A DISSERTATION FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Growth and Flowering of *Cymbidium* ‘Red Fire’ and ‘Yokihi’ in Response to Light Intensity, Temperature and Nitrogen Nutrition during Night Interruption Forcing Culture

야파 처리 시 광도, 온도, 질소 영양에 따른 심비디움의 생장과 개화 반응

BY

YOON JIN KIM

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MAJOR IN FLORICULTURE AND LANDSCAPE PLANTS

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

BY
YOON JIN KIM

MAJOR IN FLORICULTURE AND LANDSCAPE PLANTS
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Growth and Flowering of *Cymbidium* ‘Red Fire’ and ‘Yokihi’ in Response to Light Intensity, Temperature and Nitrogen Nutrition during Night Interruption Forcing Culture

Yoon Jin Kim

Department of Plant Science, Seoul National University

ABSTRACT

The effects of night interruption (NI) with different light intensities, temperature and nitrogen nutrient control were examined on vegetative and reproductive growth of *Cymbidium* ‘Red Fire’ and ‘Yokihi’. The cymbidium cultivars were grown under 9/15 h ambient light/dark (control), 9 h ambient light plus NI (22:00 to 02:00 h) with low light intensity at 3-7 μmol m^{-2} s^{-1} (LNI) or 9 h ambient light plus NI with high light intensity at 120 μmol m^{-2} s^{-1} (HNI) conditions. While none of the control plants flowered within 2 years, 100% of the ‘Yokihi’ and 80% of the ‘Red Fire’ plants grown under the HNI condition flowered. In the LNI group, 60% of the plants in both cultivars flowered. Plants in the HNI group showed a decreased time to visible inflorescence and flowering than those in the LNI group. Changes of carbohydrates including sucrose, fructose, glucose and starch were evaluated to determine the factors involved in flowering promotion in *Cymbidium* ‘Red Fire’ during a NI forcing culture. Plants grown in

the LNI and HNI had more leaves and pseudobulbs dry mass than those grown in the control group. Soluble carbohydrate concentrations in the pseudobulbs of the plants were greater in the HNI than in the LNI and control. Glucose was the most abundant soluble carbohydrate. Starch was present in the leaf exudate and was greater in the plants in the LNI than in the HNI or control. The growth and flowering of *Cymbidium* 'Red Fire' and 'Yokihi' plants were tried to improve flowering percentage during NI forcing culture with summer cooling. The greenhouses where the plants were grown were cooled by a mist system (mist) or a shade screen (shade). The temperature was approximately 2℃ lower in the mist than in the shade and the relative humidity under the mist and shade condition were 80 ± 5% and 55 ± 5%, respectively. The plants that received NI followed by the mist flowered within 2 years with different flowering percentages depending on light intensity, while none of the plants flowered with the shade condition. Photosynthetic characteristics of *Cymbidium* 'Red Fire' and 'Yokihi' were investigated when the plants were exposed to NI forcing culture in relation to leaf nitrogen content. Photoinhibition could occur when NI applied to *Cymbidium* without supplemental nitrogen. The results of this study provide information on promotion of *Cymbidium* cultivation for high value cultivars. Application of the NI improved the flower quality of *Cymbidium* by decreasing days to flower. The NI promoted *Cymbidium* flowering within 2 years. Temperature should be maintained under 27℃ by a mist system in a greenhouse cultivation to avoid heat stress and inflorescence abortion during summer growing seasons. Additional nitrogen should be fertilized when the NI is introduced in the forcing culture. The developed cultivation methods are beneficial to promote flowering and to enhance

flower quality of *Cymbidium* ‘Red Fire’ and ‘Yokihi’.

Keywords: carbohydrate, flowering, mist, night interruption, nitrogen deficiency, orchid, photoinhibition, photoperiod, photosynthesis, shade, starch, temperature, vegetative growth

Student number: 2008-30913

CONTENTS

LIST OF TABLES

LIST OF FIGURES

GENERAL INTRODUCTION

Orchidaceae constitutes one of the largest families of angiosperms. They are one of the most ecologically and evolutionarily significant plants and have successfully colonized almost every habitat on earth. *Cymbidium* is a genus of approximately 44 species native from the Himalayas to tropical regions of southeast Asia to Australia (Pridgeon, 2000). Due to the large geographic distribution of *Cymbidium*, they are often divided into three horticultural groups based on their temperature tolerance: cool, intermediate and warm. Most large-flowered *Cymbidium* species from the Himalayas and China are induced to flower by a pronounced cool period in which night temperature is kept at about 10 to 14℃ (Pridgeon, 2000; Rotor, 1952). Photosynthetic characters of *Cymbidium* have evolved differentially. The photosynthetic type is classified into strong Crassulacean acid metabolism (CAM), weak CAM and C_3 on the basis of CAM activity (Mutomura et al., 2008). CAM expression in *Cymbidium* is confined to the epiphytic and lithophytic species. All of these species from tropical to subtropical rainforest exhibit CAM activity. On the other hand, the terrestrial species always exhibit C_3 metabolism irrespective of their varied habitats.

Commercial production of potted orchids has increased throughout the world in the past quarter century. In Korea, the cultivation area of potted orchids increased 542% from 1990 to 2010. The most common genera sold in Korea from 2001 to 2010 were *Cymbidium* (62%), *Phalaenopsis* (15%) and *Dendrobium* (7%). *Cymbidium* has become most valuable potted crop sold; from 2001 to 2010, the number of potted orchids sold increased from 63,748 to 74,383 pots. From 1990

to 2010, Korea potted orchid production value increased by 673% and its wholesale value was estimated at 104 million won in 2010. Orchids are presently the most valuable flowering potted crop in Korea, of which of 33% are *Cymbidium* (Hwang, 2010).

However, *Cymbidium* grows rather slowly and generally takes 3-4 years to flower from the mericlonal stage including 2-3 years of juvenile stage. A major factor in determining flower production cost is crop time. Extended crop time increases maintenance costs including heating and cooling expenses. Reducing crop time, while maintaining a high quality product, will help to ensure a profitable crop.

Photoperiod control during the juvenile stage is an important factor for shortening crop time, thereby achieving more economical flower production. Numerous reports have described the effects of night interruption (NI) on flowering responses for herbaceous annuals and perennials (Mattson and Erwin, 2005; Oh et al., 2008; Runkle et al., 1998). However, no reports have been published on the effects of NI on the vegetative and reproductive growth of *Cymbidium*, which has a longer juvenile stage than herbaceous plants and has different physiological characteristics. Little is known of the relationship among photoperiod, temperature and nitrogen supply during NI forcing culture for *Cymbidium*.

This study was focused on the promotion of flowering in *Cymbidium* by NI treatments. The effects of NI with different light intensities with temperature, nitrogen nutrient control on vegetative growth, flowering, flower quality and crop time were determined in *Cymbidium* ‘Red Fire’ and ‘Yokihi’. The long-term,

practical aim of the present study was to describe and analyze the effects of NI and different environmental factors on the photosynthesis, growth and flowering of *Cymbidium* in order to develop practices for shortening flowering time with high crop quality.

LITERATURE REVIEW

Control of Flowering in Orchids

Light. Different species of orchids exhibit sensitivity to daylength in various ways and yet small changes in daylength are critical for the growth and flowering of *Cattleya, Dendrobium* and *Phalaenopsis* (Bhattacharjee, 1979). Flowering is also promoted when the plants are exposed to short-day (SD), even at low temperatures. In *Dendrobium pulcherrima*, spikes of 2-3 cm were initiated under 9 h SD conditions for 30 days with day/night temperature of 30/20℃ and spikes grew to 7-10 cm under SD conditions for 45 days (Wang et al., 2002). A cell division-related protein, p21, was more highly expressed in SD condition than in long-day (LD) condition (17 h) in *Doritis pulcherrima* (Wang et al., 2003). Even though most *Phalaenopsis* species are SD plants, several species such as *P. bellina* and *P. violacea* flower in summer, suggesting that they are LD plants.

Temperature. Temperature has been reported to control flowering in several orchid genera such as *Dendrobium* (Rotor, 1952), *Miltoniopsis* (Lopez and Runkle, 2006), *Phalaenopsis* (Blanchard and Runkle, 2006; Sakanishi et al., 1980) and *Zygopetalum* (Lopez et al., 2003). The promotion of flowering in these orchid genera by exposure to low temperature suggests that flowering in other orchid species could also be regulated by temperature. *Dendrobium nobile* seedlings grown at 18℃ remained vegetative, whereas those grown at 13℃ flowered regardless of the daylength (Rotor, 1952). Similarly, *Zygopetalum* plants had the highest flowering percentages when grown under a 9 h daylength followed by 8 weeks of cooling at 11 or 14℃ (Lopez et al., 2003). In some *Cymbidium* species,

the day and night temperatures likely have an effect on flower induction, as opposed to the night temperature alone (Pridgeon, 2000; Rotor, 1952; Went, 1957). The terrestrial, epiphytic, lithophytic and semi-epiphytic species require day/night temperatures of 30/25℃ for rapid growth and pseudobulb maturity (Ichihashi, 1997; Pridgeon, 2000). High light intensity and night temperature under 13℃ are required for flower bud initiation in *Cymbidium*. *Cymbidium* 'Radjah' had more inflorescences per plant when grown at 26/12℃ than at 20/12 or 26/18℃ (14 h day/10 h night) (Powell et al., 1988). *Cymbidium* included among the plants which require low temperature for flower bud initiation regardless of daylength (Rotor, 1952). During the summer in Japan, several orchid genera are commonly transported from lowland production areas to higher elevations. This strategy helps avoid high temperature stress during vegetative growth and exposed plants to cooler temperatures and higher light for flower initiation (Ichihashi, 1997).

Nitrogen nutrient. Although nitrogen assimilation in plants has been extensively studied, little information is available on uptake, transport and storage of nitrogen in orchids. There have been some studies on mineral nutrition, in *Cattleya*, *Phalaneopsis* and *Cymbidium*, in particular. These studies focused on nutrient requirements and tissue analyses (Poole and Sheehan, 1982). Generally, the rate of mineral uptake by orchids is slow in relation to that of other higher plants (Hew et al., 1993). The use of Osmocote (slow-release fertilizer) 18N-6P-12K produced more growths for *Cattleya* and larger plants of *Cymbidium* and *Phalaenopsis* (Poole and Seeley, 1977). Due to the increased growth (376 g vs. 215 g) of *Cymbidium*, the N level or release rate does not appear to be adequate for the retention of the older leaves. Either higher amounts should be given, or

supplemented with periodical liquid applications. Leaf loss in *Dendrobium* was greater in the absence of N, with only 38% leaf retention, compared to plants supplied with N and the flower number increased with increasing N concentration from 0 to 100 mg L^{-1} (Bichsel et al., 2008). For *Phalaenopsis*, maintaining N fertilization until the completion of flower initiation increased number of flowers (Wang, 2000). Nitrogen is needed for optimum flower development after flower initiation in orchids because reproductive growth is a strong nutrient sink (Bichsel et al., 2008; Yen et al., 2008). However, excess N application has been reported to adversely affect flower initiation in orchids (Bichsel et al., 2008) due to increased vegetative growth and delayed pseudobulb maturation. Conversely, prolonging nutrient application until October led to robust plants with high leaf retention in addition to accumulated reserves in the mature pseudobulbs for subsequent flower development in *Dendrobium nobile* (Yen et al., 2008).

Physiology of *Cymbidium*

Flowering in *Cymbidiums* was associated with the maturity of the pseudobulbs. Flower spikes being produced only by young developing or immature pseudobulbs (Ng and Hew, 2000). If low temperature should come at a time when the pseudobulbs had matured, it would be ineffective in the induction of flower buds and only vegetative shoots would be produced. However, some back bulbs may flower for the second time. The possibility exists, then, that flowering in *Cymbidiums* may be induced at any time the proper environmental conditions are provided. This is because several large, dormant buds are present at the basal part of every pseudobulb and if one of them develops into a flower shoot there are

other potential inflorescence bud primordia. The environmental conditions conductive to flowering may be provided either at the dormant bud stage or at the early developing bud stage, when the apical meristem of the bud primordium may differentiate either into a vegetative or a flower shoot (Rotor, 1952).

Flowering Responses to NI

Photoperiod is often manipulated to induce or prevent flowering in photoperiodic species (Blanchard and Runkle, 2010). Time to flowering was reduced by day-extension in *Eustoma grandiflorum* (Islam et al., 2005) and dwarf purple loosestrife (Kim et al., 2011a). Alternatively, NI breaks a long dark period to deliver photoperiodic lighting, resulting in modified LD conditions for plants (Vince-Prue and Canham, 1983) with low energy consumption. NI has been effective for accelerating the growth and development of LD herbaceous plants including *Campanula* (Damann and Lyons, 1996), *Coreopsis* (Runkle et al., 1998) and *Cyclamen* (Kang et al., 2008) during the SD season. In the quantitative LD plant *Cyclamen persicum*, a 4 h NI with 20 ± 3 μmol m^{-2} s^{-1} promoted growth and flowering (Oh et al., 2008). The LD herbaceous ornamentals *Asperula arvensis* and *Eschscholzia caifornica* grown under a 4 h NI with 2 μmol m^{-2} s^{-1} produced more number of leaves below the first open flowers (Mattson and Erwin, 2005). In *Lythrum salicaria*, the pattern of total dry weight distribution to leaves, stems and roots increased in photoperiod of 8 h and 1 h NI with 9,700 lux than in 9 h photoperiod (Shamsi, 1976). Whereas the flowering of SD plants, such as *Dendranthema grandiflorum* (Wilkins et al., 1990), is promoted by long night SD conditions. Most SD plants remained vegetative when illuminated for 0.5 h or less

during the middle of the night (Thomas and Vince-Prue, 1997). However, no report has been published on the effects of NI on the growth and flowering of orchids, which has a longer juvenile stage than herbaceous plants (Kim et al., 2011b).

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CHAPTER I

Night Interruption Promotes Vegetative Growth and Flowering of *Cymbidium* 'Red Fire' and 'Yokihi'

ABSTRACT

The effects of night interruption (NI) were examined on the vegetative growth and flowering of *Cymbidium* 'Red Fire' and 'Yokihi'. Plants were grown under 9/15 h ambient light/dark (control), 9 h ambient light plus NI (22:00 to 02:00 h) with low light intensity at 3-7 μmol m^{-2} s^{-1} (LNI) and 9 h ambient light plus NI with high light intensity at 120 μmol m^{-2} s^{-1} (HNI) conditions. The number of leaves, leaf length, number of pseudobulbs and pseudobulb diameter were higher in both LNI and HNI than those in controls for both cultivars. While none of the control plants flowered within 2 years, 100% of the 'Yokihi' and 80% of the 'Red Fire' plants grown under HNI condition flowered. In the LNI group, 60% of the plants flowered in both cultivars. Plants in the HNI group showed a decreased time to visible inflorescence and flowering than those in the LNI group. The number of inflorescences and florets were greater in the plants grown under HNI than those in the LNI group. The tallest plants at flowering were in the HNI group in both cultivars. NI with low light intensity can be used effectively to promote flower induction with increased growth rate during the juvenile stage in *Cymbidium.* To obtain high quality plants, however, NI with high light intensity

strategies should be considered.

Keywords: flowering, night interruption, orchid, vegetative growth

INTRODUCTION

The photoperiod regulates growth and flowering in photoperiodic plants. Plants are classified into photoperiodic response categories according to the night length that elicits a flowering response (Thomas and Vince-Prue, 1997). Long-day (LD) plants, such as *Eustoma* (Yamada et al., 2009), *Lupinus* (Cavins and Dole, 2001) and *Petunia* (Blanchard and Runkle, 2010), only flower or flower most rapidly under short night LD conditions, whereas the flowering of short-day (SD) plants, such as chrysanthemum (Wilkins et al., 1990), is promoted by long night SD conditions. Photoperiod is often manipulated to induce or prevent flowering in photoperiodic species (Blanchard and Runkle, 2010). Previous research reported reducing the time to flowering by day-extension in *Eustoma grandiflorum* (Islam et al., 2005) and dwarf purple loosestrife (Kim et al., 2011). Alternatively, night interruption (NI) breaks a long dark period to deliver photoperiodic lighting, resulting in modified LD conditions for plants (Vince-Prue and Canham, 1983) with low energy consumption. NI has been effective for accelerating the growth and development of LD herbaceous plants including *Campanula* (Damann and Lyons, 1996), *Coreopsis* (Runkle et al., 1998) and *Cyclamen* (Kang et al., 2008) during the SD season.

The juvenile stage, during which a plant is insensitive to conditions that promote floral initiation (Bernier et al., 1981), varies among different plant species. For *Brassica campestris*, the period of the juvenile stage is only a few days (Friend, 1968), whereas the average length of the juvenile stage in orchids is between 2-3 years (Hew and Yong, 2004) and that of some woody plants is 30-40

years (Hackett, 1985). If a plant is prematurely exposed to reproductive conditions before the end of the juvenile stage, the plant may not be able to support quality flowers and thereby decrease the uniformity of flowering (Cameron et al., 1996). To improve plant quality in orchids, vigorous juvenile growth producing numerous new shoots and mature pseudobulbs is desirable (Hew and Yong, 2004). Purvis (1934) reported the importance of meeting the minimum leaf formation before floral initiation for plant quality.

A major factor in determining production cost is crop time. Extended crop time increases maintenance costs including heating and cooling expenses. Reducing crop time, while maintaining a high quality product, will help to ensure a profitable crop. Photoperiod control during the juvenile stage is an important factor for shortening crop time, thereby achieving more economical production.

Cymbidium is a terrestrial orchid popularly sold as cut flowers and potted plants. However, at least 5 years are required from sowing to flower development, including 3-4 years of juvenile stage. The time to new flowering pseudobulb emergence determines the time to the number of days to flowering. Inflorescence is known to be initiated under natural LD (> 16 h) and low temperature (< 25℃) conditions after the end of juvenile growth. According to Marilyn (2002), some tropical *Cymbidium* such as *C. aurantiaca* and *C. skinneri* bloom under LD conditions.

Numerous reports have described the effects of NI on flowering responses for herbaceous annuals and perennials (Mattson and Erwin, 2005; Oh et al., 2008; Runkle et al., 1998). However, no report has been published on the effects of NI on the vegetative growth and flowering of *Cymbidium*, which has a longer

juvenile stage than herbaceous plants. For commercially viable flowering control, the method must be simple and economical and yield reproducible results (Hew and Yong, 2004). Therefore, the effects of NI were examined with different light intensities on vegetative growth, flowering, flower quality and crop time in *Cymbidium* ‘Red Fire’ and ‘Yokihi’.

MATERIALS AND METHODS

Plant and Growth Conditions

Tropical *Cymbidium* hybrids ‘Red Fire’ and ‘Yokihi’ (Mukoyama Orchids Co., Ltd., Yamanashiken, Japan) were transplanted at the mericlonal stage into 10 cm pots and then re-transplanted into 16 cm pots after 4 months of growth. The pots contained 100% chopped coconut. The plants were grown in a commercial greenhouse in Hwasung city, Republic of Korea. Average day/night temperatures for the first year (2009) and the second year (2010) of the experiments, respectively, were 27/22 and 28/24℃ in summer and 21/12 and 22/13℃ in winter. Average photosynthetic photon flux for the first and the second year of the experiments, respectively, was 563 and 500 $\mu mol\, m^{-2}\, s^{-1}$ in summer and 229 and 215 $\mu mol\, m^{-2}\, s^{-1}$ in winter. The plants were irrigated daily with tap water. Five grams of water-soluble controlled release 13N-5.7P-10.8K fertilizers (Mukoyama Orchids Co., Ltd.) were placed at the top of each pot for the control group and the group receiving a light intensity of 3-7 $\mu mol\, m^{-2}\, s^{-1}$ (LNI) and 120 $\mu mol\, m^{-2}\, s^{-1}$ (HNI). The controlled release fertilizer was applied at the three stages; transplanting, the first pseudobulb emergence and the second pseudobulb emergence. Following the second NI treatments, however, the HNI plants were additionally fertigated with NH_4NO_3 at 0.56 g L^{-1} for 1 month using a sprinkler to make them to have similar leaf N to the control and LNI plants. Without the additional N fertilization, the leaf of the plants grown under HNI condition turned yellow and had 20% less N than those of the control and the plants grown under LNI condition (data not shown). Micronutrient fertilizers were applied bimonthly

to the plants with a sprinkler. The supplemental fertilizers were composed of $Ca(NO_3)_2 \cdot 4H_2O$, Fe-EDTA, $MgSO_4 \cdot 7H_2O$, $MnSO_4$, $ZnSO_4 \cdot 7H_2O$, H_3BO_3, $CuSO_4 \cdot 5H_2O$ and $Na_2MoO_4 \cdot 2H_2O$ and provided 472, 3.44, 316, 1.63, 1.15, 1.24, 0.1 and 0.09 g m^{-3} (EC 1.0 mS cm^{-1}), respectively. Pesticides were applied at their recommended rates as needed throughout the growing period.

