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Short Communications

Low-activity cryptochrome 1 plays a role in promoting stem elongation and flower initiation of mature *Arabidopsis* under blue light associated with low phytochrome activity

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Abstract: To clarify whether cryptochrome contributes to stem elongation and flowering promoted by blue lights associated with low phytochrome activity, wild-type *Arabidopsis* was compared with its cryptochrome-deficient mutants and cryptochrome-overexpressing transgenic plants. Results indicated that the promotion effects were mainly related to low CRY1 activity, despite partial involvement of high-activity CRY2.

Key words: cryptochrome, phytochrome, *Arabidopsis,* mutants, stem elongation, flowering

Introduction

In previous studies, blue light (BL) treatments were normally created by selective solar filters or broad-band light sources such as blue fluorescent lamp, which inherently resulted in impure BL. In this case, the BL was contaminated by a low level of light from other spectral bands (Bergstrand et al. 2014). The recent development of high-power, narrow-band light emitting diodes (LEDs) provides researchers with a tool to more accurately study plant responses to pure BL (B), and impure BL by mixing B with low-level other wavelengths such as red (R) and far red (FR).

Our recent studies using LED have indicated that plant elongation mediated by BL is affected by phytochrome activity (Kong et al. 2018; Kong et al. 2020b). In these studies, pure BL (i.e., B) increased stem elongation relative to R. However, an impure BL, BR, created by adding a low level (6–10%) of R to B, reversed the promotion effect of B, and showed a similar or greater inhibitory effect compared to R. When a low level of FR was further added to BR (with a ratio of R/FR ≈ 1), the resulting another impure BL (i.e., BRF) recovered the promotion effect of B, and showed a similar promotion effect as B, compared to R. The R/FR reversibility is the classic signature of phytochrome action. Also, as an indicator of phytochrome activity, the phytochrome photostationary state (PPS) value was lower for B (0.49) and BRF (0.63) than R (0.89) and BR (0.74). When the PPS value decreases to < 0.60, most plant species show an inactive phytochrome response (Stutte 2009). It appeared that plant elongation was promoted by BL associated with lower phytochrome activity (i.e., B or BRF), but inhibited by BL associated with higher phytochrome activity (i.e., BR). The involvement of phytochrome in the BL-mediated plant elongation has been also confirmed by our recent studies on wild *Arabidopsis* and its quintuple phytochrome mutant (Kong and Zheng 2020b).

Despite the involvement of phytochrome, the action of BL photoreceptor, cryptochrome, on the mediation of plant elongation cannot be ruled out. For example, the inhibition of plant elongation by BR cannot be explained only by higher phytochrome activity, since BR, despite

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having a lower PPS, showed a greater inhibitory effect on elongation than R for some species (Kong et al. 2018). This suggests that cryptochrome also plays a role in the BR's inhibition of plant elongation. Recent study indicates that cryptochrome activity can be modified by phytochrome activity and there is a cross talk between the two photoreceptor systems (Liu et al. 2016). Possibly, cryptochrome activity was reduced in the plants under B or BRF due to low PPS values. However, the speculation about the involvement of cryptochrome in this process needs a direct proof to confirm.

Two types of cryptochromes, cryptochrome 1 (CRY1) and cryptochrome 2 (CRY2), have been discovered in *Arabidopsis* to mediate plant elongation (Yu et al. 2010). Use of cryptochrome mutants and transgenic plants in *Arabidopsis* is one of the ways to study the action of CRY1 and CRY2 on plant elongation. The *cry1* mutant impaired in BL inhibition of hypocotyl elongation (Ahmad et al. 1995). Transgenic *Arabidopsis* plants overexpressing CRY1 were found to be hypersensitive to BL with respect to inhibition of hypocotyl elongation response (Lin et al. 1996). Studies on *cry2* mutant and transgenic plants overexpressing CRY2 indicate that CRY2 also plays a role in BL-mediated inhibition of hypocotyl elongation, despite a relatively minor one compared to that of CRY1 (Lin et al. 1998). The *cry1cry2* double mutant exhibited a longer hypocotyl phenotype under BL than the *cry1* or *cry2* single mutant, suggesting a partially redundant function of the two cryptochromes in this response (Mockler et al. 1999). However, the above studies on stem elongation were performed only in de-etiolated seedlings under broad-band BL source. For mature plants, it is unclear whether CRY1 and CRY2 contribute to stem elongation response to BL with different PPS from LED lighting.

