Night Interruption and Night Temperature Regulate Flower Characteristics in Cymbidium

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Abstract. We investigated the influences of night interruption (NI) and night temperature on flowering and flower coloration in Cymbidium. Cymbidium 'Red Fire' and 'Yokihi' were grown under a 9 hours photoperiod (control), a 9 hours photoperiod with NI at a low light intensity (LNI) of 3-7 μmol·m⁻²·s⁻¹, or a 9 hours photoperiod with NI at a high light intensity (HNI) of 120 µmol·m⁻² s⁻¹ for four hours (22:00-02:00 HR) for 16 weeks during the reproductive growth stage (Experiment 1). Thirty month-old Cymbidium 'Red Fire' plants with initiated flowering buds were placed in four different growth chamber with night temperature set points of 6, 9, 12, or 15°C for 16 hours (18:00 to 09:00 HR) and a daytime temperature of 25°C (Experiment 2). In Experiment 1, the numbers of visible buds and flowers increased, and time to flowering decreased in both the LNI and HNI treatments, as compared to the control in both cultivars. Red color in Cymbidium 'Red Fire' increased by both LNI and HNI, as evidenced by an increased a* in plants grown under these conditions, relative to those grown under the control condition. Number of days to visible buds at 9-15°C ranged from 31-34 days, as compared to 39 days at 6°C in Experiment 2. Although as the temperature increased days to flowering decreased when the plant was grown at 15°C as compared to 6, 9, or 12°C, the red color (a*) also decreased. The number of flowers and percent flowering increased when the night temperature was maintained higher than 9°C. Therefore, NI treatment and maintaining the night temperature at approximately 9-12°C during the winter season after flower spike initiation in the reproductive developmental growth stage improve flower quality and controls flowering time.

Additional key words: coloration, flowering, photoperiod

Introduction

Cymbidium hybrids are one of the most popular potted orchids due to their attractive and long-lasting flowers. In Asia, during the winter season and at the Lunar New Year, there is high demand for Cymbidium. As Cymbidium blooms in winter, the plants are exposed to short-day (SD) and low night temperatures during the flower development stage. Flower spikes in Cymbidium are initiated in early summer season and full bloom was seen during the winter season when they grown under greenhouse condition.

Environmental signals, photoperiod and temperature affect flowering and phytochemical concentrations in plants (Meng et al., 2004; Torres and Lopez, 2011). The requirement for exposure to a particular photoperiod in order to effect

flowering can be modified by temperature and vice versa (Vaz et al., 2004). Increasing day length can be promoted Cymbidium growth (Hew and Yong, 2004). Long days (LD) hasten plant development and flowering also in Lythrum salicaria L. (Kim et al., 2011a), Cleome hasslerana, and Helianthus annus (Mattson and Erwin, 2005). To promote flowering in LD plants under short natural photoperiods, day length can be extended with artificial lighting. For example, flower induction occurred in Coreopsis verticillata L. 'Moonbeam' when a 9 h photoperiod was extended for 7 h with 1.0 μmol·m⁻²·s⁻¹ (Whitman et al., 1998). Night interruption (NI), the interruption of the natural dark period by artificial lighting is effective for simulating LD conditions during SD season. A ten hour photoperiod with 4 h NI was effective for accelerating the growth and flowering in many

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herbaceous plants, such as Eustoma grandiflorum (Raf.) Shinn. (Yamada et al., 2009), Cyclamen persicum (Kang et al., 2008), and Petunia × hybrida Vilm.-Andr. (Blanchard and Runkle, 2010). In our previous research, the NI effects on Cymbidium described the promotion of the vegetative growth rate (Kim et al., 2011b), however, no report determined the NI effects on flower characteristics during the reproductive stage in orchid species including Cymbidium. Temperature should drop to not less than 10°C at night in winter Cymbidium cultivation (Rittershausen and Rittershausen, 2009). Growers have been long aware that Cymbidium plants require a period of cool night, 10-13°C and warm days for flower induction (Halevy, 1985). However, detailed study of the minimum requirement of low temperature range during the period after flower spike initiation is unclear. Photoperiod and temperature also affect the flower coloration. The importance of light in regulating anthocyanin biosynthesis in flowers has been demonstrated in apple, petunia, gerbera, and rose (Biran and Halevy, 1974; Dong et al., 1998; Weiss and Halevy, 1991). In ornamental asters, more anthocyanin was accumulated at lower temperatures than at higher temperatures (Miller et al., 2011).

