國立臺灣大學生命科學院植物科學研究所

碩士論文

Institute of Plant Biology

College of Life Science

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Master's Thesis

阿拉伯芥LONG HYPOCOTYL IN FAR-RED 1參與光與FAR-RED INSENSITIVE 219/JAR1的茉莉酸訊息傳遞路徑中調控phytochrome B形成光小體之功能性研究

Functional study of *Arabidopsis* LONG HYPOCOTYL IN FAR-RED 1 in regulating the formation of phytochrome B-associated photobodies in response to light and FAR-RED INSENSITIVE 219/JAR1-mediated jasmonic acid signaling pathways

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口試委員會審定書
MASTER'S THESIS ACCEPTANCE CERTIFICATE
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本論文係葉庭妤（學號 R10B42012）在國立臺灣大學植物科學研究所完成之碩士學位論文，於民國 114 年 7 月 10 日承下列考試委員審查通過及口試及格，特此證明。

The undersigned, appointed by the Institute of Plant Biology on 10, July, 2025 have examined a Master's Thesis entitled above presented by Ting-Yu, Yeh (student ID R10B42012) candidate and hereby certify that it is worthy of acceptance.

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誌謝

碩士是個說長不長、說短不短的時光，處於學生但又算已一半踏入職場的階段，這段時期對我來說就像進入一個混沌的時空，從未知、迷茫到探索、發現來逐漸了解研究方向，並且重新認識並更了解自己。大學畢業到進入臺大植科所時剛好碰到疫情，原本正常的生活變成線上面試、上課，到後來疫情減緩後生活又慢慢回到正軌，我也換了一個新的實驗室來到 R 919，開啟一趟為期三年的新旅程。

最感謝老師給我這個機會進實驗室，讓我學習到實驗方法、解決能力之外，指引我研究和未來的方向，也讓我認識到實驗室的夥伴們。剛來這裡時由於有嘉成學長不厭其煩地為我講解各種實驗，讓我儘管較晚進實驗室也能學習到完整的內容；也很感謝凱駿學長教導我許多詳細的實驗方法，而且都非常仔細、有耐心地說明，畢業前夕也很高興可以再次見到兩位學長再次出現在生科館 9 樓。我也要謝謝坐在我兩邊的士甫學長跟晉濂學長，因為有他們讓我在實驗室多了一些樂趣跟綠葉點綴，也謝謝 Neha、Preethi 讓我認識到印度的文化與故事。還有謝謝算是一半同期的子瀚、薇涵、莉茹、翊瑄、Sushanthi 讓我的實驗室生活增加許多歡笑和回憶，也要謝謝李葳、好杰後期的加入，因為有他們這群人使得研究生生活不只是實驗與學習，更是一段值得收藏的時光。謝謝采玲、云滇、昂炘、乃樺學姐、新綱學長，雖然相處時間不算長，但是一起做實驗、去正翰參訪也讓我多了許多新的體驗與回憶。最後我也要謝謝給予我支持與照顧的家人、提供我許多經驗與建議的朋友們，還有最後這半年我的心靈支柱 BOYNEXTDOOR，因為他們六個讓我寫論文的漫長時間和實驗一直不順利的心情找到能紓解的出口，也因此才能順利完成口試並產出這篇論文。

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中文摘要

植物生長與發育會受到外界光環境與內在荷爾蒙訊息的共同調控，尤其在自然環境中光的變化與植物本身的防禦需求常會互相牴觸，因此植物必須依據環境變化，在生長與防禦之間做出平衡。茉莉酸（jasmonate，JA）作為植物重要的防禦荷爾蒙之一，其訊息傳遞會與光訊號交互作用來調節植物對逆境的反應。前人研究指出，紅光主要的接受器 phytochrome B（phyB）除了參與光形態發生外，也可與 FAR-RED INSENSITIVE 219（FIN219）結合，進一步調控茉莉酸訊息傳遞與光小體（photobody，PB）的形成。在遮陰環境下，非活化態的 phyB 會與去磷酸化的 FIN219 結合，抑制茉莉酸下游訊息傳遞路徑並使得 PB 無法形成，從而促進阿拉伯芥下胚軸的延長。此外 FIN219 亦可與轉錄因子 LONG HYPOCOTYL IN FAR-RED 1（HFR1）互相調節，共同參與遮陰反應與茉莉酸調控的訊息路徑。綜合以上研究顯示 HFR1、phyB 與 FIN219 這三者之間可能存在更廣泛的交互關係與調控網路，以及我們想進一步了解他們對於光與茉莉酸訊息路徑中的交互作用與功能。我們發現 HFR1 不僅在各種光條件下皆會與 phyB、FIN219 相互作用，並且在遠紅光、藍光與遮陰環境下三者會協同調控下胚軸的延長。此外 HFR1 與 FIN219 之間的交互作用位置會受到外源性茉莉酸（MeJA）處理所影響，反映出 HFR1 在光與荷爾蒙訊號之間的關鍵功能。本研究也首次揭示 HFR1 不僅參與光形態發生的調控，還影響 phyB 所召集形成的 PB 型態與分布而改變其數量與大小，顯示其對於 PB 組成與調控中扮演結構性角色。這些結果進一步證實 HFR1 在 phyB 介導之光訊號與 FIN219 的茉莉酸訊息路徑整合中具有多面向調節功能。總結而言，我們的研究證實 HFR1 參與光與茉莉酸訊息傳遞路徑的調控，並且與 phyB、FIN219 共同協同調控下胚軸延長與 PB 的型態，這些結果都表示 HFR1 是作為整合光與荷爾蒙訊息的核心之一，在植物生長與防禦平衡策略中扮演關鍵角色。本研究建構出 HFR1－phyB－FIN219 的模組，作為光與荷爾蒙訊號交互作用的嶄新見解，未來將可進一步探討植物生長與防禦相關的下游基因表現，以及檢測花青素與葉綠素的含量變化，以更全面了解此調控網路在植物生理上的功能意義與生態適應策略。

關鍵字：HFR1，phyB，FIN219，避蔭反應，光小體，茉莉酸

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Abstract

The interaction between light and jasmonates (JAs) is critical in regulating plant growth and defense responses. Previous studies revealed that the inactive Pr form of phytochrome B (phyB) binds to the dephosphorylated FAR-RED INSENSITIVE 219 (FIN219)/JASMONATE RESISTANT1 (JAR1), inhibiting JA signaling pathway and phyB-associated photobodies (PBs) formation under shade. Additionally, FIN219 and the transcription factor LONG HYPOCOTYL IN FAR-RED 1 (HFR1) mutually regulate each other, mediating downstream shade-inducible genes and JA signaling. According to these studies, I want to understand whether HFR1 regulates phyB-associated PBs, the relationship among these three proteins, and the role of HFR1 in light and JA signaling pathways. To figure out these questions, I generated *hfr1-201 phyB-1* double mutant, *fin219-2 phyB-1 hfr1-201* triple mutant, and overexpression lines. Current study demonstrates that HFR1 participates in far-red, blue, and shade light signaling pathways, co-regulating hypocotyl elongation with phyB and FIN219. Through the transient assay, HFR1 physically interacts with phyB and FIN219 under various light conditions, with exogenous methyl jasmonate (MeJA) influencing their interaction, which suggests that HFR1 plays a significant role in phyB-mediated light signaling and JA signaling. Importantly, we also found that HFR1 mediated phyB-associated PBs formation and pattern under the high R: FR light condition and was influenced by MeJA treatment. Taken together, HFR1 participates in the light and JA signaling pathways and plays a significant role in the trade-offs between growth and defense in *Arabidopsis*. Shortly, I will have a better understanding and insights into light and hormone-integrated regulatory mechanisms in plants.

KEYWORDS: HFR1, phyB, FIN219, shade avoidance response, photobody, JA.

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Introduction

Plants, as sessile organisms, have evolved sophisticated systems to perceive and respond to environmental cues such as light, temperature, and biotic stress (Paik and Huq, 2019; Han et al., 2024). Light serves not only as an energy source but also as a key environmental signal regulating plant development. In *Arabidopsis thaliana*, light perception is mediated by multiple classes of photoreceptors, with phytochromes playing a central role in red (R) and far-red (FR) light perception (Chen et al., 2004; Li et al., 2011; Galvao and Fankhauser, 2015; Pham et al., 2018; Luo and Shi, 2019; Tripathi et al., 2019; Willige et al., 2024). Among these, phytochrome B (phyB) is the major red-light photoreceptor and is critically involved in photomorphogenesis, shade avoidance, and integrating light with hormone signaling pathways.

Phytochrome B and Photobody Dynamics in Light Response

phyB exists in two interconvertible forms: the inactive Pr form, which resides in the cytoplasm under dark or far-red light conditions, and the active Pfr form, which translocates into the nucleus upon red light perception (Chen et al., 2005; Chen et al., 2022). Once in the nucleus, active phyB assembles into discrete subnuclear structures known as photobodies (PBs), thought to function as hubs for transcriptional regulation, protein degradation, and signal transduction (Chen and Chory, 2011; Van Buskirk et al., 2012; Kim et al., 2023; Kwon et al., 2024). These PBs are believed to be formed via liquid–liquid phase separation (LLPS), and their size, number, and persistence are dynamically influenced by light conditions and phyB's oligomeric state (Kim et al., 2021; Willige et al., 2024).

The structure of phyB consists of a N-terminal photosensory module responsible for chromophore binding and red or far-red light perception, and a C-terminal module

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containing a nuclear localization signal (NLS) that mediates nuclear import. The C-terminus has also been implicated in PB formation, likely by mediating multivalent interactions required for LLPS (Chen et al., 2022; Willige et al., 2024). Under high R: FR (unshaded) conditions, phyB Pfr accumulates in the nucleus, where large PBs are formed and promote photomorphogenesis, characterized by cotyledon expansion and inhibition of hypocotyl elongation (Peng et al., 2023). Conversely, under shade (low R: FR), PB formation is suppressed, and the seedling exhibits elongation growth typical of shade avoidance response.

FIN219 as a Converging Node in Light and Jasmonate Signaling

FAR-RED INSENSITIVE 219 (FIN219), also known as JASMONATE RESISTANT 1 (JAR1), is a GH3 family enzyme known to play dual roles in light and jasmonate (JA) signaling (Hsieh et al., 2000). In light signaling pathway, FIN219 acts downstream of phytochrome A (phyA), contributing to far-red (FR) light-induced photomorphogenesis (Kuo, 2013; Jiang et al., 2023). In the hormone pathway, FIN219 conjugates jasmonic acid (JA) to isoleucine, producing the bioactive compound JA-Ile, which plays critical roles in plant growth, defense and stress responses.

FIN219's role extends to its interaction with phyB. Under normal (high R: FR) conditions, phosphorylated FIN219 interacts with Pfr-phyB, promoting both PB formation and JA-Ile synthesis. However, under shaded environments (low R: FR), the inactive Pr-phyB interacts with dephosphorylated FIN219, suppressing both JA signaling and PB assembly. In addition, *PHYBOE-YFP* (*PHYBOE-Y*) in the *digalactosyldiacylglycerol synthase1-1* (*dgd1-1*) mutant background that contains increased levels of JA, altered the patterns of phyB-associated photobodies under shade condition. *PHYB-Y dgd1-1* showed a shorter hypocotyl phenotype than *PHYBOE-Y* under

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shade conditions. This coordination supports photomorphogenic development and integrates defense signaling. Consequently, this switch modulates the balance between growth and defense in response to environmental light cues (Peng et al., 2023).

HFR1 in Shade Avoidance and Hormone Crosstalk

LONG HYPOCOTYL IN FAR-RED 1 (HFR1) is a basic helix-loop-helix (bHLH) transcription factor and a crucial downstream regulator in phyA signaling pathway (Fankhauser and Chory, 2000; Soh et al., 2000; Desnos et al., 2001). It plays an essential role in the FR light and shade avoidance responses (Fairchild et al., 2000; Sng et al., 2023). Although HFR1 cannot bind directly to DNA, it forms heterodimers with multiple members of the PHYTOCHROME INTERACTING FACTOR (PIF) family. These heterodimers suppress the transcriptional activation of cell elongation-related genes, thereby inhibiting hypocotyl elongation and function as negative regulators of shade-induced growth (Shi et al., 2013; Paulisic et al., 2021).

