Reproductive Response of Okra and Native Rosella to Long-day Treatment with Red, Blue, and Green Light-emitting Diode Lights

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Abstract. We investigated the reproductive responses of three cultivars of short-day plants to day-extension and night-break treatment with red, blue, and green light-emitting diodes (LEDs). The plants examined were all Malvaceae species: two cultivars of okra [*Abelmoschus esculentus* (L.) Moench.] and a cultivar of native rosella [*Abelmoschus moschatus* ssp. *tuberosus* (Span.) Borss.]. To create day extension or night break, we provided supplemental light from LED panels with peak photon emissions of 470 (blue), 520 (green), or 650 (red) nm. Day-extension treatment using red or blue LEDs inhibited flower and bud appearances; the response was especially pronounced with red LEDs. Night-break treatment with red LEDs also delayed flower bud appearance, but night break with blue LEDs did not produce a clear effect. Night break with green light delayed flowering more strongly than blue light but a little less than red light. We concluded that the dark period-regulated reproductive processes of these plants are most sensitive to disruption by red light, closely followed by green light, but that they are insensitive to blue light, especially when the exposure period is short.

Long-day treatment with supplemental lighting (i.e., night break or day extension) can be used to control the flowering of plant species in which reproductive stage is influenced by photoperiod. The effect of supplemental light quality on long-day treatment has been investigated in relation to the development of suitable artificial lighting for long-day treatment. Colored supplemental lights have been investigated and used for the physical control of insect pests and diseases (Hirama et al., 2007; Schuerger and Brown, 1997) as well as for flowering control. To control flowering efficiently, and to avoid any undesirable effects such as delayed or premature flowering, it is therefore important to gain an understanding of the reproductive reactions of many species of plants to the quality of supplemental lighting.

Funke (1948) investigated the effects of day extension using supplemental lights of different wavelength ranges on the flowering of many plant species. Flowering of most of the short-day plants (SDPs) in that study was delayed as much under red light as under white light. Blue-light long-day treatment influenced the flowering of only two SDPs [*Camelia japonica* (Theaceae) and *Perilla nankinensis* (Lamiaceae)] as well as red-light long-day treatment. Cruciferous species (mostly long-day plants and *Cheiranthus allionii*, an SDP

that is used as an herb or an ornamental garden plant) displayed the different response, i.e., blue light influenced flowering, whereas red light had no effect.

The reproductive response of SDPs to long-day treatment with colored lights has been studied in many other species: chrysanthemum (Cathey and Borthwick, 1957), amaranthus (Downs, 1956), soybean (Downs, 1956; Parker et al., 1946), *Lemna paucicostata* (Lumsden et al., 1987), cosmos (Hamamoto et al., 2003; Stolwijk, 1952; Wassink et al., 1951), cocklebur (Parker et al., 1946), perilla, yellow cosmos, zinnia, and Japanese morning glory (Hamamoto et al., 2003). However, none of these studies has found an SDP that reacts to blue light in the same way as *C. allionii* in Funke's study.

Malvaceae (the mallow family) is more closely related to the Cruciferae than the families of the plants used in any of the previous studies as judged by phylogenetic relationships determined from the analysis of multiple gene sequences (Soltis et al., 1999). However, the reproductive response of mallow species to long-day treatment using monochrome light has not been investigated. The response of okra [*Abelmoschus esculentus* (L.) Moench.] to photoperiod has been investigated (Arulrajah and Ormrod, 1973; Rithichai et al., 2004), but only using white light for long-day treatment. We therefore investigated the reproductive response of three *Abelmoschus* SDPs to two types of long-day treatment with narrow-spectrum color lights to see which wavelengths were most important and to determine whether

some plants of the Malvaceae family displayed the same reproductive responses as crucifer species to different light colors.

We used light-emitting diodes (LEDs) as monochrome supplemental light sources. LEDs have narrow spectral bandwidth emissions, low energy consumption, and a long life. They are therefore useful for studies of the effects of light quality on plant growth and have the potential to be economical light sources in commercial applications.

Materials and Methods

Three experiments were carried out. Seeds of 'Clemson Spineless' and 'Emerald' (Takii Seed Co., Ltd., Kyoto, Japan) okras and 'Pontinum Red' (Sakata Seed Co., Yokohama, Japan) native rosella [*Abelmoschus moschatus* ssp. *tuberosus* (Span.) Borss.] were sown on cell trays (23-mL cells, 16 per tray) filled with growing media (Tanemakibaido; Takii Seed Co., Ltd.). The germinated seedlings were grown in three growth cabinets (KG-50HLA; Koito Industries, Ltd., Yokohama, Japan). When the plants had produced their first true leaves, nine (Expt. 1) or 11 plants (Expts. 2 and 3) per treatment were selected and transplanted into 230-mL plastic pots filled with fertilized soil (0.3N–0.8P–0.3 K g·L⁻¹) and grown in growth cabinets.