Potted Cymbidium production can be divided into two general production stages: a vegetative stage, in which growth is promoted and flowering is inhibited, and a reproductive stage, in which inflorescences are initiated and flowers develop. So far most of the reports dealt with the requirement of flower induction in response to low temperature treatment. The objective of this study was to determine how moderate range of low temperatures influence flowering of mature Cymbidium orchids during reproductive stage after flower spike initiation. By providing a minimum night temperature during this stage in winter, commercial growers could theoretically provide a cooler night temperature and still promote flowering. We also determined the effects of NI with different light intensities on flower development and coloration in Cymbidium orchid. If effective, this practice could reduce the amount of energy consumed for heating greenhouses at night and applying supplemental lighting for day-extension.

Materials and Methods

Plant and Growth Conditions

Cymbidium 'Red Fire' and 'Yokihi' (Mukoyama Orchids Co., Ltd., Yamanashiken, Japan) were transplanted at the mericlonal stage into 10 cm pots, and then re-transplanted into 16 cm pots after 4 months of growth. The pots contained 100% chopped coconut. The plants were grown for 30 months until the start of the experiments in a commercial greenhouse in Hwasung, Republic of Korea. The plants used in the experiments successfully initiated flowering inflorescence in response to night temperatures or night interruption during the flowering development stage. The average day/night temperatures for the cultivation period were 28/24°C in summer and 22/13°C in winter. The average photosynthetic photon flux for the cultivation period was 500 µmol·m⁻²·s⁻¹ in summer and 215 µmol·m⁻²·s⁻¹ in winter. The plants were irrigated daily with tap water. Five grams of water-soluble controlled release 13N-5.7P-10.8K fertilizer (Mukoyama Orchids Co., Ltd., Yamanashiken, Japan) was placed at the top of each pot. The controlled release fertilizer was applied at three stages: transplanting, first pseudobulb emergence, and second pseudobulb emergence. The plants grown were given additional 200 mg·L⁻¹ K for initiation of flower spike from June to July, 2011 and the plants were given continuously additional 200 N, 100 K, 100 P, 100 Ca, and 50 Mg (mg·L⁻¹) after the emergence of flower spike until flowering. The plants were given 300-350 mL additional nutrient solution daily by a drip irrigation system.

Micronutrient fertilizers were applied bi-monthly to the plants with a sprinkler. The micronutrient fertilizers were composed of Ca(NO₃)₂·4H₂O, Fe-EDTA, MgSO₄·7H₂O, MnSO₄, ZnSO₄·7H₂O, H₃BO₃, CuSO₄·5H₂O, and Na₂MoO₄ ·2H₂O and provided 472, 3.44, 316, 1.63, 1.15, 1.24, 0.1, and 0.09 g·m⁻³ (EC 1.0 mS·cm⁻¹), respectively. Pesticides were applied at rates recommended by the manufacturer (KyungNong Co., Ltd., Seoul, Korea) as needed throughout the growing period.

NI Treatment (Experiment 1)

The experiments were performed in a commercial greenhouse (Sang-Il Orchid Farm) in Hwasung, Republic of Korea. Thirty-month old 'Red Fire' and 'Yokihi' plants with initiated flowering spike were irradiated with high-pressure sodium lamps (SKL-01; GEO, Hwasung, Republic of Korea) from 22:00 to 02:00 HR for 16 weeks (October 2010 to January 2011). Each section was divided into three groups by placing pots at different distances from the lamps. Controls received 9 h of ambient light before plants were covered with opaque black cloth daily from 17:00 to 08:00 HR, night interruption with low light intensity (LNI) conditions were 9 h of ambient light plus NI with low light intensity of 3-7 µmol·m⁻²·s⁻¹, and night interruption with high light intensity (HNI) conditions were 9 h of ambient light plus NI with high light intensity of 120 µmol·m⁻²·s⁻¹. The average day/night temperatures during NI were 22/15°C. Environmental conditions such as temperature, relative humidity, and CO₂ concentration were uniform in individual sections of the greenhouse during the experiments. The atmospheric CO₂ concentration in the

greenhouse was maintained at approximately 800 µmol·mol⁻¹ during the NI treatment period to maximize NI effects.

