

Seasonal Variability in Dormancy and Flowering Competence in Chrysanthemum: Chilling impacts on shoot extension growth and flowering capacity

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Abstract

Chrysanthemum (*Chrysanthemum morifolium* Ramat.), an herbaceous perennial short-day plant, shows seasonality in its regulation of shoot extension growth and flowering capacity. To enhance our understanding of the physiological mechanisms of the seasonality in chrysanthemum, we investigated the changes in growth and flowering capacity, and the variation among cultivars. We also evaluated seasonal changes and the response to chilling in shoot extension and flowering capacity in chrysanthemum under controlled environmental conditions (20°C/15°C (light/dark) with a 12-h light period). Both shoot extension and flowering capacity under the subsequent growing conditions were regulated by the temperature of prior growing conditions. After the temperature increased over the summer, chrysanthemums showed a reduced ability for extension growth, and became more difficult to induce flowering. Chilling stimulated the extension growth of shoots when the meristems were entering a dormant state. Chilling had the opposite effect on two regulatory pathways involved in flowering capacity. A pathway related to the meristem dormant state was suppressed by exposure to chilling and induced flowering, and chilling activated an inhibitory regulator in parallel.

Discipline: Horticulture

Additional key words: *Chrysanthemum morifolium*, chilling, flowering

Introduction

Chrysanthemum (*Chrysanthemum morifolium* Ramat.) is an herbaceous perennial short-day (SD) plant. The effects of environmental factors on its growth and flowering are well-documented (Cathey 1969, Cockshull 1985). In late autumn, the plants form rosetted suckers from underground parts. The development of rosettes in late autumn is an adaptive response by chrysanthemum that might evolve in its environment of origin to enable survival during winter (e.g. reviewed in Vegis 1964, Thomas and Vince-Prue 1997). These suckers show little growth during winter, but begin shoot elongation in early spring and continue vegetative growth in the long summer photoperiods. Their flowering is induced when the photoperiod becomes shorter than the critical day-length

for flowering. The photoperiod is known to have a major effect on the flowering of chrysanthemum. Variation in flowering time from early summer to winter mainly reflects differences in critical day-length for flowering (Kawata 1987, Kawata et al. 1987). Temperature is also a major seasonal cue for flowering. The shoots that form a rosette lack the ability for flowering. Chilling reverses rosette stature; the plant rapidly begins extension growth, and flowering occurs under moderate temperature and short day-lengths (Schwabe 1950, Okada 1959). It has also been observed that when plants are chilled over winter, they can be insensitive to flower inductive conditions (Kawata 1987, Uda et al. 1988). Kawata (1987) introduced the concept of phasic development in chrysanthemum to offer a better understanding of their natural flowering time in Japan. Nevertheless, the terms used in the

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concept contribute to the confusion in terminology. To enhance our understanding of physiological mechanisms and molecular aspects of seasonality in chrysanthemum, the terms are increasingly important. Anyway, it can be interpreted to mean that the environmental signals of the prior season are an important factor that determines the growth and flowering capacity of chrysanthemum in subsequent seasons. To enhance our understanding of seasonality in chrysanthemums, we evaluated the effects of temperature from prior growing conditions on growth and flowering under standard climatic conditions.

Material and methods

1. Changes in shoot extension and flowering competence (Experiment 1)

Four cultivars ('City', 'Shuho-no-chikara', 'Kinyo' and 'Reagan') were used in this study. Plants that were exposed to the natural climate during winter (from December 1, 2000 to March 1, 2001) in the field ($34^{\circ}45'N$, $136^{\circ}27'E$; at Tsu, Mie, Japan; Fig. 1) were used as the mother plants and were transferred to a greenhouse maintained at temperatures above $15^{\circ}C$, with a night break (2200 to 0200) from incandescent light (K-RD100V60W; Matsushita Electric Industrial Co., Ltd., Osaka, Japan) to prevent flowering. The first and second cuttings for the experiment were harvested from the mother plants on March 15 and April 5, respectively. The rooted cuttings were transplanted to pots (12 cm in internal diameter [ID]) containing mixed soil of a fertilized medium (Kureha Engeibaido, Kureha Chemical Industry Co., Ltd., Tokyo, Japan) consisting of perlite and vermiculite (2:1:1). Liquid fertilizer containing 100 mg·L⁻¹ of N was administered as required. The rooted cuttings