NI Treatment

The plants were irradiated with high-pressure sodium (HPS) lamps (SKL-01; GEO, Hwasung city, Republic of Korea) from 22:00 to 02:00 h. The greenhouse was divided into three sections. Each section was divided into three groups by placing pots at different distances from the lamps: controls received 9 h ambient light and plants were covered with opaque black cloth daily from 17:00 to 08:00 h; LNI conditions were 9 h ambient light plus NI with low light intensity at 3-7 μmol m^{-2} s^{-1}; HNI conditions were 9 h ambient light plus NI with high light intensity at 120 μmol m^{-2} s^{-1}. The NI was employed twice during 2 years of the experimental period. The first NI treatments began right after transplanting for 16 weeks (February to May) and the second NI treatments began 38 weeks after transplanting for 16 weeks (November to February). The treatments were run on two separate batches of the plants. One batch of the plants was used for destructive sampling. The average day/night temperatures during NI were 22/15℃ and 19/12℃ for the first and the second NI treatments, respectively. The mean photosynthetic daily light integral (DLI) during the first and second NI treatments were 6.5 mol m^{-2} d^{-1} for the plants under the control, 6.5 mol m^{-2} d^{-1} for those undergoing LNI and 8.2 mol m^{-2} d^{-1} for those undergoing HNI.

The environmental conditions such as temperature, relative humidity and CO_2 concentration were uniform in individual sections of the greenhouse during the experiments. Atmospheric CO_2 concentration in the greenhouse was maintained at ambient concentration (approximately 400 μmol mol^{-1}) during the first NI treatments period, but the CO_2 concentration as elevated to approximately 800 μmol mol^{-1} during the second NI treatment period to maximize NI effects.

Data Collection and Analysis

The number of leaves, leaf length, number of pseudobulbs and pseudobulb diameter were measured monthly for each plant during the experimental period. The longest leaf measured from the base of the pseudobulb was used to assess leaf length. Pseudobulb diameter was measured at the widest point of the flowering pseudobulb by using a digital caliper (ABS Digimatic Caliper; Mitutoyo Co., Ltd., Tsukuba, Japan). Dry weights (DWs) of leaves, pseudobulbs and roots were determined after drying in an oven at 80℃ for 3 days after the first and the second NI treatments. Time to the second pseudobulb emergence from the start of the treatments was measured. The times to visible inflorescence (VI) from the start of the treatments, along with the inflorescence height above the medium at flowering and the number of inflorescences and florets, were measured. The time at which the first floret was fully open was regarded as flowering time. Pseudobulb diameter and the number of leaves at inflorescence initiation were also measured.

The experimental design was a randomized complete block with three replications with seven plants in each replication. Statistical analyses were performed using the SAS system for window V8 (SAS Inst. Inc., Cary, NC, USA).

Differences among the treatment means were assessed by Tukey's honestly significant difference test at $P < 0.05$. Regression and graph module analysis was performed using Sigma Plot (Systat Software, Inc., Chicago, IL, USA).

RESULTS

Vegetative Growth

In ‘Red Fire’ and ‘Yokihi’ cultivars, the number of leaves on plants grown under LNI and HNI conditions was higher than that of control from the start of the first NI treatments (Fig. I-1). The differences among the treatments in the number of leaves increased during the second NI treatments in both cultivars. Leaf length of ‘Red Fire’ increased at similar rates under both LNI and HNI conditions, while leaves of ‘Yokihi’ were longer under HNI conditions than under LNI or control conditions during the second NI treatments. The number of pseudobulbs increased under LNI and HNI conditions after the first NI treatments in both cultivars (Fig. I-2). At 7 weeks after post-NI treatment initiation, pseudobulbs were bigger on plants grown under both LNI and HNI conditions than those grown under control conditions in ‘Red Fire’. However, the ‘Yokihi’ cultivar exhibited bigger pseudobulbs appearing earlier in plants grown under HNI conditions than on those grown under LNI and control conditions.

Dry weights significantly ($P < 0.001$) increased under LNI and HNI after the first and the second NI treatments for both ‘Red Fire’ and ‘Yokihi’ (Table I-1), and DWs were greatest for ‘Red Fire’ grown under HNI conditions. No significant interaction between NI treatments and cultivars was found for the DWs after the second NI treatments. Time to the second pseudobulb emergence from the initial planting for ‘Red Fire’ was greatly reduced by LNI and HNI by 9 and 19 weeks compared to control, respectively (Fig. I-3). For ‘Yokihi’, the time was reduced by 8 and 12 weeks compared to control, respectively.

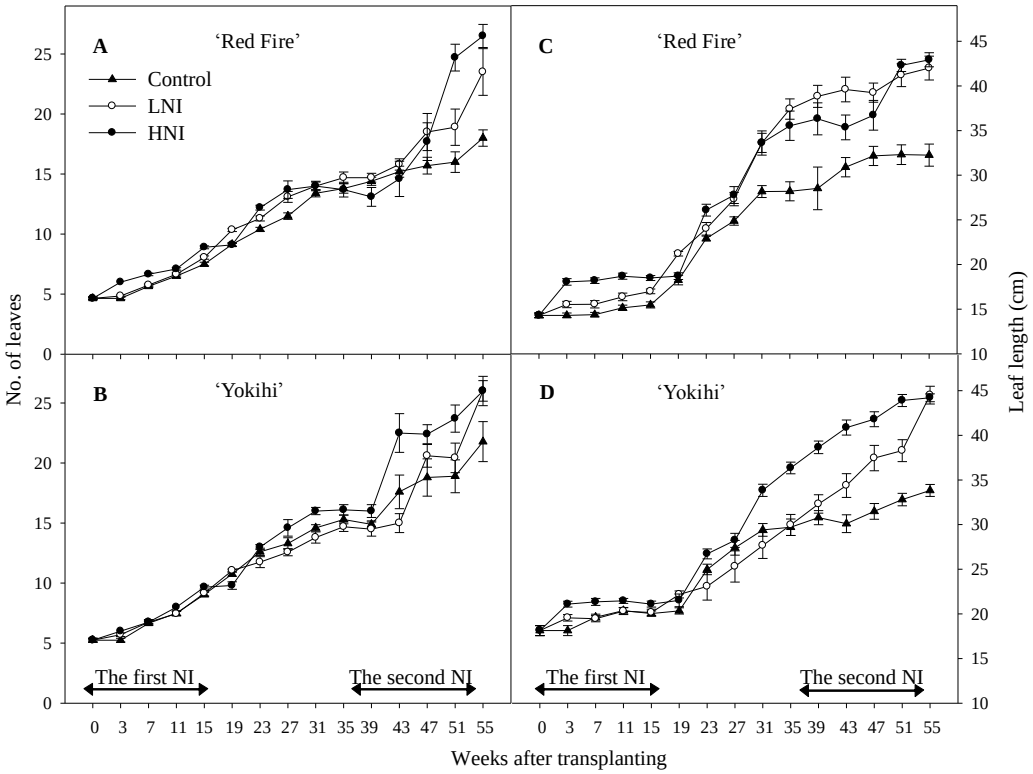

Fig. I-1. Effects of night interruption (NI) on number of leaves (A, B) and leaf length (C, D) of *Cymbidium* 'Red Fire' (A, C) and 'Yokihi' (B, D). The plants were grown under NI at 3-7 μmol m^{-2} s^{-1} (LNI) or at 120 μmol m^{-2} s^{-1} (HNI) with high-pressure sodium (HPS) lamps. Control plants were maintained under short-day condition with opaque cloth.

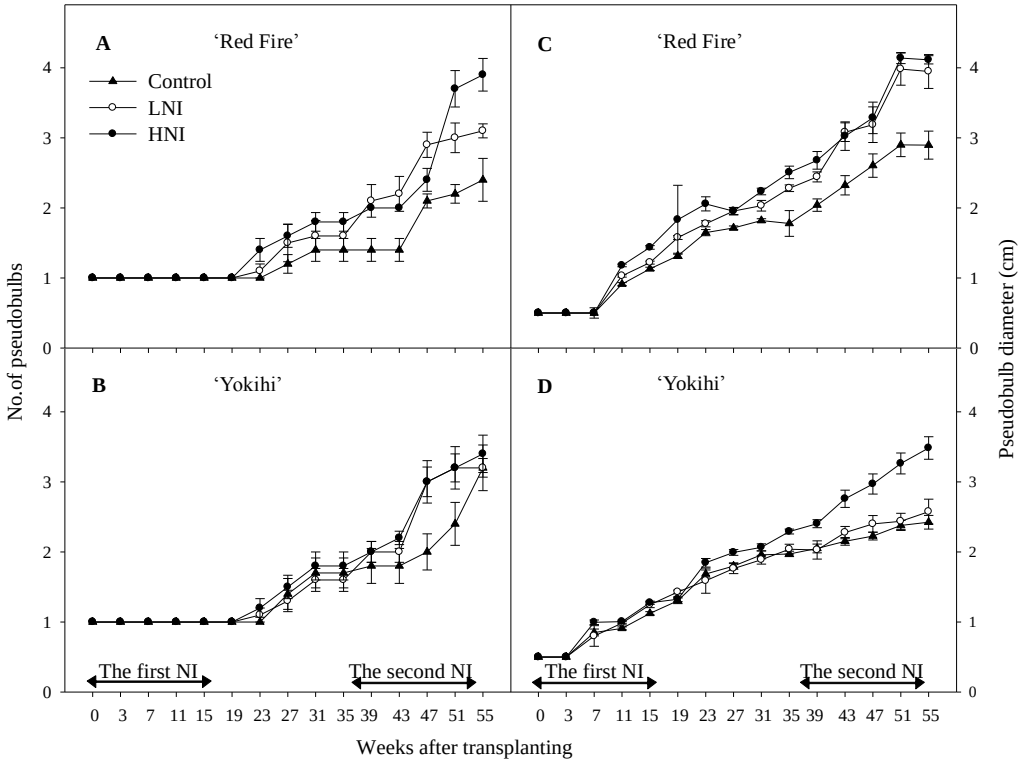

Fig. I-2. Effects of night interruption (NI) on number of pseudobulbs (A, B) and pseudobulb diameter (C, D) of *Cymbidium* 'Red Fire' (A, C) and 'Yokihi' (B, D). The plants were grown under NI at 3-7 μmol m^{-2} s^{-1} (LNI) or at 120 μmol m^{-2} s^{-1} (HNI) with high-pressure sodium (HPS) lamps. Control plants were maintained under short-day condition with opaque cloth.

Table I-1. Effects of night interruption (NI) on dry weights (DWs) of leaves, pseudobulbs and roots in *Cymbidium* 'Red Fire' and 'Yokihi' after the first and second NI treatments.

Cultivar	NI[a]	DWs after the first NI (g)[b]		DWs after the second NI (g)	
		Leaves and pseudobulbs	Roots	Leaves and pseudobulbs	Roots
'Red Fire'	Control	0.7c[c]	0.6d	9.6c	7.4bc
	LNI	1.4ab	1.0bc	14.1bc	12.1ab
	HNI	1.6a	1.4a	19.3a	14.9a
'Yokihi'	Control	1.0bc	0.8cd	8.5c	5.6c
	LNI	1.2b	1.1abc	11.4c	10.2abc
	HNI	1.3ab	1.3ab	18.7ab	14.1a
Significance					
Cultivar		NS[d]	NS	*	NS
NI		***	***	***	***
Cultivar × NI		**	NS	NS	NS

[a]The plants were grown under NI at 3-7 μmol m^{-2} s^{-1} (LNI) or at 120 μmol m^{-2} s^{-1} (HNI) with high-pressure sodium (HPS) lamps. Control plants were maintained under short-day condition with opaque cloth.

[b]The first and second NI treatments refer to Materials and Methods.

[c]Mean separation within columns by Tukey's honestly significant difference test at $P < 0.05$.

[d]NS: non-significant. [*]Significant at $P < 0.05$. [**]Significant at $P < 0.01$. [***]Significant at $P < 0.001$.

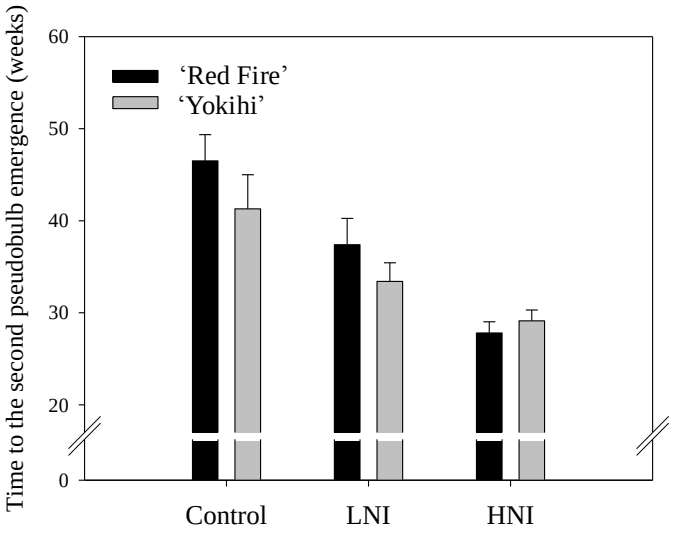

Fig. I-3. Effects of night interruption (NI) on time to the second pseudobulb emergence from the initial planting in *Cymbidium* 'Red Fire' and 'Yokihi'. The plants were grown under NI at 3-7 μmol m^{-2} s^{-1} (LNI) or at 120 μmol m^{-2} s^{-1} (HNI) with high-pressure sodium (HPS) lamps. Control plants were maintained under short-day condition with opaque cloth.

Flowering

Plants that received NI reached flowering within 2 years with different flowering percentages depending upon treatments, whereas none of the plants flowered under the control conditions in that time (Figs. I-4 and I-5). For those grown under HNI, 100% of the ‘Yokihi’ plants flowered, whereas 80% of ‘Red Fire’ flowered (Table I-2). For those grown under LNI, 60% of the plants in each cultivar flowered. There were fewer days to VI and flowering for plants in the HNI group than those in the LNI group for both cultivars (Fig. I-4). The plants under HNI conditions flowered 6 and 3 weeks earlier than plants under LNI conditions for ‘Red Fire’ and ‘Yokihi’, respectively. The number of inflorescences and florets were greater for the plants grown under HNI compared to those grown under LNI (Table I-2). The number of florets was significantly ($P < 0.01$) higher in ‘Yokihi’ than in ‘Red Fire’ under both LNI and HNI conditions. The plants under HNI were 1.1 and 11.3 cm taller at flowering than those under LNI for ‘Red Fire’ and ‘Yokihi’, respectively. More leaves developed in ‘Yokihi’ than in ‘Red Fire’ in any NI treatments (Fig. I-6). For both cultivars, more leaves also developed in the HNI, LNI and control conditions, in that order. When VI appeared, pseudobulb diameters were 5.2 and 5.9 cm in ‘Red Fire’ and 3.8 and 4.0 cm in ‘Yokihi,’ in the plants grown under LNI and HNI, respectively (Fig. I-6). The pseudobulbs of ‘Red Fire’ and ‘Yokihi’ grown under control conditions were 3.6 and 3.4 cm, respectively.

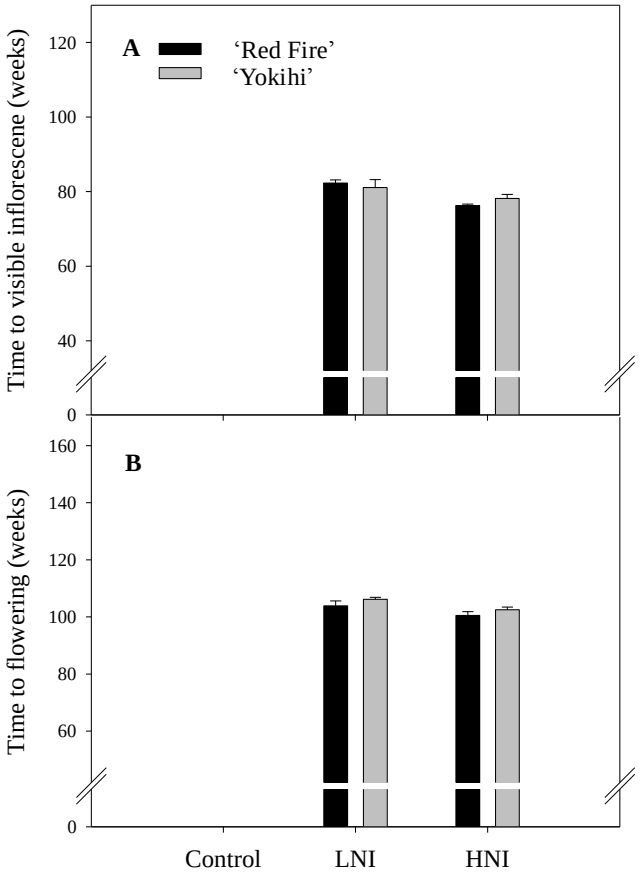

Fig. I-4. Effects of night interruption (NI) on time to visible inflorescence (A) and time to flowering from the initial planting (B) in *Cymbidium* 'Red Fire' and 'Yokihi'. The time to visible inflorescence is based on an inflorescence emergence time from any flowering pseudobulbs. The plants were grown under NI at 3-7 μmol m^{-2} s^{-1} (LNI) or at 120 μmol m^{-2} s^{-1} (HNI) with high-pressure sodium (HPS) lamps. Control plants were maintained under short-day condition with opaque cloth and did not show visible inflorescence by the end of the experiments.

Fig. I-5. Effects of night interruption (NI) on flowering in *Cymbidium* 'Red Fire' (A) and 'Yokihi' (B) at 104 weeks after transplanting. The plants were grown under NI at 3-7 μmol m^{-2} s^{-1} (LNI) or at 120 μmol m^{-2} s^{-1} (HNI) with high-pressure sodium (HPS) lamps. Control plants were maintained under short-day condition with opaque cloth.

Table I-2. Effects of night interruption (NI) on flowering of *Cymbidium* 'Red Fire' and 'Yokihi'. Treatments with < 20% flowering were not included in statistical analysis. The experiment was finished at 2 years after the start of the treatments.

Cultivar	NI[a]	Flowering (%)	No. of inflorescences	No. of florets	Height[b] at flowering (cm)
'Red Fire'	Control	0	-[c]	-	-
	LNI	60	1.4b[d]	9.6b	76.8ab
	HNI	80	2.2ab	14.8b	77.9ab
'Yokihi'	Control	0	-	-	-
	LNI	60	1.6ab	18.8ab	73.0b
	HNI	100	2.8a	30.0a	84.3a
Significance					
Cultivar			NS[e]	**	NS
NI			**	*	*
Cultivar × NI			NS	NS	NS

[a]The plants were grown under NI at 3-7 μmol m^{-2} s^{-1} (LNI) or at 120 μmol m^{-2} s^{-1} (HNI) with high-pressure sodium (HPS) lamps. Control plants were maintained under short-day condition with opaque cloth.

[b]Height was measured by the inflorescence height above the medium at flowering.

[c]Data not included in analysis because < 20% of plants flowered within 2 years.

[d]Mean separation within columns by Tukey's honestly significant difference test at $P < 0.05$.

[e]NS: non-significant. *Significant at $P < 0.05$. **Significant at $P < 0.01$.

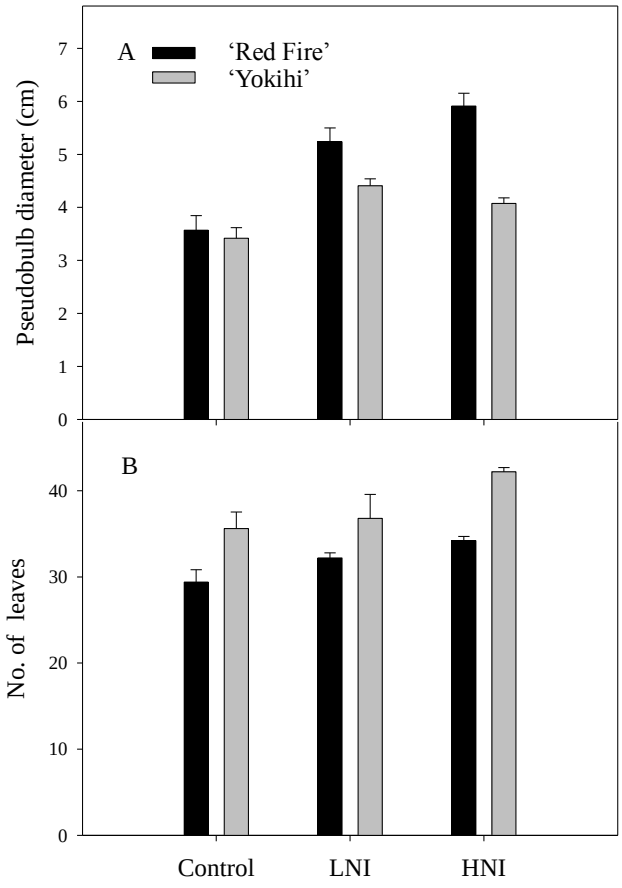

Fig. I-6. Effects of night interruption (NI) on pseudobulb diameter (A) and number of leaves (B) in *Cymbidium* 'Red Fire' and 'Yokihi' at inflorescence initiation. The plants were grown under NI at 3-7 μmol m^{-2} s^{-1} (LNI) or at 120 μmol m^{-2} s^{-1} (HNI) with high-pressure sodium (HPS) lamps. Control plants were maintained under short-day condition with opaque cloth.

