Delay of Flowering at High Temperature in Chrysanthemum: Duration of Darkness and Transitions in Lighting Determine Daily Peak Heat Sensitivity

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Heat-induced flowering delay of Chrysanthemum morifolium is a major problem affecting the production of cut flowers in Japan. Understanding the delay mechanism is indispensable to achieve stable production. Heat sensitivity has been shown to fluctuate throughout the day, as if it is regulated by a circadian clock. This paper studied the involvement of a circadian clock and photoperiod in the fluctuation of heat sensitivity throughout the day by applying pulses of heat at different times to Chrysanthemum seticuspe and Chrysanthemum morifolium under different regimens of light and dark. One experiment examined the elevation of heat sensitivity under different photoperiods in order to determine whether the transition from light to dark or vice versa serves as a signal for a clock-like regulation of heat sensitivity. Maximum heat sensitivity was frequently observed at a constant interval after light-off, but not after light-on, identifying the transition to darkness as the signal that initiates the elevation mechanism. We also examined the descending phase of heat sensitivity. Heat sensitivity peaked approximately 16 h after light-off and then gradually declined under dark conditions, suggesting an endogenous clock-controlled fluctuation. We further studied the effect of light on elevated sensitivity by conducting trials with dark periods of 13 or 16 h. Sensitivity peaked at both 10–13 and 13–16 h after light-off under the 16 h-dark condition, but only at 10–13 h after light-off under the 13 h-dark condition, indicating a rapid decline in heat sensitivity induced by light supplied 13 h after light-off. Results of the three experiments suggest that the daily elevation in heat sensitivity is potentially controlled by an internal clock that is reset by a transition from light to dark. A subsequent transition to light appears to eliminate elevated sensitivity. From this, we conclude that heat sensitivity is maximized toward the end of the night, irrespective of photoperiod, in chrysanthemums.

Key Words: flowering delay, heat sensitivity, night temperature, photoperiod, short-day.

Introduction

One of the most popular ornamental plants worldwide, Chrysanthemum morifolium Ramat. is especially popular in Japan, where it accounts for 40% of cut flower production (Ministry of Agriculture, Forestry and Fisheries, 2019). Heat-induced flowering delay of chrysanthemum has been a major problem since the introduction of year-round-production techniques in Japan. Since the mid-20th century, Western countries have artificially extended the flowering season of chrysanthemum, a short-day (SD) plant, from autumn to all year round by using light extension to inhibit flowering and by dark extension to promote flowering. While heat-induced flowering delay has generally not presented problems in Western countries at higher latitudes, summer temperatures have proven problematic for chrysanthemum flower production in Japan (Whealy et al., 1987; Shibata, 1997). To enable summer production, early-flowering chrysanthemums, known as summer-to-autumn-flowering cultivars, have been bred in Japan (Shibata, 1997). These cultivars can flower under longer periods of daylight and higher temperatures than traditional autumn-flowering plants (Kawata et al., 1987). Nevertheless, flowering delay still disturbs stable production, as flowering ability under high temperature varies substantially among cultivars (Shibata,
Stable production can be achieved by using suitable cultivars and appropriate environmental control. Therefore, understanding how heat affects chrysanthemum flowering is essential. Vegetative growth in chrysanthemum is facilitated rather than inhibited by high temperature, suggesting that reproductive growth is specifically inhibited by heat (Nakano et al., 2013). In chrysanthemum, the FLOWERING LOCUS T-like3 (FTL3) gene is the key promoter of reproductive growth that is expressed in leaves under SD conditions, producing a protein that acts in the shoot apex (Oda et al., 2012). One of the causes of heat-induced delay of flowering is low FTL3 induction in the leaf (Nakano et al., 2013). Induction of FTL3 under high temperature correlates with the heat tolerance of each cultivar (Nakano et al., 2013; Mori et al., 2019).

