length, and FW of flower were measured on each cultivar at harvest.

Data Analysis

Interaction effects were not observed for 'Jinba' in the greenhouse experiment, and therefore the main effects were presented in the results of the cultivar. All data were subjected to ANOVA using Minitab Software Version 14.1 (Minitab, Inc., State College, PA, USA). Means were separated by Duncan's multiple range test (DMRT) at $p \leq 0.05$. Statistics was based on Compton's method (1994).

RESULTS AND DISCUSSION

***In Vitro* Experiment**

Rooting percentage, and the number and length of roots were not significantly different for 'Baekma' and 'Jinba' chrysanthemum shoots grown under LED light sources at $p \leq 0.05$ (Table 1, Fig. 1 A–H). Leaf number of 'Baekma' shoots increased in white LEDs (11.4 each plant). Red LEDs supplemented with the blue light elevated leaf photosynthetic rates presumably due to enlarged stomata of chrysanthemum (Kim et al. 2004). However, red LEDs increased the shoot length (2.4 cm), internode length (1.75 cm), and S:R ratio (0.67) of 'Baekma' shoots, which was observed for various chrysanthemums treated with red LEDs (McMahon et al. 1991; Kim et al. 2004; Choi et al. 2012). Significant differences among the light treatments were observed for shoot length, number of nodes and stem length of 'Jinba' shoots, with the highest values observed for red LEDs. Radiation of the red region mostly

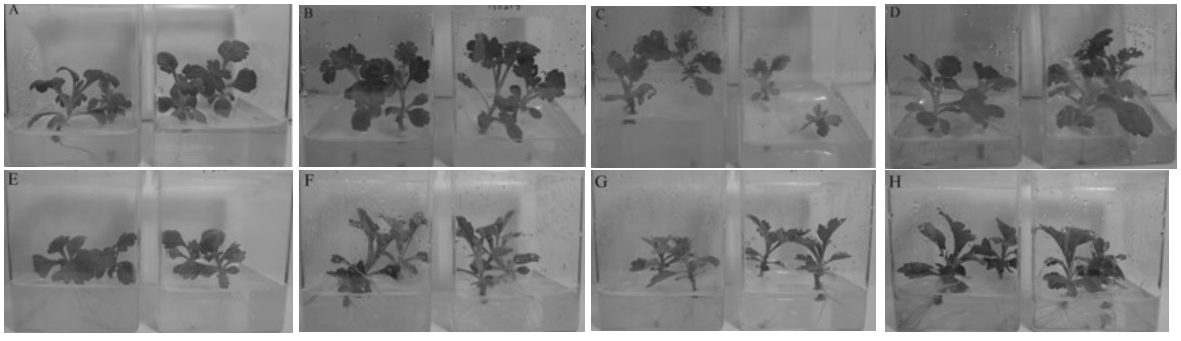

Fig. 1. *In vitro* growth of 'Baekma' (upper figures) and 'Jinba' chrysanthemum shoots (lower figures) as affected by sources of white (**A** and **E**), red (**B** and **F**), blue (**C** and **G**), and red-blue LEDs (**D** and **H**).

Table 1. *In vitro* growth of 'Baekma' and 'Jinba' chrysanthemum shoots as affected by light-emitting diode (LED) sources.

