Journal of Plant Physiology 169 (2012) 1789–1796

Contents lists available at SciVerse ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.de/jplph

Day light quality affects the night-break response in the short-day plant chrysanthemum, suggesting differential phytochrome-mediated regulation of flowering

Yohei Higuchi [a], Katsuhiko Sumitomo [a], Atsushi Oda [a], Hiroshi Shimizu [b], Tamotsu Hisamatsu [a,*]

[a] NARO Institute of Floricultural Science (NIFS), National Agriculture and Food Research Organization (NARO), 2-1 Fujimoto, Tsukuba, Ibaraki 305-8519, Japan
[b] Graduate School of Agriculture, Kyoto University, Kitashirakawa-Oiwakecho, Sakyo-ku, Kyoto 606-8502, Japan

ARTICLE INFO

Article history:
Received 6 March 2012
Received in revised form 4 July 2012
Accepted 6 July 2012

Keywords:
Chrysanthemum
Flowering
Light quality
Night break
Phytochrome

ABSTRACT

Chrysanthemum (*Chrysanthemum morifolium*) is a short-day plant, which flowers when the night length is longer than a critical minimum. Flowering is effectively inhibited when the required long-night phase is interrupted by a short period of exposure to red light (night break; NB). The reversal of this inhibition by subsequent exposure to far-red (FR) light indicates the involvement of phytochromes in the flowering response. Here, we elucidated the role of light quality in photoperiodic regulation of chrysanthemum flowering, by applying a range of different conditions. Flowering was consistently observed under short days with white light (W-SD), SD with monochromatic red light (R-SD), or SD with monochromatic blue light (B-SD). For W-SD, NB with monochromatic red light (NB-R) was most effective in inhibiting flowering, while NB with monochromatic blue light (NB-B) and NB with far-red light (NB-FR) caused little inhibition. In contrast, for B-SD, flowering was strongly inhibited by NB-B and NB-FR. However, when B-SD was supplemented with monochromatic red light (B + R-SD), no inhibition by NB-B and NB-FR was observed. Furthermore, the inhibitory effect of NB-B following B-SD was partially reversed by subsequent exposure to a FR light pulse. The conditions B-SD/NB-B (no flowering) and B + R-SD/NB-B (flowering) similarly affected the expression of circadian clock-related genes. However, only the former combination suppressed expression of the chrysanthemum orthologue of *FLOWERING LOCUS T* (*CmFTL3*). Our results suggest the involvement of at least 2 distinct phytochrome responses in the flowering response of chrysanthemum. Furthermore, it appears that the light quality supplied during the daily photoperiod affects the light quality required for effective NB.

Introduction

Photoperiod is a major environmental cue for flowering. Many plant species reproduce at favorable times of the year, by measuring seasonal changes in day length, and responding either to long days (LDs) or short days (SDs). Day-length measurement is thought to be achieved through the integration of endogenous circadian rhythms with external light signals (Bünning, 1936; Pittendrigh and Minis, 1964). Light plays 2 distinct roles in photoperiodic flowering: (1) by resetting the circadian clock that regulates the circadian phase of clock-controlled genes (CCGs); and (2) by directly modulating the activity of the CCGs (Yanovsky and Kay, 2003; Kobayashi and Weigel, 2007). In *Arabidopsis*—a facultative long-day plant (LDP)—phytochromes, cryptochromes, and ZTL/FKF1/LKP2 family proteins are important photoreceptors for the regulation of flowering (Thomas, 2006). Phytochromes are primarily red/far-red (R/FR) light receptors, and are encoded by 5 genes (*PHYA* to *PHYE*) in *Arabidopsis*. Phytochrome A (phyA) mediates the FR-light promotion of flowering (Johnson et al., 1994; Mockler et al., 2003). In contrast, phyB acts in a partially redundant manner with phyD and phyE to mediate red-light inhibition of flowering (Goto et al., 1991; Devlin et al., 1998, 1999; Mockler et al., 1999; Franklin et al., 2003). Cryptochrome 1 (cry1) and cry2 act redundantly to mediate the blue-light promotion of flowering (Guo et al., 1998; Mockler et al., 2003). Together, these photoreceptors affect flowering time by mediating light input to the circadian clock, and also by directly modulating the protein stability of CONSTANS (CO), a critical activator of *FLOWERING LOCUS T* (*FT*) (Somers et al., 1998; Valverde et al., 2004).

Abbreviations: B, monochromatic blue; CCG, clock-controlled gene; cry, cryptochrome; FR, far red; HIR, high-irradiance response; LD, long day; LDP, long-day plant; LED, light-emitting diode; LFR, low-fluence response; LL, continuous light; NB, night break; phy, phytochrome; PPFD, photosynthetic photon flux density; R, monochromatic red; RACE, rapid amplification of cDNA ends; R/FR, red/far-red; SD, short day; SDP, short-day plant; SNB, short night break; W, white.

* Corresponding author. Tel.: +81 29 838 6801; fax: +81 29 838 6841.
E-mail address: tamotsu@affrc.go.jp (T. Hisamatsu).

0176-1617/$ – see front matter © 2012 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.jplph.2012.07.003

The effects of light quality on the regulation of flowering vary according to plant species. In general, LDPs require a long daily period of exposure to light, followed by a short period of exposure to FR light, to promote flowering. Furthermore, blue light promotes flowering in cruciferous species (including *Arabidopsis*), but generally has low effectiveness in other LDP families (Thomas and Vince-Prue, 1997; Runkle and Heins, 2001). In contrast to LDPs, short-day plants (SDPs) are less sensitive to the quality of light applied during different parts of the light period, but require a long period of exposure to darkness. Thus, the majority of LDPs and SDPs are also classified as light-dominant and dark-dominant plants, respectively (Thomas and Vince-Prue, 1997).

The flowering response of SDPs to the light quality supplied during the night break (NB)—a short period of exposure to light during the required long-night phase—has been extensively studied. NB with red light was shown to be the most effective in inhibiting flowering; in many cases, this inhibitory effect was FR-light reversible (Thomas and Vince-Prue, 1997). R/FR light-absorbing phytochromes have long been considered to be important photoperiodic photoreceptors. Recent molecular genetic investigations have revealed that phytochromes are essential for photoperiodic flowering in rice—a facultative SDP. Loss-of-function of all phytochrome rice genes (in the *se5* mutant or *phyAphyBphyC* triple mutant) results in a deficient photoperiodic response, and early flowering under SD and LD conditions (Izawa et al., 2000, 2002; Takano et al., 2009). Moreover, phyB is responsible for the inhibitory effect of NB in rice (Ishikawa et al., 2005, 2009). NB causes a delay in flowering by suppressing the expression of *Heading date 3a* (*Hd3a*)—a rice orthologue of *FT*. This delay is reversed by mutations in *phyB* (Ishikawa et al., 2005). Thus, it appears that phytochromes are required for day-length recognition, and also for NB-mediated inhibition of flowering in rice.

The flowering response of SDPs to the light quality supplied during the daily photoperiod has also been investigated. For example, white- and red-light grown seedlings of *Pharbitis* were successfully induced to flower by exposure to an inductive darkness, but FR- or blue-light grown seedlings failed to flower (Takimoto and Naito, 1962). In *Xanthium pennsylvanicum*, red light supplied during the intervening light period promoted flowering, whereas FR light was inhibitory (Salisbury, 1965). Similarly, *Lemna paucicostata* T-101—a strain of SD duckweed—responded as a typical SDP when grown under a daily photoperiod of white or red light, but failed to flower when blue or FR light was initially supplied (Ohtani and Ishiguri, 1979). These findings strongly indicate that the photo-activated Pfr form of phytochrome is required for production of floral stimuli during inductive darkness (Pfr-requiring reaction). However, the molecular mechanisms underlying the flowering response of SDPs to the light quality supplied during the daily photoperiod remain to be elucidated.

