



# Both the Quality and Positioning of the Night Interruption Light are Important for Flowering and Plant Extension Growth

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## Abstract

Chrysanthemum (*Dendranthema grandiflorum* Kitamura), a qualitative short-day plant, does not produce flowers when the night length is shorter than a cultivar-dependent critical value. Flowering is effectively inhibited when the required uninterrupted night period is broken by short periods of exposure to night interruption lights (NILs). The effects of the quality and positioning of the NIL on the morphogenesis, flowering, and photoperiodic gene expressions of chrysanthemum were investigated. Blue (NI-B), red (NI-R), far-red (NI-Fr), and white (NI-W) light-emitting diodes (LEDs) at an intensity of  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetic photon flux density (PPFD) were used to interrupt the dark period or plants grown in a closed walk-in growth chamber at night for a total of 4 h. The NILs were positioned to illuminate either the shoot tip, youngest mature leaf (vigorously growing third leaf from the shoot tip), or old leaf (third leaf from the stem base). The NI-R on the shoot tip and all NI-Fr treatments increased the plant extension growth, to a similar height observed in plants in long-day (LD) treatments. Extension growth was generally suppressed for plants in the NI-B, and plant height was the smallest when NI-B was shed onto the old leaf. Plants in all NIL treatments except NI-Fr showed a negative correlation between the plant height and the age of the leaf (or tissue) exposed to the NIL. Flowering was observed for plants in all treatments except for NI-R shed onto the shoot tip. The transcriptional factor *PHYA* was slightly expressed in plants which showed a flowering response. However, the *CRY1*, *PHYB*, and *TFL* was not expressed to levels reported previously, and the reason for low expression of these genes in these plants may be due to a combined effect of these genes with other flowering promoting and/or inhibiting genes. These results suggest that both the NIL quality and the age of the leaf (or tissue) exposed to the NIL affect the morphogenesis, flowering, and expression of transcriptional factors. Moreover, the previously known effects of the NIL quality on the morphogenesis as well as the vegetative to reproductive phase transition of plants were the most pronounced when the NIL irradiated the shoot tip, and the least pronounced when the NIL was shed onto the old leaf. Therefore, it seems that not only the appropriate quality, but also the appropriate positioning of the NIL, is important for optimizing flowering and plant extension growth.

**Keywords** Blue LED · Chrysanthemum · Floral signal · Lighting position · Light quality · Photomorphogenesis

## Abbreviations

B Blue

*CRY1* *Cryptochrome 1*

*CRY2* *Cryptochrome 2*

Fr Far-red

LD Long-day

LEDs Light-emitting diodes

NI Night interruption

NIL Night interruption light

OL Old leaf

PCR Polymerase chain reaction

*PHYA* *Phytochrome A*

*PHYB* *Phytochrome B*

PPFD Photosynthetic photon flux density

R Red

SD Short-day

ST Shoot tip

*TFL1* *TERMINAL FLOWER 1*

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W White  
YL Youngest mature leaf

## Introduction

The light quality imposes important effects on the morphogenesis, photosynthesis (Kitazaki et al. 2018), and flowering (Liao et al. 2014) of plants. The growth and development of plants throughout their life cycles are controlled by photoreceptors; furthermore, photoreceptors monitor the light environment and help time key developmental transitions such as germination and flowering initiation (Kami et al. 2010). Plants sense the light quality via photoreceptors, which are categorized as phytochromes, cryptochromes, phototropins, members of the Zeidler (ZTL/FKF1/LKP2) family, and the UV-B-absorbing UVR8, which collectively control the photomorphogenesis over a wide range of wavelengths (Lin 2000; Takemiya et al. 2005). Among the photoreceptors, phytochromes are sensitive to red (R) and far-red (Fr) lights, whereas cryptochromes and phototropins absorb blue (B) light and UV-A lights (Lin 2000). Cryptochromes affect early growth, early establishment, stem elongation, and the control of seedlings' photoperiodic responses (Kimura and Kagawa 2006; López-Juez et al. 2007). Phototropins (two exist in *Arabidopsis thaliana* L.) play roles in the phototropism, control of chloroplast light-avoidance and accumulation movements, inhibition of rapid hypocotyl growth, stomatal aperture, and leaf expansion (Kimura and Kagawa 2006; López-Juez et al. 2007).

Night interruption (NI) during short-day (SD) seasons effectively accelerated the flowering of long-day (LD) plants that allowed for an earlier marketing or seed production and, under naturally LD conditions, delayed the flowering of SD plants (Hamamoto et al. 2005; Oh et al. 2013). Recent studies reported that even low-intensity NI was effective in controlling the flowering of certain plants (Kim et al. 2011; Ochiai et al. 2015; Park et al. 2015). According to the literature, *Cymbidium aloifolium* L. photosynthesized during a low-intensity, 4-h NI with 3–5  $\mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetic photon flux density (PPFD), an intensity slightly higher than that of moonlight, suggesting that the increased growth and accelerated flowering with NI are attributed to the increased net photosynthesis (Kim et al. 2011).

It is reported that some species display varying flowering responses to the NIL quality. In the LD plant *Petunia hybrida* Vilmorin, flowering was promoted by green, R, Fr, and white (W) lights, and inhibited by B light, implying that the light quality has different effects on the NI-induced day extension (Park et al. 2016). In the day neutral plant *Pelargonium × hortorum* L.H. Bailey, NI with Fr light delayed flowering (Park et al. 2017). In the SD plant *Dendranthema grandiflorum* Kitamura, NI with R light was the

most effective in inhibiting flowering, while NI with B light and NI with Fr light did little to inhibit flowering (Higuchi et al. 2012). Previous studies irradiated the NIL only onto the plant canopy or shoot tips to control flowering. No reports have yet been published on the effects of position of the NIL shed on the plant on the morphogenesis and flowering in any plants including SD plants. And this study was conducted with a typical SD plant as a model as compared to our previous studies conducted with LD and day neutral plants as the models.

