Involvement of the ethylene response pathway in dormancy induction in chrysanthemum

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Abstract

Temperature plays a significant role in the annual cycling between growth and dormancy of the herbaceous perennial chrysanthemum (Chrysanthemum morifolium Ramat.). After exposure to high summer temperatures, cool temperature triggers dormancy. The cessation of flowering and rosette formation by the cessation of elongation are characteristic of dormant plants, and can be stimulated by exogenous ethylene. Thus, the ethylene response pathway may be involved in temperature-induced dormancy of chrysanthemum. Transgenic chrysanthemums expressing a mutated ethylene receptor gene were used to assess this involvement. The transgenic lines showed reduced ethylene sensitivity: ethylene causes leaf yellowing in wild-type chrysanthemums, but leaves remained green in the transgenic lines. Extension growth and flowering of wild-type and transgenic lines varied between temperatures: at 20 °C176°C, the transgenic lines showed the same stem elongation and flowering as the wild type; at cooler temperatures, the wild type formed rosettes with an inability to flower and entered dormancy, but some transgenic lines continued to elongate and flower. This supports the involvement of the ethylene response pathway in the temperature-induced dormancy of chrysanthemum. At the highest dosage of ethephon, an ethylene-releasing agent, wild-type plants formed rosettes with an inability to flower and became dormant, but one transgenic line did not. This confirms that dormancy is induced via the ethylene response pathway.

Key words: Chrysanthemum, dormancy, ethylene, flowering, rosette formation.

Introduction

Chrysanthemums (Chrysanthemum morifolium Ramat.) are important ornamental plants around the world. Seasonal changes in extension growth and flowering of this short-day (SD) herbaceous perennial are an adaptation to a temperate climate in middle latitudes. Growth and flowering depend on the combination of growing temperature, daylength, and the environment of the previous season (Schwabe, 1950, 1955; Sumitomo et al., 2008; T Hisamatsu, unpublished data). Temperature is significant, and the temperature of the previous season is an important factor in determining the capacity for growth and flowering in the subsequent season. Because long-term exposure to chilling in winter increases this capacity, plants with a high capacity show strong positive growth in spring and summer. Exposure to summer heat reduces this capacity. This decrease narrows the temperature range over which extension growth and flowering are possible (Vegis, 1964), and chrysanthemum plants show slower extension growth and flowering in autumn than in spring at the same growing temperature (Sumitomo et al., 2008; T Hisamatsu, unpublished data). For example, at growing temperatures of 15/10 °C (light/dark), ‘Sei-marine’ plants showed rapid extension and flowering in April (spring), but showed neither late in August (autumn) after exposure to high temperatures (Sumitomo et al., 2008). Plants
exposed to summer heat form rosettes by a cessation of elongation, never flower, and become dormant under subsequent cool temperature (<15 °C) in late autumn. They continue leaf expansion in winter, although much more slowly. Thus, the dormant state in chrysanthemum plants is quantitative and can be considered a state of semi-dormancy, as in strawberry (*Fragaria × ananassa*; Sønstebey and Heide, 2006). The dormant plants have a large chilling requirement for the subsequent resumption of elongation (Schwabe, 1950).

Growth cessation and dormancy are adaptive responses enabling survival during seasons when environmental conditions are most threatening (Vegis, 1964). Rosette formation is the main adaptive response, maintaining the viability of meristems under winter cold, similar to the formation of terminal buds in woody perennials. In chrysanthemum, first high temperatures and then cool temperatures are significant environmental cues for the formation of rosettes before winter (Schwabe, 1955; Sumitomo et al., 2008; T Hisamatsu, unpublished data).