DISCUSSION

The juvenile stage is a phase of growth during which flowering cannot be induced by any treatment. Sexual reproduction is delayed until plants reach sizes sufficient to maintain the energetic demands of flowering (Thomas and Vince-Prue, 1997). Compared to plants in the control group, those grown under LNI and HNI conditions had greater numbers of leaves and pseudobulbs, along with increased leaf length and pseudobulb diameter during the juvenile stage (Figs. I- 1 and I-2). Size appears to be important in the transition to maturity. According to Thomas and Vince-Prue (1997), if a plant of sufficient size transmits one or more signals to the apex, it undergoes a phase change. While *Cymbidium* plants did not flower within 2 years when grown under control conditions, VI initiation in *Cymbidium* occurred in plants undergoing LNI and HNI within 2 years, and inflorescence development was more accelerated in plants under HNI (Fig. I-4). The number of VI and flowers in 'Yokihi' were greater than that of 'Red Fire' under the same NI treatments. The increase in the number of florets under HNI can be attributed to an increase in the number of inflorescences. Increasing DLI with NI increased biomass accumulation, decreased time to flowering, and improved final plant quality in *Primula* (Karlsson, 2002). This experiment shows that for *Cymbidium*, increasing DLI with HNI can be considered a method to promote the plant's growth rate and to increase biomass. Due to the translocation of carbohydrates from the leaves to the reproductive organs such as the pseudobulb, HNI may promote plant growth and development.

In commercial production, the ability to schedule potted plants to flower during

periods of high demand is desirable because it allows precise crop scheduling and improves production efficiency. Photoperiod control allows plants to continue to form leaf primordia or induce flowering, as seen in other species. For instance, plant height and fresh weight of chrysanthemum increased during the vegetative stage when plants were given supplemental lighting (Carpenter, 1976). NI promoted plant growth and flowering in many crops including cyclamen (Kang et al., 2008), pansy and hibiscus (Runkle and Heins, 2003). According to Blanchard and Runkle (2010), the threshold light intensity for flowering in coreopsis and campanula from an HPS lamp was 0.05-0.1 $\mu mol\ m^{-2}\ s^{-1}$. The threshold light intensity required to delay a photoperiodic flowering response in chrysanthemum is 1.1 $\mu mol\ m^{-2}\ s^{-1}$ (Cathey and Borthwick, 1961). In the present study, the light intensity for LNI was 3-7 $\mu mol\ m^{-2}\ s^{-1}$, which was high enough to influence merely stronger vegetative growth, thereby setting up conditions for flower initiation. Although DLI in the control and the LNI conditions was the same, the control plant did not flower. Since the NI treatments had no effects on the flower initiation itself, the NI treatments along with DLI are thought to have direct effects on vegetative growth for ensuring the flower initiation.

In this study, 60% of plants grown under LNI flowered, and the mean number of leaves for that group was 34. However, the flowering percentage increased to over 80% for plants grown under HNI and the plants formed a mean of 38 leaves. The reproductive stage was reached by the plants under LNI and HNI in the experiment and most with over 34 leaves were capable of flowering. Previous studies demonstrated that a minimum number of leaves are required for transition to the reproductive stage: 18-22 leaves for *Antirrhinum* (Cockshull, 1985), 16

leaves for *Coreopsis* (Cameron et al., 1996) and 6-7 leaves for *Pericallis* (Yeh and Atherton, 1997).

A minimum pseudobulb diameter was also required for inflorescence initiation. This study showed that pseudobulbs with a minimum diameter of 5.2 cm for ‘Red Fire’ and 4.4 cm for ‘Yokihi’ were required for the development to stage VI (Fig. II-6). Blanchard and Runkle (2008) reported that a pseudobulb diameter of 5.5 cm or greater was required for uniform inflorescence initiation of *Odontioda* orchids. The data in the present study indicate that no VI was observed in *Cymbidium* until plants formed a specific pseudobulb size even with NI. *Cymbidium* grown under HNI conditions exhibited the greatest increase in pseudobulb diameter, thus plants in this group had the greatest final pseudobulb diameter and exhibited early flowering. As such, pseudobulb size may be a good indicator to determine when *Cymbidium* is of adequate maturity for flower induction. Ichihashi (1997) and Lopez and Runkle (2006) also demonstrated the importance of mature pseudobulbs for flower initiation in *Dendrobium* and *Miltoniopsis* orchids.

In this study, NI promoted vegetative growth, as evidenced by an increased number of leaves and pseudobulbs in plants grown under these conditions relative to those under control conditions. Additionally, plants were able to reach the reproductive stage during the second year of cultivation. The plants grown under both LNI and HNI conditions initiated VI, but those grown under HNI showed VI 50 days earlier than plants grown under LNI. In *Cymbidium*, it generally takes more than 3 years after transplanting for high quality flowering to occur. However, the data in the present study suggest that if NI is used to deliver LD during the SD season, the greatest promotion in flower induction will occur within 2 years.

In conclusion, *Cymbidium* ‘Red Fire’ and ‘Yokihi’ were grown under LNI and HNI conditions to promote vegetative growth and enhance flowering. The findings in the present study suggest that plants grown under LNI and HNI should have pseudobulbs that will rapidly attain the minimum size required for flowering and have leaves greater in number than those grown under conventional conditions. HNI increased *Cymbidium* plant height and the number of florets and decreased the days to flowering from the initial planting. While the benefits of increased light intensity for NI may warrant the use of NI to improve crop quality and profitability, the initial investment in an NI system and the additional electricity expenses must be considered. *Cymbidium* passed through several SD and LD periods during vegetative growth. Although the plants had smaller inflorescences and florets under LNI than under HNI, when compared to control plants they would still result in higher profits due to decreased crop time. Commercial use of NI for *Cymbidium* cultivation will decrease crop time and increase profits. However, when higher quality flowering is needed more than the short crop time, a high light intensity for NI forcing culture should be considered.

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CHAPTER II

Carbohydrate Changes of *Cymbidium* 'Red Fire' in Response to Night Interruption with Different Light Intensities

ABSTRACT

The effects of night interruption (NI) on growth, carbon allocation and soluble carbohydrate concentrations were investigated in *Cymbidium* 'Red Fire'. The plants were vegetatively propagated and grown under three light intensity conditions for 24 months: 9/15 h ambient light/dark (control), 9 h ambient light plus NI (22:00 to 02:00 h) with low light intensity at 3-7 $\mu mol\, m^{-2}\, s^{-1}$ (LNI) and 9 h ambient light plus NI with high light intensity at 120 $\mu mol\, m^{-2}\, s^{-1}$ (HNI) during winter season. Carbohydrate contents in leaves, pseudobulbs and roots were measured during their development. The plants grown under HNI condition had higher total dry mass than those grown under control and LNI conditions. The pseudobulb diameter was bigger under both LNI and HNI conditions than under control. Soluble carbohydrate concentrations were higher under the HNI than under the LNI and control conditions. Glucose was the most abundant soluble carbohydrate in the pseudobulb of *Cymbidium* 'Red Fire' for flower initiation in the plants grown under the LNI and HNI conditions. The absolute amount of sucrose was much higher in leaves than in pseudobulbs and roots after NI treatments. The important storage organs for starch were the leaves and the roots

during vegetative growth, but the pseudobulbs during reproductive growth. The pseudobulbs of the plants acted as strong utilizing sink during flower developmental stage and preferentially metabolized carbohydrates rather than stored them. Increased carbohydrates in pseudobulb of *Cymbidium* under NI condition may have an important role in promotion of flowering than those in leaves and roots. This study showed that the promotion of flowering in the plants grown under LNI and HNI conditions may be due to the higher photosynthates in pseudobulbs.

Keywords: flowering, fructose, glucose, orchid, starch, sucrose

INTRODUCTION

Orchidaceae constitute one of the largest families of angiosperms. Orchids are marketed globally as cut flowers for corsages, floral arrangements and bouquets, and as potted flowering plants. Market demands of orchids have recently been more increased than in the past century (Lopez and Runkle, 2006).

Different species of orchids exhibit sensitivity to daylength in various ways. Long-day (LD) plants flower or flower earlier only when the night length is less than some critical duration, whereas short-day (SD) plants do so only under long nights (Runkle et al., 1998). Small changes in daylength are critical for the growth and flowering of *Cattleya, Dendrobium* and *Phalaenopsis* (Bhattacharjee, 1979). Artificial lighting during the middle of the night [night interruption (NI) lighting] regulated flowering of photoperiod-sensitive species (Blanchard and Runkle, 2010; Yamada et al., 2008). NI has been effective for accelerating the growth and development of many herbaceous plants, such as *Cyclamen persicum* and *Lythrum salicaria* (Kang et al., 2008; Kim et al., 2011a). While, the NI effects on flowering in orchid plants were rarely determined.

Carbohydrate accumulation plays an important role in flower induction. The main carbohydrate reserves in flower bulbs include starch and soluble sugars. Starch is the major storage carbohydrate in plants and may accumulate up to 70-80% of the dry weight of storage organs such as bulbs (Duffus and Duffus, 1984). Sugars play a critical role as products of photosynthesis, whereas sucrose is the main storage sugar in many plants and is the principal form in which carbon is transported through the plant (Smith, 1999). During the vegetative stage, roots and

pseudobulbs are active sinks in orchids (Pan, 1992). Studies on both *Catasetum viridiflavum* (Zimmerman, 1990) and *Oncidium* (Hew and Ng, 1996) have shown that carbohydrate reserves in orchid pseudobulbs are important in the initiation of new growth. The pseudobulbs of *Oncidium* accumulate massive amounts of carbohydrates during vegetative development. These carbohydrate reserves are subsequently remobilized to support new shoot and inflorescence development (Hew and Ng, 1996).

Cymbidium is commercially popular because of the ease of scheduling for meeting specific market demands, high wholesale value and long post-harvest life. Commercial production of potted *Cymbidium* has increased throughout the world. However, it grows rather slowly including 2-3 years of juvenile period. A recent study showed that the growth was promoted by NI and ultimately promoted flowering within 2 years compared to 3-4 years of general cultivation in *Cymbidium* ‘Red Fire’ and ‘Yokihi’ (Kim et al., 2011b). A minimum number of leaves and a minimum pseudobulb diameter were required for transition to the reproductive stage in *Cymbidium*. While plant size is an important determinant of flowering, large plants do not always flower (Snow and Whigham, 1989). The status of carbohydrate storage in the youngest corms appears to be an added determinant of flowering that is independent of plant size in *Tipularia discolor* (Tissue and Nobel, 1990).

To our knowledge, no study has been conducted on NI forcing culture effect on carbohydrate changes in *Cymbidium.* This study was conducted to determine the effects of NI forcing culture on relationships to the levels of carbohydrates in *Cymbidium.* The information obtained will help us understand the mechanisms

controlling photosynthate partitioning among sucrose, glucose, fructose and starch for flowering in *Cymbidium* under NI forcing culture.

MATERIALS AND METHODS

Plant and Growth Conditions

Cymbidium hybrids 'Red Fire' (Mukoyama Orchids Co., Ltd., Yamanashiken, Japan) were transplanted at the mericlonal stage into 10 cm pots and then re-transplanted into 16 cm pots after 4 months of growth. The pots contained 100% chopped coconut. The plants were grown in a commercial greenhouse in Hwasung, Republic of Korea. Average day/night temperatures for the first year (2009) and the second year (2010) of the experiments, were 27/22 and 28/24℃ in summer and 21/12 and 22/13℃ in winter, respectively. Average photosynthetic photon flux for the first and the second year of the experiments, was 563 and 500 μmol m^{-2} s^{-1} in summer and 229 and 215 μmol m^{-2} s^{-1} in winter, respectively. The plants were irrigated daily with tap water. Five grams of water-soluble controlled release 13N-5.7P-10.8K fertilizers (Mukoyama Orchids Co., Ltd.) were placed at the top of each pot for the control group and the group receiving a light intensity of 3-7 μmol m^{-2} s^{-1} (LNI) and 120 μmol m^{-2} s^{-1} (HNI). The controlled release fertilizer was applied at; transplanting, the first pseudobulb emergence and the second pseudobulb emergence.

NI Treatment

The plants were irradiated with high-pressure sodium (HPS) lamps (SKL-01; GEO, Hwasung, Republic of Korea) from 22:00 to 02:00 h. The greenhouse was divided into three sections. Each section was divided into different groups by placing pots at different distances from the lamps: controls received 9 h ambient

light and plants were covered with opaque black cloth daily from 17:00 to 08:00 h; LNI conditions were 9 h ambient light plus NI with low light intensity at 3-7 μmol m^{-2} s^{-1}; HNI conditions were 9 h ambient light plus NI with high light intensity at 120 μmol m^{-2} s^{-1}. The NI was employed twice during 2 years of the experimental period. The first NI treatments began right after transplanting for 16 weeks (February to May) and the second NI treatments began 38 weeks after transplanting for 16 weeks (November to February). The treatments were run on two separate batches of the plants. One batch of the plants was used for destructive sampling. The average day/night temperatures during NI were 22/15℃ and 19/12℃ for the first and the second NI treatments, respectively. The mean photosynthetic daily light integral during the first and the second NI treatments were 6.5 mol m^{-2} d^{-1} for the plants under the control, 6.5 mol m^{-2} d^{-1} for those undergoing LNI and 8.2 mol m^{-2} d^{-1} for those undergoing HNI.

Growth Measurements

Pseudobulb diameter was measured at the widest point of the flowering pseudobulb by using a digital caliper (ABS Digimatic Caliper; Mitutoyo Co., Ltd., Tsukuba, Japan). Four plants per treatment were harvested to assess dry mass production. The plants were cut at the roots and separated into leaves and pseudobulbs. The tissues were dried in an oven at 80℃ for 1 week for dry mass determination. Leaves, roots and pseudobulbs samples from four plants growing under the control, LNI and HNI treatments were collected at 60, 80 and 100 weeks after the start of the treatments.

Sugar Analysis

Four plants per treatment were employed to analyze sugar contents. Samples were immediately frozen in liquid N_2, subsequently lyophilized, ground using a mill (Thomas Wiley® Mini Mill 3383-L10, Thomas Scientific, Swedesboro, NJ, USA) with 60-mesh sieve and stored as powder at 80℃ until use. Soluble sugars were extracted using the method described by González-Rossia et al. (2008) with slight modifications. One hundred milligrams of ground powder were put in a 2-mL test tube containing 1-mL 80% ethanol and incubated at 85℃ for 15 min. After centrifugation at 15,000 g for 5 min, the supernatants were collected and the pellets were re-extracted twice as above. The combined supernatants were evaporated using a N_2 evaporator (N-EVAPTM, Organomation Associates, Inc., West Berlin, MA, USA) at 60℃; pellets were saved for further starch analysis. The ethylic solution of sugar extracts was dissolved in 3 mL of distilled water and passed through 0.45 μm nylon filter (Acrodisc® 13 mm Syringe Filter, Pall Co., Washington, NY, USA) and C18 Sep-Pak cartridge (Waters Associates, Milford, MA, USA). Sugars were analyzed using an HPLC (UltiMate 3000, Dionex, Sunnyvale, CA, USA) connected to a Shodex RI-101 detector (Showa Denko K.K., Kawasaki, Japan). Ten microliters of the filtered extracts were injected into a Sugar-Pak column whose temperature was kept at 75℃ and distilled water was used as solvent at a flow rate of 0.5 mL min^{-1}.

Starch in the remaining pellet was determined according to the method of Smith and Zeeman (2006). The pellet was dissolved in 1mL distilled water, autoclaved to gelatinize starch granules and then incubated at 55℃ for 2 h to hydrolyze 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 (A3404, Sigma-Aldrich Korea Ltd.). After centrifugation at 15,000 g for 10 min, the supernatants were collected, evaporated and dissolved in 3 mL of distilled water. The extracts were filtered through 0.45 μm nylon filter and C18 Sep-Pak cartridge and then the released glucose was determined by HPLC as mentioned above.

Statistical Analysis

Statistical analyses were performed using the SAS system for window V8 (SAS Inst. Inc., Cary, NC, USA). Differences among the treatment means were assessed by Tukey's honestly significant difference test at $P < 0.05$.

RESULTS

Pseudobulb Characteristics

The pesudobulbs were bigger in the plants grown under both LNI and HNI conditions than those grown under control condition after the first NI treatment at week 20 (Fig. II-1). The pseudobulb diameter difference between NI-treated and control plants became apparent after the second NI treatments. No significant differences were observed between the plants grown under LNI and HNI conditions in pseudobulb diameter. Dry mass of leaves, pseudobulbs and roots significantly increased under LNI and HNI conditions after the second NI treatment (after 54 weeks of transplanting) (Fig. II-2). The plants grown under LNI and HNI had pseudobulbs that would more rapidly attain the minimum size required for flowering than those grown under control condition.

Soluble Sugars

The major sugars including glucose and fructose increased in pseudobulbs under LNI and HNI conditions after the first NI treatment (Fig. II-3). The total soluble sugar content in the plants under HNI condition was approximately 2-fold higher than that in the plants grown under control condition. The amounts of sucrose, glucose and fructose were summed and expressed as total soluble sugar content by developmental stage after the second NI treatment (Fig. II-4). The plants were in the vegetative growth stage during experimental period in control group, while the plants under the LNI and HNI were in vegetative growth stage until week 60 and were turned over to the reproductive stage, which has mature

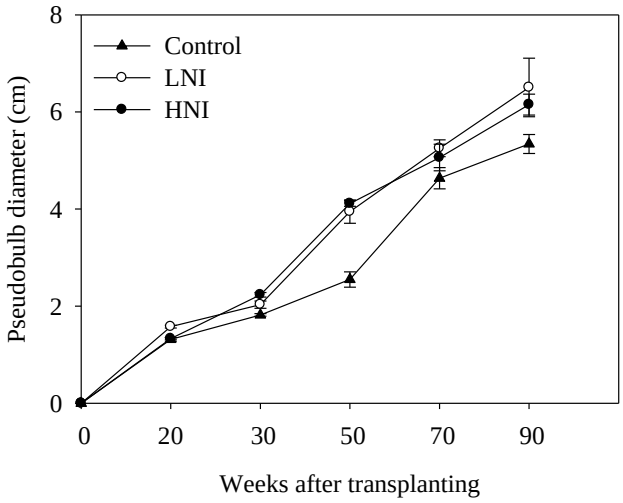

Fig. II-1. Effects of night interruption (NI) on pseudobulb diameter of *Cymbidium* 'Red Fire'. The plants were grown under NI at 3-7 μmol m^{-2} s^{-1} (LNI) or at 120 μmol m^{-2} s^{-1} (HNI) with high-pressure sodium (HPS) lamps. Control plants were maintained under short-day condition with opaque cloth. Vertical bars are means ± S.E. (n=10).

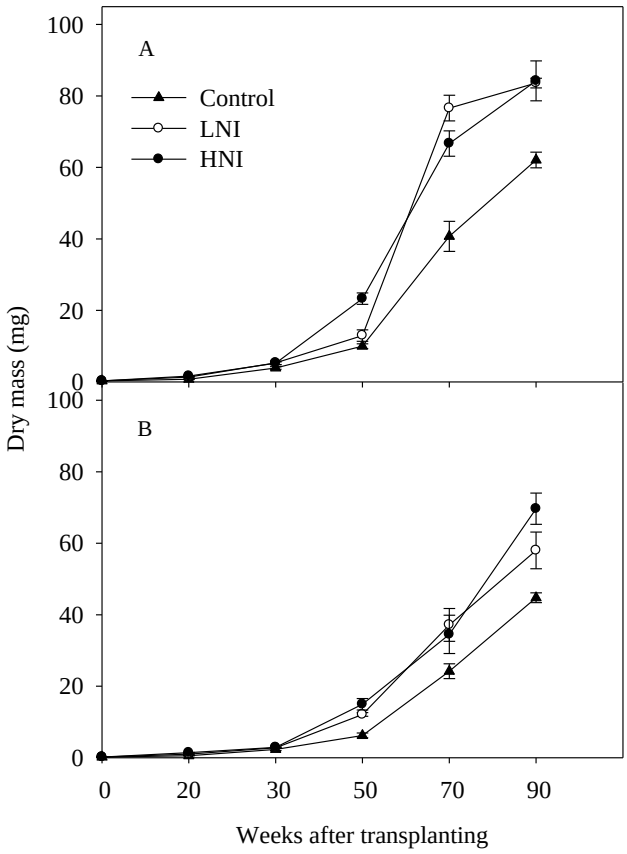

Fig. II-2. Effects of night interruption (NI) on leaves and pseudobulbs (A) and roots (B) dry mass of *Cymbidium* 'Red Fire'. The plants were grown under NI at 3-7 μmol m^{-2} s^{-1} (LNI) or at 120 μmol m^{-2} s^{-1} (HNI) with high-pressure sodium (HPS) lamps. Control plants were maintained under short-day condition with opaque cloth. Vertical bars are means ± S.E. (n=4).