Chrysanthemum (*Chrysanthemum morifolium*) is a globally important ornamental plant. It is a typical SDP, which flowers when the nights are longer than a critical minimum length. Similar to other SDPs, flowering is effectively inhibited when the required long-night phase is interrupted by a short period of exposure to red light (NB). The reversal of this inhibition by subsequent exposure to FR light (Cathey and Borthwick, 1957) indicates the involvement of phytochromes in the flowering response. Light quality and the relative amounts of red and FR light during the daily photoperiod further influence the flowering response (Kadman-Zahavi and Ephrat, 1973; McMahon and Kelly, 1999). Currently, chrysanthemum growers regulate flowering time by applying an NB of 4–5 h, using an incandescent lamp or fluorescent tube at very low levels of intensity. However, detailed investigations of the effects of light quality during the daily photoperiod and NB are required.

In the present study, we revealed that monochromatic blue light during the daily photoperiod, followed by FR or monochromatic blue light during the NB, inhibited flowering. Our findings indicate a complex interaction of light signaling between the daily photoperiod and NB. Furthermore, at least 2 distinct types of phytochromes may be involved in the regulation of chrysanthemum flowering.

Materials and methods

Plant materials and growth conditions

The chrysanthemum (*Chrysanthemum morifolium* Ramat.) cultivar 'Reagan' was used in this study. Stock plants were grown in a greenhouse, which was maintained at an air temperature above 18 °C, and ventilated when the temperature increased above 25 °C. A natural photoperiod with a 4-h night break (NB) (from 23:00 h to 03:00 h) was delivered by means of incandescent lamps (K-RD100V60W; Matsushita Electric Industrial Co. Ltd., Osaka, Japan). Rooted cuttings from the stock plants were planted into 7.5-cm plastic pots containing a commercial horticultural soil (Kureha-Engei-Baido; Kureha Chemical Industry Co. Ltd., Tochigi, Japan), and grown in the greenhouse. The plants were transferred to a growth chamber maintained at 20 °C with a 16-h photoperiod (long day (LD) conditions). Light was supplied by means of fluorescent tubes (FL40SW; Mitsubishi Co. Ltd., Tokyo, Japan), at a photosynthetic photon flux density (PPFD; 400–700 nm) of 200 μmol m^{-2} s^{-1}. After 7 d of growth under these conditions, the plants were transferred to a growth chamber maintained at 20 °C, with a range of different light conditions.

Light sources and lighting conditions

Light-emitting diode (LED) panels were used to provide monochromatic blue light (LED-B; EYELA, Tokyo, Japan), monochromatic red light (LED-R; EYELA), and far-red light (LED-FR; EYELA), with peak emissions at 465 nm, 660 nm, and 740 nm, respectively (Fig. 1A). The spectral distribution and irradiance level of each light source were measured using a spectroradiometer (LI-1800; LI-COR Inc., Lincoln, NE, USA), and a quantum sensor (LI-190SA; LI-COR Inc.) with a light meter (LI-250; LI-COR Inc.).

Different lighting conditions were used for each experiment. (i) To investigate the effects of light quality on photomorphogenesis, plants were grown under continuous light (LL) conditions at the same PPFD levels, with different wavelengths of light—blue (90 μmol m^{-2} s^{-1}), red (90 μmol m^{-2} s^{-1}), blue + red (45 μmol m^{-2} s^{-1} + 45 μmol m^{-2} s^{-1}), or blue + FR (90 μmol m^{-2} s^{-1} + 62.5 μmol m^{-2} s^{-1})—for up to 2 weeks (Fig. 1B–E). (ii) To investigate the effects of light quality on flowering response, plants were subjected to short day (SD) (12-h light/12-h dark; 12L/12D), LD (16-h light/8-h dark; 16L/8D), LL, and NB (SD with a 4-h NB) conditions, with different wavelengths of

Table 1
Effects of light quality on the flowering response of chrysanthemum under SD, LD, LL, and NB conditions.

	%Flowering (days to visible flower bud)			
	SD	LD	LL	NB
B	100 (22.1 ± 0.3 a)	0 (–)	0 (–)	0 (–)
R	100 (25.2 ± 0.4 b)	0 (–)	0 (–)	0 (–)
B + R	100 (21.9 ± 0.3 a)	0 (–)	0 (–)	0 (–)

Plants were grown under various photoperiods with monochromatic blue light (B), monochromatic red light (R), or blue plus red light (B + R) for 5 weeks.
Light intensities were set to 90 μmol m^{-2} s^{-1} (B + R = 45 μmol m^{-2} s^{-1} each).
SD, short day (12L/12D); LD, long day (16L/8D); LL, continuous light; NB, night break (12L/12D + 4-h NB). Data are represented as the mean ± SE (n = 12). Values followed by the same lower-case letter did not differ significantly ($p < 0.05$) by Tukey's honestly significant difference test.

Table 2
Effects of 4-h NB with blue, red, or far-red light on inhibition of flowering.

	Daily photoperiod	NB	Flowering (%)	Days to visible flower bud	Leaf no.
W-SD	W	–	100	22.8 ± 0.2 a	20.3 ± 0.2 a
W-SD/NB-B	W	B	100	21.8 ± 0.2 a	20.2 ± 0.3 a
W-SD/NB-R	W	R	0	–	30.5 ± 0.4 c
W-SD/NB-FR	W	FR	100	24.5 ± 0.4 b	21.5 ± 0.2 b

Data were collected 5 weeks after the start of treatment.
White (W) light was obtained by means of cool white fluorescent tubes, with light intensities of 150 μmol m^{-2} s^{-1}.
Light intensities of B, R, and FR were 39.1 μmol m^{-2} s^{-1}, 55.3 μmol m^{-2} s^{-1}, and 62.5 μmol m^{-2} s^{-1}, respectively.
Data are represented as the mean ± SE (n = 14).
Values followed by the same lower-case letter did not differ significantly (p < 0.05) by Tukey's honestly significant difference test.

Table 3
Effects of blue and red light used during the daily photoperiod on inhibition of flowering by a 4-h NB with FR light.

	Daily photoperiod	NB	Flowering (%)	Stage of flowering	Leaf no.
B-SD	B	–	100	7.0 ± 0.0 c	12.7 ± 0.2 a
B-SD/NB-B	B	B	11	0.7 ± 0.2 b	18.0 ± 0.5 b
B-SD/NB-FR	B	FR	0	0 a	17.4 ± 0.4 b
B + R-SD/NB-FR	B + R	FR	100	7.0 ± 0.0 c	12.9 ± 0.2 a

Data were collected 5 weeks after the start of treatment.
Light intensities during the light period were as follows: B, 100 μmol m^{-2} s^{-1}; and B + R, 75 μmol m^{-2} s^{-1} (B) + 25 μmol m^{-2} s^{-1} (R).
Light intensities of the NB with B and FR were 20 μmol m^{-2} s^{-1}.
Data are represented as the mean ± SE (n = 9).
Values followed by the same lower-case letter did not differ significantly (p < 0.05) by Tukey's honestly significant difference test.

