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## Phytochrome contributes to blue-light-mediated stem elongation and flower initiation in mature *Arabidopsis thaliana* plants

Yun Kong and Youbin Zheng

**Abstract:** To examine whether phytochromes contribute to blue-light-mediated stem elongation, plant phenotypic responses were investigated in wild type *Arabidopsis thaliana* (Col-0), and its quintuple phytochrome (phyA phyB phyC phyD phyE) mutant plants under the following light treatments: (1) R, a pure red light from 660-nm LED; (2) B, a pure blue light from 455-nm LED; (3) BR, a impure blue light from LED combination of 94% B and 6% R; and (4) BRF, another impure blue light from LED combination of BR and 6  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  of FR (735 nm). A photosynthetic photon flux density of  $\approx 100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  was provided for all the light treatments. The calculated phytochrome photoequilibrium was 0.89, 0.50, 0.69, and 0.60 for R, B, BR, and BRF, respectively, indicating a higher phytochrome activity under R and BR than B and BRF. After 18 days of light treatment, B or BRF increased main stem length in wild-type plants compared with R, but BR had an inhibition effect similar to R. Also, B and BRF relative to R or BR induced earlier flowering and reduced leaf size in wild type plants, showing typical shade-avoidance responses. In phytochrome-deficient mutant plants, the above shade-avoidance responses were inhibited under B or BRF. However, hypocotyl length, a growth trait characterizing the de-etiolation stage, was reduced under B, BR and BRF vs. R regardless of phytochrome absence. These findings suggest that for mature *Arabidopsis* plants, phytochrome plays a role in blue-light-mediated stem elongation and the associated shade-avoidance responses.

**Key words:** *Arabidopsis thaliana*, blue light, flowering time, hypocotyl length, leaf size, phytochrome mutant, shade-avoidance response, stem length.

**Résumé :** Pour savoir si les phytochromes favorisent l'allongement de la tige à la lumière bleue, les chercheurs ont étudié la réaction phénotypique de l'espèce sauvage d'*Arabidopsis thaliana* (Col-0) et de ses mutants à cinq phytochromes (phyA, phyB, phyC, phyD et phyE) sous les éclairages suivants : (1) R, lumière rouge pure DEL, longueur d'onde de 660 nm; (2) B, lumière bleue pure DEL, longueur d'onde de 455 nm, (3) BR, lumière bleue imparfaite DEL combinant 94 % de B et 6 % de R et (4) BRF, autre lumière bleue imparfaite DEL combinant BR et 6  $\mu\text{mol}$  par  $\text{m}^2$  par seconde de rouge lointain (FR, longueur d'onde de 735 nm). Tous les éclairages libéraient un flux de photons photosynthétiques d'une densité d'environ 100  $\mu\text{mol}$  par  $\text{m}^2$  par seconde. Le photo-équilibre du phytochrome a respectivement été établi à 0,89, à 0,50, à 0,69 et à 0,60 sous les éclairages R, B, BR et BRF, signe que le phytochrome est plus actif sous une lumière R ou BR que sous une lumière B ou BRF. Après 18 jours d'éclairage, les traitements B et BRF avaient augmenté la longueur de la tige principale de l'espèce sauvage, comparativement au traitement R, qui en inhibe la croissance, à l'instar du traitement BR. En outre, comparativement aux traitements R ou BR, les traitements B et BRF accélèrent la floraison et réduisent la taille des feuilles chez la plante du type sauvage, réaction typique d'évitement de l'ombre. Chez les mutants, sans phytochrome, la réaction d'évitement de l'ombre est inhibée par les éclairages B et BRF. Cependant, la longueur des hypocotyles, un paramètre de la croissance caractéristique au renversement de l'étiollement, est plus petite sous les éclairages B, BR et BRF que sous l'éclairage R, même en l'absence de phytochrome. Ces constatations laissent croire que les phytochromes jouent un rôle dans l'allongement de la tige à la lumière bleue chez les plants matures d'*Arabidopsis* ainsi que dans la réaction d'évitement de l'ombre qui s'y associe. [Traduit par la Rédaction]

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Mots-clés : *Arabidopsis thaliana*, lumière bleue, floraison, longueur de l'hypocotyle, taille des feuilles, phytochrome mutant, réaction d'évitement de l'ombre, longueur de la tige.

## Introduction

Previous studies using broad-band light sources indicated that blue light (BL), compared with red light (RL), inhibited shoot/leaf elongation (Cosgrove 1981; Appelgren 1991; Wheeler et al. 1991; Hoenecke et al. 1992; Brown et al. 1995; Kong et al. 2012). However, in the past decades, studies using light-emitting diode (LED) lighting have reported that stem/leaf elongation was promoted by pure BL, compared with RL, in a wide range of species (Heo et al. 2002; Hirai et al. 2006; Mizuno et al. 2011; Hata et al. 2013; Kim et al. 2014; Schwend et al. 2015; Fukuda et al. 2016; Hernandez and Kubota 2016), despite some exceptions (Chen et al. 2014; Izzo et al. 2020; Vitale et al. 2020). Unlike LED, possibly, the aforementioned non-LED light sources may have provided impure monochromatic light (Bergstrand et al. 2014). For example, the BL from monochromatic fluorescent lamp was reported to contain a low level of other wavelengths, and have a high red/far-red ratio (i.e., 1.87) which may activate phytochromes (Appelgren 1991).

