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Inhibition of Premature Flowering by High Temperature Interruption during Winter Season in *Phalaenopsis* Hybrids

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DEPARTMENT OF PLANT SCIENCE
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UNDER THE DIRECTION OF DR. KI SUN KIM
SUMMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF SEOUL NATIONAL UNIVERSITY

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ABSTRACT

High temperature above 28°C is necessary to inhibit flowering in *Phalaenopsis*. During winter season, however, growers maintain a greenhouse temperature conditions below 25°C because of greenhouse heating cost. This cultivation practice induces premature flowering. This study was conducted to develop new cultivation strategy by preventing premature flowering through energy-saving flowering inhibition using high temperature inhibition. The experiment in Chapter I was

performed to determine the growth period of the juvenile stage and low temperature duration required for inducing premature flowering. Clones of *Phalaenopsis* 'Hwasu 355' grown in a greenhouse for 2, 4, and 8 months (2, 4, and 8-month-old), which have 1-2, 2-3, or 3-4 newly developed leaves, respectively, were used in the experiment for determining the juvenile period. These plants were grown under low temperature at 25/20℃ with 7 different durations: 0, 1, 2, 3, 4, 5, and 10 weeks. After each treatment, plants were transferred to 28/28℃. In addition, to observe flower-stalk differentiation 8-month-old plants were treated with 7 weeks of low temperature. With the results of chapter I, the objective of the experiment in Chapter II was to determine the cultivation strategy for inhibition premature flowering by high temperature inhibition. Clones of 8-month-old *Phalaenopsis* 'Hwasu 355' and *Doritaenopsis* 'Mantefon' plants were cultivated at four different temperature regimes for 16 weeks: continuous low temperature (L) (no interruption), 1 week of high temperature (H) every 1 week (1L + 1H), 1 week of high temperature every 2 weeks (2L + 1H), and 2 weeks of high temperature every 2 weeks (2L + 2H). During the experiment, the low temperature and high temperature were set at 25/20℃ and 28/28℃, respectively. In Chapter I, the length of juvenile period of *Phalaenopsis* plants seemed to be between 4 and 6 months, with 3 fully developed leaves. Low temperature for 4 and 5 weeks could induce flower-stalk emergence in 8-month-old *Phalaenopsis* 'Hwasu 355', but the elongation of flower-stalks stopped. About 8 weeks of low temperature was required to induce visible inflorescences (flower-stalks longer than 0.5 cm). In chapter II, the inhibition of premature flowering was

observed in plants treated with high temperature interruption treatments. However, the flowering was slightly inhibited in *Doritaenopsis* ‘Mantefon’, whereas that was completely inhibited in *Phalaenopsis* ‘Hwasu 355’. The inhibition effects increased with shortening the interval between high temperature interruption treatments. The number of days to visible inflorescence increased with lengthening the duration of total high temperature. These results indicate that high temperature interruption can prevent premature flowering in winter. Furthermore, high temperature interruption can be used to improve previous cultivation practice through raising the flower quality by preventing premature flowering. However, because the low temperature sensitivity is different among cultivars, detailed studies are needed for specific cultivars.

Additional key words: flowering inhibition, flower-stalk differentiation, orchid, plant maturity, visible inflorescence

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GENERAL INTRODUCTION

Phalaenopsis Blume (Orchidaceae), containing approximately 66 species, is distributed throughout tropical Asia, Australia, and South Pacific Islands (Chen and Chen, 2011; Pridgeon, 2000). The orchid is epiphytic, and has thick and succulent leaves (Chen and Chen, 2011). During the past decade, *Phalaenopsis* spp. have become the most popular and economically important flowering orchids (Blanchard and Runkle, 2006). The orchids are also cultivated and traded throughout the world because of their unique flower structure, colorful flowers, and long flower longevity.

Low temperature is a primary factor for inducing flowering of *Phalaenopsis* plants; this genus is considered as having a qualitative response where the effective temperature is as high as 25°C (Blanchard and Runkle, 2006; Chen et al., 1994). Three to eight weeks of low temperature below 25°C is usually required to develop visible inflorescences with flower-stalks longer than 0.5 cm (Blanchard and Runkle, 2006; Newton and Runkle, 2009; Sakanishi et al., 1980). A diurnal temperature fluctuation is not required for flower induction, and a day temperature mainly controls flowering process (Blanchard and Runkle, 2006). Warm-day/cool-night cultivation can inhibit flowering (Blanchard and Runkle, 2006; Newton and Runkle, 2009; An et al., 2013b). However, this response appears to be cultivar dependent (Chen et al., 2008; Pollet et al., 2011). After inflorescence initiation, the development of flower-stalk is faster at a high temperature than a cooler temperature (e.g. faster at 23/21°C than at 19/17°C) (Paradiso et al., 2012).

In the juvenile stage, plants are not competent to flower, even under flower induction conditions (Amasino, 2010). The length of the juvenile stage, however, varies among species. In some herbaceous plants, the juvenile periods may last a few days to weeks, whereas the length of the juvenile stage in woody plants is longer than several years (Taiz and Zeiger, 2006). In *Phalaenopsis*, the length of the juvenile stage is unclear because of the wide range among species and cultivars. Moreover, there is several months of the period interval between physiological and commercial maturity. In commercial cultivation, the longer juvenile period is required for improving flower quality than the physiological juvenile period. Flowering in this interval period between physiological and commercial juvenile stage results in premature flowering which shows poor quality, with a short flower-stalk and low flower bud counts.

Orchids are commercially grown for 12-36 months to guarantee flower quality (Hew and Yong, 2004). *Phalaenopsis* plants are also grown for over 12 months at high temperature above 28°C to improve vegetative growth and prevent low-quality flowering. During this period, greenhouse temperature should be maintained above 28°C to prevent the development of immature inflorescence initiation (Lopez et al., 2007). *Phalaenopsis* plants go through the autumn and winter seasons more than once because of their long cultivation period. It is difficult to maintain a high temperature in a greenhouse since greenhouse heating cost is one of the largest expenses in the autumn and winter seasons (An et al., 2013b; Pollet et al., 2011). If the orchids in premature stage, which are in adult stage but not commercially mature,

are exposed to a temperature below 26°C, premature flowering can occur (Yoneda et al., 1992). For this reason, the inhibition of premature flowering is a key factor to produce high quality *Phalaenopsis* and save the heating cost.

The objective of this study was to determine the length of juvenile stage and low temperature duration to induce premature flowering in *Phalaenopsis* orchids (chapter 1), and to inhibit premature flowering using high temperature interruption (chapter 2) for developing new strategy to save heating cost during winter season.

LITERATURE REVIEW

The juvenility of orchids

The length of juvenile stage varies among orchids from one to thirteen years and the average length is between two to three years (Hew and Yong, 2004). For example, the juvenile period of *Cymbidium* 'Faridah Hashim' was about 5 years and *Dendrobium* 'Sarie Marijs' was about 3 years and 4 months (Hew and Yong, 2004). However, these data was based on the time from sowing to flowering and highly cultivar dependent. Most commercially important hybrids flower after 12-36 months (Hew and Yong, 2004; Kim et al., 2011; Lopez and Runkle, 2005). Nowadays, the juvenility based on pseudobulb size was suggested in some reports. In *Odontioda*, pseudobulbs with a diameter 5.5 cm or greater developed a visible inflorescence in 93% of plants (Blanchard and Runkle, 2008). Similarly, *Miltoniopsis* plants with a diameter smaller than 1.5 cm showed 27% of initiated flowers, whereas plants with a diameter greater than 3.1 cm showed 90% (Lopez and Runkle, 2006). These results indicate that orchids with a specific maturity are able to transit from vegetative growth to reproductive growth. *Phalaenopsis* did not have a particular pseudobulb. Therefore, the age of plants or number of leaves are commonly used to determine the maturity of *Phalaenopsis* plants.