We hypothesized that, as described in many textbooks, light quality and certain positioning only, especially a certain quality NIL irradiated the shoot tip only, at a low intensity for 4 h would affect morphogenesis and flowering, either synergistically or antagonistically. Based on the fact that flower formation is initiated by the apical meristem's transition from a vegetative to a reproductive phase and that the quality of the 4-h low-intensity NIL affects the morphogenesis and flowering of plants, this study focused on the effects of the quality and positioning (shoot tip, youngest mature leaf, and old leaf) of the NIL on the morphogenesis and flowering of a qualitative SD plant, *D. grandiflorum* Kitamura 'Gaya Yellow,' with possible applications to floricultural crop production in mind.

## Materials and Methods

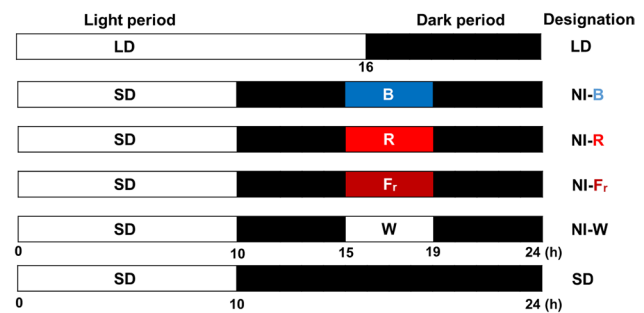
### Plant Materials and Growth Conditions

Spray-type chrysanthemum cuttings, (*Dendranthema grandiflorum* Kitamura 'Gaya Yellow,' a qualitative SD plant), were stuck in 50-cell plug trays with a commercial medium (Tosilee Medium, Shinan Grow Co., Jinju, Korea) and were placed on a glasshouse bench for rooting. Twelve days after sticking, rooted cuttings were transferred to a closed walk-in growth chamber (7700 cm long  $\times$  2500 cm wide  $\times$  2695 cm high, Green Industry Co. Ltd., Changwon, Korea) maintained at an air temperature setpoint of  $20 \pm 1$  °C, a relative humidity of  $60 \pm 10\%$ , and with a 16-h photoperiod of 140  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD (LD condition) provided by fluorescent lamps (F48T12-CW-VHO, Philips Co., Ltd., Eindhoven, The Netherlands). This growth chamber was specially designed to blow in conditioned air horizontally into the growing spaces through numerous uniformly distributed holes. The CO<sub>2</sub> concentration was maintained at atmospheric levels ( $350 \pm 50$   $\mu\text{mol mol}^{-1}$ ) by supplementation from a compressed gas tank. Following 12 days of acclimatization, the plants (approximately 10.2 cm tall) were subjected to the photoperiodic light treatments. After planting, plants were fertigated with a greenhouse multipurpose nutrient solution formulated as shown in Park et al. (2015). A randomized complete block design with 3 replications

with 2 plants each was employed. The treatment locations in a controlled environment were randomly mixed between replications to minimize position effects.

### Photoperiodic Light Treatments

A light intensity of  $180 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD via white (W) LEDs (MEF50120, More Electronics Co. Ltd., Changwon, Korea) was supplied during the photoperiod to grow the plants. Zeitgeber Time (ZT), which is a 24-h time-scale in which “light on” is ZT 0 in photoperiodic research, light was provided between ZT0 and ZT16, and between ZT0 and ZT10 in LD and SD conditions, respectively. Night interruptions were provided to SD conditions from Zt15 to Zt19. The critical day length required for flowering in the SD plant used throughout this study was 12 h, and therefore, a 14-h period of uninterrupted darkness was sufficient to initiate flowering. However, SD with 16 h of darkness was used in this study to confirm a clear flowering response. The photoperiod was started at 08:00 every day in all treatments. LD and uninterrupted SD served as the control. NI was constructed by  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD LEDs with either blue (B, 450 nm), red (R, 660 nm), far-red (Fr, 730 nm), or white (W, 400–700 nm, with 28% B, 37% R, and 15% Fr) wavelengths, which were, respectively, designated as NI-B, NI-R, NI-Fr, and NI-W (Figs. 1, 2). The average PPFD of each treatment was measured using a digital photometer (HD2102.1, Delta OHM, Padova, Italy) at a 20-cm distance above the bench top, and was adjusted to the same before NI treatments were initiated. The light spectral distribution was scanned using a spectroradiometer (USB 2000 Fiber Optic Spectrometer, Ocean Optics Inc., Dunedin, FL, USA; wavelength detection ranges of 200 to 1000 nm) 25 cm above the bench top at 1-nm wavelength intervals. The spectral distribution and

