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High Temperature Stress Prior to Vernalization Hampers Flowering Initiation and Flower-Stalk Development in *Phalaenopsis*

춘화 처리 전 고온 스트레스로 인한 호접란의 개화 개시 및 화경 발달 저해

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DEPARTMENT OF PLANT SCIENCE

THE GRADUATE SCHOOL OF SEOUL NATIONAL UNIVERSITY

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UNDER THE DIRECTION OF DR. KI SUN KIM SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF SEOUL NATIONAL UNIVERSITY

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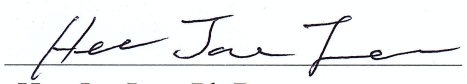
CHAIRMAN 
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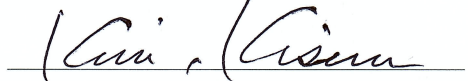
VICE-CHAIRMAN 
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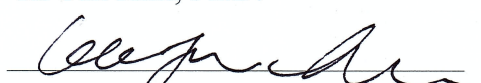
MEMBER 
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ABSTRACT

High temperature stress is an inhibitory factor in plant growth and photosynthesis. This study was conducted to determine the effects of high temperature prior to vernalization on photosynthetic capacity, carbohydrate content, and flowering of *Phalaenopsis*. High temperatures of 28 (control), 31, and 34°C were treated for 15 or 30 days before vernalization at 20°C. The experiment was conducted twice with 12-month-old *Phalaenopsis* Queen Beer 'Mantefon' (year 1 and 2) and Sogo Yukidian 'V3' (year 2) clones. During temperature treatments, relative chlorophyll content (SPAD value) and maximum quantum efficiency of photosystem II (PSII)

photochemistry (F_v/F_m) significantly decreased as temperature increased, indicating high temperature stress. CO_2 uptake, PSII operating efficiency (F_q'/F_m' or Φ_{PSII}), PSII efficiency factor (F_q'/F_v' or q_P) decreased, and non-photochemical quenching (NPQ) increased as temperature increased. In addition, sucrose content was reduced with increasing temperature. During low temperature period, similar aspects were also observed, implying the inhibitory effects of high temperature stress lasted even after the high temperature treatments. Days to visible inflorescence (>0.5cm flowering-stalk) and total days to flower significantly increased, and the total length and thickness of flower-stalk, number of buds, and flower diameter decreased by high temperature condition prior to vernalization. Exogenous sucrose supply during vernalization accelerated flowering initiation, showing significant correlation between days to visible inflorescence and sucrose contents. These results indicate that high temperature prior to vernalization hampered flowering initiation and flower-stalk development of *Phalaenopsis* by reducing photosynthetic capacity and subsequent sucrose content both during and after high temperature treatment. Furthermore, exogenous sucrose supply to high temperature-stressed plants might alleviate the damage caused by high temperature.

Additional key words: anti-vernalization, chlorophyll fluorescence, leaf color, orchid

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INTRODUCTION

Orchid family (Orchidaceae) has the most diverse species, containing over 880 genera and 25,000 species, and is distributed around the world (Givnish et al., 2016). One of the genera of Orchidaceae, *Phalaenopsis*, is an epiphytic orchid which has thick and succulent leaves (Christenson, 2001). This orchid includes approximately 66 species and is distributed throughout tropical Asia and Australia (Chen and Chen, 2011). With diverse color, longevity, and unique structure of flower, *Phalaenopsis* has high economic value and is regarded as the most commercially important orchid.

Temperature is one of the critical factors for flowering. Flowering initiation in many plants can be induced by low temperature exposure, and this process that promotes flowering is termed as vernalization (Amasino, 2005). The vernalization response was reported in *Phalaenopsis* (Sakanish et al., 1980). A low temperature below 25˚C for 3 to 7 weeks was required for flowering initiation, and a high temperature above 28˚C inhibited flowering initiation and maintained vegetative growth (Sakanish et al., 1980). Flowering initiation of *Phalaenopsis* was mainly inhibited by high daytime temperature (Blanchard and Runkle, 2006), but it was cultivar-dependent (Chen et al., 2008; Liu et al., 2013). After flowering initiation, flower-stalk development was also influenced by temperature. Days from visible inflorescence (VI) to flower under average 20˚C was longer than those under average 23˚C (Blanchard and Runkle, 2006). The number of flower buds on the main axis of the first VI increased as temperature decreased from 25.5 to 14.3˚C (Robinson, 2002).

In some species, high temperature prior to vernalization inhibited or delayed flowering initiation, and this inhibitory response is termed as anti-vernalization (Krug and Kahlen, 2008). In celery, the high temperature treatment for 10 or 20 days at 25, 30, or 35˚C before vernalization resulted in flowering inhibition, and this effect was intensified by increasing temperature and duration (Sachs and Rylski, 1980). Similarly, days to VI and total days to flower increased when the bulbs were stored at 30˚C compared to 20˚C in *Leucocoryne coquimbensis* (Ohkawa et al., 1998).

High temperature can limit plant growth, metabolism, and productivity, and high temperature stress primarily targets photosynthetic machinery (Allakhverdiev et al., 2008; Ashraf and Harris, 2013; Hasanuzzaman et al., 2013). High temperature has been reported to affect photosynthetic pigments, electron transport system (photosystem), gas exchange, and subsequent carbohydrate contents in *Phalaenopsis* (Chen et al., 2008; Guo and Lee, 2006; Jeon et al., 2006; Pollet et al., 2010). For example, the chlorophyll content was lower at 35˚C than at 25˚C (Jeon et al., 2006). The total net CO_2 uptake was significantly reduced at 33/29˚C (Guo and Lee, 2006), and temperature at 36/24˚C for 32 days significantly decreased photosynthetic efficiency (Pollet et al., 2010). In addition, plants grown at 28˚C night temperature showed the reduced carbohydrate contents than those grown at 20˚C nighttime temperature (Chen et al., 2008).

Among carbohydrates, sucrose is translocated from source to sink through the sieve element/companion cell complex of the phloem (Ruan, 2014). This material is known to act as not only energy source, but also signals in cell metabolism and plant

development (Tognetti et al., 2013). In *Phalaenopsis*, sucrose has been reported to have a crucial role in flowering initiation and flower-stalk development (Kataoka et al., 2004; Qin et al., 2012). For example, sucrose accumulation and days to visible inflorescence had a negative correlation while other soluble sugar in leaves had no correlation, indicating sucrose plays a crucial role in floral transition (Kataoka et al., 2004). Qin et al. (2012) reported that sucrose contents in leaves of *Doritaenopsis* hybrid 'Tinny Tender' increased until 28 days after low temperature treatment when bud dormancy was progressively released, and the transcription level of sucrose synthase implied that sucrose attributed to bud development and growth.

This study was performed to determine the effect of the high temperature prior to vernalization on flowering initiation and flower-stalk development and to investigate how high temperature prior to vernalization inhibits flowering through changes of photosynthetic capacity and carbohydrate accumulation.

LITERATURE REVIEW

Flowering of Orchids in Response to Temperature

Flowering responses of most plants are affected by environmental cues. Temperature is one of the critical signals for flowering. Vernalization is a process which promotes flowering by low temperature (Amasino, 2005). This vernalization-required flowering response was reported in many orchids such as *Dendrobium*, *Cattleya*, *Miltoniopsis*, *Phalaenopsis*, and *Zygopetalum* (Lopez and Runkle, 2005). For example, the flowering of *D. nobile* was induced at relatively low temperature at 13˚C regardless of photoperiod, while the vegetative growth was maintained at 18˚C (Goh and Arditti, 1985). In *C. warscewiczii*, the flowering was entirely inhibited or delayed by 2 to 3 months at short day at 18˚C, compared to short day at 13˚C (Rotor, 1952). Once flowering was initiated, flower-stalk development was also influenced by temperature. Days from VI of *Zygopetalum* Redvale 'Fire Kiss' decreased as temperature increased, with an average of 73 days at 14˚C and 30 days at 26˚C (Lopez and Runkle, 2004).

Phalaenopsis also requires the vernalization process for flowering initiation. For example, a low temperature below 25˚C induced flowering initiation (Sakanish et al., 1980), and the low temperature exposure at 25/20 (day/night) or 20/15˚C for 4 or 5 weeks was needed for uniform flowering initiation (Lee and Lin, 1984). On the contrary, a high temperature condition above 28˚C inhibited the flowering initiation

of *P. amabilis* (Sakanish et al., 1980). Similarly, flowering initiation was completely inhibited by high daytime temperature at 29˚C for above 12 h in *Phalaenopsis* cultivars (Newton and Runkle, 2009). After flowering initiation, days from VI to flower was longer in average 20˚C temperature regimes than in average 23˚C temperature regimes (Blanchard and Runkle, 2006). The number of flower buds on the main axis of the first VI increased by decreasing the temperature from 25.5 to 14.3˚C in *Phalaenopsis* Taisuco 'Smile' (Robinson, 2002).

Anti-vernalization

In some plants which require vernalization to transit from vegetative to reproductive stage, high temperature treatment before vernalization inhibited or delayed flowering initiation under even flowering-inducing temperature condition, and this phenomenon was termed as anti-vernalization (Krug and Kahlen, 2008). For example, the bolting of celery was inhibited by high temperature treatment at 25, 30, or 35˚C for 10 or 20 days before vernalization, and this inhibitory phenomenon was intensified as temperature and its duration increased (Sachs and Rylski, 1980). Similarly, delayed bolting was observed in Chinese cabbage treated by higher temperature than 18˚C before vernalization (Elers and Wiebe, 1984). *Leucocoryne coquimbensis*, a bulbuous perennial, showed longer days to VI and total days to flower when its bulbs were stored at 30˚C than at 20˚C (Ohkawa et al., 1998). In addition, the number of leaf nodes at flowering increased when the seeds were

exposed to high temperature at 30˚C before germination in *Brassica napus* var. *annua* (Dahanayake and Galwey, 1998).

Sucrose Accumulation during Flowering of *Phalaenopsis*

Sucrose is a photosynthate for translocation from source to sink through the phloem (Ruan, 2014). Sucrose accumulation is regulated by many environmental factors such as temperature, light intensity, and CO_2 concentration in *Phalaenopsis* (Kataoka et al., 2004; Liu et al., 2016; Pollet et al., 2011). For example, sucrose accumulation was maximized at about 28/22˚C and declined with temperature increase in *Phalaenopsis* hybrid 'Hercules' (Pollet et al., 2011). Under shade condition with low irradiation at 20 μmol·mol^{-1}, the sucrose content was significantly lower than that under non-shaded condition at 150 μmol·mol^{-1} in *P. aphrodite* subsp. *formosana* (Liu et al., 2016). Higher CO_2 concentration (1,000-3,000 ppm) increased the sucrose content 2-3 weeks after the CO_2 treatment compared to lower CO_2 concentration at constant 20˚C (Kataoka et al., 2004).