The physiological mechanisms involved in the development of dormancy in woody perennials have been investigated. In aspen trees (*Populus tremula × tremuloides*), a phytochrome gene is involved in photoperiodically induced dormancy (Olsen et al., 1997), and the CO/FT regulatory module controls the SD-induced growth cessation and formation of terminal buds (Böhlenius et al., 2006). Signalling mechanisms mediated by plant hormones regulate the induction of dormancy. ABA is well known to be involved in the regulation of dormancy (Horvath et al., 2003). In addition, the gaseous plant hormone ethylene has been shown to play a role in the induction of bud dormancy in birch (*Betula pendula; Ruonala et al., 2006*).

Exogenous ethylene suppresses flower initiation and internode elongation in chrysanthemum (Tjia et al., 1969), as does 2-chloroethylyphosphonic acid (ethephon), which is hydrolysed and releases to ethylene in plant tissues (Warner and Leopold, 1969; Kher et al., 1974; Cockshull and Horridge, 1978). Ethephon has been shown to induce rosette formation in chrysanthemum (Sumitomo et al., 2008). In potato (*Solanum tuberosum*), ethylene has been shown to induce bud dormancy in microtubers, and the competitive ethylene antagonist 2,5-norbornadiene has been shown to promote microtuber sprouting (Suttle, 1998). These results suggest that the ethylene response pathway may be involved in the dormancy of herbaceous perennials. However, the role of ethylene is poorly understood.

To investigate the involvement of the ethylene response pathway in temperature-induced rosette formation in chrysanthemum, the dormancy and growth of transgenic chrysanthemum plants with reduced sensitivity to ethylene under three temperature regimes were evaluated. The ethylene-low-sensitive plants elongated more than the wild type and did not form rosettes, and flowered under cooler temperatures. These findings suggest that the ethylene response pathway is involved in the induction of dormancy in chrysanthemum.

**Materials and methods**

**Effects of an ethylene precursor on growth and flowering**

Flowering of chrysanthemum is inhibited by an exposure to light given during the night (night break). Stock plants of cultivar ‘Sei-marine’ (supplied by Seikoen Co. Ltd, Hiroshima, Japan) were grown in a greenhouse (maintained at an air temperature above 18 °C, and ventilated when it rose above 25 °C) under a natural photoperiod plus a 5 h night break from 22.00 h to 03.00 h with incandescent lamps (K-RD100V60W; Matsushita Electric Industrial Co. Ltd, Osaka, Japan). Rooted cuttings from stock plants were planted in 7.5 cm plastic pots containing a commercial horticultural soil (Kureha-Engi-Baido; Kureha Chemical Co. Ltd, Tochigi, Japan) and allowed to establish under the same conditions. When plants had four or five expanded leaves, they were transferred to a growth chamber, which was controlled at 20/15 °C (light/dark) with a floral-inducing 8 h photoperiod. The light was supplied by fluorescent tubes (FL40SW; Mitsubishi Co. Ltd, Tokyo, Japan) at a photosynthetic photon flux density (PPFD) of 200 μmol m⁻² s⁻¹. On the day of transfer into the growth chamber, 1-aminocyclopropane-1-carboxylic acid (ACC; Sigma-Aldrich Japan K.K., Tokyo, Japan), an ethylene precursor, was applied to shoot tips in 10 μl of water containing 0, 10, or 50 μg μl⁻¹ active ingredient. Each treatment consisted of 13 plants. At the end of the experiment, 9 weeks after treatment had started, the number of flowering plants was recorded, and the internode length between the eighth and ninth leaves above the leaf that was the topmost expanded leaf at the beginning of the treatment was measured. Plants with no visible buds were dissected under a binocular microscope to see whether apices had initiated flower buds.

**Transgenic plants**

Transgenic lines of ‘Sei-marine’ were obtained by introducing mutated ethylene receptor genes, which were generated from the chrysanthemum ethylene receptor gene (DG-ERS1, GenBank accession no. AF547624; Narumi et al., 2005a) by introducing one-nucleotide substitutions (Narumi et al., 2005b). Nos 10, 19, 33, and 45 expressed a mutated DG-ERS1 (mDG-ERS1(etr1-4)) carrying the same dominant mutation as in Arabidopsis *etr1-4* (Chang et al., 1993). Nos 21 and 27 expressed a mutated DG-ERS1 (mDG-ERS1(Nr)) carrying the same dominant mutation as in tomato *Nr* (Wilkinson et al., 1995).