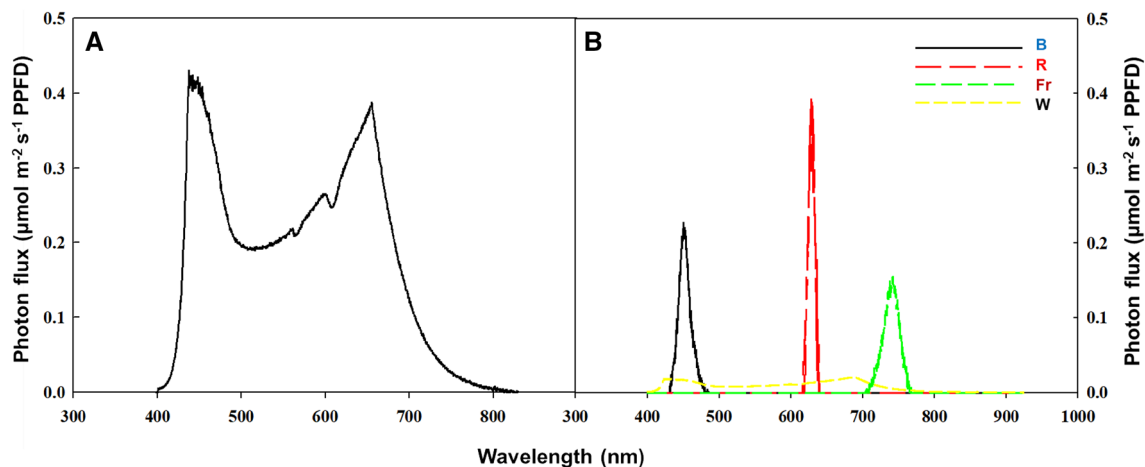


**Fig. 2** A representative image of the night interruption light (NIL) quality and positioning by light-emitting diodes (LEDs). The NI and positioning treatments, provided by LEDs, used in this study and the NIL qualities used for 4-h a day during the dark period: NI-B, blue; NI-R, red; NI-Fr, far-red; and NI-W, white. The LD and SD indicate 16-h long day and 10-h short day, respectively

characteristics measured at three locations within the plant growing areas in each treatment are shown in Fig. 1. The NIL was shed onto either the shoot tip, youngest mature leaf (vigorously growing third leaf from the shoot tip), or old leaf (third leaf from the stem base) (Fig. 3). In order to minimize the interference of other light sources and to illuminate specific areas with the NIL, a chip of specified LED light was fixed inside a rectangular column (20 mm × 20 mm × 40 mm in length) of black cardboard wrapped with reflective aluminum foil as shown in Fig. 3.

### Data Collection and Statistical Analysis

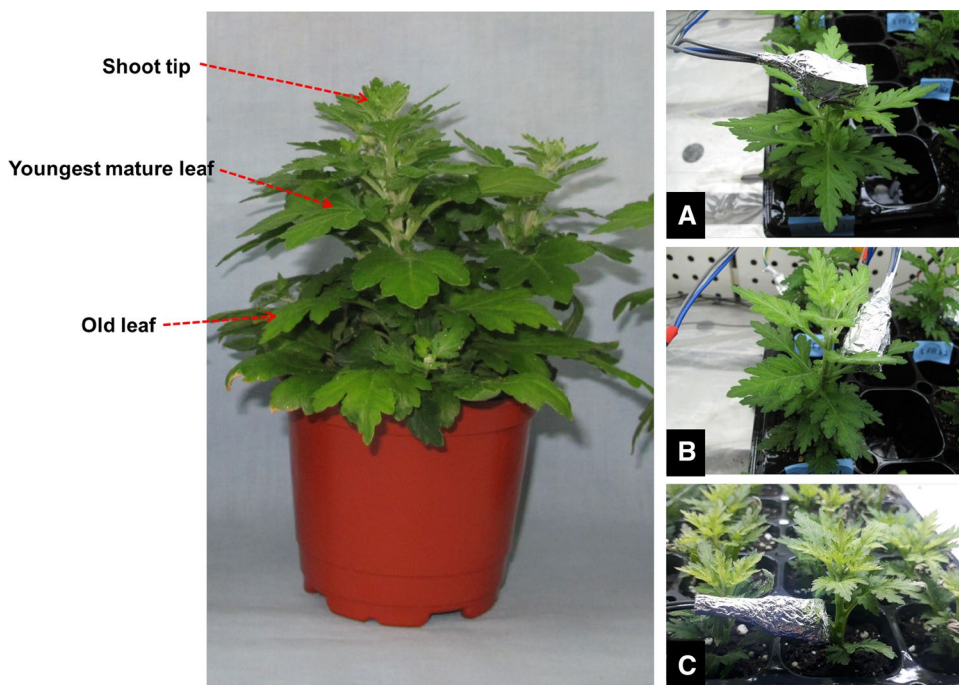
Forty-four days after initiating the photoperiodic treatments, the following data were collected: plant height, number of leaves per plant, chlorophyll content, dry mass, fresh and dry weights of the shoot and root, time from treatment initiation



**Fig. 1** Spectral distribution of the night interruption light (NIL). The spectral distribution of light used in a closed walk-in growth chamber: white light-emitting diodes (LEDs) used as daily light (a) and differ-

ent LEDs (B blue, R red, Fr far-red, W white) used as NILs (b) (Color figure online)

**Fig. 3** Photos of the night interruption light (NIL) positioning. The effects of the NIL quality and positioning provided at  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD on the morphogenesis and flowering of the photoperiodic floricultural plants: **a** shoot tip; **b** youngest mature leaf; and **c** old leaf



to visible flower buds, number of flowers and flower buds per plant (hereafter designated as the “number of flowers”), and the expression of important photoreceptor genes. The number of leaves per plant was measured by counting all leaves which are longer than 1 cm in length. The fresh weight was measured by an electronic scale (EW 220-3NM, Kern and Sohn GmbH., Balingen, Germany). Dry weight was measured after drying the divided samples of the shoot and root for 72 h in a drying oven (Venticell-222, MMM Medcenter Einrichtungen GmbH., Munich, Germany) at 70 °C. The leaf area was measured with a leaf area meter (LI-3000, LI-COR Inc., Lincoln, NE, USA). For chlorophyll content estimation, 10 mg of fresh leaf samples was taken from the young fully developed leaves. Chlorophyll was extracted using 80% 4 °C acetone. The absorbance of the supernatant was measured at 663 and 645 nm using a spectrophotometer (Biochrom Libra S22, Biochrom Co. Ltd., Holliston, MA, USA) after a centrifugation at 3000 rpm. Calculations were done according to the method by Dere et al. (1998). The statistical analysis was carried out using the statistical analysis program (SAS 9.1, SAS Institute Inc., Cary, NC, USA). The experimental

results were subjected to an analysis of variance (ANOVA) and Tukey’s multiple range test. Graphing was performed with a graphic program (SigmaPlot 12.0, Systat Software Inc., San Jose, CA, USA).