Flowering in response to low temperature in orchids

Low temperature primarily controls flowering of orchids such as *Cattleya*

(Lopez and Runkle, 2005; Rotor, 1952), *Cymbidium* (Kim et al., 2011; Lopez and Runkle, 2005), *Dendrobium* (Lin et al., 2011; Rotor, 1952; Yen et al., 2008), *Miltoniopsis* (Lopez and Runkle, 2005; 2006), *Phalaenopsis* (Blanchard and Runkle, 2006; Sakanish et al., 1980). For example, *Cymbidium* plants grown at 13°C flowered, regardless of day length, whereas none of plants flowered at about 18°C (Rotor, 1952). Similarly, in *Cattleya warscewiczii*, flowering under same photoperiod was reduced at 18°C compared with at 13°C (Rotor, 1952). In *Phalaenopsis amabilis*, flowering was observed under low temperature at 20°C and 25°C, but plants under high temperature at 28°C did not produce flower-stalks and continued vegetative growth (Sakanishi et al., 1980).

Flowering in response to low temperature can differ within the same genera, species, and cultivars because flowering process of orchids is quite complex with interaction between various factors such as environmental sensitivity, maturity, photoperiod, light intensity, and temperature (Hew and Yong, 2004; Rotor, 1952; Sanford, 1971). In *Phalaenopsis*, 75% plants of *Phalaenopsis* Brother Goldsmith '720' flowered at 26/20°C, whereas none of *Phalaenopsis* Miva Samrtissimo x Canberra '450' flowered at the same temperature conditions (Blanchard and Runkle, 2006). Moreover, inflorescence initiation of *Doritaenopsis* 'Mantefon' was inhibited under long day conditions (16/8 h) compared with short day conditions (9/15 h) even under the same temperature (An et al., 2013a). And, Wang (1995) reported that an adequate light intensity is required for rapid response to low temperatures to induce the spiking of flower-stalks.

Inhibition of premature flowering in *Phalaenopsis*

Inhibition of flowering was observed under high temperature during day time and long day length conditions (An et al., 2013a; 2013b; Blanchard and Runkle, 2006; Newton and Runkle, 2009; Pollet et al., 2011; Sakanish et al., 1980). As an example in *Phalaenopsis amabilis*, the flower-stalk emergence was delayed and the rate of the emergence decreased as hours of the high temperature at 28°C extended (Sakanishi et al., 1980). Similarly, flowering of 12-month-old *Phalaenopsis* 'Green Apple' and 6 and 12-month-old *Doritaenopsis* 'Mantefon' was inhibited by high temperature at 29°C during 12 hour daytime period, and 6 hours of high temperature completely inhibited the flowering of 6-month-old 'Green Apple' (An et al., 2013b). These results indicate that warm-day/cool-night cultivation strategy can inhibit premature flowering and save heating-cost during autumn and winter seasons (An et al., 2013b; Pollet et al., 2011). Long day length also contributed to preventing premature flowering in *Phalaenopis*. An et al. (2013a) suggested that long day length could maintain the vegetative growth and slightly inhibit premature flowering of young *Doritaenopsis* 'Mantefon' plants. However, since the photoperiod control was the secondary factor in comparison with high temperature (Ichihashi, 1997; Sakanishi et al., 1980), the approach with high temperature inhibition is more effective than photoperiod control to prevent premature flowering.

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CHAPTER I

Determination of Juvenile Period and Low temperature Duration to induce Premature Flowering in *Phalaenopsis*

ABSTRACT

This study was conducted to determine the length of the juvenile stage and the duration of low temperature required for inducing premature flowering in *Phalaenopsis*. 2, 4, and 8-month-old *Phalaenopsis* 'Hwasu 355' plants with 1-2, 2-3, and 3-4 newly developed leaves, respectively, were used in the experiments. These plants were treated with low temperature at 25/20℃ for 7 different durations starting on September 25, 2013: 0, 1, 2, 3, 4, 5, and 10 weeks. After each low temperature treatment, plants were transferred to 28/28℃. In addition, 8-month-old plants were treated with continuous low temperature to observe flower-stalk differentiation. In 2 and 4-month-old plants, even after 10 weeks of low temperature, the flower-stalk was not observed, indicating that plants younger than 4 months are not mature enough to flower. On the other hand, in 8-month-old plants, plants treated with 4 and 5 weeks of low temperature showed 30% and 20% flower-stalk emergence, respectively. However, further flower-stalk elongation of these plants was inhibited

after moving to 28/28℃ conditions, whereas the flower-stalk was consistently differentiated under continuous low temperature. 70% of plants had a visible inflorescence (flower-stalk longer than 0.5 cm) after 10 weeks of low temperature treatment, and the number of days to visible inflorescence was 53.6. These results imply that the adult stage in *Phalaenopsis* seems to begin after plants are approximately 4 months old with 2-3 newly developed leaves. In addition, premature flowering of 8-month-old 'Hwasu 355' is induced by 8 weeks of low temperature, but inflorescence initiation can be inhibited by high temperature interruption when low temperature is not enough to induce a visible inflorescence.

Additional key words: flower-stalk differentiation, orchid, plant maturity, visible inflorescence

Introduction

Phalaenopsis Blume (Orchidaceae) is the most commercially popular flowering orchids (An et al., 2013b; Blanchard and Runkle, 2006; Lopez et al., 2007). For inducing flowering of *Phalanopsis* plants, low temperature below 25°C is required and three to eight weeks of low temperature is necessary to develop visible inflorescences (Blanchard and Runkle, 2006; Newton and Runkle, 2009; Sakanishi et al., 1980). The threshold temperature for flowering inhibition is about 28°C (Sakanish etl al., 1980), and this high temperature is recommended to bring vigorous vegetative growth for flower quality (Hew and Yong, 2004; An et al., 2013b).

The juvenile period of plants varies among species. For example, the juvenile period of *Brassica campestris* is only a few days (Friend, 1968), whereas the length of the juvenile stage is longer than 4 years in trees like olives (*Olea europaea* L.) (Santos-Antunes et al., 2005). In orchids, the length of the juvenile stage is unclear because of the wide range among genera and species. Orchids are commercially grown for about 12-36 months (Hew and Yong, 2004) to support flower quality. *Phalaenopsis* plants are also grown for over 12 months at high temperature above 28°C to improve vegetative growth and prevent low-quality flowering. If the orchids, not commercially mature, are exposed to flower forcing conditions, premature flowering is induced (Yoneda et al., 1992), and the flowering of prematured plants shows a short flower-stalk and low flower bud counts in comparison with matured plants.

Vigorous vegetative growth producing numerous new and mature leaves is desirable to improve *Phalaenopsis* flower quality (Hew and Yong, 2004; An et al., 2013b). However, since commercial orchids demand long cultivation period for sufficient vegetative condition, orchids go through the autumn and winter seasons more than once. During the autumn and winter seasons, growers maintain greenhouse temperature conditions below 25°C because greenhouse heating cost is one of the largest expenses (Pollet et al., 2011; An et al., 2013b). This cultivation practice interrupts vegetative growth by inducing premature flowering. For this reason, the inhibition of premature flowering is a key factor to produce high quality *Phalaenopsis*.

Numerous reports have suggested flowering inhibition responses under high temperature and long day length conditions (Blanchard and Runkle, 2006; Newton and Runkle, 2009; Pollet et al., 2011; An et al., 2013a; An et al., 2013b). However, to our knowledge, the border of the juvenile and adult stages and the low temperature requirement for inducing premature flowering are uncertain because of limited information; thus, it is difficult to develop an appropriate strategy for preventing premature flowering of *Phalaenopsis*. This study was performed to determine the length of the juvenile stage using 2, 4, and 8-month old *Phalaenopsis* plants and the duration of low temperature for inducing premature flowering in the orchids.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Phalaenopsis 'Hwasu 355' plants grown in a greenhouse for 2, 4, and 8 months (2, 4, and 8-month-old), with 1-2, 2-3, or 3-4 newly developed leaves, respectively, were purchased from a commercial grower (Sang Mi Orchids, Taean, Korea) on 14 October 2013, and 8-month-old *Phalaenopsis* were purchased for observing flower-stalk differentiation from the same grower on 1 April 2014. The plants were transplanted into 4 cm (2-month-old plants) and 7 cm (4 and 8-month-old plants) transparent plastic pots with 100% sphagnum moss and grown in environment-controlled growth chambers. Before temperature treatment, the chamber temperature was maintained at 28°C for inhibition of flower induction and acclimation of plants to chamber conditions. The photoperiod was 12 h with 100 ± 10 μmol m^{-2} s^{-1} from high pressure metal halide lamps. The relative humidity in the growth chambers was 50% to 70%. The plants were fertigated once a week with water soluble fertilizer (EC 1.0 mS cm^{-1}; Technigro 20N−9P−20K, Sun Gro Horticulture, Bellevue, WA, USA) by hand-drip irrigation.