**Real-time PCR**

The abundance of transcripts of transgenes was investigated in shoot tips by using quantitative real-time PCR (Q-PCR). Shoot tips 5 mm long were collected from wild-type and transgenic plants grown in a closed greenhouse (maintained at an air temperature above 18 °C, and ventilated when it rose above 25 °C) under natural photoperiod plus a 5 h night break. Total RNA was extracted with an RNeasy Plant Mini Kit (Qiagen, USA), and treated with RNase-free DNase (Qiagen) according to the manufacturer’s instructions. For each sample, 500 ng of total RNA was reverse-transcribed using a Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics K.K., Tokyo, Japan) according to the manufacturer’s instructions. The cDNA was diluted to 20% of its original concentration, and 5 μl was used in a 15 μl Q-PCR reaction with SYBR Premix Ex Taq (Takara Bio Inc., Shiga, Japan) and treated with RNase-free DNase (Qiagen) according to the manufacturer’s instructions. For each sample, 500 ng of total RNA was reverse-transcribed using a Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics K.K., Tokyo, Japan) according to the manufacturer’s instructions.
Evaluation of ethylene sensitivity by leaf yellowing assay

Leaf yellowing is a typical ethylene response in chrysanthemum (Doi et al., 2003). Six stems each of wild-type and transgenic plants grown to a length of 30 cm in a closed greenhouse were harvested (maintained at an air temperature above 18 °C, and ventilated when it rose above 25 °C) under natural photoperiod plus a 5 h night break. The leaves on the basal 10 cm were removed, and stems were inserted into 300 ml glass vessels containing 200 ml of deionized water. The stems in the vessels were kept at 23 °C and 70% relative humidity, under a 12 h photoperiod at a PPFD of 10 µmol m⁻² s⁻¹ supplied by fluorescent tubes. The water was replaced every 2 d. On day 10, the stems were placed for 48 h in a 50 l acrylic resin chamber containing ethylene at 1 µl l⁻¹ (v/v) under the same conditions. The colour of the lowest leaf on the stem was determined by measurement of the L*, a*, and b* values with a spectrophotometer (CM-2600d; Konica Minolta Holdings, Inc., determined by measurement of the L*, a*, and b* values with same conditions. The colour of the lowest leaf on the stem was transferred to a growth chamber controlled at 20/15 °C (light/dark) with a floral-inducing 8 h photoperiod. The PPFD was 200 µmol m⁻² s⁻¹ supplied from fluorescent tubes.

Ethylene biosynthesis can be altered by feedback effects of reduced ethylene sensitivity in transgenic lines. So ethephon was used as an ethylene-releasing agent, because ethylene evolution by decomposition of ethephon depends only on temperature in chrysanthemums (Sumitomo et al., 2008). On the day of transfer into the growth chamber, all plants received one spray treatment of ethephon (to run-off) to all aerial parts at 0, 100, or 1000 mg l⁻¹ Ethrel-10 (Ishihara Sangyo Kaisha Ltd, Osaka, Japan) and 0.025% (v/v) Triton X-100. Each treatment consisted of 16 plants. Flowering was recorded as above, and stem length was measured twice: on transfer into the growth chamber and at the end of the experiment, 9 weeks after transfer.

Results

Effects of ACC on growth and flowering

ACC treatment decreased the percentage of flowering plants and internode length compared with plants given 0 ACC (Table 1). ACC inhibited extension growth and flowering more strongly at 500 µg per plant than at 100 µg: plants given 500 µg produced no flowers, almost ceased extension growth, and formed a rosette; thus, they entered dormancy.