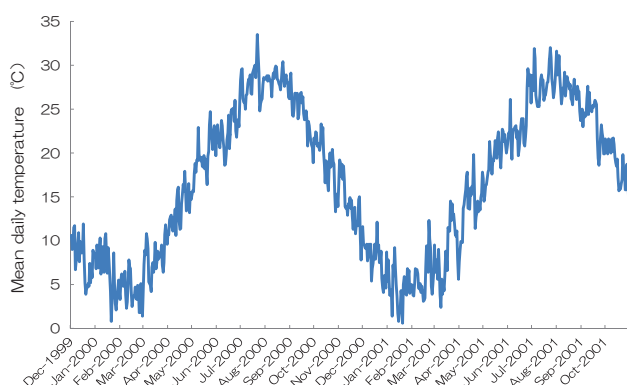


Fig. 1. Mean daily temperatures at the experimental site (Tsu, Mie, Japan) between December 1999 and October 2001.

were grown in the greenhouse as described above for six weeks after cutting before being transferred to a controlled growth room every 3 weeks on (1) April 26, (2) May 17, (3) June 7 (4) June 28 (5) July 19 (6) August 9 (7) August 30 (8) September 20, and (9) October 11, 2001. The environmental conditions of the growth room were $20^{\circ}C/15^{\circ}C$ (light/dark), with a 12-h light period during which metal halide lamps were used ($150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, D-400, Toshiba Co., Tokyo, Japan). All plants were pinched when transferred into the growth room. The pinched shoots were used as cuttings for the next set of plants (Fig. 2). Lateral shoots, except the shoot in the axil of the top-most leaf on the main shoot were removed 2 weeks later. Observations of the growth and flowering of 12 plants per treatment were made for 9 weeks. We measured the axillary bud growth, number of days until the flower buds were visible, number of nodes per shoot at flowering, length of the stem, and length of the internode (between the 4th and 5th nodes from the bottom). If no flower buds were visible after 9 weeks, the shoot tips were dissected under a stereoscopic microscope, and the number of leaf primordia and floral stages were recorded. The stage of floral development of each plant was indicated as follows: stage 0, vegetative shoot apex; stage 1, dome-shaped stage; stage 2, first stage of involucre formation; stage 3, final stage of involucre formation; stage 4, first stage of floret formation; stage 5, final stage of floret formation; stage 6, first stage of corolla formation; and stage 7, final stage of corolla formation.

2. Effect of chilling on shoot extension and flowering competence (Experiment 2)

One line and five cultivars (line 94-4008, 'City', 'Shuho-no-chikara', 'Nagano-queen', 'Kinyo' and

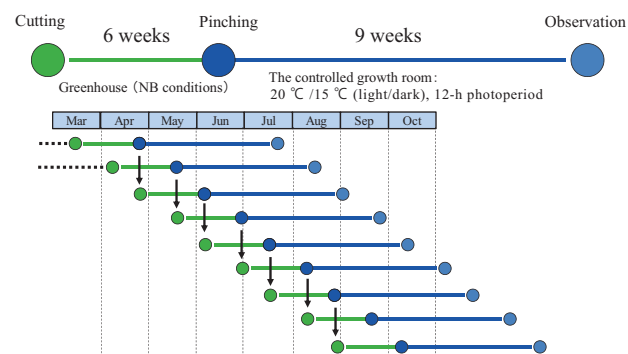


Fig. 2. Schematic representation of experimental conditions in Experiment 1.

Arrows show the pinched shoots used as cuttings for the next set of plants.