Temperature Treatment (Experiment 2)

Thirty-month old 'Red Fire' with initiated flowering spike was cultivated as described above and placed in four different growth chamber compartments with night temperature set points of 6, 9, 12, or 15°C for 16 h (18:00 to 09:00 HR) on 10 October, 2010. A 9 h photoperiod (09:00 to 18:00 HR) with a temperature of 25°C was maintained within the chamber for the day condition. The relative humidity was maintained at 80%. Experimental treatments were identical between replications. The experiment was ended either when the plants flowered or at 16 weeks, after the plants were placed into each finishing treatment.

Data Collection and Analysis

The number of days to visible buds and the first open flower were recorded. The number of visible buds of 5 mm or greater in size and the number of fully opened flowers were measured. The longest inflorescence measured from the base of the flowering pseudobulb was used to represent the inflorescence length. The percentage of the population that had visible buds or had flowered after 16 weeks was calculated by dividing the number of flowering plants in each treatment by the total number of plants in a treatment for Experiments 1 and 2. The surface colors of three different petals from each plant were measured using a colorimeter (CR-400, Minolta Co., Tokyo, Japan) and recorded using the CIE $(L^*, a^*, and b^*)$ from the uniform color space, in which the L^* scale ranged from no reflection ($L^* = 0$; black) to perfect diffuse reflection ($L^* = 100$; white), the a^* scale ranged from negative values for green to positive values for red, and the b^* scale ranged from negative values for blue to positive values for yellow. Numeric values of a^* and b^* were converted into the hue angle (H = $tan^{-1} b^*/a^*$) and chroma [chroma = $(a^{*2} + b^{*2})^{-1/2}$], which quantify the intensity or purity of the hue (Francis, 1980).

Diurnal gas exchanges were measured at 10 weeks after night temperature treatments (Experiment 2) using a portable photosynthesis system (Li 6400, Li-Cor Inc., Lincoln, NE, USA) equipped with an infrared gas analyzer. The fourth leaf from the base of the flowering pseudobulb was clamped onto a 6 cm² top clear chamber. The daytime light intensity ranged between 400 and 600 µmol·m⁻²·s⁻¹ PPF, and the block temperature was kept at 20°C during the day. The block temperature was kept at 6, 9, 12, or 15°C during the night for each treatment. The relative humidity in the leaf chamber ranged between 55 and 75%. CO₂ at 600 μmol·mol⁻¹ was supplied to the leaf chamber during the measurement. The net CO_2 assimilation rate (A_n) was recorded simultaneously during the measurement. Each measurement with three replicates was performed every hour for 5 min by using a built-in autoprogram.

The experimental design was a randomized complete block of three replications with 7 plants in each replication. Statistical analyses were performed using the SAS system for Windows V. 8 (SAS Inst. Inc., Cary, NC, USA). Differences among the treatment means were assessed by Tukey's honestly significant difference test at P < 0.05. Regression and graph module analyses were performed using Sigma Plot software (Systat Software, Inc., Chicago, IL, USA).