Sucrose is known to act as not only energy source, but also signals in cell metabolism and plant development (Tognetti et al., 2013). Flowering response is affected by sucrose accumulation in *Phalaenopsis* (Kataoka et al., 2004; Konow and Wang, 2001; Qin et al., 2012). For example, days to spiking was accelerated as the sucrose content increased by increasing light intensity and CO_2 concentration in *Phalaenopsis* 'Secret Dream', and the sucrose content significantly correlated with days to visible inflorescence while other soluble sugars in leaves had no such

correlation (Kataoka et al., 2004). Konow and Wang (2001) also reported that the increased sucrose contents by high light intensity increased flowering percentage, and accelerated days to flower in *Phalaenopsis* hybrid ‘Atien Kaala’, suggesting that sucrose is needed for both flowering initiation and flower-stalk development.

MATERIALS AND METHODS

Effect of High Temperature Prior to Vernalization on Flowering

Plant materials

In November 14, 2017, 12-month-old *Phalaenopsis* Queen Beer 'Mantefon' plants purchased from a commercial grower (Sang Mi Orchids, Taean, Korea) were potted in 10-cm transparent plastic containers filled with 100% sphagnum moss. The plants were grown in environmental-controlled growth chambers (HB-301MP, Hanbaek Scientific Co., Bucheon, Korea), and acclimated at a constant temperature of 28˚C for 5 weeks to inhibit flowering initiation before temperature treatment. The photoperiod is 12 h (8:00 to 20:00 h) with 130 ± 10 μmol·m^{-2}·s^{-1} from 250 W metal halide lamps (Han Young Electrics Co., Gwangju, Korea) and relative humidity was 60 ± 10%. The plants were fertilized weekly with water soluble fertilizer (EC 0.8 dS·cm^{-1}; Hyponex professional 20N-20P-20K, Hyponex Japan, Osaka, Japan). In April 13, 2018, two cultivars, *Phalaenopsis* Queen Beer 'Mantefon' and *Phalaenopsis* Sogo Yukidian 'V3', grown in greenhouses for 12 months (12-month-old) were purchased from a commercial grower (Sang Mi Orchids) for replication. The plants were cultivated in 10-cm transparent plastic pots with a medium composed of 100% sphagnum, and acclimated under the same condition with that of experiment in 2017 for 5 weeks. The average leaf span was 22.4 cm in *Phalaenopsis*

'Mantefon' at the beginning of year 1, and 21.9 cm and 25.7 cm in *Phalaenopsis* 'Mantefon' and 'V3', respectively, at the beginning of year 2.

Temperature treatment

Ten of each *Phalaenopsis* Queen Beer 'Mantefon' and Sogo Yukidian 'V3' plants were placed inside three different growth chambers and treated with five different temperature regimes: 28˚C for 30 days (L28; control), 31˚C for 15 days (S31), 31˚C for 30 days (L31), 34˚C for 15 days (S34), or 34˚C for 30 days (L34) (Fig. 1). Temperatures of each treatment were maintained at 28, 31, or 34˚C, and actual average air temperatures were 27.8, 30.6, or 33.7˚C and 28.0, 31.2, or 34.1˚C in year 1 and 2, respectively. After each high temperature (HT) treatment, temperature was maintained under low temperature (LT) at a constant 20˚C for vernalization and finishing which is the period for flower-stalk development.

Gas exchange measurements

Gas exchange rate was measured using a potable photosynthesis system (LI-6400, Li-Cor Inc., Lincoln, NE, USA) equipped with 2X3 clear top chamber. The measurements were carried out during nighttime (12 h, 20:00 to 08:00 h) and the uppermost mature leaf of triplicated plants were chosen for measurement. The conditions of leaf chamber, such as air temperature and relative humidity, were controlled at the same level with those of the growth chamber. CO_2 concentration was maintained at 400 μmol·mol^{-1} as a standard and flow rate was 500 μmol·m^{-2}·s^{-1}.

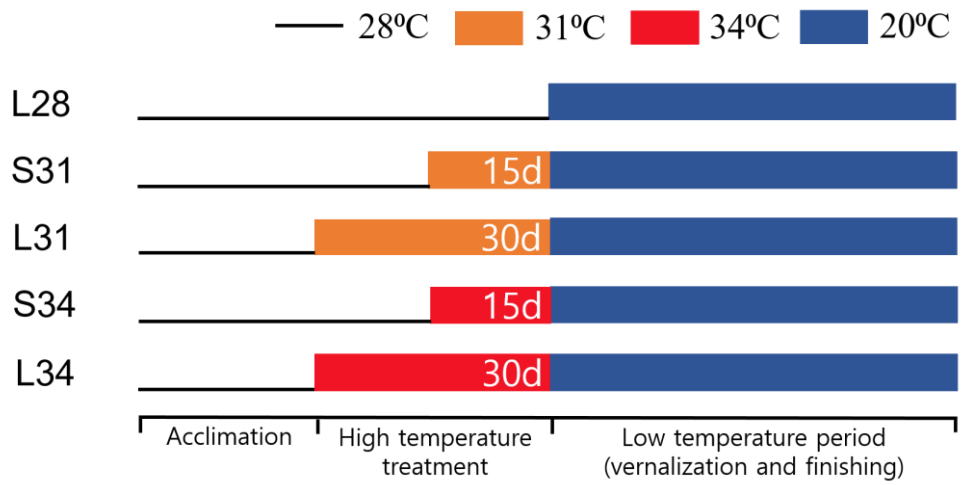

Fig. 1. Schematic diagram of temperature treatment setting. L28 (control), 28˚C for 30 days; S31, 31˚C for 15 days; L31, 31˚C for 30 days; S34, 34˚C for 15 days; L34, 34˚C for 30 days.

Chlorophyll fluorescence

Chlorophyll fluorescence was measured using a chlorophyll fluorometer (PAM-2000, Heinz Walz, Effeltrich, Germany) to monitor photosynthetic performance. The measurements were conducted from 15:00 to 17:00 h with three and six plants in year 1 and 2, respectively. To confirm adaptation to dark, the uppermost mature leaf was acclimated to dark for 30 min. The minimal fluorescence from dark-adapted leaf (F_o) was obtained by exposure to a measuring light of 0.6 kHz and less than 0.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and then a saturating pulse was irradiated for 0.8 s to determine the maximal florescence from dark-adapted leaf (F_m) for determining maximum quantum efficiency of photosystem II (PSII) photochemistry (F_v/F_m). After then, the maximal fluorescence from light-adapted leaf (F_m') was induced by illumination of continuous red, non-saturating actinic light of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and the minimal fluorescence from light-adapted leaf (F_o') were obtained by turning off actinic light and turning on far-red light for 3 s. During irradiation of continuous actinic light and saturating light pulse 20 times with a 20 s interval, F_m', F_o', and steady-state level of fluorescence (F') were recorded at the 20^{th} saturating light pulse. The PSII operating efficiency (F_q'/F_m' or Φ_{PSII}), PSII efficiency factor (F_q'/F_v' or q_P), and non-photochemical quenching (NPQ) were calculated based on the report of Baker (2008).

Carbohydrate analysis

The middle part of the uppermost mature leaf was sampled from four replications

at midday. After weighing the biomass, the leaves were frozen in liquid N_2, and stored at −80˚C until analysis.

Soluble sugar contents were determined by the method of González-Rossiaet et al. (2008) with slight modifications. About 200 mg leaf biomass were incubated in 80% ethanol at 85˚C for 15 min. After centrifugation at 3,600 g for 30 min, the supernatant was collected and this extraction was repeated twice more as described above. The sugar extracts were evaporated using a N_2 evaporator (N-EVAPTM, Organomation Associates, Inc., West Berlin, MA, USA) at 60˚C and the remaining ethanol insoluble material was dried and saved for starch analysis. The evaporated supernatant was dissolved in 3 mL of distilled water, and then passed through 0.45 μm nylon filter (Acrodisc® 13 mm syringe filter, Pall Co., Washington, NY, USA) and C18 Sep-Pak cartridge (Water Associates, Milford, MA, USA). Sugar contents were quantified by using ion chromatography with a pulsed amperometric detector (ICS-5000, Thermo Dionex, Sunnyvale, CA, USA) on Dionex CarboPac PA1 column using 50 mM NaOH (1 $mL \cdot min^{-1}$) as an eluent.

The starch, expressed as glucose equivalents, was measured according to the protocol of Smith and Zeeman (2006). The remaining sediment was dissolved in 1 mL of distilled water, and the pellets were boiled to gelatinize starch granules. Each tube was added with 0.5 mL of 0.2 M Na-acetate (pH 5.5) buffer, 15 units of amyloglucosidase (A7095, Sigma-Aldrich Korea Ltd., Yongin, Korea), and 5 units of α-amylase (A4862, Sigma-Aldrich Korea Ltd.) and incubated at 55˚C for 2 h. After centrifugation at 3,600 g for 30 min, the supernatant was collected, re-extracted

twice, and evaporated, followed by dissolution in 3 mL of distilled water. The extract was passed through 0.45 μm nylon and C18 Sep-Pak, and then the released glucose was analyzed by using an HPLC (UltiMate 3000, Dionex, Sunnyvale, CA, USA) equipped with a Shodex RI-101 detector (Showa Denko K.K., Kawasaki, Japan) on Waters Sugar-Pak column using distilled water (0.5 $mL \cdot min^{-1}$) as an eluent.

Data collection

Days to VI, and total days to flower were recorded when VI was longer than 0.5 cm and the first flower was opened, respectively. Days from VI to flower was calculated. Flowering percentage and the number of flower-stalk were also recorded. On the date of the first flower opening, total length of flower-stalk, flower-stalk thickness (from the second node of the first flower-stalk), the number of flower buds, and flower diameter (from the first flower of the first flower-stalk) were measured. The relative chlorophyll content of the uppermost mature leaf was measured using a chlorophyll meter (SPAD 502, Konica Minolta Sensing, Osaka, Japan).

Statistical analysis

The experiment was employed in a completely randomized design and ANOVA procedure of the SAS system for Windows version 9.4 (SAS Inst., Inc., Cary, NC, USA) was used for statistical analysis. Differences among the treatments were compared by Duncan's multiple range test at $P < 0.05$. Graph module analysis was

performed using SigmaPlot software version 10.0 (Systat Software, Inc., Chicago, IL, USA).

Effect of Exogenous Sucrose Supply on Flowering

Plant materials

In April 13, 2018, 12-month-old *Phalaenopsis* Queen Beer 'Mantefon' plants purchased from a commercial grower (Sang Mi Orchids, Taean, Korea) were potted in 10-cm transparent plastic containers filled with 100% sphagnum moss. The plants were grown in a closed production system at the experimental farm of Seoul National University (Suwon, Korea), and acclimated at a constant temperature of 28˚C for 7 weeks to inhibit flowering initiation before temperature treatment. The photoperiod was 12 h (8:00 to 20:00 h) with 130 ± 10 μmol·m^{-2}·s^{-1} from 250 W metal halide lamps (Han Young Electrics Co.) and relative humidity was 60 ± 10%. The plants were fertilized weekly with water soluble fertilizer (EC 0.8 mS·cm^{-1}; Hyponex professional 20N-20P-20K, Hyponex Japan). The average leaf span of *Phalaenopsis* 'Mantefon' was 22.0 cm.