Temperature Treatment

The 2, 4, and 8-month-old plants were treated with low temperature at 25/20°C for 6 different durations: 0, 1, 2, 3, 4, 5, and 10 weeks (Fig. I–1). Plants were then

transferred to 28/28°C conditions to interrupt further reproductive growth. Ten replicate plants were randomly selected for each transfer.

Flower-stalk Differentiation in 8-month-old Plants

Twenty-one plants of 8-month-old *Phalaenopsis* 'Hwasu 355' were treated with low temperature at 25/20°C for 7 weeks. Three replicates were randomly selected every week. The stem of each plant was longitudinally dissected and photographed using a mini-capture microscope (MV200UV, Cosview, Shenzhen, Guangdong, China), as described by Rotor (1952).

Data Collection and statistical analysis

The number of plants with a flower-stalk was recorded. The percentage of visible inflorescences and the date when the inflorescence length reached up to 0.5 cm were recorded for each treatment. Plants without a flower-stalk bud within 10 weeks of the temperature treatment period at 25/20°C were considered non-reproductive or too immature to flower. Plants with a visible inflorescence were regarded as having had induced flowering, whereas plants which had a flower-stalk but no visible inflorescence were considered to have had interrupted flower induction. Statistical analyses were performed using the SAS system for Windows version 9.3 (SAS Institute, Inc., Cary, NC, USA). Graph module analysis were performed using SigmaPlot software version 10.0 (Systat Software, Inc., Chicago, IL, USA).

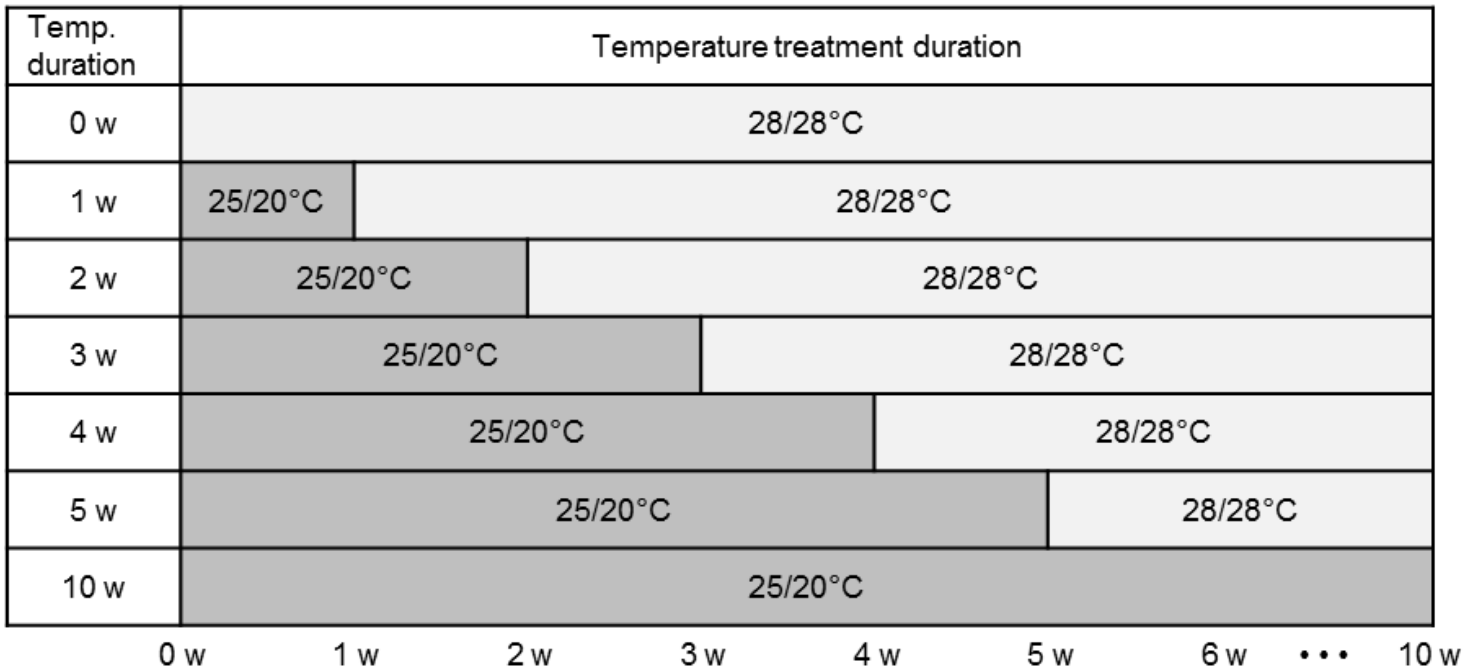

Figure I-1. Schematic diagram of temperature treatment settings in Chapter I.

RESULTS

Length of Juvenile Stage in 2, 4, and 8-month-old Plants

In 2 and 4 month-old plants, flower-stalk emergence was not observed after 10 weeks of low temperature at 25/20℃ (Fig. I–2). In 8-month-old plants, however, 70% of the plants showed flower-stalk emergence and a visible inflorescence under the same conditions (Fig. I–2 and Table I–1).

Flower-stalk Emergence and Inflorescence Initiation with Various Low temperature Duration

Inflorescence initiation was not detected in 2 and 4-month-old plants, despite receiving 10 weeks of low temperature treatment (Fig. I–2). In 8-month-old plants, all emerged flower-stalks developed into visible inflorescences which were longer than 0.5 cm (Table I–1). The number of days to visible inflorescence was 53.6 days (about 8 weeks, Table I–1). Among 8-month-old plants treated with 0, 1, 2, 3, 4, and 5 weeks of low temperature, only plants treated with 4 or 5 weeks of low temperature showed flower-stalk emergence (Fig. I–3). However, the flower-stalks were shorter than 0.5 cm, and further flower-stalk elongation was not observed after the plants were transferred to 28/28℃ conditions (Fig. I–3).

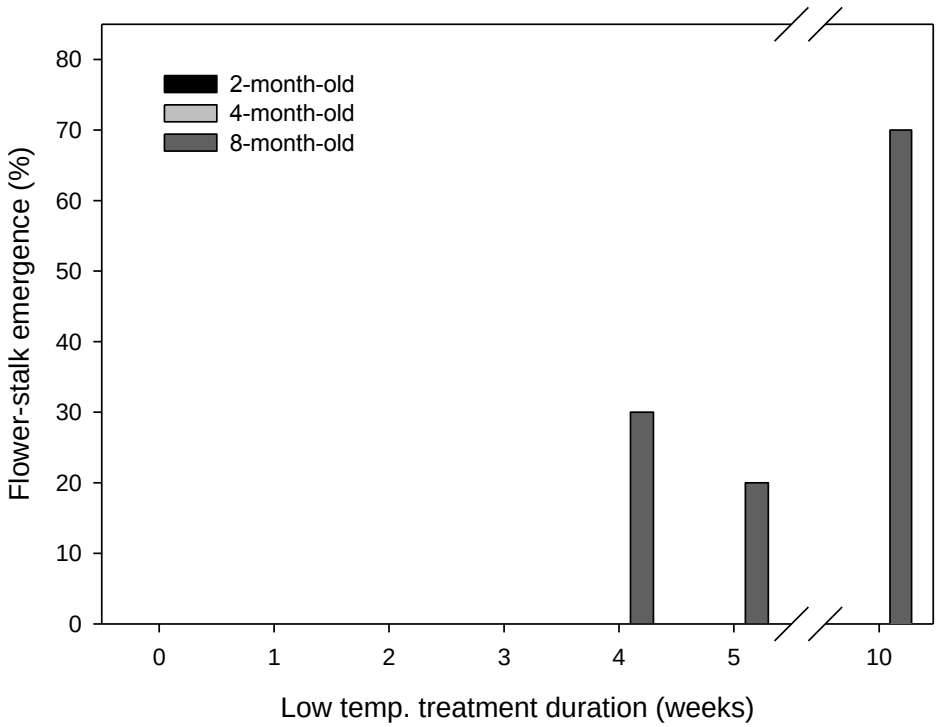

Figure I-2. Percentages of flower-stalk emergence of 2, 4, and 8-month-old *Phalaenopsis* 'Hwasu 355' as affected by low temperature treatment duration at 25/20°C. After each low temperature treatment, the plants were moved to and maintained at 28/28°C until the end of the 10-week temperature treatment period.