Cockshull and Kofranek (1994) reported that high temperature during the night affects flowering delay more significantly than daytime temperature, suggesting that temperature sensitivity fluctuates throughout a 24-hour-period. This suggestion is plausible, as the SD-sensing and temperature signal are interdependent for flowering promotion via FTL3 (Higuchi et al., 2013; Nakano et al., 2013). Heat sensitivity is not immediately prompted by a transition to darkness, but rather escalates gradually during dark periods (Nakano et al., 2015). Fluctuations in biological activity throughout the day can be regulated by endogenous circadian mechanisms (Harmer et al., 2001). In plants, light and dark transition signals are important stimuli for the entrainment of the circadian clock to a 24-h cycle by resetting it every day (Harmer et al., 2001). Elucidation of the mechanism that determines heat sensitivity in chrysanthemum will provide broadly applicable information for production that is not limited to the experimental cultivars and conditions examined here. In this study, chrysanthemum day-length and temperature treatments were performed to assess the involvement of light/dark transition signals in the fluctuation of heat sensitivity that leads to flowering delay.

Materials and Methods

Plant materials

The plants were grown in a glasshouse heated at 18°C and ventilated at 25°C. To inhibit floral initiation during plant preparation, night breaking (22:00–04:00) was performed by using incandescent lamps (KRD110V75W/D; Panasonic Corp., Kadoma, Japan) at a photosynthetic photon flux density (PPFD) of about 20 μmol·m⁻²·s⁻¹. The plants were suitably irrigated with groundwater and supplemented with 1/1000 dilution of liquid fertilizer (Kumiai Ekihi 1 gō; Katakura & Co-op Agri Corp., Tokyo, Japan) once every three weeks. Chrysanthemum seticuspe (Maxim.) Hand.-Mazz. f. boreale (Makino) H. Ohashi & Yoneke (accession NIFS-3) was used in this experiment. The flowering characteristics of this plant, which has often been used as an experimental model of the Chrysanthemum genus, are similar to autumn-flowering C. morifolium (e.g., Oda et al., 2012, Higuchi et al., 2013; Nakano et al., 2013; Hirakawa et al., 2019). The NIFS-3 stock plants were cold-treated at 5°C for a month in a chamber (LP1-PL; Nippon Medical & Chemical Instruments Co., Ltd., Osaka, Japan) to avoid dormancy. Cuttings were rooted on a 200-cell plug tray filled with soil (Metro-Mix 350; Sun-Gro Horticulture, Agawam, MA, USA). Rooted plants were transplanted into 7.5 cm plastic pots containing soil (Yokabaida; Hokkaido Peat Moss Co., Ltd., Kounosu, Japan) and established for 2–3 weeks in the glasshouse. The cultivar C. morifolium ‘Sei-shiraito’ (Inochio Seikoen Inc., Fuchu, Japan) was also used. Cuttings were delivered from the nursery and potted as described above. The apical bud was pinched just before the experiment to avoid unexpected floral initiation during shipping and preparation. One lateral shoot per plant was kept for the experiment.

Growth conditions and flowering time measurement

Changes in heat sensitivity were evaluated by exposing the plants to the same magnitude of heat at different times in the photoperiod cycle. The day-length and heat treatments were performed in growth chambers for the 24 h cycles (LPH-350SP; Nippon Medical & Chemical Instruments). The 32 h cycles were conducted in different growth chambers (FL1-2000; Tokyo Rikakikai Co., Ltd., Tokyo, Japan). Optimal and suboptimal temperatures were defined as 20°C and 30°C, respectively (Karlsson et al., 1989; Nakano et al., 2013). Rates of heating and cooling were set to approximately 0.8°C·min⁻¹. Light was supplied with fluorescent tubes (FL40SEX-N-HG; HotaluX, Ltd., Tokyo, Japan) at a PPFD of about 200 μmol·m⁻²·s⁻¹. Initial bract visibility, as viewed from the top of the plant, was identified as visible capitulum development; the first vertical ligulate flower was identified as anthesis. Significant difference tests were performed by using KyPlot software (KyensLab Inc., Tama, Japan).

Experiment 1. Comparison of heat pulse effects under 32 h and 24 h cycles

Control plants (NIFS-3) were grown at a constant temperature of 20°C. Plants were subjected to a cycle of 16 hours-light (h-L)/16 hours-dark (h-D) (Fig. 1A). A four-hour heat pulse of 30°C was applied daily from 20–24 h after light-on (L-on) or 12–16 h after light-off (L-off). Four-hour heat pulse experiments, similar to those described by Nakano et al. (2015), were also performed under 8 h-L/16 h-D conditions (Fig. 1B) to
Experiment 1. Heat pulse treatment under 16 h-L/16 h-D cycles
Heat pulse treatments were applied to NIFS-3 under 16 h-L/16 h-D cycles. Control plants were grown at a constant temperature of 20°C. A four-hour heat pulse at 30°C was applied daily at 0–4, 12–16 or 20–24 h after L-off.