Results and Discussion

Effects of Night Interruption on Flower Development (Experiment 1)

Under the LNI and HNI conditions, both the cultivars exhibited 100% flowering and reached anthesis earlier than the control photoperiod. The application of the control photoperiod greatly reduced the flowering percentage of 'Red Fire' (13.8%) and 'Yokihi' (41.5%) plants at 16 weeks after treatment (Table 1). Significantly greater numbers of visible buds were observed in Cymbidium 'Yokihi' than in 'Red Fire'. More visible buds and flowers were opened after LNI than HNI in both cultivars and the number of days to visible buds, and first open flowers were reduced for 'Red Fire' and 'Yokihi' by LNI and HNI compared with the control. Many previous photoperiod researches reported flowering control by day-extension. Flower induction occurred in Salvia farinacea, Limonium sinuate, and Linaria maroccana (Mattson and Erwin, 2005) when natural photoperiod was extended for 6 h with 150 µmol·m⁻²·s⁻¹. In this study, NI with different light intensity was applied to the plants as a method of day-extension in Cymbidium cultivation. The light intensity for LNI was 3-7 µmol·m⁻²·s⁻¹, which was influenced merely stronger flowering characteristics compared to HNI. NI has also been shown to accelerate flowering in Cyclamen persicum (Oh et al., 2008) and Lythrum salicaria L. (Kim et al., 2011a). Long days induced accumulation of carbohydrates in Psygmorchis pusilla orchid plants when compared with those cultivated under short photoperiods (Vaz et al., 2004).

Increasing the irradiance from 150 to 900 µmol·m⁻²·s⁻¹ reduced the number of days to first open flower of Limnanthes alba Hartweg 'Mermaid' from 104 to 73 days (Seddigh and Jolliff, 1994). Increased irradiance can also hasten flowering of Viola wittrockiana Gams. and Petunia hybrida 'Fantasy Pink Morn', which flowered in as little as 28 days after germination when grown at constant 24°C under 8-9 h of

Table 1. Effect of night interruption (NI) during the finishing stage on flowering percentage, numbers of days to visible buds and the first open flower, numbers of visible buds and open flowers, and inflorescence length at the first open flower of Cymbidium 'Red Fire' and 'Yokihi'.

Cultivar	NI ^z	Days to visible buds	No. of visible buds	Days to first open flower	No. of flowers ^y	Inflorescence length (cm)	Percent flowering
'Red Fire'	Control	45.2 a [×]	40.3 ab	_w	-	72.6 a	13.8
	LNI	38.0 ab	49.0 ab	67.3 b	35.5 ab	81.6 a	100
	HNI	28.0 b	36.7 b	71.5 b	31.2 b	82.1 a	100
'Yokihi'	Control	40.7 a	53.7 ab	85.3 a	7.3 c	75.8 a	41.5
	LNI	40.0 a	61.5 a	65.7 b	49.0 a	79.4 a	100
	HNI	28.0 b	56.3 ab	71.5 b	31.2 b	80.5 a	100
Significance Cultivar		NS	***	NS	**	NS	
NI		***	NS	***	***	**	
Cultivar × NI		NS	NS	NS	NS	NS	

²The plants were grown under NI at 3-7 μmol·m⁻²·s⁻¹ (LNI) or at 120 μmol·m⁻²·s⁻¹ (HNI) from 22:00 to 02:00 HR with high-pressure sodium (HPS) lamps. Control plants were not treated with NI.

Table 2. Effect of night interruption (NI) during the finishing stage on the external petal color of Cymbidium 'Red Fire' and 'Yokihi' at the first open flowers.

Cultivar	NI ^z	L*	a*	b*	Hue (°)	Chroma
'Red Fire'	Control	36.8 b ^y	18.3 b	14.9 b	38.5 a	24.6 a
	LNI	40.5 b	25.0 ab	7.2 c	16.8 a	26.4 a
	HNI	38.5 b	31.1 a	7.2 c	13.0 a	32.0 a
'Yokihi'	Control	63.5 a	1.8 c	45.6 a	41.9 a	36.6 a
	LNI	66.1 a	1.8 c	48.4 a	41.0 a	41.0 a
	HNI	67.3 a	3.5 c	49.7 a	70.4 a	41.6 a
Significance						
Cultivar		***	***	***	*	**
NI		NS	***	NS	NS	NS
Cultivar × NI		NS	**	**	NS	NS

²Plants were grown under night interruption conditions at 3-7 μmol·m⁻²·s⁻¹ (LNI) or 120 μmol·m⁻²·s⁻¹ (HNI) from 22:00 to 02:00 HR with high-pressure sodium (HPS) lamps. Control plants were maintained under the short-day condition with black opaque cloth. ^yMean separation within the columns by Tukey's honestly significant difference test at P < 0.05.

ambient daylight conditions plus continuous 100 μmol·m⁻²·s⁻¹ HPS lighting (Erwin et al., 1997). In some orchids, high light intensity stimulates flowering and increases assimilate concentrations, suggesting a relationship between flowering and the reservation of assimilates (Kubota et al., 2005).

