JARQ 44 (1), 67 - 72 (2010) http://www.jircas.affrc.go.jp

Lavandula × *intermedia* is a Vernalization Type Plant

Satoshi KUBOTA[1], Hirofumi MOMOSE[1], Kazuo YONEDA[1,2] and Masaji KOSHIOKA[1]*

[1] Department of Plant Science and Resources, College of Bioresource Sciences, Nihon University (Fujisawa, Kanagawa 252-8510, Japan)

Abstract

Lavandula × *intermedia* is cultivated as an ornamental plant in Japan but its flowering characteristics have not been elucidated. So, a series of experiments were conducted to determine the influence of cold treatment and day length on flowering. An exposure to a 14-week cold treatment (CT) under natural winter condition induced flowering. An increasing duration of day length was associated with an increase in flowering rates and with a decrease in days to the first visible bud (VB) and the first open flower (FLW). This suggests that *L.* × *intermedia* may be an essential quantitative long-day plant. When the plants were treated with a low temperature in a cold room at 5℃, a 10-week CT resulted in perfect flowering. Plants without CT and plants after a 6-week CT did not initiate floral buds. An increasing duration of CT was also associated with an increase in the rate of flowering shoots and with a decrease in days to VB and FLW. Thus, exposure to a period of low temperature is the primary factor promoting flowering in *L.* × *intermedia*. Apical dissections and scanning electron microscope (SEM) observations showed vegetative meristems at the end of a 10-week CT at 5℃. Then, dome formation and sepal initiation of the first floret of each shoot apex were observed at 7 and 21 days after CT, respectively. This suggests that *L.* × *intermedia* is a so-called "after effect" flowering plant. Thus, we conclude that *L.* × *intermedia* is a typical vernalization type plant.

Discipline: Horticulture
Additional key words: cold treatment, day length, flowering

Introduction

Lavandula × *intermedia* is a sterile hybrid between *L. angustifolia* and *L. latifolia*[3], which is hardy and considered a shrub as well as *L. angustifolia*, but is commonly produced and marketed as a herbaceous perennial. *Lavandula* × *intermedia* is cultivated in Japan as an ornamental plant and for its oil, which is used in perfumery, and as a dry flower.

Some species of herbaceous perennials require a cold treatment for subsequent flowering, while low temperature hastens or improves uniformity of flowering in others[2]. In *L. angustifolia* 'Munstead', exposure to periods of low temperatures was shown to be the primary factor promoting flowering[7]. Flowering of the plant was hastened by a period of cold temperature at 5℃ for a minimum of 10 weeks. However, whether floral initiation occurred during or after cold treatments had not been determined. A study on *L. angustifolia* 'Hidcote' reported that a period of 9 weeks cold treatment was shown to satisfy flowering and that the duration of cold treatment had no visible effect on dissected meristems on removal from the cold treatment[4]. This suggests that *L. angustifolia* may be an obligate vernalization type plant, although the initiation of floral primordia has not been elucidated. In our preliminary experiments, we found that plants cultivated from cuttings of *L.* × *intermedia* 'Super Sevillian Blue' required a 113-day low temperature exposure under natural winter condition for subsequent flowering under natural day length and that a 14-h day length hastened flowering of the plants (unpublished data). We also found that floral buds of the plants never initiated during a 130-day low temperature exposure under natural winter condition but initiated 3 weeks after the end of low temperature exposure (unpublished data). This suggests that the ecological nature of *L.* × *intermedia* may be similar to that of *L. angustifolia*, because one parent of *L.* × *intermedia* is *L. angustifolia*. However, we have been unable to find any information in the literature on the response of *L.* × *intermedia* to low temperature for subsequent flowering and on the initiation of floral primordia.

[2] Deceased

*Corresponding author: e-mail koshioka.masaji@nihon-u.ac.jp

Received 17 February 2009 ; accepted 21 April 2009.

The aims of this study were to determine the influence of cold treatment and day length on flowering of *L.* × *intermedia* and to find whether the flowering was induced by a vernalization requirement or by a direct low temperature requirement.