Table 4
Effects of a 15-min NB with blue, red, and FR light on inhibition of flowering.

	Daily photoperiod	NB	Flowering (%)	Days to visible flower bud	Leaf no.	Stage of flowering	Diameter of flower bud (mm)
B-SD	B	–	100	22.6 ± 0.6 a	10.0 ± 0.2 a	7.0 ± 0.0 c	3.6 ± 0.2 b
B-SD/SNB-B	B	B	100	26.3 ± 0.5 b	11.1 ± 0.3 b	6.0 ± 0.4 b	1.8 ± 0.2 a
B-SD/SNB-R	B	R	0	–	14.6 ± 0.2 c	0 a	–
B-SD/SNB-FR	B	FR	100	24.5 ± 0.5 ab	9.9 ± 0.2 a	7.0 ± 0.0 c	2.3 ± 0.2 a

Data were collected 4 weeks after the start of treatment.
Light intensity during the light period was 100 μmol m^{-2} s^{-1}.
Light intensity of the NB was 25 μmol m^{-2} s^{-1}.
Data are represented as the mean ± SE (n = 9 or n = 10).
Values followed by the same lower-case letter did not differ significantly (p < 0.05) by Tukey's honestly significant difference test.

light: blue (90 μmol m^{-2} s^{-1}), red (90 μmol m^{-2} s^{-1}), or blue + red (45 μmol m^{-2} s^{-1} + 45 μmol m^{-2} s^{-1}) for up to 5 weeks (Table 1). (iii) To investigate the effects of NB light quality on flowering response, plants were grown under white fluorescent tubes (150 μmol m^{-2} s^{-1}) for 12 h (W-SD), and NB was provided with different wavelengths of light—NB-B (10 W m^{-2}, 39.1 μmol m^{-2} s^{-1}; W-SD/NB-B), NB-R (10 W m^{-2}, 55.3 μmol m^{-2} s^{-1}; W-SD/NB-R), or NB-FR (10 W m^{-2}, 62.5 μmol m^{-2} s^{-1}; W-SD/NB-FR)—for up to 5 weeks (Table 2). (iv) To investigate the effects of SD light quality on flowering response and gene expression, plants were subjected to 12-h blue light (90 μmol m^{-2} s^{-1})/12-h dark; B-SD), B-SD plus NB-B (90 μmol m^{-2} s^{-1}; B-SD/NB-B), or B-SD supplemented with red light (65 μmol m^{-2} s^{-1} blue + 25 μmol m^{-2} s^{-1} red; B + R-SD) plus NB-B (B + R-SD/NB-B) for 5 weeks (Figs. 2 and 3). For RNA extraction and subsequent RT-PCR analysis, the leaves and shoot apex were harvested 7 d and 3 weeks after the start of treatment, respectively (Figs. 2 and 3). (v) To investigate the combined effects of SD and NB light quality on flowering response, plants were subjected to B-SD (100 μmol m^{-2} s^{-1}), B-SD plus NB-B (20 μmol m^{-2} s^{-1}; B-SD/NB-B), B-SD plus NB-FR (20 μmol m^{-2} s^{-1}; B-SD/NB-FR), or B + R-SD (75 μmol m^{-2} s^{-1} + 25 μmol m^{-2} s^{-1}) plus NB-FR (B + R-SD/NB-FR) for 4 weeks (Table 3). (vi) To investigate the combined effects of a short NB period (SNB; 15 min) and light quality on flowering response, plants were subjected to B-SD (100 μmol m^{-2} s^{-1}), B-SD plus SNB-B (25 μmol m^{-2} s^{-1}; B-SD/SNB-B), B-SD plus SNB-R (25 μmol m^{-2} s^{-1}; B-SD/SNB-R), or B-SD plus SNB-FR (25 μmol m^{-2} s^{-1}; B-SD/SNB-FR) light for up to 4 weeks (Table 4).

Table 5
Reversibility of NB-B-induced inhibition of flowering by subsequent exposure to FR.

	Daily photoperiod	NB	Days to visible flower bud	Leaf no.	Diameter of flower bud (mm)
B-SD	B	–	22.5 ± 0.3 a	16.2 ± 0.2 a	7.0 ± 0.2 c
B-SD/SNB-B	B	B	28.5 ± 0.5 c	16.6 ± 0.1 a	2.9 ± 0.1 a
B-SD/SNB-B/FR	B	B/FR	25.6 ± 0.3 b	16.1 ± 0.2 a	4.0 ± 0.2 b

Light intensities of B and FR were 90 μmol m^{-2} s^{-1} and 62.5 μmol m^{-2} s^{-1}, respectively.
A 15-min period of FR light was delivered immediately after a 15-min NB with B light.
Data are represented as the mean ± SE (n = 12).
Values followed by the same lower-case letter did not differ significantly (p < 0.05) by Tukey's honestly significant difference test.

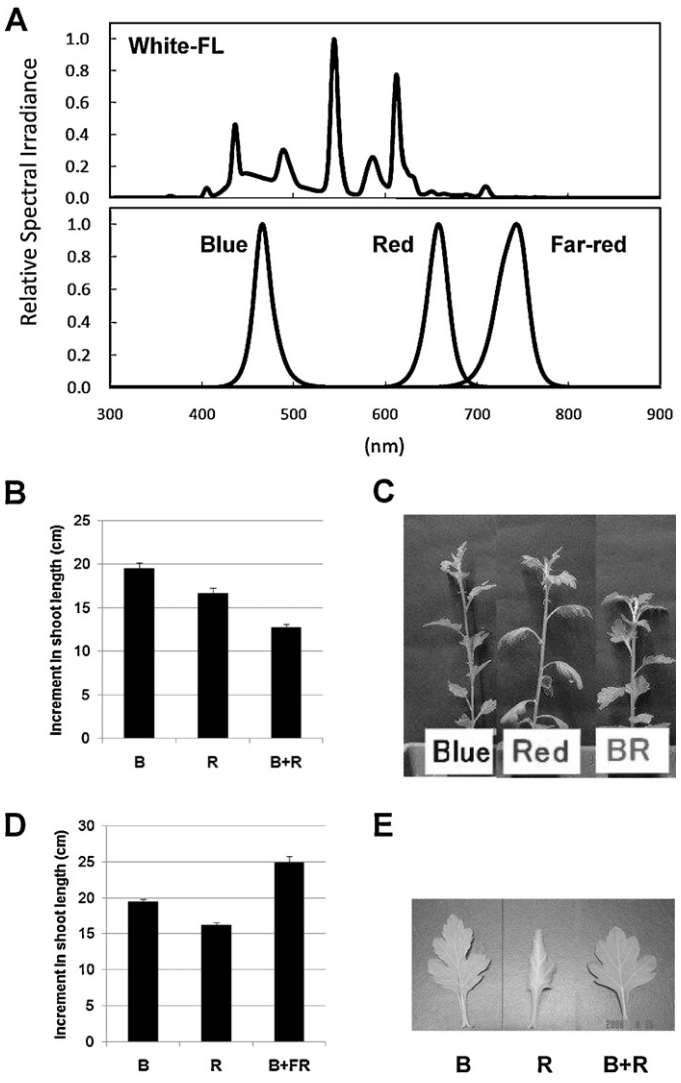

Fig. 1. Effects of light quality on growth of chrysanthemum. (A) The spectra of artificial light sources used in this study (white-FL, blue, red, far red) are shown as relative spectral irradiance in the wavelength range of 300–900 nm. (B–E) Effects of red (R), blue (B), and far-red (FR) light on shoot extension and leaf expansion of chrysanthemum. Plants were grown under continuous light with B, R, B + R, or B + FR light sources for 2 weeks. Light intensities were as follows: B, 90 μmol m^{-2} s^{-1}; R, 90 μmol m^{-2} s^{-1}; B + R, 45 μmol m^{-2} s^{-1} each; and B + FR, 90 μmol m^{-2} s^{-1} (B) and 62.5 μmol m^{-2} s^{-1} (FR). Data are represented as mean ± SE (n = 12).