'Reagan') were used in this experiment. Plants that were exposed to the natural climate during winter in the field (from December 1, 1999 to March 20, 2000; Fig. 1) were used as mother plants and transferred to a greenhouse, as described in Experiment 1. Cuttings for this experiment were harvested from the mother plants on August 24, 2000. The rooted cuttings were transplanted into pots (9 cm in ID) containing mixed soil and then grown in the greenhouse for 6 weeks after cutting, before being transferred to a controlled cold growth room maintained at 3°C for 0, 4, 8, or 12 weeks. Cold treatments began on October 6, 2000. During cold treatment, the plants were kept under night break conditions (a main 12-h light period with a 4-h night break supplied by fluorescent tubes, FL40SW; Mitsubishi Co., Ltd., Tokyo, Japan). After cold treatment, the plants were transplanted into pots (12 cm in ID) containing mixed soil and then placed

in a controlled growth room, as described in Experiment 1. The plants were grown in a growth room for 8 weeks. Growth and flowering were assessed as described in Experiment 1.

Results

1. Changes in shoot extension and flowering competence (Experiment 1)

The total stem length was not a suitable index for shoot extension growth because the number of nodes at flowering affected the stem length. Therefore, the length of the internode (between the 4th and 5th nodes from the bottom) was used as an index for shoot extension growth (Fig. 3). The number of nodes at flowering was used as an index for flowering.

In all of the cultivars tested, the internode extension growth showed the same seasonal pattern. Raising the temperature during summer reduced the internode extension (Figs. 3 and 4). The cultivars differed in their internode lengths, which under growing conditions that supported the greatest elongation ranged from 4.3

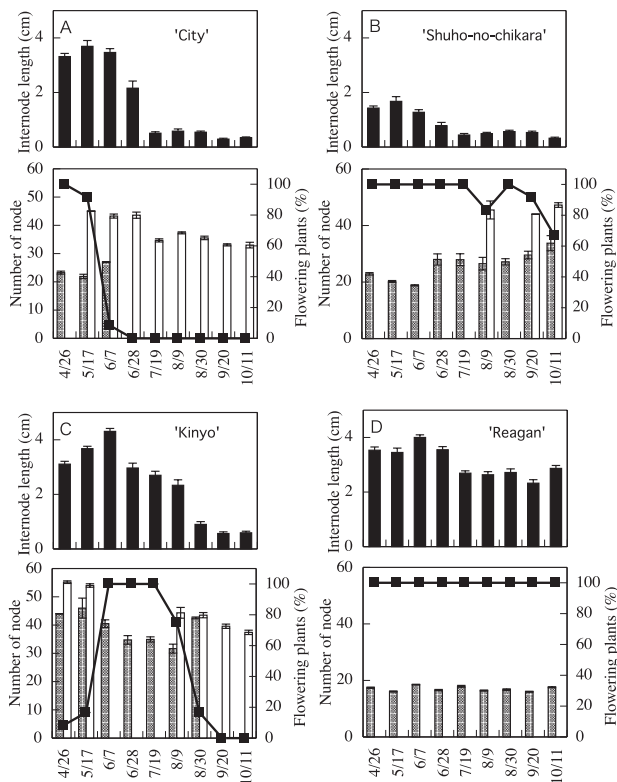


Fig. 3. Changes in growth and flowering competence in four chrysanthemum cultivars (A-D).

The x axes show the date of transfer from the greenhouse to control environments. Black bars in the upper panels show internode lengths. Grey and white bars in the lower panels show the number of nodes in flowering and non-flowering plants, respectively. Line shows the percentage of flowering plants. Plants were grown at 20°C/15°C (light/dark), with a 12-h light period for 9 weeks. Data are mean $\pm$ SE values ($n = 12$ plants).