Materials and methods

Experiment 1. Photoperiod on flowering

Micro-propagated young plants of *L.* × *intermedia* 'Super Sevillian Blue' supplied from the Nisshin OilliO Group Ltd. were transplanted into 12-cm pots with a mix of sand, MetroMix (Sun Gro Horticulture Canada Ltd.) and perlite (1:1:1) and pruned as each plant had five lateral shoots on 6th December, 2002. Plants were placed in a greenhouse maintained below 25℃ in day and above 15℃ in night (25℃day/15℃night). Plants were top-watered as necessary with a water-soluble fertilizer (Hyponex: HYPONeX Japan Co. Ltd.) in moderate dilution. Sixty plants were then treated with cold temperature under natural winter condition at the agricultural experimental field of Nihon University (Fujisawa, Kanagawa) for 14 weeks from 17th December, 2002 until 15th March, 2003. After the cold treatment (CT), plants were returned to the greenhouse maintained at 25℃day/15℃night. Plants on all benches were covered with black cloth at 1700HR and opened at 0900HR. Each set of fifteen plants was grown under an 8-h photoperiod of natural day light with 3-h, 4-h, 5-h, or 6-h of supplemental lighting by 60-watt incandescent lamps. Watering and fertilization were done if necessary. Days from the end of CT to the first visible bud (VB) when the inflorescence was identified and to the first open flower (FLW) were calculated for each plant. Number of florets was counted.

Experiment 2. Confirmation of cold treatment

Micro-propagated young plants of *L.* × *intermedia* 'Super Sevillian Blue' purchased from a commercial supplier were transplanted into 12-cm pots on 10th December, 2005 and pruned on 26th December, 2005 as described in Experiment 1. Plants were placed in a greenhouse maintained at 25℃day/15℃night. Thirty-six plants were transferred to a cold room maintained at 5℃ under an 8-h photoperiod by 10-watt fluorescent lamps on 3rd August, 2006. Each set of 9 plants was moved to a phytotron from the cold room at 0, 6, 8, or 10 weeks after CT started. The phytotron (FR-535A-S6: Koito Industries Ltd., Japan) was maintained at 23℃ in day and 15℃ in night under a 16-h photoperiod with metal halide lamps at 489μmol/m²/s. Watering and fertilization were done if necessary. Days to VB and FLW and number of florets were obtained as described above.

Experiment 3. Initiation and development of floral buds

Micro-propagated young plants of *L.* × *intermedia* 'Super Sevillian Blue' supplied from the Nisshin OilliO Group Ltd. were transplanted into 12-cm pots and pruned on 21st May, 2004 as described in Experiment 1. Plants were placed in a greenhouse maintained at 25℃day/15℃night. Forty-five plants were transferred to a cold room maintained at 2℃ under an 8-h photoperiod by 10-watt fluorescent lamps on 9th July and kept for 10 weeks until 17th September, 2004. After 10-week CT, plants were moved to a natural light phytotron maintained at 23℃day/15℃night under a 16-h photoperiod by natural day light with 4-h of supplemental lighting by 60-watt incandescent lamps in the morning (0400HR-0800HR) and in the evening (1600HR-2000HR), respectively. Watering and fertilization were done if necessary. Two lateral shoot apices and 3 lateral shoot apices from each set of five plants were collected for scanning electron microscope (SEM) and optical microscope analyses on 17th September (0 day after CT), 24th September (7 days after CT), 1st October (14 days after CT), 8th October (21 days after CT), 11th October (24 days after CT), 14th October (27 days after CT), 17th October (30 days after CT), 20th October (33 days after CT), and 23rd October (36 days after CT), respectively. For SEM analyses, shoot apices were previewed under a stereoscopic microscope and then viewed in SEM. The samples were dehydrated using an ethyl alcohol (EtOH) series: 85%, 95% and 100% each for 20 min, and then an EtOH/isoamyl acetate series: 3:1, 1:1 and 1:3 each for 20 min, and finally only isoamyl acetate for 20 min. The specimens were dried in a critical-point drier (HCP-2, Hitachi High-Technologies Corporation, Japan), and were then coated with carbon in an ion coater (H-1010, Hitachi High-Technologies Corporation, Japan). Observations were made using a SEM (S-3500N: High-Technologies Corporation, Japan) at 5 kV accelerating voltage. For optical microscope analyses, shoot apices were embedded in plastic resins by using Technovit 7100 (Heraeus Kulzer, Wehrheim, Germany). The embedded tissues were sliced into 6-μm sections. The sections were then dyed with 0.1 % toluidine blue solution. Observations were made using an optical microscope (BX41, Olympus Corporation, Japan).

