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A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

Breaking Bud Dormancy in *Erythronium japonicum* Decne. (Liliaceae) by Natural and Artificial Chilling

자연 저온과 인공 저온 처리에 따른 얼레지 눈의 휴면 타파

BY

SHIN YOUNG KIM

FEBRUARY, 2014

MAJOR IN FLORICULTURE AND LANDSCAPE PLANTS
DEPARTMENT OF HORTICULTURAL SCIENCE AND BIOTECHNOLOGY
THE GRADUATE SCHOOL OF SEOUL NATIONAL UNIVERSITY

Breaking Bud Dormancy in *Erythronium japonicum* Decne. (Liliaceae) by Natural and Artificial Chilling

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

BY
SHIN YOUNG KIM

MAJOR IN FLORICULTURE AND LANDSCAPE PLANTS
DEPARTMENT OF HORTICULTURAL SCIENCE AND BIOTECHNOLOGY
THE GRADUATE SCHOOL OF SEOUL NATIONAL UNIVERSISTY

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APPROVED AS A QUALIFIED THESIS OF SHIN YOUNG KIM
FOR THE DEGREE OF MASTER OF SCIENCE
BY THE COMMITTEE MEMBERS

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Breaking Bud Dormancy in *Erythronium japonicum* Decne. (Liliaceae) by Natural and Artificial Chilling

Shin Young Kim

Department of Horticultural Science and Biotechnology
The Graduate School of Seoul National University

ABSTRACT

This study was conducted to investigate flower bud differentiation and to determine the chilling requirements for bud dormancy breaking and sprouting in *Erythronium japonicum* Decne. (Liliaceae). The dormant bulbs grown in an open field were randomly selected from June to December, 2012 for observation of flower bud differentiation and development. The 3-4 year old bulbs and >10 year old bulbs were used for vegetative development and flower bud development, respectively. For natural chilling treatments, the plants were transferred from an experimental field to a growth module every month from September to February. Artificial chilling treatments were applied at 5℃ for 0, 2, 4, 8, and 12 weeks in October and November. Modified Chilling Hours Model (MCHM) and Modified Utah Model (MUM) were used for calculating the chilling hours. Flower bud initiation and differentiation in *E. japonicum* were observed in May, and from June to July, respectively, followed by the flower bud elongation after August.

Sprouting and bud break did not occur throughout the experiment when dormant bulbs were transferred from September (0 MCH and 0 MCU) to November (612 MCH and 839.5 MCU). However, all plants sprouted when the dormant bulbs were transferred after January (1,956 MCH and 1,914.7 MCU). Prolonged cold storage also promoted sprouting of dormant *E. japonicum*. No or 4 weeks (672 MCH and 684 MCU) of cold storage at 5℃ in October did not induce bud break and sprouting. However, when the dormant bulbs were stored at 5℃ for 12 weeks (2,016 MCH and 2,030 MCU) in October, percent sprouting increased to 72.7%. The chilling treatment for 8 weeks (1,571 MCH and 1,886 MCU) or 12 weeks (2,243 MCH and 2,558 MCU) at 5℃ in November resulted in 72.7% and 100% of sprouting. In conclusion, at least 1,956 MCH or 1,914 MCU in natural chilling and 1,571 MCH or 1,886 MCU of cold storage could be recommended for dormancy breaking in forcing culture of *E. japonicum*.

Additional key words: chill unit, differentiation, dormancy breaking, flower bud, flowering, perennial plants

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INTRODUCTION

Erythronium japonicum Decne. (Liliaceae), as a herbaceous perennial native to Korea, is the only species of the genus *Erythronium* in the north-eastern Asia (Shoichi, 2005). *E. japonicum,* with the characteristics of a typical vernal plant that inhabits the cool-temperate mesic deciduous-forest floor, flowers in early spring from March to April (Tetsuya et al., 2002). Since native stands of Korea rarely have flowering plants in this early spring period, *E. japonicum* is high in scarcity value. It has beautiful deep pink flowers and elegant foliage providing ground cover. Recently, a demand for *E. japonicum* as a garden and landscape plant has increased due to this distinctive flowering period as well as its great aesthetic appeal. However, limited flowering period and a lack of the information on established cultivation method are problems for growing of *E. japonicum* as the horticultural plant. Therefore, controlling the growth and flowering time of *E. japonicum* is critical for forcing and retarding cultures.

After herbaceous perennial species enter dormancy during the winter, the dormancy breaking and sprouting correspond closely to seasonal variations in temperature regimes (Shoichi, 2005). Chilling treatment has been used for dormancy breaking or controlling sprouting of perennial herbaceous plants, such as *Paeonia lactiflora* (Fulton et al., 2001; Rhie et al., 2012), *Adonis amurensis* (Jung and Kim, 2009), and *Polygonatum odoratum* (Yun et al., 2011). The duration and intensity of chilling requirement for dormancy breaking vary among species according to their natural origins (Rogers, 1995). However, there is little information about chilling requirement for dormancy breaking and

flowering of *E. japonicum.*

Chilling requirements have widely been used to model the dates for breaking dormancy in many plant species (Campoy et al., 2012). Numerous models have been developed to estimate the amount of chill exposure. For example, the Chilling Hours Model (CHM), which is widely known by growers, is frequently used for comparative chill model studies. The Utah Model (UM) has been applied in temperate zone (Alburquerque et al., 2008). As these models are cultivar-specific, they are useful for predicting the probability of the successful adaptation of a cultivar to a pre-determined environment. Therefore, these chill unit models can be used for predicting sprouting and chilling requirements of herbaceous plants.

In this study, the chilling requirements for dormancy breaking and sprouting of *E. japonicum* were investigated. The specific objectives were (1) to observe the differentiation and development of flower buds (2) to determine the natural and artificial chilling requirements for dormancy breaking.

LITERATURE REVIEW

Characteristics of *E. japonicum*

The genus *Erythronium* includes 20-30 species of bulbous plants in the north temperate zone, one of which occurs from the Pyrenees, across France, southern Europe, and Asia, to Korea and Japan (Frederick, 1910). It is a typical vernal plant that inhabits the cool-temperate mesic deciduous-forest floor (Tetsuya et al., 2002). *Erythronium japonicum* Decne. (Liliaceae) (Eolleji in Korean) is the only species of the genus *Erythronium* in the north-eastern Asia (Shoichi, 2005). In Asia, *E. japonicum* has been used as an ornamental and medicinal plant for many years.