Beyond its role at the transcriptional level, HFR1 is regulated post-translationally by CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1), a nucleocytoplasmic E3 ubiquitin ligase. Under dark or low-light conditions, COP1 localizes to the nucleus and targets HFR1 for ubiquitination and subsequent degradation (Jang et al., 2005; Yang et al., 2005; Zhang et al., 2008). In contrast, under light conditions, COP1 is inactivated by photoreceptors such as phyB and cryptochromes, resulting in its exclusion from the nucleus and reduced degradation of HFR1 and other positive regulators of photomorphogenesis such as ELONGATED HYPOCOTYL 5 (HY5) (Luo et al., 2014; Lu et al., 2015).

HY5 is a bZIP-type transcription factor that positively regulates light responses by binding to the promoters of a wide range of light-inducible genes, activating their

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transcription (Lee et al., 2007). It plays significant roles in red, blue, and ultraviolet light signaling pathways and promotes photomorphogenic features such as chlorophyll biosynthesis, antioxidant responses, flavonoid accumulation, and anthocyanin production(Stawska and Oracz, 2019; Kelly et al., 2021). Like HFR1, HY5 is also a target of COP1-mediated degradation. Despite belonging to different transcription factor families, HY5 and HFR1 often function synergistically under most light conditions (Choi et al., 2024). Both are suppressed by COP1 and act together to activate light-responsive gene expression, positioning them as central nodes in light signaling integration(Malakar et al., 2025).

In addition to its role in light signaling, HFR1 is involved in the jasmonate (JA) signaling pathway. Previous studies showed that HFR1 protein and gene expression levels are regulated by FAR-RED INSENSITIVE 219 (FIN219), which in turn modulates JA-responsive gene expression under shade conditions (Kuo, 2013). FIN219 acts as a signaling hub integrating both light and JA pathways, showing physical interaction with phyB and cooperative regulation with HFR1 in response to shade or exogenous methyl jasmonate (MeJA) treatment (Peng et al., 2023).

Integrative Interactions Among HFR1, PHYB, and FIN219

According to previous studies, the active Pfr phyB interacted with phosphorylated FIN219 under ambient light conditions and Pr form of phyB with dephosphorylated FIN219 under shade light. The dephosphorylated, active form of FIN219 facilitates the conversion of jasmonic acid (JA) to jasmonoyl-isoleucine (JA-Ile), thereby activating the JA signaling pathway. At the same time, this interaction regulates the formation of photobodies (PBs) in the nucleus, promoting the assembly of a small number of large PBs. This photobody configuration is associated with the promotion of photomorphogenesis

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in *A. thaliana*, leading to expanded cotyledons and suppressed hypocotyl elongation. In contrast, under shaded conditions, the inactive form of phyB binds to the active FIN219. This interaction results in the suppression of JA signaling, the absence of PB formation in the nucleus, and the manifestation of typical shade avoidance phenotypes, such as upward leaf petiole positioning and elongated hypocotyls. Our previous results also indicated that the gene expression of *HFR1* was co-regulated by phyB and FIN219 (Peng et al., 2023). It is known that HFR1 and FIN219 mutually regulate each other under shade conditions; however, no direct physical interaction between these two had been confirmed (Kuo, 2013). Based on these findings, this study aims to further investigate the interrelationship among HFR1, phyB, and FIN219, and to explore how these factors integrate light and jasmonate signaling pathways in the regulation of plant growth and development.

Significance and Objectives of This Study

This study aims to clarify the triadic interactions among HFR1, phyB, and FIN219 in regulating plant growth under fluctuating light conditions and hormonal cues. Specifically, I seek to: **1.** Dissect the physical and functional relationships among these three proteins using genetic and molecular approaches. **2.** Examine how MeJA treatments and shade environments influence the subcellular dynamics and functional outputs of HFR1–phyB–FIN219 interactions. **3.** Explore the physiological relevance of photobody formation and nuclear speckle localization in integrating light and hormone signaling. **4.** Establish whether these interactions constitute a cooperative complex, a hierarchical regulatory cascade, or a feedback loop involved in fine-tuning *Arabidopsis* development.

By addressing these questions, we aim to deepen our understanding of how light signaling integrates with hormonal pathways to regulate plant morphology and defense.

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This integrative view has broader implications for plant adaptation to natural environments, crop improvement, and synthetic regulation of growth–defense trade-offs.

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Materials and Methods

Plant materials and growth conditions

Arabidopsis mutants *hfr1-201* (Kuo, 2013), *fin219-2* (Hsieh et al., 2000), *phyB-1* (Hirschfeld et al., 1998) are in the Columbia-6 (Col-6), Col-0, and Landsberg *erecta* (L*er*) ecotypes backgrounds, respectively. The double mutants *fin219-2 hfr1-201* (Kuo, 2013), *fin219-2 phyB-1* (Peng et al., 2023), *hfr1-201 phyB-1*, and the triple mutant *fin219-2 phyB-1 hfr1-201* were generated by crossing, and homozygous lines were confirmed by genotyping. Seeds were stratified at 4℃ in the dark for three days and then grown on 1/2 Murashige and Skoog (MS) medium containing 0.3% sucrose and 0.9% agar at 22℃ in a growth chamber under continuous white light. To generate *$35S_{pro}$: HFR1-CFP-HA/hfr1-201* (*HFR1-CFP*) transgenic plants, the full-length coding sequence of *HFR1* was cloned into pEarlygate 102 vectors by using the Gateway system (Invitrogen). The resulting construct introduced into *Agrobacterium* strain GV3101 and then transformed into the *hfr1-201* mutant by floral dipping. The selection marker for the transgenic plant H*FR1-CFP* was BASTA.

Measurement of the hypocotyl lengths

The growth chambers were maintained at 22℃ during the experiment. Seeds were stratified for 3 days at 4℃ and then grown under various light conditions, including far-red light, blue light, and red light for 3 days. Shading and non-shading used white light with or without extra far-red LED light. In this study, the experimental light intensity of far-red light is 2 μmol m^{-2} s^{-1} and 10 μmol m^{-2} s^{-1}; blue light is 2 μmol m^{-2} s^{-1} and 10 μmol m^{-2} s^{-1}; red light is 2 μmol m^{-2} s^{-1} and 10 μmol m^{-2} s^{-1}; white light is 20 μmol m^{-2} s^{-1}. Wild-type and mutant seedlings were grown under high R: FR (ratio =7) for two days and then

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transferred to high R: FR or low R: FR (ratio =0.3) for another four days at 22℃. Light intensity was approximately 60 μmol m^{-2} s^{-1}. The hypocotyl lengths of seedlings (n≥25) were measured by using ImageJ software.

Bimolecular fluorescence complementation (BiFC)

The constructs, *$35S_{pro}$: HFR1-YN*, *$35S_{pro}$: PHYB-YC*, and *$35S_{pro}$: FIN219-YC* generated with pEarlygate 201 (*YFP*-N terminal) and pEarlygate 202 (*YFP*-C terminal) vectors were transiently expressed in 3-week-old *Nicotiana benthamiana* by agroinfiltration with the GV3101 strain. After 2-day incubation under white light conditions, plants were transferred to far-red light, blue light, red light, dark, high R: FR or low R: FR light conditions for 4 hours at 25℃. Then I examined the YFP fluorescence signal in the epidermal cells of *Nicotiana* leaves by using a Nikon H600L microscope.

Protein extraction and Western blotting

Seedlings were ground into powder with liquid nitrogen, and soluble proteins were extracted with the protein extraction buffer (Supplemental Table 4) and clarified by centrifugation. The protein concentration of the supernatant was determined by using Bradford reagent (Bio-Red Protein Assay, Bio-Red, Munich, Germany). A total of 100-200 μg of plant proteins was separated by SDS-PAGE and transferred onto the PVDF membrane. After blotting, HFR1, FIN219, and PHYB proteins were detected by specific HFR1, monoclonal FIN219, and PHYB antibodies with 1:200, 1:120, and 1:5000 dilutions, respectively. After washing off the primary antibody, the membranes were detected by the secondary antibodies with a 1:5000 dilution. We used KETA CL to visualize the chemiluminescence signal.

Total RNA Isolation and RT-PCR

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The RNA extraction was done by the RNA Plus mini kit (LabPrep), and DNA contamination in samples was eliminated by using TURBO^{TM} DNase (ABI). The total RNA, 1 μg, was converted to the first-strand cDNA pool by adding oligo-dT primers, RNasin (Promega), and reverse transcriptase (Applied Biosystems). After reverse transcription, I used a 1μL aliquot of 2-fold diluted cDNA from RT-PCR for gene expression analysis. Gene-specific primers and PCR conditions used in the RT-PCR experiments are listed in Supplemental Table 3.

Assays of phyB-associated photobodies formation

PHYB-YFP, *PHYB-YFP fin219-2*, *PHYB-YFP hfr1-201*, *PHYB-YFP fin219-2 hfr1-201*, *PHYB-YFP dgd1-1*, and *PHYB-YFP dgd1-1 hfr1-201* plants were grown under high R: FR for two days and then transferred to high R: FR or low R: FR for another four days on the MS plate or with and without MeJA treatment before examination of PHYB-YFP-associated photobodies with a Nikon H600L microscope.

Statistical analysis

We performed statistical data analyses by using one-way ANOVA with post hoc Tukey's test in SPSS software and Excel software to evaluate significant differences between samples. The difference is significant at the *P*-value < 0.05.

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Results

Regulatory Relationships Between HFR1 and phyB or FIN219 Under Different Monochromatic Light Conditions

Previous studies established that phyB, FIN219, and HFR1 played critical roles in light signaling pathways of *Arabidopsis* seedlings. To further investigate the regulatory relationships among these three components, we analyzed hypocotyl phenotypes under various monochromatic light conditions using wild-type plants, three single mutants, and all combinations of the double mutants. Consistent with prior findings, the single mutant *fin219-2* had a longer hypocotyl than Col-0, which suggests that FIN219 plays a critical role under low far-red light conditions (Figure 1). However the single mutant *phyB-1* had a significantly long hypocotyl compared to L*er*, implying that the photoreceptor phyB impacts light signaling, particularly in red light (Figure 3). Through the comparison of the hypocotyl length between the single mutant *hfr1-201* and wild-type, HFR1 is involved in signaling under both strong and weak far-red light, as well as under blue light conditions (Figure 1 and 2), but it appears to play an antagonistic role under red light and darkness (Figure 3 and 4). HFR1 is a negative regulator of photomorphogenesis under red light. Analysis of the double mutants compared to respective single mutants revealed that HFR1 acts downstream of phyB and FIN219 in both far-red and blue light signaling, functioning cooperatively to regulate hypocotyl elongation of seedling development (Figure 1 and 2). In contrast, under red light and darkness, the *hfr1-201* mutant displays a shorter hypocotyl than wild-type Col-0, suggesting that HFR1 may play a negative role, and its functions depend on phyB and FIN219 (Figure 3 and 4). According to the hypocotyl phenotype under darkness, the double mutant *fin219-2 hfr1 201* is similar to the single mutant *fin219-2* and the wild type Col-0, suggesting that FIN219 is in the

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downstream of HFR1 in regulating hypocotyl elongation (Figure 4). In brief summary, HFR1 participates in the far-red light and blue light signaling pathways to act as a positive regulator and synergistically regulates hypocotyl elongation with FIN219 and phyB.