LED Source	Root (R)			Leaf No.	Shoot Length (cm)	Stem (S)			S:R Ratio	Fresh Weight (g)
	Rooting (%)	No.	Length (cm)			Node No.	Internode (cm)	Length (cm)		
					'Baekma'					
White	100 ± 0	10.0 ± 1.2	6.1 ± 1.6	11.4 ± 2.2 a	1.8 ± 0.3 ab	3.8 ± 0.6	0.92 ± 0.17 b	2.3 ± 0.3 b	0.43 ± 0.10 b	0.78 ± 0.06
Red	100 ± 0	7.5 ± 3.7	4.7 ± 0.9	7.3 ± 1.2 b	2.4 ± 0.3 a	2.8 ± 0.8	1.75 ± 0.42 a	2.9 ± 0.3 a	0.67 ± 0.10 a	0.58 ± 0.14
Blue	100 ± 0	6.3 ± 2.1	6.0 ± 0.9	9.2 ± 0.4 ab	1.4 ± 0.4 b	3.0 ± 0.8	0.90 ± 0.06 b	2.0 ± 0.6 b	0.32 ± 0.06 b	0.65 ± 0.16
Red-blue	100 ± 0	7.5 ± 2.9	5.9 ± 0.8	9.5 ± 0.5 ab	1.8 ± 0.2 ab	3.2 ± 0.6	1.12 ± 0.18 b	2.2 ± 0.3 b	0.39 ± 0.08 b	0.84 ± 0.18
Significance	ns	ns	ns	≤ 0.05	≤ 0.05	ns	≤ 0.01	≤ 0.05	≤ 0.01	ns
					'Jinba'					
White	100 ± 0	14.0 ± 2.7	10.0 ± 1.3	7.8 ± 0.4	1.7 ± 0.1 b	2.2 ± 0.2 b	1.96 ± 0.23	2.2 ± 0.2 b	0.23 ± 0.02	1.30 ± 0.41
Red	100 ± 0	12.8 ± 2.3	11.0 ± 1.9	9.7 ± 1.1	3.1 ± 0.3 a	4.0 ± 0.4 a	1.29 ± 0.17	3.7 ± 0.2 a	0.34 ± 0.03	1.61 ± 0.26
Blue	100 ± 0	13.8 ± 3.4	9.7 ± 2.2	9.3 ± 1.6	1.6 ± 0.3 b	2.5 ± 0.3 b	1.96 ± 0.69	2.4 ± 0.4 b	0.25 ± 0.05	0.99 ± 0.20
Red-blue	100 ± 0	12.0 ± 4.4	11.0 ± 1.5	11.8 ± 2.3	1.8 ± 0.4 b	3.2 ± 0.9 ab	1.76 ± 0.60	3.1 ± 0.3 a	0.28 ± 0.04	1.42 ± 0.40
Significance	ns	ns	ns	ns	≤ 0.001	≤ 0.05	ns	≤ 0.001	ns	ns

Means (n = 6) ± standard error within a column of each cultivar followed by a different letter are significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$). ns: not significantly different.

promoted net photosynthesis and partitioning of assimilates through the improved absorption of the red wavelengths (600–700 nm) and absorption peak of chlorophyll (Massa et al. 2008), thereby stimulating plant growth (Barnes and Bugbee 1992; Okamoto 1996; Baroli et al. 2008).

Greenhouse Experiment

Interaction effects between source and time of light were significant for growth of leaf and stem in 'Baekma' at $p \leq 0.05$ (Table 2). Leaf number of 'Baekma' increased in lighting with incandescent bulb at 22:00 h (49) and 0:00 h (47), and with white LEDs at 22:00 h (47), while the lowest value was observed for red LED-treated flowers at 20:00 h (39). Length and width of leaves were mostly enlarged by white LEDs at 20:00 h, with the lowest value observed for red LEDs at 22:00 h. Leaf FW and chlorophyll contents using a SPAD meter increased in white LEDs at 20:00 and 22:00 h. Leaf stem diameter expanded with all light sources at 20:00 h or with white LEDs at 22:00 h. The largest stem cavity was observed for flowers treated with red LEDs at 00:00 h or white LEDs at 22:00 h. Although white LEDs at 20:00 h increased stem cavity, cavity area ratio (cavity size/stem diameter) was the lowest, with the highest ratio observed for red-LED treated flowers at