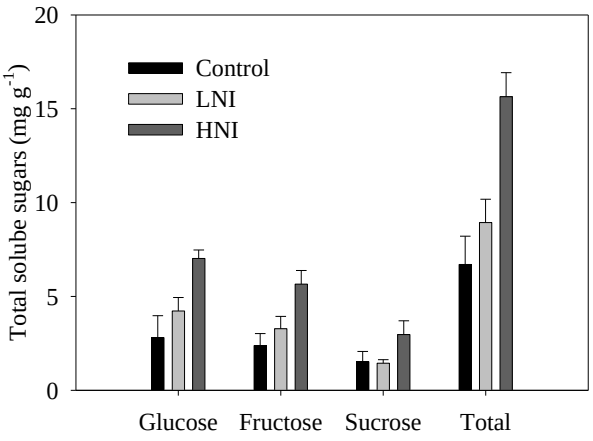

Fig. II-3. Effects of the first night interruption (NI) on soluble sugar contents in pseudobulbs of *Cymbidium* 'Red Fire' at 20 weeks after transplanting. The plants were grown under NI at 3-7 μmol m^{-2} s^{-1} (LNI) or at 120 μmol m^{-2} s^{-1} (HNI) with high-pressure sodium (HPS) lamps. Control plants were maintained under short-day condition with opaque cloth. Vertical bars are means ± S.E. (n=4).

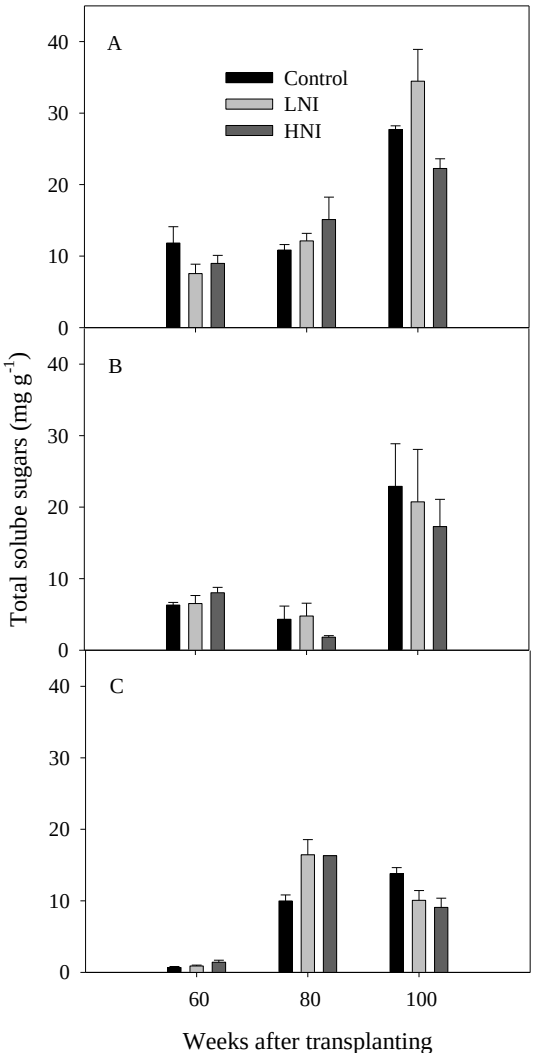

Fig. II-4. Effects of the second night interruption (NI) on soluble sugar contents of *Cymbidium* 'Red Fire' leaves (A), roots (B) and pseudobulbs (C). The plants were grown under NI at 3-7 μmol m^{-2} s^{-1} (LNI) or at 120 μmol m^{-2} s^{-1} (HNI) with high-pressure sodium (HPS) lamps. Control plants were maintained under short-day condition with opaque cloth. Vertical bars are means ± S.E. (n=4).

for flower initiation evidenced by certain number of leaves and size of pseudobulb diameter after week 80. Total soluble sugars decreased in the pseudobulb between week 80 and 100 in the plants grown under the LNI and HNI conditions, while it increased in the plants grown under the control condition (Fig. II-4C). The main soluble sugars of the pseudobulb were glucose, followed by fructose and sucrose (Fig. II-5). However, the absolute amount of sucrose in leaves was much higher than that in pseudobulbs at vegetative growth stage, week 60 (Fig. II-5A). Little amounts of sucrose were accumulated under all treatments in the pseudobulbs at vegetative growth stage (week 60), but it increased before flower initiation in the plants grown under LNI and HNI conditions (week 80). The sucrose concentration in roots was significantly increased in the plants grown under the LNI and HNI conditions at week 100, the inflorescence emergence stage (Fig. II-5B). A reduction in total soluble sugar content was observed during plant development (week 100) (Fig. II-4) to accompany the decreases in sucrose, glucose and fructose contents (Fig. II-5).

Starch

The important storage organs for starch were the pseudobulbs and leaves in *Cymbidium* ‘Red Fire’, which was indicated by the low starch concentrations in the roots after the second NI treatments, at week 80 (Fig. II-6). The starch concentration in the pseudobulbs in the plants grown under LNI and HNI conditions at week 100, which was in inflorescence emergence and flowering stage, was lower than that in the plants grown under the control condition, which was in vegetative growth stage (Fig. II-6C). The decreases of starch concentration

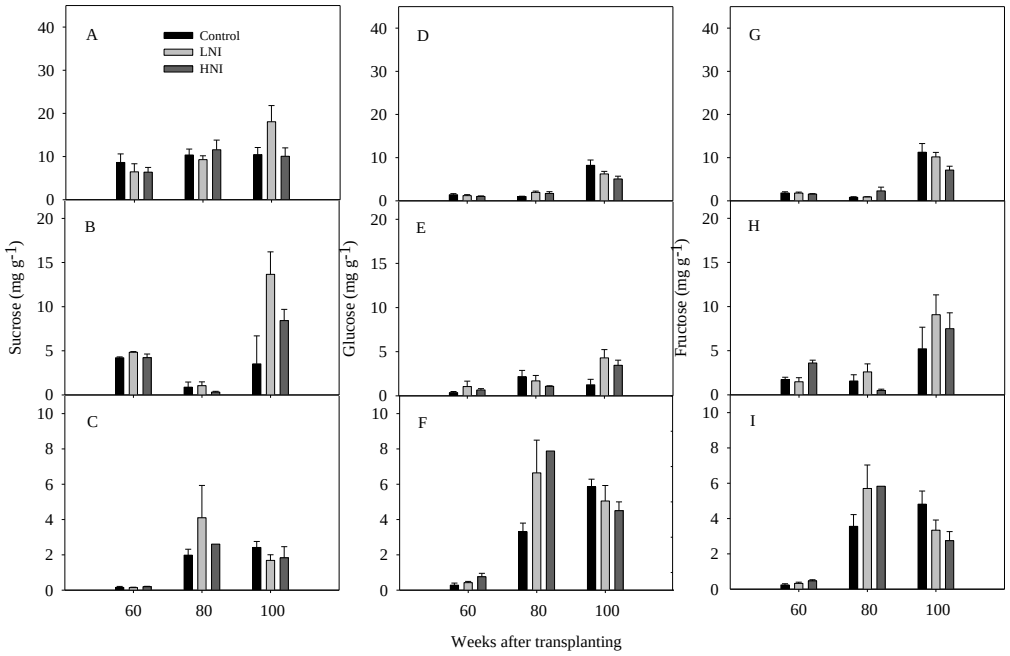

Fig. II-5. Effects of the second night interruption (NI) on sucrose, glucose and fructose contents in *Cymbidium* 'Red Fire' leaves (A, D, G), roots (B, E, H) and pseudobulbs (C, F, I). The plants were grown under NI at 3-7 μmol m^{-2} s^{-1} (LNI) or at 120 μmol m^{-2} s^{-1} (HNI) with high-pressure sodium (HPS) lamps. Control plants were maintained under short-day condition with opaque cloth. Vertical bars are means ± S.E. (n=4).

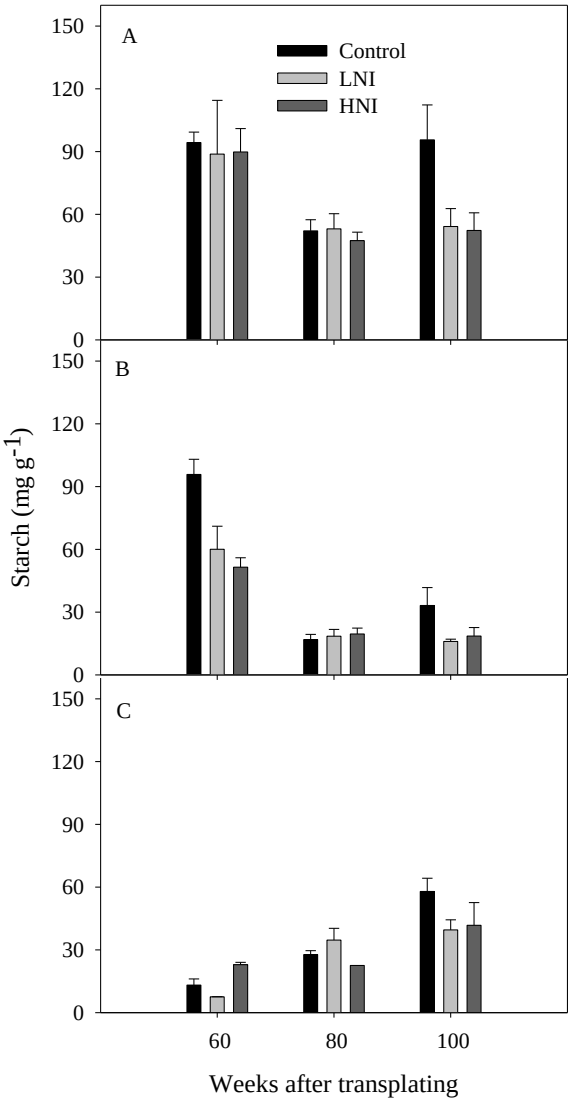

Fig. II-6. Effects of the second night interruption (NI) on starch contents in *Cymbidium* 'Red Fire' leaves (A), roots (B) and pseudobulbs (C). The plants were grown under NI at 3-7 μmol m^{-2} s^{-1} (LNI) or at 120 μmol m^{-2} s^{-1} (HNI) with high-pressure sodium (HPS) lamps. Control plants were maintained under short-day condition with opaque cloth. Vertical bars are means ± S.E. (n=4).

in leaves and roots were subsequently accompanied by the decrease in pseudobulbs in the plants grown under LNI and HNI conditions. The important storage organs for starch were leaves and roots at 60 weeks after the transplanting, but became pseudobulbs at week 80. The starch concentration in pseudobulbs was also highly correlated ($R = 0.91$; $P < 0.001$) with total pseudobulbs dry mass.

DISCUSSION

The pseudobulb of *Cymbidium* orchid is a strong sink for partitioning the photosynthates during vegetative development. During its growth, the photosynthates produced in the upper younger leaves are transported to the developing pseudobulb sink. In our present study, carbohydrates in *Cymbidium* 'Red Fire' pseudobulbs of different developmental stages were analyzed by HPLC. Glucose and fructose are abundant in young and developing pseudobulbs (Figs. II-3 and II-5) at the inflorescence pre-initiation stage (week 80) in the plants grown under the LNI and HNI conditions. It is well known that plants at vegetative stage always convert sugar photosynthates into polysaccharides and accumulate them in sinks as nutritional and energy sources (Wang et al., 2008). As the inflorescence initiates from the pseudobulb base (designated as week 80), *Cymbidium* switches its life cycle from the vegetative stage to the reproductive stage in the plants grown under the LNI and HNI conditions. Concurrently with inflorescence initiation, glucose and fructose started to mobilize in pseudobulbs (Fig. II-5). Starch increased in pseudobulbs from the early inflorescence developing stage, week 80 (Fig. II-6). Concomitantly with the growth and development of inflorescence in the plants grown under the LNI and HNI conditions, starch was gradually degraded for energy supply and eventually used up in the late inflorescence stage (week 100) during which the floral organs were completely developed.

The roots and pseudobulbs were found to be the main starch sinks, while the leaves were the main source for soluble sugars. The high sucrose concentration in

roots before flowering stage in the plants grown under the LNI and HNI conditions, at week 100 (Fig. II-5) implies that the leaves are the main manufacturer of carbohydrates. The roots retained their sugars and prevented the converted starch to break down into sugars. The majority of soluble sugars were found in the pseudobulbs rather than in the leaves and roots in terrestrial orchid *Spathoglottis unguiculata* (Hew et al., 1998). At 100 weeks after transplanting, when the flowers were initiated in the plants grown under the LNI and HNI conditions, the starch concentration of pseudobulbs dropped (Fig. II-5), probably due to carbohydrate mobilization to inflorescence. Once starch has been converted, it normally remains unchanged until the next growing season, when it is rapidly mobilized to support new growth. This metabolic network implicates that sucrose plays a functional role to regulate starch biosynthesis during the inflorescence developmental stage of pseudobulbs.

The simultaneous increases of soluble sugars and starch in pseudobulbs (Figs. II-4C and II-6C) indicated that the leaf-producing photosynthates become available for inflorescence growth, thus ensuring high starch concentrations in pseudobulbs. Assimilate of photosynthesis is suggested to be related to flower development (Kataoka et al., 2004). If flower induction of *Cymbidium* 'Red Fire' is triggered by the excess of photosynthate under the LNI and HNI conditions, the flower induction and development can be controlled by means of increasing photosynthesis, decreasing consumption or exogenous application of sugars. The assimilation or mobilization of photosynthates is associated with the induction of reproductive growth in *Cymbidium*. Before the transition from vegetative to floral differentiation, starch grains accumulated in cells of the quiescent zone but

disappeared during differentiation of floral organs in pear plants (Peng and Iwahori, 1994), suggesting that abundant carbohydrates are consumed for the initiation and consequent development of floral organs. The increases of sucrose contents in leaves before spiking have also been reported in *Phalaenopsis* (Kataoka et al., 2004) and *Oncidium* (Wang et al., 2003).

Large pseudobulbs of *Cymbidium* constituted almost two-third of the total biomass in the plants grown under the LNI and HNI conditions. Maintaining such a large biomass of pseudobulbs requires substantial amount of energy in the form of carbohydrates, which plays an important role in regulation of leaf photosynthesis and thus, the carbon balance of orchids (He et al., 2011). In *Cymbidium*, it generally takes more than 3 years after transplanting for high quality flowering to occur. However, our previous data suggest that if NI is used to deliver LD during the SD season, the greatest promotion in flower induction will occur within 2 years (Kim et al., 2011b). This study showed that the promotion of flowering in the plants grown under the LNI and HNI conditions may be due to the higher photosynthates in pseudobulbs.

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CHAPTER III

Growth and Flowering of *Cymbidium* 'Red Fire' and 'Yokihi' during Night Interruption Forcing Culture with Mist and Shade Systems

ABSTRACT

Growth and flowering of *Cymbidium* 'Red Fire' and 'Yokihi' plants were examined in a greenhouse with cooling systems in the summer and night interruption (NI) lighting in the winter during a forcing culture. The greenhouse was divided into two sections for separate cooling controls during the summer season. One section was cooled by a mist system (mist), while the other was cooled by a shade screen (shade). During the winter, the plants were grown with NI either at a low light intensity at 3-7 μmol m^{-2} s^{-1} (LNI) and at 120 μmol m^{-2} s^{-1} (HNI) with high-pressure sodium lamps during 22:00-02:00 h, whereas the control plants were grown under a 9 h photoperiod condition. NI for 16 weeks and cooling for 9 weeks were employed twice during the experimental period of 2 years. The air temperature was approximately 2℃ lower in the mist than in the shade and the relative humidity of the mist was 80 ± 5% as compared to 55 ± 5% the shade. The daily light integral of the shade sections was 48% of the mist sections. The time from initial planting to flowering pseudobulb emergence for 'Red Fire' and 'Yokihi' decreased by LNI and HNI, respectively, regardless of the

cooling treatments. However, the flowering promotion effect was greater in the mist than in the shade. Over 60% of the plants of ‘Yokihi’ and ‘Red Fire’ flowered in the NI in the mist, whereas none of the ‘Red Fire’ flowered in the shade and 20% of the shade-cooled ‘Yokihi’ plants in the LNI flowered within 2 years. The number of flowers was greater in the plants grown under the HNI compared to those grown under the LNI with the mist. In conclusion, NI during the short-day season and mist during summer season are recommended to promote the growth and flowering of *Cymbidium* ‘Red Fire’ and ‘Yokihi’. Commercial use of NI for *Cymbidium* forcing culture will decrease crop time within 2 years and increase profits when mist system was employed.

Keywords: mist, photoperiod, relative humidity, shade, temperature

INTRODUCTION

Cymbidium, a tropical terrestrial orchid species, is commonly grown as a commercial potted plant because of the ease of scheduling for meeting specific market demands and high wholesale value. However, it grows rather slowly and generally takes 3-4 years to flower from the mericlonal stage.

Flowering in plants is regulated by both internal factors and environmental signals such as daylength and temperature (Cerdan and Chory, 2003). Different species of orchids exhibit sensitivity to daylength in various ways and yet small changes in daylength are critical for the growth and flowering of *Cattleya*, *Dendrobium* and *Phalaenopsis* (Bhattacharjee, 1979). Artificial lighting during the middle of the night [night interruption (NI) lighting] regulated flowering of daylength-sensitive species (Blanchard and Runkle, 2010; Yamada et al., 2008). The growth rate of *Cymbidium* 'Red Fire' and 'Yokihi' was promoted by NI as a method of delivering long-days during the short-day period (Kim et al., 2011).

Seedlings of *Dendrobium nobile* grown at 18℃ remained vegetative, whereas those grown at 13℃ flowered regardless of daylength (Rotor, 1952). *Zygopetalum* 'Fire Kiss' had the highest flowering percentages when they grown under a 9 h photoperiod followed by 8 weeks of cooling at 11 or 14℃ (Lopez et al., 2003). The temperature tolerance of *Cymbidium*, which is native from the Himalayas to tropical regions, varies depending on its origin. The growth of *Cymbidium* originating in China was accelerated at a day/night temperature of 30/25℃, but a positive diurnal fluctuation of 10-14℃ was required for flower initiation in the large-flowered *Cymbidium* species. Day and night temperatures likely have an

effect on flower induction in some *Cymbidium* species (Pridgeon, 2000; Rotor, 1952; Went, 1957). *Cymbidium* ‘Radjah’ had more inflorescences per plant when grown at 26/12℃ than at 20/12 or 26/18℃ (14 h day/10 h night) (Powell et al., 1988). Low temperatures are required for flower bud initiation regardless of daylength in *Cymbidium* (Rotor, 1952).

During summer, several *Cymbidium* hybrids are commonly transported from lowland production areas to higher elevations. This strategy helps avoid high temperature stress during vegetative growth and exposed plants to cooler temperatures and higher light for flower initiation (Ichihashi, 1997). However, plant quality is often reduced during transportation and this can be costly for growers. Although flowering of many *Cymbidium* species can be manipulated by temperature during summer season, crop cultivation in summer in a greenhouse is negatively affected by high temperature, which decreases yield and product quality. A number of cooling techniques, such as natural ventilation, white-shading, shade screens and evaporative cooling (i.e., wet pad-fan, misting and fogging systems) are used to efficiently maintain the temperature and humidity of a greenhouse at acceptable levels during hot periods (Perdigones et al., 2008) instead of the transportation.

NI promoted growth and flowering in *Cymbidium* ‘Red Fire’ and ‘Yokihi’ by increased biomass in the previous research (Kim et al., 2011). Reducing crop time, within 2 years for flowering by NI will ensure a profitable crop, thereby achieving more economical production in *Cymbidium*. However, the methods of maintaining crops during summer for flower initiation in a greenhouse have not been determined under NI forcing culture. In this study, the effects of two cooling

techniques, mist and shade systems in a greenhouse, were determined on the growth and flowering of *Cymbidium* ‘Red Fire’ and ‘Yokihi’ when NI was applied in a forcing culture.

MATERIALS AND METHODS

Plant and Growth Conditions

Cymbidium ‘Red Fire’ and ‘Yokihi’ (Mukoyama Orchids Co., Ltd., Yamanashiken, Japan) were transplanted into 10 cm pots containing 100% chopped coconut and then re-transplanted into 16 cm pots after 4 months of growth. The plants were grown in a commercial greenhouse (Sang-Il Orchid Farm) in Hwasung, Republic of Korea. The plants were irrigated daily with tap water. Five grams of water-soluble controlled release 13N-5.7P-10.8K (Mukoyama Orchids Co., Ltd.) were placed on the top of each pot. The controlled release fertilizer was applied different times during production: at transplanting and upon emergence of the first and the second flowering pseudobulbs for 3-4 months, each. Micronutrient fertilizers were applied bimonthly to the plants using a sprinkler. The supplemental fertilizers were composed of $Ca(NO_3)_2 \cdot 4H_2O$, Fe-EDTA, $MgSO_4 \cdot 7H_2O$, $MnSO_4$, $ZnSO_4 \cdot 7H_2O$, H_3BO_3, $CuSO_4 \cdot 5H_2O$ and $Na_2MoO_4 \cdot 2H_2O$ and provided 472, 3.44, 316, 1.63, 1.15, 1.24, 0.1 and 0.09 g m^{-3} (EC 1.0 mS cm^{-1}), respectively.