Physical Interactions Between HFR1 and phyB or FIN219 Under Different Light Conditions

Having characterized the regulatory relationships between HFR1 and phyB or FIN219, we sought to determine whether these relationships involve direct physical interactions. Previous studies provided no evidence for a physical interaction between HFR1 and phyB, and Kuo (2013) reported that HFR1 does not physically interact with FIN219. To re-examine this, we performed bimolecular fluorescence complementation (BiFC) assays in *Nicotiana benthamiana*. HFR1 was fused with the N-terminal region of YFP (YN), while phyB and FIN219 were fused with the C-terminal region of YFP (YC) (Figure 5A). Negative controls included HFR1-YN paired with empty YC, while FIN219-YN and FIN219-YC were used as positive controls (Figure 5B). Our results showed that HFR1 interacts physically with both phyB and FIN219 under all light conditions tested (Figure 5C). The HFR1-phyB interaction localized exclusively to the nucleus, whereas the HFR1-FIN219 interaction was observed in both the nucleus and the cytoplasm. These results suggest that HFR1, phyB, and FIN219 not only regulate hypocotyl elongation in *Arabidopsis* but also potentially act together to co-regulate protein functions in downstream signaling pathways.

Regulatory Relationships Among HFR1, phyB, and FIN219 in Shade Avoidance Responses

Beyond far-red and blue light signaling, HFR1 is also involved in the critical shade avoidance response pathway. phyB and FIN219 have also been implicated in the

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cooperative regulation of hypocotyl elongation under shade conditions. To investigate this, I generated overexpression lines *HFR1-CFP #1* and *HFR1-CFP #2* in the *hfr1-201* mutant background via floral dipping. These lines were used to determine whether *HFR1* overexpression could rescue the shade phenotype of *hfr1-201* mutant (Figure 6A and 6B). Phenotypic and transcript analyses showed that *HFR1-CFP #1* more effectively rescued the hypocotyl elongation phenotype under low R: FR conditions compared to *HFR1-CFP #2*, despite similar transcript levels (Figure 6C). To further confirm the function of these overexpression lines, I included the previously established *3xFlag-HFR1 #1* for phenotypic comparisons (Supplemental Figure 2). Under low R: FR, *HFR1-CFP #1* displayed a phenotype similar to that of the wild-type Col-0 (Figure 6A and 6B).

To assess whether HFR1, phyB, and FIN219 also act together under shade conditions, we generated a triple mutant by genetic crossing, incorporating the Col-6 ecotype of HFR1 as a control for ecotypic background effects. Under simulated shade (low R: FR, ratio = 0.3), all mutants displayed statistically significant hypocotyl elongation relative to their respective ecotype controls (Col-6, L*er*, and Col-0), while under non-shade conditions (high R: FR, ratio = 8.3), only *phyB-1* mutant showed a significant phenotype (Figure 7). In both light conditions, the double and triple mutants confirmed that HFR1, phyB, and FIN219 function synergistically in regulating hypocotyl elongation (Figure 7). Interestingly, statistical analyses revealed that the phenotypes of the double and triple mutants more closely resembled *phyB-1* than *hfr1-201* or *fin219-2*, especially under shade conditions. This suggests that while all three regulators are involved in the shade response, phyB plays a primary regulatory role, and additional unidentified components may contribute, particularly given the slightly shorter hypocotyls observed under shade compared to non-shade environments.

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Physical Interactions Among HFR1, phyB, and FIN219 in Shade Avoidance Responses

Since physical interactions were observed between HFR1 and phyB or FIN219 under monochromatic light, I extended my BiFC assays in *N. benthamiana* to simulated shade environments by applying varying intensities of supplemental far-red light. Using the same construct combinations, I confirmed that HFR1 physically interacts with phyB (nuclear) and FIN219 (nuclear and cytoplasmic) under all simulated shade conditions (Figure 8). Increasing far-red intensity had no substantial impact on interaction strength, although the subcellular localization of HFR1-FIN219 interactions showed slight variations under different shade levels.

Combined Effects of Jasmonic Acid Signaling and Shade Avoidance on HFR1 Interactions with phyB and FIN219

Previous research showed that FIN219 plays dual roles: it regulates light signaling. Also, it catalyzes the conversion of jasmonic acid (JA) into its bioactive form, JA-Ile, making it a central player in JA signaling. To assess potential crosstalk between JA and light signaling pathways, and their impact on HFR1 interactions with phyB and FIN219, we conducted BiFC assays in *N. benthamiana* with and without exogenous methyl jasmonate (MeJA) under both shade and non-shade conditions. HFR1-YN paired with empty YC was used as a negative control, and NLS-mCherry was used to mark the nuclear localization.

The results indicated that MeJA application did not alter the interaction between HFR1 and phyB (Figure 9). However, MeJA significantly affected the subcellular localization of the HFR1-FIN219 interaction, shifting fluorescence from both the nucleus and cytoplasm to predominantly cytoplasmic. This occurred under both shade and non-

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shade conditions. Phenotypic data showed that HFR1 participates in both shade light signaling and JA signaling pathways in hypocotyl elongation, modulated by phyB and exogenous MeJA (Figure 10).

Functional Roles of HFR1 in phyB-Associated Photobody (PB) Formation and Regulation by the JA Pathway

In addition to their effects on phenotype, I investigated whether HFR1 influences the formation of phyB-associated photobodies (PBs), and how this correlates with physiological function. I used *Arabidopsis* lines expressing *PHYB-YFP* crossed into *hfr1-201*, *fin219-2*, and the double mutant backgrounds to generate *PHYB-YFP hfr1-201*, *PHYB-YFP fin219-2*, and *PHYB-YFP fin219-2 hfr1-201* lines. Under high R: FR conditions, *PHYB-YFP* forms a few large nuclear PBs (Figure 11). In *PHYB-YFP fin219-2*, many small PBs are observed. In *PHYB-YFP hfr1-201*, PBs resemble the wild-type but are more small ones, while in the double mutant, PBs are also more abundant than in *PHYB-YFP fin219-2*. Under low R: FR, PHYB-YFP fluorescence was diffuse in the nucleus with no PB formation across all genotypes.

To further explore HFR1's role in light and JA pathways, I generated *PHYB-YFP dgd1-1 hfr1-201* plants and examined them under both light conditions. Under high R: FR, MeJA decreased the PB numbers and increased their size in *PHYB-YFP hfr1-201* (Figure 12). In *PHYB-YFP fin219-2 hfr1-201*, PB formation appeared enhanced by MeJA. Under low R: FR, MeJA induced PB formation in all lines, but the PBs were faint or scattered. *PHYB-YFP dgd1-1 hfr1-201* showed PB patterns similar to MeJA-treated *PHYB-YFP hfr1-201* and *PHYB-YFP fin219-2 hfr1-201* under respective conditions. Phenotypic data further confirmed HFR1’s essential role in hypocotyl elongation, modulated by phyB overexpression and exogenous MeJA (Figure 13).

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In summary, HFR1 physically and functionally interacts with both phyB and FIN219, contributing to hypocotyl elongation under far-red light, blue light, and shade conditions. These interactions are further modulated by the jasmonic acid signaling pathway. HFR1 is also involved in the formation of phyB-associated photobodies, particularly under high R: FR conditions, indicating its broader role in integrating light and hormone signaling to fine-tune plant development.

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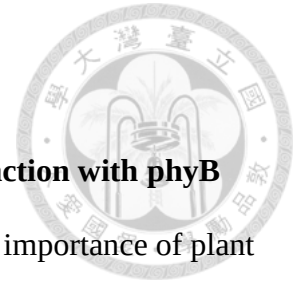
Discussion



The Multifaceted Role of HFR1 in Light Signaling and Its Interaction with phyB

Recent advances in light signaling studies have emphasized the importance of plant responses under complex light environments that mimic natural shading conditions (Martinez-Garcia and Rodriguez-Concepcion, 2023). In *A. thaliana*, HFR1 is a basic helix-loop-helix transcription factor known to participate in far-red and blue light signaling pathways. Although no definitive physical interaction between HFR1 and phyB has been confirmed until now, functional evidence strongly supports that HFR1 acts downstream of phyB or in a parallel regulatory module. In our study, BiFC assays revealed physical interaction between HFR1 and phyB localized in the nucleus across various light conditions, including high and low R: FR ratios and blue light exposure (Figure 5 and 8).

Phenotypically, our single and double mutant analyses showed that HFR1 and phyB synergistically suppress hypocotyl elongation, particularly under shaded conditions (Figure 7 and 9). This suggests that HFR1 functions either downstream of or cooperatively with phyB to mediate photomorphogenesis. The regulatory relationship becomes less evident under red light or darkness, where PHYB remains the dominant repressor, while HFR1's involvement appears minimal (Figure 3 and 4). It has been documented previously that HFR1 is less involved in the regulation of hypocotyl elongation in darkness and red light, and has comparable hypocotyl phenotype with wild-type plants (Fairchild et al., 2000). Studies showed that HFR1 positively regulates phyB-dependent seed germination, and prevents PIF1 from binding to its target genes *in vivo* by forming heterodimers with PIF1 in darkness (Shi et al., 2013). Thus, HFR1 may regulate the external phenotype in response to darkness and red light by interacting with

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different partners.

In high R: FR conditions, our data show that phyB forms large photobodies (PBs) in the nucleus, which associated with short hypocotyls and photomorphogenic traits. Mutants lacking HFR1 presented increased PB numbers, suggesting that HFR1 may influence the spatial organization or dynamics of phyB-associated PBs (Figure 11 and 12).

Integration of Hormonal Signals: The Role of HFR1 and FIN219 in JA Signaling

HFR1 is also implicated in jasmonic acid (JA) signaling, a pathway known to regulate defense responses and growth inhibition. FIN219/JAR1, a GH3-family protein involved in JA-Ile biosynthesis, has previously been shown to interact with phyB and mediate JA-light signal integration (Kuo, 2013). In this study, we extended our investigation to the relationship between HFR1 and FIN219.

Despite earlier suggestions that HFR1 and FIN219 do not physically interact, here BiFC data show a clear interaction signal in both the nucleus and the cytoplasm under multiple light conditions (Figure 5 and 8). This spatial localization pattern remained stable under shaded and non-shaded conditions, although slight variations were noted upon MeJA treatment. Exogenous MeJA appeared to increase the cytoplasmic localization of the HFR1–FIN219 complex, suggesting a hormonal influence on protein–protein interactions.

These results indicate that HFR1 may serve as a signaling node integrating FIN219-mediated JA responses and light perception pathways, facilitating fine-tuned regulation of hypocotyl elongation and defense gene expression under shade.

Coordination Between HFR1, phyB, and FIN219 in Shade and JA-Mediated Growth Regulation

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To understand whether HFR1, phyB, and FIN219 collaboratively regulate plant growth under shade and JA signaling, I analyzed hypocotyl phenotypes across various single, double, and triple mutant combinations. Among the three various wild types, Col-0 and Col-6 present similar phenotypes regardless of the light conditions. Under low R: FR conditions mimicking shade, I observed a pronounced hypocotyl elongation in the triple mutant (Figure 7). This supports the hypothesis that these three factors act in a synergistic manner or in parallel to suppress excessive elongation of plants under shade stress. In the future, it may be possible to observe the interaction and levels of the three endogenous proteins in *Arabidopsis* through co-immunoprecipitation (Co-IP) assays to better understand the regulatory relationship among them.

Interestingly, in high R: FR light conditions, only *phyB-1* mutants showed significant elongation, suggesting that phyB plays a dominant role in light conditions mimicking full sunlight. However, under low R: FR or with additional MeJA treatment, *hfr1-201* and *fin219-2* single and double mutants also exhibited elongated hypocotyls, further implying their role in integrating shade and hormonal cues (Figure 10).

In transgenic overexpression lines of *HFR1-CFP* and *3xFlag-HFR1*, partial or full phenotypic rescue of *hfr1-201* mutants was achieved under shade (Supplemental Figure 2). This supports the functional relevance of HFR1 in attenuating hypocotyl elongation under reduced R: FR ratios and reinforces its interaction with upstream regulators.