E. japonicum, as a herbaceous perennial flowers in early spring from March. The dormant stage in this species apparently occurs in summer, lasting for at least 5 months from May to September. New roots sproute in October. Subsequently, in November, new aerial shoots are formed within the bulb, remaining underground the coming spring (Shoichi, 2005).

Flower Bud Differentiation

Flower buds could be initiated prior to the dormancy, and the bud development continued throughout the dormant period with an increasing complexity in the floral organs within a dormant bud (Takeda and Wisniewski, 1989). The differentiation time and developmental stage of flower bud are considered to be cultivar specific. In a wide range of winter and spring flowering perennials, flowers are typically initiated within the renewal bud during late

summer or autumn (Christiaens et al., 2012). This pattern is generally found in temperate fruit species (Faust et al., 1997), woody ornamentals such as *Rhododendron* (Criley, 1985), and herbaceous perennials such as *Tulipa*, *Hyacinth*, and *Liatris* (Le Nard and De Hertogh, 1993), *Fragaria* (Hancock, 1999), and *Paeonia* (Hall et al., 2007). The early spring flowering perennials in Korea such as *Pulsatilla* (Ha et al., 1988), *Lycoris* (Park et al., 1991), *Cymbidium* (Park et al., 2001), and *Hepatica* (Lee et al., 2005), generally initiated the flower buds in April to June. However, there is little information on the differentiation of flower bud in *E. japonicum* native to Korea.

Chilling Requirement for Dormancy Breaking

Dormancy is a phase of development that occurs annually for next year's growth in temperate perennials (Saure, 1985). To break dormancy, plants require a certain amount of winter chilling (Alburquerque et al., 2008). Duration and intensity of the chilling requirement for dormancy breaking vary among species (Cesaraccio et al., 2004). For example, the effective chilling period ranged from 250 to 1,000 h in woody plants (Vegis, 1964). If chilling requirements are not fulfilled in plants, uneven and delayed bud break, reduced shoot vigor, limited anthesis, and poor flower development could occur (Cambell and Sugano, 1975).

While the optimum chilling temperature is usually around 2-10℃ for perennials distributed in temperate zone (Erez and Lavee, 1971), threshold temperature affects the sufficient chilling accumulation for dormancy breaking. The temperatures above a certain threshold contribute to the chilling requirement but others do not (Cesaraccio et al., 2004). For example, bud dormancy in

woody plants is released by non-freezing chilling temperatures for a long-term exposure, whereas freezing temperatures have been considered to have little or no effect on dormancy breaking.

Chill Unit Models for Predicting Chilling Requirement

Chill unit models has been developed for measuring the quantity of cold requirement for overcoming dormancy in plant species. In order to determine what temperature ranges are more effective than other temperature under field conditions, many chill unit models have been proposed to predict the response of buds of perennial plants to chilling (Dennis, 2003). For example, the CHM is the one of the simplest and oldest models. It quantifies chilling as the number of hours during the low temperature season, when temperatures are between 0 and 7.2℃ (Bennett, 1949; Weinberger, 1950). UM assign chill unit values to different temperature ranges (Richardson et al., 1974). This model considers the temperatures noticeably higher than 7℃ as useful for overcoming dormancy and the negative effect of temperatures above a certain threshold on the chill accumulated. Thus, UM has been adapted in various ways to adjust to varying climatic conditions (Norvell and Moore, 1982; Shaltout and Unrath, 1983).

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Bulbs of 3- or 4-year-old *E. japonicum* were purchased from the private farm (Namusarang, Gwacheon; Pyeongchang Gosan Plants, Pyeongchang, Korea) on April 30, 2012. The dormant bulbs was 5-7 cm long and had one bud each. The bulbs were transplanted into 12 cm plastic pots filled with commercial potting medium (Sunshine Mix #4, Sun-Gro Horticulture, Bellevue, WA, USA) and then grown in an experimental plot (Fig. 1A) in the Experimental Farm, Seoul National University, Suwon, Korea. Daily average soil temperature at a depth of 5 cm were monitored every 30 min for calculating chilling accumulation under natural environment using a thermo data logger (Watch Dog Model 450, Spectrum Technologies, Inc., Plainfield, IL, USA). The plants were moved to forcing conditions in a growth module (Fig. 1B) or subjected to a chilling treatment at 5℃ in a dark storage room. Forcing conditions in a growth module were 18℃/12℃, day/night temperature with a photoperiod of 12 h at 200 μmol m^{-2} s^{-1} at plant canopy level (HPI-T Plus Philips, 400 W lamps, Philips, Eindhoven, Netherlands). Each pot in open field or in a growth module was weekly received 50 mL (pH 5.5, EC 1.5 $dS \cdot m^{-1}$) of a water soluble fertilizer, Technigro® 16N-17P-17K (Sungro Ltd., Vancouver, BC, Canada). The plants were watered when the surface of the potting medium was dry.

Fig. 1. An experimnetal plot (A) and a growth module (B) used in this study.

Microscope Examination on Vegetative and Flower Buds

In 2012, the vegetative bulbs of five plants grown in open field were used for microscopic observation of bud. The bulbs of the plants were randomly selected and dissected at seven times (June 29, July 27, August 31, September 28, October 26, November 30 and December 28 in 2012).

In 2013, the dormant flower bulbs of about 10-year-old *E. japonicum* obtained from Hantaek Botanical Garden (Yongin, Korea 37° 05' S, 127° 24' E) were additionally dissected at five times in 2013 (June 28, July 26, August 30, September 27, October 31, November 29 and December 27). The dissected tissues were investigated and photographed by using a dissecting microscope (SteREO Discovery.V12, Car Zeiss, Jena, Germany).

Chilling Treatments

For natural chilling treatments, plants had been grown in an open field and were transferred to a growth module at six different transferring dates (September 28, October 26, November 30, and December 28 in 2012; January 25 and February. 22 in 2013).

For artificial chilling treatment, plants were transferred from an open field to a cold storage room at October 12 and November 16. In the cold storage room, dormant bulbs were exposed to 5℃ for 0, 2, 4, 8, and 12 weeks from October or from November. The chilling treated plants were moved to a growth module for forcing.