NI Treatment

Cymbidium ‘Red Fire’ and ‘Yokihi’ received supplemental light from high-pressure sodium (HPS) lamps (SKL-01, GEO, Hwasung, Republic of Korea) from 22:00 to 02:00 h. The greenhouse was divided into three experimental sections in which potted plants were placed at different distances from the lamps. The control plants received 9 h of ambient light and were then covered with an opaque black

cloth daily from 17:00 to 08:00 h. The low light intensity of NI (LNI) conditions were 9 h of ambient light plus NI at 3-7 μmol m^{-2} s^{-1} and high light intensity of NI (HNI) conditions were 9 h of ambient light plus NI at 120 μmol m^{-2} s^{-1}. The average day/night temperatures during NI treatment were 22/15℃ and 19/12℃ for the first and the second year, respectively. Environmental conditions such as air temperature, relative humidity (RH) and CO_2 concentration were kept the same in all sections of the greenhouse during the experimental period. The first NI treatment (year 1) began immediately after transplanting and lasted for 16 weeks (February to May 2009) and the second NI treatments (year 2) began 38 weeks after transplanting and lasted for another 16 weeks (November 2009 to February 2010).

Summer Cooling Treatment

Cooling treatments were delivered twice during 2 years of the experimental period. The plants were subjected to the summer cooling treatments in the middle of the each NI period, from June to August for 9 weeks each in 2009 and 2010. The 80-m long greenhouse was divided into two identical sections. One of the sections was cooled by a shade screen (shade), while the other was cooled by a mist system (mist). The shade screen was polyester with nominal values of 50% shade. The greenhouse was ventilated through roof and side openings. The greenhouse was equipped with a single continuous roof vent per span 79.5-m long and 4-m wide, located on the north roof side and two side vents. All vents were completely open during the experimental period.

The mist cooling equipment comprised of a high-pressure water vapor

distribution system, consisting of three mist and fan modules, each module with a capacity to cover 100 m^2. Vertical air mixing fans were used to facilitate homogeneous water vapor distribution inside the greenhouse. A circular ring with eight nozzles for the distribution of mist droplets was placed above the fan outlet. Each module had a total capacity of approximately 160 L h^{-1} at a working pressure of approximately 40 bars. Based on previous experimental data showing that greenhouse air temperature levels before 10:00 or after 16:00 h were below the optimal temperature (25 ± 1℃) necessary for the crop, the mist system and shade screen were adjusted to operate continuously between 10:00 h and 16:00 h.

Dry and wet temperature sensors placed close to the plant canopy in each greenhouse section were used to monitor the air temperatures and RH. A Nongjungcyber greenhouse computer with a data logger (NJ2005, Nongjung, Seoul, Korea) for temperature and another data logger (M100, Hanyoung, Seoul, Korea) for RH were connected for the recording and storage of climatic data. The average photosynthetic photon flux (PPF) was measured every 30 min using a Li-Cor 1400 portable sensor (Li-Cor, Co., Inc., Lincoln, NE, USA) from 09:00 to 17:00 h.

Data Collection

The climatic data collected in the summer were averaged over 30 min intervals, covering the period from 05:00 to 21:00 h. Due to similar responses to temperature, RH and PPF in most days, only selected data collected during representative sunny days were presented. The data selected for analysis were recorded on July 5, July 23 and August 13 in 2009 and June 23, July 13 and

August 19 in 2010.

The number of leaves, leaf length, leaf width, number of pseudobulbs and pseudobulb diameter were measured monthly. The longest leaf measured from the base of the pseudobulb was used to represent leaf length. Pseudobulb diameter was measured at the widest point of the flowering pseudobulb using a digital caliper (ABS Digimatic Caliper; Mitutoyo Co., Ltd., Tsukuba, Japan). The time from the start of the treatments to flowering pseudobulb emergence and flowering percentage after the NI and cooling treatments were recorded. The time to visible inflorescence (VI) from the start of the treatments and the number of flowers were also measured. The time at which the first floret was fully open was regarded as the flowering time.

Experimental Design and Statistical Analysis

The NI and cooling treatments were employed twice during the 2 years of experimental period. In year 1 (2009), eight plants per NI treatment group were grown in three replicates. The experimental design was a randomized complete block. Four plants in three replicates from each NI treatment group were moved to the mist condition, while the other four plants in three replicates were placed under the shade condition. In year 2 (2010), all of the plants treated with both the NI and summer cooling were re-exposed to the same NI and summer cooling in year 1. The experiments were conducted on the same plants for 2 years.

Statistical analyses were performed using SAS for Windows V8 (SAS Institute Inc., Cary, NC, USA). Differences in growth and flowering among the treatments were assessed by the Tukey's honestly significant difference test at $P < 0.05$.

Regression and graph module analysis were performed using the Sigma Plot (Systat Software, Inc., Chicago, IL, USA).

RESULTS

Greenhouse Microclimate in the Summer

The average greenhouse air temperature between 10:00 and 16:00 h was 27.6 and 28.6℃ under the mist and 29.9 and 31.3℃ under the shade in 2009 and 2010, respectively. The night air temperature was not controlled, yet naturally remained at 20 ± 2℃. The average day air temperature in the mist-cooled section was 2.3 and 2.7℃ lower than that in the shade section in 2009 and 2010, respectively (Fig. III-1). In the mist-cooled section, the air temperature reached to about 28℃ after 11:00 h, remained at this level until 14:00 h and then started to decrease in 2010 (Fig. III-1B). However, the air temperature reached to a mean maximum value of 35.9℃ at 15:00 h in 2010 in the shade. The average leaf temperature between 10:00 and 16:00 h was 29.2℃ in the mist and 31.8℃ in the shade in 2010 (data not shown). The analysis of variance revealed significant differences between the different cooling treatments. RH was higher in the misting system (80 ± 5%) than in the shade screen (55 ± 5%) in both years (Fig. III-2). The average PPF values in the mist and shade sections during the summer were 305 and 142 μmol m^{-2} s^{-1}, respectively (Fig. III-3). The mean photosynthetic daily light integral (DLI) during the summer period of 2010 was 8.8 and 4.1 mol m^{-2} d^{-1} at plant height in the mist and shade conditions, respectively.

Plant Growth and Flowering

The numbers of leaves and pseudobulbs, leaf length and pseudobulb diameter in ‘Red Fire’ plants grown in the LNI and HNI were greater than those in the

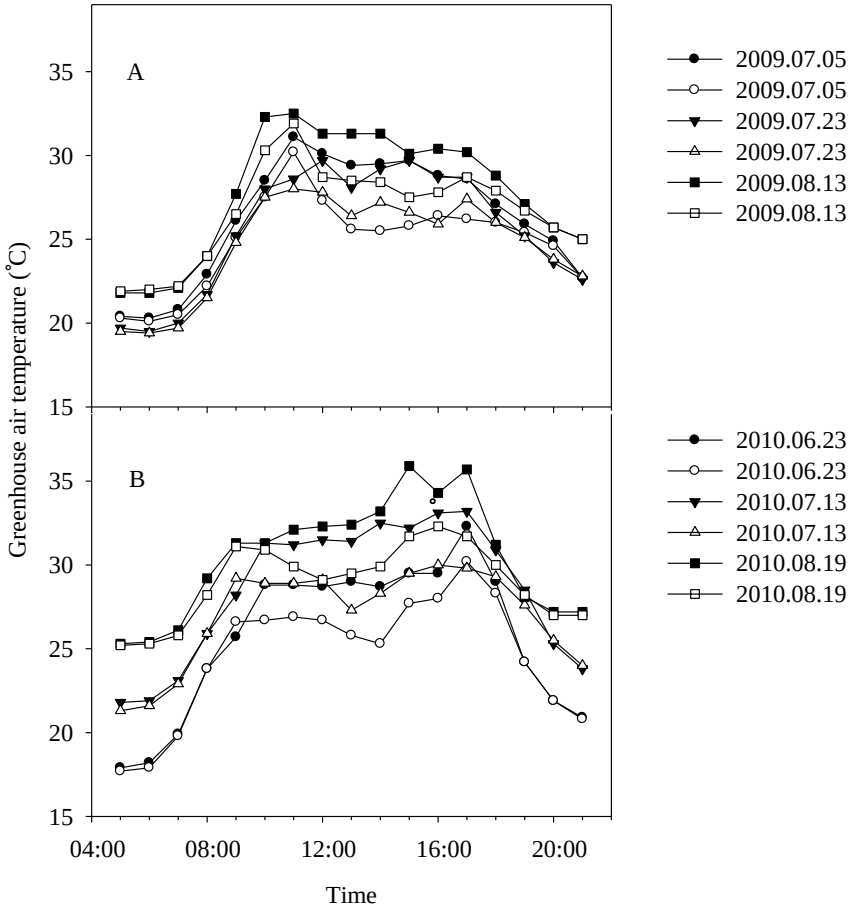

Fig. III-1. Air temperature inside the two greenhouse sections in the summer of 2009 (A) and 2010 (B). open symbol = mist condition; closed symbol = shade condition; A: circle = July 5; triangle = July 23; square = August 13; B: circle = June 23; triangle = July 13; and square = August 19.

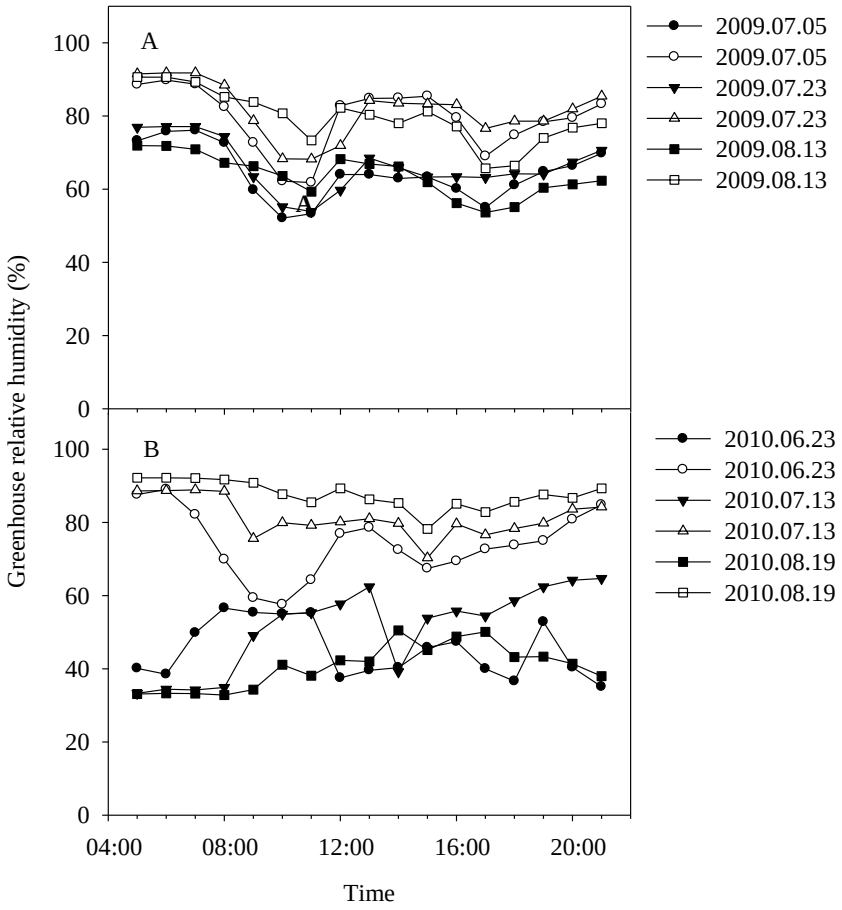

Fig. III-2. Relative humidity inside the two greenhouse sections in the summer of 2009 (A) and 2010 (B). open symbol = mist condition; closed symbol = shade condition; A: circle = July 5; triangle = July 23; square = August 13; B: circle = June 23; triangle = July 13; and square = August 19.

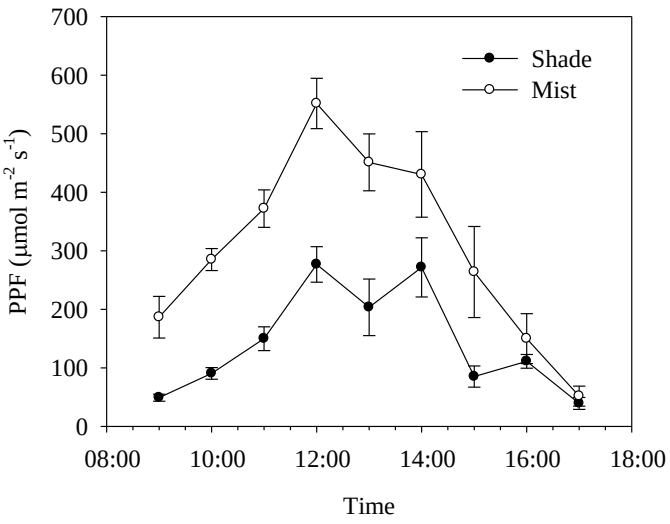

Fig. III-3. Changes in the greenhouse average photosynthetic photon flux (PPF) in the mist and shade condition during 09:00-17:00 h in 2010. open symbol = mist condition and closed symbol = shade condition.

control after the first year NI treatment, regardless of the types of cooling treatment (Table III-1). The leaf length of ‘Red Fire’ grown in the HNI was significantly ($P < 0.001$) longer under the shade (37.3 and 70.0 cm) as compared to the mist (32.9 and 60.8 cm) in years 1 and 2, respectively. The number of pseudobulbs increased in the LNI and HNI conditions after the first year of NI treatment, but increased more significantly after the second year of NI treatment (Table III-1). After year 1, the pseudobulbs were bigger in the plants grown in both LNI and HNI conditions as compared to those grown in the control irrespective of the cooling methods. However, the pseudobulbs were significantly bigger in the plants grown in the NI with mist than those in plants grown in the shade in year 2. In ‘Yokihi’, the pseudobulb diameter was the largest at 5.89 cm with HNI in the mist condition (Table III-2). The leaf length and width of ‘Yokihi’ significantly increased under both LNI and HNI and leaves were longer and wider in the shade than in the mist in both years. Since all of the ‘Yokihi’ plants grown in the HNI with shade died as a result of soft rot disease in August 2010, no more data were collected for this treatment.

The time from the initial planting to flowering pseudobulb emergence in ‘Red Fire’ decreased by more than 16 weeks in the LNI and 25 weeks in the HNI in the mist. However, the promotional effect of reduction of the time to emergence of flowering pseudobulb was significantly higher in the mist (Table III-3). Over 60% of the plants of ‘Yokihi’ and ‘Red Fire’ flowered in the NI in the mist, whereas none of the ‘Red Fire’ flowered in the shade and 20% of the shade-cooled ‘Yokihi’ plants in the LNI flowered (Table III-3). The number of flowers was greater in the plants grown under the HNI compared to those grown under the LNI with the mist.

Table III-1. Effects of cooling system and night interruption (NI) on number of leaves, leaf length, leaf width, number of pseudobulbs and pseudobulb diameter in *Cymbidium* 'Red Fire' after 2 years of treatments.

Cooling system[a]	NI	No. of leaves	Leaf length (cm)	Leaf width (cm)	No. of pseudobulbs	Pseudobulb diameter (cm)
Year 1						
Mist	Control	13.5 ab[b]	28.5 c	1.79 a	1.33 ab	1.81 c
	LNI	14.1 a	33.9 ab	2.05 a	1.67 ab	2.06 ab
	HNI	13.9 a	32.9 abc	2.78 a	1.83 a	2.22 a
Shade	Control	12.3 b	32.4 bc	1.87 a	1.08 b	1.90 bc
	LNI	13.3 ab	35.4 ab	1.99 a	1.33 ab	1.90 b
	HNI	14.0 a	37.3 a	1.95 a	1.50 ab	2.08 ab
Significance						
NI		**[c]	***	NS	*	***
Cooling		*	**	NS	*	NS
Cooling × NI		NS	NS	NS	NS	*
Year 2						
Mist	Control	30.1 a	62.9 bc	2.14 a	2.42 bc	3.21 ab
	LNI	29.0 a	71.0 a	2.42 a	2.67 b	4.86 a
	HNI	30.4 a	60.8 c	2.44 a	4.58 a	4.86 a
Shade	Control	31.5 a	70.5 ab	2.15 a	1.83 c	3.32 b
	LNI	31.7 a	77.3 a	2.38 a	2.41 bc	4.18 ab
	HNI	31.1 a	70.0 c	2.38 a	3.00 b	4.06 b
Significance						
NI		NS	***	**	***	***
Cooling		*	***	NS	***	**
Cooling × NI		NS	NS	NS	**	*

[a]The plants were grown under mist or shade cooling systems for 9 weeks for each year from June to August 2009 and 2010 summer season.

[b]Mean separation within columns by Tukey's honestly significant difference test at $P < 0.05$.

[c]NS: non-significant. [*]Significant at $P < 0.05$. [**]Significant at $P < 0.01$. [***]Significant at $P < 0.001$.

Table III-2. Effects of cooling system and night interruption (NI) on number of leaves, leaf length, leaf width, number of pseudobulbs and pseudobulb diameter in *Cymbidium* 'Yokihi' after 2 years of treatments.

Cooling system[a]	NI	No. of leaves	Leaf length (cm)	Leaf width (cm)	No. of pseudobulbs	Pseudobulb diameter (cm)
Year 1						
Mist	Control	14.6 abc[b]	29.7 c	1.77 b	1.53 ab	1.95 a
	LNI	14.0 c	28.0 c	1.75 b	1.62 ab	1.89 a
	HNI	15.9 a	34.1 b	2.05 a	1.77 a	2.07 a
Shade	Control	15.8 ab	36.2 ab	1.89 ab	1.32 ab	1.95 a
	LNI	15.5 abc	36.7 a	1.84 b	1.32 ab	1.99 a
	HNI	14.2 bc	35.7 ab	1.84 b	1.17 b	1.88 a
Significance						
NI		NS[c]	***	**	NS	NS
Cooling		NS	***	*	**	NS
Cooling × NI		***	***	***	NS	NS
Year 2						
Mist	Control	33.8 ab	65.6 c	2.36 c	2.83 a	4.20 b
	LNI	34.5 ab	71.5 bc	2.41 bc	2.83 a	4.45 b
	HNI	37.8 a	74.3 b	2.75 a	2.83 a	5.89 a
Shade	Control	31.5 b	70.5 bc	2.47 bc	1.83 a	3.32 c
	LNI	37.8 a	82.0 a	2.68 ab	2.08 a	3.83 bc
	HNI	-[d]	-	-	-	-
Significance						
NI		**	***	**	NS	***
Cooling		NS	***	**	NS	***
Cooling × NI		*	NS	NS	NS	NS

[a]The plants were grown under mist or shade cooling systems for 9 weeks for each year from June to August 2009 and 2010 summer season.

[b]Mean separation within columns by Tukey's honestly significant difference test at $P < 0.05$.

[c]NS: non-significant. [*]Significant at $P < 0.05$. [**]Significant at $P < 0.01$. [***]Significant at $P < 0.001$.

[d]The plants showing lower than 30% survival rate were not included in the analysis.

Table III-3. Effects of cooling system and night interruption (NI) on time to emergence of flowering pseudobulbs, visible inflorescence (VI) and flower, number of flowers and flower percentage in *Cymbidium* 'Red Fire' and 'Yokihi' after 2 years of treatments.

Cooling system[a]	NI	Time to emergence of flowering pseudobulb (weeks)	Time to VI (weeks)	Time to flower	No.of flowers /pot	Flowering percentage
'Red Fire'						
Mist	Control	51.0 a[b]	-[c]	-	-	0
	LNI	34.3 bc	82.3 a	103.9 a	9.6 a	60
	HNI	26.0 c	76.3 b	100.5 a	14.8 a	80
Shade	Control	47.1 a	-	-	-	0
	LNI	46.4 a	-	-	-	0
	HNI	43.4 ab	-	-	-	0
Significance						
NI		***[d]	***	NS	NS	
Cooling		***	-	-	-	
Cooling × NI		**	-	-	-	
'Yokihi'						
Mist	Control	45.0 a	-	-	-	0
	LNI	30.6 b	81.1 a	106.2 a	18.8 a	60
	HNI	27.9 b	78.2 a	102.5 a	30.0 a	100
Shade	Control	46.6 a	-	-	-	0
	LNI	40.4 a	-	-	-	20
	HNI	37.4 ab	-	-	-	0
Significance						
NI		***	NS	NS	NS	
Cooling		***	-	-	-	
Cooling × NI		NS	-	-	-	

[a]The plants were grown in the mist and shade for 9 weeks in each year from June to August in year 2009 and 2010.