Our recent studies on ornamental plants indicate that in addition to stem elongation, plant flowering also showed similar response to BL with different PPS (Kong et al. 2020a). In other words, plant flowering was promoted by BL associated with lower phytochrome activity (i.e., B or BRF), but inhibited by BL associated with higher phytochrome activity (i.e., BR). The involvement of phytochromes in the actions of these BLs has been confirmed in our recent study on phytochrome mutant of *Arabidopsis* (Kong and Zheng 2020b). Whether cryptochrome, as BL receptor, was also involved in this process is unclear. Previous studies on *Arabidopsis* indicated that *cry1cry2* double mutant showed delayed flowering than the wild type or the *cry1 and cry2* monogenic mutants under broad-band BL (Guo et al. 1998; Mockler et al. 1999). These observations suggest that CRY2 acts redundantly with CRY1 in promoting flowering induction. However, only a broad-band BL from non-LED lighting was used in the above studies. Further study is needed to investigate whether CRY1 and CRY2 contribute to flowering response to BL with different PPS from LED lighting.

The objective of this study was to examine whether cryptochromes (CRY1 and CRY2) contribute to BL-mediated stem elongation and flower initiation by comparing the phenotypic responses among wild type, cryptochrome-deficient mutant and cryptochrome-overexpressing transgenic plants of *Arabidopsis* under R, B, BR, and BRF LEDs.

Materials and Methods

The experiment was conducted in a walk-in growth chamber at the University of Guelph, Guelph, ON, Canada. Six genotypes of *Arabidopsis*, one wild type (Col-0), three cryptochrome-deficient mutants (*cry1, cry2*, and *cry1cry2*) and two cryptochrome-overexpressing transgenic lines (*CRY1-OX* and *CRY2-OX*) were used for the experiment. Seeds were stratified for

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3 days at 4 °C and then sown on agar which was placed on top of rockwool cube a hydroponic system as described by Kong and Zheng (2020b). The six genotypes were evenly and randomly distributed in different rows (i.e., four rows for each genotype and 12 rockwool cubes for each row) within each tray. The sown trays were placed under the light treatments in the growth chamber. The fertigation method and the environment condition for growing the plants were the same as described in Kong and Zheng (2020a).

Light treatments included: (1) R, a pure red light from 660 nm LED; (2) B, a pure BL from 455 nm LED; (3) BR, an impure BL from LED with combination of a photon flux of 94% B and 6% R; and (4) BRF, another impure BL from LED with combination of BR and 6 μmol m^{-2} s^{-1} of FR (735 nm). For each light treatment, a photosynthetic photon flux density (PPFD) of around 100 μmol m^{-2} s^{-1} was achieved at the plant canopy level. The calculated PPS values were 0.89, 0.69, 0.60, and 0.50 for R, BR, BRF, and B, respectively. The setting up of the four light treatments and the calculation method of PPS values were the same as described in Kong and Zheng (2020a).

Once over 50% of the seeds were germinated for each genotype under each light treatment, the cumulative germination percentages were determined. After 18-d lighting, twelve plants from each genotype per light treatment (i.e., three plants from each row in each tray) were randomly selected for plant morphology measurements. The measured plant traits included main stem length, hypocotyl length, and flowering index. The flowering index was defined as follows: 0, flower stalk invisible; 1, flower stalk visible; 2, flower petal visible; 3, flower(s) opened.