Floral stages were classified into 8 stages as follows based on morphological observations under microscopes; 0, vegetative; Ⅰ, dome formation; Ⅱ, sepal initiation; Ⅲ, petal initiation; Ⅳ, petal formation; Ⅴ, stamen initiation; Ⅵ, stamen formation; and Ⅶ, pistil initiation (Fig. 1). Stage 0 was the vegetative control. As *L.* × *intermedia* has an indeterminate inflorescence, the first axillary bud in the shoot apex will grow as the first floret in a normal flowering process. Thus, the floral stage of each shoot was decided based on that of the first floret in the shoot apex. Two shoots

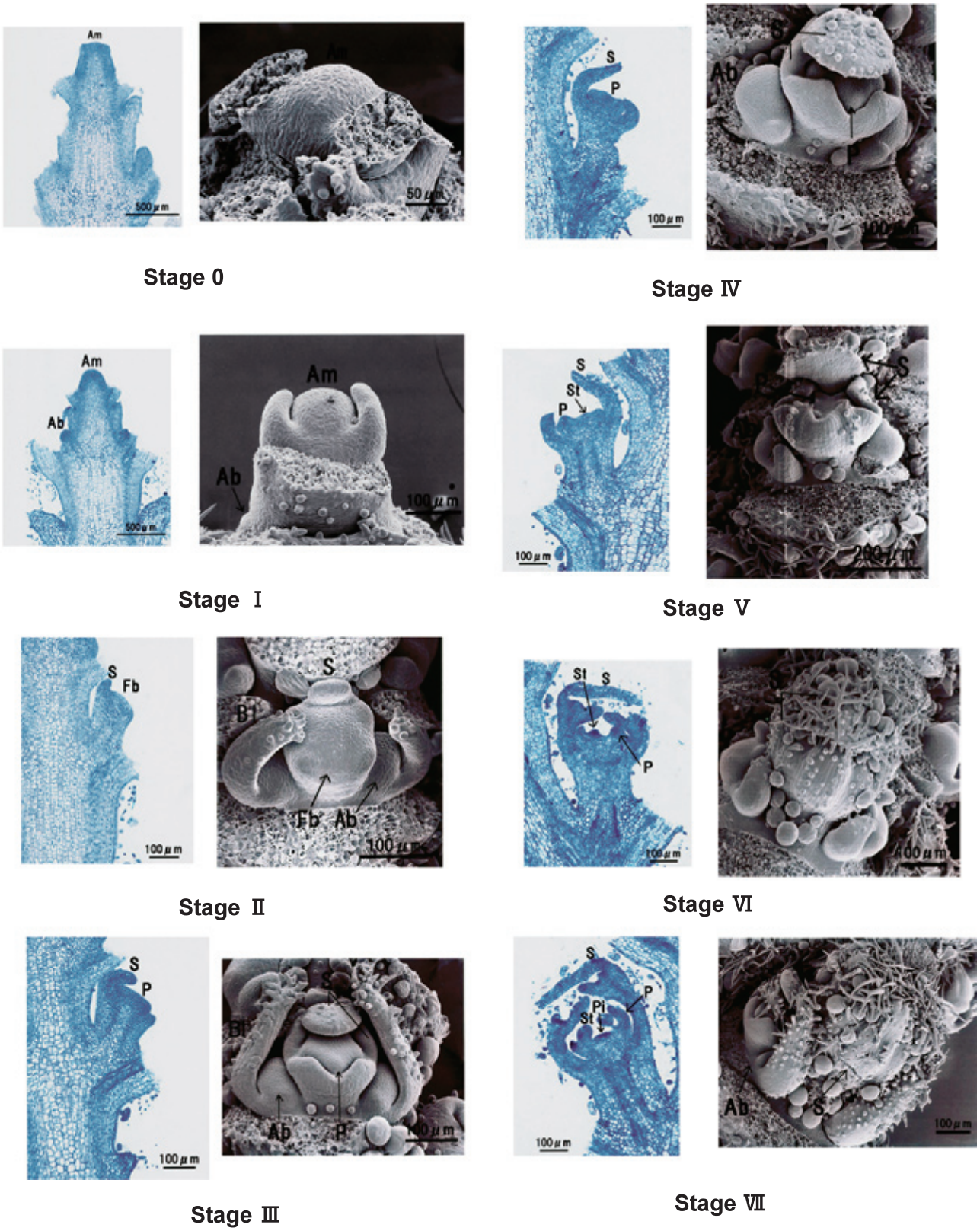

Fig. 1. Microscopic observations of floral stages in *L.* × *intermedia* 'Super Sevillian Blue'
Stage 0, vegetative; Stage Ⅰ, dome formation; Stage Ⅱ, sepal initiation; Stage Ⅲ, petal initiation; Stage Ⅳ, petal formation; Stage Ⅴ, stamen initiation; Stage Ⅵ, stamen formation; Stage Ⅶ, pistil initiation. Am: apical meristem; Ab: axillary bud; Bl: bract leaf; Fb: flower bud; P: petal; Pi: pistil; S: sepal; St: stamen.

of each plant were used for determining the floral stage.

Results

1. Photoperiod on flowering

Diurnal temperatures during the 14-week CT under natural winter condition changed between 10℃ and 15℃ in day and between -2℃ and 3℃ in night, respectively. As shown in Table 1, flowering was observed in plants treated with a 14-week CT. The rate of flowering plants increased as day length increased. Those with 13-h and 14-h day lengths were both 100%. Similarly, the rate of flowering shoots increased as day length increased, although that did not reach 100% even with a 14-h day length. On the other hand, days to VB decreased as day length increased. Similarly, days to FLW decreased as day length increased. There were significant differences in rate of flowering plants, rate of flowering shoots, days to VB and days to FLW among day lengths. There was no significant difference in number of florets among day lengths.

2. Cold treatment on flowering