Foliar spray of sucrose

Plants received a foliar spray (25 mL per plant) of sucrose (S7903, Sigma-Aldrich Korea Ltd.) at 0, 20, 40, and 80 g·L^{-1} (0, 2, 4, and 8%) with 0.1% Tween-20 (P1379, Sigma-Aldrich Korea Ltd.). The sucrose solution was treated during

vegetative growth (28˚C), and before and during vernalization (20˚C) (Fig. 2). The sucrose solutions were sprayed twice a month on all leaves, and fifteen replications was used for each treatment.

Sucrose analysis

The plants received foliar sucrose spray under 20˚C was sampled 15 and 30 days after vernalization. The middle part of the uppermost mature leaf was sampled from three replications at midday. After weighing the biomass, the leaves were frozen in liquid N_2, and stored at −80˚C until analysis. The analysis was performed as previously described.

Data collection and statistical analysis

The vegetative growth parameters such as leaf span, and width, length, thickness, and relative chlorophyll content of the uppermost mature leaf were measured from the plants sprayed during vegetative growth.

Days to VI was recorded when VI was longer than 0.5 cm. Flowering percentage and number of flower-stalks were also recorded. The experiment was employed in a completely randomized design and ANOVA procedure of the SAS system for Windows version 9.4 (SAS Inst. Inc.) was used for statistical analysis. Differences among the treatments were compared by Duncan's multiple range test at $P < 0.05$. Graph module analysis was performed using SigmaPlot software version 10.0 (Systat Software, Inc.)

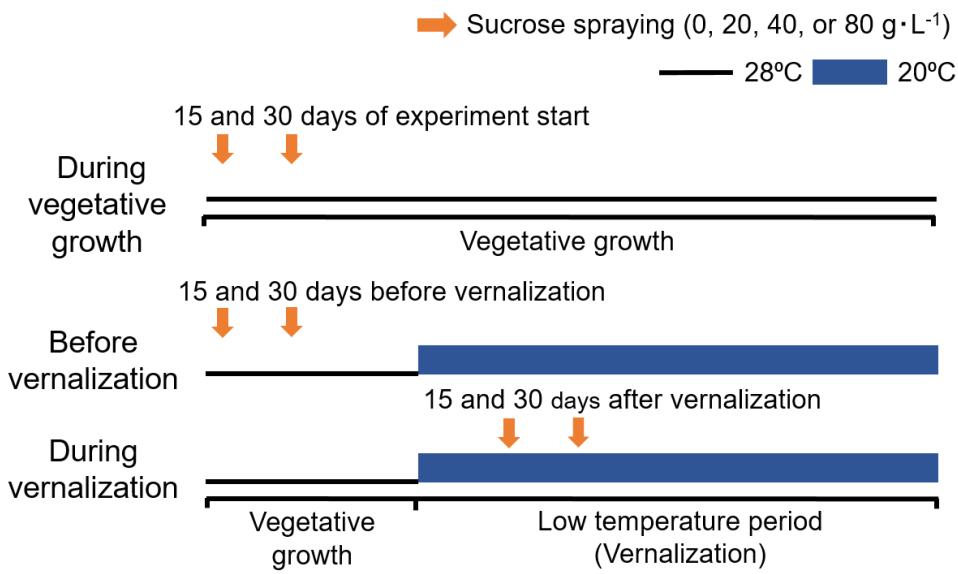

Fig. 2. Schematic diagram of exogenous sucrose supply.

RESULTS

Effect of High Temperature Prior to Vernalization on Flowering

Relative chlorophyll contents

High temperature decreased relative chlorophyll contents (SPAD value) and this thermal response was intensified with increasing temperature and treatment duration (Fig. 3). These high temperature effects were observed regardless of replication and cultivars. The SPAD value decreased by 13.8, 17.2, and 26.5% as temperature increased in *Phalaenopsis* 'Mantefon' in both year 1 and 2, and *Phalaenopsis* 'V3' in year 2, respectively. The treatment duration also intensified these effects. In year 2, plants of both cultivars under L34 had significantly lower SPAD value compared to those under S34. Fig. 4 shows that leaves, especially new leaves, of plants under higher temperature condition were brighter than those in L28.

Chlorophyll fluorescence

F_v/F_m showed decreasing trends as treatment temperature increased (Fig. 5A). While the plants of L28 had a minimal F_v/F_m value of 0.79 and 0.80 at 15 and 30 days after high temperature treatment, respectively, those of L31 and L34 showed 0.77 and 0.76 at 15 days after high temperature treatment, and 0.77 and 0.75 at 30 days after high temperature treatment, respectively. After high temperature

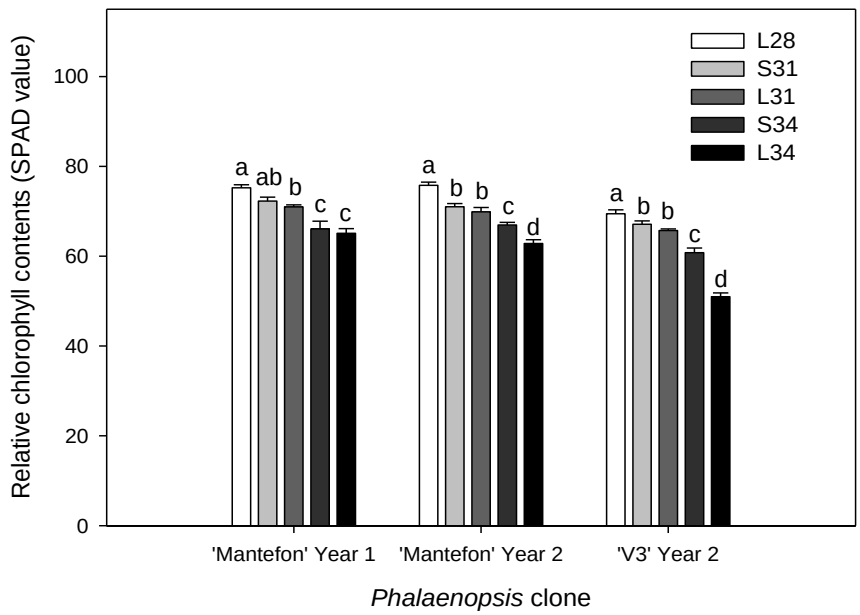

Fig. 3. Relative chlorophyll contents (SPAD value) of *Phalaenopsis* 'Mantefon' leaves in year 1 and 2, and 'V3' in year 2 after high temperature treatment. L28 (control), 28˚C for 30 days; S31, 31˚C for 15 days; L31, 31˚C for 30 days; S34, 34˚C for 15 days; L34, 34˚C for 30 days. Letters indicate mean separation by Duncan's multiple range test at $P < 0.05$. Vertical bars represent the SEs of the means (n=10).

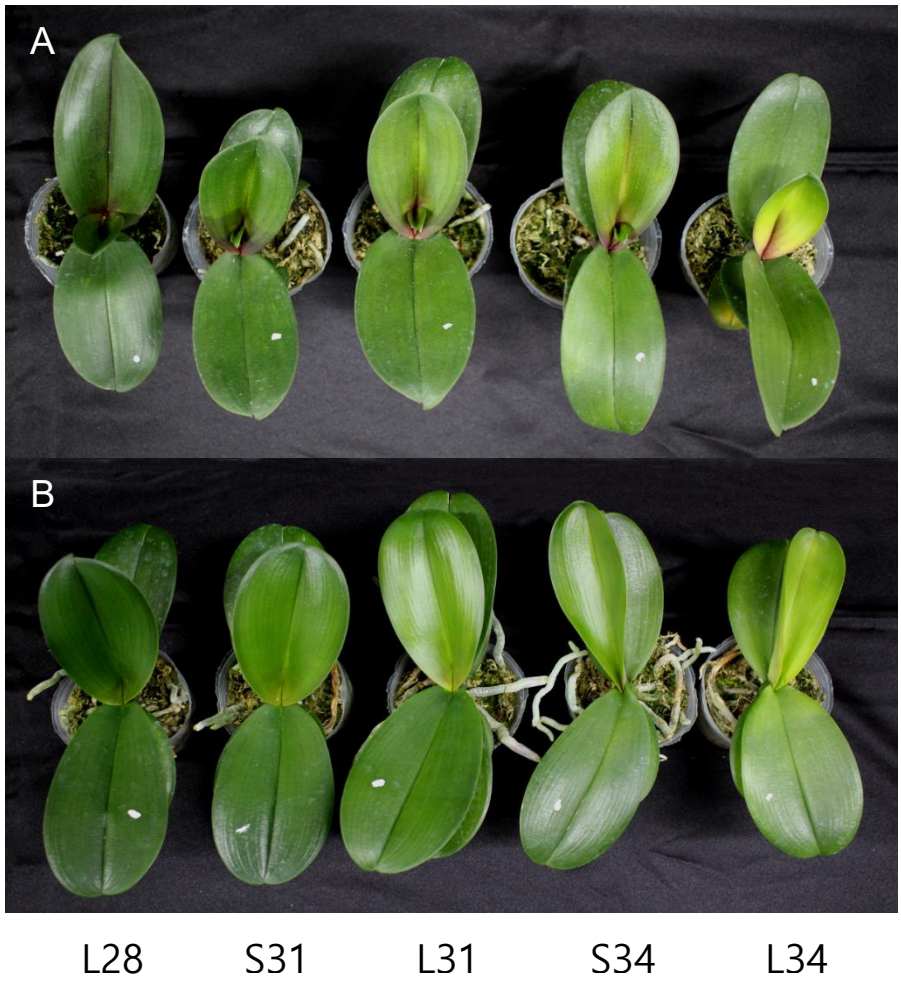

Fig. 4. *Phalaenopsis* 'Mantefon' (A) and 'V3' (B) in year 2 after high temperature treatment. L28 (control), 28˚C for 30 days; S31, 31˚C for 15 days; L31, 31˚C for 30 days; S34, 34˚C for 15 days; L34, 34˚C for 30 days.