Data analysis was performed using the Data Processing System software (DPS, version 7.05; Refine Information Tech. Co., Hangzhou, China). In this experiment, the growth chamber had uniform environmental conditions except for light treatments, and four rows of plants were randomly allocated to each combination of light treatments × *Arabidopsis* genotypes. In this case, the experimental arrangement was considered as a Completely Random Design with two factors and four replicates. Two-way ANOVA was used to determine the effects of each factor (i.e., light treatment, or genotype), and their interaction. Data were presented as means ± SE (standard error; n = 4). The means were separated using Duncan's new multiple range test at the $P \leq 0.05$ level.

Results and Discussion

The main stem elongation promoted by BLs with low PPS is related to low-activity CRY1

In the wild-type *Arabidopsis* plants, B and BRF increased main stem length compared with R, and their promotion effect was eliminated by over expression of CRY1, but not CRY2 although over expression of CRY2 reduced the promotion effect of BRF (Fig.1A). This suggests that the promotion effects of B and BRF on main stem elongation were mainly related to low activity of CRY1 rather than CRY2. A previous study on de-etiolated seedlings of *Arabidopsis* also indicates that CRY1 plays a main role in mediating inhibition of stem elongation (Lin et al. 1998). In the wild-type plants, BR showed similar inhibition effects as R, and over expression of both CRY1 and CRY2 did not change the BR's inhibition effects, indicating a higher cryptochrome activity under BR than B and BRF. In the present study, B and BRF had a lower PPS than BR, so the low CRY1 activity under B and BRF appeared to be caused by low phytochrome activity under the two BLs. In other words, there is a crosstalk between cryptochrome and phytochrome (Su et al. 2017). For example, the co-action between CRY1 and phyB has been observed under suboptimal light conditions (Casal 2000). In this study, B and BRF had a low PPS values (<0.6), which can induce shade response in plants, and can be considered as suboptimal light.

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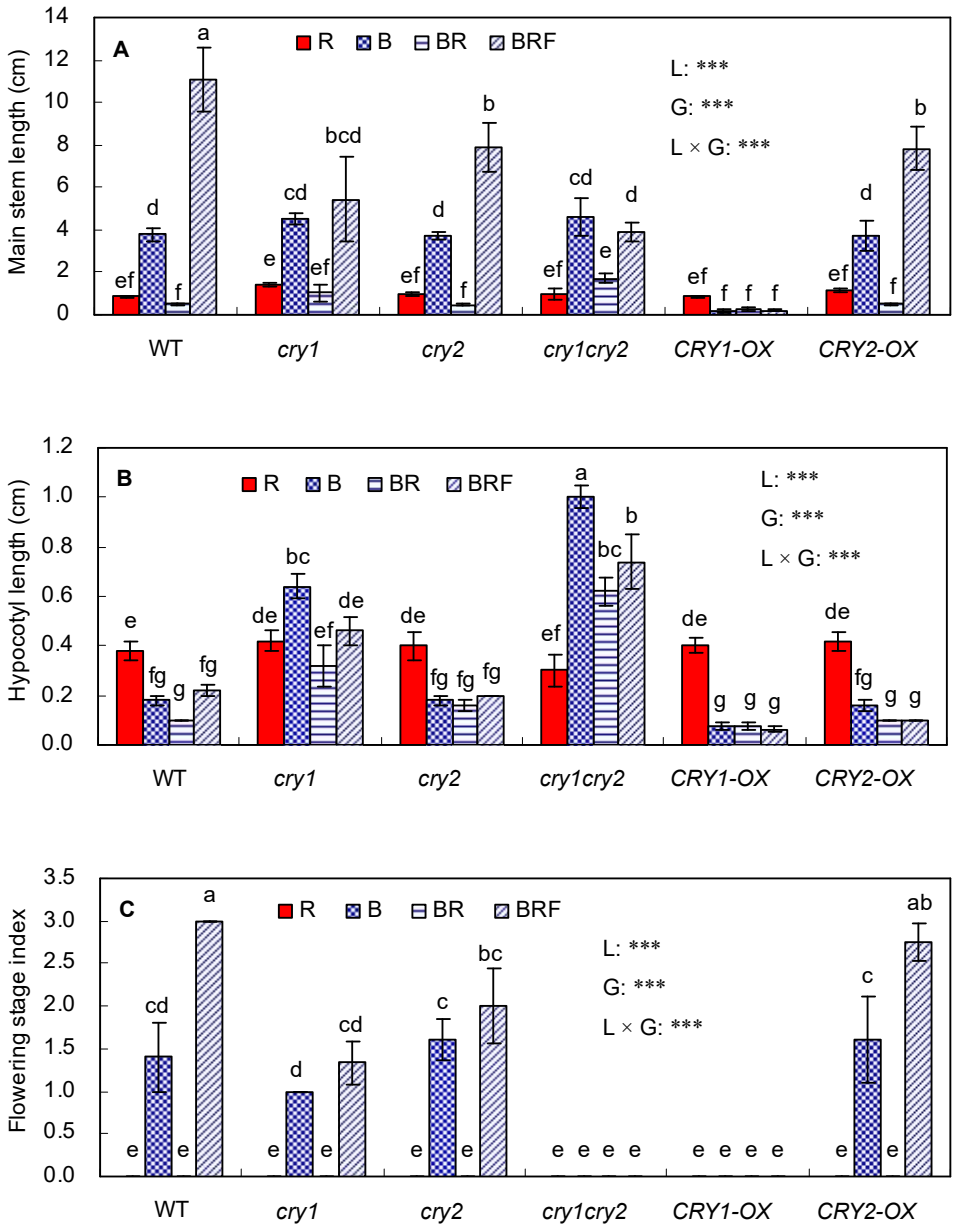