Table I-1. Percentage of visible inflorescence (VI) and days to VI in 8-month-old *Phalaenopsis* 'Hwasu 355' as affected by low temperature treatment duration. The percentage of VI was recorded after the end of the 10-week low temperature treatment period and the number of days to visible inflorescence (length longer than 0.5 cm) was counted from the start of the temperature treatment period.

Low temp. treatment (weeks)	VI (%)	Days to VI
0	0	-
1	0	-
2	0	-
3	0	-
4	0	-
5	0	-
10	70	53.6
Significance	***	

***, significant at $P < 0.001$; –, VI was not observed.

Figure I-3. Flower-stalk emergence in 8-month-old *Phalaenopsis* 'Hwasu 355' treated with 0, 1, 2, 3, 4, 5, and 10 weeks of low temperature. After each low temperature treatment, the plants were moved to and maintained at 28/28°C. Flower-stalk elongation in plants treated with 4 or 5 weeks of low temperature stopped when the plants were transferred to 28/28°C conditions. Photographs were taken at 10 weeks after the start of the low temperature treatment. Close-up photos show flower-stalks emergence (4 and 5 weeks) and visible inflorescence (10 weeks).

Flower-stalk Differentiation under Low temperature Conditions in 8-month-old Plants

A flower-stalk emerged at the axil of the leaves, and the flower-stalk meristem was enveloped by several bracts and epidermal tissues in the differentiated inflorescence (Fig. I–4). The apical tip of the flower-stalks differentiated with low temperature treatment (Fig. I–4F). In addition, the flower-stalks extended, and grew into the epidermal tissue as the low temperature treatment was prolonged. The size of the flower-stalks was similar among plants treated with 0 to 4 weeks of low temperature (Figs. I–4A, B, and C). On the other hand, when the low temperature treatment was longer than 5 weeks, the size of the flower-stalks was dramatically enlarged (Figs. I–4D, E, and F).

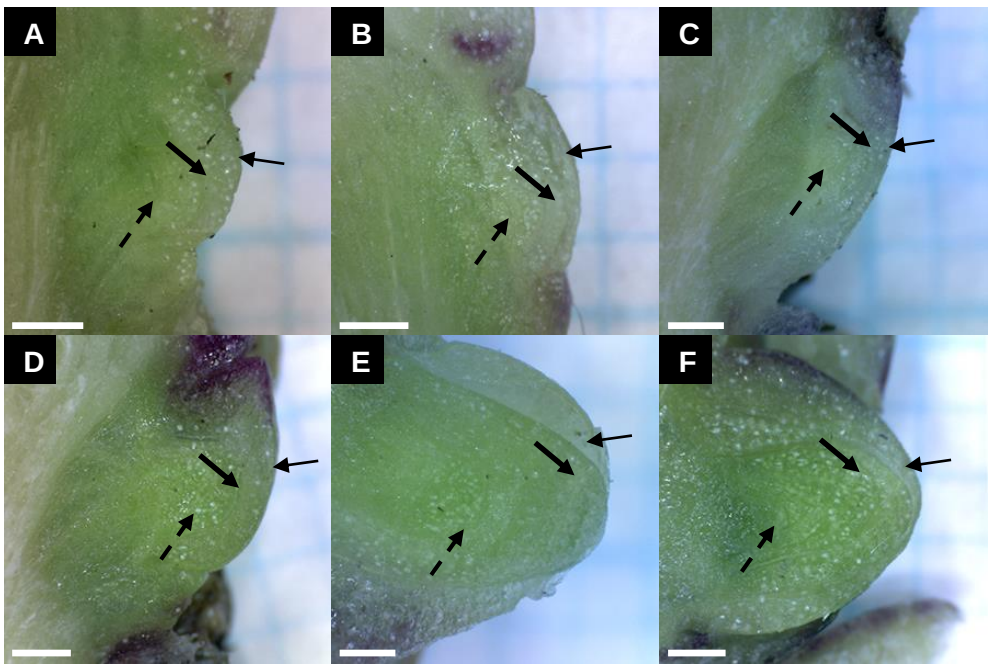

Figure I-4. Flower-stalk differentiation in 8-month-old plants during the low temperature treatment period at 25/20°C. Photographs were taken after 0 (A), 3 (B), 4 (C), 5 (D), 6 (E), and 7 (F) weeks from the start of the low temperature treatment on 6 June 2014. Thick arrow: bract; thin arrow: epidermal tissue; dash arrow: flower-stalk meristem. Scale bar = 1 mm.

DISCUSSION

Flowering cannot be induced in the juvenile phase. Reproductive growth is delayed until plants reach any stage sufficient to maintain the demands for flowering (Thomas and Vince-Prue, 1997; Kim et al., 2011). 6-month-old plants of *Doritaenopsis* 'Mantefon' and *Phalaenopsis* 'Green Apple' with 3-4 fully developed leaves flowered (An et al., 2013a; 2013b), and inflorescence initiation was induced in plants of *Phalaenopsis* 'Hercules' with 4-5 fully developed leaves under flower-inducing conditions (Pollet et al., 2011). These reports indicate that *Phalaenopsis* plants older than about 6 months or with about 4 fully developed leaves are in the adult phase already. However, whether plants younger than 6 months old are reproductive or non-reproductive is uncertain in these studies. In our result, *Phalaenopsis* 'Hwasu' younger than 4 months old with 2-3 fully developed leaves didn't flower, although they were treated with low temperature at 25/20℃ for 10 weeks (Fig. 2). Therefore, the border between the juvenile and adult phases of *Phalaenopsis* plants is assumed to be between 4 and 6 months, when plants have 3 fully developed leaves. Nevertheless, since the maturity of plants differs among species and cultivars, more studies are necessary.

Flower-stalks were observed in 8-month-old plants treated with 4 or 5 weeks of low temperature at 30% and 20%, respectively (Figs. 2 and 3). However, further elongation of the flower-stalks was not observed in plants treated with 5 weeks of low temperature, whereas flower-stalk elongation was maintained under continuous

low temperature conditions (Figs. 4E and F). These results indicate that 5 weeks of low temperature is not enough to induce non-reversible flower induction, even in 8-month-old *Phalaenopsis* ‘Hwasu 355’ plants. This phenomenon is probably because reproductive growth was interrupted when the plants were moved to 28/28℃ conditions. Similar results were suggested by Wiebe et al. (1992), who reported that high temperature treatment after vernalization (flower induction by low temperature) reduced bolting in kohlrabi (*Brassica oleracea* L. var. *gongylodes*).

In addition, Malik and Perez (2011) reported that high temperature interruption under flower-inducing conditions inhibited inflorescence development in olive. However, high temperature after inflorescence initiation where the flower-stalk length was about 5-10 cm was not harmful to the further development of flower-stalks in *Phalaenopsis*, but rather it accelerated their growth (Lopez et al., 2007; Sakanishi et al., 1980). Flowering was considered to be induced in *Phalaenopsis* when the flower-stalk length reached 0.5 cm (visible inflorescence) (Blanchard and Runkle, 2006). Therefore, the stage where the flower-stalk length was shorter than 0.5 cm is regarded as reversible flower induction.

The number of days to visible inflorescence was 53.6 days (about 8 weeks). Therefore, the low temperature duration requirement for inducing premature flowering in 8-month-old *Phalaenopsis* ‘Hwasu 355’ plants is about 8 weeks on the basis of visible inflorescence. However, the low temperature duration for inducing flowering varies according to different cultivars, treatment temperature, and plant maturity. Generally young *Phalaenopsis* plants require longer low temperature

duration for flowering than mature plants. In the study by Blanchard and Runkle (2006), the days to visible inflorescence of *Phalaenopsis* Miva Smartissimo × Canberra '450' was shorter than that of the previous year at the same low temperature conditions. Thus, the duration for inducing premature flowering in young *Phalaenopsis* 'Hwasu 355' plants is assumed to be longer than the common duration for flower induction in commercially mature plants.