[b]Mean separation within columns by Tukey's honestly significant difference test at $P < 0.05$.

[c]Plants did not flower within 2 years after the start of the treatments. The plants with showing lower than 30% flowering were not included in the analysis.

[d]NS: non-significant. [*]Significant at $P < 0.05$. [**]Significant at $P < 0.01$. [***]Significant at $P < 0.001$.

The number of flowers was significantly ($P < 0.01$) higher in ‘Yokihi’ than in ‘Red Fire’ under both LNI and HNI conditions. *Cymbidium* grown at a day temperature of 28℃ or higher in the shade condition exhibited leaf necrosis and inflorescences abortion in both cultivars. In addition, the mature ‘Yokihi’ plants grown under HNI died due to soft rot in early August 2010 (Table III-3).

DISCUSSION

The average day air temperature in the mist-cooled section was 2.5℃ lower than that in the shade section (Fig. III-1), while the RH was 25% higher in the mist section than in the shade section (Fig. III-2) in this experiment. Evaporative cooling systems not only decrease air temperature also increase absolute humidity in the greenhouse (Arbel et al., 1999; Katsoulas et al., 2001). The advantage of the mist is the establishment of uniform temperature and humidity conditions throughout the greenhouse (Perdigones et al., 2008). In *Dendrobidum nobile*, the plants had the greatest numbers of inflorescences and flowers when they were grown in wet soil (Kosugi et al., 1971). Withner (1974) reported that mist cooling is effective due to both the cool initial temperature of the water and its evaporative effects on the leaf surfaces in *Cymbidium*.

Ventilation in the shaded condition decreased greenhouse PPF and DLI, approximately by 48% as compared to the mist condition in this study during the daytime (Fig. III-3). Natural ventilation with shade screen is usually the easiest cooling method due to its low cost and simplicity, but it is generally not sufficient for extracting excess energy during sunny summer days (Sethi and Sharma, 2007).

The plants grown in the mist had larger pseudobulb size than those grown in the shade in both NI treatments (Tables III-1 and III-2). Ichihashi (1997) reported that flower initiation in *Dendrobium nobile* occurred on mature pseudobulbs only when they were exposed to temperatures of 7.5-20℃. In *Miltoniopsis* Augres ‘Trinity’, 90% of the pseudobulbs initiated inflorescences when the pseudobulb diameter was 3.1 cm or greater (Lopez and Runkle, 2006) and a temperature

maintained at 17℃ yielded the greatest increase in pseudobulb diameter in *Odontioda* (Kubota et al., 2005).

No plants flowered when they grown in the shade condition during the summer even though they were grown in the NI condition (Table III-3). Although the parents of ‘Red Fire’ and ‘Yokihi’ have not been reported, these cultivars required relative high temperatures for flower initiation as compared to other *Cymbidium* species. Inflorescence initiation in summer and flower development before winter also occurred in *Cymbidium* ‘Shiratama-Nishiki’ (Kosugi et al., 1971). Therefore, prolonging the photoperiods in winter promoted vegetative growth, but lowering the temperature in summer required inflorescence initiation in *Cymbidium* ‘Red Fire’ and ‘Yokihi’. In general, flowering occurs only after the termination of vegetative growth in pseudobulbs and is regulated by temperature thereafter as opposed to photoperiod in many orchid species (Chae, 2002; Ng and Hew, 2000). *Miltoniopsis* ‘Trinity’ had the highest flowering percentage (> 90%) when they grown under a 9 h photoperiod at 20℃ for 4-8 weeks and subsequently transferred to 14℃ for 8 weeks (Lopez and Runkle, 2006). *Odontioda* orchids should be grown at temperatures of < 26℃ to prevent heat stress and inflorescence abortion (Blanchard and Runkle, 2008). *Epidendrum, Phalaenopsis* and *Dendrobium* required relative or absolute low temperatures for flower initiation (Chae, 2002).

Cymbidium ‘Yokihi’ grown under HNI died due to soft rot in the second year of the treatment (Table IV-3). In *Odontioda* ‘Velano’ grown at 28/18°C (12 h day/12 h night) developed yellow leaves within 8 weeks and died within 24 weeks (Kubota et al., 2005). A similar response to high temperature has been reported in

Zygopetalum ‘Fire Kiss’, in which the flower buds on plants grown at temperatures greater than 25℃ developed necrotic lesions and aborted within 20 days (Lopez and Runkle, 2004). Although plants can tolerate high temperatures (> 27℃), such conditions favor soft rot infection (Wright and Triggs, 2009). Klement (1990) stated that high temperatures (> 30℃) can deplete O_2 stored in plant tissues due to respiration. Control (without NI) plants did not flower within 2 years under either the mist or shade conditions, because they were still in a juvenile stage.

In conclusions, NI promoted vegetative growth with both the mist and shade systems during the early growth stage, but flower initiation occurred within 2 years only when the plants were grown in the NI with the mist system. These results indicate that lowering the temperature by approximately 2℃ and raising RH by the mist system could be important in encouraging flower initiation during summer season in *Cymbidium*, as opposed to moving the plants from a low to high elevation areas. *Cymbidium* ‘Red Fire’ and ‘Yokihi’ should be grown at temperatures less than 27℃ in summer, where pseudobulbs will attain the minimum size required for uniform flowering. Therefore, NI during the short-day season and mist during summer season are recommended to promote the growth and flowering of *Cymbidium* ‘Red Fire’ and ‘Yokihi’. Our data suggest that commercial use of NI for *Cymbidium* forcing culture will decrease crop time within 2 years and increase profits when mist system was employed.

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CHAPTER IV

Photosynthetic Characteristics of *Cymbidium* 'Red Fire' and 'Yokihi' in Response to Night Interruption and Nitrogen Nutrition

ABSTRACT

Cymbidium has a long vegetative growth period, thus shortening of total cultivation duration is desired for commercial cultivation. Flowering of *Cymbidium* can be promoted by night interruption (NI). In this study, photosynthetic characteristics of *Cymbidium* 'Red Fire' and 'Yokihi' were determined in relation to leaf nitrogen (N) content when the plants were exposed to NI (22:00 to 02:00 h) for forcing cultures. The plants exposed to 9 h ambient light (control), ambient light plus a NI at 3-7 $\mu mol\, m^{-2}\, s^{-1}$ (LNI) and ambient light plus a NI at 120 $\mu mol\, m^{-2}\, s^{-1}$ (HNI). Net CO_2 assimilation (A_n) in both LNI and HNI increased during NI, more with HNI. However, midday depression in A_n after 2 years of NI treatments, which was attributed to N deficiency as represented by leaf chlorosis, appeared on the plants in the HNI with low chlorophyll contents in leaves. F_v/F_m ratio remained constant in leaves grown in the control and LNI during the daytime, but leaves exposed to the HNI exhibited a decline in F_v/F_m. No photoinhibition was observed in the plants in the control and LNI conditions. *Cymbidium* 'Red Fire' was supplied with different N concentrations, 0, 100, 200 or 400 mg L^{-1}, during the LNI and HNI cultures. In the HNI group, the leaves of

N 0 had significantly lower A_n than other N treatments during the daytime, while no significant difference was showed among N 100, 200 and 400 treatments. However, no statistically significant difference was observed in A_n between the N treatments during the day and night in the LNI plants. It is assumed that photoinhibition could occur when the HNI is applied to *Cymbidium* without supplemental N. Additional N fertilization should be followed when the HNI is introduced in *Cymbidium* forcing culture to enhance photosynthesis.

Keywords: nitrogen deficiency, photosynthesis, photoinhibition

INTRODUCTION

Cymbidium are marketed globally as cut or potted flowering plants. However, growth is rather slow and it takes several years to flower. The shortening of total period of cultivation is of demand to produce commercial cultivars economically.

Daylength regulates growth and flowering in photoperiodic plants. Different species of orchids exhibit sensitivity to daylength in various ways. Long-day (LD) condition promoted *Cymbidium* growth (Hew and Yong, 2004), yet small changes in daylength are critical for the growth and flowering of *Cattleya, Dendrobium* and *Phalaenopsis* (Bhattacharjee, 1979). Flowering in orchids is also promoted when the plants are exposed to short-day (SD). In *Dendrobium pulcherrima*, spikes of 2-3 cm were initiated under 9 h SD conditions for 30 days with day/night temperature of 30/20℃ and spikes grew to 7-10 cm under SD conditions for 45 days (Wang et al., 2002).

Photosynthesis is decreased by strong solar radiation or long light exposure duration, so that shade might benefit production in some orchids (He et al., 1998; Pan et al., 1997; Powles, 1984). *Cymbidium* is a shade plant and the photosynthetic rate of *C. sinense* is low and ranges between 2.0 and 2.6 μmol CO_2 m^{-2} s^{-1} which is about 1/5 that of most C_3 plants (Pan et al., 1997). Light intensity is important because of photoinhibition (PI) (Mohotti and Lawlor, 2002). The PI decreases the capacity for net photosynthetic rate per unit leaf area (A_n) in orchid plants including *Dendrobium* and *Oncidium* (He et al., 1998) and A_n responds similarly to radiation and shade. In general, the photosynthetic capacity of *Cymbidium* is greater for leaves grown in the shade than those grown in unshaded

condition (Pan et al., 1997).

The photosynthetic capacity of leaves depends on the characteristics and amounts of the components of the photosynthetic machinery and the availability of nutrients. Nitrogen (N) is particularly important, as it is required for the synthesis of cellular components, including chlorophyll (Chl), thus related to capture of photosynthetically active radiation and ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) (Lawlor, 2001). Nitrogen deficiency decreases both source and sink capacity, by decreasing the formation of photosynthetic components and thus A_n and shortening the productive life-span of leaves. Also, the number and size of organs are decreased, limiting sink capacity for the utilization of assimilates so that carbohydrates accumulate and may led to feedback inhibition of A_n and PI (Baker and Bowyer, 1994; Melis, 1999).

Flowering was promoted in *Cymbidium* ‘Red Fire’ under a night interruption (NI), as a method to breaks a long dark period to deliver photoperiodic lighting condition (Kim et al., 2011b). In the previous study, however, *Cymbidium* ‘Red Fire’ and ‘Yokihi’ under NI forcing culture were required additional N fertilization to maintain leaf N contents. Without the additional N fertilization, the leaf of the plants grown under NI condition turned yellow and had 20% less N than those of the plant grown under no NI condition. Little is known of the relationship among photoperiod, nitrogen supply and photosynthetic capacity for *Cymbidium*. In this study, therefore, the photosynthetic characteristics and the effects of N fertilization during NI forcing culture were determined in *Cymbidium* ‘Red Fire’ and ‘Yokihi’.

MATERIALS AND METHODS

Photosynthetic Characteristics of *Cymbidium* 'Red Fire' and 'Yokihi' during NI Forcing Culture (Experiment 1)

Plant materials and growth conditions. *Cymbidium* hybrids 'Red Fire' and 'Yokihi' (Mukoyama Orchids Co., Ltd., Yamanashiken, Japan) were transplanted into 10 cm pots containing 100% chopped coconut and then re-transplanted into 16 cm pots after four months of culture. The plants were grown in a commercial greenhouse of Sang-Il Orchid Farm, Hwasung, Republic of Korea. The plants were irrigated daily with tap water. Five grams of water-soluble controlled release 13N-5.7P-10.8K (Mukoyama Orchids Co., Ltd.) were placed on the top of each pot. The controlled release fertilizer was applied different times: at transplanting, the first pseudobulb emergence and the second pseudobulb emergence. Micronutrient fertilizers were applied bimonthly to the plants using a sprinkler. The supplemental fertilizers were composed of $Ca(NO_3)_2 \cdot 4H_2O$, Fe-EDTA, $MgSO_4 \cdot 7H_2O$, $MnSO_4$, $ZnSO_4 \cdot 7H_2O$, H_3BO_3, $CuSO_4 \cdot 5H_2O$ and $Na_2MoO_4 \cdot 2H_2O$, and they provided 472, 3.44, 316, 1.63, 1.15, 1.24, 0.1 and 0.09 g m^{-3} (EC 1.0 dS m^{-1}), respectively. Average day/night temperatures for the first year (2009) and the second year (2010) of the experiments, respectively, were 27/22 and 28/24℃ in summer and 21/12 and 22/13℃ in winter. Average photosynthetic photon flux PPF) for the first and second year of the experiments, respectively, was 563 and 500 μmol m^{-2} s^{-1} in summer and 229 and 215 μmol m^{-2} s^{-1} in winter.

Light treatment. The plants were irradiated daily with high-pressure sodium (HPS) lamps (SKL-01, GEO, Hwasung, Republic of Korea) from 22:00-02:00 h.

The greenhouse was divided into three groups for experiments; 1) control (9 h ambient light; plants were covered with opaque black cloth daily from 17:00 to 08:00 h), 2) LNI (9 h ambient light plus a 'low light intensity NI' where PPF at the top of the plants was maintained at 3-7 μmol m^{-2} s^{-1}) and 3) HNI (9 h ambient light plus a 'high light intensity NI' where PPF at the top of the plants was set at 120 μmol m^{-2} s^{-1}). Each treatment was applied to the *Cymbidium* 'Red Fire' and 'Yokihi' twice during total experimental period. The first treatment began right after transplanting (weeks 1-16) and the second treatment began from 38 weeks after transplanting (weeks 38-54).

Measurements. Gas exchanges were measured with three replicated plants at 48 weeks after transplanting in January 2010 (during the second NI treatment) using a portable photosynthesis system (Li 6400, Li-Cor Co., Inc., Lincoln, NE, USA) equipped with an infrared gas analyzer. Fourth mature leaf from the base of the flowering pseudobulb was clamped on to a 6 cm^2 top clear chamber. The light intensity illuminated in the daytime ranged between 400 and 600 μmol m^{-2} s^{-1} PPF and block temperature was kept at 20 and 15°C during the day and night, respectively. Relative humidity in the leaf chamber ranged between 55 and 75%. The CO_2 concentration in the greenhouse was approximately 400 and 800 μmol mol^{-1} during the day and night, respectively, and the same amount of CO_2 was supplied to the leaf chamber during the measurement. Net CO_2 assimilation rate (A_n), stomatal conductance (g_s) and transpiration rate (E) were recorded simultaneously during the measurement. Each measurement was taken at every hour for 5 min by using a built-in autoprogram. Measurements were taken at irradiance levels of 1,000, 800, 600, 400, 300, 200, 100, 50 and 0 μmol m^{-2} s^{-1}

PPF for A_n in response to light intensity. This decreasing irradiance through each step enabled a rapid stabilization of gas exchange. A minimum and maximum wait time for each step were set to be 8 and 10 min, respectively. The light-response curves were fitted into the exponential model; $y = a + be^{cx}$ (Constable and Rawson, 1980; Iqbal et al., 1996) to estimate several parameters.

Chl a fluorescence was measured by using a portable pulse amplitude modulation fluorometer (PAM-2000, Walz, Effeltrich, Germany). After 30 min dark adaption, a measuring light of 0.6 kHz and less than 0.1 μmol m^{-2} s^{-1} PPF was irradiated to obtain minimum fluorescence in dark-adapted state (F_o) and then a saturating light pulse at about 8,000 μmol m^{-2} s^{-1} PPF was irradiated for 0.8 s to induce maximum fluorescence in dark-adapted stated (F_m). After the first saturating light pulse, an actinic light intensity of 200 μmol mol^{-1}, a light-saturated PPF of *Cymbidium* hybrids, was irradiated to gain maximum (F_m') and minimum fluorescence (F_o') in light-adapted state.

During actinic light adaptation, the saturating light pulse was irradiated 20 times with a 20 s interval. Then, F_m', F_o', and fluorescence at steady state (F_s) at the 20 th saturating light pulse were recorded, regarding it as a steady state of fluorescence. With these fluorescence parameters, photosystem II (PSII) activities were estimated. Potential quantum yields in the dark- and light-adapted states were estimated from $(F_m–F_o)/F_m = F_v/F_m$ and $(F_m'–F_o')/F_m' = F_v'/F_m'$, respectively, representing the efficiency of energy captured by open PSII (Genty et al., 1989). Quenching due to non-photochemical dissipation of absorbed photon energy (q_N) was calculated at the end of each irradiation period, according to the equation $q_N = (F_m – F_m')/(F_m – F_0')$. The coefficient for photochemical quenching,

q_P, represents the fraction of open PSII reaction center and was calculated as (F_m' – F_s)/(F_m' – F_0'). Actual quantum yield of PSII (Φ_{PSII}), described as the fraction of absorbed light utilized through photochemistry, was estimated from (F_m'–F_s)/F_m' (Genty et al., 1989). Electron transport rate (ETR) was derived from Φ_{PSII} × 0.5 × PPF of actinic light × 0.84.

The SPAD value of the fourth expanded mature leaf from the base of the flowering pseudobulb was measured with a SPAD 502 in seven replicated plants (Minolta Camera Co. Ltd., Osaka, Japan). Leaf nutrient content was analyzed in leaves at week 57 (after the second NI treatment). A standard micro-Kjeldahl procedures was used to determine total N content (Nelson and Sommers, 1972) and inductively coupled plasma spectroscope (ICPS-7510, Shimadzu Co., Kyoto, Japan**)** was used to analyze phosphorus (P) and potassium (K) content (Lim, 2000).

Effect of NI with Different N Fertilization Levels on Photosynthetic Characteristics of *Cymbidium* 'Red Fire' (Experiment 2)

Plant materials and growth conditions. *Cymbidium* 'Red Fire' (Mukoyama Orchids Co., Ltd.) were transplanted at the mericlonal stage into 10 cm pots and then re-transplanted into 16 cm pots after 4 months of culture. The pots contained 100% chopped coconut. The plants were grown in a greenhouse at the experimental farm of the College of Agriculture and Life Sciences, Seoul National University in Suwon, Republic of Korea. The average day/night air temperatures of the greenhouse was 25/18℃ and the average PPF was 561 μmol m^{-2} s^{-1} during the day experimental period. The plants were irrigated daily with tap water. Five

grams of water-soluble controlled release 13N-5.7P-10.8K fertilizers (Mukoyama Orchids Co., Ltd.) were placed at the top of each pot. The controlled release fertilizer was applied at the different stages; transplanting, the first pseudobulb emergence and the second pseudobulb emergence.

Light treatment. The NI was applied to the plants 1 year after transplanting for *Cymbidium* 'Red Fire'. The experiment was laid out in a randomized complete block design with three replications. There were seven plants in each replication. The environmental conditions such as air temperature, relative humidity and CO_2 concentration during the experiment were maintained the same among sections of a greenhouse. Atmospheric CO_2 concentration in a greenhouse was maintained at 800 μmol mol^{-1} during the NI treatment to maximize NI effect.

Nitrogen treatment. During the NI treatments, the plants were treated with four levels of N, 0, 100, 200 or 400 mg L^{-1}. The treatments consisted of control 0, 100, 200, 400, LN 0, LN 100, LN 200, LN 400, HN 0, HN 100, HN 200 or HN 400. NH_4NO_3 (2:8 ratio) 0, 0.28, 0.56 or 1.12 g L^{-1} was used to add nutrients to the fertilizer solutions. 100 K, 100 P, 100 Ca and 50 Mg (mg L^{-1}) were held constant. Plants were given 300-350 mL fertilizer solutions per day by a drip irrigation system. The average electrical conductivity (EC) values for the solutions ranged from 0.7, 0.9, 1.3 and 1.7 dS m^{-1}, depending on the N level and the pH of all solutions was adjusted to 6.1 ± 0.1. The N treatments began 1 year after transplanting and lasted for 16 weeks (November 2011 to February 2011).

Measurements. Gas exchanges were measured in January 2011 (during NI treatment) using a portable photosynthesis system (Li 6400, Li-Cor Co., Inc.) equipped with an infrared gas analyzer. Plants of each NI treatment were grown

under NI with different N levels, N 0, 100, 200 and 400 mg L^{-1}. Fourth leaf from the base of the flowering pseudobulb was clamped on to a 6 cm^2 top clear chamber. The light intensity illuminated in the daytime ranged between 400 and 600 μmol m^{-2} s^{-1} and block temperature was kept at 20 and 15°C during the day and the night, respectively. Relative humidity in the leaf chamber ranged between 55 and 75%. The CO_2 concentration in the greenhouse was approximately 400 and 800 μmol mol^{-1} during the day and night, respectively, and the same amount of CO_2 was supplied to the leaf chamber during the measurement. The number of leaves, leaf length, leaf width, pseudobulb diameter and the SPAD value were measured monthly. The longest leaf measured from the base of the pseudobulb was used to represent leaf length. Pseudobulb diameter was measured at the widest point of the flowering pseudobulb using a digital caliper (ABS Digimatic Caliper; Mitutoyo Co., Ltd., Tsukuba, Japan). The SPAD value of the fourth expanded mature leaf from the base of the flowering pseudobulb was measured with a SPAD 502 (Minolta Camera Co. Ltd., Osaka, Japan) in seven plants per each replication.

