Shade avoidance responses are changes in plant architecture to reduce the part of a body that is in the shade in natural habitats. The most common warning signal that induces shade avoidance responses is reduction of red/far-red light ratio perceived by phytochromes. A pair of basic helix–loop–helix transcription factors, named PHYTOCHROME-INTERACTING FACTOR 4 (PIF4) and PIF5, is crucially involved in the shade avoidance-induced hypocotyl elongation in Arabidopsis thaliana. It has been recently reported that PIF7 also plays a role in this event. Here, we examined the involvement of these PIFs in end-of-day far-red light (EODFR) responses under light and dark cycle conditions. It was shown that PIF7 played a predominant role in the EODFR-dependent hypocotyl elongation. We propose the mechanism by which PIF7 together with PIF4 and PIF5 coordinate and transcribes a set of downstream genes to promote elongation of hypocotyls in response to the EODFR treatment.

Key words: Arabidopsis thaliana; circadian clock; end-of-day far-red light responses; phytochrome-interacting factor; shade avoidance

Sessile plants must continuously adjust their growth and development to optimize photosynthetic activity in ever-changing conditions of light. In this respect, shade avoidance responses are changes in plant body form to reduce the degree of shade caused by canopy of competitive neighborhoods. The molecular mechanism of shade avoidance responses has been extensively studied particularly in Arabidopsis thaliana.1-2)

The availability of photosynthetically active radiation (PAR) for carbohydrate productions is crucial for plants in natural habitats. For plants growing under sunlight, PAR ranges from 400 nm (blue light) to 700 nm (red light). Plants growing in canopy (or shading neighbors) experience relatively higher levels of far-red light (ca. 735 nm). Hence, the most common warning signal to induce shade avoidance responses is the reduction of red/far-red ratio (low R:FR) perceived by phytochromes (mainly phyB). In natural fields, shade light signals are complex due to their spatial and/or temporal variations. Under laboratory conditions, however, shade light signals can only be supplied by adding FR light to a source of white light (e.g., fluorescent light). Numerous experiments under such artificial conditions have made it possible to characterize phenotypes of shade avoidance syndromes (SAS) in A. thaliana, namely repression of germination in seed stage, hypocotyl elongation in seedling stage, petiole extension in rosette stage, and acceleration of flowering in transition to the reproductive stage.3,4) Among SAS, hypocotyl growth is the most conventional hallmark to explore shade avoidance responses in laboratory.3-6)

It is well known that phyB signaling is mediated by PHYTOCHROME-INTERACTING FACTOR (PIF) family (including PIL1, PIL2, PIF1, PIF3, PIF4, PIF5, PIF7, and PIF8) to regulate germination, skotomorphogenesis, and chlorophyll synthesis.7-8) In particular, a pair of closely related homologs PIF4 and PIF5 is involved in shade avoidance-induced hypocotyl elongation.9-11) They are also responsible for the short day-induced and/or warm night-induced hypocotyl elongation.12-16) PIF4 and PIF5 belong to a subfamily of basic helix–loop–helix transcription factors that interact with the active form of phyB in the nucleus.7,17,18) Upon the binding to phyB, PIF4, and PIF5 are rapidly phosphorylated and finally degraded in the proteasome in the daytime. In a low R:FR condition, however, the resulting inactivated phyB is unable to degrade these proteins, and the accumulated PIF4 and PIF5 proteins transcribe a number of downstream genes involving in expansion of cells in hypocotyls.19-21) Hence, PIF4 and PIF5 have been cited frequently in the literatures as central players in shade avoidance responses.9-11) However, it was recently reported that not only PIF4 and PIF5 but also PIF7 plays a role in the shade avoidance-induced hypocotyl elongation.22) PIF7 is unique in that although it interacts with phyB and becomes an inactive phosphorylated form, unlike
PIF4 and PIF5, it is not degraded in the daytime.\textsuperscript{22,23}) PIF7 appears to function as a negative regulator of the CBF (also known as DREB) in a manner dependent on photoperiod.\textsuperscript{24,25}) CBFs are responsible for cold acclimation and freeze tolerance. Currently, it remains to address the issue of which PIF factor is more critically involved in the shade avoidance-induced hypocotyl elongation.

Surprisingly, the results of this study suggested that PIF7 plays a predominant role in the shade avoidance-induced hypocotyl elongation under our experimental conditions, while PIF4 and PIF5 play relatively minor roles. We propose the mechanism by which PIF7 together with PIF4 and PIF5 coordinate to transcribe a set of downstream genes to promote elongation of hypocotyls under the end-of-day far-red light (EODFR) conditions in \textit{A. thaliana}.