### Total RNA Isolation, cDNA Synthesis, and Real-Time Polymerase Chain Reaction (PCR) of Selected Genes

For total RNA extraction, the most recently matured leaf was collected 30 days after the initiation of the photoperiodic light treatments, when plants had already developed visible flower buds, unless they were grown in LD or with NI-R shed onto the shoot tip. The most recently matured leaf was collected at 09:00, 1 h after the daily light treatment initiation. This decision was based on the fact that at this time of the day, photosynthetic rates are high. Independent PCRs with equal amounts of cDNA were performed using primers of *Phytochrome A* (*PHYA*), *Phytochrome B* (*PHYB*), *cryptochrome 1* (*cry1*), and *TERMINAL FLOWER 1* (*TFL1*) genes of the sequence (Table 1). *Actin* was used as an internal control, as it is commonly used to normalize

**Table 1** List of the primers used to quantify the levels of gene expression

Gene	Accession no.	Forward primer	Reverse primer
<i>PHYA</i>	EU915082	5'-GACAGTGTTCAGGCTTCAACAAG-3'	5'-ACCACCAGTGTGTGTTATCCTG-3'
<i>PHYB</i>	NM_127435	5'-GTGCTAGGGAGATTACGCTTTC-3'	5'-CCAGCTTCTGAGACTGAACAGA-3'
<i>CRY1</i>	NM_116961	5'-CGTAAGGGATCACCGAGTAAAG-3'	5'-CTTTAGGTGGGAGTTGTGGAG-3'
<i>TFL1</i>	AB839767	5'-ACAACGGACTCCTCATTTGG-3'	5'-CGCGAAACTACGAGTGTGA-3'
<i>Actin</i>	AB205087	5'-CGTTTGATCTTGCTGGTCG-3'	5'-CAGGACATCTGAAACGCTCA-3'



molecular expression studies. The relative expression of each gene was determined using the  $2^{-\Delta\Delta C_t}$  method (Livak and Schmittgen 2001). Then, the individual gene expression values of the treated plants were divided by the mean gene expression level for plants in SD at each sampling date. The methods used for total RNA isolation and semi-quantitative real-time PCR analysis of selected genes were the same as those described in Park et al. (2015).

## Results

### Morphogenesis

Plants grown in LD were taller than those in SD (Fig. 4a). Compared to plants grown in SD, plants in NI-R and NI-Fr were taller, and plants in NI-B were shorter. In all NI treatments except NI-Fr, the plant height was negatively correlated with the age of the leaf (or tissue) exposed to the NIL. The greatest plant height was observed for plants in NI-Fr applications, regardless of where the NIL was shed onto. Plant height was the smallest when NI-B was shed onto the old leaf as compared to SD.

For all NI treatments except NI-R, the dry mass of plant was negatively correlated with the age of the leaf (or tissue) where the NIL was applied (Fig. 4b). The dry mass of plants grown with NI-B or NI-Fr shed onto the old leaf was 26% or 31% lower, respectively, than that of plants grown in SD. The lower dry mass of plants grown with NI-B shed onto the old leaf (Fig. 4b) may be related to the distinctively shorter plants (Fig. 4a). Plants with NI-Fr shed onto the old leaf had the lowest dry mass, which may be related to the overgrowth or stretchiness.

The highest number of leaves was observed for plants in the LD, and plants in NI-W had the highest number of leaves of all plants in NI treatments (Fig. 4c). The lowest number of leaves per plant was observed for plants whose old leaves were illuminated by NI-B. For NI-R and NI-W, illumination of the shoot tip resulted in the greatest observed leaf areas (Fig. 4d), but when shed onto the youngest mature leaf or old leaf, leaf expansion or mean leaf area was drastically suppressed (Fig. 4e). The NI-B and NI-Fr positioning did not seem to affect the leaf area. Of all NIL qualities and positioning considered in the experiment, the chlorophyll content was the lowest when NI-R was shed onto the shoot tip (Fig. 4f). The NI-Fr seemed to have no significant effects on the chlorophyll content regardless of where on the plant it was shed onto.

### Flowering

Flowering was induced by all treatments except LD and NI-R shed onto the shoot tip (Figs. 4g, h, 5a, b). Moreover,

the time from treatment initiation to visible flower buds was shorter for plants whose old leaves were illuminated by NI-Fr or NI-B as compared to SD, and was longer for plants shed by NI-R on the youngest mature leaf or old leaf and NI-W shed onto the shoot tip, youngest mature leaf, or old leaf, than plants in SD (Fig. 4g). When NI-R was shed onto the youngest mature leaf, the number of flowers per plant was 63% higher than that observed in SD (Fig. 4h).