Chill Unit Model

Effects of chilling treatments in terms of amounts of chilling units were calculated according to the Modified Chilling Hours Model (MCHM) and Modified Utah Model (MUM) (Table 1). The MCHM and MUM were developed based on the Chilling Hours Model (CHM) (Weinberger, 1950) and Utah Model (UM) (Richardson et al., 1974), respectively. Since these two models did not assign chill unit per hour to temperature below 0℃, 1 chill unit per hour was assigned to temperature ranging from −5℃ to 7℃ in MCHM; 0.8 and 1 chill unit per hour was assigned to temperature ranging from −5℃ to 1.4℃ and from 1.4℃ to 2.4℃, respectively, in MUM.

Data Collection and Analyses

Percent sprouting, days to sprouting, leaf width, and leaf length were recorded weekly. The experiment was conducted as a completely randomized design with twelve plants replicates per treatment during the forcing. The SAS version 9.2 (SAS Institute Inc., Cary, NC, USA) was used to perform analysis of variance (ANOVA) and general linear models (GLM). When significant differences occurred, the means were separated using Tukey's studentized range tests at $P = 0.05$. Analysis and graph modules were made using Sigma Plot 10.0 program (Systat Software, Inc., Chicago, IL, USA).

Table 1. Temperature ranges for calculating chill unit in Chilling Hours Model (CHM), Utah Model (UM), Modified Chilling Hours Model (MCHM), and Modified Utah Model (MUM).

Model	Temperature range
CHM	0 ℃ < T < 7 ℃ = 1, else : 0
UM	T ≤ 1.4 ℃ = 0
	1.4 ℃ < T ≤ 2.4 ℃ = 0.5
	2.4 ℃ < T ≤ 9.1 ℃ = 1
	9.1 ℃ < T ≤ 12.4 ℃ = 0.5
	12.4 ℃ < T ≤ 15.9 ℃ = 0
	15.9 ℃ < T ≤ 18.0 ℃ = -0.5
	T ≥ 18.0 ℃ = -1
MCHM	-5℃ <T < 7 ℃ = 1, else : 0
MUM	-5℃>T = 0
	-5℃ < T ≤ 1.4 ℃ = 0.8
	1.4 ℃ < T ≤ 2.4 ℃ = 1
	2.4 ℃ < T ≤ 9.1 ℃ = 1
	9.1 ℃ < T ≤ 12.4 ℃ = 0.5
	12.4 ℃ < T ≤ 15.9 ℃ = 0
	15.9 ℃ < T ≤ 18.0 ℃ = - 0.5
	T ≥ 18.0 ℃ = -1

RESULTS AND DISCUSSION

Initiation and Differentiation of Flower Buds

The vegetative bud development was observed in the bulbs of 3- or 4- year-old *E. japonicum* plants (Fig. 2). The ratio for bud/bulb increased from 0.01 on June 29 to 0.89 on December 28 (Fig. 3). This ratio increased rapidly by 0.32 from September 28 to October 26 and 0.30 from November 30 to December 28 (Fig. 3). This increase indicates that vegetative bud growth in the bulb was affected by low temperature from September to October in autumn, and that the bulbs were ready to sprout in December. In *Cymbidium goeringii*, similarly, shoot apical meristem was enlarged when the temperatures dropped gradually (Park et al., 2001). In *E. japonicum*, new aerial shoots were formed within the bulb in November, remaining underground until the sprouting (Shoichi, 2005). Leaves of *Lycoris* were also formed in mid-September (Park et al., 1991). These results indicate that the vegetative bud of *E. japonicum* is initiated in before June, elongated by low temperature from September 28 to October 26, and completed on December 28.

Flower buds in the bulbs of 10-year-old plants was developed (Fig. 4). Renewal buds in bulb were protected by two specialized cover leaves firmly enveloping the inside of the bud (Fig. 4). On June 28, the carpel, stamens, and petals in flower bud were observed (Fig. 4A, B), indicating that flower buds had already been initiated in May. On July 26, the differentiation of each organ was observed (Fig. 4C,D). On July 26, the ratio of the flower bud/bulb was 0.07 and the carpel, stamens, and petals of flower bud was developed further compared to

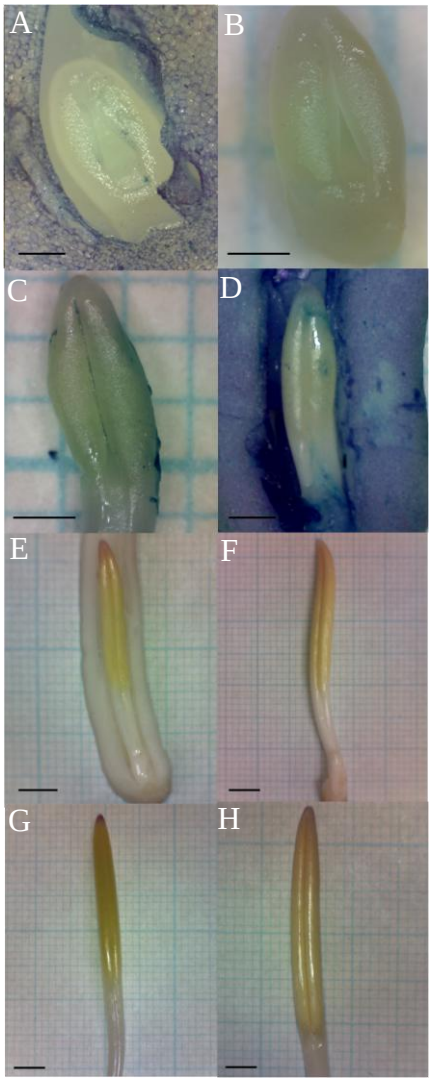

Fig. 2. Development of vegetative buds of 3- or 4- year old *E. japonicum*. The buds were sampled on June 29 (A), July 27 (B), Aug. 31 (C), Sept. 28 (D), Oct. 26 (E), Nov. 30 (F), Dec. 28 (G) in 2012, and Jan 25 (H) in 2013, respectively. Scale bars are 0.5 mm, except (C) = 1 mm.