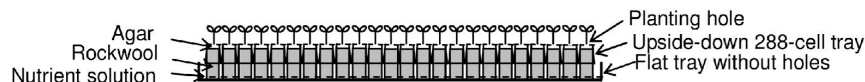
The promotion effects of pure BL on stem elongation have been also found in our recent studies on ornamental plants and microgreens under LED lighting, and these phenomena have been concluded as related to lower phytochrome activity (Kong et al. 2018, 2019a, 2019b, 2020). In these LED studies, pure BL (B) promoted stem elongation compared with RL (R). However, when a small portion (6% or 10%) of R was added to B, the impure BL (BR) reversed the B promotion effect on elongation, and had similar or greater inhibition effect relative to R. After further adding a low level of far-red light (FR) to BR (R/FR  $\approx$  1), the resulting impure BL (BRF) recovered the promotion effect similar to B, compared with 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 (0.5) and BRF (0.6) than R (0.9) and BR (0.7). When the PPS value decreases to 0.6, most plant species show an inactive phytochrome response (Stutte 2009). Since B reduces PPS below 0.6, possibly the B-promoted elongation is related to low phytochrome activity, and under certain conditions B might need to co-act with R to inhibit elongation growth by increasing phytochrome activity. However, the speculation about the involvement of phytochrome in BL action was only based on reversal response to R/FR and calculated PPS values. It needs further confirmation from direct evidence such as a comparison of phenotypic responses to the above light treatments between wild *Arabidopsis thaliana* and its phytochrome mutant plants.

For wild type *Arabidopsis*, co-action with RL was found to increase BL's inhibition effect on hypocotyl elongation of de-etiolated seedlings in a previous study

(Ahmad and Cashmore 1997), where the inhibitory effect was enhanced by 10 min RL pulses following 10 min BL pulses, but partially reversed by a subsequent 10 min FR pulses. It was concluded that active phytochrome is required for full expression of cryptochrome activity, which mediates BL's inhibition effect on hypocotyl elongation (Ahmad and Cashmore 1997). However, differing from our recent study on bedding plants, in the study on *Arabidopsis* by Ahmad and Cashmore (1997), BL alone inhibited hypocotyl elongation relative to RL, and co-action with RL only strengthened the BL's inhibition effect. The different result about the BL response may be due to different lighting source (non-LED vs. LED), different plant species (*Arabidopsis* vs. bedding plants), and different growth stage (de-etiolation stage vs. vegetative stage). In this case, for mature plants of wild *Arabidopsis* under LED lighting treatments similar to our previous studies, whether B and BRF, relative to R and BR, can promote plant elongation similarly to bedding plants needs further study. Also, phytochrome was only shown to be involved in BL's inhibition effect on plant elongation in the study by Ahmad and Cashmore (1997). However, it is unknown whether phytochrome also contributes to stem elongation promoted by B or BRF from LED lighting in our previous studies.

In contrast to the above opinion that active phytochrome is required for BL-mediated inhibition effect, some earlier studies on phytochrome-deficient *Arabidopsis* mutants (phyA and phyB) showed little impairment in BL-dependent inhibition of hypocotyl elongation (Koornneef et al. 1980; Young et al. 1992). However, it has been shown that considerable residual phytochrome responses are retained in all the above phytochrome-deficient mutants (Chory et al. 1989; Reed et al. 1994; Ahmad and Cashmore 1997). The phytochrome family in *Arabidopsis* has five members: phyA, phyB, phyC, phyD, and phyE, and they have partially overlapping functions (Strasser et al. 2010). Although phyA and phyB are the most important two phytochrome family members, the other three members, phyC, phyD, and phyE, can co-action with phyA or phyB to regulate plant growth and development (Legris et al. 2019). For example, phyA, phyB, phyC, phyD, and phyE can regulate seedling de-etiolation; phyA, phyB, and phyE can suppress stem elongation; and phyB, phyD, and phyE can suppress shade avoidance (Franklin and Quail 2010). In this case, the possibility that other phytochrome family members (e.g., phyC, phyD and (or) phyE) may also contribute to BL-mediated inhibition of hypocotyl elongation cannot be ruled out (Strasser et al. 2010). A recent study on the quintuple phytochrome mutant (phyA phyB phyC phyD phyE) indicated that BL alone inhibited hypocotyl elongation of de-etiolated *Arabidopsis* seedlings, suggesting that

**Fig. 1.** Side-view diagram of a hydroponic system used for growing *Arabidopsis* plants in this experiment.



cryptochrome can operate in the absence of phytochrome (Strasser et al. 2010). However, in the above studies, the investigation of elongation growth was focused only on hypocotyl length of de-etiolated seedlings and was performed under non-LED lighting which might provide impure BL in many cases. Therefore, the stem elongation response of mature *Arabidopsis* plants needs to be further tested in quintuple phytochrome mutant under BL from LED lighting.