### Gene Expression Analysis

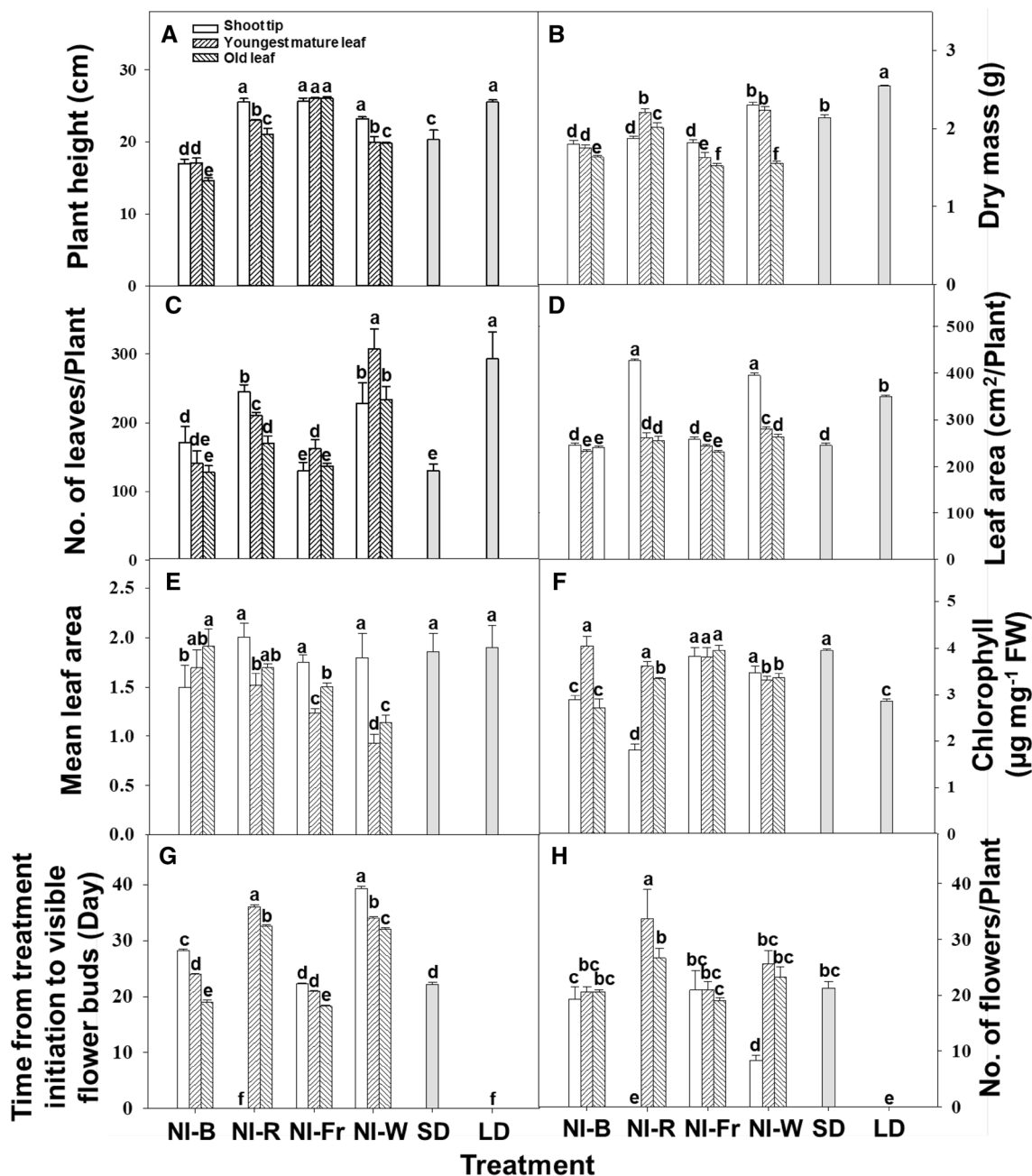
The expression of *PHYA* was significantly reduced by most of the treatments as compared to SD (Fig. 6a), whereas expression of *PHYB* was enhanced by most of the treatments as compared to SD (Fig. 6b). For the plants in SD, the expressions of *PHYA* and *PHYB* were the greatest and lowest, respectively (Fig. 6a, b). *CRY1* was highly expressed in plants in SD and reduced in all NI treatments except NI-W shed onto the old leaf (Fig. 6c). *TFL1* was enhanced in all NI treatments as well as LD, and the highest level of *TFL1* expression was observed in plants grown with NI-B shed onto the young mature leaf (Fig. 6d).

## Discussion

### Plant Height

Light capture is strongly dependent on the light absorption characteristics of leaves; pigment composition, thickness, tissue porosity, chloroplast orientation at different positions (ages) on the stem, and optical properties of leaves all affect the light absorption characteristics (van Ieperen 2012). Among the plants grown in NI-B, NI-R, and NI-W, but NI-Fr, it was observed that the younger the leaves that were exposed to the NIL (Fig. 4a), the incrementally taller the plants were. This means that NIL applied to younger leaves promotes, while that applied to older leaves suppresses stem elongation, suggesting different preferable sites of NIL application depending on the type of crops produced such as cut flowers and potted flowers. These changes in stem elongation were caused by decreased number of leaves or shortened time from the treatment initiation to visible flower buds in all, but NI-W shed onto the shoot tip, the reason for which is not clearly explainable.