**Materials and methods**

\textbf{Plant materials and growth conditions.} All plant materials used in this study originated in the Columbia (Col-0) genetic background, except for phyA phyB mutant (Landsberg erecta, Ler). The mutants, phyA phyB,\textsuperscript{26} cry1 cry2,\textsuperscript{27} elf3-8,\textsuperscript{28} pif7-2,\textsuperscript{23} and pif4-101 pif5-1,\textsuperscript{11} have been described previously. Seeds were surface sterilized and stratified at 4°C for a few days and then they were germinated and grown on 0.3% (w/v) gelan gum plate containing Murashige and Skoog medium (pH is adjusted to 5.7) and 1.0% (w/v) sucrose in climate-controlled growth chambers at 20°C. Other detailed growth conditions were given in each section. Photosynthetic photon (400–700 nm) flux density (PPFD) was measured with a Light Meter Li-250 equipped with a Quantum sensor Li-190 (Li-COR) and was set to 80 \(\mu\text{mol m}^{-2} \text{s}^{-1}\). When necessary, far-red light was supplemented by light-emitting diodes (LED \(\lambda_{\text{max}} = 735 \text{ nm, YEELA}\) equipped in the chamber (R: FR ratio, 0.4; intensity, 220 \(\mu\text{mol m}^{-2} \text{s}^{-1}\)). Light spectra were scanned with a Sun Spectroradiometer S-2440 (Soma Optics) and processed with the SpectIR Software. Wavelength range of light components described in this study is defined as follows: blue light, 420–470 nm; red light, 635–685; far-red light, 710–760 nm.

\textbf{Preparation of RNA and qRT-PCR.} Total RNA was purified from frozen plant materials (the aerial part of 7- or 8-d-old seedlings) with the RNeasy plant mini kit (Qiagen, Venlo). To synthesize cDNA, RNA (1 \(\mu\)g of each) was converted to cDNA with ReverTra Ace (TOYOBO, Osaka) and oligo-dT primer. The synthesized cDNAs were amplified with SYBR Premix Ex Taq II (Takara Bio, Kyoto) and the primer set for each target gene, analyzed using a Stepone Plus™ real-time PCR System (Life technologies, CA). The following standard thermal cycling program was used for all PCR: 95°C for 120 s, 40 cycles of 95°C for 10 s, and 60°C for 60 s. Ct value for individual reactions was determined by analysis of raw fluorescence data (without baseline correction) using the freely available software PCR Miner (http://www.miner.ewindup.info).\textsuperscript{29}) Based on the comparative Ct method, relative expression level was calculated. The APX3 encoding an ascorbate peroxidase isozyme was used as an internal reference. The primer set used in this study was listed in Supplementary Table 1.

**Results and discussion**

\textbf{Setup of experimental conditions} \textit{Arabidopsis} seedlings were grown in a growth chamber with neutral white fluorescent lights (the photosynthetic photon flux density (PPFD) of 80–100 \(\mu\text{mol m}^{-2} \text{s}^{-1}\)) as a light source for normal high red (R): far-red (FR) conditions. Its radiating wavelength spectrum was measured (Fig. 1(A), upper panel). The data indicated that the light is relatively enriched with blue and red light, while it contains a very low level of far-red light. To reduce the R: FR ratio, arrays of FR light-emitting diodes (LED) with \(\lambda_{\text{max}} = 735 \text{ nm}\) were equipped for a lower shelf in the same growth chamber. The resulting light spectrum of the lower part of chamber was also measured (Fig. 1(A), lower panel). The FR-LED supplementation in the lower part reduced the R: FR ratio to 0.4, as compared with 7.4 in the upper part, without altering PPFD.

With this conditioned growth chamber, we examined hypocotyl elongation (i.e., a hallmark of SAS) under four different growth conditions. \textit{Arabidopsis} seedlings (accession Columbia-0, Col-0) were grown at 20°C in a 15-h light/9-h dark cycle, during which FR was supplied every day for 3 h at three different timings (zeitgeber time (ZT)): ZT0–ZT3, ZT6–ZT9, ZT12–ZT15, in addition to the reference without FR-supplementation (Fig. 1(B)). After incubation for 7d, the resulting hypocotyl lengths were compared among them (Fig. 1(C)). It was shown only the condition-d was effective to enhance the hypocotyl elongation, suggesting that, only when FR is supplied at the end-of-day, it causes the shade avoidance response. The result is in good agreement with previous study on gating of the rapid shade avoidance response by Salter et al.\textsuperscript{25}) The experimental procedure (condition-d) to induce shade avoidance responses will be referred to as the end-of-day far-red light condition (EODFR condition) hereafter.