The Role of HFR1 in Photobody Formation and Downstream Signal Modulation

Photobodies, phase-separated nuclear structures assembled by PHYB, have been proposed to function as platforms for transcriptional control and protein turnover (Chen et al., 2022; Willige et al., 2024). Our observations in *PHYB-YFP* transgenic lines revealed that in *hfr1-201* backgrounds, the number of PBs was significantly increased,

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while PB size appeared reduced. This suggests that HFR1 may be involved in regulating PB size and number, potentially by modulating protein aggregation dynamics or nuclear retention mechanisms.

Interestingly, MeJA treatment caused an increase in PB formation in *PHYB-YFP hfr1-201 fin219-2* backgrounds, suggesting that JA signaling, likely mediated through FIN219, enhances PB aggregation when HFR1 is absent. This implies that HFR1 might limit JA-enhanced PB formation, acting as a repressor in hormone-induced PB remodeling.

Triple mutant and transgenic lines also revealed that the formation of PBs is not only regulated by red light or phyB activation but also influenced by JA-related components and transcriptional repressors such as HFR1. The ability of MeJA to restore PB formation under shade conditions in HFR1-deficient plants highlights the integrative nature of these signaling pathways.

Toward a Unified Model for Light and Hormonal Signal Integration

Based on phenotypic and molecular evidence, I propose that HFR1 is a key integrator in the phyB–FIN219 regulatory network (Figure 14). Under shaded conditions, phyB is converted into its inactive Pr form and remains cytoplasmic. FIN219, depending on its phosphorylation state, modulates JA-Ile levels and indirectly affects PB formation. HFR1, interacting with both phyB and FIN219, contributes to fine-tuning downstream transcriptional responses and growth regulation.

The physical interactions of HFR1 with both phyB and FIN219 under a wide range of environmental conditions suggest a modular complex in which HFR1 bridges light and hormone pathways to maintain developmental plasticity. Furthermore, MeJA-induced spatial redistribution of HFR1–FIN219 complexes supports the hypothesis that JA

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signaling can dynamically alter regulatory modules in response to environmental stimuli.

My findings present new insights into how plants balance growth and defense through a tri-component signaling hub involving HFR1, phyB, and FIN219. Future studies focusing on the expression of shade- and JA-responsive genes, as well as anthocyanin and chlorophyll content, will further clarify the physiological relevance of this regulatory network.

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Figures

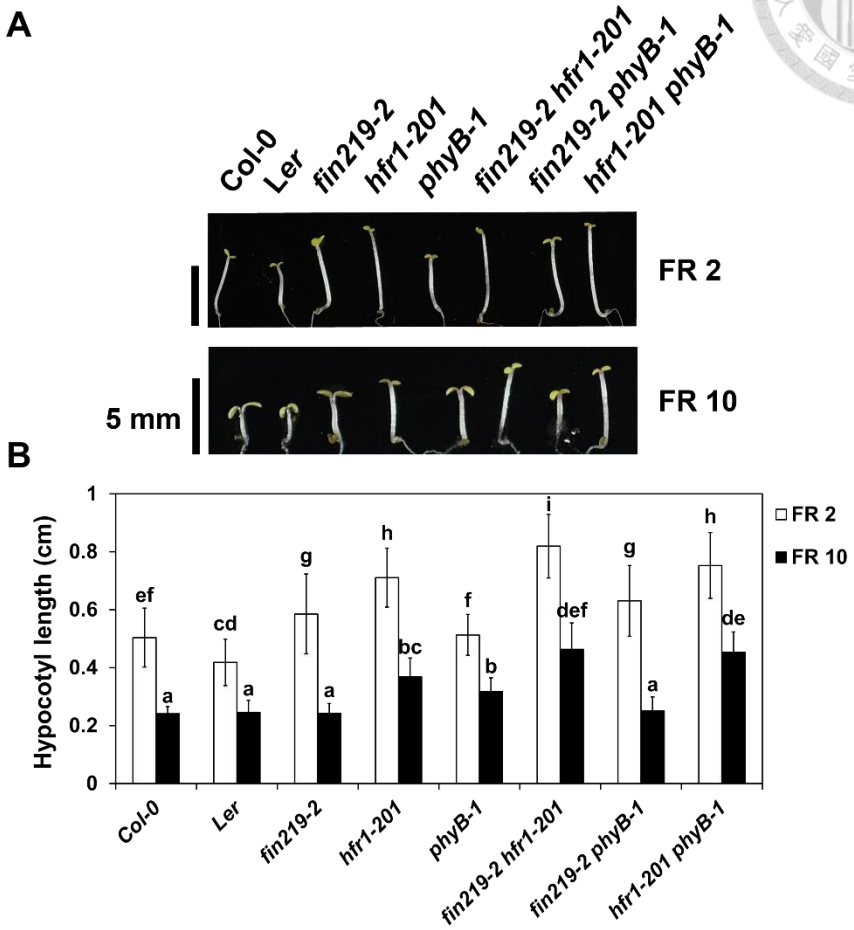

Figure 1. HFR1 and FIN219 synergistically regulate hypocotyl elongation under far-red light. HFR1 is epistatic to phyB, and they coordinately regulate hypocotyl length under high far-red light.

A, The *A. thaliana* hypocotyl phenotype of Col-0, L*er*, *fin219-2*, *hfr1-201*, *phyB-1*, *fin219-2 hfr1-201*, *fin219-2 phyB-1*, and *hfr1-201 phyB-1* seedlings grown under low (2 μmol m^{-2} s^{-1}) and high (10 μmol m^{-2} s^{-1}) far-red (FR) light conditions for 3 days. Scale bar = 5 mm.

B, Quantification of hypocotyl lengths of the seedlings shown in (A). Data are presented as mean ± SE ($n \geq 30$). Different lowercase letters represent a statistically significant difference in hypocotyl lengths by one-way ANOVA, Tukey's HSD, $P < 0.05$.

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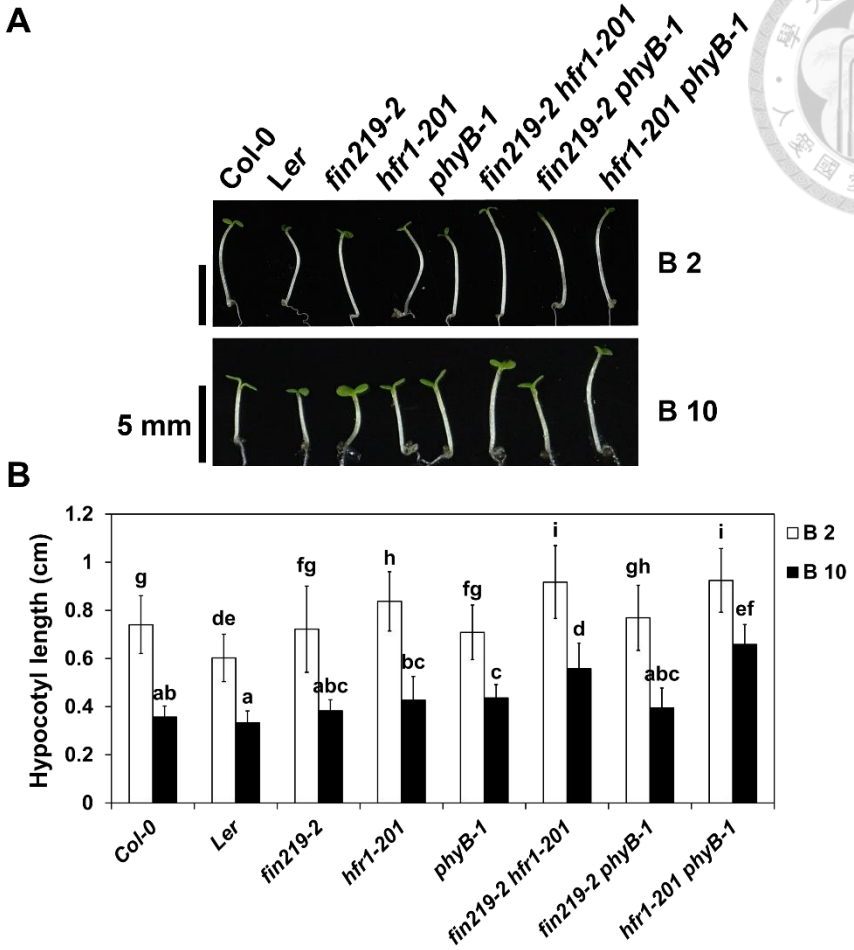

Figure 2. HFR1 participates in the blue light signaling pathway to positively regulate the photomorphogenic development of seedlings.

A, The *A. thaliana* hypocotyl phenotype of Col-0, L*er*, *fin219-2*, *hfr1-201*, *phyB-1*, *fin219-2 hfr1-201*, *fin219-2 phyB-1*, and *hfr1-201 phyB-1* seedlings grown under low (2 μmol m^{-2} s^{-1}) and high (10 μmol m^{-2} s^{-1}) blue (B) light conditions for 3 days. Scale bar = 5 mm.

B, Quantification of hypocotyl lengths of the seedlings shown in (A). Data are presented as mean ± SE ($n \geq 30$). Different lowercase letters represent a statistically significant difference in hypocotyl lengths by one-way ANOVA, Tukey's HSD, $P < 0.05$.

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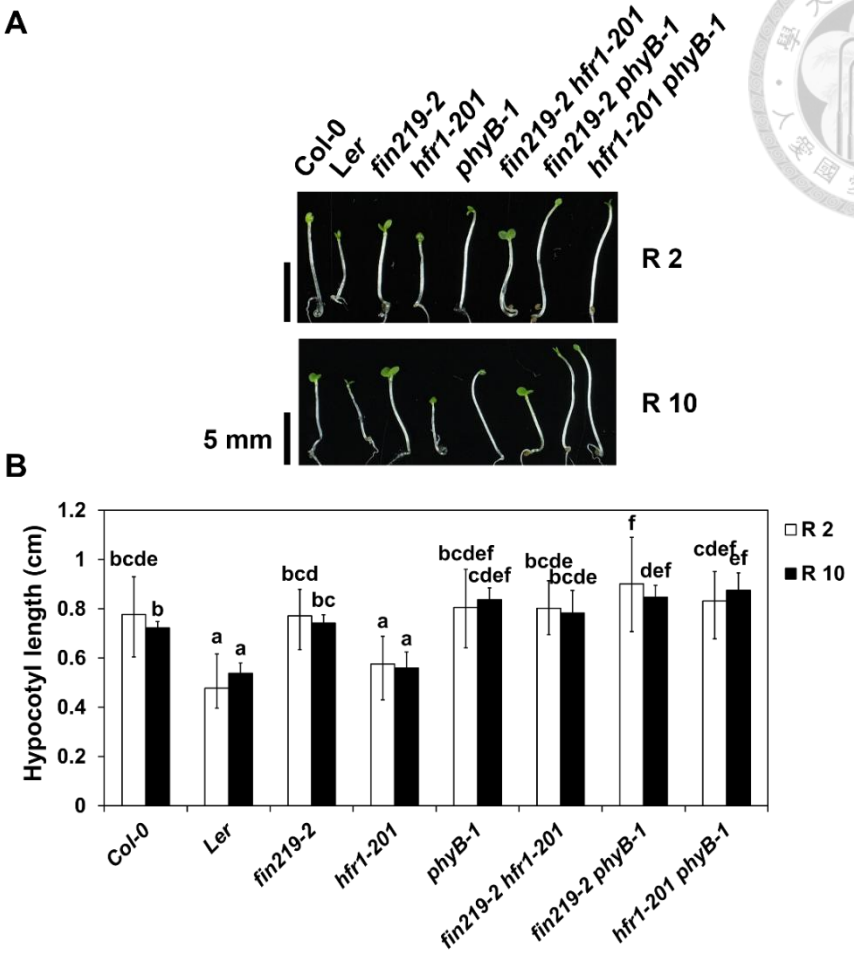

Figure 3. HFR1 is hypostatic to FIN219 and phyB and acts as a negative regulator of photomorphogenesis in the red-light signaling pathway.