This study shows that plants grown in NI-Fr were taller regardless of the location of light exposure, indicating that the effects of Fr light may not depend strongly on its positioning on the plant (Fig. 4a). Many species develop elongated stems and smaller leaves when they are grown in close proximity to neighboring plants, and these characteristics are known as the shade avoidance response (Hersch et al. 2014). The shade avoidance response improves a plant's reach to



**Fig. 4** The effects of the night interruption light (NIL) quality and its positioning on the plant height (a), dry mass (b), number of leaves per plant (c), leaf area per plant (d), mean leaf area (e), chlorophyll content (f), time from treatment initiation to visible flower buds (g), and number of flowers per plant (h) in *Dendranthema grandiflorum* 'Gaya Yellow.' The NIL qualities used for 4-h a day during the dark

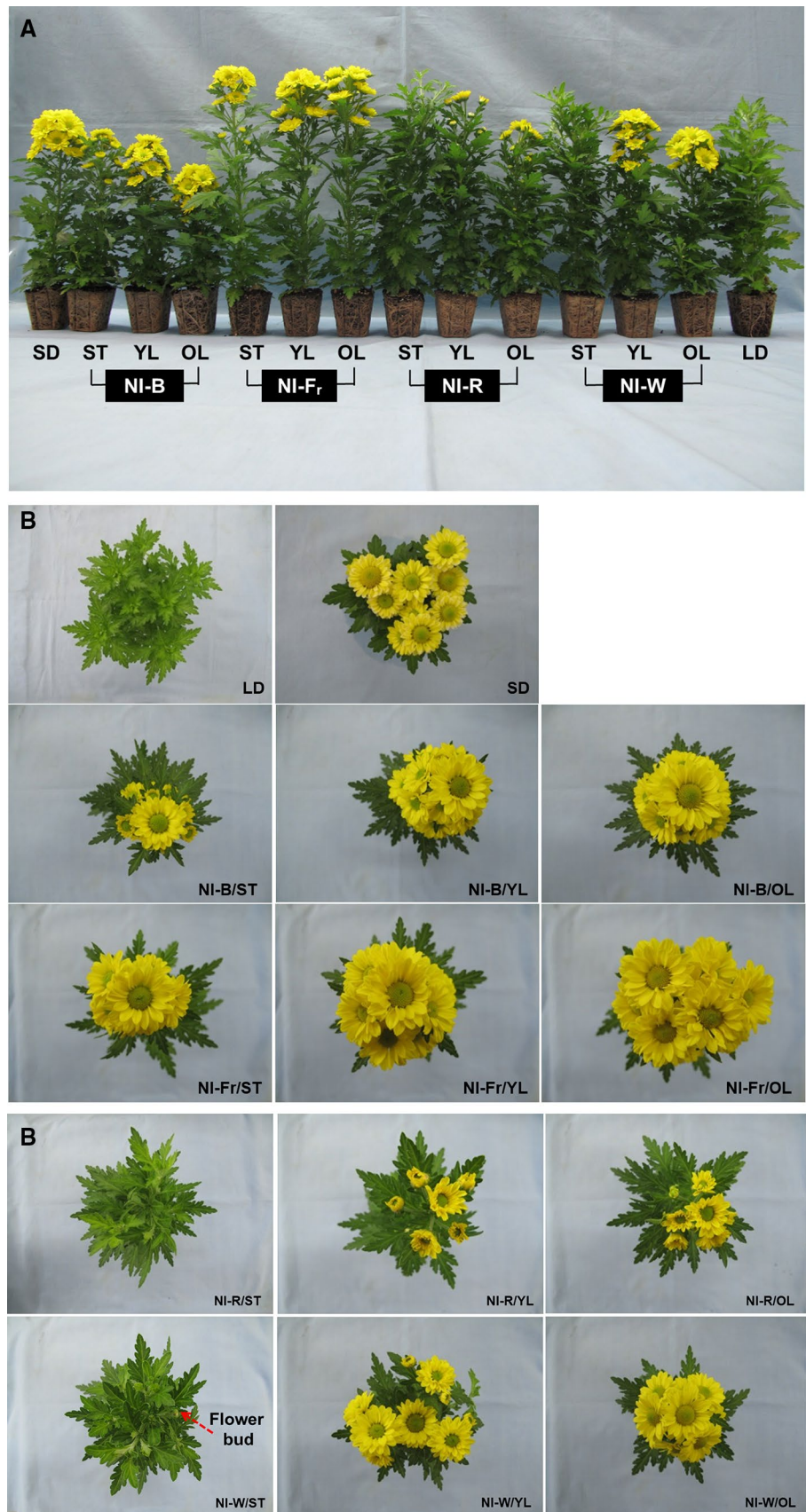
period: NI-B, blue; NI-R, red; NI-Fr, far-red; and NI-W, white. The LD and SD indicate 16-h long day and 10-h short day, respectively. Data are the mean  $\pm$  S.E. of the three biological replicates. Means accompanied by different letters are significantly different ( $P < 0.05$ ) according to the Tukey's studentized range test at 5% significance level

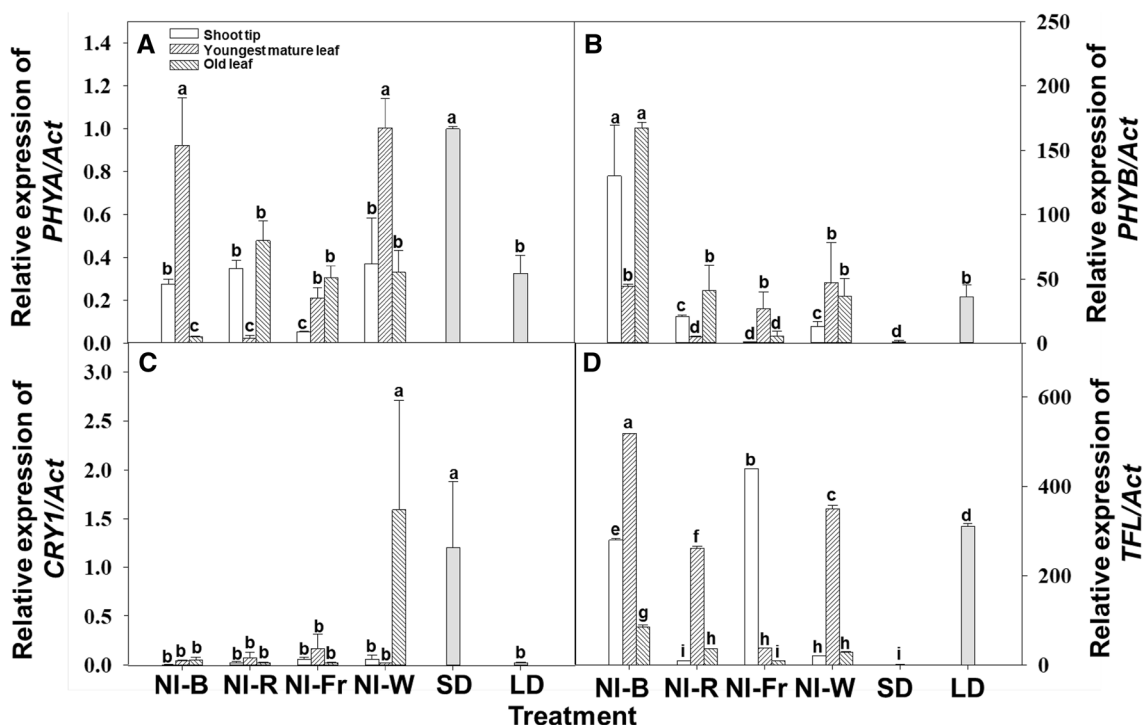
light above other plants and provides a competitive advantage (Hersch et al. 2014).