(vii) To investigate the reversibility of NB-B-induced inhibition of flowering by subsequent exposure to FR, plants were subjected to B-SD (90 μmol m^{-2} s^{-1}), B-SD plus a SNB-B (90 μmol m^{-2} s^{-1}; B-SD/SNB-B), or B-SD/SNB-B followed by 15 min of FR light (10 W m^{-2}, 62.5 μmol m^{-2} s^{-1}; B-SD/SNB-B/FR) (Table 5). The light conditions used are summarized in Supplementary Fig. S1.

Data collection and analysis

The following measurements were recorded: length of stem and leaf number at the start and end of experiments; days until flower buds became visible; and number of nodes per shoot at flowering. If no flower buds were visible by the end of the experiment, shoot tips were dissected under a stereoscopic microscope, and the number of leaf primordia and floral stage 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

A

	Daily photoperiod	NB	Flowering (%)	Stage of Flowering	Leaf No.
B-SD	B	-	100	6.0±0.2 c	24.0±0.2 a
B-SD/NB-B	B	B	0	0 a	28.0±0.4 b
B+R-SD/NB-B	B+R	B	100	2.9±0.1 b	27.8±0.2 b

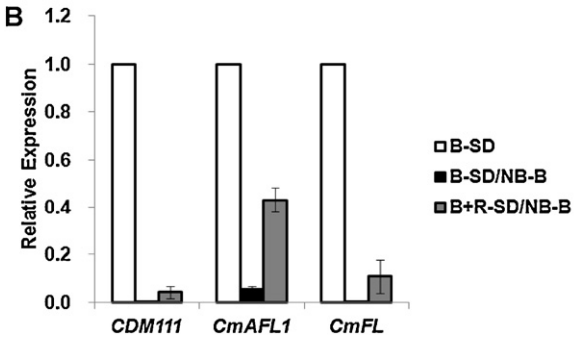

Fig. 2. Effects of blue and red light during the daily photoperiod on inhibition of flowering by a 4-h NB with blue light. (A) Flowering response under B-SD (12-h blue light/12-h D), B-SD/NB-B (B-SD with 4-h NB-B), and B + R-SD/NB-B (B + R-SD with 4-h NB-B). Data were collected 3 weeks after the start of the experiment. Light intensities were B, 90 μmol m^{-2} s^{-1} and B + R, 65 μmol m^{-2} s^{-1} (B) + 25 μmol m^{-2} s^{-1} (R). Data are represented as the mean ± SE (n = 22). Values followed by the same lower-case letter did not differ significantly (p < 0.05) by Tukey's honestly significant difference test. (B) Expression levels of floral meristem identity genes in shoot apices. Plants were grown under B-SD, B-SD/NB-B, and B + R-SD/NB-B for 3 weeks, after which shoot tips were harvested for RNA extraction and RT-PCR. Data were normalized against *CmACTIN* expression. The maximum value in each experiment was set to 1. Data are represented as the mean ± SE of 3 biological replicates, each consisting of pooled material from 3 plants.

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. Analyses of variance and mean separation by Tukey's honestly significant difference test (p < 0.05) were performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Gene expression analysis was conducted on 3 biological replicates using RNA from separate plants.

Isolation of full-length cDNA from chrysanthemum

A full-length cDNA library was constructed using the leaves and shoot apex of a diploid wild chrysanthemum, *Chrysanthemum seticuspe* f. *boreale* (accession: NIFS-3), grown under various photoperiodic and temperature conditions, and sequenced using an FLX genome sequencer (Roche Diagnostics K.K., Tokyo, Japan) to obtain >2,700,000 sequence tags (Hisamatsu et al., unpublished data). More than 60,000 contig sequences were screened, and several clones showing high homology to *Arabidopsis LHY*, *TOC1*, *GI*, *CO*, *PHYA*, and *PHYB* were identified. Sequence analysis revealed that the cDNA clones for *TOC1*, *GI*, *CO*, and *PHYA* homologues contained entire coding sequences. The open reading frame of the chrysanthemum homologue of *LHY* was determined by 3′- and 5′-rapid amplification of cDNA ends (RACE)-PCR, using a 5′/3′-RACE second-generation kit (Roche Diagnostics K.K.) according to the manufacturer's instructions. The 5′ region of the chrysanthemum *PHYB* gene was isolated by TAIL-PCR, using a random primer (5′-NGTCGASWGANAWGAA-3′) and gene-specific primers (5′-TCCTCAGTCCTCACAGGCTCTAAGTCA-3′, 5′-ATCCCCACATCAATCCTATGCAGAATC-3′, and 5′-AAGCGTAAT CTCCCTAGCACGAAAAGC-3′). Genomic DNA was used as the template for TAIL-PCR. Sequences data obtained in this study have been deposited at DDBJ under the following accession numbers: *CsLHY*, AB733625; *CsTOC1*, AB733626; *CsGI*, AB733627; *CsCOL1*, AB733628; *CsPHYA*, AB733629; and *CsPHYB*, AB733630.

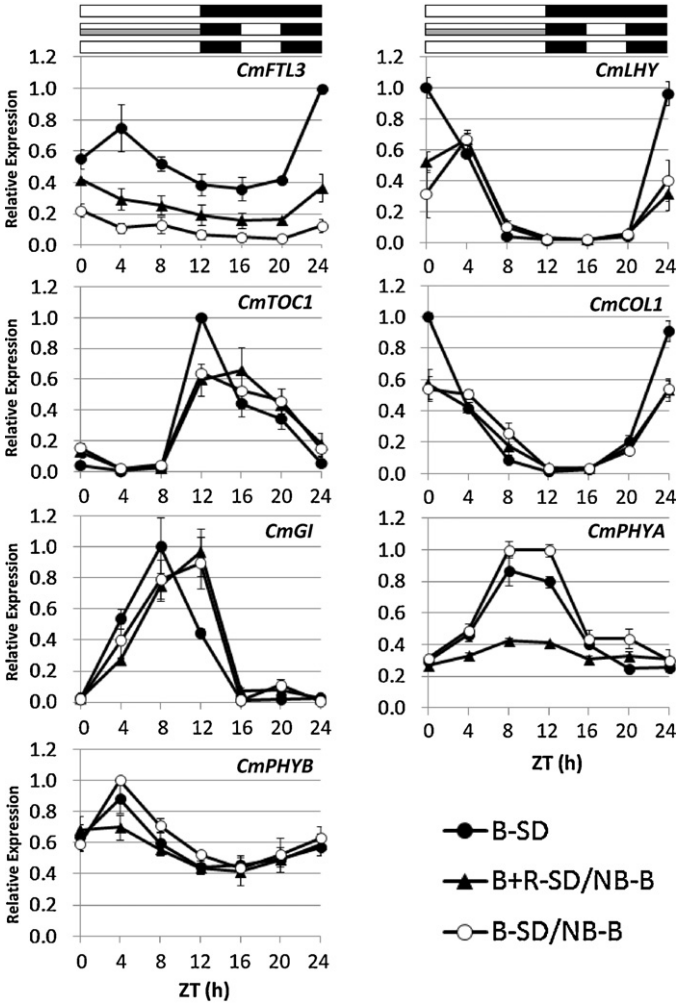

Fig. 3. Temporal expression patterns of flowering-related genes in chrysanthemum leaves. Plants were grown under B-SD (12-h blue light/12-h D), B-SD/NB-B (B-SD with 4-h NB-B), and B + R-SD/NB-B (B + R-SD with 4-h NB-B) for 7 d. The horizontal white and gray bars represent the blue and red light periods, respectively. The black bar represents the period of darkness. Data were normalized against *EF1α* expression. The maximum value in each experiment was set to 1. Data are represented as the mean ± SE of 3 biological replicates, each consisting of pooled material from 2 or 3 plants. Error bars, when not evident, were smaller than the symbols used.