A, The *A. thaliana* hypocotyl phenotype of Col-0, L*er*, *fin219-2*, *hfr1-201*, *phyB-1*, *fin219-2 hfr1-201*, *fin219-2 phyB-1*, and *hfr1-201 phyB-1* seedlings grown under low (2 μmol m^{-2} s^{-1}) and high (10 μmol m^{-2} s^{-1}) red (R) light conditions for 3 days. Scale bar = 5 mm.

B, Quantification of hypocotyl lengths of the seedlings shown in (A). Data are presented as mean ± SE (n ≥ 30). Different lowercase letters represent a statistically significant difference in hypocotyl lengths by one-way ANOVA, Tukey's HSD, $P < 0.05$.

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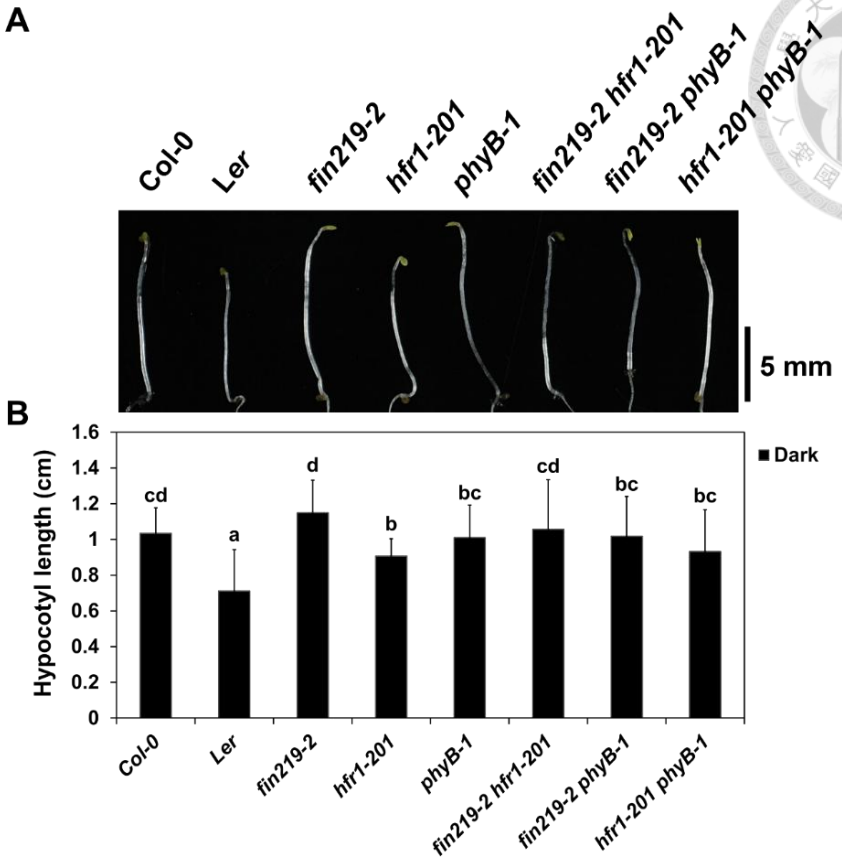

Figure 4. FIN219 may act downstream of HFR1 under the dark condition.

A, The *A. thaliana* hypocotyl phenotype of Col-0, L*er*, *fin219-2*, *hfr1-201*, *phyB-1*, *fin219-2 hfr1-201*, *fin219-2 phyB-1*, and *hfr1-201 phyB-1* seedlings grown under the dark condition for 3 days. Scale bar = 5 mm.

B, Quantification of hypocotyl lengths of the seedlings shown in (A). Data are presented as mean ± SE (n ≥ 30). Different lowercase letters represent a statistically significant difference in hypocotyl lengths by one-way ANOVA, Tukey's HSD, $P < 0.05$.

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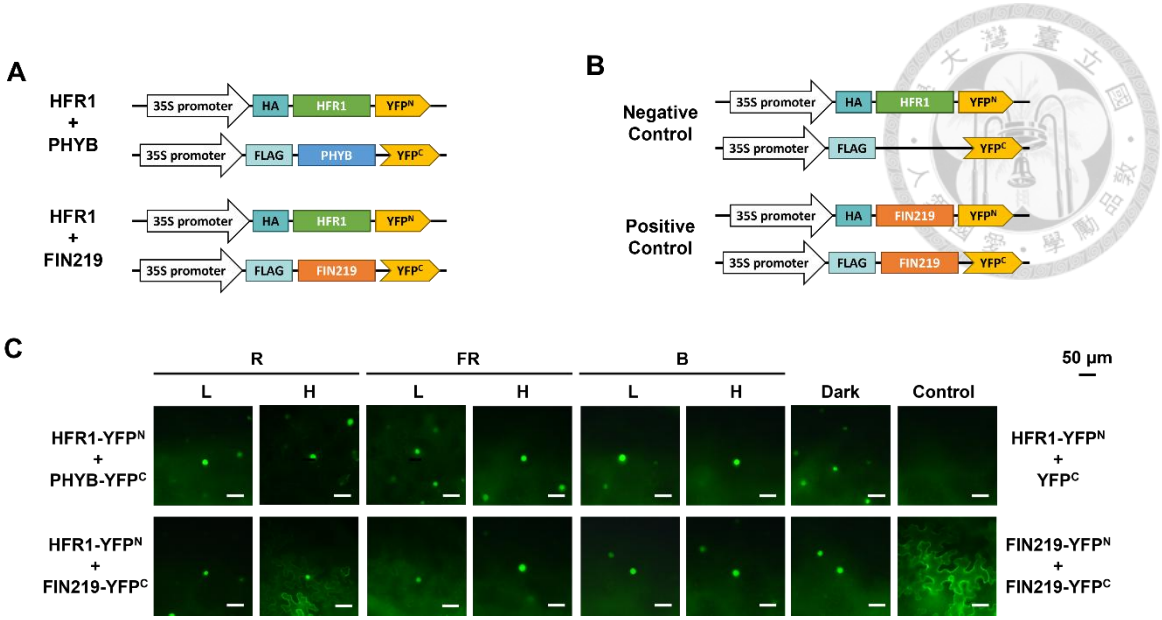

Figure 5. HFR1 interacts with phyB and FIN219 under different light conditions.

A, Schematic diagram of the constructs for bimolecular fluorescence complementation (BiFC) analysis. HFR1 fused with YFP^N (*$35S_{pro}$: HA-HFR1-YFP^N*), phyB fused with YFP^C (*$35S_{pro}$: FLAG-PHYB-YFP^C*), and FIN219 fused with YFP^C (*$35S_{pro}$: FLAG-FIN219-YFP^C*).

B, The interaction of HFR1-YFP^N + YFP^C and FIN219-YFP^N + FIN219-YFP^C is performed as negative and positive controls, respectively.

C, The constructs of HFR1 and phyB, HFR1 and FIN219 were introduced in *Nicotiana benthamiana* leaves under low intensity (L) light (2 μmol m^{-2} s^{-1}) or high intensity (H) light (10 μmol m^{-2} s^{-1}) in different light conditions (R, FR, B, Dark) for 4 hours. Scale bar = 50 μm.

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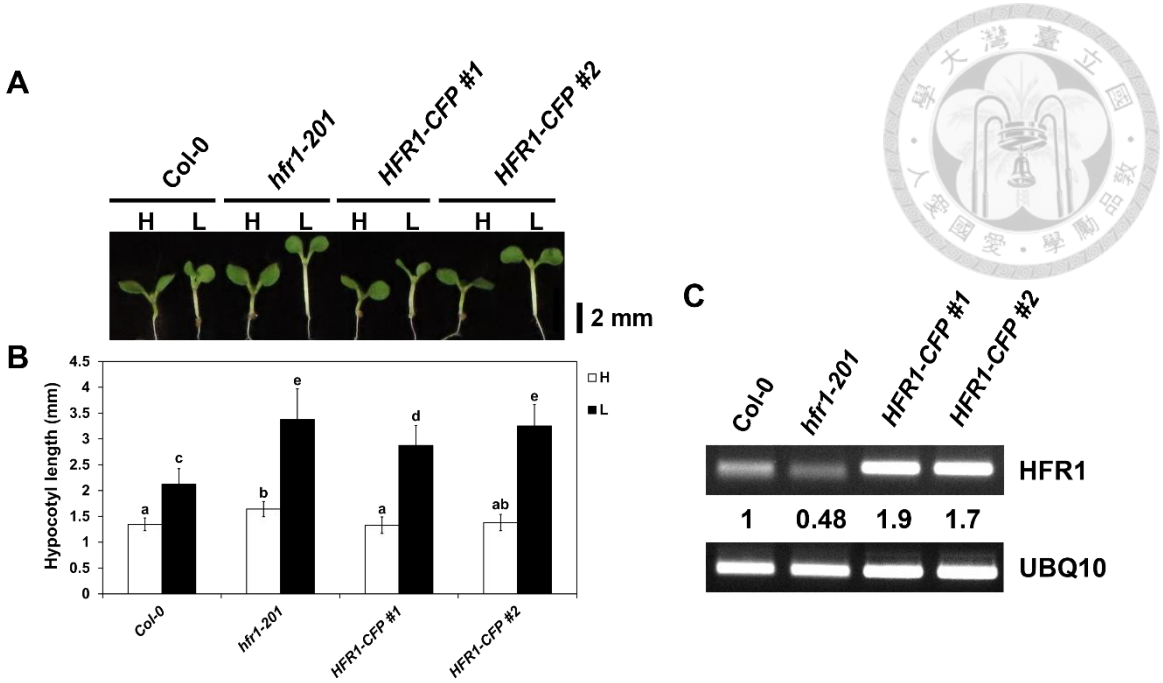

Figure 6. HFR1 participates in the shade avoidance signaling pathway to positively regulate photomorphogenic development of seedlings.

A, *A. thaliana* seedlings of Col-0, *hfr1-201*, *35S*$_{pro}$*: HFR1-CFP/hfr1-201* (*HFR1-CFP #1, #2*) were grown under high (H) R: FR light for 2 days and then transferred to high R: FR or low (L) R: FR light for 4 days. Scale bar = 2 mm.

B, Quantification of hypocotyl lengths of the seedlings shown in (A). Data are presented as mean ± SE (n ≥ 25). Different lowercase letters represent a statistically significant difference in hypocotyl lengths by one-way ANOVA, Tukey's HSD, $P < 0.05$.

C, Analysis of *HFR1* expression by RT-PCR in Col-0, *hfr1-201*, and two *HFR1* transgenic plants. Samples were grown under white light for 6 days before collection. *UBQ10* was included as an internal control.

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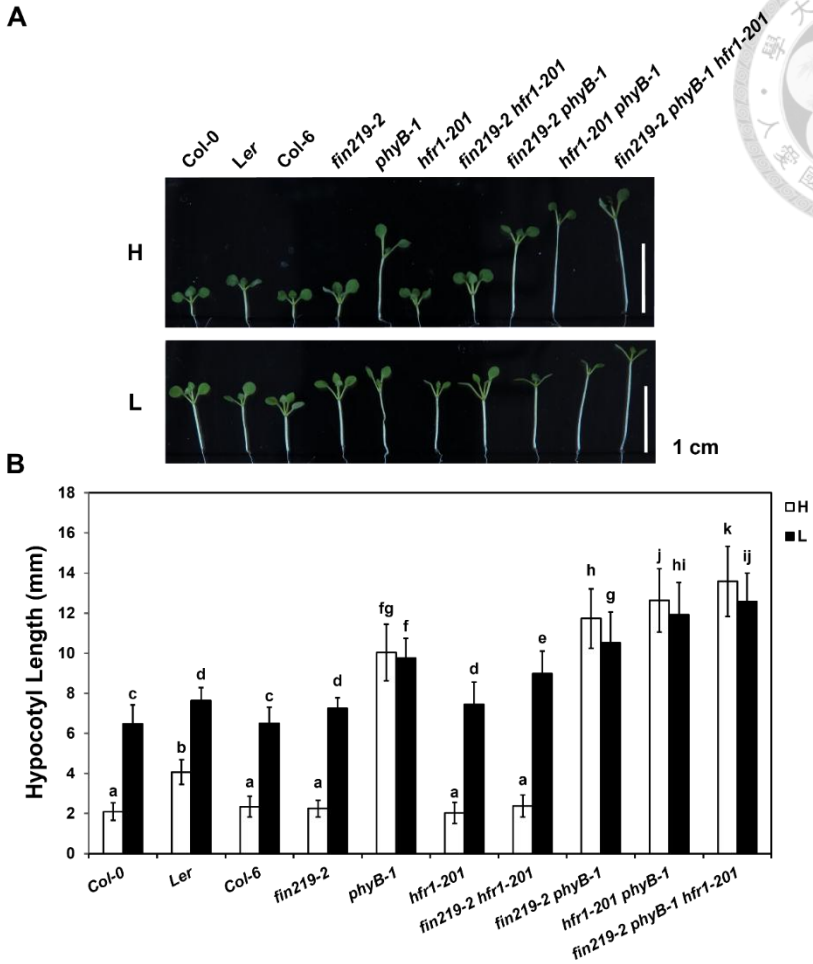

Figure 7. HFR1 shows a synergistic relationship with phyB and FIN219 in hypocotyl elongation under low R: FR light.