PCR analysis

Shoot tips of all transgenic lines expressed the transgenes (Fig. 1A). The relative expression level of endogenous DG-ERS1 to CmACTIN was the same as or higher than the wild-type level in shoot tips of transgenic lines (Fig. 1B). Expression of the transgenes up-regulated endogenous DG-ERS1 transcription in lines Nos 10, 21, and 27.

| Table 1. Effects of ACC on flowering and internode length of chrysanthemum ‘Sei-marine’ |
|---------------------------------|-----------------|-----------------|
| Total ACC dose (µg plant⁻¹)      | Number of flowering plants (%) | Internode lengthc (mm) |
|---------------------------------|-----------------|-----------------|
| 0                               | 61.5            | 10.1±0.6        |
| 100                             | 15.4            | 2.8±0.4         |
| 500                             | 0               | 1.1±0.1         |

* Length between eighth and ninth leaves above the leaf that was the topmost expanded leaf at the beginning of treatment.
ethylene starting on day 10, the colour value in the wild type increased sharply at day 18, and leaves on the stems showed yellowing. On the other hand, the increase in the colour values of the transgenic lines was very small, and leaves remained green after exposure to ethylene.

Growth and flowering of transgenic lines under lower temperatures

The percentage of flowering plants of wild-type and transgenic chrysanthemum 'Sei-marine' was 100% at 20 °C (Fig. 3A, C, D). It reduced to 75% at 17.5 °C and 0% at 15 °C in the wild type, but remained at 100% in the transgenic lines at 17.5 °C and between 10% (No. 10) and 100% (No. 33) at 15 °C except in No. 19, which did not flower. All transgenic lines but No. 10 showed the same elongation as the wild type at 20 °C (Fig. 3B). Stem elongation in all lines showed a consistent tendency to decrease as the temperature dropped. Wild-type and transgenic lines showed no difference in stem elongation at 17.5 °C. Stem elongation in wild-type plants decreased to 4 cm and plants formed rosettes at 15 °C. Nos 10, 19, 45, and 27 also ceased stem elongation and formed rosettes, but Nos 21 and 33 kept growing (Fig. 3B–D).

Reduced effects of ethephon on growth and flowering of transgenic line No. 33

In the wild type, ethephon treatment decreased the percentage of flowering plants and stem elongation (Table 2; Fig. 4). The suppression was stronger as the concentration increased; no plants that received 1000 mg l⁻¹ ethephon flowered. On the other hand, in No. 33, ethephon treatment did not reduce the percentage of flowering plants, although the number of days to the appearance of flower buds increased (data not shown). Stem elongation in both lines decreased as the concentration of ethephon increased. The wild-type plants that received 1000 mg l⁻¹ ethephon formed upper rosettes (Fig. 4). Decreases in stem elongation were smaller in No. 33 than in the wild type.

Discussion

The growing temperature and the temperature of the previous season are important factors determining seasonality in growth and flowering in chrysanthemum (Schwabe, 1950, 1955; Konishi, 1980; Sumitomo et al., 2008; T Hisamatsu, unpublished data). Under cool temperatures, plants previously exposed to high temperatures form rosettes by ceasing extension growth, and
failed to flower. This can be regarded as a kind of dormancy.

Exogenous ethylene and ethephon reduce internode elongation and inhibit flower induction (Tjia et al., 1969; Kher et al., 1974; Cockshull and Horridge, 1978; Konishi et al., 1985). Our results confirm these responses. The plants that received the highest dosage of ACC and ethephon formed rosettes with an inability to flower and entered dormancy (Tables 1, 2). This result suggests that ethylene is involved in the entry to dormancy. Therefore, the involvement of the ethylene response pathway in the temperature-induced dormancy of transgenic chrysanthemums with reduced ethylene sensitivity was investigated (Narumi et al., 2005b).
analysis confirmed the expression of transgenes and the up-regulated expression of endogenous *DG-ERS1* mRNA in shoot tips of the transgenic lines (Fig. 1). This increased *DG-ERS1* expression might increase the level of ethylene receptor proteins, and cause an additive reduction of ethylene sensitivity, since ethylene receptors negatively regulate the ethylene response (Hua and Meyerowitz, 1998). The transgenic lines apparently showed reduced ethylene sensitivity compared with the wild type in the present conditions (Fig. 2) with the leaf yellowing assay established by Doi *et al.* (2004). There was little variation in the leaf yellowing response among the selected transgenic lines, although the variation has been observed on primitive screening with the *in vitro* transgenic plants (Narumi *et al.*, 2005b).