In conclusion, the adult phase of *Phalaenopsis* plants seems to begin between the 4 and 6 months old stage, with 3 fully developed leaves. Low temperature treatment for 4 and 5 weeks could induce flower-stalk emergence in 8-month-old *Phalaenopsis* 'Hwasu 355', but is not enough to induce non-reversible flowering. The duration that induced a visible inflorescence in 8-month-old *Phalaenopsis* 'Hwasu 355' is about 8 weeks, which is assumed to be longer than mature plants require. However, more detailed studies are needed because the maturity of *Phalaenopsis* plants differs among species and cultivars.

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CHAPTER II

Inhibition of premature flowering of *Phalaenopsis* orchids by high temperature interruption

ABSTRACT

This study was conducted to determine the flowering inhibition effect on premature flowering by high temperature interruption. 8-month-old young *Phalaenopsis* 'Hwasu 355' and *Doritaenopsis* 'Mantefon' plants were treated with four different temperature treatments: continuous low temperature (no interruption), 1 week of high temperature every 1 week of low temperature (1L + 1H), 1 week of high temperature every 2 weeks of low temperature (2L + 1H), and 2 weeks of high temperature every 2 weeks of low temperature (2L + 2H). The low temperature and high temperature were set at 25/20℃ and 28/28℃, respectively, and maintained for 16 weeks. In *Phalaenopsis* 'Hwasu 355', flower-stalks of plants grown at no interruption conditions emerged, whereas flower-stalk emergence of plants treated with three high temperature interruption was not observed. However, *Doritaenopsis* 'Mantefon' plants showed flower-stalk emergence in all treatments. The percentage of visible inflorescence, flower-stalk longer than 0.5 cm, increased with increasing

the interval between high temperature interruptions. The number of days to visible inflorescence was increased with increasing total high temperature duration during cultivation. High temperature during interruption promoted the vegetative growth with increasing the number of new leaves, leaf span, length and width of the uppermost mature leaf in *Phalaenopsis* ‘Hwasu 355’. However, only the number of new leaves increased in *Doritaenopsis* ‘Mantefon’ because inflorescence initiation was induced even in high temperature interruption; the effects of high temperature on vegetative growth were lowered by the reproductive growth. These results indicate that high temperature interruption can prevent premature flowering and promote vegetative growth compared with low temperature cultivation. However, since *Doritaenopsis* ‘Mantefon’ plants were more sensitive to low temperature and emerged in interruption treatments, the modification of interruption method is needed in order to inhibit premature flowering more effectively.

Additional key words: flowering inhibition, flower-stalk, orchid, visible inflorescence

INTRODUCTION

Low temperature is the most important factor for flower induction of *Phalaenopsis* plants (Blanchard and Runkle, 2006; Sakanishi et al., 1980). The orchids require low temperature below 25℃ for flowering (Chen et al., 1994; Sakanishi etl al., 1980), and are classified as responding to low temperature qualitatively (Blanchard and Runkle, 2006). However, if the orchids are exposed to high temperature above 28℃, the reproductive growth is inhibited and the vegetative growth is maintained (An et al., 2013b; Newton and Runkle, 2009; Sakanishi et al., 1980). The day and night temperatures affect separately on flower induction (Blanchard and Runkle, 2006), and the flower induction is particularly controlled by a day temperature (An et al., 2013b; Blanchard and Runkle, 2006; Newton and Runkle, 2009).

Phalaenopsis plants normally flower from late summer to early spring (Lopez et al., 2007; Rotor, 1952; Sakanishi et al., 1980). During this period, the orchids are exposed to continuous short photoperiod. Research by Rotor (1952) revealed that short photoperiod induced inflorescence stalks and lateral flowering branches, whereas long photoperiod slightly prevented or delayed flowering. Similarly, short photoperiod with 8/16 h promoted flower-stalk emergence by 5 to 7 days in 3 and 6-year-old *Phalaenopsis* plants grown at 23℃ as compared with natural photoperiod (Yoneda et al., 1991). Sufficient light intensity is also required for inducing inflorescence initiation. For example, the flower-stalk emergence of *Phalaenopsis*

plants was observed in 28 days when grown at 20/15℃ with 160 μmol·m^{-2}·s^{-1}, whereas plants with 0 or 8 μmol·m^{-2}·s^{-1} did not spike the flower-stalk within 6 weeks (Wang, 1995).

Temperature, day length, and light intensity controls can inhibit flower induction of *Phalaenopsis* plants (An et al., 2013a; 2013b; Blanchard and Runkle, 2006; Konow and Wang, 2001; Newton and Runkle, 2009; Pollet et al., 2011; Sakanish et al., 1980; Wang, 1998). For example, the rate of flower-stalk emergence in *Phalaenopsis amabilis* plants was declined under high temperature conditions at 28°C (Sakanishi et al., 1980). Similarly, *Phalaenopsis hybrida* (Minho Princess × *Phalaenopsis equestris*) did not flower at 30/25℃, whereas it did flower under 25/20℃ (Su *et al.*, 2001). Long photoperiod could slightly inhibit the flowering of *Doritaenopsis* 'Mantefon' plants, and maintained the vegetative growth (An et al., 2013a). The rate of Bloomed plants in *Phalaenopsis* Atien Kaala seedlings was 2%, 77%, and 98%, respectively, under low, medium, and high PPF, representing from about 30 to 100, 60 to 160, and 150 to 330 μmol·m^{-2}·s^{-1} (Konow and Wang, 2001). However, because the photoperiod effect was the secondary factor as compared with temperature effect (Ichihashi, 1997; Sakanishi et al., 1980), high temperature control is efficient to inhibit flower induction.

The temperature duration for flower induction varies among species. If plants are exposed to high temperature periods during or after receiving the low temperature, devernalization can occur (Dole and Wilkins, 2005; Chintraruck and Ketellapper, 1969). For example, in kohlrabi (*Brassica oleracea* L. var. *gongylodes*),

the rate of bolting decreased when high temperature was treated after vernalization (flower induction by low temperature) (Wiebe and Liebig, 1992). Similarly, the inflorescence development was inhibited by high temperature interruption during flower forcing treatment in olive (*Olea europaea* L.) (Malik and Perez, 2011). However, to our knowledge, no data have been reported on the effect of devernalization in long-term cultivation of orchids. The objective of this study was to develop new cultivation method for preventing premature flowering by high temperature interruption.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Phalaenopsis 'Hwasu 355' and *Doritaenopsis* 'Mantefon' plants grown in a greenhouse for 8 months (8-month-old), with 3−4 newly developed leaves, were purchased from a commercial grower (Sang Mi Orchids, Taean, Korea) on 1 April 2014. Plants were transplanted to 12 cm transparent plastic pots filled with 100% peat moss on 14 July 2014. Ten plants of each treatment and cultivar were placed inside four different growth chambers (HB-301MP, Hanbaek Scientific Co., Buchen, Gyeonggi, Korea) in a completely randomized block design. Before temperature treatment, the chamber temperature was maintained at 28°C for 5 weeks in order to inhibit flower induction and acclimate plants to chamber conditions. The photoperiod was 12 h with 100 ± 10 μmol m^{-2} s^{-1}. The relative humidity in the growth chambers was 60 to 80%. The plants were fertigated once a week with water soluble fertilizer (EC 1.0 mS cm^{-1}; Technigro 20N−9P−20K, Sun Gro Horticulture, Bellevue, WA, USA) by hand-drip irrigation. The mean of leaf span was 24.7 and 24.0 cm in *Phalaenopsis* 'Hwasu 355' and *Doritaenopsis* 'Mantefon', respectively.