The primers used in expression analysis were designed from these sequences, and we confirmed the amplification of a single amplicon in each primer set (Supplementary Table S1).

Gene expression analysis by quantitative real-time PCR

Total RNA was extracted from the chrysanthemum leaves or shoot apex using an RNeasy Plant Mini Kit (Qiagen K.K., Tokyo, Japan), and treated with RNase-free DNase (Qiagen K.K.) according to the manufacturer's instructions. A Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics K.K.) was used to synthesize cDNA from 1 μg of total RNA, in accordance with the manufacturer's instructions. The cDNA was diluted 10-fold, and 5 μL was used in 15-μL quantitative RT-PCR (qRT-PCR) reactions with SYBR Premix *Ex Taq* (Takara Bio Inc., Shiga, Japan), performed in a LightCycler system (Roche Diagnostics K.K.). PCR products were quantified against a standard curve using a plasmid containing the genes of interest. For each experiment, 2 technical and 3 biological replicates were performed. The primer sequences and PCR conditions used in the analyses are listed in Supplementary Table S1.

Results

Effects of light quality on photomorphogenesis and flowering of chrysanthemum

To investigate the effects of light quality on photomorphogenesis of chrysanthemum, plants were grown under LL conditions at the same PPFD levels, with different wavelengths of light: blue, red, blue + red, or blue + FR. In comparison with blue or red light alone, the blue + red combination significantly suppressed shoot extension (Fig. 1B and C). In contrast, exposure to blue + FR light promoted shoot extension (Fig. 1D). Plants grown under red light exhibited downward-curling leaves, whereas those grown under blue, or blue + red light showed normally expanded leaves (Fig. 1E).

To investigate the effects of light quality on flowering response, plants were grown under SD, LD, LL, or NB conditions, with blue, red, or blue + red light. The critical day length required for flowering in the cultivar 'Reagan' used throughout the present study is ~13 h, and therefore a 12-h period of darkness is sufficient to initiate flowering and subsequent capitulum development (Hisamatsu et al., unpublished; Li et al., 2009). All of the plants subjected to the SD treatment formed flower buds, regardless of the different light sources (Table 1). In contrast, plants grown under the LD, LL, and NB conditions failed to form flower buds, irrespective of the light source (Table 1).

Effects of NB light quality on flowering response

To investigate the effects of NB light quality on flowering response, plants were grown under the following conditions: W-SD, W-SD/NB-B, W-SD/NB-R, or W-SD/NB-FR (Fig. 1A and Table 2). Under W-SD, W-SD/NB-B, and W-SD/NB-FR conditions, all of the plants formed flower buds. In contrast, plants grown under the W-SD/NB-R treatment failed to form flower buds (Table 2).

Effects of SD light quality on flowering response and gene expression

To investigate the effects of SD light quality on flowering response and gene expression, plants were subjected to the following conditions: B-SD, B-SD/NB-B, or B + R-SD/NB-B. Under B-SD conditions, flowering was effectively inhibited by a 4-h NB with blue light (B-SD/NB-B). However, when B-SD was supplemented with monochromatic red light (B + R-SD), no inhibition by NB-B was observed (B + R-SD/NB-B; Fig. 2A). Chrysanthemum homologues of the well-characterized floral meristem identity genes *APETALA1* (*CDM111*), *FRUITFULL* (*CmAFL1*), and *LEAFY* (*CmFL*) were selected for expression analysis by qRT-PCR (Shchennikova et al., 2004; Li et al., 2009). Under B-SD conditions, *CDM111*, *CmAFL1*, and *CmFL* were highly expressed in shoot apices, which developed flower buds. In contrast, under B-SD/NB-B conditions, expression of these genes was very low or barely detectable (Fig. 2B). Under B + R-SD/NB-B conditions, transcripts of *CDM111*, *CmAFL1*, and *CmFL* were detected, but in lower amounts than under B-SD conditions (Fig. 2B). Moreover, the progression of flower development under B + R-SD/NB-B conditions was slightly delayed compared with that under B-SD conditions (Fig. 2A).

Possible involvement of phytochromes in NB-induced inhibition of flowering by blue and FR light

To investigate the combined effects of SD and NB light quality on flowering response, plants were subjected to the following conditions: B-SD, B-SD/NB-B, B-SD/NB-FR, or B + R-SD/NB-FR (Table 3). Under B-SD conditions, flowering was effectively inhibited by a 4-h

NB with FR light (B-SD/NB-FR). However, when B-SD was supplemented with monochromatic red light (B + R-SD), no inhibition by NB-FR was observed (B + R-SD/NB-FR; Table 3).

To investigate the combined effects of an SNB and light quality on flowering response, plants were subjected to the following conditions: B-SD, B-SD/SNB-B, B-SD/SNB-R, or B-SD/SNB-FR. Under B-SD conditions, flowering was completely inhibited by a 15-min NB with red light (B-SD/SNB-R) (Table 4). Under the B-SD/SNB-B and B-SD/SNB-FR conditions, flowering was partially inhibited. Moreover, B-SD/SNB-B suppressed flowering more effectively than did B-SD/SNB-FR (Table 4).

To investigate the reversibility of NB-B-induced inhibition of flowering by subsequent exposure to FR, plants were subjected to the following conditions: B-SD, B-SD/SNB-B, or B-SD/SNB-B/FR (Table 5 and Supplementary Fig. S2). In comparison with the B-SD conditions, flowering under the B-SD/SNB-B conditions was delayed. This effect was partially reversed by subsequent exposure to a FR light pulse (B-SD/SNB-B/FR; Table 5 and Supplementary Fig. S2).