It has been well noted that the phytohormones auxin, brassinosteroid, and gibberellin are involved in shade avoidance responses.\textsuperscript{3,2} NPA (1-N-naphthylphthalamic acid), PPZ (propiconazole), and PAC (paclobutrazol) are the widely used specific inhibitors for the actions of these phytohormones, respectively. Hence, the EODFR-induced hypocotyl elongation should be compromised by the application of these inhibitors in growth medium. These critical experiments were carried out to test the rationality of our experimental conditions. The results showed that these chemicals effectively inhibited the EODFR-induced hypocotyl elongation (Fig. 2). Based on these observations, we established the proper method to analyze the effect of low R: FR on \textit{Arabidopsis} architectures such as hypocotyl elongation.

\textbf{PIF7 is predominantly responsible for EODFR-induced shade avoidance responses} It is widely accepted that both PIF4 and PIF5 are crucially responsible for the FR-induced hypocotyl
Recently, it was claimed that PIF7 is also important for the phenomenon. Hence, we first wanted to examine which PIF factor is predominantly implicated in the EODFR-induced shade avoidance responses. For this purpose, we employed a pif4 pif5 double loss-of-function mutant together with a pif7 single mutant to quantitatively examine their phenotypes with references to the EODFR-induced hypocotyl elongation. 

Fig. 1. Experimental conditions of the EODFR treatment of seedlings. Notes: (A) Profiles of light spectra. Photon flux density irradiated from fluorescent light (FL) with or without far-red LED used in this study was analyzed. (B) Diurnal light conditions for the far-red light (FR) treatment. White area denotes a FL period. Black area denotes a dark period. Hatched area denotes a FL + FR period (3 h). (C) Phenotype of FR responses. Seedlings were grown at 20 °C in 15 h light/9 h dark cycles with or without FR treatment. Timings of low R:FR correspond to the experimental condition a-d in panel B, respectively. The resulting lengths of hypocotyls were measured (n = 20).
elongation (Fig. 3(A)). The results suggest that PIF7 is predominantly responsible for the EODFR-induced hypocotyl elongation, although PIF4/PIF5 also contribute to the event. It was also found that (i) seedlings of red light photoreceptor double mutant phyA phyB were no longer sensitive to the EOFFR treatment, compared with the wild-type parental strain, Landsberg erecta (Ler); (ii) seedlings of blue light photoreceptor mutant cry1 cry2 were still sensitive to it, suggesting the red light receptors (phyA and phyB) but not the blue light receptors (cry1 and cry2) are involved in the EODFR-induced hypocotyl elongation; (iii) a representative circadian clock mutant (elf3) does not compromise the EODFR-induced hypocotyl elongation significantly (Fig. 3(B)). The PIF7-dependent and EODFR-induced hypocotyl elongation of young seedlings was readily recognized with naked eyes (Fig. 3(C)). Another hallmark of the EODFR-induced shade avoidance responses is petiole elongation in rosette stage. This was examined after a prolonged incubation of the mutant plants together with wild-type Col-0. The results also suggest that PIF7 is mainly responsible for the EODFR-induced petiole elongation (Fig. 3(D)).

We previously found that PIF4 and PIF5 were significantly implicated in the thermo-sensitive photoperiodic control of plant architecture.\(^{13}\) We then asked whether PIF7 is also involved in the regulation. As opposed to the EODFR responses, PIF7 played a minor role in the warm temperature-induced hypocotyl elongation, while PIF4 and PIF5 were predominantly involved in the event (Supplementary Fig. S1). Considering these, we concluded that PIF7 is mainly responsible for the EODFR-induced shade avoidance response, while PIF4 and PIF5 play relatively minor roles in it at least under our experimental conditions.