Statistical Analysis

Statistical analyses were performed using the SAS system for window V8 (SAS Institute Inc., Cary, NC, USA). Differences among the treatments means were assessed by Tukey's honestly significant difference test at $P < 0.05$. Regression and graph modules analysis was made by Sigma Plot (SPSS, Inc., Chicago, IL, USA).

RESULTS

Experiment 1

Photosynthetic CO_2 assimilation. Net CO_2 assimilation rates (A_n) in response to PPF of 1-year-old *Cymbidium* were 6.11 μmol $CO_2\,m^{-2}\,s^{-1}$ at 700 μmol $m^{-2}\,s^{-1}$ PPF and 4.9 μmol $CO_2\,m^{-2}\,s^{-1}$ at 500 μmol $m^{-2}\,s^{-1}$ PPF in 'Red Fire' and 'Yokihi', respectively, and a light compensation point was at about 13-25 μmol $m^{-2}\,s^{-1}$ in both cultivars (Fig. IV-1). Leaf photosynthesis in *Cymbidium* 'Red Fire' and 'Yokihi' grown in the NI was monitored for 24 h at the vegetative growth stage (Fig. IV-2). Net CO_2 assimilation rate, transpiration rate and stomatal conductance in the daytime were higher in the control and LNI than in the HNI treatment during the second NI treatments (Figs. IV-2 and IV-3). The means of A_n during NI in the control leaves was approximately -0.29 and -0.18 μmol $CO_2\,m^{-2}\,s^{-1}$ in 'Red Fire' and 'Yokihi', respectively. The maximum A_n during NI was 1.99 μmol $CO_2\,m^{-2}\,s^{-1}$ in 'Red Fire' and 3.81 μmol $CO_2\,m^{-2}\,s^{-1}$ in 'Yokihi' at 01:00 h in HNI. In leaves of the plants exposed to the HNI condition, a marked decrease in the photosynthetic rate per unit leaf area was observed in the daytime in both cultivars. In the plants exposed to the LNI, the means of A_n was 0.28 and 0.61 μmol $CO_2\,m^{-2}\,s^{-1}$ in 'Red Fire' and 'Yokihi' during NI, respectively. The plants which received HNI photosynthesized only 45.4% as compared to the control in 'Red Fire', whereas the plants photosynthesized 77.4% as compared to the control in 'Yokihi'. Total net photosynthetic rate of the plants in the HNI was 56.3% of the control plants, whereas similar photosynthetic rate of the control plants was shown in the LNI in 'Red Fire'. The 'Yokihi' plants photosynthesized more in the LNI and

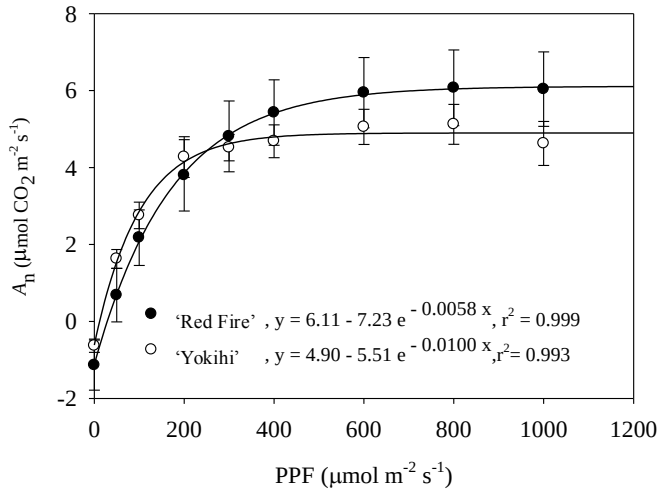

Fig. IV-1. Net CO_2 assimilation rate (A_n) in response to incident PPF in 1-year-old 'Red Fire' and 'Yokihi' leaves when CO_2 concentration was at 600 μmol CO_2 mol^{-1} air.

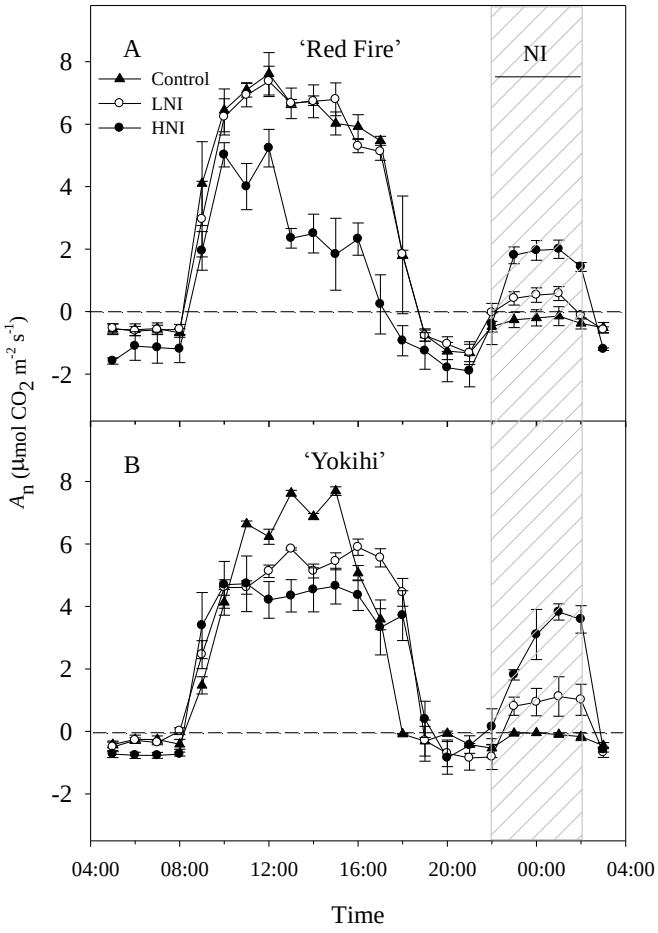

Fig. IV-2. Diurnal changes in photosynthetic rate (A_n) of *Cymbidium* 'Red Fire' (A) and 'Yokihi' (B) grown under NI at 3-7 μmol m^{-2} s^{-1} (LNI) or at 120 μmol m^{-2} s^{-1} (HNI) with high-pressure sodium (HPS) lamps. Control plants were grown under an uninterrupted 15 h skotoperiod. Measurements were taken 48 weeks after transplanting (during the second NI treatment). Vertical bars are means ± S.E. (n=3).

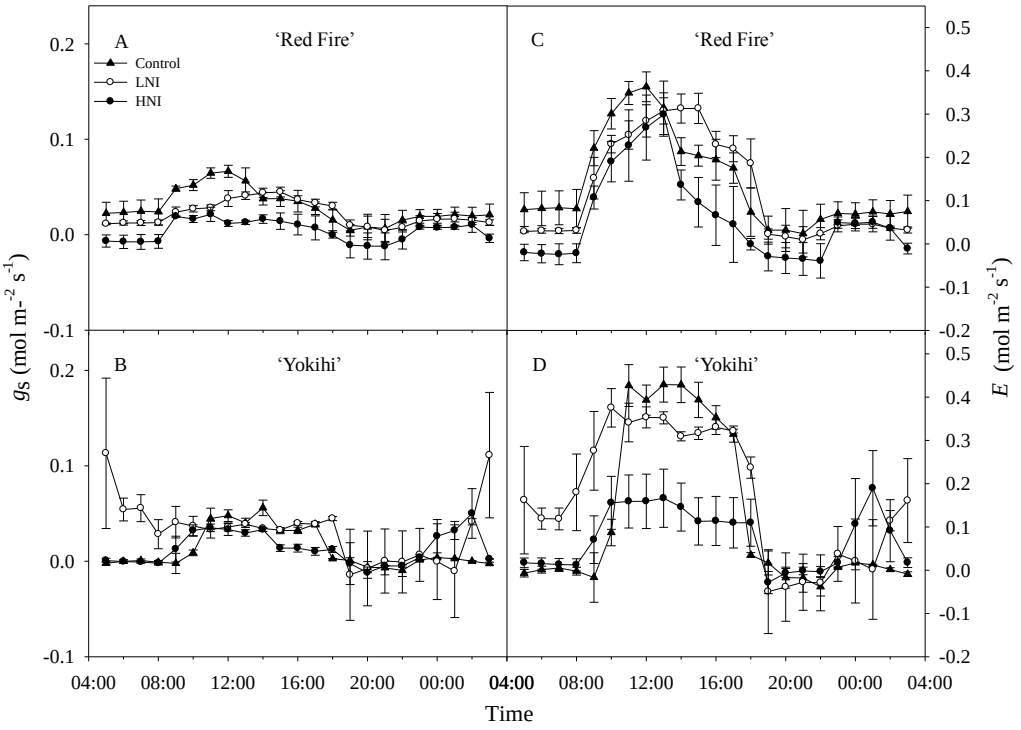

Fig. IV-3. Diurnal changes in stomatal conductance (*g*s) and transpiration (*E*) of *Cymbidium* 'Red Fire' (A, C) and 'Yokihi' (B, D) grown under NI at 3-7 μmol m^{-2} s^{-1} (LNI) or at 120 μmol m^{-2} s^{-1} (HNI) with high-pressure sodium (HPS) lamps. Control plants were grown under an uninterrupted 15 h skotoperiod. Measurements were taken 48 weeks after transplanting (during the second NI treatment) for Exp.1. Vertical bars are means ± S.E. (n=3).

HNI than in the control condition. Stomatal conductance and leaf transpiration decreased strongly in leaves exposed to the HNI condition during daytime, while no changes were observed in the control and LNI conditions.

Chl a fluorescence measurements. The F_v/F_m ratio remained very constant in leaves grown in the NI in the daytime in 'Yokihi'. Leaves exposed to the HNI condition, however, exhibited a decline in a F_v/F_m ratio in the daytime in 'Red Fire' (Fig. IV-4). The proportion of open PS II centers (PQ) decreased and excess light energy was dissipated through q_N in the plants under the HNI. Non-photochemical quenching increased in the plants grown under the HNI in 'Yokihi'. The HNI led to a decrease in yield in both cultivars (Fig. IV-4). The ETR responses to PPF followed similar exponential increases with increased in PPF for all treatments (Fig. IV-5). The differences among the plants grown under the control, LNI and HLI conditions in leaf ETR increased as PPF increased. When PPF was 500 μmol m^{-2} s^{-1}, the plants grown under the HNI had a 15.1, a 27.6% lower ETR as compared to the control plants of 'Red Fire' and 'Yokihi', respectively, and 29.4, 22.3% lower than in the LNI in 'Red Fire' and 'Yokihi', respectively.

Chl content. Chl content decreased in leaves under the HNI condition in both cultivars and increased in leaves under the LNI in 'Red Fire' as compared to control plants. In the LNI leaves, SPAD value remained at approximately 76 and 68, but it dropped to about 44 and 50 in the HNI leaves of 'Red Fire' and 'Yokihi', respectively (Fig. IV-6). Leaves of the plants grown under HNI condition gradually turned yellow after the second NI treatment (Fig. IV-7). These findings are consistent with a leaf yellowing index in both cultivars and a significant

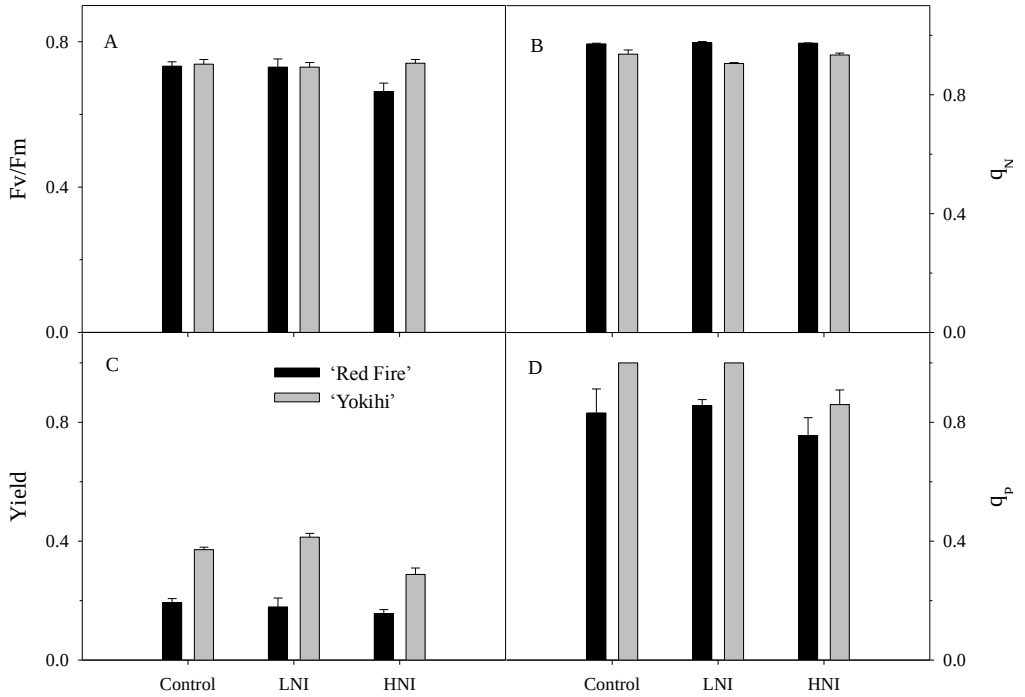

Fig. IV-4. The F_v/F_m ratio (A), quenching due to non-photochemical dissipation of absorbed photon energy (q_N) (B), quantum yields (C) and coefficient for photochemical quenching (q_P) (D) in *Cymbidium* 'Red Fire' and 'Yokihi' grown under NI at 3-7 μmol m^{-2} s^{-1} (LNI) or at 120 μmol m^{-2} s^{-1} (HNI) with high-pressure sodium (HPS) lamps. Control plants were grown under an uninterrupted 9 h photoperiod. Measurements were taken 48 weeks (during the second NI treatments) after transplanting for Expt. 1. Vertical bars are means ± S.E. (n=3).

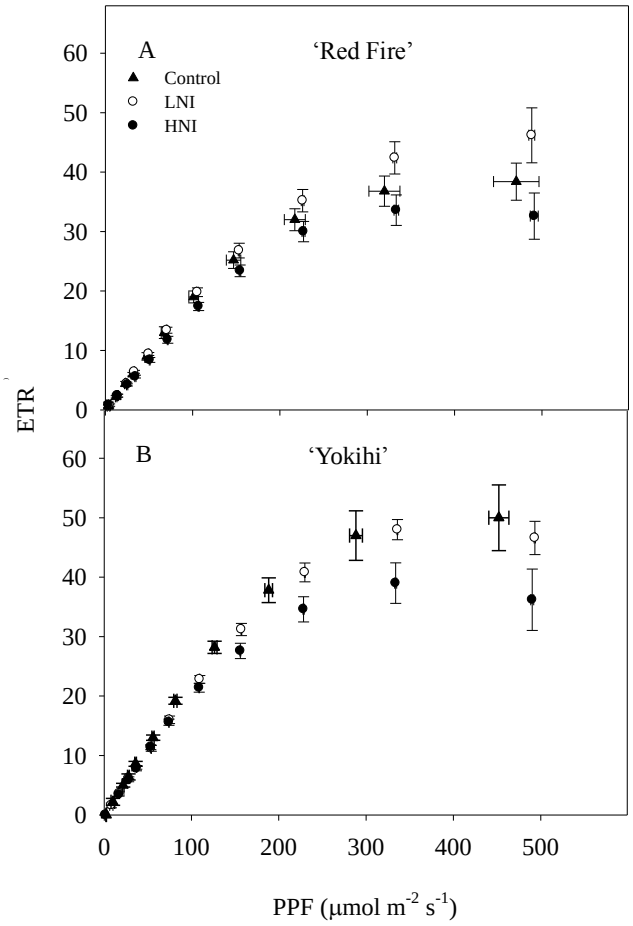

Fig. IV-5. Electron transport rate (ETR) responses to PPF of *Cymbidium* 'Red Fire' (A) and 'Yokihi' (B) grown under NI at 3-7 μmol m^{-2} s^{-1} (LNI) or at 120 μmol m^{-2} s^{-1} (HNI) with high-pressure sodium (HPS) lamps. Control plants were grown under an uninterrupted 15 h skotoperiod. Measurements were taken 48 weeks after transplanting (during the second NI treatments) for Expt. 1. Vertical bars are means ± S.E. (n=3).

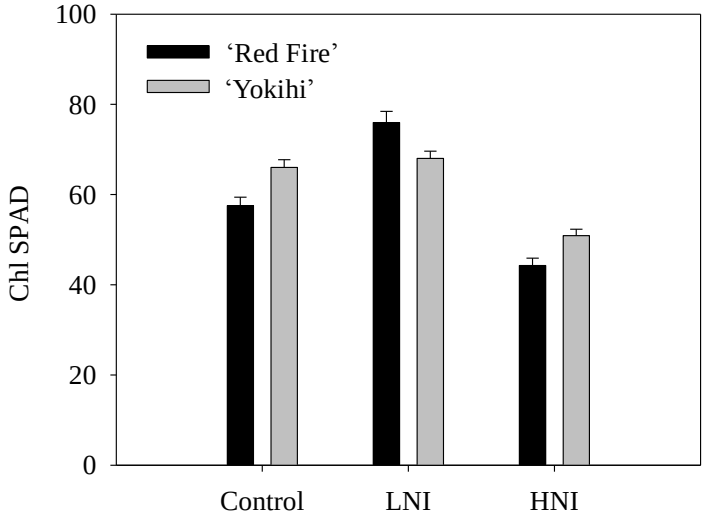

Fig. IV-6. Chlorophyll (Chl) SPAD readings in *Cymbidium* ‘Red Fire’ and ‘Yokihi’ grown under NI at 3-7 μmol m^{-2} s^{-1} (LNI) or at 120 μmol m^{-2} s^{-1} (HNI) with high-pressure sodium (HPS) lamps. Control plants were grown under an uninterrupted 15 h skotoperiod. Measurements were taken 48 weeks after transplanting (during the second NI treatments) in Expt. 1.

Fig. IV-7. Effects of night interruption (NI) on leaf appearance of *Cymbidium* 'Red Fire' and 'Yokihi'. Control plants were maintained under a short-day condition using opaque cloth (A). The plants were grown under NI at 3-7 μmol m^{-2} s^{-1} (LNI) (B) or at 120 μmol m^{-2} s^{-1} (HNI) (C) with high-pressure sodium (HPS) lamps. Photographs were taken 55 weeks after transplanting (after the second NI treatment) in Expt. 1.

difference ($P < 0.05$) between SPAD value and net photosynthetic rate was observed.

Leaf N content. Total N content per unit dry matter (% N) of leaves in the LNI and control conditions were higher in N content than those in the HNI condition in 'Yokihi' (Table IV-1). Those in the HNI decreased in 'Red Fire' although there was no significant difference. No significant difference ($P < 0.05$) was shown in phosphorus and potassium content in all three treatments. A decline in leaf N content in the HNI was more pronounced in 'Red Fire' than in 'Yokihi' as similarly shown in A_n and F_v/F_m.

Experiment 2

The F_v/F_m ratio at midday was significantly lower in the HNI leaves than in the LNI and control conditions at N 0 treatment (data not shown). The F_v/F_m was higher with abundant N treatment than with low N treatment. The average *A*n of leaves for the different treatments increased from zero at dawn to a maximum between 11:00 and 13:00 h (Fig. IV-8). In the LNI plants, no statistically significant difference was observed in A_n between the day and night time in the N treatments. A_n tended to be slightly higher in N 100 and N 400 than in N 0 and N 200 during the day in the control plants. Leaves of N 0 treatment in the plants grown under the HNI had significantly lower A_n than in other N treatments during the day. This was consistent with the A_n characteristics in Experiment 1, where the HNI leaves with N 0 treatment had lower rate than the LNI and control leaves throughout the day. The decrease in midday A_n of the plants grown with N 0 in the HNI group after 2 years of NI treatment was statistically significant as

Table IV-1. Effects of night interruption (NI) lighting on leaf nitrogen (N), phosphorus (P) and potassium (K) contents in *Cymbidium* 'Red Fire' and 'Yokihi'. Plants were grown under NI at 3-7 μmol m^{-2} s^{-1} (LNI) or at 120 μmol m^{-2} s^{-1} (HNI) with high-pressure sodium (HPS) lamps. The control plants were grown under an uninterrupted 15 h skotoperiod.

Cultivar	Light	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})
'Red Fire'	Control	19.2b[a]	1.7a	11.8a
	LNI	17.2b	2.2a	8.5a
	HNI	14.6b	1.7a	9.3a
'Yokihi'	Control	20.0b	2.0a	8.7a
	LNI	26.7a	2.1a	8.3a
	HNI	18.5b	2.1a	8.5a
Significance				
Cultivar		**	NS	NS
Light		***	NS	*
Cultivar × Light		**	NS	NS

[a] Mean separation within colums by Tukey's honestly significant difference test at $P < 0.05$. Data are means ± S.E. (n=4) for leaf N, P and K contents.