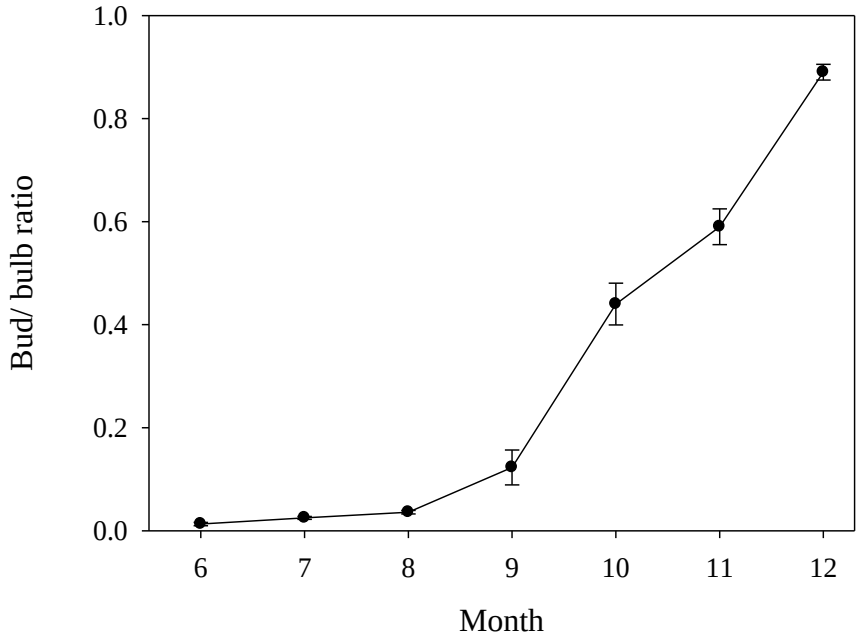

Fig. 3. Changes in vegetative bud/bulb ratio of *Erythronium japonicum* from June to December, 2012. Vertical bars indicate the standard errors of the means (n = 3).

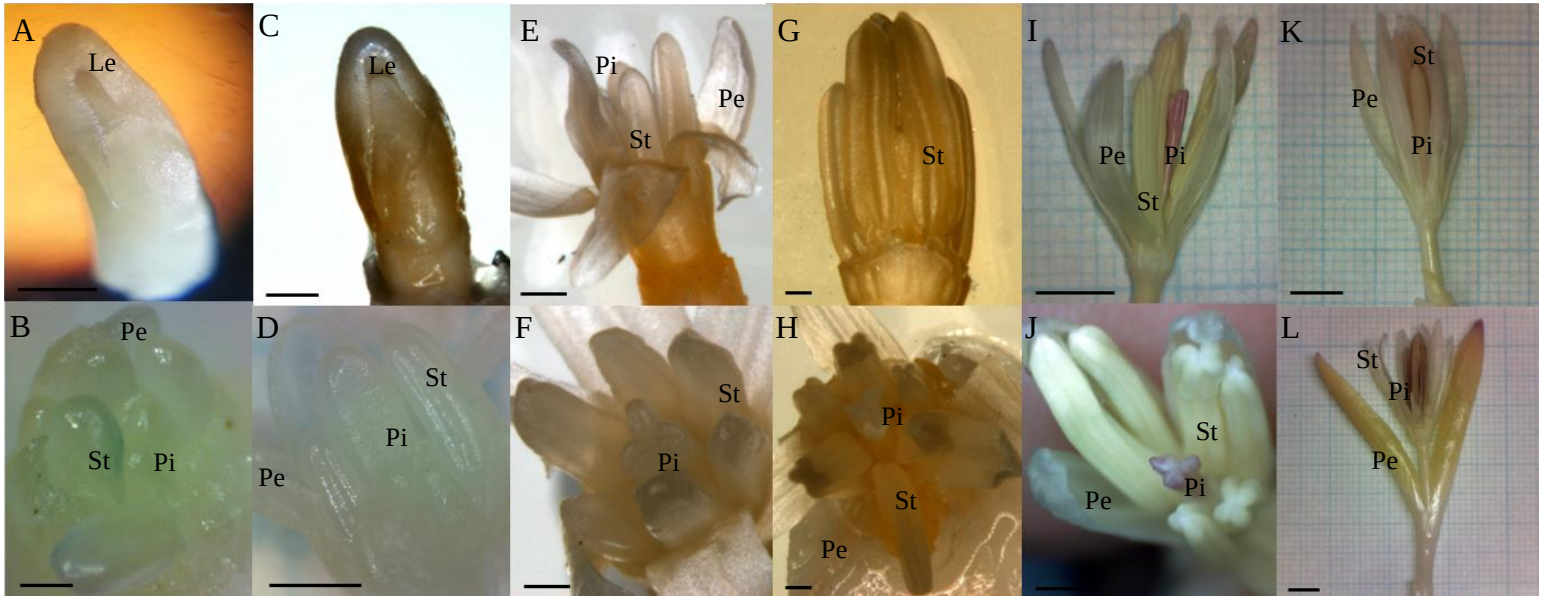

Fig. 4. Development of flower buds of *Erythronium japonicum*. The flower buds were observed on June 28 at start (A and B), July 26 (C and D), August 30 (E and F), September 27 (G and H), and October 25 (I and J), November 29 (K), December 27 (L) respectively. Scale bars are 1 mm for A, C, G, and H, 0.2 mm for B and J, and 0.5 mm for D, E, F, I, K and L. Pi, Pistil; St, Stamen; Pe, Petal; Le, Leaf.

those on June 28 (Fig. 4B-D). On September 27, the flower bud was 1 cm longer than that on August 31. The ratio of the flower bud/bulb increased from 0.10 on August 31 to 0.26 on September 28 (Fig. 5). At the end of October, the carpels, stamens, and petals were almost completely developed (Fig. 2I-J). Yoon and Lee (1998) found that the renewal bud in *Convallaria majalis* began to be initiated in April and continued to develop until the onset of dormancy on September 26. Flower buds of *Pulsatilla koreana* were also initiated in late June and continued to develop until mid October (Ha, 1988). These results indicate that the flower buds of *E. japonicum* are initiated in May and completed after October under natural condition.