Three specific issues to be addressed in this study

It should be noted that PIF7 is inactivated without degradation during the daytime by phosphorylation through the interaction with phyB, whereas an exposure to FR results in a rapid PIF7-activation through dephosphorylation.\(^{12}\) On the contrary, the PIF4 and PIF5 proteins are degraded by phyB during the daytime.\(^{5,10}\) Then, the first question was raised. How are functionally active PIF4 and PIF5 proteins accumulated by EODFR treatment? Second, what are the downstream target genes of PIF-dependent EODFR signaling? Finally, how do PIF7/PIF4/PIF5 coordinately regulate these downstream target genes? In the following sections, these issues were addressed, one by one.

EODFR enhances the transcription of PIF4/PIF5

The diurnal expression profiles of PIF4, PIF5, and PIF7 genes were first analyzed for seedlings grown under the EODFR conditions (Supplementary Fig. S2). All of them showed a diurnal rhythm with a peak at the daytime. Then, the diurnal expression profiles of these PIF genes under EODFR conditions were compared with reference conditions (Fig. 4(A)–(C)). The results showed that PIF4 showed a robust oscillation, while the diurnal oscillation of PIF5 and PIF7 is less robust than that of PIF4. In any case, it was revealed that no obvious difference was observed for the diurnal expression profiles of PIF7 between both the growth conditions (Fig. 4(A)). On the contrary, it was noticed that the EODFR treatment of seedlings enhanced expression of PIF4 and PIF5 (Fig. 4(B) and (C), see arrows). To confirm these, the expression profiles of these genes during the exposure to FR were followed with short intervals (Fig. 4(D)–(F)). The transcription of PIF4 and PIF5 was significantly up-regulated by the EODFR treatment (Fig. 4(E) and (F)), while PIF7 did not respond at all (Fig. 4(D)). Hence, the EODFR treatment enhances the transcription of PIF4 and PIF5.

![Fig. 3. Involvement of PIF4, PIF5, and PIF7 in EODFR responses.](image-url)

Notes: (A) Hypocotyl length of seedlings in response to the EODFR treatment. Seedlings (Col-0, pif4/5, and pif7) were grown under the EODFR conditions, and the resulting lengths of hypocotyls were measured (n = 20). The pif4 pif5 mutant showed an impaired response with reference to the EODFR-induced hypocotyl elongation (The asterisk denotes \(p < 0.1\) in t-test). The pif7 mutant showed a more severely compromised phenotype. (B) Box shade plot of the result of EODFR responses. The experiment (A) was reproduced, employing the cry1 cry2, phyA phyB, elf3 mutants in addition to pif4 pif5 and pif7. (C) Representative phenotype of the EODFR responses in wild-type (Col-0) and the pif7 mutant. (D) Phenotype of EODFR responses at the rosette stage. Seedlings (Col-0, pif4 pif5, pif7) were kept growing under the same EODFR conditions for 10d and photographed.
A wide variety of downstream gene was implicated in the EODFR-induced shade avoidance responses.

Kozuka et al. carried out DNA microarray analyses to identify the genes induced by FR. More recently, Li et al. carried out RNA sequence analyses to identify the candidates of PIF7-target genes. We inspected these genome-wide databases to gain hints with regard to the genes implicated in the EODFR-induced shade avoidance responses under our experimental conditions. The diurnal expression profiles of selected genes were analyzed for Col-0 seedlings in the presence and absence of EODFR treatment. This approach allowed us to identify several EODFR-associated genes (Fig. 5(A)–(F)). The EODFR-induced genes identified in this study were YUC8 (auxin synthesis), IAA29 (auxin signaling), ATHB2 (transcription factor), XTH15 (cell wall synthesis), BIM1 (brassinosteroid signaling), and GAI (gibberellic acid signaling), although the latter two showed a relatively higher level of EODFR-independent basal-level expression. Hence, a variety of downstream genes were implicated in the EODFR-induced shade avoidance responses.

Both PIF7 and PIF4/PIF5 contribute to transcription of the downstream genes in a manner different from each other.