Fig. 1 Elongation growth and flowering in wild-type, cryptochrome-deficient mutants, and cryptochrome-overexpressing transgenic plants of *Arabidopsis* under different light treatments. For the X-axis labels, WT: wild-type; *cry1*: CRY1-deficient mutant; *cry2*: CRY2-deficient mutant; *cry1cry2*: both CRY1- and CRY2-deficient double mutant; *CRY1-OX*: CRY1-overexpressing transgenic plants; *CRY2-OX*: CRY2-overexpressing transgenic plants. R: pure red light; B: pure blue light; BR: impure blue light created by mixing B with low-level (6 %) R; and BRF: impure blue light created by mixing BR with low-level far-red light (red/far-red ≈ 1). Symbols for light quality (L), plant genotype (G), or the interaction of light quality and plant genotype (L × G) followed by ns, *, **, or *** denote that treatment effects are not significant or significant at $P \leq$ 0.05, 0.01, or 0.001, respectively. Bars are means ± SE (n = 4). Bars bearing the same letter are not significantly different at $P \leq 0.05$, according to Duncan's new multiple range test.

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Although the promotion effects of B and BRF on main stem elongation are mainly related to low activity of CRY1, the action of cryptochrome on this process seems to be different for B and BRF. In the present study, deficiency of either CRY1 or CRY2, or both, reduced the stem elongation promoted by BRF, but not by B. This indicates that both CRY1 and CRY2 contributed partly to BRF-promoted main stem elongation, but neither CRY1 nor CRY2 was directly involved in B-promoted main stem elongation. It has been confirmed that active phytochrome or cryptochrome inhibits plant elongation by binding downstream transcription factors and/or regulators such as PHYTOCHROME INTERACTING FACTORs (PIFs) (Leivar and Monte 2014; Wang and Lin 2020). Possibly, main stem elongation promoted by B was through only one signal pathway including phytochrome, but that by BRF was through at least two pathways including both phytochrome and cryptochrome. This speculation is partly supported by greater promotion on stem elongation of the wild-type plants under BRF than B, and the deficiency of cryptochrome reduced BRF's promotion effect down close to B's effect. Also, B can directly affect phytochrome activity, since phytochromes have a secondary peak of absorption in BL, and both phyA and phyB are BL photoreceptors (Casal 2000).