Our recent studies on bedding plants and microgreens indicate that the plant elongation promoted by B or BRF is a shade-avoidance response (Kong et al. 2018; Kong et al. 2019b). Besides increased stem elongation, B or BRF, relative to R or BR, caused earlier flowering, smaller cotyledon, longer petiole, and lighter leaf greenness, which varied sensitivity among different species. Possibly, under the same BL treatments with low PPS (i.e., B or BRF) as our recent study, there is a similar shade-avoidance response in the wild-type *Arabidopsis* plants. Since the shade-avoidance response was mediated by BL associated with low phytochrome activity (i.e., B or BRF), it is possible that the quintuple phytochrome mutant may differ from wild type in the response to these BL treatments.

Based on the above information, when the light treatments (R, B, BR, and BRF) similar to our recent study on bedding plants were used for wild-type *Arabidopsis* and quintuple phytochrome mutant, three hypotheses were proposed as follows: (1) wild-type plants show an elongation response pattern similar to bedding plants; (2) quintuple phytochrome mutants differ from wild type in their elongation responses to light treatments; (3) B or BRF, compared with R or BR, can induce some shade-avoidance responses in the wild-type, but quintuple phytochrome mutant can change this response. The objective of this study was to explore the involved mechanism for BL action by testing the above hypotheses.

## Materials and Methods

### Plant materials and maintenance

The experiment was performed at the University of Guelph, Guelph, ON, Canada. Two genotypes of *Arabidopsis*, wild type (Col-0) and quintuple phytochrome (phyA phyB phyC phyD phyE) mutant (Strasser et al. 2010), were used for this experiment. Taking into account the low seed germination capacity of this phytochrome-deficient mutant, before seeding, seeds were suspended in GA<sub>4+7</sub> (Duchefa Biochemie, Haarlem, the Netherlands) solution of 100  $\mu$ M, and were stratified at 4 °C for 3 d. After rinsing in deionized water three

times, the stratified seeds were sown in planting holes (one seed per hole) of a hydroponic system (Fig. 1), with 0.7% Plant Agar (Fisher Scientific, Geel, Belgium), and rockwool cubes (Starter Plugs, Grodan Inc., Ontario, Canada). The two genotypes were evenly and randomly distributed in different rows (i.e., five rows for each genotype) within each tray. The sown trays were placed under the light treatments in a walk-in growth chamber. The fertigation method and the environment condition for growing the plants followed the way by Kong and Zheng (2020).

### Light treatments and arrangement

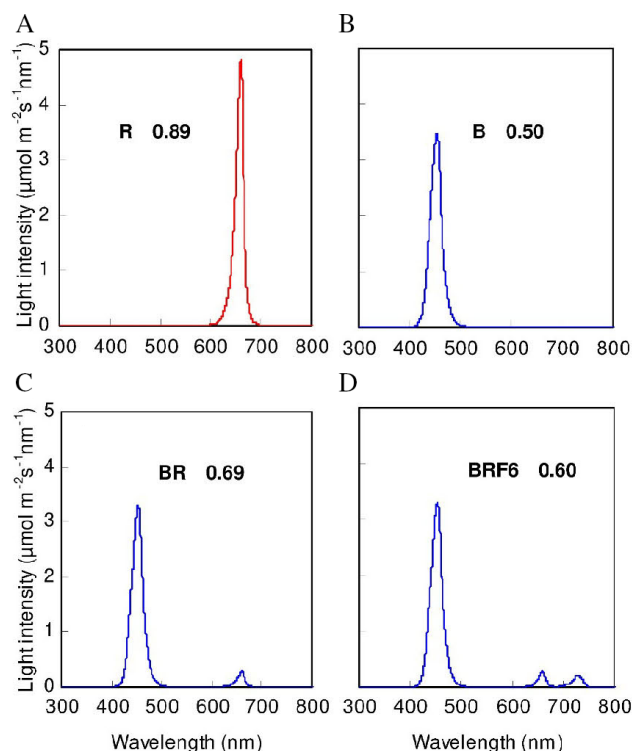
Light treatments included: (1) R, a pure RL from 660 nm LED; (2) B, a pure BL from 455 nm LED; (3) BR, a impure BL from LED combination of 94% B and 6% R; and (4) BRF, another impure BL from LED combination of BR and 6  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup> of FR (735 nm)(Fig. 2). Based on the light spectral distribution, the phytochrome photostationary state (PPS), also called phytochrome photoequilibrium (i.e., the ratio of active phytochrome to total phytochrome), was calculated for each of the four light treatments according to Sager et al. (1988). The calculated PPS values, indicators of phytochrome activity, were 0.89, 0.69, 0.60, and 0.50 for R, BR, BRF, and B, respectively. The light treatments were achieved by adjusting the intensities and spectra of a LX602C LED lighting system (Heliospectra AB, Gothenburg, Sweden) using System Assistant 2.0.1 (Heliospectra AB). In the chamber, the four light treatments were arranged to four divided compartments randomly. Opaque curtains were used to separate these compartments to avoid neighboring light pollution. For each light treatment, a photosynthetic photon flux density (PPFD) of around 100  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup> was achieved at the plant canopy level. Light quality and intensity were set up and verified for the light treatments using a USB2000 + UV/VIS spectrometer (Ocean Optics, Inc., Dunedin, FL, USA).