Temperature treatment

The 8-month-old *Phalaenopsis* 'Hwasu 355' and *Doritaenopsis* 'mantefon' plants were treated with four different temperature regimes: continuous low temperature (no interruption), 1 week of high temperature every 1 week of low

temperature (1L + 1H), 1 week of high temperature every 2 weeks of low temperature (2L + 1H), and 2 weeks of high temperature every 2 weeks of low temperature (2L + 2H). The low temperature and high temperature were set at 25/20℃ and 28/28℃, respectively, and temperature treatments were maintained for 16 weeks.

Data Collection and statistical analysis

The percentage of visible inflorescences and the date when the inflorescence length reached up to 0.5 cm were recorded for each treatment. Days to visible inflorescence were calculated for each plant. The number of new leaves, leaf span (the length from one end of a leaf to the opposite leaf end), and the leaf length and width of the uppermost mature leaf were measured every 4 weeks for each plant. Statistical analyses were performed using the SAS system for Windows version 9.3 (SAS Institute, Inc., Cary, NC, USA). Comparisons between treatments and cultivars were performed using Duncan's honest significant difference test at $P < 0.05$. Graph module analysis were performed using SigmaPlot software version 10.0 (Systat Software, Inc., Chicago, IL, USA).

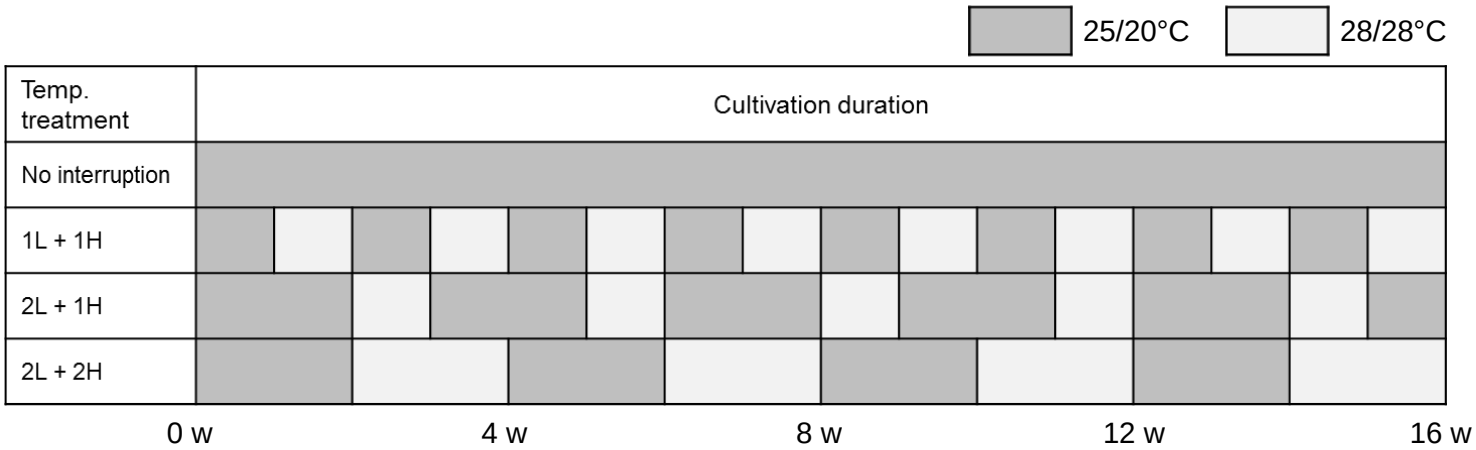

Figure II-1. Schematic diagram of temperature treatment settings in Chapter II. No interruption, 16 weeks of continuous low temperature; 1L + 1H, 1 week of high temperature every 1 week of low temperature; 2L + 1H, 1 week of high temperature every 2 weeks of low temperature; 2L + 2H, 2 weeks of high temperature every 2 weeks of low temperature.

RESULTS

Vegetative growth of 8-month-old *Phalaenopsis* Plants under Four Different Temperature Treatments

***Phalaenopsis* 'Hwasu 355'**: High temperature interruption maintained the vegetative growth in *Phalaenopsis* 'Hwasu 355' during 16 weeks of cultivation period (Fig. II-2A). The number of new leaves and length of the uppermost mature leaf increased in plants treated with high temperature interruption ($P \leq 0.01$) (Table II-1). The significant difference was not observed among three high temperature interruption treatments. Other vegetative growth indices slightly increased except the thickness of the uppermost mature leaf, but not significant. The thickness of the uppermost mature leaf became thinner when treated with high temperature interruption.

***Doritaenopsis* 'Mantefon'**: *Doritaenopsis* 'Mantefon' plants grown at all cultivation conditions spiked and the reproductive growth were induced (Fig. II-2B). The number of new leaves increased in plants treated with high temperature interruption ($P \leq 0.01$) and there was no difference among three high temperature interruption treatments. However, other indices were similar in all treatments in contrast to *Phalaenopsis* 'Hwasu 355' plants.

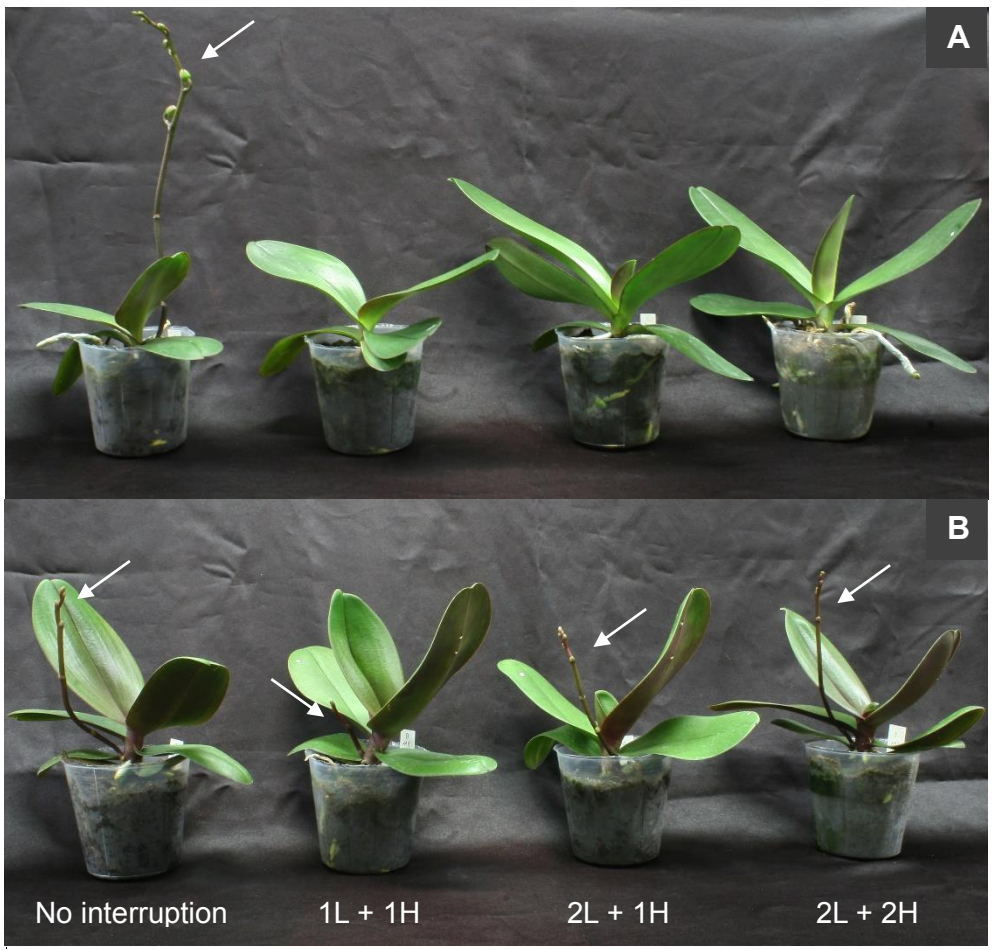

Figure II-2. Flower-stalk emergence and vegetative growth of 8-month-old *Phalaenopsis* 'Hwasu 355' (A) and *Doritaenopsis* 'Mantefon' (B) plants after 16 weeks of temperature treatments. No interruption, 16 weeks of continuous low temperature; 1L + 1H, 1 week of high temperature every 1 week of low temperature; 2L + 1H, 1 week of high temperature every 2 weeks of low temperature; 2L + 2H, 2 weeks of high temperature every 2 weeks of low temperature. Arrows indicate flower-stalks.