The wild-type plants showed reduced flowering and extension growth as the growing temperature dropped, and at 15 °C, they entered dormancy, forming rosettes with an inability to flower (Fig. 3). Some transgenic lines showed greater extension growth and flowering than the wild type. This difference suggests that the ethylene response pathway is involved in the temperature-induced inhibition of extension growth and flowering. In contrast to the leaf yellowing response, the transgenic lines showed variation in the response of extension growth and flowering to temperature: *No. 33* showed no reduction in flowering and a smaller decrease in stem elongation than the other transgenic lines as the temperature dropped. Although Q-PCR analysis showed variation in the expression levels of transgenes and endogenous *DG-ERS1* mRNA in the transgenic lines, there was no quantitative correspondence to the suppression of dormancy. Aida *et al.* (2008) reported that the levels of GUS activity did not quantitatively correspond to the expression levels of GUS mRNA, but depended on the translational efficiency of transgenes in a GUS assay of chrysanthemum transformants. Post-transcriptional regulation might alter the level of protein production of transgenes in this experiment. To confirm that dormancy is induced via the ethylene response pathway, the effect of exogenous ethylene in *No. 33*, which showed no entry to dormancy at 15 °C was investigated. *No. 33* showed reduced responses to ethephon, and did not enter dormancy even at the highest dosage, although ethephon induced dormancy in the wild type (Table 2; Fig. 4). These results confirm that the ethylene response pathway is involved in dormancy of the chrysanthemum.

In herbaceous perennial chrysanthemums, low temperature controls both dormancy induction (in autumn) and its release (in winter), as reported for the woody Rosaceae family (Heide and Prestrud, 2005; Heide, 2008). Thus, our results strongly suggest that the ethylene response pathway is at least involved in dormancy induction in chrysanthemum, as reported in potato microtuber dormancy (Suttle, 1998) and in birch bud dormancy (Ruonala *et al.*, 2006). So far, there is no evidence for its involvement in the dormancy release processes during low temperature in chrysanthemum.

### Table 2. Effects of ethephon on flowering and stem elongation of wild type and transgenic line *No. 33* of chrysanthemum ‘Sei-marine’

When plants were transferred to a growth chamber controlled at 20/15 °C (light/dark) with an 8 h photoperiod, one spray treatment of ethephon (0, 100, or 1000 mg l⁻¹) was applied. Data were collected 6 weeks after ethephon treatment. Values are means ±SE (n=16). **, Significant differences between wild type and *No. 33* at the same rate of ethephon (ANOVA: P < 0.01).

| Ethephon content (mg l⁻¹) | Percentage of flowering plants | Wild type | No.33 |
|--------------------------|-------------------------------|-----------|-------|
| 0                        | 100                           | 100       |       |
| 100                      | 31.3                          | 100       |       |
| 1000                     | 0                             | 100       |       |
| Stem elongation (cm)     |                               |           |       |
| 0                        | 21.5±0.3                      | 22.6±0.3  |       |
| 100                      | 11.4±0.8                      | 20.6±0.8**|       |
| 1000                     | 6.6±0.2                       | 15.5±0.9**|       |