Effects of Natural Cumulative Chilling on Sprouting

E. japonicum plants sprouted completely when they were transferred on January 25 and February 22, but bud sprouting did not occur throughout the experiment when the plants were transferred on September 28, October 26, and November 30 (Table 2, Fig. 6). When they were transferred on December 28, percent sprouting was only 15.3% (Table 2). These results indicate that chilling requirement for dormancy breaking was satisfied between December 28, 2012 and January 25, 2013. In *Polygonatum odoratum*, percent sprouting increased up to 83% as the plants transferred after December 7 (Yun et al., 2011). Jung and Kim (2009) also reported that 90% of rootstocks broke buds when transferred on December 5. Days to sprouting were 14.4 days when plants were transferred on February 22, whereas it was 28 days when transferred on December 28 (Table 2). Similarly, the delayed transferring date was more effective for breaking

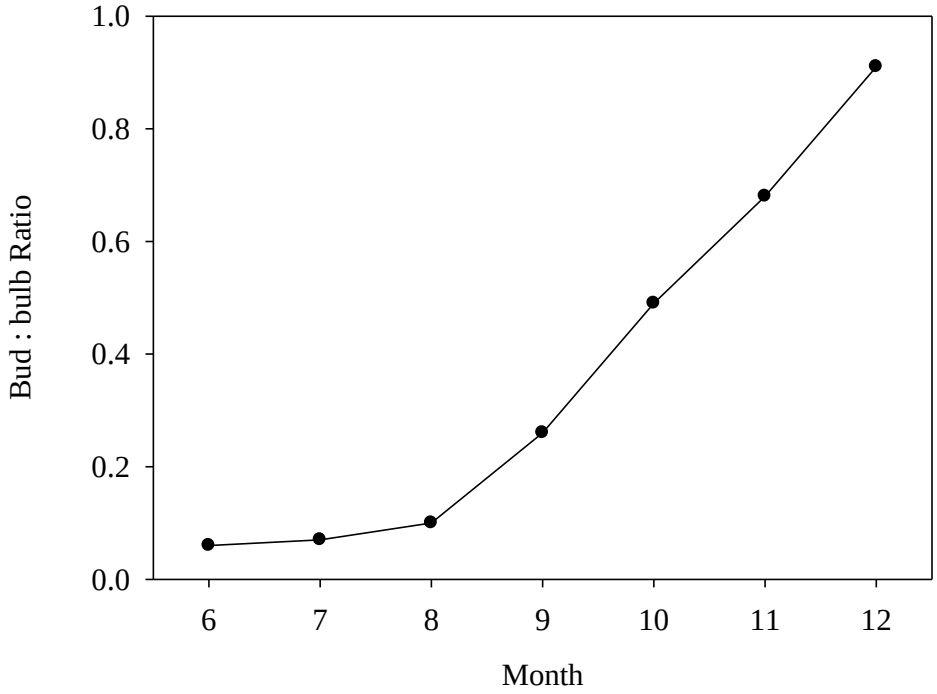

Fig. 5. Changes in flower bud/bulb ratio of *Erythronium japonicum* from June to October, 2012.

Table 2. Effects of transferring date from field to growth module on days to sprouting, sprouting rate, leaf length, and leaf width of *Erythronium japonicum*.

Transferring date	Days to sprouting	Percent sprouting	Leaf	
			Length (cm)	Width (cm)
September 28	-[z]	-	-	-
October 26	-	-	-	-
November 30	-	-	-	-
December 28	28.0 a[y]	15.3	6.50 a	4.40 a
January 25	19.6 ab	100	7.49 a	4.13 a
February 22	14.1 b	100	8.56 a	4.35 a
Significance	**		NS	NS

[z] Not detected (not shown until the end of experiment).

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

NS, ** Non-significant or significant at $P < 0.01$, respectively.

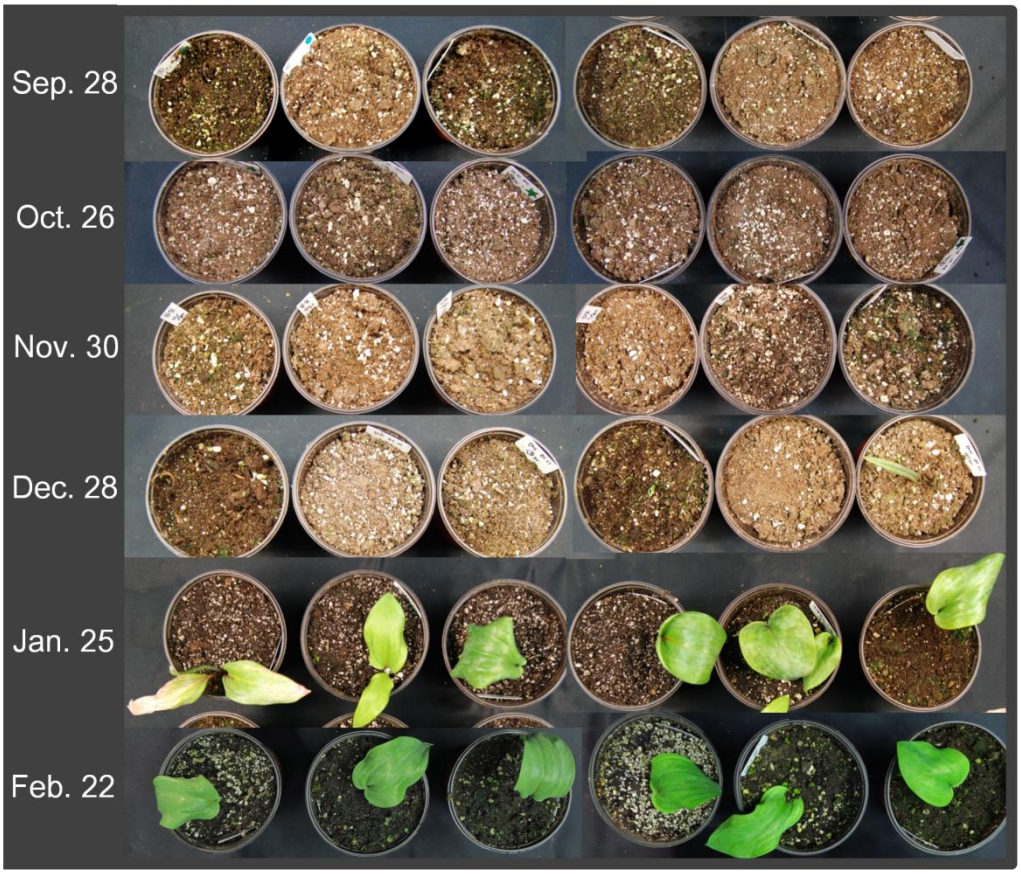

Fig. 6. Effects of transferring date from field to growth module on sprouting of *Erythronium japonicum*. Pots were photographed after 56 days of forcing.

dormancy and sprouting of *Polygonatum odoratum* (Yun et al., 2011), *Adonis amurensis* (Jung and Kim, 2009), and *Paeonia* (Yeo et al., 2012). However, transferring date had no significant effect on leaf width and length (Table 2), suggesting that in *E. japonicum* the optimum transferring date for dormancy breaking may be after January 25.