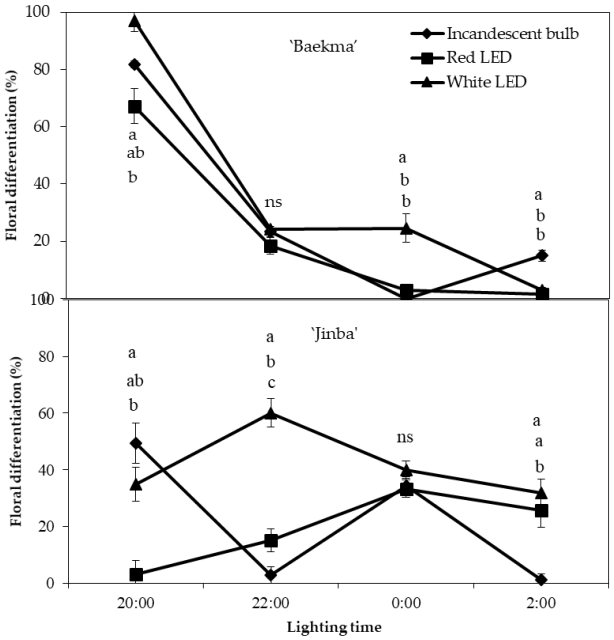

Fig. 2. Floral differentiation of 'Baekma' and 'Jinba' chrysanthemum as affected by sources and time of supplemental lighting. Different lower-case letters on each datum point for each phase indicate significant differences as determined by Duncan's multiple range test at $p \leq 0.05$. ns: not significantly different. Bars represent error of the means.

02:00 h (68%; data not shown). Light source affected FW and chlorophyll contents of leaves, and diameter, cavity, and FW of stems, which were significantly increased by white LEDs compared with incandescent bulb and red LEDs (Table 2), as previously found in *Chrysanthemum morifolium* (Jeong et al. 2012). For the effects of light time, light starting at 20:00 h increased length, width, and chlorophyll contents of leaves, with enlarging stem diameter. It is probable that early night break may have been effective in extending day length in chrysanthemum flowers, thereby causing long-day effect.

Leaf width of 'Jinba' flowers increased in lighting only at 00:00 h (6.1 cm) and 02:00 h (6.1 cm) compared with that of 'Jinba' flowers at 20:00 h (5.8 cm; Table 3). This result could be due to long-day conditions maintained during the summer and fall, possibly diminishing treatment effects of lights from August to October.

Floral differentiation of 'Baekma' was above 60% for all light treatments at 20:00 h but below 60% in 'Jinba' flowers (Fig. 2). However, floral differentiation of 'Baekma' rapidly was reduced as a result of lighting from 22:00 h to 02:00 h, while differentiation in 'Jinba' varied among light time treatments. White LEDs induced mostly overall floral differentiation in both flower cultivars, which was previously observed (Kwon et al. 2013).

Table 2. Growth of leaf and stem in 'Baekma' chrysanthemum as affected by sources and time of supplemental lighting.