![Fig. 4](https://academic.oup.com/jxb/article-abstract/59/15/4075/516069/4080-Sumitomo-et-al)
Flower bud initiation on the main stem is induced by shortening of the photoperiod under cooling temperatures (18–23 °C) in late summer. The main stem flowers, fruits, and then dies before winter. Suckers emerge from its base in response to the disappearance of its apical dominance in mid to late autumn. These suckers soon enter dormancy and form rosettes with an inability to flower, even under SD floral-inducing conditions, in response to cool temperatures (Fig. 5). Here, there were differences between extension growth and flowering responses to decreasing growing temperature. Although the reduction of extension growth in Nos 10, 19, 27, and 45 was similar to that in the wild type, the inhibition of flowering as temperature dropped was smaller than in the wild type (Fig. 3A, B). Moreover, No. 33 showed no inhibition of flowering, even though extension growth decreased as temperature dropped. These results suggest that the reduced ethylene sensitivity was responsible more for the inhibition of the temperature-induced reduction of flowering than for the inhibition of elongation under lower temperatures. In birch, SD triggers the cessation of elongation growth and the formation of terminal buds. In ethylene-insensitive transgenic birches, the formation of terminal buds was inhibited, but elongation was still reduced under SD, suggesting that ethylene facilitates the SD-induced terminal bud formation (Ruonala et al., 2006). Our results suggest that the ethylene response pathway facilitates the temperature-induced inhibition of flowering in apical buds, not the reduction of stem elongation, associated with dormancy of chrysanthemums. The dormant buds are thus saved from flowering. This is very important behaviour to allow chrysanthemums to survive during winter. If flowering were induced in all buds on suckers in late autumn, the following cold would seriously damage them, and the plants could not resume growth the next year. Our results suggest that the ethylene response pathway plays a role in saving buds by inhibiting floral induction under cool SD conditions. Ethylene might therefore play an important role as a gaseous signal molecule in characterizing a perennial’s growth behaviour (the annual cycling between growth and dormancy) that permits survival in winter.

**Supplementary data**

Supplementary data can be found at JXB online.

**Fig. S1.** Average daily air temperatures in a closed greenhouse, where stock plants of wild-type and transgenic chrysanthemum ‘Sei-marine’ were grown during summer.

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**References**

Aida R, Narumi T, Ohtsubo N, Yamaguchi H, Kato K, Shinmyo A, Shibata M. 2008. Improved translation efficiency in chrysanthemum and torenia with a translational enhancer derived from the tobacco alcohol dehydrogenase gene. *Plant Biotechnology* 25, 69–75.

Bohlenius H, Huang T, Charbonnel-Campaa L, Brunner AM, Jansson S, Strauss SH, Nilsson O. 2006. *CO/FT* regulatory module controls timing of flowering and seasonal growth cessation in trees. *Science* 312, 1040–1043.

Chang C, Kwok SF, Bleecker AB, Meyerowitz EM. 1993. *Arabidopsis* ethylene response gene *ETR1*: similarity of product to two-component regulators. *Science* 252, 539–544.

Cockshull KE, Horridge JS. 1978. 2-Chloroethylphosphonic acid and flower initiation by *Chrysanthemum morifolium* Ramat. in short days and in long days. *Journal of Horticultural Science* 53, 85–90.

Doi M, Nakagawa Y, Watabe S, Aoe K, Inamoto K, Imanishi H. 2003. Ethylene-induced leaf yellowing in cut chrysanthemums (*Dendranthema grandiflora* Kitamura). *Journal of the Japanese Society for Horticultural Science* 72, 533–535.

Doi M, Watabe S, Aoe K, Inamoto K, Imanishi H. 2004. Leaf yellowing of cut chrysanthemum (*Dendranthema grandiflora* Kitamura) ‘Shuho-no-chikara’ induced by ethylene and the post-harvest increase in ethylene sensitivity. *Journal of the Japanese Society for Horticultural Science* 73, 229–234.

Heide OM. 2008. Interaction of photoperiod and temperature in the control of growth and dormancy of *Prunus* species. *Scientia Horticulturae* 115, 309–314.

Heide OM, Prestrud AK. 2005. Low temperature, but not photoperiod, controls growth cessation and dormancy induction and release in apple and pear. *Tree Physiology* 25, 109–114.