Table II-1. Vegetative growth of 8-month-old *Phalaenopsis* 'Hwasu 355' and *Doritaenopsis* 'Mantefon' plants after 16 weeks of temperature treatments.

Temperature treatment	No. of new leaves	Leaf span (cm)	Uppermost mature leaf		
			Length (cm)	Width (cm)	Thickness (mm)
		Phalaenopsis 'Hwasu 355'			
No interruption[z]	1.33 b[y]	26.05 a	14.37 b	6.33 a	2.32 a
1L + 1H	2.50 a	28.88 a	16.88 a	6.87 a	2.17 a
2L + 1H	2.17 a	28.32 a	17.05 a	6.95 a	2.10 a
2L + 2H	2.33 a	27.88 a	18.22 a	6.77 a	2.08 a
Significance	**	NS	**	NS	NS
		Doritaenopsis 'Mantefon'			
No interruption	1.33 b	25.98 a	15.40 a	7.40 a	2.36 a
1L + 1H	2.17 a	25.82 a	15.02 a	7.75 a	2.25 a
2L + 1H	2.00 a	26.68 a	15.87 a	7.67 a	2.31 a
2L + 2H	2.50 a	28.28 a	15.80 a	7.53 a	2.16 a
Significance	**	NS	NS	NS	NS

[z]No interruption, 16 weeks of continuous low temperature; 1L + 1H , 1 week of high temperature every 1 week of low temperature; 2L + 1H, 1 week of high temperature every 2 weeks of low temperature; 2L + 2H, 2 weeks of high temperature every 2 weeks of low temperature.

[y]Means within columns followed by different letters are significantly different by Duncan's honest significant difference test at $P \leq 0.05$.

NS, non-significant; **, significant at $P \leq 0.01$.

Flower Induction and Inhibition of 8-month-old *Phalaenopsis* Plants under Four Different Temperature Treatments

***Phalaenopsis* 'Hwasu 355'**: Flower-stalk emergence of *Phalaenopsis* 'Hwasu 355' plants was observed only under 16 weeks of low temperature treatment (Fig. II-2A and II-3A). After 16 weeks, 80% of plants grown at the continuous low temperature conditions had visible inflorescences (Table II-2). Visible inflorescence was first recorded after about 7 weeks (48 days) of temperature treatment (Fig. II-3A). The number of days to visible inflorescence was 64.6 (Table II-2).

***Doritaenopsis* 'Mantefon'**: Flower-stalk emergence of *Doritaenopsis* 'Mantefon' plants was observed under all temperature treatments (Fig. II-2B and II-3B). During the experiment, 100% of plants showed visible inflorescences when grown at the continuous low temperature conditions (Table II-2). However, 30%, 70%, and 60% of plants had visible inflorescences when treated with 1 week of high temperature every 1 week of low temperature, 1 week of high temperature every 2 weeks of low temperature, 2 weeks of high temperature every 2 weeks of low temperature, respectively. The percentage of visible inflorescence increased when the interval between interruption treatments extended, and these results were highly significant ($P \leq 0.01$). The date for the first visible inflorescence was delayed when the total high temperature duration increased (1 L + 1 H and 2 L + 2 H) (Fig. II-3B). Similarly, the number of days to visible inflorescence was extended when the total high temperature duration increased (Table II-2).

Figure II-3. Percentages of visible inflorescences of 8-month-old *Phalaenopsis* 'Hwasu 355' (A) and *Doritaenopsis* 'Mantefon' (B) as affected by four different temperature treatments. No interruption, 16 weeks of continuous low temperature; 1L + 1H, 1 week of high temperature every 1 week of low temperature; 2L + 1H, 1 week of high temperature every 2 weeks of low temperature; 2L + 2H, 2 weeks of high temperature every 2 weeks of low temperature.

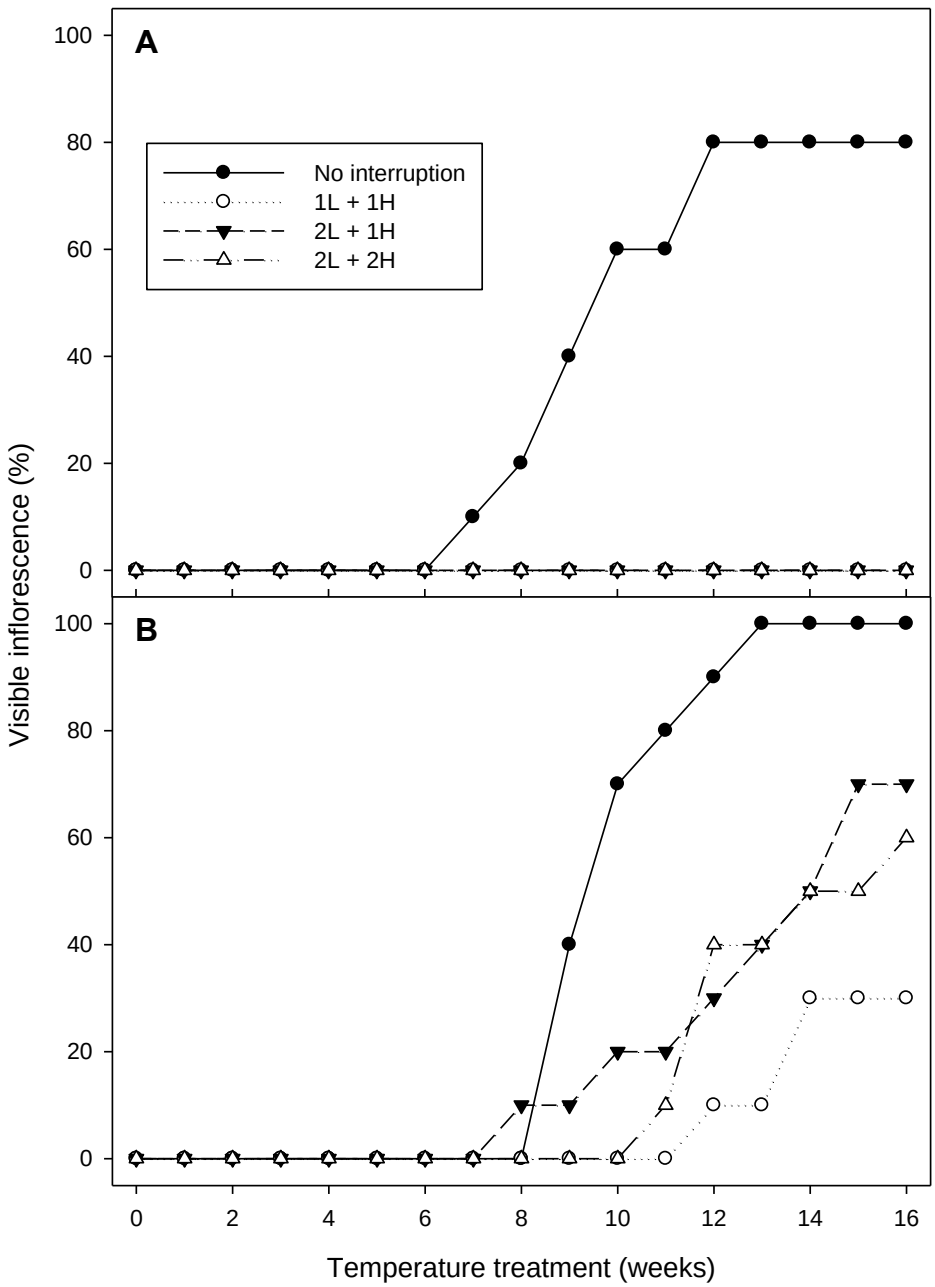
A
B
No interruption
1L + 1H
2L + 1H
2L + 2H
Visible inflorescence (%)
Temperature treatment (weeks)
0
20
40
60
80
100
0
2
4
6
8
10
12
14
16

Table II-2. Flowering of 8-month-old *Phalaenopsis* 'Hwasu 355' and *Doritaenopsis* 'Mantefon' plants after 16 weeks of temperature treatments.