Light		Leaf					Stem			
Source	Time	No.	Length (cm)	Width (cm)	FW (g)	Chlorophyll Content	Diameter (mm)	Cavity (mm)	Length (cm)	FW (g)
Incandescent bulb	20:00	41.4 ± 0.8 de	10.2 ± 0.2 b	4.5 ± 0.1 b	37.0 ± 1.4 ab	43.0 ± 0.7 bcde	5.3 ± 0.1 ab	2.9 ± 0.1 abc	107 ± 1 cd	21.6 ± 0.7 a
	22:00	48.7 ± 0.7 a	9.7 ± 0.2 bcd	4.2 ± 0.1 b	35.3 ± 1.0 abcd	41.1 ± 0.6 e	4.7 ± 0.2 cde	2.6 ± 0.1 c	120 ± 1 a	19.8 ± 0.7 ab
	00:00	47.1 ± 0.6 ab	9.3 ± 0.2 cd	4.5 ± 0.1 b	36.3 ± 1.1 abc	44.4 ± 1.0 abc	4.6 ± 0.2 de	2.8 ± 0.1 abc	117 ± 2 ab	19.7 ± 0.6 ab
	02:00	43.4 ± 0.9 cd	9.3 ± 0.1 cd	4.3 ± 0.1 b	29.6 ± 1.7 e	40.7 ± 0.7 e	4.4 ± 0.1 e	2.5 ± 0.1 c	110 ± 2 c	15.7 ± 0.7 d
Red LED	20:00	38.9 ± 0.8 f	9.8 ± 0.4 bcd	4.4 ± 0.1 b	29.9 ± 2.9 de	44.2 ± 0.7 abcd	5.3 ± 0.2 ab	3.2 ± 0.3 ab	100 ± 1 e	18.1 ± 0.8 bc
	22:00	45.5 ± 0.5 bc	9.2 ± 0.2 d	4.2 ± 0.2 b	33.5 ± 2.1 abcd	42.3 ± 1.6 cde	5.1 ± 0.2 abc	3.0 ± 0.2 abc	114 ± 1 b	19.2 ± 1.2 abc
	00:00	46.1 ± 0.6 b	9.8 ± 0.2 bcd	4.3 ± 0.1 b	30.9 ± 2.0 cde	41.1 ± 1.0 e	5.1 ± 0.1 bcd	3.3 ± 0.1 a	115 ± 1 b	18.1 ± 0.7 bc
	02:00	44.7 ± 0.6 bc	10.0 ± 0.2 bc	4.3 ± 0.1 b	28.8 ± 1.9 e	41.7 ± 0.6 cde	4.4 ± 0.1 e	3.0 ± 0.1 abc	115 ± 1 b	17.1 ± 0.5 cd
White LED	20:00	40.1 ± 0.7 ef	10.9 ± 0.3 a	5.0 ± 0.2 a	37.5 ± 1.9 ab	45.4 ± 1.1 ab	5.6 ± 0.1 a	2.7 ± 0.1 bc	106 ± 1 d	20.8 ± 0.7 a
	22:00	46.8 ± 1.0 ab	9.5 ± 0.1 cd	4.2 ± 0.1 b	38.8 ± 0.9 a	45.8 ± 0.5 a	5.3 ± 0.1 ab	3.3 ± 0.2 a	119 ± 1 ab	20.8 ± 0.5 a
	00:00	45.5 ± 1.2 bc	9.4 ± 0.2 cd	4.4 ± 0.1 b	36.6 ± 1.9 ab	41.5 ± 0.8 de	5.0 ± 0.1 bcd	2.8 ± 0.1 abc	116 ± 1 ab	21.1 ± 1.0 a
	02:00	45.4 ± 0.9 bc	9.5 ± 0.2 cd	4.3 ± 0.1 b	32.6 ± 1.3 bcd	42.6 ± 0.8 cde	4.7 ± 0.2 cde	2.9 ± 0.2 abc	117 ± 1 ab	17.2 ± 0.6 cd
Source (S)		ns	ns	ns	≤ 0.001	≤ 0.05	≤ 0.05	≤ 0.01	ns	≤ 0.05
Time (T)		≤ 0.001	≤ 0.001	≤ 0.001	ns	≤ 0.01	≤ 0.001	ns	≤ 0.001	ns
S × T		≤ 0.001	≤ 0.001	≤ 0.01	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.05	≤ 0.001	≤ 0.001

Means (n = 10) ± standard error within each column followed by a different letter are significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$). ns: not significantly different. FW: fresh weight, LED: light-emitting diode

Table 3. Growth of leaf and stem in 'Jinba' chrysanthemum as affected by sources and time of supplemental lighting.

Light	Leaf					Stem		
	No.	Length (cm)	Width (cm)	FW (g)	Chlorophyll Content	Diameter (mm)	Length (cm)	FW (g)
				Source				
Incandescent	53.0 ± 0.5	11.6 ± 0.1	6.0 ± 0.1	50.3 ± 1.5	61.0 ± 0.5	5.9 ± 0.1	108 ± 1	32.7 ± 1.2
Red LED	53.5 ± 0.4	11.6 ± 0.1	5.9 ± 0.1	53.1 ± 1.4	61.1 ± 0.4	6.0 ± 0.1	108 ± 1	33.6 ± 0.9
White LED	53.5 ± 0.5	11.8 ± 0.1	6.0 ± 0.1	51.9 ± 1.5	60.1 ± 0.4	6.0 ± 0.1	109 ± 1	33.5 ± 0.9
Significance	ns	ns	ns	ns	ns	ns	ns	ns
				Time (h)				
20:00	53.3 ± 0.5	11.5 ± 0.1	5.8 ± 0.1 b	49.4 ± 1.6	60.5 ± 0.5	5.8 ± 0.1	108 ± 1	33.1 ± 1.4
22:00	53.4 ± 0.6	11.5 ± 0.1	5.9 ± 0.1ab	50.8 ± 1.5	60.9 ± 0.4	5.9 ± 0.1	109 ± 1	32.8 ± 1.1
00:00	53.1 ± 0.6	11.9 ± 0.1	6.1 ± 0.1 a	55.2 ± 1.7	61.6 ± 0.5	6.3 ± 0.1	107 ± 1	34.7 ± 0.9
02:00	53.4 ± 0.5	11.7 ± 0.1	6.1 ± 0.1 a	51.7 ± 1.8	60.0 ± 0.5	5.9 ± 0.1	109 ± 1	32.5 ± 1.2
Significance	ns	ns	≤ 0.05	ns	ns	ns	ns	ns