The use of B light during the photoperiod to inhibit plant elongation was previously documented in chrysanthemums (Oyaert et al. 1999; Kim et al. 2004; Shimizu et al. 2006). However, the growth-suppressing effects of

the NIL, especially B light, are not well known. Schuenger et al. (1997) noted that B light plays a part in inhibiting the growth, changing the stem anatomy, and morphologically developing the leaves in pepper plants. Furthermore, Khatkhat and Pearson (2006) reported that a low level B light during the photoperiod suppressed plant growth. Our finding

**Fig. 5** The effects of the night interruption light (NIL) quality and positioning provided at  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD on the flowering of *Dendranthema grandiflorum* ‘Gaya Yellow’ measured 44 days after treatment: side view (a) and top view (b) (See Fig. 3 for details on the NIL quality). The lighting positions were *ST* shoot tip, *YL* youngest mature leaf, *OL* old leaf





**Fig. 6** The effects of the night interruption light (NIL) quality and positioning provided at  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD on the relative gene expression by real-time PCR of *PHYA* (a), *PHYB* (b), *CRY1* (c), and *TFL* (d) of *Dendranthema grandiflorum* ‘Gaya Yellow’ (See Fig. 1 for details on the NIL quality). The relative expression of each gene was determined using the  $2^{-\Delta\Delta C_t}$  method. Then, the individual gene

expression values of the treated plants were divided by the mean gene expression level for plants in SD at each sampling date. Data are the mean  $\pm$  S.E. of the three biological replicates. Means accompanied by different letters are significantly different ( $P < 0.05$ ) according to the Tukey’s studentized range test at 5% significance level

that NI-B may limit plant height (Fig. 4a) can help growers achieve desired compact growth, i.e., in potted flowering plants. Tewolde et al. (2016) reported that the node length of tomato plants was reduced when R and B LEDs were used in conjunction for inter-lighting on the lower canopy. Inter-lighting, which refers to directing light to lower parts of plants, on the lower plant canopy may be a possible practical method to suppress plant height, and control flowering. The B light inter-lighting could be more effective than combined R and B light inter-lighting in controlling the plant height, and NI-B may be a good, environmentally sound alternative to plant growth retardants which are widely used in the production of potted flowering plants.

### Leaf Growth

Plants shed with NI-B or NI-Fr onto the youngest mature leaf or old leaf resulted in smaller leaf area as compared to plants in SD (Fig. 4d). This was due to the rapid switch from vegetative growth to reproductive growth in all, but NI-W shed onto the shoot tip, as the sensitivity of old leaves to NI seemed negligible in comparison to that of the shoot tip, which is in agreement with the established theory (Zeevaart

2008) who reported that florigen was the leaf-produced signal that induces floral initiation at the shoot tip.

Chlorophyll content decreased in non-flowering treatments such as plants grown with NI-R shed onto the shoot tip and LD condition in this study (Fig. 4f). Gang et al. (1992) reported that the chlorophyll content increased during the transition from vegetative to reproductive growth and decreased during maturation. Thus, in this study, the decrease in the chlorophyll content of plants in LD and plants grown with NI-R shed onto the shoot tip as compared to other treatments may be due to continued vegetative growth. This may apply to the NI-B shed onto the youngest mature leaf (Fig. 4). However, in other NI-B treatments such as NI-B shed onto the shoot tip or old leaf, chlorophyll content decreased, although flowering was observed. The real reason for these decreased chlorophyll contents in plants grown with NI-B shed onto the shoot tip or old leaf is not clearly explainable.

### Flowering and Gene Expression

The expression of *PHYA* slightly promotes flowering in plants grown with NI-B and NI-W shed onto the youngest



mature leaf, and SD (Fig. 6a). It was speculated that *PHYA* can promote flowering by suppressing *PHYB* function and/or by independent mechanisms. The non-flowering that was observed in LD despite high level of *PHYA* expression may be due to the high expression levels of *PHYB* and *TFL* observed in that treatment (Fig. 6a, b, d). *PHYB* acts in a partially redundant manner with *PHYD* and *PHYE* to mediate the R light-dependent inhibition of flowering (Franklin et al. 2003; Mockler et al. 1999). However, *PHYB* may serve a more complex role in floral initiation than just as a simple inhibitor (Lin 2000). The *CRY1* and *CRY2* act redundantly to mediate the B light-dependent promotion of flowering (Mockler et al. 2003). Because the *phyA* mutant flowered late in response to a day extension with the Fr-rich incandescent light (Johnson et al. 1994), it may be speculated that *PHYA* may mediate the Fr light-dependent inhibition of the *PHYB* function (Lin 2000), analogous to the antagonistic relationship of *CRY2* and *PHYB*, and to the Fr light-dependent *PHYA* function in the hypocotyl development inhibition. The *CRY2*, in addition to its antagonism to *PHYB*, also mediates the B light-dependent promotion of floral initiation. The *CRY2*'s mediation of the B light-dependent flowering promotion is redundant with that of *CRY1* (Mockler et al. 1999).