Color attributes of the effects of light on commercial Cymbidium flowers are expressed in terms of hue angle (h_{ab}) , lightness (L^*) , and chroma (saturation or brightness, C^*) (Table 2). The evolution of the hue angle has been observed between the plants of the control treatment and NI-treated flowers with a numerical expression for the qualitatively measured chromaticity. The red flowering group ('Red Fire') features tonalities (hue angles) ranging from 13.0° to 38.5°, coupled with low chroma (24.6-32.0) and low lightness (36.8-40.5) levels. The hue angle of the yellow flowering group ('Yokihi') ranged from 41.0° to 70.4°, joining with high L^* values from 63.5 to 67.3. A high value for a^* was observed in 'Red Fire', especially in HNI, which showed dark red color among all of the treatments. Anthocyanin accumulation is closely correlated with flower development during in vivo photoregulation (Meng et al., 2004). Weiss and Halevy (1991) proposed that photosynthesis might play a role in the light regulation of petunia corolla pigmentation. However, in Gerbera, photosynthesis may not be involved in the light regulated pigmentation, especially in the later stages of flower development (Meng et al., 2004). In 'Red

^yNo. of flowers was measured at 16 weeks after the treatment.

^xMean separation within the columns by Tukey's honestly significant difference test at P < 0.05.

WIndicates plants did not flower at 16 weeks after treatment.

Nonsignificant or significant at P < 0.01 or 0.001, respectively.

Nonsignificant or significant at P < 0.05, 0.01, or 0.001, respectively.

Fire', photosynthesis might play a role in the light regulation of cymbidium pigmentation under LNI and HNI. However, there were no significant differences in the color parameters among light treatments in 'Yokihi' (Table 2). Although the improvement of color pigmentation in the plants grown under LNI was lower than the plants grown under HNI, they would still result in higher benefits due to the promoted flowering and increased number of flowers compared to the plants grown under control. This information will enable growers to produce more diverse flowering Cymbidium plants for specific market dates with low energy consumption when they use artificial lighting during winter season.

Effects of Night Temperature on Flower Development (Experiment 2)

After 16 weeks, all Cymbidium 'Red Fire' plants flowered when grown at night temperatures of 9, 12, or 15°C, whereas 67% of the plants flowered when grown at a temperature of 6°C (Table 3). The number of flowers per plant in Cymbidium 'Red Fire' was the lowest at 6°C. Cymbidium 'Red Fire' flowered under a wider range of night temperatures, but the plants flowered earliest when exposed to 15°C. 'Red Fire' treated with 6°C reached flowering an average of 15 days later than plants at 9 or 12°C, and 20 days later than plants at 15°C. In Odontioda George McMahon

'Fortuna' and *Odontioda* Lovely Penguin 'Emperor', the percentage of plants that initiated an inflorescence was greatest (> 90%) when the plants were grown at 14 and 17°C, compared to 20 or 23°C (Blanchard and Runkle, 2008). The number of days to visible buds at 9-15°C ranged from 31-34 days compared to 39 days at 6°C (Table 3). The numbers of flowers and buds per plant decreased linearly from over 170 to fewer than 30 as the temperature increased from 15.2 to 29.8°C, representing an average reduction of nine flowers for every 1°C increase in *Oenothera fruticosa* (Clough et al., 2001). Therefore, temperatures which are increased to over 20°C could represent the high range to plants during the flower development stage in many flowering plants. The height at first flower was 35% shorter as the temperature increased from 15.2 to 23.8°C in Oenothera fruticosa (Clough et al., 2001). However, there was no change in plant height in this study as the forcing temperature increased from 6 to 15°C (Table 3).