Temperature treatment	Visible inflorescence (%)	Days to visible inflorescence
	Phalaenopsis 'Hwasu 355'	
No interruption[z]	80 a[y]	64.6
1L + 1H	0 b	-
2L + 1H	0 b	-
2L + 2H	0 b	-
Significance	***	
	Doritaenopsis 'Mantefon'	
No interruption	100 a	68.1 b
1L + 1H	30 b	90.7 a
2L + 1H	70 ab	83.6 ab
2L + 2H	60 ab	86.2 a
Significance	**	*

[z] No interruption, 16 weeks of continuous low temperature; 1L + 1H, 1 week of high temperature every 1 week of low temperature; 2L + 1H, 1 week of high temperature every 2 weeks of low temperature; 2L + 2H, 2 weeks of high temperature every 2 weeks of low temperature.

[y]Means within columns followed by different letters are significantly different by Duncan's honest significant difference test at $P \leq 0.05$.

*, **, ***, significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

DISCUSSIONS

High temperature interruption treatment promoted vegetative growth, especially the number of new leaves (Table II-1). In *Phalaenopsis* 'Hwasu 355', other vegetative growth indices also increased slightly. However, only new leaves were more developed in *Doritaenopsis* 'Mantefon'. These results could be attributed to the inhibition of premature flowering in *Phalaenopsis* 'Hwasu 355' by high temperature interruption, whereas the reproductive growth was induced in *Doritaenopsis* 'Mantefon' (Fig. II-2). Similarly to our results, in *Doriaenopsis* 'Mantefon' grown at warm-day/cool-night conditions with 6 hours of heating, leaf span and length of the uppermost mature leaf were shorter than those heated for 12, 18, and 24 hours; plants at 6 hours heating spiked and plants at 12, 18, and 24 heating did not spike (An et al., 2013b). In addition, the number of new leaves and leaf span increased and flowering rate decreased with increasing daily heating duration in *Phalaenopsis* 'Mosella' and 'Golden Treasure' (Newton and Runkle, 2009). Thus, high temperature during interruption can promote the vegetative growth, especially in developing new leaves, and the further promotion of the vegetative growth is induced when premature flowering is prevented.

Premature flowering of *Doritaenopsis* 'Mantefon' was slightly inhibited and that of *Phalaenopsis* 'Hwasu 355' was completely inhibited by high temperature interruption (Fig. II-2 and 3). These results are similar to the research of Malik and Perez (2011), who reported that inflorescence development of olive flowers was

inhibited by high temperature interruption. Pollet et al. (2011) suggested that the sensitivity to low temperature conditions differed among *Phalaenopsis* cultivars; *Phalaenopsis* 'Fire Fly', 'Precious' and 'Vivaldi' were more sensitive than 'Boston', 'Bristol', 'Chalk Dust', 'Lennestadt', and 'Liverpool'. Similar results were reported by Blanchard and Runkle (2006), Newton and Runkle (2009), and An et al., (2013b). In our experiment, *Doritaenopsis* 'Mantefon' was more sensitive to low temperature than *Phalaenopsis* 'Hwasu 355'. Therefore, in order to practice this high temperature interruption to commercial cultivation, detailed study is needed to determine when, how long, and how high the high temperature interruption should be for each cultivars under cultivation.

The number of days to visible inflorescence in *Phalaenopsis* 'Hwasu 355' and *Doritaenopsis* 'Mantefon' was 64.6 and 68.1, respectively, at 25/20℃ (no interruption) (Table II-2). In the previous experiment (Chapter I), the number of days to visible inflorescence in *Phalaenopsis* 'Hwasu 355' was 53.6 (Table I-1). The percentage of visible inflorescence and days to visible inflorescence slightly increased in *Phalaenopsis* 'Hwasu 355', but not significant (data not shown). In *Doritaenopsis* 'Mantefon', the percentage of visible inflorescence decreased with decreasing the interval between interruption treatments. However, days to the first visible inflorescence and the number of days to visible inflorescence increased with increasing total high temperature duration. These results indicate that the interval between interruption treatments should be shorter and the total duration of high temperature should be longer to inhibit premature flowering more effectively.

In conclusion, high temperature interruption can inhibit premature flowering in *Phalaenopsis* plants. In addition, high temperature during interruption also can promote the vegetative growth in comparison with current cultivation practice (no interruption). However, because the sensitivity among cultivars differs, the interval between high temperature interruptions and high temperature duration should be modified depending on the sensitivity of cultivars to low temperature

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ABSTRACT IN KOREAN

팔레놉시스의 개화를 억제시키기 위해서는 28도 이상의 고온이 필요하다. 하지만 겨울철 온실 내 온도를 고온으로 유지하는 것은 난방비가 많이 들기 때문에 재배자들은 보통 25도 이하로 온도를 유지한다. 이러한 재배 방법은 조기 불시 개화를 유도한다. 이번 연구는 겨울철 난방에 대한 부담을 줄이면서 조기 불시 개화를 억제시킬 수 있는 고온 교란을 이용한 새로운 재배 방법을 알아보기 위해 수행하였다. 첫 번째 실험에서는 팔레놉시스의 유년기를 알아보고, 조기 개화가 일어나는 나이대에서는 어느 정도의 저온에 노출되어야 개화가 일어나는 지 알아보았다. 유년기 길이를 알아보기 위해서 배양병에서 꺼낸 후 1-2, 2-3, 3-4장의 잎이 나온 '화수 355' 품종 2, 4, 8개월묘를 이용하였고, 각 식물체를 주, 야간 25/20도 저온을 0, 1, 2, 3, 4, 5, 10주 동안 다르게 처리게 처리하였다. 저온 처리가 끝난 식물체는 28/28도로 옮겼다. 또한 조기 개화가 유도된 8개월묘에서 화경이 분화하는 양상을 알아보기 위해 8개월묘를 7주간 저온 처리하였고, 1주일 간격으로 선발하여 분화 양상을 관찰하였다. 첫 번째 실험 결과를 바탕으로 두 번째 실험에서는 고온 교란을 통해 팔레놉시스의 조기 개화를 억제시킬 수 있는 재배 방법을 알아보았다. 두 번째 실험에서는 첫 번째 실험에서 조기 개화가 발생한다고 나타난 8개월묘를 이용하였고, 품종은

‘화수 355’와 ‘만천홍’을 이용하였다. 고온 교란이 없는 처리구, 1주일 저온 마다 1주일 고온 교란, 2주일 저온 마다 1주일 고온 교란, 2주일 저온 마다 2주일 고온 교란 처리구를 두었고, 각 처리구에서 16주간 재배 하였다. 저온 처리 온도는 25/20도 고온 교란 온도는 28/28도로 설정하였다. 첫 번째 실험에서 팔레놉시스의 유년기 길이는 충분히 발단된 잎이 3장 정도 있는 4개월에서 6개월 사이인 것으로 나타났다. 저온 처리를 4주와 5주 동안 하여도 화경은 분화하였으나 저온 처리 후 고온에 노출되었을 때 화경 신장이 정지하였다. ‘화수 355’ 품종의 8개월 묘의 경우 개화를 유도하기 위해서는 0.5센치 이상인 화경을 기준으로 대략 8주의 25/20도 저온이 필요한 것으로 나타났다. 두 번째 실험에서는 ‘화수 355’ 품종의 경우 고온 교란을 한 세 처리구 모두에서 개화가 억제되었으나, ‘만천홍’ 품종의 경우에는 고온 교란을 통해 일부만 개화를 억제시켰다. 개화 억제 효과는 고온 교란 사이의 기간이 짧을수록 큰 것으로 나타났고, 개화 유도에 필요한 기간은 재배 기간 중 총 고온 기간이 길수록 길게 나타났다. 이러한 결과는 겨울철 고온 교란이 기존 재배 방법을 개선하여 조기 개화를 억제시킴으로써 나중의 개화 품질을 증진시킬 수 있다는 것을 뜻한다. 하지만 품종 마다 저온에 대한 민감도가 다르기 때문에 품종별로 얼마 동안의 저온 기간 마다 고온 기간을 어떻게 조절하는 가에 대한 구체적인 실험이 추가적으로 필요하다.