### Biometric measurements

Once seed germination was over 50% for each genotype under each light treatment, the cumulative germination percentages were determined. After 18-d lighting, five plants from each genotype in each of light treatments (i.e., one plant from each row in each tray) were randomly selected for investigating plant morphology. The observed plant traits included main stem length, hypocotyl length, rosette leaf number, total leaf number, flowering index, and leaf morphology (size and color). The values of flowering index (ranging from 0–3) were defined as the same as our previous study



**Fig. 2.** The spectral distribution and PPS (phytochrome photostationary state) values of four light treatments delivered by light emitting diodes (LEDs). R = a pure red light with a peak at 660 nm; B = a pure blue light with a peak at 455 nm; BR = an impure blue light with a combination of 94% B and 6% R; and BRF = another impure blue light with a combination of BR and 6  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  of FR (735 nm). The numbers inside the figures are PPS values estimated according to Sager et al. (1988). [Colour online.]



(Kong and Zheng 2020). Leaf size and color were observed following the method by Kong et al. (2019b) and Karcher and Richardson (2003).

#### Statistical analysis

DPS 7.05 Software (Refine Information Tech. Co., Hangzhou, China), a data processing System, was used for the data analysis. In this experiment, the chamber environment conditions were uniform except for light treatments, and five rows of plants in growing trays were randomly distributed to each combination of light treatments  $\times$  *Arabidopsis* genotypes. In this case, the experimental arrangement can be considered as a completely random design with two factors and five replicates. Two-way ANOVA was used to determine the effects of each factor (i.e., light treatment, or *Arabidopsis* genotype), and their interaction. For each plant trait, means separation for different treatments were determined using Duncan's new multiple range test ( $P \leq 0.05$ ).

## Results

Cumulative germination percentage was not different among the different treatments (Supplementary Fig. S1<sup>1</sup>). Under the light treatments, main stem length differed in response pattern between phytochrome mutant and wild type plants (Fig. 3A). For wild type plants, B and BRF promoted main stem elongation relative to R or BR, and BR showed an inhibitory effect similar to R, but BRF vs. B had a greater promotion effect. For the phytochrome mutant, plants under B and R showed a similar height, but were shorter than those under BR and BRF, and plants were taller under BR than BRF. Phytochrome mutant had reduced main stem length under B or BRF, but increased main stem length under BR compared with wild type. It suggested that the absence of phytochromes attenuated the enhancement effect of B or BRF and removed the inhibition effect of BR on main stem elongation.

For hypocotyl length, the light response pattern of phytochrome mutant was similar to that of wild type; B, BR, and BRF reduced this trait compared with R, while the three BL treatments were not different from each other (Fig. 3B). The different response in hypocotyl from main stem suggests that BL-mediated elongation growth differed during the early and late growth stages. Under R, phytochrome mutant showed greater hypocotyl length than wild type plants. Hypocotyl was longer under BR for phytochrome mutant than wild type. It suggested that in this case during early growth stage, BL was more effective to inhibit elongation growth than RL, showing an inhibition effect independent of phytochrome.

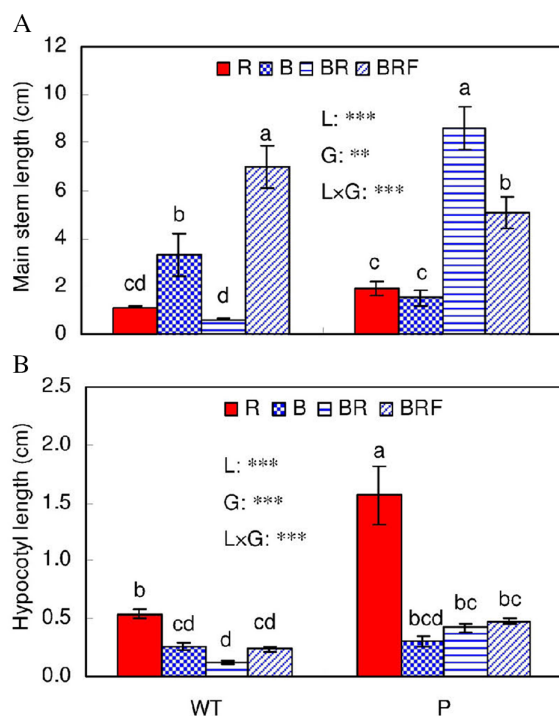
For total leaf number, the light response pattern of phytochrome mutant was different from that of wild type. B, BR, and BRF, compared with R, did not change total leaf number for wild type, but increased this trait for phytochrome mutant (Fig. 4A). Under R, despite promoting hypocotyl elongation during early growth stage, the quintuple phytochrome mutant was not able to develop beyond some rudimentary leaves at the late stage. This might contribute to the different response of total leaf number between wild and mutant plants.