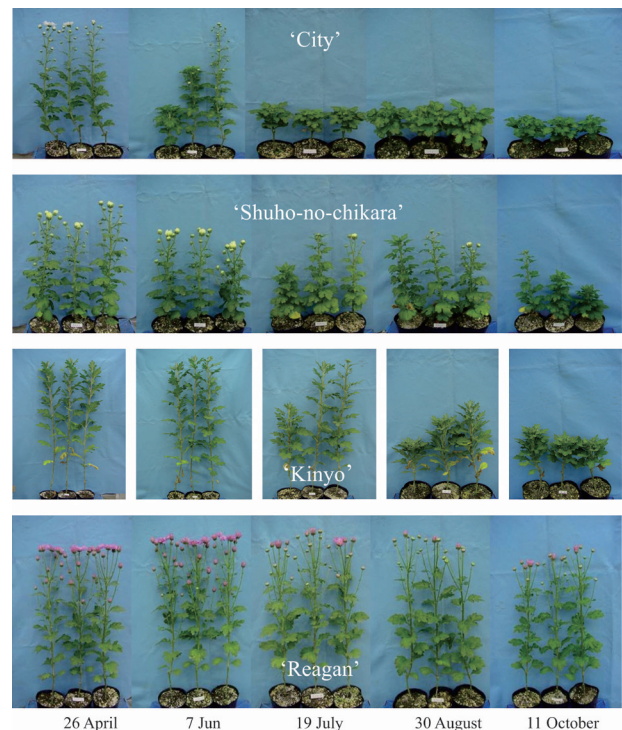


Fig. 4. Seasonal changes in shoot extension growth and flowering competence in four chrysanthemum cultivars (Experiment 1).

The date is the date of transfer from the greenhouse to control environments. Plants were grown at 20 °C / 15 °C (light/dark), with a 12- h light period for 9 weeks.

cm ('Kinyo') to 1.7 cm ('Shuho-no-chikara'). Under minimum growth conditions, internode lengths varied from 0.3 cm ('City' and 'Shuho-no-chikara') to 2.3 cm ('Reagan'). The cultivars with the shortest internode lengths of about 0.5 cm formed a rosette.

The cultivars also demonstrated different flowering responses (Figs. 3 and 4). All plants from cultivar 'City' (Fig. 3A) flowered when transferred on April 26. The rate of flowering rapidly decreased compared to 'Shuho-no-chikara'. After June 28, there was no flowering. The number of nodes in non-flowering plants at the time of harvest decreased after July 19. The decrease in the flowering rate in 'Shuho-no-chikara' (Fig. 3B) was evident as late as August, with the first substantial decrease in transfers observed on August 9. Nevertheless, 66% of 'Shuho-no-chikara' plants were flowering on October 11. The number of nodes at flowering decreased slightly until June 7, and then increased. The cultivar 'Kinyo' (Fig. 3C) showed a flowering pattern different from those previously described. Its flowering was low on April 26, reached 100% on June 7, and then decreased

after August 9. The number of nodes at flowering decreased until August 9, and then increased. Flowering in 'Reagan' (Fig. 3D) was not affected by the growing conditions before transfer to the growth room. Its flowering was consistently high during the experimental period. The number of nodes at flowering also showed no changes during the experimental period.

2. Effect of chilling on shoot extension and flowering competence (Experiment 2)

In this experiment, we began cold treatment on October 6. According to the results of Experiment 1, if a cultivar has a rosette habit, it should form a rosette under our experimental conditions at this time.

The cultivars tested showed different shoot extension and flowering responses (Figs. 5 and 6). Line 94-4008 and 'City' demonstrated a strong rosette habit without chilling, and all plants formed a rosette. Chilling reversed internode extension and promoted flowering (Fig. 5A and B). 'Shuho-no-chikara' also illustrated a rosette habit, although weaker than that of line 94-4008 or 'City'