A, The *A. thaliana* hypocotyl phenotype of Col-0, L*er*, Col-6, *fin219-2*, *hfr1-201*, *phyB-1*, *fin219-2 hfr1-201*, *fin219-2 phyB-1*, *hfr1-201 phyB-1*, and *fin219-2 phyB-1 hfr1-201* seedlings grown under high R: FR light for 2 days and then transferred to high R: FR or low R: FR light for 4 days. Scale bar = 1 cm.

B, Quantification of hypocotyl lengths of the seedlings shown in (A). Data are presented as mean ± SE (n ≥ 30). Different lowercase letters represent a statistically significant difference in hypocotyl lengths by one-way ANOVA, Tukey's HSD, $P < 0.05$.

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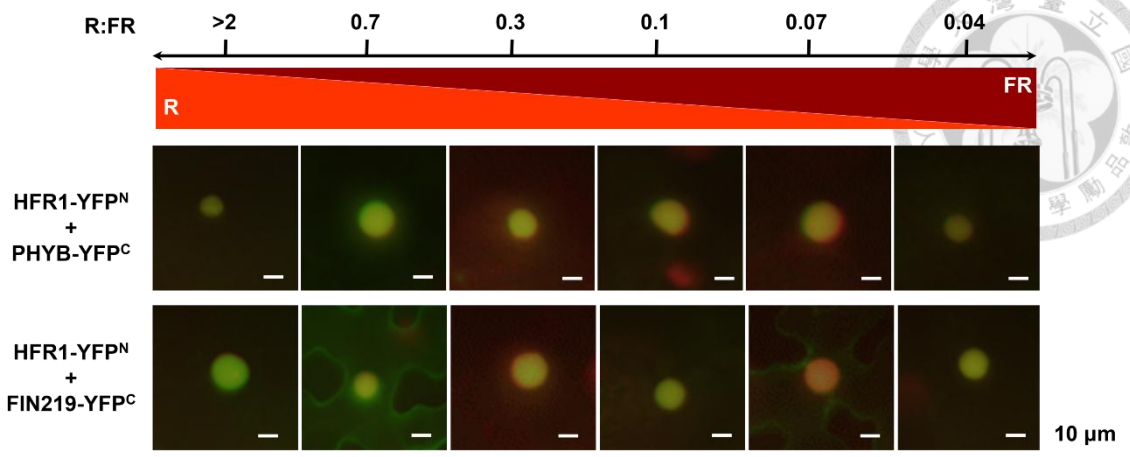

Figure 8. HFR1 interacts with phyB and FIN219 under high R: FR and low R: FR light.

The BiFC interaction analysis of HFR1-YFP^{N} + PHYB-YFP^{C} and HFR1-YFP^{N} + FIN219-YFP^{C} was introduced in *Nicotiana benthamiana* leaves under high R: FR light for 2 days and then transferred to different ratios of high R: FR or low R: FR light for 4 hours. Scale bar = 10 μm.

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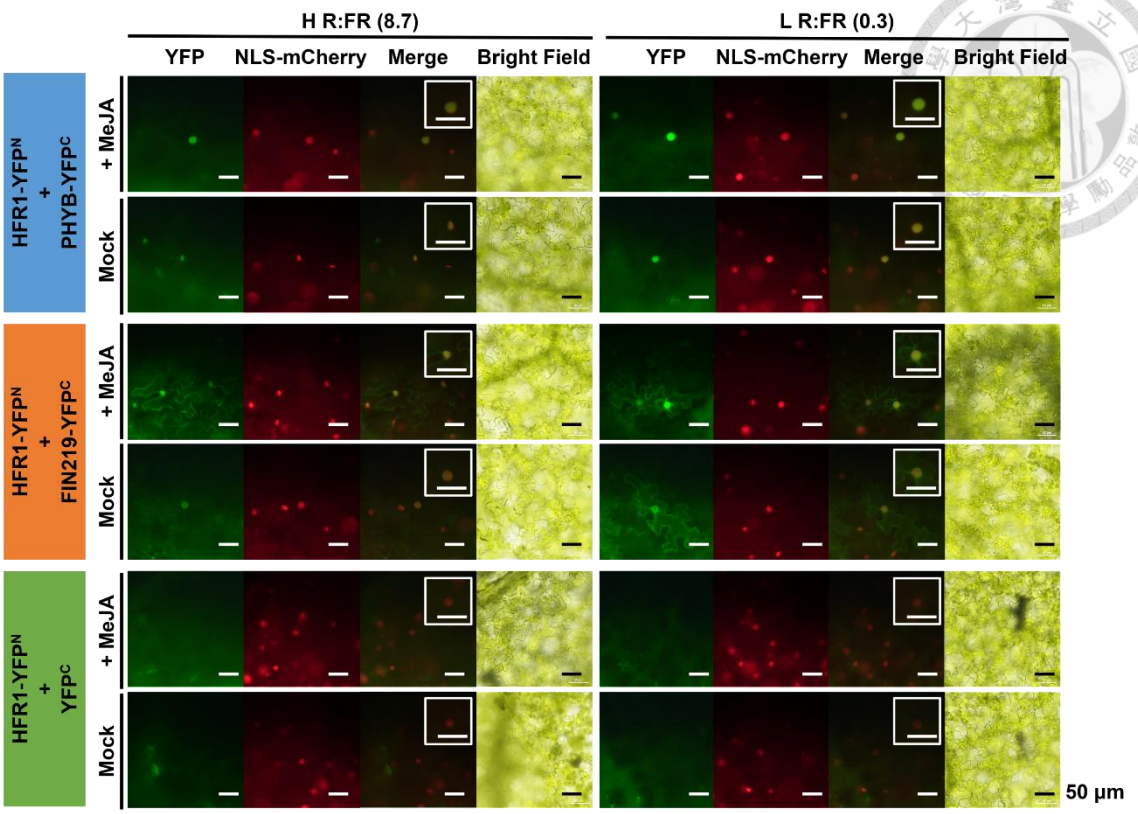

Figure 9. HFR1 and phyB have a physical interaction in the nucleus under low R: FR and high R: FR light conditions.

The interaction of HFR1 and phyB, HFR1 and FIN219 by BiFC analysis in *N. benthamiana* under high (H) R: FR light for 2 days, and then transferred to high R: FR light or low (L) R: FR light for 4 hours in the absence (Mock) or in the presence of methyl jasmonate (MeJA) treatments. The red fluorescent signal of NLS-mCherry (*35S$_{pro}$: NLS-mCherry*) was introduced to indicate the localization of the nucleus. Interaction of HFR1-YFP^N + YFP^C is performed as a negative control. Scale bar = 50 μm.

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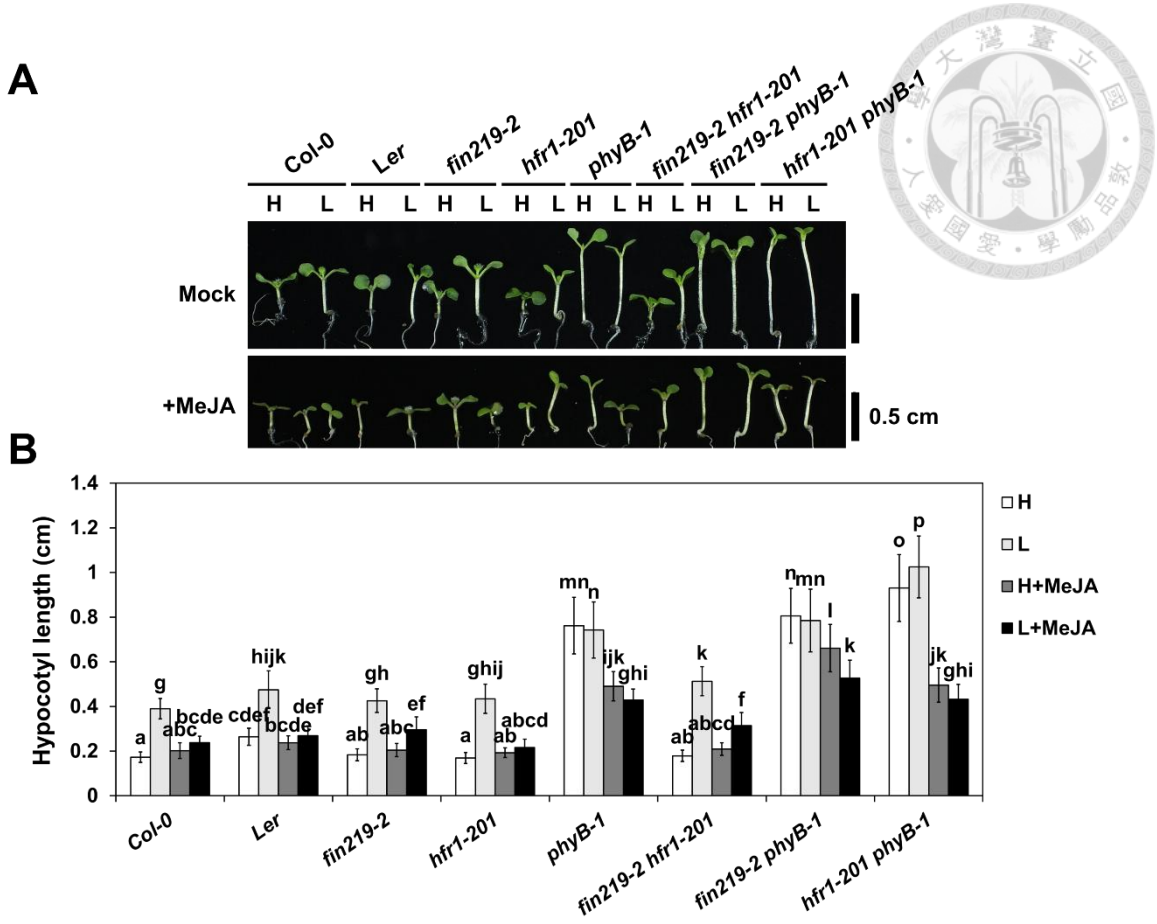

Figure 10. HFR1 shows a synergistic relationship with phyB and FIN219 in response to JA-regulated hypocotyl elongation under low R: FR light.

A, The *A. thaliana* hypocotyl phenotype of Col-0, L*er*, *fin219-2*, *hfr1-201*, *phyB-1*, *fin219-2 hfr1-201*, *fin219-2 phyB-1*, and *hfr1-201 phyB-1* seedlings grown under high (H) R: FR light for 2 days and then transferred to high R: FR or low (L) R: FR light for 4 days in the presence or absence of MeJA treatments in the growth medium. Scale bar = 0.5 cm.