Effects of Artificial Chilling on Sprouting

When *E. japonicum* bulbs were stored at 5℃ for 8 or 12 weeks in October, 18.1 and 72.7% of them were sprouted, respectively (Fig. 7). The plant treated with chilling for 0, 2, and 4 weeks in October failed to sprout under forcing condition until the end of experiment. However, the chilling treatment for 2, 4, 8, or 12 weeks at 5℃ in November resulted in 9, 18.1, 72.7, or 100% of sprouting, respectively (Figs. 7, 8). The plants without chilling treatment did not sprout until the end of experiment in November (Fig. 7). These results indicated that chilling treatments for 12 weeks from October or 8 weeks from November are effective for dormancy breaking. Chilling accumulated in the natural environment before the beginning of the artificial chilling treatment in November caused the longer exposure to low temperature than in October. In October, the days to sprouting were 36 and 56 days with chilling treatments at 5 ℃, for 12 and 8 weeks, respectively. Similarly in November, as the chilling duration increased from 2 to 12 weeks, the days to sprouting decreased from 78 to 20.6 days. As the duration of chilling requirement extended, days to sprouting were decreased greatly in both October and November. Yoshie and Fukuda (1994) also showed similar results in *E. japonicum*, the 120 days chilling

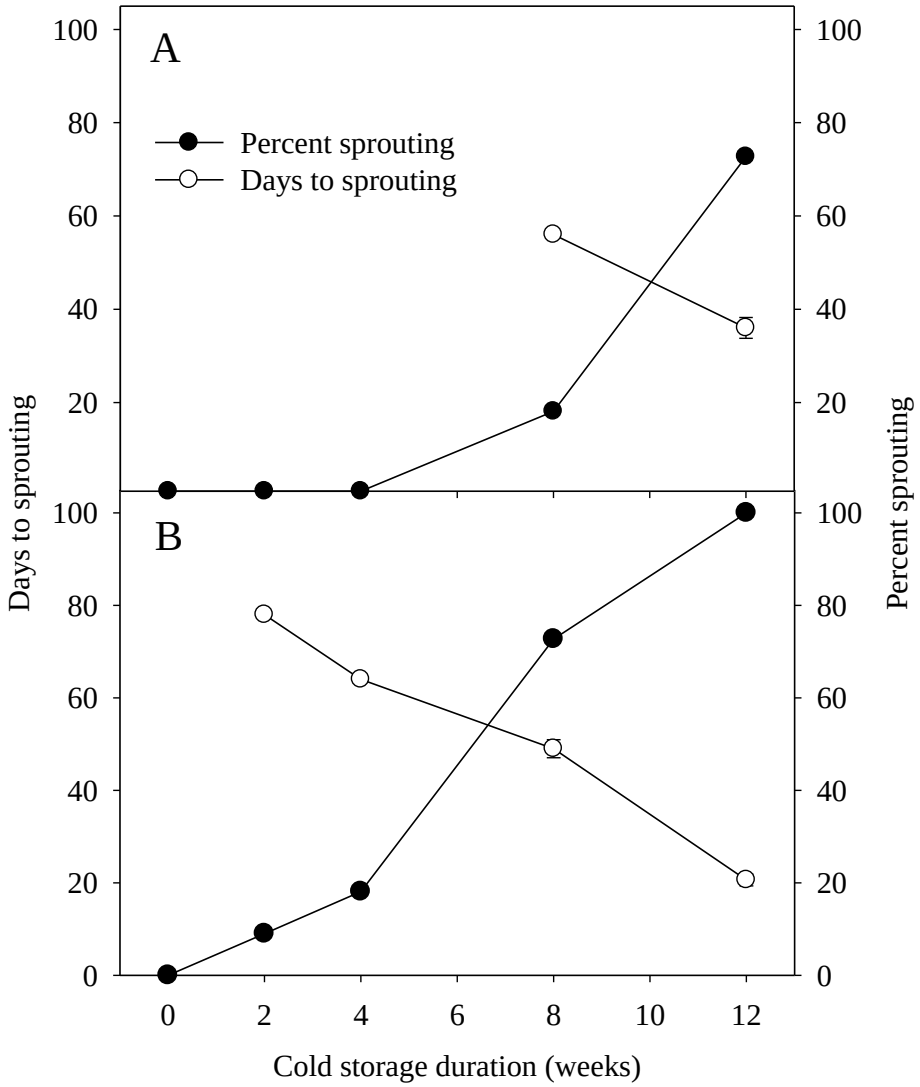

Fig. 7. Effects of cold storage duration on days to sprouting and percent sprouting of *Erythronium japonicum*. The dormant bulbs were subjected to 5℃ for 0, 2, 4, 8, and 12 weeks in October (A) and November (B). Vertical bars indicate the standard errors of the means. (n=2 for 8weeks, n=8 for 12weeks in October; n=2 for 4 weeks, n=8 for 8 weeks, n=11 for 12weeks in November).