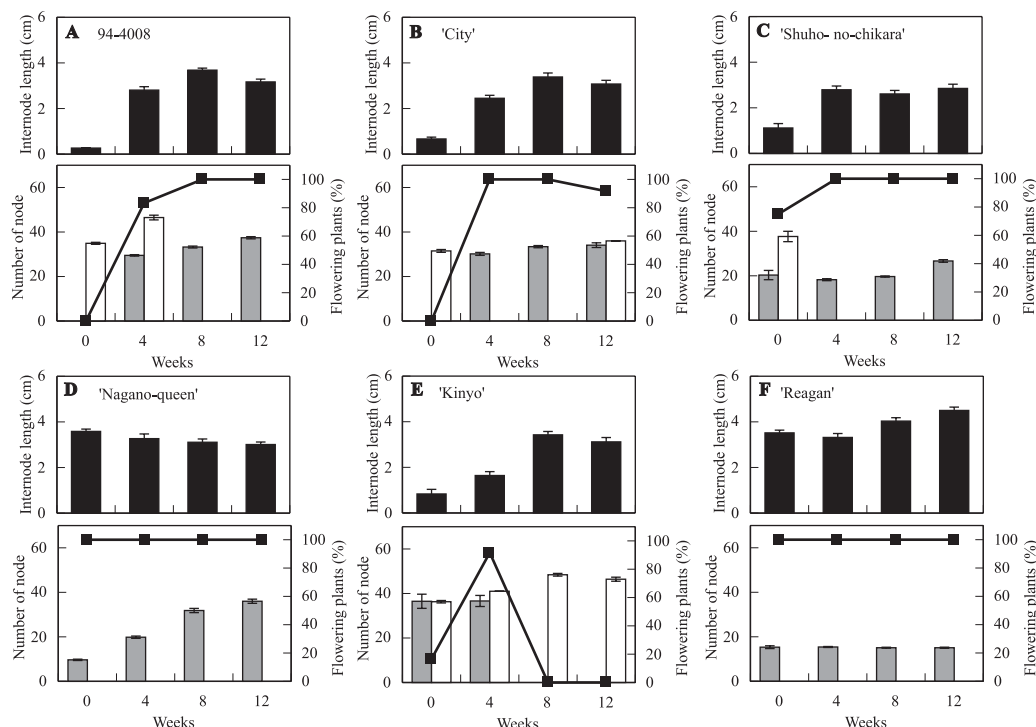


Fig. 5. Effect of pre-chilling (3°C) duration on growth and flowering competence in one line and five chrysanthemum cultivars (A-F).

The x axes show the duration of pre-chilling. Black bars in the upper panels show internode lengths. Grey and white bars in the lower panels show the number of nodes in flowering and non-flowering plants, respectively. Line shows the percentage of flowering plants. Plants were grown at 20°C/15°C (light/dark), with a 12-h light period for 8 weeks. Data are mean $\pm$ SE values (n = 12 plants).

(Fig. 5C). Though 75% of the ‘Shuho-no-chikara’ plants flowered without chilling, chilling promoted internode extension and flowering, but the number of nodes at flowering during the 12-week chilling period was slightly higher than that during the 4- or 8-week chilling period. ‘Nagano-queen’ did not show a rosette habit; the cultivar could flower without chilling (Fig. 5D). However, the number of nodes at flowering in ‘Nagano-queen’ increased with longer exposure to chilling. There was no effect of chilling on internode extension. The cultivar

‘Kinyo’ had a rosette habit with a complex response (Fig. 5E). Chilling both promoted and inhibited flowering. Only 17% of the plants flowered without chilling. All plants initiated flowering after 4 weeks of chilling, but no flowering was observed after 8 and 12 weeks of chilling. The internode extension was promoted by chilling, and longer chilling treatments had stronger effects than short chilling periods. ‘Reagan’ did not show changes in flower induction capacity (Fig. 5F) in response to chilling, which slightly promoted the internode extension of ‘Reagan’.