Plants without CT and plants after a 6-week CT did not initiate floral buds in a subsequent forcing condition as shown in Table 2. On the other hand, plants after a 10-week CT showed 100% in both rates of flowering plants and flowering shoots. Plants after an 8-week CT showed 100% in rate of flowering plants but 75.6% in rate of flowering shoots. The days to VB and FLW in plants after an 8-week CT were 10.7 days and 13.3 days more than those after a 10-week CT, respectively. There were significant differences in rate of flowering shoots, days to VB, days to FLW and number of florets between an 8-week CT and a 10-week CT.

3. Initiation and development of floral buds

As shown in Fig. 2, all shoot apices were vegetable (Stage 0) at the end of a 10-week CT. Although the first floret of the shoot apex formed a dome shape at Stage Ⅰ, it was considered that the first floret had clearly initiated reproductive development when it had reached Stage Ⅱ, in which it had begun to initiate sepals. At 7 days after CT, 80% of the first floret of each shoot apex was still in Stage 0, whereas the first floret of each shoot apex reached either Stage Ⅰ or Stage Ⅱ at 14 days after CT. At 21 days after CT, 90% of the first floret of each shoot apex reached reproductive developmental stages, either Stage Ⅱ or Stage Ⅲ. Then, the first floret of each shoot apex gradually initiated floral leaves and reached either Stage Ⅶ or Stage Ⅵ at 36 days after CT.

Discussion

Many woody perennials do not flower until they reach a certain stage of maturity; up to that stage they are said to

Table 1. Effect of day length on flowering of *L.* × *intermedia* 'Super Sevillian Blue' after cold treatment in Experiment 1

Day Length (hr)	Rate of flowering plants (%)	Rate of flowering shoots (%)	Days to visible bud	Days to first open flower	Number of florets
11	66.6a	57.8a	49a	101a	59.8a
12	93.3b	74.3ab	44b	96b	72.2a
13	100c	76.0ab	41b	93b	69.5a
14	100c	90.7b	34c	86c	57.3a

Values labeled with the same letter are not different at the 5% significant level.

Table 2. Effect of cold treatment length on flowering of *L.* × *intermedia* 'Super Sevillian Blue' in Experiment 2

Length of cold treatment (Weeks)	Rate of flowering plants (%)	Rate of flowering shoots (%)	Days to visible bud	Days to first open flower	Number of florets
0	0	0	-	-	-
6	0	0	-	-	-
8	100	75.6	35.6	77.9	73.1
10	100	100	24.9	64.6	90.4
Significant difference beween 8-weeks and 10-weeks	NS	**	**	**	*

*,**: Significant treatment effects at P<0.01 and P<0.05, respectively.
NS: not significant.