The temperature and relative humidity in the growth cabinets were controlled at 23 °C and 70%, respectively. Light was provided by 14 fluorescent lamps (EPR96EX-N/A; Matsushita Electric Industrial Co., Ltd., Osaka, Japan) at a photosynthetic photon flux density (PPFD) of 200 μmol·m⁻²·s⁻¹ at floor level. The PPFD was measured with a quantum sensor (LI-190SA; LI-COR, Lincoln, NE). In Expt. 1, the light treatments consisted of a 6-h photoperiod followed by either 1) a continuous 18-h dark period; or 2) a 12-h day extension with supplemental lighting. In Expts. 2 and 3, the light treatments consisted of an 8-h photoperiod followed by either 1) an uninterrupted 16-h dark period; or 2) a 4-h night break of supplemental lighting preceded and followed by a 6-h dark period (Fig. 1A). In each experiment, the day-extension or night-break treatments for each cultivar were continued until flower buds were found in all plants grown in all three growth cabinets. The plants were then moved into a glasshouse.

The supplemental light for day extension or night break was provided by two handmade 75 × 95-mm LED panels for each of three light colors (blue, green, and red); peak photon emission wavelengths were 470 nm for blue, 520 nm for green, and 650 nm for red (Fig. 1B). Each LED panel consisted of 15 individual LEDs hung from a pole set horizontally at a height of 60 cm above the floor of the growth cabinet. In Expts. 1 and 2, day-elongation or night-break treatments involved one treatment with blue LED panels and one with red LED panels. In Expt. 3, three LED treatments were used: blue panels, red panels, and green panels. Electrical controllers kept the PPFD at floor level

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below the center of each LED panel at 4 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

In Expt. 1, day-extension treatment continued for 43 d for 'Clemson Spineless' okra, 51 d for 'Emerald' okra, and 64 d for native rosella plants. After the light treatment, the plants were moved into a glasshouse with natural daylength of less than 10.5 h, where they were grown for 9 ('Emerald'), 10 ('Clemson Spineless'), and 28 (native rosella) days.

In Expt. 2, night-break treatment was carried out for 48 d in both cultivars of okra and for 68 d in native rosella. The plants were then exposed to natural short daylength (less than 11.5 h) in a glasshouse for 13 d for okra and 14 d for native rosella.

In Expt. 3, night-break treatment was carried out for 56 (okra) or 74 (native rosella) days. The night-break treatment was followed by exposure to short daylength (less than 11 h) in a glasshouse for 15 d for okra and 25 d for native rosella.

In all three experiments, we assessed reproductive development by the position of the lowest node bearing a flower bud on individual plants and from the total number of opened flowers per treatment (Expt. 1) or individual plant (Expts. 2 and 3). Statistical analysis was performed with Excel 2003 (Microsoft Corporation, Redmond, WA) with the add-in software Excel Tokei 2006 (Social Survey Research Information Co., Ltd., Tokyo, Japan).

Results and Discussion

In Expt. 1, the control treatment (i.e., without day extension) tended to result in flower buds appearing on lower nodes and in faster flowering than with day extension by blue or red LEDs (Fig. 2). However, the inhibition of flower opening and bud appearance was more pronounced with red LEDs than with blue LEDs.

In Expt. 2, night break with red LEDs delayed the appearance of flower buds and flowering to about the same extent as day extension with red LEDs in Expt. 1. However, night break with blue LEDs did not inhibit flower and bud appearance in any plant type and may have caused slightly earlier flowering in the 'Emerald' okra (Fig. 3). Red light suppressed flowering in both the 12-h day-extension treatment and the 4-h night-break treatment, and blue light had a weaker effect on flowering in the night-break treatment. This suggests that shorter periods of exposure to red light than to blue light would be required to suppress flowering.

Expt. 3 compared the effects of night-break treatment with those of three different LEDs (blue, green, and red) on reproduction. In the two okras, flower buds developed faster and on lower nodes in the order of blue, green, and red LED treatments (Fig. 4). Although the native rosella displayed the same trend, the differences were not significant. Night break with red light suppressed flowering more effectively than night break

Fig. 1. (A) Daily photoperiods (white zones) and supplemental lighting periods (shaded zones) used in Expts. 1, 2, and 3 to study the effects of light-extension and night-break treatments with different colored lights on the reproductive responses of three plant cultivars belonging to the family Malvaceae. Black zones indicate dark periods. (B) Relative photon fluxes (peak = 1) of three types of light-emitting diodes used for supplemental lighting. Spectral distribution scans were taken from wavelengths of 300 to 800 nm with a spectroradiometer (LI-1800; LI-COR, Lincoln, NE).