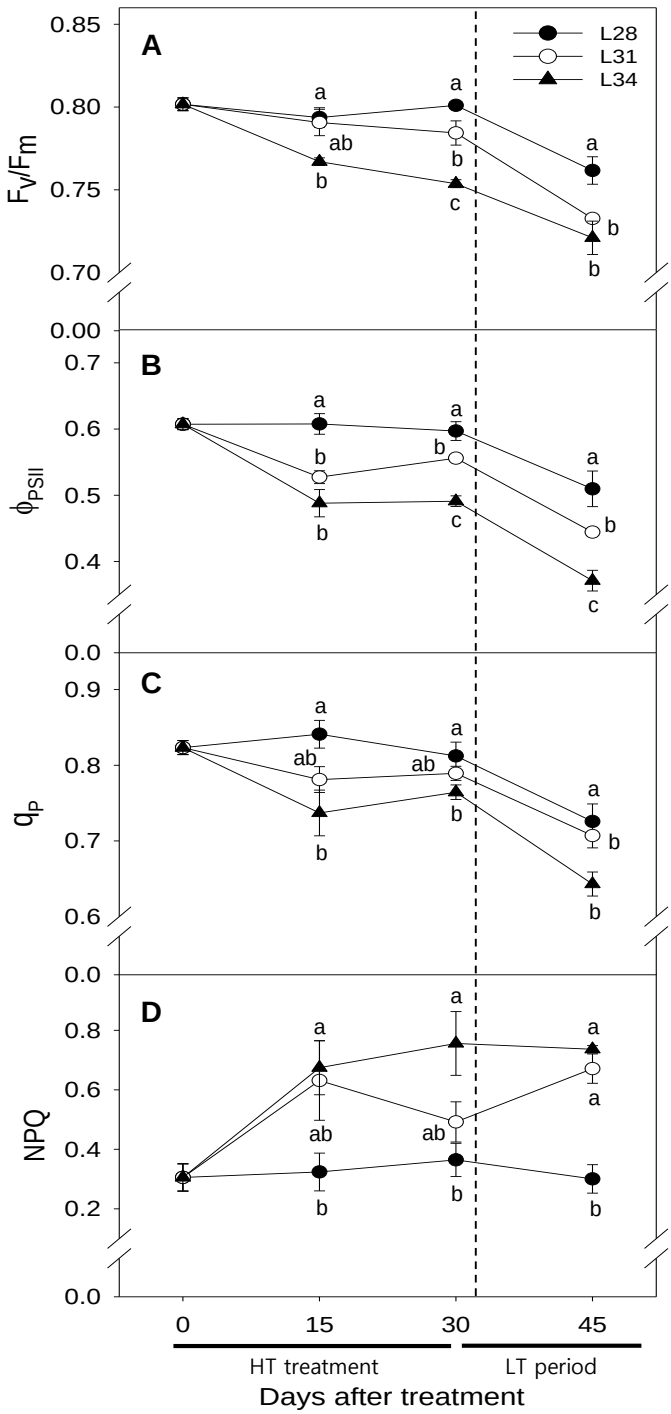
A
B
C
D
L28
L31
L34
F_V/F_m
ϕ_{PSII}
q_P
NPQ
0.85
0.80
0.75
0.70
0.00
0.7
0.6
0.5
0.4
0.0
0.9
0.8
0.7
0.6
0.0
0.8
0.6
0.4
0.2
0.0
a
ab
b
c
0
15
30
45
HT treatment
LT period
Days after treatment

Fig. 5. Maximum quantum efficiency (F_v/F_m) (A), PSII operating efficiency (Φ_{PSII}) (B), PSII efficiency factor (q_P) (C), and non-photochemical quenching (NPQ) (D) of *Phalaenopsis* 'Mantefon' leaves in year 1. L28 (control), 28˚C for 30 days; L31, 31˚C for 30 days; L34, 34˚C for 30 days. Letters indicate mean separation by Duncan's multiple range test at $P < 0.05$. Vertical bars represent the SEs of the means (n=3).

treatment, these significant decreases lasted during vernalization peiord. Φ_{PSII} and q_P significantly decreased with increasing temperature during both high temperature treatment and vernalization (Fig. 5B, C). In contrast, NPQ significantly increased with increasing temperature regardless of measurement timing (Fig. 5D).

CO_2 uptake

During high temperature treatment, the increasing temperature significantly decreased CO_2 uptake during nighttime (Fig. 6). The CO_2 uptake of the plants of L31 and L34 were lower by 31.2 and 49.6%, respectively, at 15 days after high temperature treatment than those of L28. At 30 days after high temperature treatment, the plants of L31 and L34 had lower CO_2 uptake by 20.4 and 49.6%, respectively, than those of L28. During vernalization, the plants of L31 and L34 still showed lower CO_2 uptake by 13.8 and 28.0%, compared to those of L28.

Carbohydrate contents

Glucose and fructose contents were not significantly different among the treatments, and no trend was found during both high temperature treatment and vernalization (Fig. 7A, B). Starch contents tended to decrease as temperature increased, although there was no significant difference (Fig. 7D). Among carbohydrates, only sucrose showed a significant difference (Fig. 7C). The sucrose contents were reduced by increasing temperature during both high temperature treatment and vernalization.

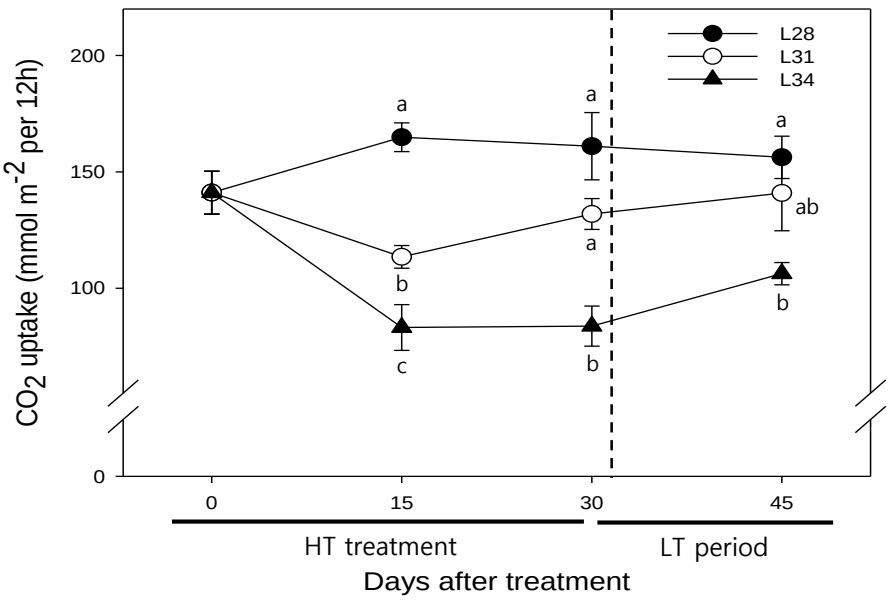

Fig. 6. CO_2 uptake during nighttime of *Phalaenopsis* 'Mantefon' leaves in year 1. L28 (control), 28˚C for 30 days; L31, 31˚C for 30 days; L34, 34˚C for 30 days. Letters indicate mean separation by Duncan's multiple range test at $P < 0.05$. Vertical bars represent the SEs of the means (n=3).

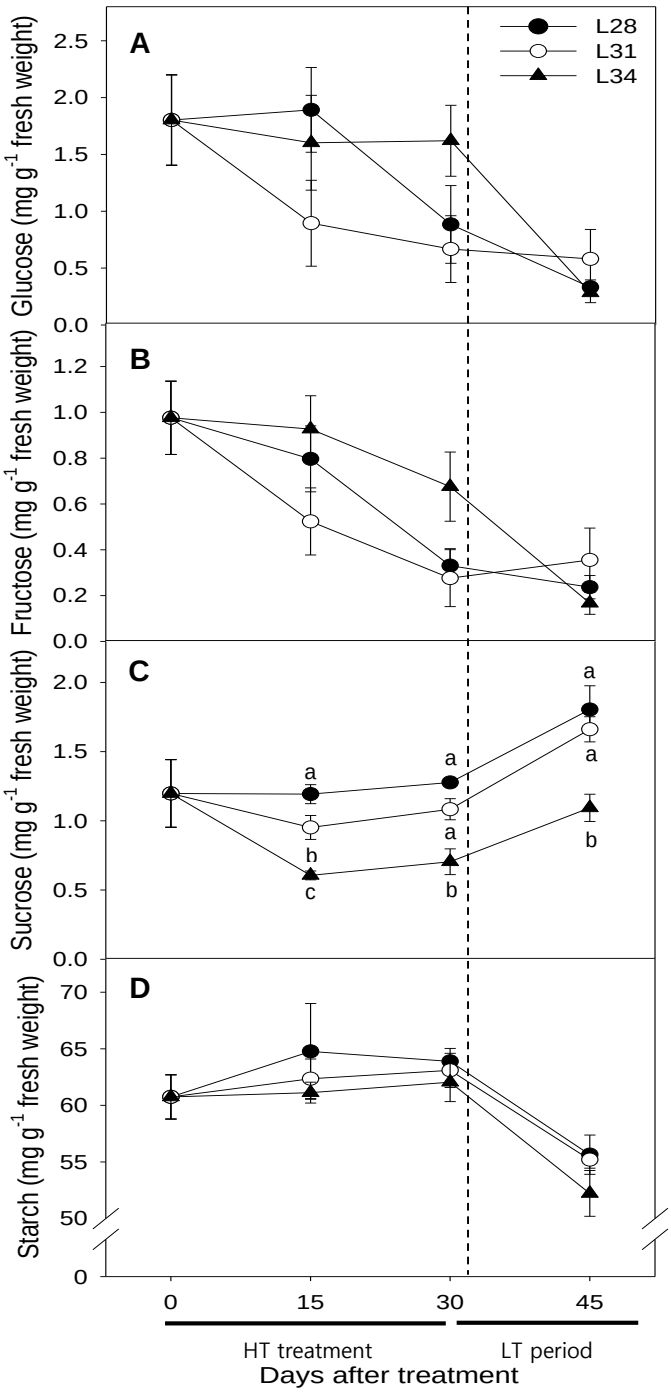
A
B
C
D
L28
L31
L34
Glucose (mg g-1 fresh weight)
Fructose (mg g-1 fresh weight)
Sucrose (mg g-1 fresh weight)
Starch (mg g-1 fresh weight)
0
15
30
45
HT treatment
LT period
Days after treatment

Fig. 7. Carbohydrate contents in *Phalaenopsis* 'Mantefon' leaves in year 1. L28 (control), 28˚C for 30 days; L31, 31˚C for 30 days; L34, 34˚C for 30 days. All values are mean ± SE of three individual plants. Letters indicate mean separation by Duncan's multiple range test at $P < 0.05$. Vertical bars represent the SEs of the means (n=4).

Recovery of thermal stress

Relative chlorophyll contents and the maximum quantum efficiency of PSII photochemistry (F_v/F_m) were measured as indicators of thermal stress during high temperature treatment. These measurements were also made during low temperature period to investigate the recovery aspects of thermal stress. The decreased SPAD values during high temperature treatment were slightly recovered during low temperature period, and F_v/F_m values also showed the similar trend to SPAD value (Fig. 8A, B). However, there were still significant differences among treatments until 150 days after start of treatment in both SPAD and F_v/F_m value, showing these thermal stresses also lasted during low temperature period.