Experiment 2. Heat pulse treatment under 24 h-D/8 h-L cycles
Heat pulse treatments were applied to NIFS-3 under 24 h-D/8 h-L cycles (Fig. 2A). Control plants were grown at a constant temperature of 20°C. A four-hour heat pulse at 30°C was applied daily at 0–4, 12–16 or 20–24 h after L-on.

Experiment 3. Heat pulse at the same time after L-off under different dark/light ratios over 24 h
Heat pulse treatments were applied to NIFS-3 under 13 h-D/11 h-L or 16 h-D/8 h-L conditions (Fig. 3A, B). Control plants were grown at a constant temperature of 20°C. A three-hour heat pulse at 30°C was applied daily at 10–13 or 13–16 h after L-off. ‘Sei-shiraito’ is a summer-to-autumn-flowering C. morifolium cultivar that can flower under a shorter period of darkness than NIFS-3 (Mori et al., 2017). Heat pulse treatments of ‘Sei-shiraito’ were performed under 10 h-D/14 h-L or 15 h-D/9 h-L conditions (Fig. 4A, B). A five-hour heat pulse at 30°C was applied daily at 5–10 or 10–15 h after L-off.

Results
Experiment 1
This experiment studied the relationship between flowering delay and heating time from the moment the light was turned on (L-on) or off (L-off). This was done in order to elucidate the role of the light-to-dark or the dark-to-light transition signal and determine the time at which the heat-sensitivity responsible for flowering delay was highest. Consistent with the fact that the absolute length of darkness is important for flowering (Higuchi et al., 2013), the results showed that the 16 h-D condition was sufficiently long to induce anthesis of NIFS-3 in combination with 16 h-L. The time to visible capitulum, an accepted proxy for the time of floral initiation, did not significantly differ among the temperature conditions under 16 h-D/16 h-D cycles (Fig. 1C), confirming that heat has a lower impact on floral initiation than floral development (Nozaki and Fukai, 2008; Nakano et al., 2013). The heat pulse applied at 20–24 h after L-on did not affect anthesis under 16 h-D/16 h-D cycles. Anthesis was delayed for 8.4 cycles in plants which were heat-treated at 12–16 h after L-off. Under 8 h-L/16 h-D cycles, the number of days to visible capitulum development was slightly higher by heating at 20–24 h after L-on (Fig. 1D). The heat pulse applied at 12–16 h or 20–24 h after L-on delayed anthesis for 4.9 and 13.3 days, respectively. The effect of a heat pulse applied at 20–24 h after L-on was the most significant.

Experiment 2
This experiment was designed to study the dynamics...
of heat sensitivity for up to 24 h beginning at L-off. Temperature conditions did not affect the time to visible capitulum development (Fig. 2B). All of the experimental plants underwent anthesis under 24 h-D/8 h-L conditions. The heat pulse treatments performed at 12–16 h and 20–24 h after L-off delayed anthesis for 4.2 and 1.5 cycles, respectively, compared to the control. The plants treated with heat at 12–16 h after L-off showed an increased delay of anthesis relative to those treated at 20–24 h after L-off.

Experiment 3

In this experiment, a heat pulse was applied at a constant time after L-off (either with or without supplementary lighting) to study the effect of the dawn signal on the fluctuation in heat sensitivity. The control