For rosette leaf number, its light response pattern was similar to total leaf number for the phytochrome mutant, but different from total leaf number for wild type (Fig. 4B). All the BLs (i.e., B, BR, and BRF), relative to R, increased rosette leaf number in phytochrome mutant, but reduced this trait in wild type. For BL, rosette leaves under BR were increased compared with B and BRF for wild-type plants, but were reduced compared with B for phytochrome-deficient mutants. Also, under BR, the phytochrome mutant had less rosette leaves than wild type.

For flowering index, its light response pattern was generally similar to the response of main stem length, but

<sup>1</sup>Supplementary data are available with the article at <https://doi.org/10.1139/cjps-2021-0018>.

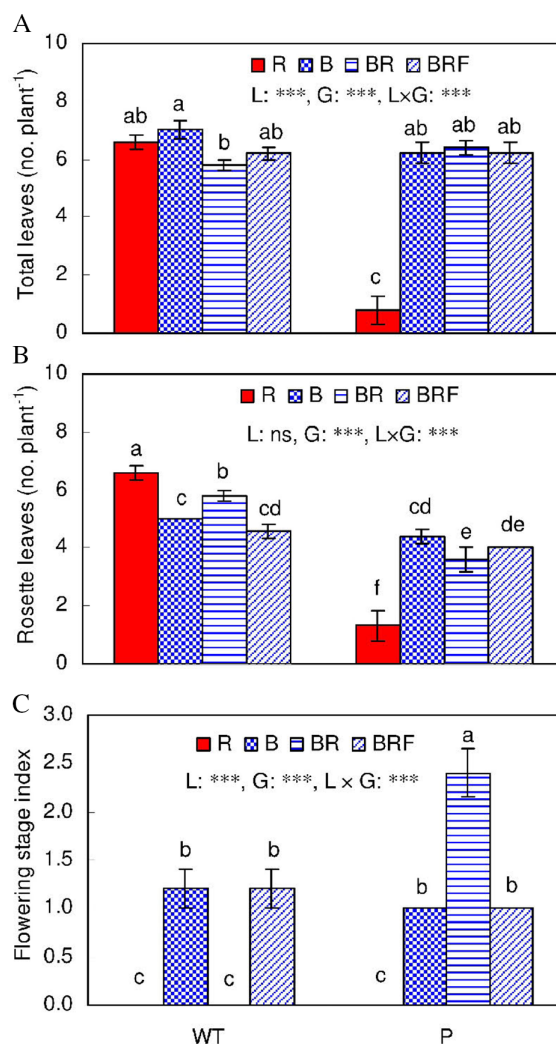
**Fig. 3.** Stem elongation of wild-type *Arabidopsis* and its phytochrome-deficient mutant growing under different light spectra. WT = wild type; P = quintuple phytochrome (phyA phyB phyC phyD phyE) mutant. For the four light treatments, R = a pure red light from 660 nm LED; B = a pure blue light from 455 nm LED; BR = an impure blue light from LED combination of 94% B and 6% R; and BRF = another impure blue light from LED combination of BR and  $6 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  of FR (735 nm). Data are presented as means  $\pm$  SE ( $n = 5$ ). The symbols inside the chart, i.e., L, G and L  $\times$  G denote light treatment, plant genotype, and their interaction, respectively. Behind the symbols, ns, \*, \*\*, or \*\*\* indicate no significance or significance at a level of 0.05, 0.01, or 0.001, respectively, for the effect of treatment on the plant trait. Different letters on the data indicate significant difference (Duncan's new multiple range test,  $P \leq 0.05$ ). [Colour online.]



different between wild type and phytochrome mutant (Fig. 4C). In wild type, flowering index was increased under B and BRF, compared with R or BR, but was not different between BR and R, or between BRF and B. In phytochrome mutant, flowering index was increased under B, BR, and BRF compared with R, and the promotion effect was greater for BR than B and BRF, and was similar for B and BRF. Under BR, phytochrome mutant showed a much greater flowering index than wild type. It suggested that for wild *Arabidopsis*, low-PPS BL (i.e., B or BRF) promoted flowering, but high-PPS BL (i.e., BR) inhibited flowering. However, in the absence of phytochromes, BR lost flowering inhibition effect, and promoted flowering to a greater degree than B or BRF.