There were no significant differences in b^* , hue, and chroma values according to night temperature in 'Red Fire' (Table 4). However, a* increased in plants grown at 6°C and significantly decreased at 15°C, compared to 9 or 12°C, indicating that the color was vivid under low temperature conditions. Temperature is the most important external factor influencing dark respiration (Levitt, 1980). High night

Table 3. Effects of night temperature on the numbers of days to visible buds and the first open flower, numbers of visible buds and open flowers, inflorescence length, and flowering percentage at the first open flower in Cymbidium 'Red Fire'.

Night temperature (°C)	Days to visible buds	No. of visible buds	Days to the first open flower	No. of flowers ^z	Inflorescence length (cm)	Percent flowering
6	39.0 a ^y	51.3 a	109.2 a	4.0 b	80.3 a	67
9	32.5 b	41.0 a	93.2 ab	33.5 a	78.5 a	100
12	33.8 ab	54.7 a	94.8 ab	36.8 a	78.4 a	100
15	31.5 b	44.3 a	88.8 b	37.0 a	78.3 a	100
Significance						
Temperature	*	NS	**	***	NS	

²No. of flowers, inflorescence length, and percent flowering were measured at 16 weeks after treatment.

Table 4. Effect of night temperature during the finishing stage on external petal color of Cymbidium 'Red Fire' at the first open flowers.

Night temperature (°C)	L*	a*	b*	Hue (°)	Chroma
6	34.4 b ^z	17.7 a	9.9 a	24.2 a	17.5 a
9	41.6 a	16.3 ab	7.5 a	22.9 a	18.4 a
12	40.4 ab	15.9 ab	8.3 a	24.1 a	15.1 a
15	38.6 ab	13.2 b	6.7 a	25.8 a	15.6 a
Significance					
Temperature	*	*	NS	NS	NS

^zMean separation within the columns by Tukey's honestly significant difference test at P < 0.05.

YMean separation within the columns by Tukey's honestly significant difference test at P < 0.05.

[&]quot;Nonsignificant or significant at P < 0.05, 0.01, or 0.001, respectively.

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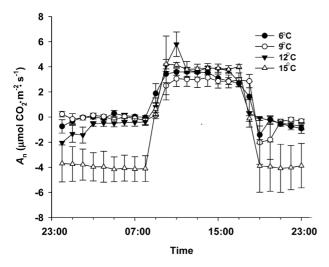


Fig. 1. Diurnal changes in net photosynthetic rate (A_n) of Cymbidium 'Red Fire' grown under 6, 9, 12, or 15°C night temperature. Measurements were taken at 10 weeks after temperature treatment (Experiment 2). Vertical bars are S.E. of the means (n = 3).

temperatures (15°C) increased dark respiration compared to 6, 9, and 12°C (Fig. 1), perhaps decreasing the carbon pool available for growth and anthocyanin production. The total net photosynthetic rate of the plants during the daytime (09:00-17:00 HR) was not significantly different among the night temperature treatments. Increasing the night temperatures might cause a higher respiration rate. Jiao et al. (1997) stated that the dark respiration rate was more sensitive to changes in temperature than leaf photosynthesis. Low night temperatures should minimize the loss of carbohydrates associated with maintenance and uncoupled respiration, leaving more for growth, storage, and pigment production (Deal et al., 1990).

In conclusion, both LNI and HNI hastened flowering and increased the percentage of flowering and number of flowers in Cymbidium 'Red Fire' and 'Yokihi' during their flowering development stage. HNI increased reddish flower color more than LNI at this stage. Increasing the night temperatures up to 15°C decreased the days to flowering, but also decreased red flower color with increased dark respiration. While maintaining night temperatures of 6°C increased the flower color, it also delayed flowering. Therefore, it is recommended that a NI system and/or maintaining night temperatures of 9°C for Cymbidium flowering after flower spike initiation to decrease flowering time and increase red flower coloration to with low energy consumption.

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