NIFS-3 plants flowered earlier under 16 h-D/8 h-L than under 13 h-D/11 h-L (Student’s t-test, $P < 0.05$, Fig. 3C, D), confirming the typical SD-induced promotion of anthesis in chrysanthemums. Under the 13 h-D/11 h-L protocol, anthesis was delayed for 12.8 days by a heat pulse applied at 10–13 h after L-off (Fig. 3C). Under 16 h-D/8 h-L conditions, heat pulse treatment applied at 10–13 h and 13–16 h after L-off delayed anthesis for 6.8 and 7 days, respectively (Fig. 3D). There was no difference between these two treatments. The control plants of C. morifolium ‘Sei-shiraito’ flowered earlier under 15 h-D/9 h-L than under 10 h-D/14 h-L (Student’s $t$-test, $P < 0.05$, Fig. 4C, D). Anthesis was delayed for 11.8 days by a heat pulse applied at 5–10 h after L-off under 10 h-D/14 h-L treatment (Fig. 4C). Under 15 h-D/9 h-L treatment, anthesis was delayed for 5.1 and 8.7 days.
days by heat pulses applied at 5–10 h and 10–15 h after L-off, respectively (Fig. 4D). A heat pulse applied at 10–15 h after L-off significantly delayed anthesis, compared to a heat pulse applied at 5–10 h after L-off. Days to visible capitulum development was not affected by temperature in any photoperiod in either NIFS-3 or ‘Sei-shiraito’ (data not shown).

Discussion

This study aimed to understand the mechanisms regulating the fluctuation in heat sensitivity for flowering inhibition in chrysanthemums. The first experiment examined which transition signal (from light to dark or vice versa) resets the clock mechanism involved in the fluctuation of heat sensitivity. The second considered the effect of an extended dark period on heat sensitivity and the third experiment investigated the effect of light on the continuity of elevated heat sensitivity. The data suggest that a transition-to-dark signal, a clock mechanism, and a transition-to-light signal maintain the daily fluctuation in sensitivity. These findings will make it possible to predict sensitivity under various growing conditions.

Transition to a dark signal resets the clock mechanism regulating heat sensitivity

In plants, daily light/dark transitions are important signals that reset the circadian mechanisms regulating biological events (Harmer et al., 2001). Data obtained under 8 h-L/16 h-D conditions implied that heat sensitivity is controlled by a clock mechanism measuring one day (24 h) after L-on or 16 h after L-off in NIFS-3 (Nakano et al., 2015). However, it remained unclear whether the transition from dark to light or vice versa was the signal responsible for resetting the endogenous clock-like mechanism regulating heat sensitivity. In order to resolve this question, we measured the time between each transition and the peak of heat sensitivity under different photoperiods. Under 8 h-L/16 h-D cycles, heat sensitivity was highest at 20–24 h after L-on (Nakano et al., 2015). We hypothesized that peak heat sensitivity would stay constant relative to the initiating signal regardless of photoperiod. The trial we chose to employ was a 16 h-L/16 h-D that accounted for the fact that the dark period must be at least 14 h to induce flowering of NIFS-3 regardless of the light period during non-24 h cycles (Higuchi et al., 2013). Because heat treatment at 20–24 h after L-on resulted in low heat sensitivity under this regimen, we concluded that maximum sensitivity did not occur at a constant time after the L-on signal (Fig. 1C). Elevated temperature sensitivity was observed at 10–24 h after L-off under 32 h and 24 h conditions (Fig. 1D, D). This indicated that the clock mechanism regulating fluctuations in sensitivity is reset by the transition from light to dark.

Transition to light signal eliminates heat sensitivity

In addition to understanding the mechanism underlying peak heat sensitivity, it is also important to understand how this sensitivity diminishes. As extended light or dark conditions are often used to study the characteristics of circadian events in plants, we employed a
24 h-D/8 h-L cycle to observe the heat sensitivity behavior (Fig. 2A). The heat pulse applied at 12–16 h after L-off caused a more significant delay of anthesis than that applied at 20–24 h after L-off (Fig. 2B), showing that sensitivity does not continue to rise during an extended period of darkness. Rather, heat sensitivity appears to be maximal approximately 16 h after the transition to darkness, and then declines gradually. This result suggests that a clock-controlled 24 h fluctuation cycle of heat sensitivity peaks around 16 h after dark transition, although further studies under more varied conditions are needed to confirm this. In contrast to the 24 h-D condition (Fig. 2B), heat-induced flowering delay was not observed with the pulse applied at 16–20 h after L-off (0–4 h after L-on) under the 16 h-D condition (Fig. 1D). This suggests that elevated sensitivity may be suspended by the application of light. To study the daily terminus of peak sensitivity under 24 h cycles, we compared the effects of heat pulses applied for the same number of hours after L-off under different photoperiods (Fig. 3A, B). Plants were sensitive to heat pulses applied at both 10–13 h and 13–16 h after L-off under the 16 h-D condition (Fig. 3D). However, a heat pulse at 13–16 h after L-off had no effect when applied in conjunction with light under the 13 h-D condition (Fig. 3C). These results show that heat sensitivity elevation during a dark period is cancelled out by a transition to light at a time that correlates with the dawn in a natural 24 h light cycle.