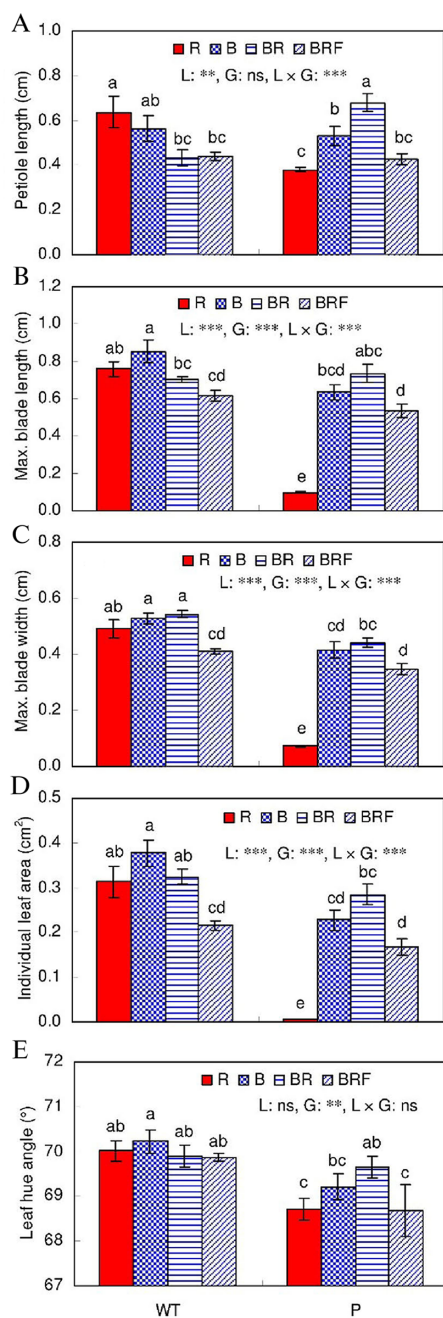
For petiole length, the light response pattern of wild type was different from that of phytochrome

**Fig. 4.** Leaf number and plant flowering of wild-type *Arabidopsis* and its phytochrome-deficient mutant growing under different light spectra. WT = wild type; P = quintuple phytochrome (phyA phyB phyC phyD phyE) mutant. For the four light treatments, R = a pure red light from 660 nm LED; B = a pure blue light from 455 nm LED; BR = an impure blue light from LED combination of 94% B and 6% R; and BRF = another impure blue light from LED combination of BR and  $6 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  of FR (735 nm). Data are presented as means  $\pm$  SE ( $n = 5$ ). The symbols inside the chart, i.e., L, G and L  $\times$  G denote light treatment, plant genotype, and their interaction, respectively. Behind the symbols, ns, \*, \*\*, or \*\*\* indicate no significance or significance at a level of 0.05, 0.01, or 0.001, respectively, for the effect of treatment on the plant trait. Different letters on the data indicate significant difference (Duncan's new multiple range test,  $P \leq 0.05$ ). [Colour online.]



mutant (Fig. 5A). In wild type, BR and BRF reduced petiole length compared with R, but for the phytochrome mutant, BR vs. R increased this trait, and there was no difference between BRF and R. Compared with wild type, phytochrome mutant had a longer petiole under BR, but a shorter petiole under R. This indicated

**Fig. 5.** Leaf size and color of wild-type *Arabidopsis* and its phytochrome-deficient mutant growing under different light spectra. WT = wild-type; P = quintuple phytochrome (phyA phyB phyC phyD phyE) mutant. For the four light treatments, R = a pure red light from 660 nm LED; B = a pure blue light from 455 nm LED; BR = an impure blue light from LED combination of 94% B and 6% R; and BRF = another impure blue light from LED combination of BR and 6  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  of FR (735 nm). Data are presented as means  $\pm$  SE ( $n = 5$ ). The symbols inside the chart, i.e., L, G and L  $\times$  G denote light treatment, plant genotype, and their interaction, respectively. Behind the symbols, ns, \*, \*\*, or \*\*\* indicate no significance or significance at a level of 0.05, 0.01, or 0.001, respectively, for the effect of treatment on the plant trait. Different letters on the data indicate significant difference (Duncan's new multiple range test,  $P \leq 0.05$ ). [Colour online.]



that in the absence of phytochromes BR vs. R lost the inhibition effect and showed a promotion effect on petiole elongation.

For blade size (i.e., maximum blade length and width) and leaf area, the light response pattern of wild type was different from that of phytochrome mutant (Figs. 5B–5D). In wild type, BRF, compared with R or B, reduced blade size and leaf area, but B or BR had similar effects as R on these traits. In the phytochrome mutant, the blade size and leaf area were increased under B, BR, or BRF relative to R, and were reduced under BRF vs. BR. Under both B and R, phytochrome mutant, compared with wild type, had reduced blade size and leaf area. This indicates that the absence of phytochrome inhibited the leaf expansion under B or R.