Flower-stalk emergence

Although high temperature treatment prior to vernalization had no effect on percentage of visible inflorescence, it made a significant delay in flower-stalk emergence. As temperature and duration of treatment increased, days to visible inflorescence showed increasing trends by 53.6, 50.5, and 55.5% in 'Mantefon' in year 1 and 2, and 'V3' in year 2, respectively (Fig. 9). These trends were observed regardless of replication and cultivars.

Flower-stalk development

In addition to flower-stalk emergence, flower-stalk development was influenced

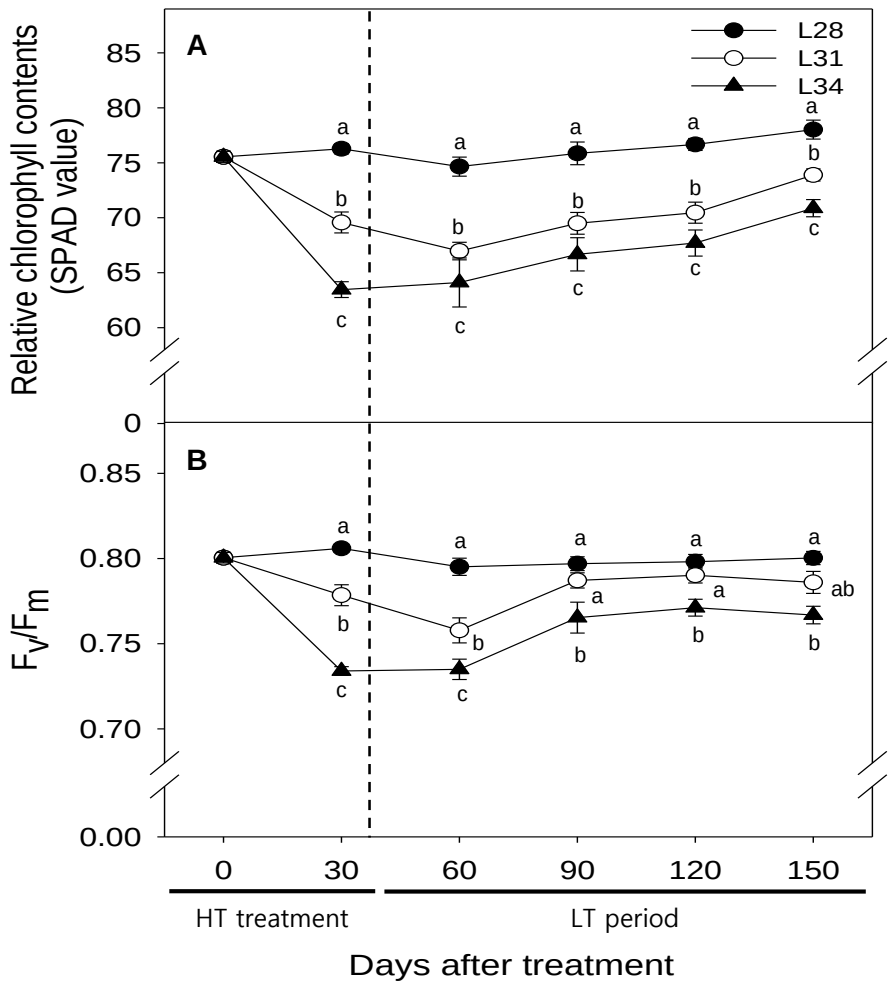

Fig. 8. Recovery of relative chlorophyll contents (SPAD value) and maximum quantum efficiency (F_v/F_m) during low temperature period of *Phalaenopsis* 'Mantefon' leaves in year 1. L28 (control), 28˚C for 30 days; L31, 31˚C for 30 days; L34, 34˚C for 30 days. Letters indicate mean separation by Duncan's multiple range test at $P < 0.05$. Vertical bars represent the SEs of the means (N>6).

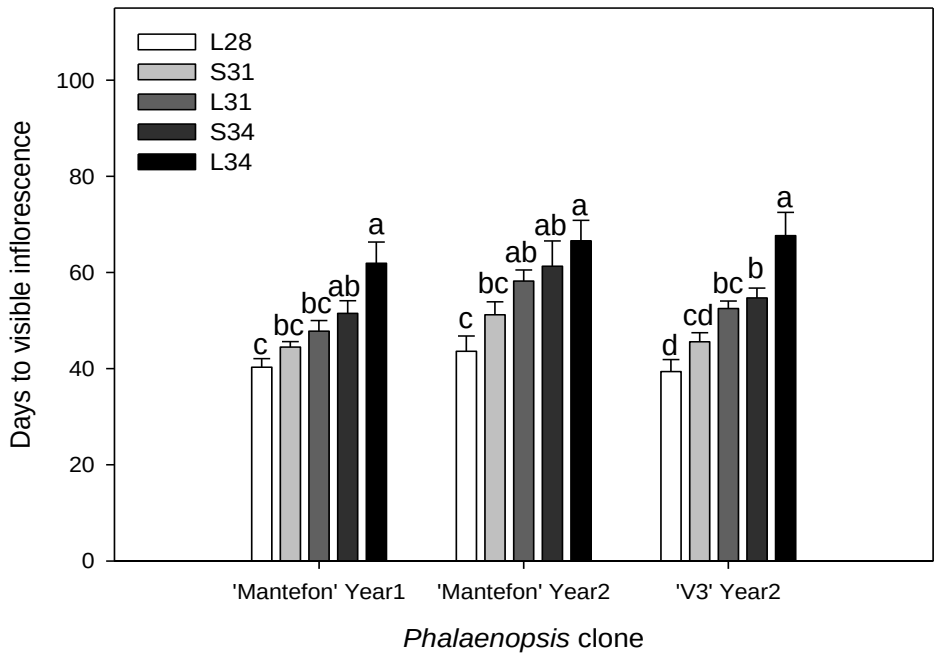

Fig. 9. Days to visible inflorescence of *Phalaenopsis* 'Mantefon' in year 1 and 2, and 'V3' in year 2. L28 (control), 28˚C for 30 days; S31, 31˚C for 15 days; L31, 31˚C for 30 days; S34, 34˚C for 15 days; L34, 34˚C for 30 days. Letters indicate mean separation by Duncan's multiple range test at $P < 0.05$. Vertical bars represent the SEs of the means (N=10).

by high temperature treatment (Table 1). Days from VI to flower increased by 12.5 days and total days to flower also significantly increased by 33.6 days as temperature increased. Total length of flower-stalk, flower-stalk thickness, number of flower buds, and flower diameter decreased with increasing temperature, although there was no effect on the number of flower-stalk. Fig. 10 showed the flowering characteristics of *Phalaenopsis* 'Mantefon' in response to different temperature prior to vernalization.

Effect of Exogenous Sucrose Supply on Flowering

Effect of exogenous sucrose supply under high temperature

During vegetative growth, the spraying sucrose could not induce flower-stalk emergence (Table 2). The number of new leaves, leaf span, and length and thickness of the uppermost mature leaves were not affected by exogenous sucrose supply. The width of the uppermost mature leaves showed increase in 20 and 40 $g \cdot L^{-1}$ treatments compared to 80 $g \cdot L^{-1}$ treatment, although there was no significant difference compared to 0 $g \cdot L^{-1}$ treatment. However, sucrose-treated plants had significantly higher SPAD value than 0 $g \cdot L^{-1}$ treatment.

Effect of exogenous sucrose supply before and during vernalization

All of the *Phalaenopsis* 'Mantefon' plants showed flower-stalk emergence regardless of both sucrose concentration and treatment timing (Table 3). In response

Table 1. Flowering characteristics of *Phalaenopsis* 'Mantefon' as influenced by high temperature prior to vernalization in year 1.

Treatment	Flowering (%)	Days from VI to flower	Total days to flower	No. of flower-stalks	Total flower-stalk length (cm)	Flower-stalk thickness (mm)	No. of flower buds	Flower diameter (cm)
L28[z]	100	117.1 b[y]	157.9 c	2.2 a	40.0 a	5.45 a	30.5 a	5.72 a
S31	100	122.8 ab	167.3 bc	2.2 a	38.8 a	5.25 ab	26.0 ab	5.37 b
L31	100	125.6 ab	173.4 b	2.1 a	32.6 ab	4.92 ab	27.0 ab	5.08 bc
S34	100	123.4 ab	173.3 b	2.1 a	35.6 ab	4.60 b	21.9 ab	4.79 cd
L34	100	129.6 a	191.5 a	1.8 a	29.8 b	4.69 b	19.9 b	4.75 d
Significance		*	***	NS	*	*	*	***

[z]L28 (control), 28˚C for 30 days; S31, 31˚C for 15 days; L31, 31˚C for 30 days; S34, 34˚C for 15 days; L34, 34˚C for 30 days.

[y]Means within columns followed by different letters are significantly different by Duncan's multiple range test at $P < 0.05$.

NS indicates non-significant; * or *** indicates significance at $P < 0.05$ or 0.001, respectively.

Fig. 10. Flowerings of *Phalaenopsis* 'Mantefon' as influenced by high temperature prior to vernalization in year 1 after 27 weeks of low temperature period. L28 (control), 28˚C for 30 days; S31, 31˚C for 15 days; L31, 31˚C for 30 days; S34, 34˚C for 15 days; L34, 34˚C for 30 days.

Table 2. Effect of exogenous sucrose supply during vegetative growth on flowering and vegetative growth of *Phalaenopsis* 'Mantefon' at 8 weeks after treatment.

Sucrose concentration ($g \cdot L^{-1}$)	Flowering	Vegetative growth					
	Visible Inflorescence (%)	No. of new leaves	Leaf span (cm)	Uppermost mature leaf			
				Length (cm)	Width (cm)	Thickness (mm)	SPAD
0	0	1.8	27.0	16.9	6.9 ab[z]	2.18	73.7 b
20	0	1.8	27.2	16.5	7.2 a	2.32	76.6 a
40	0	1.9	25.9	16.6	7.3 a	2.20	76.0 a
80	0	1.8	25.9	17.5	6.7 b	2.18	76.0 a
Significance	NS	NS	NS	NS	*	NS	*

[z]Means within columns followed by different letters are significantly different by Duncans's multiple range test at $P < 0.05$.
NS indicates non-significant; * indicates significance at $P < 0.05$.

Table 3. Effect of exogenous sucrose supply before and during vernalization on percentage of visible inflorescence (VI) and days to VI of *Phalaenopsis* 'Mantefon'.