Heat sensitivity in C. morifolium

We studied the similarities between effects of photoperiod on heat sensitivity in NIFS-3 plants with the more economically important C. morifolium cultivars. Gradual elevation of heat sensitivity toward the end of the dark period was observed in ‘Sei-shiraito’ under the 15 h-D condition (Fig. 4D). Heat sensitivity was eliminated by a transition to light in ‘Sei-shiraito’ under the 10 h-D condition (Fig. 4C, D). These responses were observed in other cultivars of various genetic backgrounds (Table S1). These results suggest that the mechanism revealed by using NIFS-3 could be applied to produce various C. morifolium cultivars.

Conclusion

This study demonstrated the importance of photoperiod variations in heat sensitivity fluctuations that affect flowering in chrysanthemums (Fig. 5). The findings indicate that diurnal fluctuation in heat sensitivity reaches a maximum during the night irrespective of photoperiod. Heat-induced flowering delay is caused by suppression of FTL3 expression, a chrysanthemum florigen (Nakano et al., 2013). Further, SD induction of the FTL3 gene is significantly inhibited by heating towards the second half of the night (Nakano et al., 2015), suggesting that daily fluctuation in heat sensitivity may correlate with the rhythm of FTL3 regulation. It has been suggested that FTL3 expression is regulated by a circadian clock gene (Oda et al., 2017, 2020), daylength (Oda et al., 2012; Nakano et al., 2019) and self-induction of FTL3 (Nakano et al., 2019), as well as temperature. The relationship among these regulatory factors and the mechanisms responsible for maximum heat-sensitivity warrant further study. Previous reports have shown that the reproductive stage is dependent on fluctuations in heat sensitivity: the middle phases of capitulum development (e.g., differentiation and development of the florets) are more heat-sensitive than the transition of a vegetative shoot meristem to an inflorescence meristem and subsequent capitulum growth (Nozaki and Fukai, 2008; Nakano et al., 2013). Knowledge of variations in heat sensitivity throughout the day and reproduction will help determine the optimal timing for environmental control techniques such as cooling and dark extension. Such knowledge will also enable efficient characterization and selection of cultivars. The data collected in this and previous studies can provide guidance; however, they may not fully reflect actual growing conditions. Heat treatment of NIFS-3 during periods of relatively low heat sensitivity coupled with exposure to light resulted in a slight, but significant, delay in flowering (Nakano et al., 2015). Extremely high temperatures during the daytime may have much greater effects on summer flowering. Therefore, fluctuations in heat sensitivity in chrysanthemum need to be considered in the context of daily temperature fluctuations.
Supplements

Supplemental material is available at <https://doi.org/10.2503/hortj.UTD-192>.

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Literature Cited

Cockshull, K. E. and A. M. Kofranek. 1994. High night temperatures delay flowering, produce abnormal flowers and retard stem growth of cut-flower chrysanthemums. Sci. Hort. 56: 217–234.

Harmer, S. L., S. Panda and S. A. Kay. 2001. Molecular basis of circadian rhythms. Ann. Rev. Cell Dev. Biol. 17: 215–253.

Higuchi, Y., T. Narumi, A. Oda, Y. Nakano, K. Sumitomo, S. Fukai and T. Hisamatsu. 2013. The gated induction of a systemic floral inhibitor, antiflorigen, determines obligate short-day flowering in chrysanthemums. Proc. Natl. Acad. Sci. USA 110: 17137–17142.

Hirakawa, H., K. Sumitomo, T. Hisamatsu, S. Nagano, K. Shirasawa, Y. Higuchi, M. Kusaba, M. Koshioka, Y. Nakano, M. Yagi, H. Yamaguchi, K. Taniguchi, M. Nakano and S. Isobe. 2019. De novo whole-genome assembly in Chrysanthemum seticuspe, a model species of Chrysanthemums, and its application to genetic and gene discovery analysis. DNA Res. 26: 195–203.