Effects of SD light quality on expression patterns of flowering-related genes in chrysanthemum leaves

To investigate the expression patterns of flowering-related genes in the leaves of chrysanthemum, plants were subjected to the following conditions: B-SD, B-SD/NB-B, or B + R-SD/NB-B. After 7 d, the leaves were harvested for RNA extraction and RT-PCR analysis. Expression of *CmFTL3* was highest under B-SD conditions, lowest under B-SD/NB-B conditions, and intermediate under B + R-SD/NB-B conditions (Fig. 3). Furthermore, these expression levels were correlated with the extent of flower induction. Under B-SD conditions, the expression patterns of 2 putative orthologues of circadian clock-related genes—*TIMING OF CAB EXPRESSION 1* (*CmTOC1*) and *LATE ELONGATED HYPOCOTYL* (*CmLHY*)—showed clear diurnal rhythms, which peaked at dusk and dawn, respectively. Under the B-SD/NB-B and B + R-SD/NB-B conditions, the amplitude of this rhythmic expression was slightly reduced, and the peak phase was delayed (Fig. 3). The expression patterns of 2 chrysanthemum orthologues of flowering-related genes that act downstream of the circadian clock—*GIGANTEA* (*CmGI*) and *CONSTANS* (*CmCOL1*)—and 2 chrysanthemum photoreceptor genes—*CmPHYA* and *CmPHYB*—also showed clear diurnal rhythms (Fig. 3). Under the B-SD conditions, the expression of *CmGI* peaked 8 h after dawn. Under B-SD/NB-B and B + R-SD/NB-B conditions, this peak was shifted approximately 4 h toward the evening. Meanwhile, *CmCOL1* was highly expressed in the morning. However, under B-SD/NB-B and B + R-SD/NB-B conditions, the amplitude of this rhythmic expression was slightly reduced. Under B-SD and B-SD/NB-B conditions, the expression of *CmPHYA* peaked in the evening. However, under B + R-SD/NB-B conditions, this peak was slightly reduced. Meanwhile, under B-SD and B-SD/NB-B conditions, the expression level of the *CmPHYB* transcript increased 4 h after dawn. However, under B + R-SD/NB-B conditions, this peak was slightly reduced.

Discussion

Effects of light quality on shoot extension and flowering response of chrysanthemum

Genetic investigations using model plants have demonstrated that phytochrome and cryptochrome family members display synergistic and also antagonistic behavior (Lin, 2000; Neff et al., 2000). In the present study, the blue + red light combination significantly suppressed shoot extension compared to blue or red light alone (Fig. 1B and C). This finding suggests a synergistic effect of blue and red light receptors in the inhibition of chrysanthemum shoot extension. Changes in light quality in the red and FR regions of the spectrum are detected by phytochromes, and phyB is the major regulator of this response (Franklin and Whitelam, 2005). In addition to red-light responses, phyA and phyB regulate various blue light-mediated physiological responses, in an overlapping manner with cryptochrome (Shinomura et al., 1996; Neff and Chory, 1998; Usami et al., 2007). In the present study, the suppression of shoot extension under monochromatic blue light conditions was inhibited by supplementation with FR light (Fig. 1D). This finding suggests that shoot extension in chrysanthemum is partly regulated by blue light-stimulated phytochromes.

In *Arabidopsis*, blue light plays an important role in the promotion of flowering under LD conditions. Wild-type plants grown under a LD photoperiod with monochromatic red light flowered much later than did those grown under a LD photoperiod with red + blue light (Mockler et al., 2003). Blue-light promotion of flowering is mediated by 3 photoreceptors—phyA, cry1, and cry2 (Mockler et al., 2003). In contrast to *Arabidopsis*, chrysanthemum showed no remarkable change in flowering response under SD, LD, LL, or NB conditions, irrespective of the different light sources tested (Table 1 and Li et al., 2009). Thus, it appears that each red- and blue-light signal is sufficient for day-length recognition of chrysanthemum.

Effects of SD light quality on NB-mediated inhibition of flowering

In the present study, NB-R showed the strongest inhibition of flowering under W-SD conditions (Table 2). However, when monochromatic blue and monochromatic red light were used during the daily photoperiod and for NB, NB-B and NB-R were similarly effective in inhibiting flowering (Table 1). Furthermore, NB-FR inhibited flowering of chrysanthemum grown under a daily photoperiod with monochromatic blue light, but not white light (Tables 1 and 2). We postulated that these discrepancies were caused by the difference in light quality provided during the daily photoperiod. Physiological studies on SDPs have frequently indicated that light has 2 distinct roles in photoperiodic flowering: (1) by counteracting the effect of darkness, i.e. the 'night-break' reaction; and (2) by promoting the magnitude of flowering, i.e. the 'main-light-period' reaction (Pfr-requiring reaction; Thomas and Vince-Prue, 1997). For instance, in *Pharbitis*, *Xanthium*, and *Lemna*, red-light exposure preceding a period of inductive darkness promoted flowering, whereas blue or FR light did not (Takimoto and Naito, 1962; Salisbury, 1965; Ohtani and Ishiguri, 1979). Meanwhile, in chrysanthemum, FR light exposure at the end of a short light period (8-h sunlight; R/FR ratio of ~1.15) inhibited flowering, and also affected sensitivity to NB with red light (Kadman-Zahavi and Ephrat, 1973). These findings suggest that the relative amount of Pfr at the end of the light period is important for the flowering-inducing capacity during darkness. In the present study, the inhibitory effect of NB-B and NB-FR was observed only following a daily photoperiod with monochromatic blue, and was canceled by supplementation with red light (Fig. 2 and Table 3). It is well known that blue and red light establish *in vitro* photoequilibria for phytochrome (Pfr/Pr + Pfr) of 0.3–0.5 and 0.8, respectively (Butler et al., 1964). In the present study, the estimated phytochrome photoequilibria (Pfr/Pr + Pfr) maintained during the daily photoperiods under B-SD and B + R-SD conditions were 0.54 and 0.85, respectively (Fig. 2A and Table 3; Sager et al., 1988). Therefore, it is likely that the ratio of Pfr/Pr maintained throughout the blue photoperiod was much lower than that maintained under the blue + red photoperiod. It is possible that the red-light-activated Pfr strengthened the floral-inducer activity, resulting in a higher

energy requirement to abolish flowering. Alternatively, red-light exposure during the daily photoperiod may have induced desensitization of the blue/FR-sensing receptor during NB. We believe that the latter hypothesis is more likely because, under SD with blue, red, or blue + red photoperiods, flowering was not promoted by red-light supplementation compared to that with monochromatic blue light—in fact, it was slightly delayed in monochromatic red light (Table 1).

Characterization of the molecular mechanisms underlying the flowering response of chrysanthemum to light quality supplied during the daily photoperiod

Expression analyses of flowering-related genes in shoot tips and leaves have facilitated an understanding of how to regulate chrysanthemum flowering. Recently, 3 chrysanthemum orthologues of *FT*—*CsFTL1*, *CsFTL2*, and *CsFTL3*—were identified. The gene product of *CsFTL3* was shown to be a key regulator of photoperiodic flowering in diploid wild chrysanthemum, *Chrysanthemum seticuspe* (Oda et al., 2012). *CsFTL3* was induced in the leaves, and its up-regulation was correlated with the events occurring in the shoot apical meristem (i.e. floral evocation), via the activation of floral-integrator and/or floral-identity genes under flower-inductive SD conditions (Oda et al., 2012). Moreover, overexpression of *CsFTL3* induced flowering under LD conditions, indicating that the gene product has the potential to induce chrysanthemum flowering. In the present study, the expression of *CmFTL3* was strongly correlated with the extent of flower induction, i.e. highest under B-SD conditions, lowest under B-SD/NB-B conditions, and intermediate under B + R-SD/NB-B conditions (Fig. 3). This finding suggests that regulation of *CmFTL3* expression in the leaves is a key regulator of photoperiodic flowering under the monochromatic blue-light conditions used in the present study. Furthermore, derepression of *CmFTL3* expression by red-light supplementation of the blue-light photoperiod may have promoted flower induction under B + R-SD/NB-B conditions.