Means (n = 10) ± standard error within each column followed by the same letter are not significantly different according to Duncan's New Multiple Range Test ($p \leq 0.05$). Because the interaction effect is not significant, the results of the main effects are given in the table. FW: fresh weight; ns: not significantly different

Table 4. Flower characteristics of 'Baekma' chrysanthemum as affected by sources and time of supplemental lighting.

Light		Flowering	No. of Petals	Peduncle Length (cm)	FW (g)
Source	Time				
Incandescent bulb	20:00	–	0 ± 0 c	6.0 ± 0.5 a	0.0 ± 0.0 f
	22:00	27 July	343 ± 2 a	4.5 ± 0.2 b	10.3 ± 0.6 bc
	00:00	26 July	335 ± 3 a	3.3 ± 0.3 c	11.7 ± 0.6 b
	02:00	29 July	334 ± 5 a	4.6 ± 0.3 b	8.4 ± 0.5 d
Red LED	20:00	–	0 ± 0 c	5.6 ± 0.4 a	0.0 ± 0.0 f
	22:00	29 July	325 ± 6 b	3.4 ± 0.2 c	8.0 ± 0.7 d
	00:00	27 July	329 ± 4 b	3.7 ± 0.2 bc	8.8 ± 0.7 cd
	02:00	29 July	323 ± 2 b	4.0 ± 0.2 bc	6.5 ± 0.2 e
White LED	20:00	–	0 ± 0 c	5.6 ± 0.3 a	0.0 ± 0.0 f
	22:00	29 July	334 ± 6 a	3.4 ± 0.2 c	13.5 ± 0.5 a
	00:00	29 July	343 ± 5 a	4.2 ± 0.2 bc	11.7 ± 0.8 b
	02:00	31 July	335 ± 5 a	3.3 ± 0.2 c	8.7 ± 0.6 d
Source (S)		–	ns	ns	≤ 0.05
Time (T)		–	≤ 0.001	≤ 0.001	≤ 0.001
S × T		–	≤ 0.001	≤ 0.001	≤ 0.001

Means (n = 10) ± standard error within each column followed by a different letter are significantly different according to Duncan's New Multiple Range Test ($p \leq 0.05$). FW: fresh weight; ns: not significantly different
Means (n = 10) ± standard error within each column followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).

The first flowers appeared on 31 July in 'Baekma' treated with white LEDs at 02:00 h and on 26 July when treated with incandescent bulb at 00:00 h (Table 4). White LEDs caused overall late flowering, similar to the results on freesia (Lee and Hwang 2014), chrysanthemum (Jeong et al. 2012), and *Chenopodium ambrosioides* L. (Bavrina et al. 2002). Regardless of light sources, no plants flowered with treatments starting at 20:00 h, although floral differentiation was high (Fig. 2), which resulted in the greatest peduncle length under each light source. Lighting at 20:00 h partially overlapped with the natural light. The effects of light treatments could have diminished floral development and flowering. Light starting at 20:00 h stimulated vegetative growth (Table 2), which may have partially delayed flowering (Adams et al. 2003). Incandescent bulb and white LEDs increased number of petals and FW at 22:00 h, 00:00 h, and 02:00 h compared with the red LEDs as reported in a previous study (Kwon et al. 2013).

Earliest flowering in 'Jinba' occurred under white LEDs on 28 November, 3 d earlier compared with the use of incandescent bulb and red LEDs (Table 5). As shown on the leaves and stems of 'Jinba', light sources did not significantly affect flower characteristics, whereas lighting at 00:00 h reduced the number of petals and peduncle length.