For leaf color, the light response pattern of wild type was different from that of phytochrome mutant (Fig. 5E). In wild type, leaf hue angle was not different among the light treatments. In phytochrome mutant, leaf hue angle was increased under BR, compared with R or BRF, but there was no difference among R, B and BRF. Under R, B or BRF, phytochrome mutant, compared with wild type, had decreased leaf hue angle. This indicates that in the absence of phytochromes, leaves under R, B or BRF reduced greenness.

## Discussion

### BL's effect on stem elongation of mature *Arabidopsis* plants is related to phytochrome activity

Similar to bedding plants (Kong et al. 2018), compared with R, wild *Arabidopsis* plants, showed longer main stem under BL with lower PPS (i.e., B or BRF), rather than BL with higher PPS (i.e., BR), suggesting that BL's effect is related to phytochrome activity. Also, in wild type *Arabidopsis*, BRF promoted stem elongation to a larger degree compared with B, possibly due to an additive promotion effect of FR (Kusuma and Bugbee 2021). This differed from bedding plants where BRF showed a similar promotion effect as B (Kong et al. 2018). The mechanism underlying the different responses among plant species needs further study. Unlike main stem, hypocotyl elongation responses to BLs did not vary with different phytochrome activity, indicating by the different PPS values of BLs. For hypocotyl elongation, all the BL treatments (B, BR, and BRF) showed similar inhibitory effects compared with R. This also indicates that in mature plants only, our first hypothesis that wild *Arabidopsis* under BL show a stem elongation response pattern similar to bedding plants cannot be rejected. Similar inhibitory effects of BL vs. RL on hypocotyl elongation has been found in a previous study on *Arabidopsis* (Ahmad and Cashmore 1997). However, differing from our current study, the BL's inhibition effect was strengthened when followed by a RL pulse and weakened when followed by a FR pulse, suggesting the contribution of phytochrome activity to BL-mediated hypocotyl



elongation. The inconsistency of the results may be partly explained by the different light intensity employed in the two studies: a much lower BL intensity was used in the previous study ( $\approx 30 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) than in our present study ( $\approx 100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ). At least for some species, inhibitory effect on elongation by either pure or impure BL strengths with light intensity increasing (Cope and Bugbee 2013; Johnson et al. 2019). Therefore, in the present study, regardless of phytochrome activity, BL at a PPFD of  $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  might be strong enough to inhibit hypocotyl elongation during de-etiolation stage, rather than stem elongation in mature plants for *Arabidopsis*. The stronger effect of light intensity than phytochrome activity on BL-mediated elongation may also help explain shorter plant stems developed under BL vs. RL even in some recent studies using LED lighting (Chen et al. 2014; Izzo et al. 2020; Vitale et al. 2020).

Although the PPS values can be easily used to indicate the phytochrome activities induced by different BL treatments, they may not reflect the real situation, and thus may lack accuracy. The reason lies in that the PPS value is calculated phytochrome photoequilibrium according to absorption of light spectrum, which was measured in the solution with isolated and purified phytochrome, rather than in plant leaves (Sager et al. 1988). Masking pigments (predominantly chlorophyll), and leaf structure can alter intracellular light regimes around phytochrome (Gardner and Graceffo 1982). Studying the difference between phytochrome mutants and wild type is another way to explore the involvement of phytochrome in BL-mediated elongation growth. However, many early studies on phyA phyB mutants cannot exclude the involvement of other residual phytochrome species (Strasser et al. 2010). In this case, quintuple phytochrome mutant, which is deficient of all the currently known phytochrome species, may provide a new plant material to study the mechanism of BL's action on stem elongation.

#### **Phytochrome play an active role in BL-mediated stem elongation of mature *Arabidopsis***

The pattern of main stem length response to the BL treatments was totally different between the phytochrome mutant and the wild *Arabidopsis* in the present study. For plants under the light treatments following an order of, R, B, BR, and BRF, main stem was short-tall-short-tall for wild type, but was short-short-tall-short for phytochrome mutant. The different response between the wild type and phytochrome mutant suggests that phytochrome is actively involved in the BL-mediated main stem elongation. Recent studies on *Arabidopsis* indicates that transcriptional changes in response to BL can be coordinately regulated by a cross talk at least between cryptochrome and phytochrome due to some of the shared signaling pathways (Liu et al. 2016; Pedmale et al. 2016; Mishra and Khurana 2017;

Su et al. 2017; Yang et al. 2017). Possibly, phytochrome activity can modify the function of cryptochrome, the BL receptor, on main stem elongation (Liu et al. 2016).

Obviously, the above difference in main stem length between wild type and phytochrome mutant plants resulted from their different responses to each of the three BL treatments. Under BR (i.e., BL with a higher PPS value), main stem was the tallest for phytochrome mutant, but was the shortest for wild type among the light treatments. The reversal effect of BR on elongation in the presence or absence of phytochrome indicated that active phytochrome played an important role in the inhibitory effect of BR on stem elongation for wild *Arabidopsis*. Under B or BRF (i.e., BL with lower PPS values), phytochrome mutant reduced main stem length compared with wild type. In the absence of phytochrome, the promotion effect on main stem elongation was eliminated under B and reduced under BRF relative to R. The reduced elongation response in phytochrome mutant indicated that low-activity phytochrome might contribute partly to increased main stem elongation under BL associated with low PPS for wild types. Possibly, some other photoreceptors (e.g., phototropins), in addition to phytochromes, were also partly involved in the BL-promoted elongation (Kong and Zheng 2020).