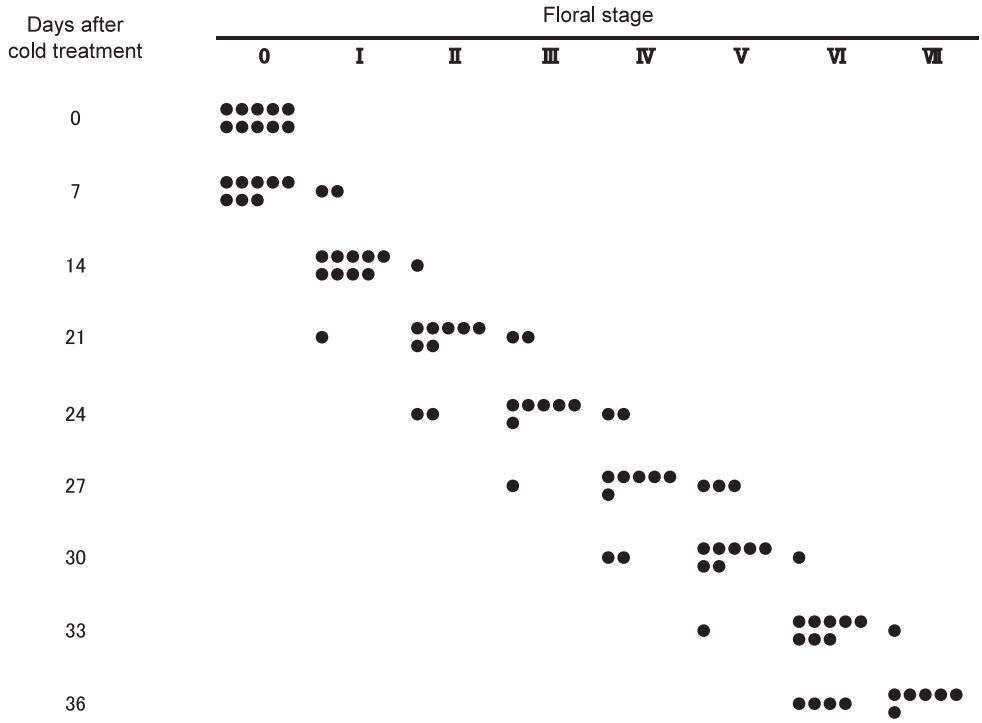

Fig. 2. Floral stages of *L.* × *intermedia* 'Super Sevillian Blue'in days after 10 weeks cold treatment at 5℃
Microscopic observation of floral stage is shown in Fig. 1. Stage 0, vegetative; Stage Ⅰ, dome formation; Stage Ⅱ, sepal initiation; Stage Ⅲ, petal initiation; Stage Ⅳ, petal formation; Stage Ⅴ, stamen initiation; Stage Ⅵ, stamen formation; Stage Ⅶ, pistil initiation. One circle indicates one shoot.

be juvenile[5]. As *L. angustifolia* 'Munstead' which is one parent of *L.* × *intermedia* has been shown to have a juvenile phase which appeared to end after the plants had about 8 to 11 nodes[7], *L.* × *intermedia* may have the same nature as *L. angustifolia*. Therefore, we used plants of *L.* × *intermedia* propagated from cuttings but not seedlings for excluding juvenility.

Low temperatures are required for flowering of many herbaceous perennials. The requirement for low temperature differs among plant species. Some species require a period of low temperature for initiation of development of floral primordia in subsequent appropriate condition after exposure to low temperature and some species initiate floral leaves during exposure to low temperature. Furthermore, floral initiation will not occur until photoperiodic requirements are met as well in some species[1]. Our preliminary experiments showed that a low temperature requirement was stringent for flowering of *L.* × *intermedia*, that the plants propagated from cuttings required a 113-day CT under natural winter condition for subsequent flowering in a greenhouse maintained at 25℃day/15℃night under natural day length, and that a 14-h day length hastened flowering of the plants in a subsequent forcing condition (unpublished data). In the present experiments, the exposure to a 14-week CT under natural winter condition seemed to be enough to induce flowering, although flowering ratios varied among day lengths (Table 1). Increasing duration of day length was significantly associated with an increase in rates of flowering plants and flowering shoots and with a decrease in days to VB and FLW. However, there was no difference in days between VB and FLW among day lengths (Table 1). Thus, day length affected floral initiation but not floral development. Besides, we confirmed that longer day length (16-h) promoted more flowering in a different experiment done at the same time and under the same environmental conditions. Plants under an 8-h photoperiod of natural day light with 8-h of supplemental lighting by 60-watt incandescent lamps showed 100% in rate of flowering plants, 100% in rate of flowering shoots, 27 days to VB, 77 days to FLW and a floret number of 105, respectively. These suggest that *L.* × *intermedia* 'Super Sevillian Blue' may be an essential quantitative long-day plant. Therefore, we decided to choose a 16-h day length in Experiments 2 and 3.