To determine the involvement of CCGs, we analyzed the expression patterns of *CmTOC1* and *CmLHY*, and also of 2 chrysanthemum orthologues of flowering-related genes that act downstream of the circadian clock—*CmGI* and *CmCOL1*. The expression of CCGs was similarly affected by B-SD/NB-B and B + R-SD/NB-B conditions; however, only B-SD/NB-B conditions suppressed flowering (Figs. 2A and 3; Supplementary Fig. S2). Therefore, differences in flowering response between B-SD/NB-B and B + R-SD/NB-B may not be caused by the altered circadian phase of CCGs. Lumsden and Furuya (1986) investigated the effects of NB on the phase setting of circadian rhythms in *Pharbitis*, and demonstrated that a very short NB (6–200 s of red light) inhibited flowering without affecting the circadian rhythm. However, longer exposures to light resulted in shifting of the circadian phase (Lumsden and Furuya, 1986). Similarly, the expression patterns of *PnGI* and *PnCO* were not altered by $\leq$10 min of NB (Liu et al., 2001; Higuchi et al., 2011). However, the expression of *PnFT1* was completely suppressed (Hayama et al., 2007). In rice, NB with 10-min white light had no major effect on the expression of *OsGI* and *Hd1* (an ortholog of *CO*), but strongly suppressed the expression of *Hd3a* (Ishikawa et al., 2005). Taken together, these findings indicate that NB has 2 distinct roles depending on light intensity and duration: (1) by resetting the circadian rhythm; and (2) by directly abolishing the production of floral stimuli. Our present data suggest that both events occur during the 4-h NB for chrysanthemum, but that the latter is more important for inhibition of flowering. Moreover, the expression of an *FT*-like gene is regulated independently of the phase shift in the circadian rhythm.

Possible involvement of two distinct phytochrome-mediated regulation systems in the flowering response of chrysanthemum

Cathey and Borthwick (1957) demonstrated that the NB-mediated inhibition of flowering by red light is FR-light reversible. This R/FR reversibility is known as the low-fluence response (LFR), and is mediated by phyB-type receptors (Casal et al., 1998). In the present study, under W-SD conditions, red light showed the strongest inhibition of flowering, whereas blue and FR light had minimal inhibitory effects (Table 2). These findings confirmed that NB-mediated inhibition of chrysanthemum flowering is clearly dependent on the red-light-activated Pfr form of phytochrome, and may be mediated by phyB-type receptors. When monochromatic blue light was supplied during the daily photoperiod, flowering was inhibited by NB-FR, and also by NB-B (Fig. 2 and Table 3). Although NB-FR and NB-B appear to have similar effects, they probably comprise 2 distinct types of phytochrome response. The effects of SNB-B and SNB-FR were much weaker than were those of SNB-R; however, SNB-B suppressed flowering more effectively than did SNB-FR (Table 4). Moreover, the inhibitory effect of SNB-B was partially reversed by subsequent exposure to an FR light pulse (Table 5 and Supplementary Fig. S2). This finding suggests that a photoconvertible type of phytochrome, such as phyB, is involved in the NB-B reaction in chrysanthemum. In *Arabidopsis*, phyA and phyB have been shown to function under blue light (Shinomura et al., 1996; Neff and Chory, 1998). In rice, an NB with blue light suppressed flowering, but this delay in flowering was lost in the *phyB-1* mutant (Ishikawa et al., 2009). Therefore, similar mechanisms for NB-B-induced inhibition of flowering may operate in chrysanthemum and rice.

In addition to the R/FR-reversible LFR, 2 other phytochrome responses exist: (1) the very-low-fluence response; and (2) the high-irradiance response (HIR). In contrast to the LFR, the HIR occurs primarily under conditions of FR light, and is mediated by phyA-type receptors (Casal et al., 1998). In the present study, the inhibition of flowering by SNB-FR was weak (Table 4), but became more prominent under longer exposure (>4 h) to FR light (Table 3). This response may be categorized as an HIR. The inhibitory effects of NB-B and NB-FR were similarly abolished by red-light supplementation of the blue-light photoperiod (Fig. 2 and Table 3). The effects of red-light supplementation on flowering were stronger than were those of FR reversibility in the 'night-break' reaction (Supplementary Fig. S2). Thus, it appears that the mechanism involved in the 'main-light-period' reaction may be important in elucidating the NB response of chrysanthemum. The expression of *CmPHYA* mRNA was negatively regulated by red-light supplementation of blue light (Fig. 3). Furthermore, in *Arabidopsis*, phyA protein accumulates during the dark period, and rapidly degrades upon exposure to red light (Sharrock and Clack, 2002). Recently, Kong et al. (2010) demonstrated that phyA plays a dominant role in inhibiting flowering of soy bean (*Glycine max*; an SDP) under LD conditions. These findings are in accordance with our present hypothesis that the regulation of *CmFTL3* expression in chrysanthemum leaves under B-SD/NB-B conditions may be partly regulated by a phyA-mediated photoperiodic regulation system. Nevertheless, we cannot exclude the possibility that the NB-B response pathway in chrysanthemum is mediated by cryptochromes.

In conclusion, our present findings suggest the involvement of 2 distinct phytochrome-mediated regulation systems in the flowering response of chrysanthemum. Moreover, we have shown that red light supplied during the daily photoperiod affects sensitivity to light irradiation during the flower-inductive dark period. Further studies, including our ongoing loss-of-function analyses of *PHYA* and *PHYB*, are required to elucidate the molecular mechanisms involved in these photoreceptor-mediated photoperiodic regulation systems.

Acknowledgments

The authors thank Ms N. Tozawa for help with plant growth analysis. This work was supported by the grant 'Elucidation of biological mechanisms of photoresponse and development of advanced technologies utilizing light' from the Ministry of Agriculture, Forestry and Fisheries.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jplph.2012.07.003.