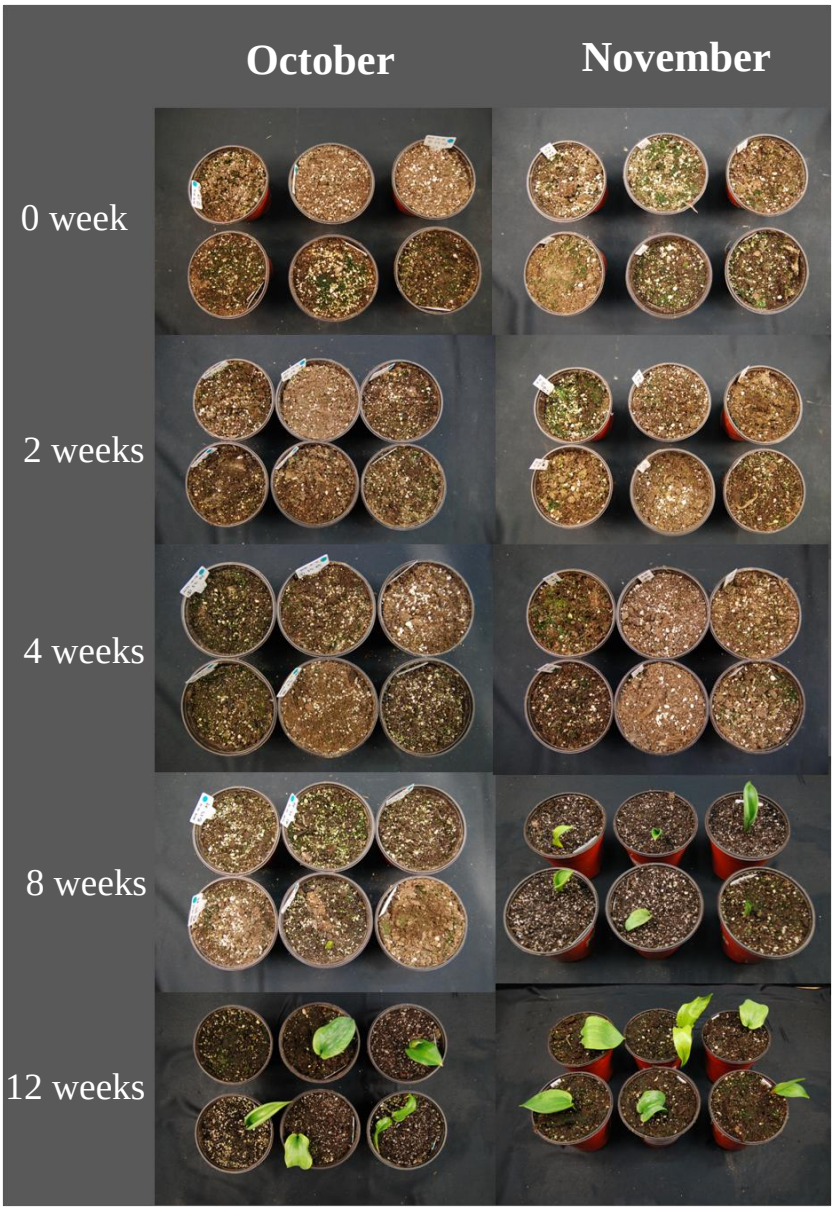

Fig. 8. Effects of cold storage duration from cold storage room to growth module on sprouting of *Erythronium japonicum*. Dormant bulbs of *E. japonicum* were subjected to 5℃ for 0, 2, 4, 8, and 12 weeks in October and November.

treatment at 3℃ was more effective on earlier leaf emergence compared to 60 days chilling treatment. These results indicated that the effect of chilling on dormancy breaking increase as the chilling treatment duration is extended.

Chill Unit for Dormancy Breaking

The amounts of chilling, in terms of the chill unit, required for dormancy breaking were calculated by using Chilling Hour Model (CHM) and Utah Model (UM). Chill unit increased to 1025 CH until December 19 and to 590 CU until November 23 when the soil temperature decreased below 5℃ (Fig. 9). After the soil temperature decreased below 0℃ from December 19, chill unit maintained at 1025 CH and 590 CU (Fig. 9). This was because CHM and UM did not assign chill unit per hour to temperature below 0℃ (Table 1). Thus, the chill units on December 28 when the sprouting rate was 15% and on January 25 when it was 100% were the same as 1,025 CH and 590 CU (Table 2, Fig. 9). These results indicate that the chill unit calculated with CHM and UM did not reflect the physiological change. Temperatures below 0℃ were not considered as effective for chilling treatment in woody species (Shaultout and Unrath, 1983). In herbaceous perennials, however, freezing induced dormancy breaking of storage organs like bulls, tuberous root, and root crown (Dole, 2003). In *Betula pubescens* and *B. pendula*, the exposure to freeze released bud dormancy (Rinne et al., 1997).

Thus, CHM and UM were modified to consider the temperature below 0℃ (Table 1). To assign chill unit per hour to temperature below 0℃, 1 chill unit per hour was assigned to temperatures ranging from −5 to 7℃ in Modified Chilling