To answer the last question how PIF7/PIF4/PIF5 coordinately regulate the downstream genes, we again employed pif7 single and pif4 pif5 double mutants. The diurnal expression profiles of target genes in the pif7 mutant are expected to give us a hint for PIF4/PIF5-dependent expression of the target genes, whereas those in the pif4 pif5 mutant are expected to become a hint for PIF7-dependent expression. It was found that rapid induction of downstream genes in response to FR was no longer observed in pif7. But EODFR-induced expression was still observed specifically at the end of night in the pif7 mutant, suggesting that PIF4/PIF5 is involved in transcription of the downstream genes at that time (Fig. 5(G)–(L)). On the other hand, the EODFR-induced expression of downstream genes at the end of night was impaired in pif4 pif5, compared with wild-type Col-0. But the pif4 pif5 mutant was not deficient in the rapid response to FR, suggesting that PIF7 starts to function immediately after the EODFR treatment and keeps working throughout the dark period, giving rise to trapezoidal profiles (Fig. 5(M)–(R)). It is found that PIF7 and PIF4/PIF5 act independently in an additive manner, as judged by the result that the expression profile of each target gene in Col-0 is equivalent to the combination of two profiles in pif7 and pif4 pif5 mutants. For example, the expression of YUC8 is mainly dependent on PIF7, while that of ATHB2 is dependent on both PIF7 and PIF4/PIF5 equally. Hence, PIF7 and PIF4/PIF5 contribute to the activation of downstream genes in a manner different from each other.
Insight into the mechanism of EODFR-induced shade avoidance responses

The mechanism of EODFR-induced shade avoidance responses implicated in this study was summarized schematically (Fig. 6). PIF7 plays a predominant role in the EODFR-induced shade avoidance responses, and PIF4/PIF5 play a relatively supporting role. The latter notion is consistent with that from other group.31) This

Fig. 5. EODFR-induced transcription of a set of downstream target genes of PIFs.
Diurnal expression profile of the downstream target genes of PIF7, which were identified in the previous study,22) with or without the EODFR treatment. RNA samples were prepared from the aerial part of 8-d-old seedlings of Col-0 (A–F), pif7 (G–L), and pif4 pif5 (M–R) at 3-h intervals. Expression of YUC8 (A, G, M), IAA29 (B, H, N), ATHB2 (C, I, O), XTH15 (D, J, P), BIM1 (E, K, Q), and GAI (F, L, R) was analyzed by qRT-PCR. White area denotes a FL period (12 h). Shaded area, a dark period (9 h). Hatched area, a FL + FR period (3 h).
view does not rule out the possibility that other PIF factors (e.g., PIF1 and PIF3) might be involved in the EODFR-induced shade avoidance responses in part. PIF7 and PIF4/PIF5 work for the EODFR-induced shade avoidance responses through different ways from each other. PIF7 is subjected to the phosphorylation and inactivated by phyB during daytime. The EODFR stimulus inactivates phyB, thereby resulting in an immediate dephosphorylation and activation of PIF7. Hence, PIF7 is rapidly activated by EODFR treatment as the transcription factor for the downstream target genes, such as YUC8 and IAA29. The activated PIF7 works throughout the night, giving rise to trapezoidal expression profiles of target genes. On the other hand, the activity of PIF4/PIF5 proteins during the daytime is inhibited by a phyB-dependent degradation and inactivation. It is found in this study that the EODFR stimulus first induces the transcription of PIF4/PIF5. Hence, it takes a time to be translated into the active proteins under dark conditions. As a result, PIF4 and PIF5 proteins become functional only at the end of night under EODFR conditions, giving rise to triangular expression profiles of target genes. Taken together, PIF7 and PIF4/PIF5 are responsible for EODFR-dependent elongation of hypocotyls by regulating their downstream target genes independently in an additive manner (Fig. 6). In this study, it should be emphasized that the EODFR stimulus is immediately followed by darkness. Otherwise, the active PIF7/PIF4/PIF5 would be reversibly inactivated by light. Neither morning-FR nor noon-FR stimuli are effective to induce elongation of hypocotyls (Fig. 1). The FR stimulus only at the end-of-day induces a variety of downstream genes through the activation of PIF7/PIF4/PIF5. We identified some of the representative downstream genes in our experimental conditions, which are also listed in Fig. 6.

The molecular mechanism underlying shade avoidance responses is a long-standing subject in plant biology. Here, we answered several previously unresolved questions with regard to this field. The findings in this study will provide us with new insights into the EODFR-induced shade avoidance responses.

### Supplementary material

The supplementary material for this paper is available online at http://dx.doi.org/10.1080/09168451.2015.1065171.

### Acknowledgments

We are grateful to Dr. Whitelam (University of Leicester, UK) for *A. thaliana* seeds of the phyhl-201 phyB-5 mutant, Dr. Lin (University of California at Los Angeles, CA) for the cryl-304 cry2-1 mutant, Dr. Millar (University of Edinburgh, UK) for the elf3-8 mutant, and Dr. Prat (Campus University) for the pif4-101 pif5-1 mutant. This work was supported by the Japan Society for the Promotion of Science under Grant (No. 23580133 and No. 20370018); and Naito Foundation under Grant (The Naito Foundation Subsidy for Promotion of Specific Research Projects).