Sucrose concentration ($g \cdot L^{-1}$)	VI (%)	Days to VI
Before vernalization		
0	100	50
20	100	45
40	100	49
80	100	53
Significance		NS
During vernalization		
0	100	52 ab[z]
20	100	40 c
40	100	46 b
80	100	52 a
Significance		***

[Z]Means within columns followed by different letters are significantly different by Duncans's multiple range test at $P < 0.05$.
NS indicates non-significant; *** indicates significance at $P < 0.001$, respectively.

to spraying sucrose solution before vernalization, days to VI slightly decreased in plants which were treated with 20 $g \cdot L^{-1}$ of sucrose solution, but the difference had no significance. However, the exogenous sucrose supply during vernalization significantly accelerated the flowering initiation. At the first sucrose treatment (15^{th} day of vernalization), the significant increase of sucrose contents in leaves were observed in plants treated with 20 $g \cdot L^{-1}$ of sucrose solution, and there was no significant difference among plants treated with 0, 40, and 80 $g \cdot L^{-1}$ (Fig. 11A). At the second time of sucrose treatment (30^{th} days of vernalization), the difference among treatments was also significant, and the trend of sucrose contents change was similar to those of first sucrose treatment (Fig. 11B). Moreover, there was significant correlation between sucrose contents and days to visible inflorescence (Fig. 12).

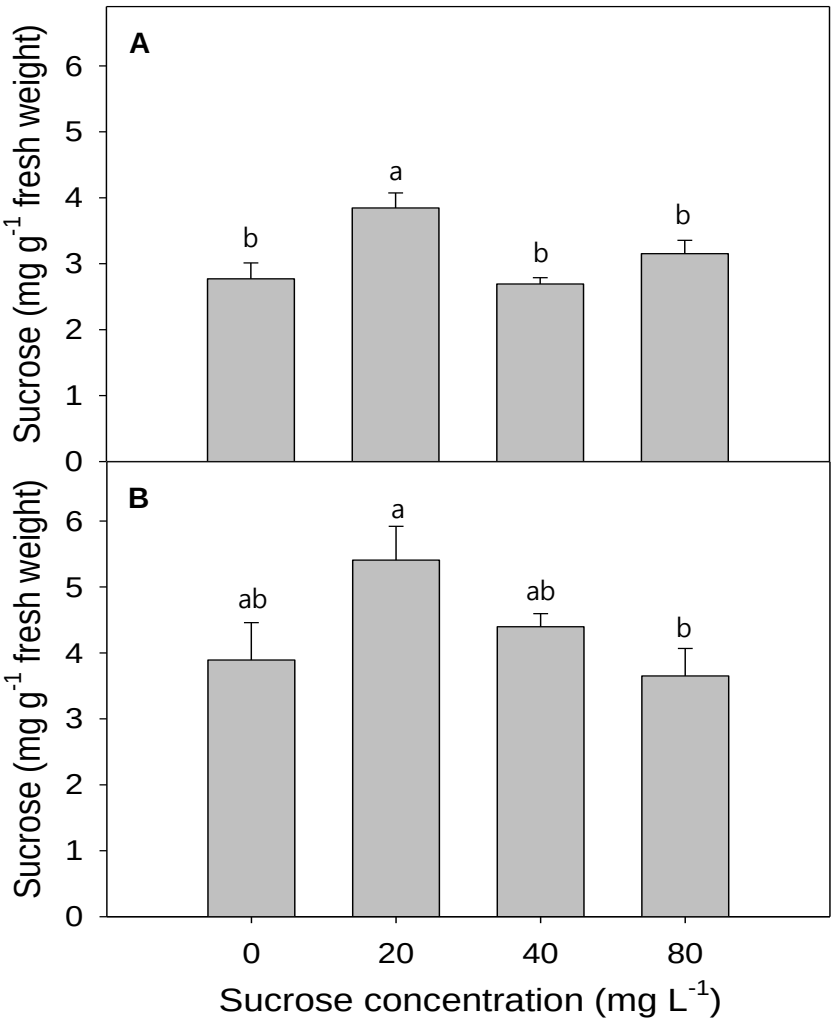

Fig. 11. Sucrose contents in *Phalaenopsis* 'Manteton' leaves after spraying sucrose at 15 (A) and 30 (B) days under low temperature condition. Letters indicate mean separation by Duncan's multiple range test at $P < 0.05$. Vertical bars represent the SEs of the means (n=3).

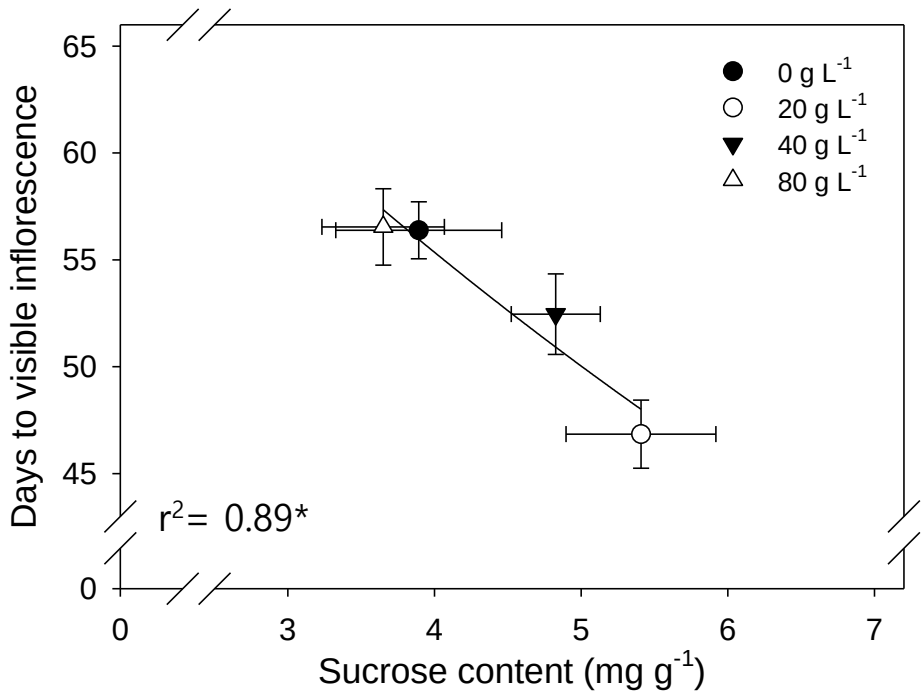

Fig. 12. Relationship between days to visible inflorescence and sucrose content in *Phalaenopsis* 'Mantefon' leaves at 30 days after treatment. Vertical and horizontal bars represent the SEs of the means. Equation for regression lines are presented for significant correlations with corresponding r^2. * indicates significance at $P < 0.05$.

DISCUSSION

Effect of High Temperature Prior to Vernalization on Flowering

High temperature stress primarily targets the photosynthetic machinery (Allakhverdiev et al., 2008). Therefore, photosynthesis is one of the physiological processes which are most sensitive to high temperature in plants (Crafts-Brandner and Salvucci, 2002). Various components involved in photosynthesis such as photosynthetic pigment, electron transport system, and gas exchange system can be severely affected by high temperature stress (Ashraf and Harris, 2013). In this study, the photosynthetic capability such as relative chlorophyll contents, electron transport, and CO_2 uptake were reduced.

As one of the thermal stress parameters, relative chlorophyll contents (SPAD value) were significantly reduced when temperature increased during high temperature treatment, and treatment duration made a significant difference in SPAD value between S34 and L34 of both *Phalaenopsis* cultivars in year 2 (Fig. 3). Many researches already showed that the chlorophyll contents decreased under high temperature condition, and the effects were intensified by longer duration of treatment in many plants such as *Capsicum annuum*, *Sorghum bicolor*, *Triticum aestivum*, and *Phalaenopsis* (Bhandari et al., 2018; Cha-um et al., 2010; Djanaguiraman et al., 2010, Efeoglu and Terzioglu, 2009). The reason for lesser

accumulation of chlorophyll by high temperature stress is the reduced chlorophyll biosynthesis, resulting from destruction of numerous enzymes in chlorophyll biosynthesis process (Ashraf and Harris, 2013). For example, the 5-aminolevulinic acid dehydratase (ALAD) and porphobilinogen deaminase (PBGD) which are the enzymes in chlorophyll biosynthesis pathway decreased under high temperature condition in *Cucumis sativus* (Tewari and Tripathy, 1998).

Chlorophyll fluorescence parameters are also good indicators for thermal stress under high temperature condition because PSII is the most susceptible component to high temperature stress (Mathur et al., 2014). F_v/F_m decreased as temperature increased, indicating thermal stress by high temperature (Fig. 5A). Considering that F_v/F_m of non-stressed leaves is consistent with values of around 0.83, the minimal F_v/F_m values of 0.791 (L31) and 0.767 (L34) in year 1, and 0.779 (L31) and 0.735 (L34) in year 2 were the evidence of damage in PSII electron transport ability (Murchie and Lawson, 2013). However, high temperature stress in this study was not severe stress, but moderate stress, because the values above 0.74 are regarded as the regular value of F_v/F_m (Lichtenthaler et al., 2005; Pollet et al., 2011). Φ_{PSII} was also reduced with temperature increase, and it means increasing temperature decreased proportion of the light absorbed by PSII photochemistry (Fig. 5B). The reason for reduced Φ_{PSII} was speculated with the decrease of q_p, photochemical quenching, and the increase of NPQ, non-photochemical quenching (Fig. 5C, D). Edwards and Baker (1993) showed PSII electron transport was correlated very well with CO_2 fixation. Likewise, the CO_2 uptake was reduced with increasing temperature as Φ_{PSII} did in

this study (Figs. 5, 6). The decrease of photosynthetic efficiency of PSII and gas exchange by high temperature was also observed in *Phalaenopsis* (Hsu, 2007; Jeon et al., 2006; Pollet et al., 2011). *Phalaenopsis* hybrid 'New candy' had the decreased F_v/F_m under 36.1˚C day temperature condition, and *Phalaenopsis* hybrid 'Hercules' showed decreasing aspects of F_q'/F_m' and CO_2 uptake with increasing temperature (Pollet et al., 2010, 2011).

Consistent with the decrease of photosynthetic capacity, although other carbohydrates such as glucose, fructose, and starch contents were not significantly different among treatments, sucrose content was significantly decreased by increasing temperature (Fig. 7). As one of the end-product of photosynthesis, sucrose is exported from leaves (source) to non-photosynthetic tissues (sink) through phloem (Ainsworth and Bush 2011). The carbohydrates that derived from sucrose constitute ~ 90% of plant biomass, making sucrose a crucial determinant (Ruan, 2014), and the development processes including plant growth and flowering are dependent on the sucrose signaling (Tognetti et al., 2013). In *Phalaenopsis*, the potential role of phloem-mobile sucrose was reported in controlling the flowering response (Konow and Wang, 2001; Qin et al., 2012). For example, the sucrose increased when bud dormancy is progressively released, and the authors suggested that it sustains bud development and growth considering the difference in transcription level of sucrose synthase (Qin et al., 2012).