Horvath DP, Anderson JV, Chao WS, Foley ME. 2003. Knowing when to grow: signals regulating bud dormancy. *Trends in Plant Science* 8, 534–540.

Hua J, Meyerowitz EM. 1998. Ethylene responses are negatively regulated by a receptor gene family in *Arabidopsis thaliana*. *Cell* 94, 261–271.

Kher MA, Yokoi M, Kosugi K. 1974. Effect of Ethrel on the growth and flower formation in pot chrysanthemums. *Journal of the Japanese Society for Horticultural Science* 43, 91–96.

Konishi K. 1980. On the rosetting of chrysanthemum plants. *Journal of the Japanese Society for Horticultural Science* 49, 107–113 (In Japanese with an English summary).

Konishi K, Kajihara S, Kageyama Y. 1985. Inducing rosette formation in chrysanthemum plants by ethephon treatment. *Journal of the Japanese Society for Horticultural Science* 54, 89–93 (In Japanese with an English summary).
Narumi T, Aida R, Ohmiya A, Satoh S. 2005b. Transformation of chrysanthemum with mutated ethylene receptor genes: mDG-ERS1 transgenes conferring reduced ethylene sensitivity and characterization of the transformants. Postharvest Biology and Technology 37, 101–110.

Narumi T, Kanno Y, Suzuki M, Kishimoto S, Ohmiya A, Satoh S. 2005a. Cloning of a cDNA encoding an ethylene receptor (DG-ERS1) from chrysanthemum and comparison of its mRNA level in ethylene-sensitive and -insensitive cultivars. Postharvest Biology and Technology 36, 21–30.

Olsen JE, Junntila O, Nilsen J, Eriksson ME, Martinussen I, Olsson O, Sandberg G, Moritz T. 1997. Ectopic expression of oat phytochrome A in hybrid aspen changes critical daylength for growth and prevents cold acclimatization. The Plant Journal 12, 1339–1350.

Ruonala R, Rinne PLH, Baghour M, Moritz T, Tuominen H, Kangasjärvi J. 2006. Transitions in the functioning of the shoot apical meristem in birch (Betula pendula) involve ethylene. The Plant Journal 46, 628–640.

Schwabe WW. 1950. Factors controlling flowering of the chrysanthemum. I. The effects of photoperiod and temporary chilling. Journal of Experimental Botany 1, 329–343.

Schwabe WW. 1955. Factors controlling flowering of the chrysanthemum. V. De-vernalization in relation to high temperature and low light intensity treatments. Journal of Experimental Botany 6, 435–450.

Sønsteby A, Heide OM. 2006. Dormancy relations and flowering of the strawberry cultivars Korona and Elsanta as influenced by photoperiod and temperature. Scientia Horticulturae 110, 57–67.

Sumitomo K, Kunitake T, Douzono M, Onozaki T, Shibata M, Hisamatsu T. 2008. Variation in the effects of ethephon on flowering and extension growth in chrysanthemum as a function of temperature, season, and genetics. Journal of Horticultural Science and Biotechnology 83, (in press).

Suttle JC. 1998. Involvement of ethylene in potato microtuber dormancy. Plant Physiology 118, 843–848.

Tjia BOS, Rogers MN, Hartley DE. 1969. Effects of ethylene on morphology and flowering of Chrysanthemum morifolium Ramat. Journal of the American Society for Horticultural Science 94, 35–39.

Vegis A. 1964. Dormancy in higher plants. Annual Review of Plant Physiology 15, 185–224.

Warner HL, Leopold AC. 1969. Ethylene evolution from 2-chloroethylphosphonic acid. Plant Physiology 44, 156–158.

Wilkinson JQ, Lanahan MB, Yen H-C, Giovannoni JJ, Klee HJ. 1995. An ethylene-inducible component of signal transduction encoded by Never-ripe. Science 270, 1807–1809.