The flowering is theorized to be induced when one or more of the flowering promotor genes (*PHYA* and *CRY1*) are expressed in high levels (Lin 2000; Song et al. 2013). In general, B light enhances flowering by the photoregulation of several floral induction genes (Fukuda et al. 2008). In *Arabidopsis thaliana*, the B light signal perceived by *CRY1* or *CRY2* stimulates the expression of *FT* and *SUPPRESSOR OF OVEREXPRESSION OF CO 1 (SOC1)* via increased *CONSTANS (CO)* protein stabilization, which subsequently induces differentiation of the meristematic tissue and early flowering (Fukuda 2013). Although it is not known whether it applies to chrysanthemums in the same manner, florigen is the hypothetical signal produced at the leaf that initiates the shoot apex flowering (Zeevaert 2008). Our findings indicate that flowering was not observed for at least 44 days of treatment for plants with NI-R shed onto the shoot tip (Fig. 5b). Unexpectedly, flowering was induced in plants with NI-R shed onto the youngest mature leaf or old leaf, indicating that the commonly known R light suppression of flowering during NI in SD plants may not necessarily apply in the case of NIL irradiation onto young and old leaves. Light signals stimulate photoreceptors, thereby controlling the floral induction genes (Cerdán and Chory 2003). Flowers bloom early when the 'gene switch' to initiate floral buds is turned on by a light of a specific quality. According to Higuchi et al. (2012), NI-Fr for chrysanthemum has reversed the flowering response inhibited by NI-R. Further reverse genetic approaches have elucidated the *PHYB*-mediated inhibition of flowering in plants under NI-R and the subsequent reversion of flowering response by NI-Fr in chrysanthemum

(Higuchi et al. 2012). In addition, the knock-down of the *PHYB* gene resulted in the up-regulation of the flowering inducer (florigen) and down-regulation of the flowering inhibitor (antiflorigen) in chrysanthemum, and led to the extremely early flowering response (Higuchi et al. 2013). However, *PHYB* and *TFL*, both flowering inhibitors, were not highly expressed in plants in NI-R shed onto the shoot tip, although flowering was not observed (Fig. 6b). The real reason for low expression of these genes in these plants may be a combined effect of these genes with other flowering inhibiting genes such as *Anti-florigenic FT/TFL1* family protein (*AFT*), which were not investigated in this study.

The transcriptional factor *PHYA*, a flowering promotor gene, was slightly expressed, while *CRY1*, another flowering promotor gene, was not expressed to an expected level in such treatments as SD and all NI treatments except NI-R shed onto the shoot tip. The overall expression patterns of photomorphogenic genes observed in this study differed from those described in previous studies (Higuchi et al. 2012, 2013). Possible explanations for this disparity are differences in the chrysanthemum genotypes used, the experimental environment, the sample collection time, the plant part from which the samples were collected, and the specific wavelengths of LEDs used in the studies.

In this study, the time after treatment initiation to visible flower buds was shortened by NI-Fr or NI-B shed onto the old leaf, and was lengthened by NI-R shed onto the youngest mature leaf or old leaf and by NI-W shed onto the shoot tip, youngest mature leaf, or old leaf. In the experiment on the effects of the NIL quality, flowering was inhibited for plants in NI-R and NI-W (Park 2014), while flowering was interrupted by NI-R treatments in the experiment on the effects of the NIL quality and positioning.

Interestingly, our experiment shows that plants with NI-R shed onto the youngest mature leaf had more flowers per plant than those in SD. This agrees with the overflow metabolism concept, where the carbon demands associated with plant growth are exceeded by the carbohydrate production (Matsuki 1996; Park et al. 2013), and is expressed in ways such as a higher number of flowers per plant due to the relatively longer duration of vegetative growth.

Our results indicated that the quality of the NIL and the age of the leaf (or tissue) exposed to the NIL affected the morphogenesis, flowering, and transcriptional factors of chrysanthemum. The plant height was related to the time from treatment initiation to visible flower buds. Leaf growth was affected when NIL was targeted onto the shoot tip. The previously known effects of the NIL quality on the vegetative to reproductive phase transition of plants were pronounced when the NIL was shed onto the shoot apex. The transcriptional factor *PHYA* was slightly expressed in plants which showed a flowering response. However, the *CRY1*, *PHYB*, and *TFL* were not expressed to levels reported previously,

and the reason for low expression of these genes in these plants may be due to a combined effect of these genes with other flowering promoting and/or inhibiting genes. The NI-R, NI-Fr, and NI-B shed onto the youngest mature leaf or old leaf could potentially control the flowering of SD plants. The NI-B shed onto the old leaf could potentially help produce desirably compact potted SD plants due to its height-suppressing characteristics. Inter-lighting the lower plant canopy could be a practical application of the results of this study to control the plant height and flowering in the production of floricultural crops.

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**Author Contributions** Designed the project: BRJ and YGP; Performed the experiments: YGP; Analyzed the data: YGP; Maintained the plant factory: YGP; Took care of plants: YGP; Wrote paper: YGP and BRJ.

## Compliance with Ethical Standards

**Conflicts of interest** The authors declare that they have no conflict of interest.

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