References

Bünning E. Die endogene Tagesrhythmik als Grundlage der photoperiodischen Reaktion. Ber Deutsch Bot Ges 1936;54:590–607.
Butler WL, Hendricks SB, Siegelman HW. Action spectra of phytochrome in vitro. Photochem Photobiol 1964;3:521–8.
Casal JJ, Sanchez RA, Botto JF. Modes of action of phytochromes. J Exp Bot 1998;49:127–38.
Cathey HM, Borthwick HA. Photoreversibility of floral initiation in Chrysanthemum. Bot Gaz 1957;119:71–6.
Devlin PF, Patel SR, Whitelam GC. Phytochrome E influences internode elongation and flowering time in *Arabidopsis*. Plant Cell 1998;10:1479–88.
Devlin PF, Robson PR, Patel SR, Goosey L, Sharrock RA, Whitelam GC. Phytochrome D acts in the shade avoidance syndrome in *Arabidopsis* by controlling elongation growth and flowering time. Plant Physiol 1999;119:909–15.
Franklin KA, Whitelam GC. Phytochromes and shade-avoidance responses in plants. Ann Bot 2005;96:169–75.
Franklin KA, Praekelt U, Stoddart WM, Billingham OE, Halliday KJ, Whitelam GC. Phytochromes B, D, and E act redundantly to control multiple physiological responses in Arabidopsis. Plant Physiol 2003;131:1340–6.
Goto N, Kumagai T, Koornneef M. Flowering responses to light-breaks in photomorphogenic mutants of *Arabidopsis thaliana*, a long day plant. Physiol Plant 1991;83:209–15.
Guo H, Yang H, Mockler TC, Lin C. Regulation of flowering time by *Arabidopsis* photoreceptors. Science 1998;279:1360–3.
Hayama R, Aagashe B, Luley E, King R, Coupland G. A circadian rhythm set by dusk determines the expression of *FT* homologs and the short-day photoperiodic flowering response in *Pharbitis*. Plant Cell 2007;19:2988–3000.
Higuchi Y, Sage-Ono K, Sasaki R, Ohtsuki N, Hoshino A, Iida S, et al. Constitutive expression of the *GIGANTEA* ortholog affects circadian rhythms and suppresses one-shot induction of flowering in *Pharbitis nil*, a typical short-day plant. Plant Cell Physiol 2011;52:638–50.
Ishikawa R, Tamaki S, Yokoi S, Inagaki N, Shinomura T, Takano M, et al. Suppression of the floral activator *Hd3a* is the principal cause of the night break effect in rice. Plant Cell 2005;17:3326–36.
Ishikawa R, Shinomura T, Takano M, Shimamoto K. Phytochrome dependent quantitative control of *Hd3a* transcription is the basis of the night break effect in rice flowering. Genes Genet Syst 2009;84(179):84.
Izawa T, Oikawa T, Tokutomi S, Okuno K, Shimamoto K. Phytochromes confer the photoperiodic control of flowering in rice (a short-day plant). Plant J 2000;22:391–9.
Izawa T, Oikawa T, Sugiyama N, Tanisaka T, Yano M, Shimamoto K. Phytochrome mediates the external light signal to repress *FT* orthologs in photoperiodic flowering of rice. Genes Dev 2002;16:2006–20.
Johnson E, Bradley M, Harberd NP, Whitelam GC. Photoresponses of light-grown *phyA* mutants of *Arabidopsis* (phytochrome A is required for the perception of daylength extensions). Plant Physiol 1994;105:141–9.
Kadman-Zahavi A, Ephrat E. Effect of red and far-red illuminations at the end of short days and their interaction with night-break illuminations, on flowering of *Chrysanthemum morifolium* plants. Plant Cell Physiol 1973;14:409–11.
Kobayashi Y, Weigel D. Move on up, it's time for change—mobile signals controlling photoperiod-dependent flowering. Genes Dev 2007;21:2371–84.
Kong F, Liu B, Xia Z, Sato S, Kim BM, Watanabe S, et al. Two coordinately regulated homologs of *FLOWERING LOCUS T* are involved in the control of photoperiodic flowering in soybean. Plant Physiol 2010;154:1220–31.
Li T, Niki T, Nishijima T, Douzono M, Koshioka M, Hisamatsu T. Roles of *CmFL* CmAFL1, and CmSOC1 in the transition from vegetative to reproductive growth in *Chrysanthemum morifolium* Ramat. J Hortic Sci Biotechnol 2009;84:447–53.
Lin CT. Plant blue-light receptors. Trends Plant Sci 2000;5:337–42.
Liu J, Yu J, McIntosh L, Kende H, Zeevaart JAD. Isolation of a CONSTANS ortholog from Pharbitis nil and its role in flowering. Plant Physiol 2001;125(1821):30.
Lumsden PJ, Furuya M. Evidence for two actions of light in the photoperiodic induction of flowering in *Pharbitis nil*. Plant Cell Physiol 1986;27:1541–51.
McMahon MJ, Kelly JW. $CuSO_4$ filters influence flowering of *Chrysanthemum* cv. Spears. Sci Hortic 1999;79:207–15.
Mockler TC, Guo H, Yang H, Duong H, Lin C. Antagonistic actions of *Arabidopsis* cryptochromes and phytochrome B in the regulation of floral induction. Development 1999;126:2073–82.
Mockler T, Yang H, Yu X, Parikh D, Cheng Y, Dolan S, et al. Regulation of photoperiodic flowering by *Arabidopsis* photoreceptors. Proc Natl Acad Sci USA 2003;100:2140–5.
Neff MM, Chory J. Genetic interactions between phytochrome A, phytochrome B, and cryptochrome 1 during Arabidopsis development. Plant Physiol 1998;118:27–36.
Neff MM, Fankhauser C, Chory J. Light: an indicator of time and place. Genes Dev 2000;14:257–71.
Oda A, Narumi T, Li T, Kando T, Higuchi Y, Sumitomo K, et al. *CsFTL3*, a chrysanthemum *FLOWERING LOCUS T-like* gene, is a key regulator of photoperiodic flowering in chrysanthemums. J Exp Bot 2012;63:1461–77.
Ohtani T, Ishiguri Y. Inhibitory action of blue and far-red light in the flowering of *Lemna paucicostata*. Physiol Plant 1979;47:255–9.
Pittendrigh CS, Minis DH. The entrainment of circadian oscillations by light and their role as photoperiodic clocks. Am Nat 1964;108:261–95.
Runkle ES, Heins RD. Specific functions of red, far red, and blue light in flowering and stem extension of long-day plants. J Am Soc Hortic Sci 2001;126:275–82.
Sager JC, Smith WO, Edwards LL, Cyr KL. Use of spectral data to determine photosynthetic efficiency and phytochrome photoequilibria. Trans Am Soc Agric Eng 1988;31:1882–9.
Salisbury FB. Time measurement and the light period in flowering. Planta 1965;66:1–26.
Sharrock RA, Clack T. Patterns of expression and normalized levels of the five *Arabidopsis* phytochromes. Plant Physiol 2002;130:442–56.
Shchennikova AV, Shulga OA, Immink R, Skryabin KG, Angenent GC. Identification and characterization of four chrysanthemum MADS-box genes, belonging to the *APETALA1/FRUITFULL* and *SEPALLATA3* subfamilies. Plant Physiol 2004;134:1632–41.
Shinomura T, Nagatani A, Hanzawa H, Kubota M, Watanabe M, Furuya M. Action spectra for phytochrome A- and B-specific photoinduction of seed germination in *Arabidopsis thaliana*. Proc Natl Acad Sci U S A 1996;93:8129–33.
Somers DE, Devlin PF, Kay SA. Phytochromes and cryptochromes in the entrainment of the *Arabidopsis* circadian clock. Science 1998;282:1488–90.
Takano M, Inagaki N, Xie X, Kiyota S, Baba-Kasai A, Tanabata T, et al. Phytochromes are the sole photoreceptors for perceiving red/far-red light in rice. Proc Natl Acad Sci USA 2009;106:14705–10.
Takimoto A, Naito Y. Studies on the light controlling flower initiation of *Pharbitis nil* X. Photoperiodic responses of the seedlings grown under various light conditions. Bot Mag Tokyo 1962;75:255–63.
Thomas B. Light signals flowering. J Exp Bot 2006;57:3387–93.
Thomas B, Vince-Prue D. Photoperiodism in plants. London: Academic Press; 1997. pp. 85–142.
Usami T, Mochizuki N, Kondo M, Nishimura M, Nagatani A. Cryptochromes and phytochromes synergistically regulate *Arabidopsis* root greening under blue light. Plant Cell Physiol 2007;45:1798–808.
Valverde F, Mouradov A, Soppe W, Ravenscroft D, Samach A, Coupland G. Photoreceptor regulation of CONSTANS protein in photoperiodic flowering. Science 2004;303:965–6.
Yanovsky MJ, Kay SA. Living by the calendar: how plants know when to flower. Nat Rev Mol Cell Biol 2003;4:265–75.