On the other hand, when plants were constantly treated with low temperature in a cold room at 5℃, even a duration of 10-week CT resulted in perfect flowering, namely, 100% in both rates of flowering plants and flowering shoots (Table 2). Plants without CT and plants after a 6-week CT did

not initiate floral buds. Plants after an 8-week CT showed 100% in rate of flowering plants, although a lower rate of flowering shoots was observed than that after a 10-week CT. Increasing durations of CT was associated with an increase in rate of flowering shoots and with a decrease in days to VB and FLW (Table 2). These suggest that exposure to a period of low temperature is the primary factor promoting flowering in *L.* × *intermedia* 'Super Sevillian Blue' and mainly affects the floral initiation but not floral development and that a critical duration of CT for perfect flowering might be in between 8 and 10 weeks in the present experiments.

L. angustifolia 'Hidcote' showed no visible changes in the meristems during CT at 4℃, although histological data have not yet been demonstrated[4]. In the present experiments, apical dissections and SEM showed vegetative meristems at the end of 10-week CT at 5℃, and then dome formation and sepal formation of the first floret of each shoot apex were observed at 7 and 21 days after CT, respectively (Figs. 1 & 2). Two effects of cold treatment on flowering are known[6]. In the typical vernalization "after effect", floral primordial of cold-treated plants are not present at the time cold treatment is completed, and they differentiate later only when the plants are returned to higher temperatures. In the second type of cold-treatment effect, the so-called "direct effect", floral primordial are actually initiated during the period of slow growth with cold treatment. In the present experiment, we confirmed that *L.* × *intermedia* 'Super Sevillian Blue' showed an "after effect" flowering but not a "direct effect" flowering based on the histological results. Therefore, we conclude that *L.* × *intermedia* 'Super Sevillian Blue' is a typical vernalization type plant and may be an essential quantitative long-day plant.

Acknowledgments

We thank the Nisshin OilliO Group Ltd. for supplying plant materials, and Mrs. Daichi Muneoka, Katsuhisa Takaku and Souhei Miyake, and Misses Miho Arai, Kaori Miura, Sawako Kamoi, Nana Tsukahara, Tomoyo Numagami, Asami Komoda, Natsumi Kobayashi, and Emi Haga for their technical assistances.

References

1. Chouard, P. (1960) Vernalization and its relations to dormancy. *Ann. Rev. Plant Physiol.,* **11**, 191-238.
2. Iversen, R. & Weiler, T. (1994) Strategies to force flowering of six herbaceous garden perennials. *HortTechnology*, **4**, 61-65.
3. McNaughton, V. (2000) Lavender. Timber Press, Inc., Portland, Oregon, pp.107.
4. Monaghan, J. M., Wurr, D. C. E. & Fellows, J. R. (2004) The effects of temperature and lighting on flowering of lavender (*Lavandula angustifolia* 'Hidcote'). *J. Hort. Sci. & Biotech.,* **79**, 811-817.
5. Taiz, L. & Zeiger, E. (2006) Plant physiology. Sinauer Associate Inc., Massachusetts, pp.512.
6. Thomas, B. & Vince-Prue, D. (1984). Juvenility, photoperiodism and vernalization. *In* Advanced plant physiology, ed. Wilkins, M. B., Longman Scientific & Technical, Essex, 408-439.
7. Whitman, C. M. et al. (1996) Cold treatments, photoperiod, and forcing temperature influence flowering of *Lavandula angustifolia*. *HortScience*, **31**, 1150-1153.