Reduced photosynthesis by abiotic stress was gradually recovered under non-stress condition in many plants (Liu et al., 2012; Miyashita et al., 2005). High

temperature stress in photosynthesis caused both reversible decline and irreversible damage, and the reversible changes explains how photosynthesis tolerates and recovers from high temperature stress (Sharkey and Zhang, 2010). In *Phalaenopsis*, the recovery of damaged photosynthetic capacity by high temperature stress was reported (Cha-um et al., 2010; Seubma et al., 2012). For example, leaves exposed to 47˚C for 60 min of high temperature showed 45% reduction F_v/F_m, and 22% recovery was observed 24 hours after transfer to 28˚C condition in *Phalaenopsis* 'Sweetheart' (Seubma et al., 2012). Based on the recovery aspects of the SPAD values and F_v/F_m in this study, the thermal stress was gradually recovered under 20˚C condition. However, the significant decrease in both parameters by higher temperature was still observed until 150 days after high temperature treatment, implying irreversible damaged by high temperature on photosynthetic capacity (Fig. 8). Such recovery trends suggested that the decrease of photosynthetic capacity by high temperature stress might be attributed to subsequent flowering processes.

The flower-stalk emergence was significantly delayed with increasing temperature and duration (Fig. 9), and days from VI to flower and total days to flower were also increased as temperature increased (Table 1). Considering the high temperature stress continued during both vernalization and long-term finishing period (Fig. 8), this results indicated that high temperature prior to vernalization have inhibitory effects on both flowering initiation and flower-stalk development. Consistently, besides timing of flowering, other flowering parameters such as total flower-stalk length, flower-stalk thickness, the number of flower bud, and flower

diameter decreased as temperature increased (Table 1). Given that the high temperature resulted in the decrease in photosynthesis capacity and consequent sucrose contents not only during high temperature treatment, but also after high temperature treatment, it is reasonable to suggest that the reduced sucrose contents by high temperature stress prior to vernalization hampered flowering initiation and flower-stalk development. Actually, the roles of sucrose on flowering has been reported (Kataoka et al., 2004; Lee et al., 2004). For example, *Phalaenopsis* hybrid 'Secret Dream', showed a significant correlation between sucrose contents and the days to visible inflorescence in various environmental conditions (Kataoka et al., 2004). In chrysanthemum, exogenous sucrose supply also improved the flower diameter and flower-stalk length, suggesting that higher sucrose contents promote flower development (Lee et al., 2004).

In conclusion, high temperature prior to vernalization reduced photosynthetic capacity and sucrose contents, and these effects lasted during low temperature period. Consequently, the high temperature stress hampered the flowering initiation and flower-stalk development of *Phalaenopsis* plants, and sucrose could be attributed to this phenomenon. However, it remained unclear whether sucrose is a crucial factor which can influence the flowering response alone in *Phalaenopsis*, because high temperature stress may affect various physiological processes such as plant hormones (Mathur et al., 2014). Therefore, the effect of sucrose accumulation on flowering should be studied further in *Phalaenopsis*.

Effect of Exogenous Sucrose Supply on Flowering

Exogenous sucrose was sprayed during vegetative stage to confirm whether sucrose is a critical factor for flowering initiation or not. The results in this study showed 0% flower-stalk emergence, indicating that sucrose application cannot substitute for the effect of vernalization (Table 2). Similarly, sucrose treatment was not sufficient to maintain flower-stalk development in *Phalaenopsis amabilis* Blume with about 8 cm flower-stalk (Chen et al., 1994). While sucrose treatment could not affect flowering of *Phalaenopsis* under 28˚C condition, it made significant difference in chlorophyll contents of the uppermost mature leaves (Table 2). In *Phaseolus vulgaris*, chlorophyll-synthesizing ability of leaves declined by the dark incubation, but only carbohydrate, especially sucrose, was efficient to preserve the pigment contents or rather to increase (Wolff and Price, 1960). In potato (cv. Sandy), 20 $g \cdot L^{-1}$ of sucrose concentration in medium significantly increased chlorophyll contents as well as plant growth parameters (Mohamed and Alsadon, 2010).

When sucrose solution was sprayed before and during vernalization, flower-stalk emergence of all plants was induced by vernalization regardless of both the timing and concentration of sucrose application (Table 3). However, only spraying 20 $g \cdot L^{-1}$ sucrose solution during vernalization significantly accelerated the flower-stalk emergence (Table 3). In sucrose contents in leaves sprayed during vernalization, only the spraying 20 $g \cdot L^{-1}$ significantly increased the sucrose contents in leaves of *Phalaenopsis* plants, while higher concentration showed no significant difference

from 0 $g \cdot L^{-1}$ (Fig. 11). Considering that foliar uptake rate of potassium (K) and chelated iron (Fe) decreased as concentration increased (Chamel, 1988; Schlegel et al., 2006) and high viscosity by lower temperature decreased absorption of zinc (Zn) (Rathore et al., 1970), reduced sucrose uptake in leaves sprayed with 40 and 80 $g \cdot L^{-1}$ may be because of higher concentration and viscosity than 20 $g \cdot L^{-1}$ sucrose solution. Consistent with the change of days to VI according to sucrose concentration, the sucrose contents in leaves showed a significant correlation with days to visible inflorescence (Fig. 12). The relationship between sucrose contents and flowering was observed in many plants such as *Sinapis alba*, *Arabidopsis thaliana*, *Xanthium strumarin*, *Phalaenopsis* (Bodson and Outlaw, 1985; Corbesier et al., 1998; Houssa et al., 1991; Kataoka et al., 2004). For example, a significant correlation between sucrose content in leaves and days to flower-stalk emergence was observed under various environment conditions in *Phalaenopsis* (Kataoka et al., 2004). In addition, many paper reported that sucrose application affected flowering (Friend et al., 1984; Roldán et al 1999; Sun et al., 2017). In *Brassica campestris*, the sucrose application promoted flowering initiation, and this phenomenon was not due to osmotic stress (Friend et al., 1984). Recently, the spraying of 50mM sucrose accelerated flowering of chrysanthemum 'Floral Yuuka' under short day plus night break condition, and increased *CmFTL2*, *FT-like* gene, expression level, suggesting that sucrose promoted flowering through the *FT* genes (Sun et al., 2017). Therefore, the results in this study indicate that the increased sucrose contents of leaves can promote flowering initiation.

In conclusion, the exogenous sucrose application during vernalization significantly promoted flower-stalk emergence with increase of sucrose content in leaves, and there was a significant relationship between sucrose content and flower initiation. These results can confirm that the reduced sucrose content by high temperature prior to vernalization is the reason for the hampered flowering initiation and flower-stalk development. Also, sucrose application to plants stressed by high temperature might alleviate the effect of high temperature on flowering.

LITERATURE CITED

Ainsworth EA, Bush DR (2011) Carbohydrate export from the leaf: a highly regulated process and target to enhance photosynthesis and productivity. Plant Physiol 155:64-69

Allakhverdiev SI, Kreslavski VD, Klimov VV, Los DA, Carpentier R, Mohanty P (2008) Heat stress: an overview of molecular responses in photosynthesis. Photosynth Res 98:541

Amasino RM (2005) Vernalization and flowering time. Curr Opin Biotechnol 16:154-158

Ashraf MHPJC, Harris PJC (2013) Photosynthesis under stressful environments: an overview. Photosynthetica 51:163-190

Baker NR (2008) Chlorophyll fluorescence: a probe of photosynthesis in vivo. Annu Rev Plant Biol 59:89-113

Bhandari SR, Kim YH, Lee JG (2018) Detection of temperature stress using chlorophyll fluorescence parameters and stress-related chlorophyll and proline content in paprika (*Capsicum annuum* L.) seedlings. Hort Sci Technol 36: 619-629

Blanchard MG, Runkle ES (2006) Temperature during the day, but not during the night, controls flowering of *Phalaenopsis* orchids. J Exp Bot 57:4043-4049

Bodson M, Outlaw WH (1985) Elevation in the sucrose content of the shoot apical meristem of *Sinapis alba* at floral evocation. Plant Physiol 79:420-424

Cha-um S, Ulziibat B, Kirdmanee C (2010) Effects of temperature and relative humidity during in vitro acclimatization, on physiological changes and growth characters of *Phalaenopsis* adapted to in vivo. Aust J Crop Sci 4:750

Chamel A (1988) Foliar uptake of chemicals studied with whole plants and isolated cuticles, p. 27-48. In: Neuman PM (Ed.). Plant growth and leaf-applied chemicals. CRC Press, Boca Raton, FL, USA,

Chen WH, Chen HH (2011) Orchid biotechnology II, p. 1-2. 1st Edn. World Sci. Publ. Co. Pte. Ltd., Singapore, Doi:10.1142/7982

Chen WH, Tseng YC, Liu YC, Chuo CM, Chen PT, Tseng KM, Yeh YC, Ger, MJ, Wang HL (2008) Cool-night temperature induces spike emergence and affects photosynthetic efficiency and metabolizable carbohydrate and organic acid pools in *Phalaenopsis aphrodite*. Plant Cell Rep 27:1667-1675

Chen WS, Liu HY, Liu ZH, Yang L, Chen WH (1994) Gibberellin and temperature influence carbohydrate content and flowering in *Phalaenopsis*. Physiol Plant 90:391-395

Christenson EA (2001) *Phalaenopsis*: a monograph, 1st Edn. Timber Press, Portland, OR, USA, p. 24-25

Corbesier L, Lejeune P, Bernier G (1998) The role of carbohydrates in the induction of flowering in *Arabidopsis thaliana*: comparison between the wild type and a starchless mutant. Planta 206:131-137

Crafts-Brandner SJ, Salvucci ME (2002) Sensitivity of photosynthesis in a C4 plant, maize, to heat stress. Plant Physiol 129:1773-1780

Dahanayake SR, Galwey NW (1998) Effects of interactions between low and high temperature treatments on flowering of spring rape (*Brassica napus* var. *annua*). Ann Bot 81:609-617

Djanaguiraman M, Prasad PV, Seppanen M (2010) Selenium protects sorghum leaves from oxidative damage under high temperature stress by enhancing antioxidant defense system. Plant Physiol Biochem 48:999-1007

Edwards GE, Baker NR (1993) Can CO_2 assimilation in maize leaves be predicted accurately from chlorophyll fluorescence analysis? Photosynth Res 37:89-102

Efeoglu B, Terzioglu S (2009) Photosynthetic responses of two wheat varieties to high temperature. EurAsia J BioSci 3:97-106

Elers B, Wiebe HJ (1984) Flower formation of Chinese cabbage. II. Anti-vernalization and short-day treatment. Sci Hort 22:327-332

Friend DJ, Bodson M, Bernier G (1984) Promotion of flowering in *Brassica campestris* L. cv. Ceres by sucrose. Plant Physiol 75:1085-1089

Givnish TJ, Spalink D, Ames M, Lyon, SP, Hunter SJ, Zuluaga A, Doucette A, Caro GG, McDaniel J, Clements MA, Arroyo MTK, Endara L, Kriebel R, Williams NH, Arroyo MT (2016) Orchid historical biogeography, diversification, Antarctica and the paradox of orchid dispersal. J Biogeogr 43:1905-1916