Moderate-intensity BLs with low PPS can activate CRY1 to inhibit hypocotyl elongation

Differing from main stem, hypocotyl elongation of wild-type *Arabidopsis* plants was similarly inhibited by B, BR and BRF, compared with R, and over express of CRY1 or CRY2 did not increase the inhibitory effect of the three BLs (Fig. 1B). This suggests that the wild-type plants had a high activity of cryptochrome during hypocotyl growth under B, BR and BRF, despite their differences in PPS values. In this case, the cryptochrome activity appeared to be not affected by phytochrome activity for hypocotyl elongation, which differed from main stem. Previous studies indicate that cryptochrome activity is positively related to BL intensity (Liu et al. 2016), and the same-intensity BL may trigger contrasting response in different organs in a single plant due to different threshold values (Yu et al. 2010). Possibly, in our study, the cryptochrome activity under B and BRF at a light intensity of 100 μmol m^{-2} s^{-1} was high enough to inhibit elongation of hypocotyl rather than main stem.

Similar to main stem, CRY1 played a main role in mediating hypocotyl elongation under the three BLs (B, BR, and BRF). Deficiency of CRY1 eliminated and even reversed the inhibitory effect of the three BLs as observed in wild-type plants; however, deficiency of CRY2 did not (Fig.1B). Despite a minimal effect of CRY2 on the inhibition of hypocotyl elongation, the inhibition effect of CRY1 can be strengthened by its co-action with CRY2. In the present study, the deficiency of both CRY1 and CRY2 modified the plant response under the three BLs to a larger degree than the deficiency of only CRY1. The roles of CRY1 and CRY2 and their co-action were also supported by a previous study on de-etiolated *Arabidopsis* seedlings under BL of 60 μmol m^{-2} s^{-1} that a *cry2* mutant does not have elongated hypocotyl, but the *cry1cry2* mutant shows a longer hypocotyl than the *cry1* mutant (Mockler et al. 1999).

Low-activity CRY1 and high-activity CRY 2 together contributes to flowering promotion by BLs with low PPS

In the wild-type plants, B and BRF promoted flowering compared with R, and their promotion effect was not eliminated by the deficiency of either CRY1 or CRY2, but by the deficiency of both CRY1 and CRY2 (Fig.1C), suggesting that CRY1 acts redundantly with CRY2 to promote

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flowering under these conditions. Similar result has been found in a previous study on *Arabidopsis* under broad-band BL (Mockler et al. 1999). It is worthwhile to note that in the present study CRY1 and CRY2 acted redundantly to different degree under B and BRF: CRY1 and CRY2 had nearly overlapping function in B-promoted flowering, but they had only partially overlapping function in BRF-promoted flowering. The underlying mechanisms of the difference between B and BRF still need a further study.

Although CRY1 and CRY2 have overlapping functions in regulating flowering, their involved activity is different in the same physiological process. In the present study, the promotion effect of B and BRF on flowering was eliminated by over expression of CRY1, but was not changed by over expression of CRY2 (Fig. 1C). This suggests that the promotion effect of B and BRF on flowering in the wild-type plants was related to low activity of CRY1 and high activity of CRY2. Although the involvement of low-activity CRY1 in flowering regulation is easily understandable, it is difficult to explain how CRY2 showed high activity if without abundant CRY2 protein in wild-type *Arabidopsis* plants under continuous B and BRF at a level of 100 μmol m^{-2} s^{-1}. Normally, CRY2 protein is stable only under low intensity of BL (<10 μmol m^{-2} s^{-1}), and gets degraded at higher intensity, especially under continuous illumination (Lin et al. 1998; Yu et al. 2007). However, a previous study suggests that under the narrow- vs. broad-band BL, CRY2 protein appears to be more stable and accumulates even at relatively high BL intensities (Ahmad et al. 2002). Possibly, in the present study, narrow-band BL from LED lighting contributed to the abundant CRY2 protein.

In summary, the stem elongation and flower initiation promoted by BL with low PPS values were mainly related to low CRY1 activity, although high-activity CRY2 was also partly involved in these processes.

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