Karlsson, M. G., R. D. Heins, J. E. Erwin, R. D. Berghage, W. H. Carlson and J. A. Biernbaum. 1989. Irradiance and temperature effects on time of development and flower size in chrysanthemums. Sci. Hort. 39: 257–267.

Kawata, J., T. Toyoda, M. Uda, M. Okimura, M. Shibata, T. Kameno, M. Amano, Y. Nakamura and T. Matsuda. 1987. Factors controlling the flowering time of chrysanthemums. Bull. Natl. Res. Inst. Veg. Ornam. Plants Tea A1: 187–222 (In Japanese with English abstract).

Ministry of Agriculture, Forestry and Fisheries. 2019. Heisei 30 nendo kaki seisan shukka toukei. Ministry of Agriculture, Forestry and Fisheries. <http://www.maff.go.jp/j/tokei/kouhyou/sakumotou/sakkyou_kaki/index.html> (In Japanese).

Mori, Y., Y. Nakano, Y. Hayashi, S. Takahashi, T. Hisamatsu and K. Sumitomo. 2019. Genetic variation in the effect of high temperature on flowering and FLOWERING LOCUS T-like 3 gene repression in summer-to-autumn-flowering small-flowered spray-type chrysanthemums. Hort. Res. (Japan) 18: 381–390 (In Japanese with English abstract).

Mori, Y., Y. Suzuki, A. Yamagata, S. Murazaki, M. Takada, T. Yabuki, N. Yokoi, M. Mato, H. Tazuke, T. Nagai, S. Yano, T. Ogawa, N. Hirose, A. Oda, Y. Nakano, T. Hisamatsu and K. Sumitomo. 2017. Selection of summer-to-autumn-flowering small-flowered spray-type chrysanthemums for fine control of flowering using light culture. Hort. Res. (Japan) 16: 27–39 (In Japanese with English abstract).

Nakano, Y., Y. Higuchi, K. Sumitomo and T. Hisamatsu. 2013. Flowering retardation by high temperature in chrysanthemums: involvement of FLOWERING LOCUS T-like 3 gene repression. J. Exp. Bot. 64: 909–920.

Nakano, Y., Y. Higuchi, K. Sumitomo and T. Hisamatsu. 2015. Delay of flowering by high temperature in chrysanthemum: heat-sensitive time-of-day and heat effects on CsFTL3 and CsAFT gene expression. J. Hort. Sci. Biotech. 90: 143–149.

Nakano, Y., T. Takase, S. Takahashi, Y. Higuchi, K. Sumitomo and T. Hisamatsu. 2019. Chrysanthemum requires short-day repeats for anthesis: Gradual CsFTL3 induction through a feedback loop under short-day conditions. Plant Sci. 283: 247–255.

Nozaki, K. and S. Fukai. 2008. Effects of high temperature on floral development and flowering in spray chrysanthemum. J. App. Hort. 10: 8–14.

Oda, A., Y. Higuchi and T. Hisamatsu. 2017. Photoperiod-insensitive floral transition in chrysanthemum induced by constitutive expression of chimeric repressor CsLHY-SRDX. Plant Sci. 259: 86–93.

Oda, A., Y. Higuchi and T. Hisamatsu. 2020. Constitutive expression of CsGI alters critical night length for flowering by changing the photo-sensitive phase of anti-florigen induction in chrysanthemum. Plant Sci. DOI: 10.1016/j.plantsci.2020.110417.

Oda, A., T. Narumi, T. Li, T. Kando, Y. Higuchi, K. Sumitomo, S. Fukai and T. Hisamatsu. 2012. CsFTL3, a chrysanthemum FLOWERING LOCUS T-like gene, is a key regulator of photoperiodic flowering in chrysanthemums. J. Exp. Bot. 63: 1461–1477.

Shibata, M. 1997. Studies on responses to temperature and photoperiod and breeding of spray type chrysanthemums with summer-to-autumn flowering. Bull. Natl. Res. Inst. Veg. Ornam. Plants Tea 12: 1–71 (In Japanese with English abstract).

Whealy, C. A., T. A. Nell, J. E. Barrett and R. A. Larson. 1987. High temperature effects on growth and floral development of chrysanthemum. J. Am. Soc. Hort. Sci. 112: 464–468.