Goh CJ, Arditti J (1985) Orchidaceae, p. 309-336. In: Halevy AH (ed.). Handbook of flowering. Vol I. CRC Press. Boca Raton, FL, USA

González-Rossia D, Reig C, Dovis V, Gariglio N, Agustí M (2008) Changes on carbohydrates and nitrogen content in the bark tissues induced by artificial

chilling and its relationship with dormancy bud break in *Prunus* sp. Sci Hort 118:275-281

Guo WJ, Lee N (2006) Effect of leaf and plant age, and day/night temperature on net CO_2 uptake in *Phalaenopsis amabilis* var. *formosa*. J Am Soc Hort Sci 131:320-326

Hasanuzzaman M, Nahar K, Alam M, Roychowdhury R, Fujita M (2013) Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. Intl J Mol Sci 14:9643-9684

Houssa P, Bernier G, Kinet JM (1991) Qualitative and quantitative analysis of carbohydrates in leaf exudate of the short-day plant, *Xanthium strumarium* L. during floral transition. J Plant Physiol 138:24-28

Hsu BD (2007) On the possibility of using a chlorophyll fluorescence parameter as an indirect indicator for the growth of *Phalaenopsis* seedlings. Plant Sci 172:604-608

Jeon MW, Ali MB, Hahn EJ, Paek KY (2006) Photosynthetic pigments, morphology, and leaf gas exchange during ex vitro acclimatization of micropropagated CAM *Doritaenopsis* plantlets under relative humidity and air temperature. Environ Exp Bot 55:183-194

Kataoka K, Sumitomo K, Fudano T, Kawase K (2004) Changes in sugar content of *Phalaenopsis* leaves before floral transition. Sci Hort 102:121-132

Konow EA, Wang YT (2001) Irradiance levels affect in vitro and greenhouse growth, flowering, and photosynthetic behavior of a hybrid *Phalaenopsis* orchid. J Am Soc Hort Sci 126:531-536

Krug H, Kahlen K (2008) Modelling production subsystems at high abstraction level: a review. III. Development - Vernalization. Eur J Hort Sci 73:183-187

Lee BJ, Won MK, Park HS, Yang ES, Lee JS, Atherton JG (2004) Effects of exogenous sucrose supply on growth and flowering of chrysanthemum. Hort Environ Biotechnol 45:365-369

Lee N, Lin GM (1984) Effect of temperature on growth and flowering of *Phalaenopsis* white hybrid. J Chinese Soc Hort Sci 30:223-131

Lichtenthaler HK, Buschmann C, Knapp M (2005) How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll fluorescence decrease ratio R Fd of leaves with the PAM fluorometer. Photosynthetica 43:379-393

Liu YC, Liu CH, Lin CY, Lu CH, Chen WH, Wang HL (2016) Effect of lowing irradiance on the photosynthetic performance and spiking of *Phalaenopsis*. Photosynthetica 54:259-266

Liu YC, Tseng KM, Chen CC, Tsai YT, Liu CH, Chen WH, Wang HL (2013) Warm-night temperature delays spike emergence and alters carbon pool metabolism in the stem and leaves of *Phalaenopsis aphroide*. Sci Hort 161:198-203

Liu YF, Qi MF, Li TL (2012) Photosynthesis, photoinhibition, and antioxidant system in tomato leaves stressed by low night temperature and their subsequent recovery. Plant Sci 196:8-17

Lopez RG, Runkle ES (2004) The effect of temperature on leaf and flower development and flower longevity of *Zygopetalum* Redvale 'Fire Kiss' orchid. HortScience 39:1630-1634

Lopez RG, Runkle ES (2005) Environmental physiology of growth and flowering of orchids. HortScience 40:1969-1973

Mathur S, Agrawal D, Jajoo A (2014) Photosynthesis: response to high temperature stress. J Photochem Photobiol B 137:116-126

Miyashita K, Tanakamaru S, Maitani T, Kimura K (2005) Recovery responses of photosynthesis, transpiration, and stomatal conductance in kidney bean following drought stress. Environ Exp Bot 53:205-214

Mohamed MH, Alsadon AA (2010) Influence of ventilation and sucrose on growth and leaf anatomy of micropropagated potato plantlets. Sci Hort 123:295-300

Murchie EH, Lawson T (2013) Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. J Exp Bot 64:3983-3998

Newton LA, Runkle ES (2009) High-temperature inhibition of flowering of *Phalaenopsis* and *Doritaenopsis* orchids. HortScience 44:1271-1276

Ohkawa K, Kim HH, Nitta E, Fukazawa Y (1998) Storage temperature and duration affect flower bud development, shoot emergence, and flowering *of Leucocoryne coquimbensis* F. Phil. J Am Soc Hort Sci 123:586-591

Pollet B, Kromwijk A, Vanhaecke L, Dambre P, Van Labeke MC, Marcelis LFM, Steppe K (2011) A new method to determine the energy saving night temperature for vegetative growth of *Phalaenopsis*. Ann Appl Biol 158:331-345

Pollet B, Steppe K, Dambre P, Van Labeke MC, Lemeur R (2010) Seasonal variation of photosynthesis and photosynthetic efficiency in *Phalaenopsis*. Photosynthetica 48:580-588

Qin Q, Kaas Q, Zhang C, Zhou L, Luo X, Zhou M, Sun X, Zhang L, Paek KY, Cui Y (2012) The cold awakening of *Doritaenopsis* 'Tinny Tender'orchid flowers: the role of leaves in cold-induced bud dormancy release. J Plant Growth Regul 31:139-155

Rathore VS, Wittwer SH, Jyung WH, Bajaj YPS, Adams MW (1970) Mechanism of zinc uptake in bean (*Phaseolus vulgaris*) tissues. Physiol Plant 23:908-919

Robinson KA (2002) Effects of temperature on the flower development rate and morphology of *Phalaenopsis* orchid. MS Thesis. Michigan State Univ., East Lansing, MI, USA

Roldán M, Gómez-Mena C, Ruiz-García L, Salinas J, Martínez-Zapater JM (1999) Sucrose availability on the aerial part of the plant promotes morphogenesis and flowering of *Arabidopsis* in the dark. Plant J 20:581-590

Rotor GB (1952) Daylength and temperature in relation to growth and flowering of orchids. Cornell Univ Agr Exp Sta Bul 885:3-47

Ruan YL (2014) Sucrose metabolism: gateway to diverse carbon use and sugar signaling. Annu Rev Plant Biol 65:33-67

Sachs M, Rylski I (1980) The effects of temperature and daylength during the seedling stage on flower-stalk formation in field-grown celery. Sci Hort 12:231-242

Sakanish Y, Imanishi H, Isida G (1980) Effect of temperature on growth and flowering of *Phalaenopsis amabilis*. Bul Univ Osaka Prefecture Ser B 32:1-9

Schlegel TK, Schönherr J, Schreiber L (2006) Rates of foliar penetration of chelated Fe (III): role of light, stomata, species, and leaf age. J Agric Food Chem 54: 6809-6813

Seubma P, Kasemsap P, van Kooten O, Harbinson J (2012) Effects of high temperature exposure on chlorophyll fluorescence of *Phalaenopsis* leaves. CMUJ Nat Sci Special Issue on Agricultural & Natural Resources 11:409-420

Sharkey TD, Zhang R (2010) High temperature effects on electron and proton circuits of photosynthesis. J Integr Plant Biol 52:712-722

Smith AM, Zeeman SC (2006) Quantification of starch in plant tissues. Nat Protoc 1:1342

Sun J, Wang H, Ren L, Chen S, Chen F, Jiang J (2017) *CmFTL2* is involved in the photoperiod-and sucrose-mediated control of flowering time in chrysanthemum. Hort Res 4:17001

Tewari AK, Tripathy BC (1998) Temperature-stress-induced impairment of chlorophyll biosynthetic reactions in cucumber and wheat. Plant Physiol 117:851-858

Tognetti JA, Pontis HG, Martínez-Noël GMA (2013) Sucrose signaling in plants: a world yet to be explored. Plant Signal Behav 8:1-10

Wolff JB, Price L (1960) The effect of sugars on chlorophyll biosynthesis in higher plants. J Biol Chem 235:1603-1608

ABSTRACT IN KOREAN

호접란은 28°C 이상의 온도에서 개화가 억제되고 영양 생장을 지속한다. 하지만 영양 생장 기간 동안 계절의 변화에 따라 특히 여름철에 고온 환경에 노출되고, 이로 인한 고온 스트레스는 식물의 생장 및 광합성에 부정적인 영향을 준다. 본 실험은 춘화 처리 전 고온의 정도와 기간이 호접란의 광합성 능력 및 탄수화물 함량, 그리고 개화에 어떠한 영향을 미치는지에 대해 알아보기 위해 수행되었다. 온도 처리는 28°C를 대조구로 하여 15 또는 30일간 31 또는 34°C로 처리하였으며, 이후 춘화 처리 및 화경 발달 기간은 20°C에서 진행하였다. 실험에는 12개월된 호접란을 사용하였고, 1년차에는 만천홍 품종, 2년차에서는 만천홍과 V3 품종을 사용하였다. 온도 처리 기간 동안 처리 온도가 증가함에 따라 엽록소 함량과 광계2의 최대 양자 수율이 유의하게 감소하며 고온으로 인한 스트레스 반응을 보였고, 마찬가지로 처리 온도가 증가함에 따라 CO_2 흡수량과 광계2의 양자 수율, 전자 전달률은 감소하였으며, 비광화학적 소멸은 증가하였다. Sucrose 함량의 경우 처리 온도가 증가함에 따라 유의한 감소를 보였다. 고온 스트레스로 인한 광합성 능력과 sucrose 함량의 감소는 저온 기간에도 지속되었으며, 이는 고온으로 인한 스트레스 반응이 고온 처리 이후에도 지속된다는 것을

보여 준다. 또한 화경 출현 일수 및 화경 출현에서 개화까지의 소요 일수, 그리고 총 개화 소요 일수가 모두 유의하게 증가하였으며, 총 화경 길이 및 화경 두께, 소화 수, 그리고 소화 크기도 춘화 처리 전 고온 환경으로 인해 감소하는 경향을 보였다. 더욱이 춘화 처리 동안의 외부에서의 sucrose 처리는 화경 출현 일수를 유의하게 단축시켰고, 잎에서의 sucrose 함량과 화경 출현 일수는 유의한 상관 관계를 가졌다. 이러한 결과들을 종합해보면 춘화 처리 전 고온 환경으로 인한 고온 스트레스는 광합성 능력 및 sucrose 함량을 감소시키고, 이러한 영향은 저온 처리 기간 동안에도 지속되며, 그로 인해 개화 개시 및 화경의 발달을 저해한다고 추정할 수 있다. 또한 이러한 춘화 처리 전 고온 스트레스로 인한 개화 저해 반응은 외부의 sucrose 처리를 통해 그 정도를 경감시킬 수 있을 것으로 보인다.