Discussion

1. Changes in shoot extension and flowering potential

Although it was known that chrysanthemum shows seasonal changes in shoot extension and flowering competence, the mechanism of these changes remains unclear (Mason and Vince 1962, Konishi 1980, Kawata 1987, Kawata et al. 1987, Shibata 1997). In chrysanthemum, differences in growing conditions prior to transfer to standard climatic conditions had clear effects on shoot extension and flowering competence (Figs. 3, 4, 5 and 6). All the cultivars tested demonstrated the same tendency; after the temperature increased over summer, a reduced potential for internode extension was observed, and it became more difficult to induce flowering. Such reduction in internode extension led to rosette formation. Conversely, it has been shown that chrysanthemum plants that were isolated from high summer-temperature conditions did not exhibit a rosette type of growth (Konishi 1980). This raises the question, is rosette growth behaviour in chrysanthemums their dormant state? In strawberry (*Fragaria* × *ananassa*), an herbaceous perennial plant, a decrease in the potential for vegetative growth (leaf and petiole growth) is observed after exposure to natural summer and autumnal climates (Robert et. al. 1999, Sønsteby and Heide 2006). In both chrysanthemum and strawberry, the development of a rosette type of growth (or stunted growth) is an apparent adaptive response for survival in winter. Dormancy is considered an adaptive response for survival under unfavourable conditions (e.g. reviewed in Vegis 1964, Thomas and Vince-Prue 1997). In strawberry, although the dormant state is only quantitative, stunted plants are generally considered to be dormant (Vegis 1964, Guttridge 1969 & 1985, Sønsteby and Heide 2006). In several species from high latitudes, dormancy release requires exposure to chilling temperature. The decrease in the potential for vegetative growth of strawberry is released by exposure to chilling (Guttridge 1985). In chrysanthemum, chilling temperature stimulates the capacity for stem extension growth and prevents or



Fig. 6. Effect of pre-chilling (3°C) duration on growth and flowering competence in one line and four chrysanthemum cultivars (Experiment 2).

Plants were grown at 20°C/15°C (light/dark), with a 12-h light period for 8 weeks.

releases plants from a rosette type of growth (Schwabe 1950, Okada 1959, Konishi 1975; Fig. 5). Thus, chilling restores growth ability and releases the plant from the dormant state in the apical buds. Taken together, the rosette growth behaviour in chrysanthemum is similar to the dormant state in strawberry. The environmental factors controlling the induction of dormancy, including photoperiod and/or temperature, have been reported (Lang et al. 1987). In this study, all the plants were grown under the same night break conditions, so the effect of photoperiod could be eliminated. Thus, our results suggest that a high temperature condition over summer is one of the important environmental signals that enable a decrease in extension growth potential and an induction of the dormant condition in chrysanthemum.

2. Chilling has two principal effects on flowering capacity

In chrysanthemum, once the apical buds enter a dormant state, they require exposure to chilling to become responsive to short photoperiods and induce flowering. The chilling requirement for flowering competence in chrysanthemum was called “vernalization” by Schwabe (1950), and it emphasizes the promotive effects of chilling on flowering that were confirmed in these experiments (Fig. 5). A recent understanding of the molecular basis of vernalization in *Arabidopsis* and temperate cereals supports the hypothesis. The expression of the floral repressor *FLOWERING LOCUS C* (for *Arabidopsis*) or *VERNALIZATION2* (for temperate cereals), is repressed by chilling (Greenup et al. 2009). The reduction of the floral repressor induced by chilling accelerates flowering. Presumably, the response to chilling in chrysanthemum may overcome a block that prevents flowering (Fig. 5). The floral repressor related to the dormant state (inhibitory factor I in Fig. 7B) may be developed under a high temperature condition over summer. However, chilling can also have the opposite effect on flowering in chrysanthemum.

Our results clearly showed the existence of a response to chilling that inhibited flowering, as well as the existence of genetic variations (Fig. 5). The existence of a second inhibitory factor was apparent in cultivars such as ‘Nagano queen’ that lacked the inhibiting regulation related to the dormant state in the meristem (related to rosette growth habit). In these plants, it became more difficult to induce flowering (increasing the number of nodes at flowering) when exposed to chilling under our experimental conditions (Fig. 5). The inhibitory effect of chilling became stronger as the duration of chilling increased. Chilled plants appeared to be insensitive to the floral-inductive short photoperiod. Such inhibition of

flowering by chilling, first reported for chrysanthemum by Higuchi and Hara (1975), leads to an insensitivity to flower in inductive short photoperiods in early spring (Uda et al. 1988, Shibata 1997). This inhibition by chilling was proposed to reflect “the juvenile phase” (Kawata 1987). Comparable inhibition of flowering after breaking dormancy by exposure to chilling has also been reported in strawberry (Guttridge 1985, Battey 2000). This inhibition offers an explanation for the failure of plants to initiate flowering during spring when the photoperiod conditions are short enough to induce flowering. The genetic variation of this inhibition of flowering could be involved in the determination of flowering time in nature, as proposed by Kawata (1987).