Differing from main stem length, hypocotyl length was reduced under B, BR, and BRF relative to R for both phytochrome mutant and wild type, showing inhibitory hypocotyl elongation response to the BLs for the two *Arabidopsis* genotypes. It appears that phytochrome is not required for cryptochrome to inhibit hypocotyl elongation under BL in some cases (Strasser et al. 2010). It is well known that hypocotyl elongation occurs only at early growth stage, but main stem elongation lasts until late growth stage. Possibly, the involvement of phytochromes in the BL-mediated elongation was less significant during early vs. late growth stage for *Arabidopsis* under the conditions (e.g.,  $\approx 100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) in the present study. Consequently, the second hypothesis that quintuple phytochrome mutants differ from wild-type plants in their elongation responses to light treatments cannot be rejected only at late growth stage. However, the BL's inhibition effect on hypocotyl length was greater for phytochrome mutant than wild type. This was mainly due to the failed inhibition of hypocotyl elongation growth by R for the phytochrome mutant rather than wild type. Similar stretching hypocotyl response to RL has been found in phyA phyB double mutant of *Arabidopsis* (Reed et al. 1994), phyA phyB phyC triple mutant of rice (Takano et al. 2009), and quintuple phytochrome mutant of *Arabidopsis* (Strasser et al. 2010).

#### **BL-promoted elongation growth of mature *Arabidopsis* plants is a shade-avoidance response partly mediated by phytochrome**

In the current study, for the wild type plants, B or BRF, compared with R or BR, not only increased main stem



length, but also promoted flowering and reduced leaf size (including leaf area, and maximum blade length and width), showing typical shade-avoidance responses (Casal 2012). Similar shade-avoidance responses have been also observed under B or BRF in our recent study on bedding plants (Kong et al. 2018). It is worthwhile to note that, for wild type *Arabidopsis*, plants under B or BRF relative to R or BR did not show shade-avoidance responses in some traits such as hypocotyl length, petiole length, and leaf color. It appeared that for the same plant, different plant traits had varied sensitivity in shade-avoidance response to BL with low PPS. This was supported by our previous studies on other plant species (Kong et al. 2018; Kong et al. 2019b). Hypocotyl length and leaf morphology showed a lower sensitivity in shade-avoidance response to BL with low PPS than main stem length and flowering index. This possibly resulted partly from varied threshold levels for BL to induce the shade-avoidance response at different stages or in different cells (Mishra and Khurana 2017).

Differing from wild type plants, phytochrome-deficient plants showed some antagonized shade-avoidance responses under B or BRF rather than BR or R. For phytochrome mutant, B or BRF increased leaf size relative to R, delayed flowering, reduced petiole length and main stem length relative to BR. It appears that the absence of phytochromes can prevent the shade-avoidance responses induced in wild types under BL with low PPS (i.e., B or BRF), suggesting a role played by phytochromes in the responses. Obviously, the third hypothesis that quintuple phytochrome mutant can change the shade-avoidance response induced in wild types under B or BRF relative to BR or R cannot be rejected. However, even in absence of phytochromes, some shade-avoidance responses (e.g., reduced leaf size and greenness) were still found under BRF vs. BR. This suggests that some other photoreceptors (e.g., phototropins), in addition to phytochrome, might be partly involved in the shade-avoidance response of wild *Arabidopsis* induced by BL with low PPS (Kong and Zheng 2020).

## Conclusion

Overall, for wild type *Arabidopsis* plants under 24-h LED lighting at a PPFD of  $\approx 100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , BL with low PPS (i.e., B or BRF), relative to R, promoted main stem elongation, but BL with high PPS (i.e., BR) showed a similar inhibition effect as R. The absence of phytochrome reduced and even eliminated the promotion effect of B and BRF, and reversed BR effect from inhibition to promotion. However, regardless of PPS values, all the BLs (i.e., B, BR and BRF) relative to R reduced hypocotyl length in both wild types and phytochrome mutants. This suggests that it is in mature *Arabidopsis* plants that BL's effect on stem elongation is related to

phytochrome activity, and phytochrome is actively involved in BL-mediated stem elongation. Along with enhanced main stem elongation, B and BRF, compared with R or BR, also induced earlier flowering and reduced leaf size in wild type plants, showing typical shade-avoidance responses. In the absence of phytochrome, the above shade-avoidance responses were prevented under B or BRF, and induced under BR. Therefore, phytochrome contributes to BL-mediated stem elongation and the associated shade-avoidance responses in mature *Arabidopsis* plants.

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