NS: non-significant. *Significant at $P < 0.05$. **Significant at $P < 0.01$. ***Significant at $P < 0.001$.

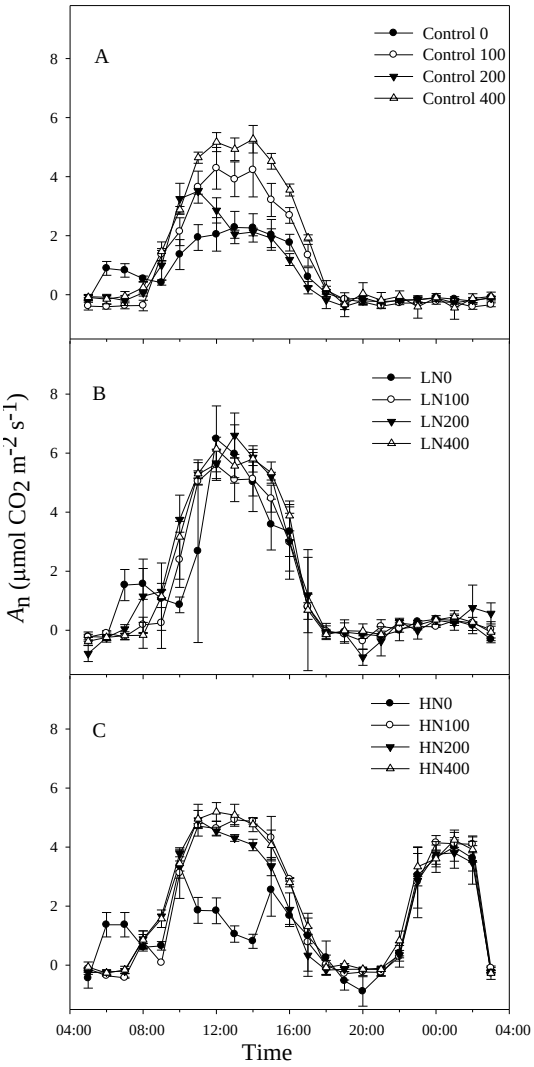

Fig. IV-8. Diurnal changes in photosynthetic rate (A_n) of *Cymbidium* 'Red Fire' grown under NI at 3-7 μmol m^{-2} s^{-1} (LNI) (B) or at 120 μmol m^{-2} s^{-1} (HNI) (C) with high-pressure sodium (HPS) lamps. The control plants were grown under an uninterrupted 9 h photoperiod (A). The plants of each NI treatment were grown under the NI with different N fertilization levels, N 0, 100, 200 and 400 mg L^{-1}. Measurements were taken during the NI treatments in Expt. 2.

compared to the control, LNI or HNI with supplement N treatments. No significant difference in A_n was observed among the HNI plants with N 100, 200 and 400 treatments during the day and night.

The numbers of leaves, leaf length, leaf width and pseudobulb diameter in ‘Red Fire’ plants grown in the LNI and HNI were greater than those in the control after the first NI and N treatment, regardless of the levels of N (Table IV-2). The leaf Chl contents was lower in the plants grown under the HNI with N 0, but those were similar with the LNI and control leaves in HN 400. Although the biomass characteristics were not statistically difference among N treatments under NI condition, the photographs showed the growth of roots and shoots increased more in N 100 treatment under the LNI condition and N 200 or 400 treatments under the HNI condition (Fig. IV-9). However, no difference in biomass characteristics was observed in control condition regardless of N concentration.

Table IV-2. Effects of night interruption (NI) and different N fertilization levels on number of leaves, leaf length, leaf width, pseudobulb diameter and leaf chlorophyll contents in *Cymbidium* 'Red Fire'.

Light	N conc.[a] ($mg \cdot L^{-1}$)	No. of leaves	Leaf length (cm)	Leaf width (cm)	Pseudobulb diameter (cm)	Leaf chlorophyll (SPAD)
Control	0	21.6	45.9	2.75	3.45	58.4
	100	22.4	45.5	2.73	3.46	61.3
	200	22.3	44.9	2.60	3.47	61.9
	400	23.9	45.9	2.65	3.39	62.7
LNI	0	19.7	45.8	1.95	3.37	67.6
	100	21.3	46.5	2.06	3.35	68.9
	200	20.3	47.3	1.98	3.44	68.3
	400	20.5	46.7	2.18	3.38	63.5
HNI	0	21.5	45.8	2.66	3.45	56.7
	100	23.5	42.5	2.71	3.24	62.5
	200	22.7	42.7	2.55	3.15	59.8
	400	22.8	44.3	2.80	2.85	63.8
Significance						
Light		***[b]	**	***	**	NS
N conc.		NS	NS	**	NS	***
Light × N conc.		NS	NS	*	NS	*

[a]N conc.: nitrogen (N) concentration.

[b]NS: non-significant. *Significant at $P < 0.05$. **Significant at $P < 0.01$. ***Significant at $P < 0.001$.

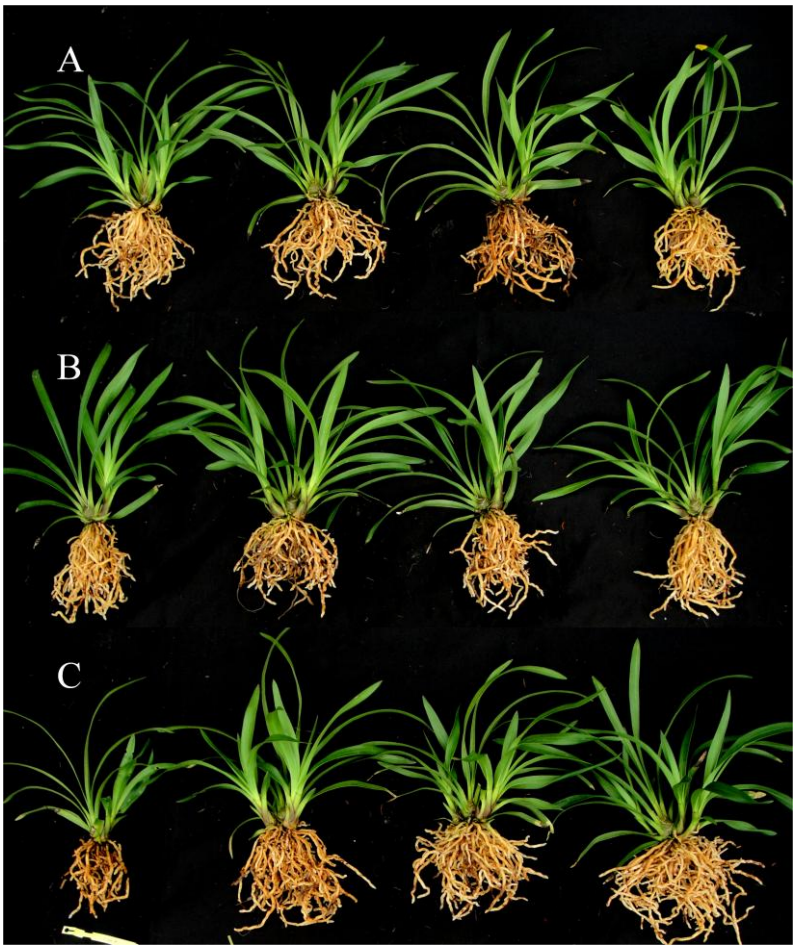

Fig. IV-9. Effects of night interruption (NI) and N fertilization on biomass characteristics of *Cymbidium* 'Red Fire'. Control plants were maintained under a short-day condition using opaque cloth (A). The plants were grown under NI at 3-7 μmol m^{-2} s^{-1} (LNI) (B) or at 120 μmol m^{-2} s^{-1} (HNI) (C) with high-pressure sodium (HPS) lamps. The plants of each NI treatment were grown under the NI with different N fertilization levels, N 0, 100, 200 and 400 mg L^{-1} (left to right). Measurements were taken after the NI treatment in Expt. 2.

DISCUSSION

Even though the A_n was lower in the plants grown under the LNI condition than in the HNI condition, the plants photosynthesized slightly but consistently during 16 weeks of NI for each treatment (Fig. IV-2). The plants gradually accumulated photosynthate and it increased the plant growth and development. In *Cymbidium* 'Red Fire' and 'Yokihi', increased growth under NI (Kim et al., 2011b) may have been due to a direct effect of LD lighting through NI treatment on photosynthesis. This is contrary to the generally held view that light of approximately 3-4 $\mu mol\ m^{-2}\ s^{-1}$ is unlikely to have any significant impact on net photosynthesis. Adams et al. (2008) reported that the relationship between low PPF (3-4 $\mu mol\ m^{-2}\ s^{-1}$) and net photosynthesis is non-linear and therefore low intensity LD lighting can offset respiration very efficiently in *Petunia*. Hofstra et al. (1969) also reported that low intensity light can be used efficiently to offset respiration. Their experiment showed that 13 $\mu mol\ m^{-2}\ s^{-1}$ during the night was five times more efficient than it was to add this during the day in cocksfoot photosynthesis. A small increase in photosynthesis will have a greater impact when ambient light levels are low.

Exposure of leaves to excess light may cause PI of photosynthesis (Reichenauer et al., 1997). This is typically manifested as a decrease in quantum yield of CO_2 assimilation and a reduction in the rate of light-saturated photosynthesis and it is often linearly related with inhibition of PSII photochemistry (Long et al., 1994). In this study, however, the plants were not exposed to high light intensity during day or night. The plants were exposed to

longer photoperiod than the commercial light condition in NI treatment. A reduction in flower production occurred when the annual total sunshine hours was high in *Oncidium* Goldiana (Ding et al., 1980). The extent of PI was determined by the duration and the PPF throughout the exposure period (Powles, 1984). The PI is not linked only to excess light, also to stress factors such as chilling temperatures, drought and/or air pollutants and nutrient deficiency (Guidi et al., 2000; Li et al., 2009).

The depression of A_n in the plants grown under the HNI condition after the NI could be attributed to N deficiency and decreased *g*s and *E* seemed to be the major cause of limiting leaf A_n because *g*s decreased concurrently with the decrease in leaf A_n in the HNI condition (Figs. IV-2 and IV-3). The lower F_v/F_m values indicate that a decrease in the PSII efficiency occurred in the plants grown under the HNI condition (Fig. IV-4). The decline in photosynthetic gas exchange in the plants under the HNI condition during the day can be a phenomenon with decreased Chl content (Figs. IV-6 and IV-7), which is the result of N deficiency associated with increased leaf area production and plant energy conversions in photosynthesis during NI (Table IV-1). Plants need more nutrients for its vegetative growth and plants protect the photosynthetic apparatus from 'over-energization' (Dewir et al., 2005). Lower rates of photosynthesis under conditions of N limitation are often attributed to reduction in Chl content and RuBisCo activity. N deficiency reduced leaf Chl content and A_n, resulting in lower biomass production in other crops (Bottrill et al., 1970; Cechin and Fumis, 2004; Khamis et al., 1990). Different sensitivity shown in F_v/F_m consisted with the different aspect of leaf N content and A_n between cultivars.

NI has long been applied to many floricultural crops to control the flowering responses (Kang et al., 2008; Oh et al., 2008; Runkle and Heins, 2003), however, few studies reported the effects of NI with nutrient control on flowering. This is assumed that the NI methods effectively promoted flowering in herbaceous plants, which has short life cycle, such as *Lythrum salicaria* (Kim et al., 2011a), *Cyclamen* (Oh et al., 2008) and *Eustoma* (Islam et al., 2005), not in orchid plants, which has long juvenile period. One way to enhance the harvestable yield in the tropical orchid is to increase the photosynthetic rates of source leaves by providing higher growth irradiances under well-watered and well-supplied mineral nutrients environment (He et al., 1998). However, increasing the photosynthetic rate of source leaves by increasing irradiance is not feasible because many tropical orchids are shade plants (Hew and Yong, 2004). For instance, light saturation of *Oncidium* Goldiana leaves occurs at between 80 and 100 μmol m^{-2} s^{-1} for all the different stages of development. Plants grown under high irradiance may lead to PI of the leaves. *Cymbidium* is also classified as shade plants, thus other cultural practice should be considered when photoperiod control was used (Menard and Dansereau, 1995).

Although N assimilation in plants has been extensively studied, little information was reported on uptake, transport and storage of N in orchids. There have been some studies on mineral nutrition, in *Cattleya*, *Phalaneopsis* and *Cymbidium*, in particular. These studies focused on nutrient requirements and tissue analyses (Poole and Sheehan, 1982). Generally, the rate of mineral uptake by orchids is slow in relation to that of other higher plants (Hew et al., 1993). The use of Osmocote (slow-release fertilizer) 18N-6P-12K produced more growths for

Cattleya and larger plants of *Cymbidium* and *Phalaenopsis* (Poole and Seeley, 1977). Due to the increased growth (376 g vs. 215 g) of *Cymbidium*, the N level or release rate does not appear to be adequate for the retention of the older leaves. Either higher amounts should be given, or supplemented with liquid applications periodically. Leaf loss in *Dendrobium* Red Emperor 'Prince' was greater in the absence of N with only 38% leaf retention, compared to the plants supplied with N. The flower number increased with increasing N concentration from 0 to 100 mg L^{-1} (Bichsel et al., 2008). For *Phalaenopsis*, maintaining N fertilization until the completion of flower initiation increased flower number (Wang et al., 2002). The highest yields was shown in 'Caliente' roses under ambient light with N applications at 200 mg L^{-1}, while 300 mg L^{-1} was optimal under HPS supplementary lighting (Armitage and Tsujita, 1979b). N levels at 100 and 200 mg L^{-1} were insufficient to maintain foliar N at an optimum level when using supplementary HPS and optimum level of foliar N was restored with N at 400 mg L^{-1} in 'Forever Yours' roses (Armitage and Tsujita, 1979a). This study showed that the plants grown under the LNI and HNI required more than 100 and 200 mg L^{-1} additional N supply, respectively, with fertilizer which contains 0.65 g N, 0.35 g P and 0.54 g K in 5 g of water soluble controlled release (Fig. IV-9). Although supplementary lighting produces higher yields and enhances plant growth, fertilization must also be optimized in orchids.

In conclusion, *Cymbidium* photosynthesized even under low PPF range, 3-4 $\mu mol\ m^{-2}\ s^{-1}$ in NI forcing culture. However, prolonging photoperiod with NI with high growth irradiances can at as an abiotic stress depending on plant species and/or other abiotic factor such as plant nutrition. The results showed that the

same N supply as in the control and LNI conditions was insufficient for the plants under the HNI because the plants photosynthesized more in the HNI than in the plants grown under the control and LNI. This means that plants need more N nutrient to support more photosynthesis. Additional N supply, higher than 100 mg L^{-1} is needed when the use of NI lighting is introduced in *Cymbidium* cultivation to shorten the flowering time without PI.

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CONCLUSION

This study was focused the effects of NI at different light intensities with temperature and N nutrient control on vegetative growth, flowering, flower quality and crop time in *Cymbidium* ‘Red Fire’ and ‘Yokihi’. *Cymbidium* ‘Red Fire’ and ‘Yokihi’ were grown under the LNI and HNI conditions to promote vegetative growth and to hasten flowering. The HNI increased *Cymbidium* plant height and the number of florets and decreased the days to flowering from the initial planting. While the benefits of increased light intensity for the NI may warrant the use of NI to improve crop quality and profitability, the initial investment in an NI system and the additional electricity expenses must be considered. Although the plants had smaller inflorescences and florets in the LNI than in the HNI, when compared to the control plants they would still result in higher profits due to decreased crop time. Commercial use of NI for *Cymbidium* cultivation can be recommended to decrease crop time and increase profits. However, the transition to flowering of *Cymbidium* does not occur with such a strict environmental cue, low temperature before the plant matures. *Cymbidiums* ‘Red Fire’ and ‘Yokihi’ could be force to flower within 2 years by having both summer cooling with mist and winter forcing by NI treatment. Temperature should be maintained at 27℃ to avoid heat stress and inflorescence abortion during the summer growing seasons in the greenhouse. This study also showed the effects of NI on carbohydrate concentrations and photosynthetic characteristics in *Cymbidium* ‘Red Fire’ and ‘Yokihi’. The pseudobulbs of the plants acted as strong utilizing sink during flower developmental stage and

preferentially metabolized carbohydrates rather than stored them. Increased carbohydrates in pseudobulb of *Cymbidium* under NI condition may have an important role in promotion of flowering than those in leaves and roots. The promotion of flowering in the plants grown under the LNI and HNI conditions compared to control condition may be due to the higher photosynthates in pseudobulbs. *Cymbidium* photosynthesized even under low PPF range, 3-4 μmol m^{-2} s^{-1} in NI forcing culture. However, prolonging photoperiod with NI with high growth irradiances can act as an abiotic stress depending on plant species and/or other abiotic factor such as plant nutrition. The same N supply as in the control and LNI conditions was insufficient for the plants under the HNI because the plants photosynthesized more in the HNI than in the plants under the LNI. This means that plants need more N nutrient to support more photosynthesis. Additional N supply, more than 100 mg L^{-1} is needed when the use of the HNI lighting is introduced in *Cymbidium* cultivation to shorten the flowering time. The long-term, practical aim of the present study was to describe the effects of NI and different environmental factors, such temperature and nitrogen nutrient on the forcing culture of *Cymbidium* in order to guide the development of floricultural practices for shortening flowering time with high crop quality.

ABSTRACT IN KOREAN

본 논문은 심비디움 재배 시 야파 처리와 온도, 질소 관리 등의 환경 조절을 통한 개화 촉진 기술의 개발과 그 원인 구명에 목적을 두었다. 겨울철의 야간(22:00-02:00)에 인공광을 조명하여 최소한의 난방으로 개화를 유도할 수 있었다. *Cymbidium* ‘Red Fire’와 ‘Yokihi’의 야파 처리 효과를 알아보기 위하여 저광도(3 μmol m^{-2} s^{-1})와 고광도(120 μmol m^{-2} s^{-1}) 야파 처리(22:00-02:00)를 실시한 결과, 엽수와 엽장, 위구경의 수와 단경 등은 야파 처리 시 무처리구보다 증가하였다. 무처리구에서는 2년 안에 전혀 개화를 하지 않은 반면, 저광도 야파 처리구에서는 60%, 고광도 야파 처리구에서 ‘Red Fire’는 80%, ‘Yokihi’는 100%가 개화하였으며 화서나 꽃의 수는 고광도 야파 처리구에서 저광도 야파 처리구보다 증가하였다. 야파 처리와 함께 여름철의 온도 저하 효과를 구명하기 위하여 온실에 미스트 시스템과 차광 커튼을 설치한 구역으로 나누어 실험을 실시한 결과, 미스트 시스템을 가동한 것이 차광을 한 처리구에서보다 약 2℃정도 낮아졌고, 습도는 차광 구역이 55 ± 5%인데 비해 미스트 구역은 80 ± 5%이었다. 1일 적산 광량은 온도 조절 방식에 따라서 차이가 있었는데 차광 구역은 미스트 구역의 약 48%이었다. *Cymbidium* ‘Red Fire’와 ‘Yokihi’ 두 품종 모두 개화 가능한 위구경 신초의 출현일수는 야파 처리에 의해 단축되었는데 그 효과는 미스트 시스템을 적용하였을 때에 현저하게 촉진되었다. *Cymbidium* ‘Red Fire’와 ‘Yokihi’ 두 품종에서 겨울철에 야파 처리와 함께 여름철에 미스트 시스템을 병행해야 60% 이상의 개화를 2년 안에 유도할 수 있었으며 여름철

에 차광 처리만 하였을 경우에는 조기 개화를 유도 할 수 없었다. 또한 야파 처리 시 추가적인 질소 시비가 권장되며, 저광도 야파 처리구에서 질소 농도를 100mg L^{-1}으로 유지하였을 때 꽃의 직경, 화서의 직경과 길이 등이 증가되었다. 고광도의 야파 처리구에서는 질소 농도를 200mg L^{-1}이상으로 유지하였을 때 꽃의 품질이 향상되었다. 이러한 야파 처리를 통한 심비디움 개화 촉진의 원인을 구명하기 위하여 식물체 내 탄수화물과 광합성 특성을 조사한 결과 심비디움 조기 개화에는 위구경 내의 축적 탄수화물이 잎이나 뿌리의 탄수화물보다 큰 영향을 미쳤으며, 이는 야파 처리구에서 광합성 산물의 축적이 원인인 것으로 판단된다. 광합성률은 고광도 야파 처리구에서는 주간의 2/3 정도이었으며 저광도 야파 처리구에서 미미하지만 꾸준한 광합성을 하는 것을 조사하여 조기 개화의 원인을 더 명확히 하였다. 즉 심비디움 개화 촉진을 위한 야파 처리는 여름철 온도 관리, 질소 비료 관리를 함께 고려할 때 광합성 및 탄수화물의 증가로 인하여 고품질의 심비디움을 2년 안에 개화시키는 것이 가능하다.