Typically, chrysanthemum cultivars showed both the promotion and inhibition of flowering, depending on the duration of chilling (Fig. 5). We propose that there are at least two important regulators that contribute to flowering competence. The genetic variations observed in this study indicate that these two forms of regulation are apparently distinct, and that the chilling temperature independently regulated both factors. For example, in ‘Kinyo’ we observed a drastic change in flowering capacity from April to October (Fig. 3). This can be explained because the regulation developed by chilling dominantly suppresses flowering, without a reduction in extension growth in spring, and the other form of regulation related to dormancy dominantly suppresses flowering, with a reduction in extension growth from late summer to autumn. These proposed regulators suggest that chilling exposure during winter induces flowering competency by removing the inhibition related to dormancy (Fig. 7B). The other form of inhibition appears to be coincident with the decrease in inhibition related to dormancy. ‘Reagan’ might not have either of these inhibitory regulators because it showed stable flowering throughout the experimental periods (Fig. 5).

3. Conclusion

In chrysanthemum, the dormant meristem lacks the ability for shoot extension and flowering. Chilling had principal effects on the three regulatory networks, namely, the network that regulates extension growth competence and the two networks involved in flowering competence (Fig. 7). First, chilling stimulates the capacity of shoot extension growth when the apical buds are entering a dormant condition (dormancy release). Second, somewhat like a vernalization response, chrysanthemum requires chilling to remove the action of the floral repressor to initiate flowering competence when the apical buds enter a dormant condition. The commonalities between the signals that regulate endodormancy release and the

chilling requirement for flowering (vernalization) provide the hypothesis that these two processes might share the mechanisms of action (Horvath 2009). Third, chilling prevents flowering competence. The inhibitory effect of chilling became stronger as the duration of chilling increased. Chilled plants appeared to be less sensitive to the subsequent floral inductive short photoperiod. The balance of these three responses determines seasonality in the regulation of extension growth and flowering competence in chrysanthemum. This balance thus enables plants to align their life cycle with favourable environmental conditions.

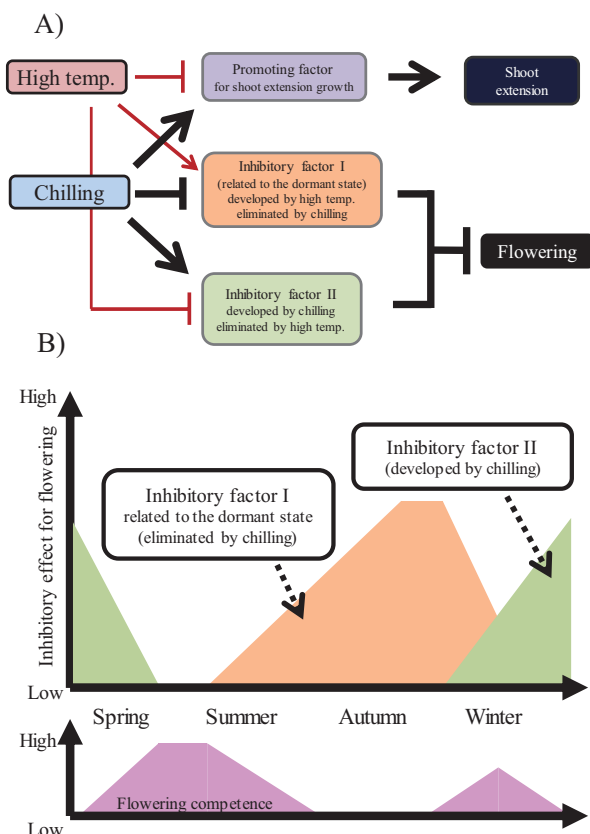


Fig. 7. (A) A model for the interactions between chilling, shoot extension, and flowering in chrysanthemum. Chilling has principal effects on three regulatory networks, namely, the network involved in the competence for extension growth and the two networks that play a role in flowering competence. Arrows represent promotion. T bars represent inhibition. (B) A model for seasonal change in flowering competence in chrysanthemum (case example based on 'Kinyo').

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