Total FW of stem + leaf + flower was higher in 'Baekma' flowers treated with white LEDs at 22:00

Table 5. Flower characteristics of 'Jinba' chrysanthemum as affected by sources and time of supplemental lighting.

Light	Flowering	No. of Petals	Peduncle Length (cm)	Fresh Weight (g)
		Source		
Incandescent bulb	01 December	202 ± 2	3.8 ± 0.1	30.5 ± 1.0
Red LED	01 December	200 ± 2	3.7 ± 0.1	32.6 ± 1.0
White LED	28 November	199 ± 2	3.7 ± 0.1	31.9 ± 1.2
Significance	–	ns	ns	ns
		Time (h)		
20:00	30 November	203 ± 3 a	3.7 ± 0.1 ab	29.8 ± 1.0
22:00	30 November	202 ± 2 a	3.8 ± 0.1 a	31.2 ± 0.8
00:00	30 November	193 ± 2 b	3.4 ± 0.1 b	33.8 ± 1.7
02:00	30 November	204 ± 3 a	4.0 ± 0.2 a	31.8 ± 1.0
Significance	–	≤ 0.01	≤ 0.05	ns

Means (n = 10) ± standard error within each column followed by a different letter are significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$). Because the interaction effect is not significant, the results of the main effects are given in the table. FW: fresh weight; ns: not significantly different

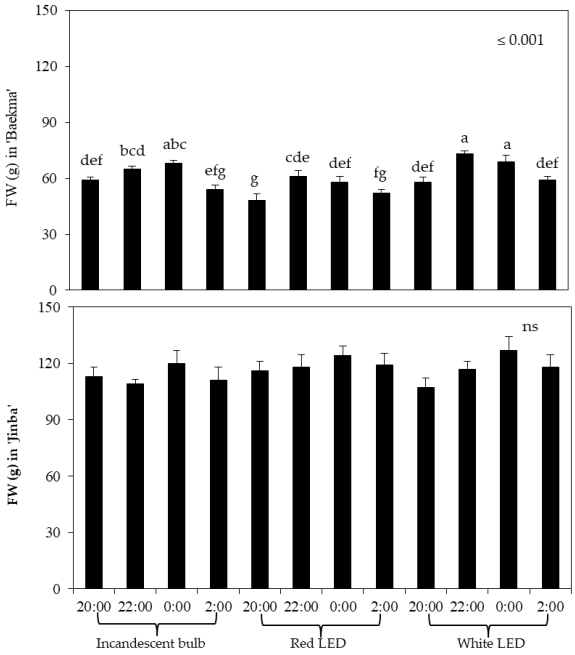

Fig. 3. Total fresh weight (FW) of 'Baekma' and 'Jinba' chrysanthemum as affected by sources and time of supplemental lighting. Different lower-case letters on each datum point for each phase indicate significant differences as determined by Duncan's multiple range test at $p \geq 0.05$. ns: not significantly different. Bars represent error of the means.

h (73 g) and 00:00 h (69 g), with the lowest values observed for those under red LEDs at 20:00 h (48 g) and 02:00 h (52 g; Fig. 3). Total FW of stem + leaf + flower in 'Jinba' was not significantly different among the treatments (Fig. 3); day length and flowering time of each cultivar should be taken into account.

CONCLUSION

Results from this current in vitro study confirmed previous findings that plant growth increased under red LEDs but was reduced under blue LEDs, which was not observed in a greenhouse study. Total FW of 'Baekma' flowers was high under white LEDs at 22:00 h in the greenhouse, which successfully delayed flowering and enlarged flower size. Lighting at 20:00 h was not recommended for any light sources in the experiment due to little flower production in 'Baekma'. In 'Jinba', light treatments did not change growth and flowering significantly, probably because the treatment duration overlapped with the natural light, which should be applied with caution on the day-length responses of 'Baekma' and 'Jinba' flowering. Both light sources, red and white, could be comparable to incandescent bulb, and in particular, white LEDs were effective in balancing vegetative and reproductive growth of 'Baekma', as well as illuminating spaces inside.

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