B, Quantification of hypocotyl lengths of the seedlings shown in (A). Data are presented as mean ± SE (n ≥ 30). Different lowercase letters represent a statistically significant difference in hypocotyl lengths by one-way ANOVA, Tukey's HSD, $P < 0.05$.

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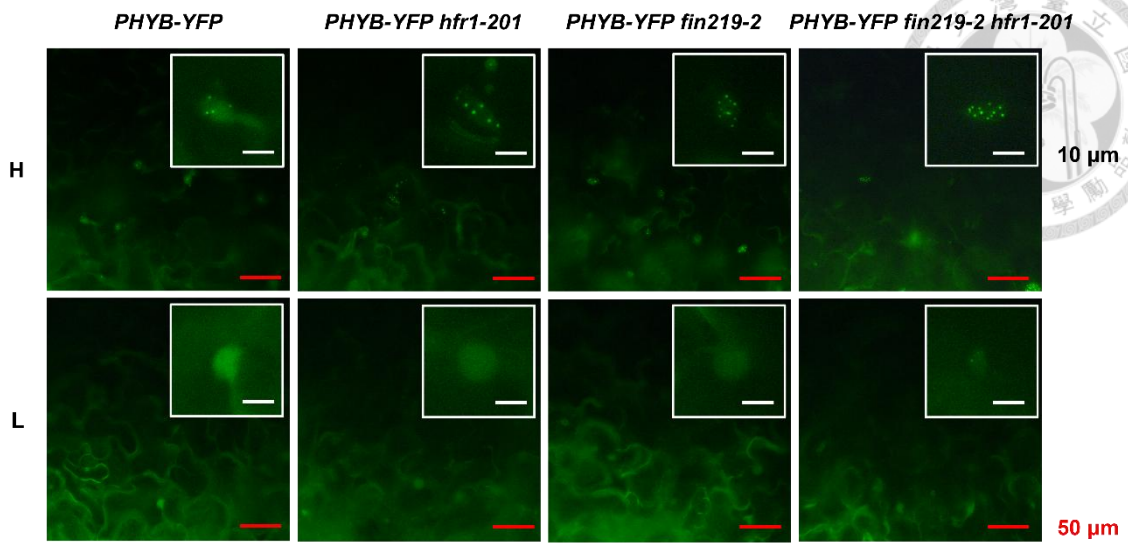

Figure 11. HFR1 is involved in phyB-associated photobodies and regulates the formation under high R: FR light.

phyB-associated photobodies formed under high R: FR light. The *A. thaliana* seedlings of *PHYB-YFP*, *PHYB-YFP/hfr1-201*, *PHYB-YFP/fin219-2*, and *PHYB-YFP/fin219-2 hfr1-201* were grown under high (H) R: FR light for 2 days and then transferred to high R: FR or low (L) R: FR light for 4 days. White scale bar = 10 μm. Red scale bar = 50 μm.

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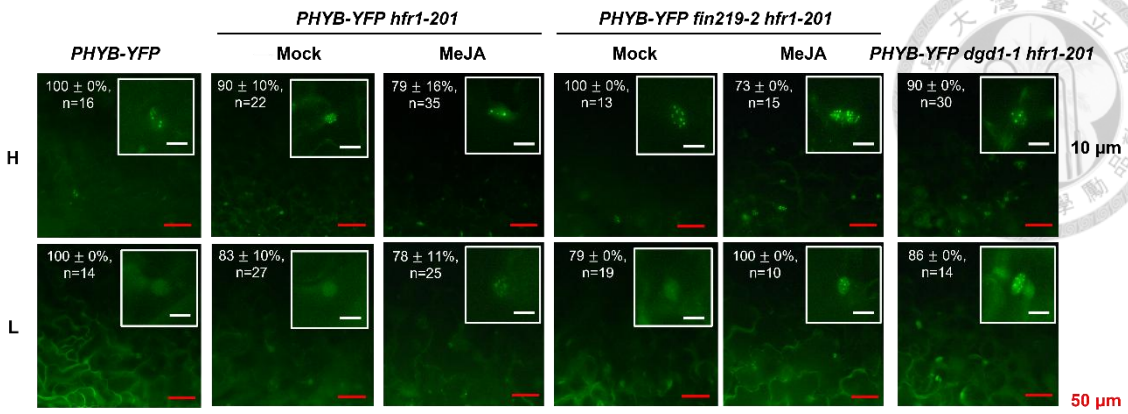

Figure 12. A high level of endogenous JA and additional MeJA increased phyB-associated photobodies in HFR1 reducing lines under high R: FR and low R: FR light.

phyB-associated photobodies formation enhanced by MeJA and *A. thaliana* endogenous JA line (*dgd1-1*) under high (H) R: FR and low (L) R: FR light. The *A. thaliana* seedlings of *PHYB-YFP/hfr1-201*, *PHYB-YFP/fin219-2 hfr1-201*, *PHYB-YFP/dgd1-1 hfr1-201* were grown under high R: FR light for 2 days and then transferred to high R: FR or low R: FR light for 4 days in the absence (Mock) or presence of MeJA treatments in the growth medium. Scale bar = 2 μm.

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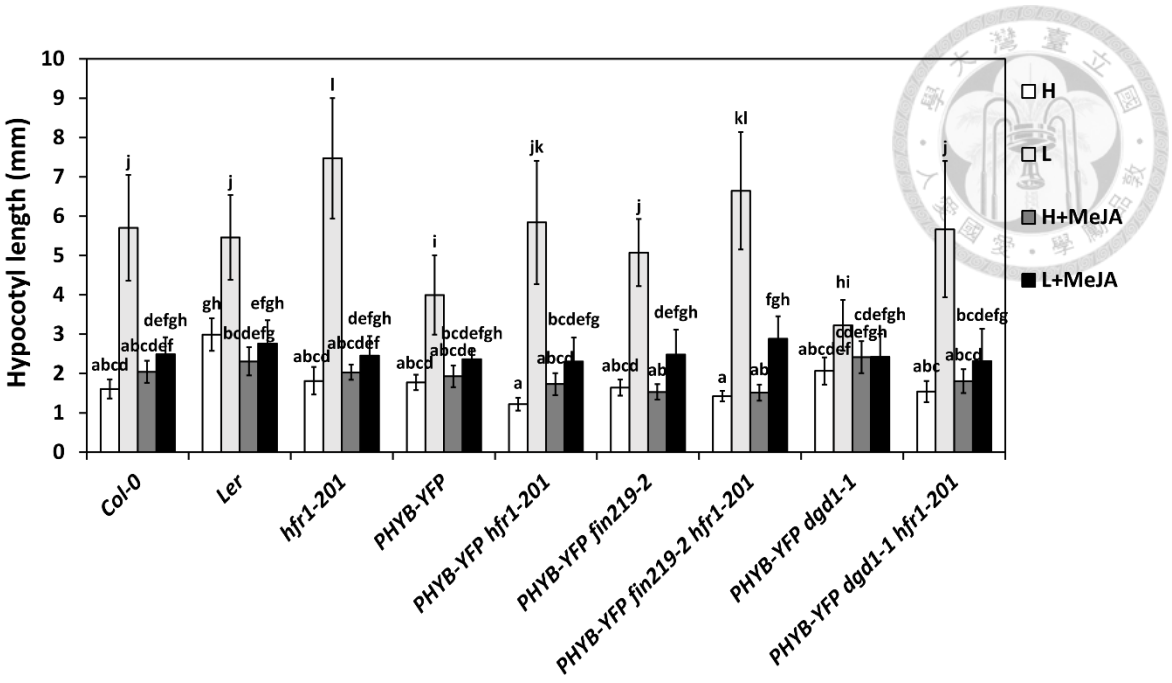

Figure 13. HFR1 shows a strong influence on hypocotyl length and is inhibited by MeJA under low R: FR light.

The *A. thaliana* seedlings of Col-0, L*er*, *hfr1-201*, *PHYB-YFP*, *PHYB-YFP/hfr1-201*, *PHYB-YFP/fin219-2*, *PHYB-YFP/fin219-2 hfr1-201*, *PHYB-YFP/dgd1-1*, and *PHYB-YFP/dgd1-1 hfr1-201* were grown under high (H) R: FR light for 2 days and then transferred to high R: FR or low (L) R: FR light for 4 days in the presence or absence of MeJA treatments in the growth medium. Quantification of hypocotyl lengths of the seedlings is presented as mean ± SE ($n \geq 25$). Different lowercase letters represent a statistically significant difference in hypocotyl lengths by one-way ANOVA, Tukey's HSD, $P < 0.05$.

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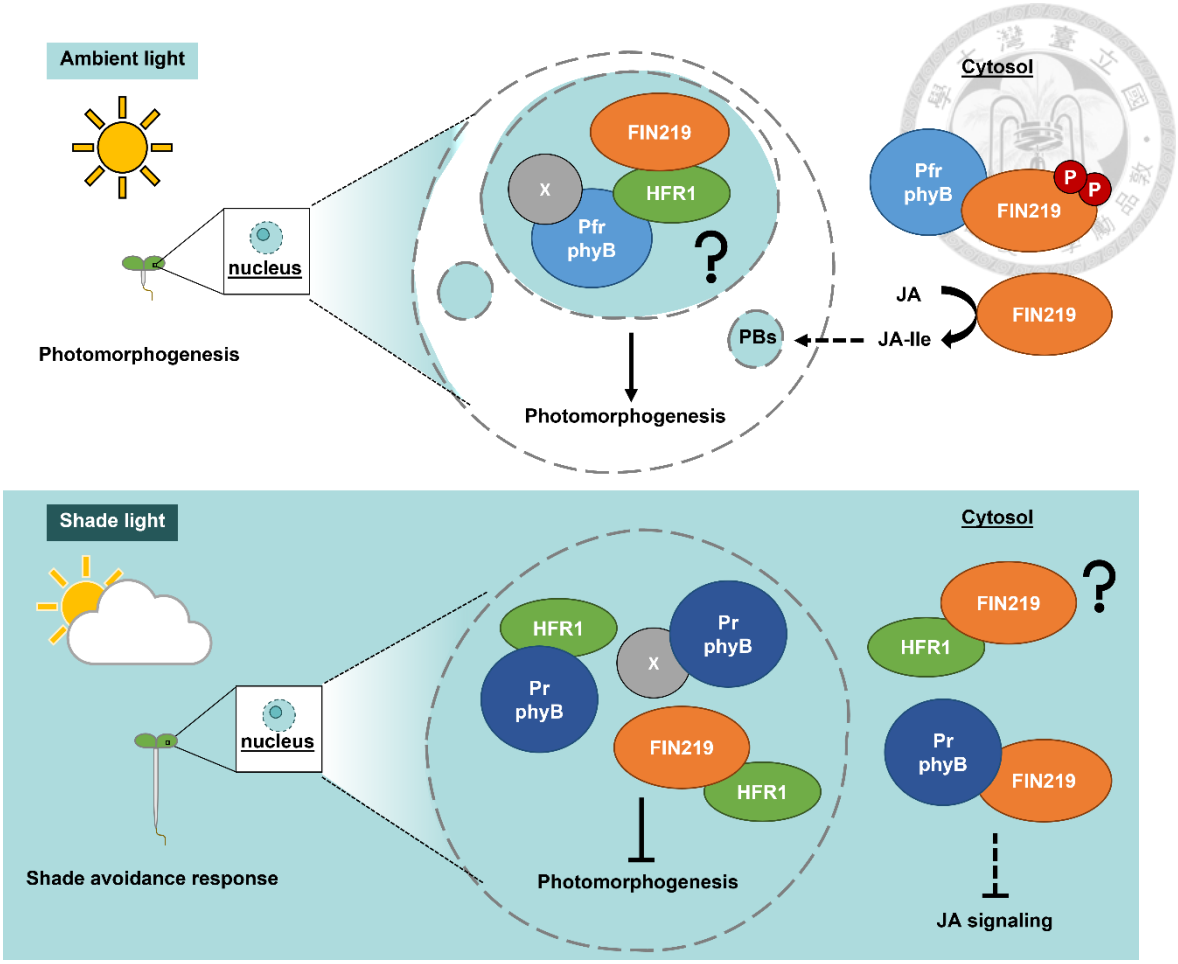

Figure 14. A model illustrates the relationship among HFR1, FIN219, and phyB under ambient and shade light conditions.