Fig. 2. Node position bearing the first flower bud (top graphs) and mean number of opened flowers (bottom graphs) in two cultivars of okra ('Clemson Spineless' and 'Emerald') and native rosella plants subjected to day extension with blue or red light-emitting diode lights or without day-extension treatment (Dark) (Expt. 1). Numbers of opened flowers were counted per treatment (nine plants per treatment) and were averaged. Vertical bars indicate SES. Treatments marked with different letters are significantly different according to Tukey's test at $P \leq 0.05$.

with blue light, like in Expt. 2. In the two cultivars of okra, green light also suppressed flowering more effectively than blue light but slightly less than red light, whereas in native rosella, there was no difference among the three light types. In these experiments, the two cultivars of okra perceived red light more strongly than they perceived the other two wavelengths. Green light was also perceived, but to a lesser extent than red light. Blue light, however, was only weakly perceived. Although native rosella also tended to perceive red light, the effect on reproduction was

far less pronounced than in the two cultivars of okra, and there was very little evidence that native rosella perceived either blue light or green light.

Many previous studies on plants from families other than the Malvaceae and Cruciferae agree with the results of the present study. Night break with red light delayed floral initiation in chrysanthemum (Asteraceae; SDP; Cathey and Borthwick, 1957), amaranthus (Amaranthaceae; SDP), and soybean (Leguminosae; SDP) (Downs, 1956). The inhibition of flowering in *Lemna paucicostata*,

Fig. 3. Node position bearing the first flower bud (top graphs) and number of opened flowers (bottom graphs) in two cultivars of okra ('Clemson Spineless' and 'Emerald') and native rosella plants subjected to night-break treatment with blue or red light-emitting diode lights or without night-break treatment (Dark) (Expt. 2). Vertical bars indicate SEs. Treatments marked with different letters are significantly different according to Tukey's test at $P \leq 0.05$.

Fig. 4. Node position bearing the first flower bud (top graphs) and number of opened flowers (bottom graphs) in two cultivars of okra ('Clemson Spineless' and 'Emerald') and native rosella plants subjected to night-break treatment with blue, green, or red light-emitting diode lights (Expt. 3). Vertical bars indicate SEs. Treatments marked with different letters are significantly different according to Tukey's test at $P \leq 0.05$.

an SDP species of the family Lemnaceae, was greatest with red (≈ 650 nm in wavelength) light followed by green (≈ 550 nm) and then blue (≈ 450 nm) light (Lumsden et al., 1987). Parker et al. (1946) studied the suppression of floral initiation by night break in soybean and cocklebur (Asteraceae). Both plants showed maximum sensitivity at ≈ 650 nm wavelength (red light), whereas sensitivity to green light (500 to 600 nm) was less than to red light but greater than to blue light (400 to 500 nm).

In our previous study (Hamamoto et al., 2003), budding and flowering of perilla (Lamiaceae), Japanese morning glory (Con-

volvulaceae), and zinnia, cosmos, and yellow cosmos (Asteraceae) were delayed more strongly by red-light than by blue-light night-break treatment. We also found in that study that night break with green light produced a similar level of delay to budding and flowering as night break with red light in perilla, zinnia, and cosmos, but the effect was not quite as pronounced in Japanese morning glory and yellow cosmos. Cosmos has been reported in other studies to respond to green light to the same degree as to red light (Stolwijk, 1952; Wassink et al., 1951).

There are few reports of marked delay in flowering of SDPs exposed to long-day treat-

ment with blue light. Although flowering is delayed in *Camelia japonica* and *Perilla nankinensis* by day extension with blue light, it is delayed to the same degree by red light (Funke, 1948).

A stated aim of this study was to examine whether SDP mallows respond to long-day treatment with red and blue light in the same way as the SDP crucifer *C. allionii* responded in Funke's (1948) study. The plant species used in the present study and other studies described did not respond to red and blue light in the same way as *C. allionii*; instead, their responses either followed or did not contradict the more usual pattern of a reproductive delay by red light and little or no response to blue light. If there are SDPs that react in the same way as *C. allionii* in plant families other than the Cruciferae, then they are yet to be identified.

Considering the results of the present and earlier studies, green light would be effective in providing a long-day treatment for many SDPs, but the strength of the effect would depend on the plant species concerned and cannot be predicted simply from the family to which the species belongs.

As suggested by Guo et al. (1998) and Thomas and Vince-Prue (1997), inhibition of flowering with red light is affected by red-light receptors such as phytochromes, which absorb well light at wavelengths above 600 nm (Kelly and Lagarias, 1985), and inhibition of flowering with blue light is influenced by blue-light receptors such as cryptochromes, which absorb well light at wavelengths below 500 nm (Banerjee et al., 2007; Lin et al., 1995). However, the red- and blue-light receptors absorb green light poorly (Banerjee et al., 2007; Kelly and Lagarias, 1985; Lin et al., 1995), suggesting that the response to green light may depend on any other photoreceptors.

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