### Disclosure statement

No potential conflict of interest was reported by the authors.

### Funding

This work was supported by the Japan Society for the Promotion of Science [grant number 23580133], [grant number 20370018]; Naito Foundation under Grant (The Naito Foundation Subsidy for Promotion of Specific Research Projects).

### References

[1] Pierik R, de Wit M. Shade avoidance: phytochrome signalling and other aboveground neighbour detection cues. J. Exp. Bot. 2014;65:2815–2824.

[2] Casal JJ. Shade avoidance. Arabidopsis Book. 2012;10:e0157.

[3] Martinez-Garcia JF, Galleni M, Molina-Contreras MJ, Llorente B, Bevilaqua MR, Quail PH. The shade avoidance syndrome in Arabidopsis: the antagonistic role of phytochrome A and B differentiates vegetation proximity and canopy shade. PLoS ONE. 2014;9:e109275.

[4] Ciolfi A, Sessa G, Sassi M, et al. Dynamics of the shade-avoidance response in Arabidopsis. Plant Physiol. 2013;163:331–353.

[5] Cole B, Kay SA, Chory J. Automated analysis of hypocotyl growth dynamics during shade avoidance in Arabidopsis. Plant J. 2011;65:991–1000.

[6] Saltar MG, Franklin KA, Whitelam GC. Gating of the rapid shade-avoidance response by the circadian clock in plants. Nature. 2003;426:680–683.

[7] Leivar P, Quail PH. PIFs: pivotal components in a cellular signaling hub. Trends Plant Sci. 2011;16:19–28.
Oh E, Zhu JY, Bai MY, Arenhart RA, Sun Y, Wang ZY. Interactions between HLH and bHLH factors modulate light-regulated plant development. Mol. Plant. 2012;5:688–697.

Lorrain S, Allen T, Dued P, Whitelam GC, Fankhueter C. Phytochrome-mediated inhibition of shade avoidance involves degradation of growth-promoting bHLH transcription factors. Plant J. 2008;53:312–323.

Nomoto Y, Kubozono S, Miyachi M, Yamashino T, Nakamichi N, Mizuno T. Circadian clock- and PIF4-mediated external coincidence mechanism coordinately integrates both of the cues from seasonal changes in photoperiod and temperature to regulate plant growth in Arabidopsis thaliana. Plant Signaling Behav. 2013;8:e22863.

Nomoto Y, Kubozono S, Miyachi M, Yamashino T, Nakamichi N, Mizuno T. A circadian clock- and PIF4-mediated double coincidence mechanism is implicated in the thermosensitive photoperiodic control of plant architectures in Arabidopsis thaliana. Plant Cell Physiol. 2012;53:1965–1973.

Nomoto Y, Kubozono S, Yamashino T, Nakamichi N, Mizuno T. Circadian clock- and PIF4-controlled plant growth: a coincidence mechanism directly integrates a hormone signaling network into the photoperiodic control of plant architectures in Arabidopsis thaliana. Plant Cell Physiol. 2012;53:1950–1964.

Kunihiro A, Yamashino T, Nakamichi N, Niwa Y, Nakashima K, et al. The phyB-PIF feedback loop. Mol. Plant. 2012;5:734–749.

Hao Y, Oh E, Choi G, Liang Z, Wang ZY. Phytochrome interacting factors: a novel molecular recognition motif necessary for targeting basic helix-loop-helix transcription factors. Plant Cell. 2004;16:3033–3044.

Zhao S, Fernald RD. Comprehensive algorithm for quantitative real-time polymerase chain reaction. J. Comput. Biol. 2005;12:1047–1064.

Kozuka T, Kobayashi J, Horiguchi G, et al. Involvement of auxin and brassinosteroid in the regulation of petiole elongation under the shade. Plant Physiol. 2010;153:1608–1618.

Sellaro R, Pacini M, Casal JJ. Diurnal dependence of growth responses to shade in Arabidopsis: role of hormone, clock, and light signaling. Mol. Plant. 2012;5:619–628.

Yamashino T, Nomoto Y, Lorrain S, et al. Verification at the protein level of the PIF4-mediated external coincidence model for the temperature-adaptive photoperiodic control of plant growth in Arabidopsis thaliana. Plant Signaling Behav. 2013;8:e23390.