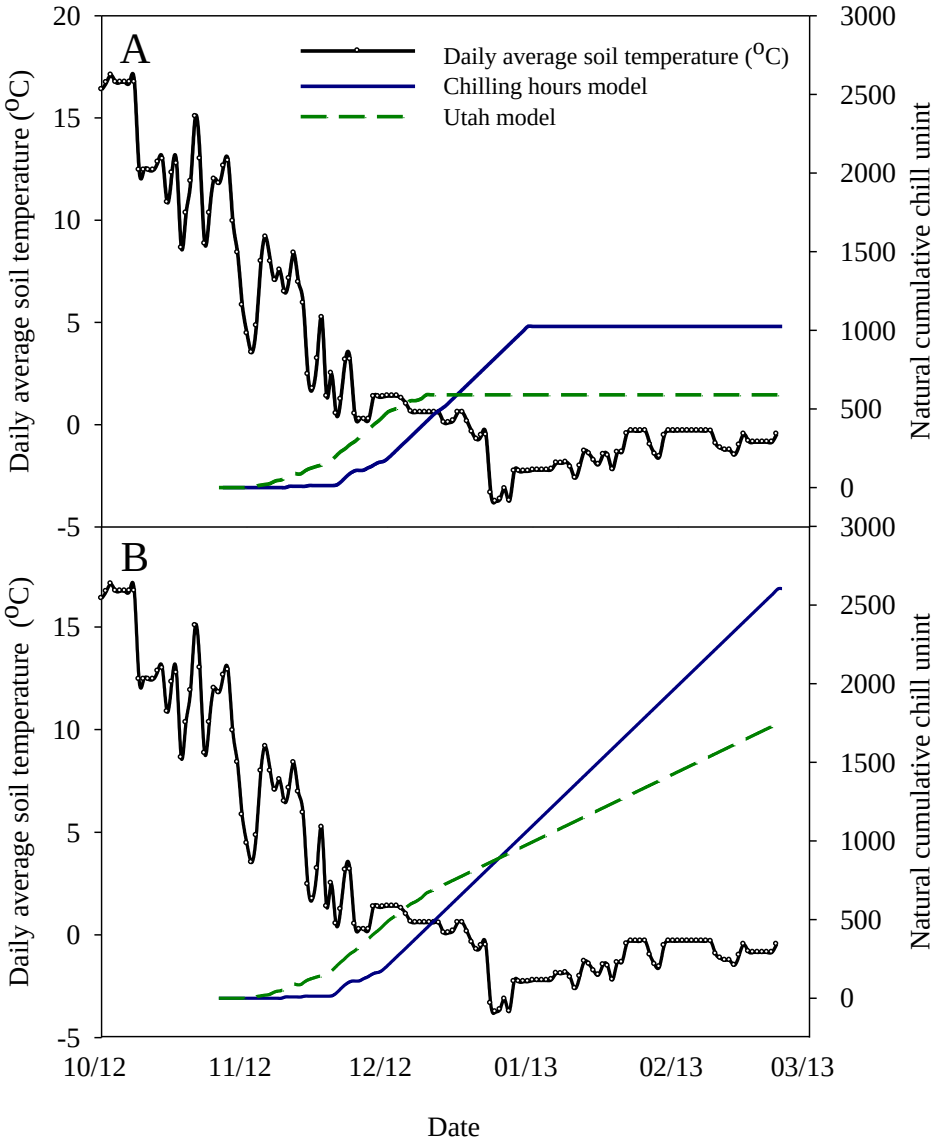

Fig. 9. Changes in daily average soil temperature and cumulative chill unit from October, 2012 to March, 2013 in an experimental plot. Cumulative chill units were calculated using chill unit model (A) and modified chill unit model (B).

Hour Model (MCHM); 0.8 and 1 chill unit per hour was assigned to temperatures ranging from −5 to 1.4℃ and from 1.4 to 2.4℃, respectively, in Modified Utah Model (MUM). In MCHM and MUM, the chill unit increased from 1,284 MCH/1,377 MCU on December 28 to 1,956 MCH/1,914 MCU on January 25 (Fig. 9B). In artificial chilling experiment, the chill units with 12 week chilling treatment from October, 8 and 12 weeks in November were 2,016 MCH/2,030 MCU, and 1,571 MCH/1,886 MCU, 2,243 MCH/ 2,558 MCU respectively. These chill units between natural chilling and artificial chilling had similar values for dormancy breaking.

In conclusion, at least 1900 MCH/MCU was required for dormancy breaking and sprouting of *E. japonicum* in forcing culture. When calculating chill unit for herbaceous perennials, the chill unit models might be need to be modified, with the regional temperature conditions considered. However, further studies should be conducted to determine the optimum weighted value for low temperature calculated in modified chill unit models.

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ABSTRACT IN KOREAN

본 실험은 한국 자생 식물인 얼레지의 촉성 재배를 위하여 꽃눈 분화와 휴면 타파를 위한 저온 요구도를 알아보고자 수행하였다. 화아 분화 시기를 규명하기 위하여 3-4년생 얼레지 구근은 2012년 6월부터 12월까지, 10년 이상된 얼레지는 2013년 6월부터 12월까지 관찰하였다. 3-4년생 얼레지는 꽃눈이 아닌 잎눈이 형성되었다. 잎눈의 분화는 5월에 시작되어, 6월에서 7월 사이에 신장하였다. 특히 8월에서 12월까지 잎눈의 크기가 두드러지게 신장하였다. 10년 이상된 얼레지 구근에서는 꽃눈을 관찰할 수 있었다. 5월에 꽃눈 분화가 시작되었고, 암술, 수술, 꽃잎을 관찰할 수 있었다. 6월에는 분화된 각 기관의 모습이 뚜렷해졌고, 7월 이후엔 크기가 커졌다. 얼레지의 휴면 타파를 위한 저온 요구도를 알아보기 위하여 9월부터 2월까지 매달 생장 모듈에 입실하였다. 자연 저온 축적이 이루어지지 않은 9월 28일, 10월 26일, 11월 30일에 입실한 경우 실험이 종료될 때까지 맹아가 전혀 이루어지지 않았다. 이는 휴면 타파에 필요한 저온이 충족되지 않았음을 의미하였다. 하지만 12월 28일에 입실한 경우 맹아율이 15%, 1월 25일에 입실한 경우 맹아율이 100%였으며, 맹아 소요 일수는 입실시기가 늦어질수록 감소하였다. 또한 얼레지를 10월 12일과 11월 15일부터 각각 0, 2, 4, 8, 및 12주 동안 5℃에 저장한 후, 생장 모듈에 입실한 결과, 10월 12일부터 4주 이하로 저온 저장한 경우 실험을 종료할 때까지 맹아가 전혀 이루어지지 않았다. 그러나 8주 저온 저장한 얼레지는 18%, 12주 저온 저장한 경우에는 72% 이상 맹아되었다. 11월 15일부터 저온 처리한 실험에서는 0주 저온 저장의 경우 맹아가 이루어지지 않았다. 이는 휴면 타파를 위한 저온이 충족되지 않았음을 의미한다. 하지만 2주, 4주, 8주, 12주

저장한 경우 맹아율이 각각 9, 18, 72, 100%였으며, 저온 저장 기간이 길어질수록 맹아 소요 일수가 감소하였다. 따라서 얼레지의 경우 10월에는 12주, 11월에는 8주 이상 저온처리를 해야 내재 휴면 타파를 위한 저온이 충족된다. 저온 요구도를 알아보기 위해 Chilling hour 모델(CHM)과 Utah 모델 (UM)을 이용하여 저온 요구도를 계산하였다. 11월 토양 온도가 5℃ 이하로 떨어지면서 Chill unit 값이 증가하였지만, 12월 이후 토양 온도가 0℃ 이하로 떨어지면서 Chill unit 값이 증가하지 않았다. 이는 각각의 모델 계산식이 온도가 0℃ 이하 범위를 축적되는 저온량으로 계산하지 않기 때문이다. 하지만 자연 입실 실험에서 12월과 1월에 입실한 식물체가 맹아율과 맹아 소요 일수의 차이를 보였으며, 이는 각 모델이 식물의 생리적 변화를 반영하지 못하기 때문이라고 판단되었다. 이를 위해 각 모델의 0℃ 이하 범위의 Chill unit 값을 수정한 Modified Chilling Hour 모델(MCHM)과 Modified Utah 모델(MUM) 사용하였다. 이를 통해 자연 저온은, 1월 25일 입실의 경우 1956MCH, 1914.7MCU이며 인공 저온은, 10월 12주 저온 저장은 1571MCH, 1886MCU이고, 11월 8주 저온 저장은 2243MCH, 2558 MCU이다. 따라서 얼레지의 휴면 타파를 위한 저온 요구도가 약 1900MCH, MCU가 충족되어야 한다. 그러나 겨울철 영하의 온도로 내려가는 중부 지방에서는 기존의 모델로 다년생 초화류의 저온 요구도를 구할 때 Chill unit 모델의 수정이 필요할 것으로 판단된다.