This study shows that HFR1 is involved in the shade avoidance pathway and regulates hypocotyl elongation with phyB and FIN219. HFR1 physically interacts with phyB and FIN219 under ambient light and shade conditions. HFR1 is also involved in phyB-associated photobodies (PBs) and changes the photobody pattern under high R: FR light. Combined with previous studies, photoactivated (Pfr) phyB interacts with phosphorylated FIN219 under ambient light, converts more JA-Ile through dephosphorylated FIN219, and then promotes the formation of PBs in the nucleus; HFR1 in the nucleus binds to phyB and FIN219, and HFR1 may play the role of a connector. It can also be inferred from other studies that other members (X) may be involved in the formation and regulation of phyB-associated PBs, leading to photomorphogenesis (top panel). In the

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shaded environment, phyB is inactive form (Pr) and binds to dephosphorylated FIN219 in the cytosol, thereby inhibiting the JA signaling pathway. HFR1 also interacts with FIN219 in the cytosol, but its role and impact remain unknown. HFR1 in the nucleus binds to phyB and FIN219 individually. At this time, phyB spreads in the nucleus and no PBs are formed, promoting the shade avoidance response (lower panel). In summary, this emphasizes the important role of HFR1 in phyB-mediated light signaling and JA signaling.

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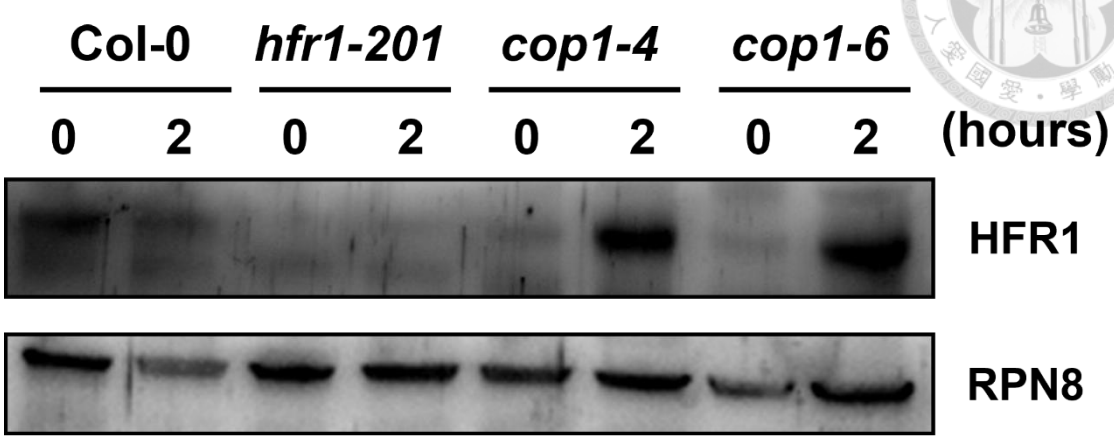
Supplemental Figures and Tables



Supplemental Figure 1. HFR1 is ubiquitinated and degraded via the E3 ubiquitin ligase COP1, particularly under low light conditions.

Immunoblot analysis of HFR1 proteins in *A. thaliana* seedlings of Col-0, *hfr1-201*, *cop1-4*, and *cop1-6* under the dark condition for 4 days, and then transferred to low far-red light condition (2 μmol m^{-2} s^{-1}) for 2 hours with 50 μM MG132 treatment. RPN8 serves as a loading control.

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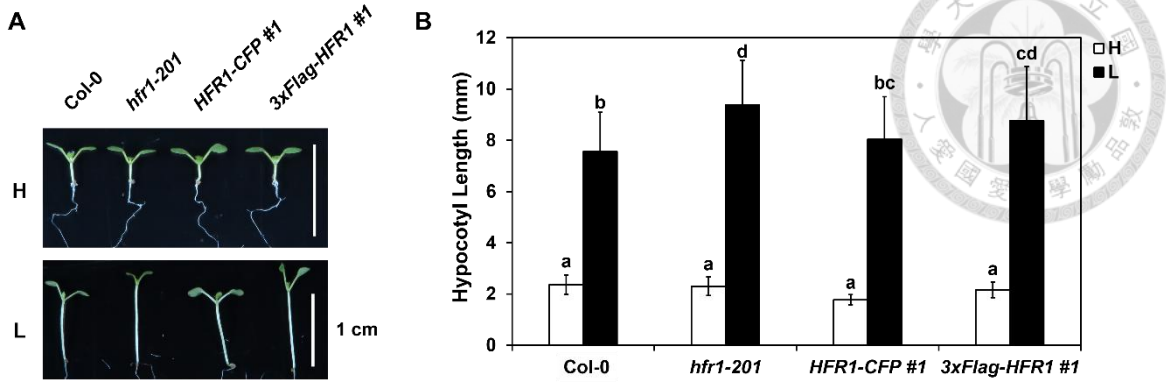

Supplemental Figure 2. HFR1 participates in the shade avoidance signaling pathway to positively regulate photomorphogenic development of seedlings.

A, The seedlings of Col-0, *hfr1-201*, *35Spro: HFR1-CFP/hfr1-201* (*HFR1-CFP#1*, *#2*) were grown under high R: FR light for 2 days and then transferred to high R: FR or low R: FR light for 4 days. Scale bar = 2 mm.

B, Quantification of hypocotyl lengths of the seedlings shown in (A). Data are presented as mean ± SE ($n \geq 25$). Different lowercase letters represent a statistically significant difference in hypocotyl lengths by one-way ANOVA, Tukey's HSD, $P < 0.05$.

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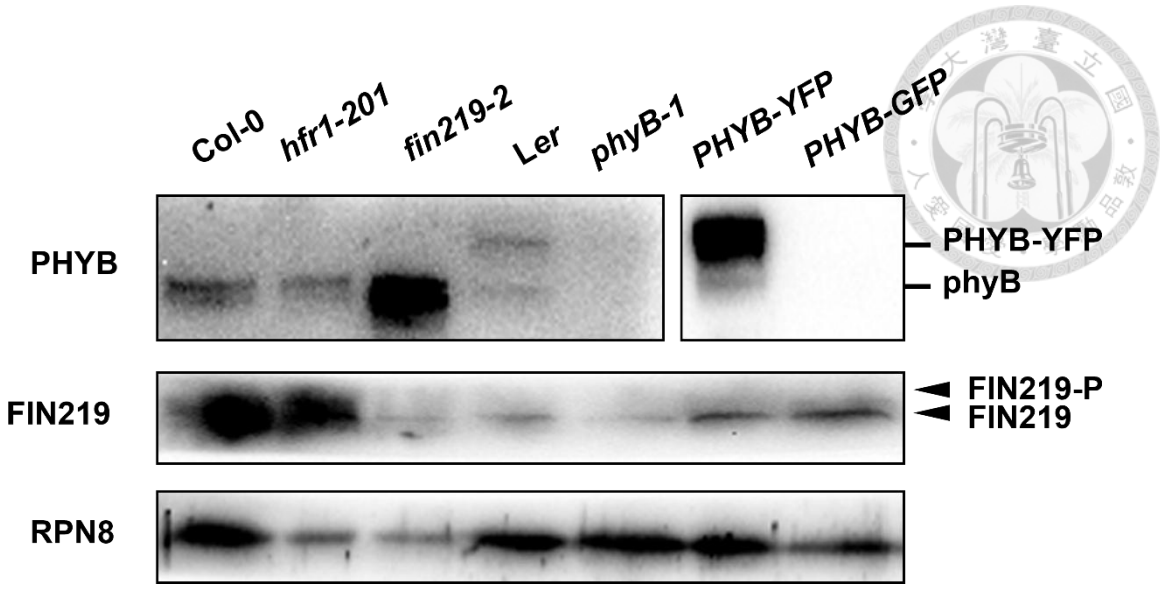

Supplemental Figure 3. HFR1 acts as a negative regulator to inhibit both FIN219 and phyB protein level under WL condition.

Immunoblot analysis of PHYB and FIN219 proteins in Col-0, L*er*, *hfr1-201*, *fin219-2*, *phyB-1*, *35Spro: PHYB-YFP/phyB-1*, and *35Spro: PHYB-GFP/phyB-1* plants under white light condition for 4 days. The upper to lower arrows indicate the phosphorylated and dephosphorylated forms of FIN219, respectively. RPN8 serves as a loading control.

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Supplemental Table 1. Genotyping Primers and PCR Conditions

Primer List

Gene (Accession No.)	Primer No.	Primer Name Sequence (5' to 3')
HFR1 (AT1G02340)	1553	HFR1 Fw AGAGGTCGTTGTCGTGAAG
	1554	HFR1 Rv TCAGCTTTGTCCGAGAAAATG
	1555	RB 86 TCGGGCCTAACTTTTGGTG
PHYB (AT2G18790)	2840	PHYB-cDNA-FP GATGATTCACCCTAAATCCTTCC
	2890	PHYB-1342-R-GT AGAAGCATATCACATAACAGTGTCTG
	2891	phyB-1-1342-R-GT AGAAGCATATCACATAACAGTGTCTA
FIN219 (AT2G46370)	809	LBb1.3 ATTTTGCCGATTTCGGAAC
	823	FIN219-F-SalI GCAGTCGACATGTTGGAGAAGGTTGAAACTTTC
	824	FIN219-R-nstop+1-BamHI GGCGGATCCAAAACGCTGTGCTGAAGTAGCT
DGD1 (AT3G11670)	2877	DGD1-GT-R-1690-3 TATGCACCTTTTGAGAAAGCTTG

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	2878	dgd1-1-GT-R-1690-3 TATGCACCTTTTGAGAAAGCTTA
	2885	DGD1-F-1 ATGGTAAAGGAAACTCTAATTCCT

PCR conditions include number of cycles, annealing temperature, and extension time.

Gene	**Cycles**	**Temperature (℃)**	**Extension Time (min)**
HFR1	35	60	1
PHYB	35	65	2.5
FIN219	35	60	1.5
DGD1	35	63	1.5

PCR Reaction

2X Master Mix	**(Total 10 μL)**
2X Mix Buffer	5
ddH_2O	3.5
Fw-Primer (10 μM)	0.5
Rv-Primer (10 μM)	0.5
Template DNA	0.5

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Supplemental Table 2. Constructs Used in This Study

Clone List

Clone No.	Clone Name	Strain	Resistance
HHL1807	HFR1/pEG201	DH5α	Kan
HHL1808	HFR1/pEG201	GV3101	Kan
HHL1813	HFR1/ pEG102	DH5α	Kan
HHL1814	HFR1/pEG102	GV3101	Kan
HHL1618	PHYB/pEG202	GV3101	Kan
HHL1600	FIN219/pEG201	GV3101	Kan
HHL1601	FIN219/pEG202	GV3101	Kan
HHL1821	pEarleygate 201	GV3101	Kan
HHL1822	pEarleygate 202	GV3101	Kan

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Supplemental Table 3. Sequences of Primers and RT-PCR Cycling Parameters

Gene	Accession No.	Primer No.	Primer Name	Sequence (5' to 3')
HFR1	AT1G02340	2857	HFR1-qPCR-F	TTCAGTTACTCGAAAAGGTTCCA
		2858	HFR1-qPCR-R	CGAAACCTTGTCCGTCTTG
UBQ10	AT4G05320	1379	UBQ10-FQ	TCCGGATCAGCAGAGGCTTA
		1380	UBQ10-RQ	TCAGAACTCTCCACCTCAAG

Number of cycles and annealing temperature used in RT-PCR are listed below.

Gene	Cycles	Temperature (℃)
HFR1	30	51.5
UBQ10	30	60

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Supplemental Table 4. Buffer Recipes and Preparation Conditions

The following table summarizes the buffer solutions used in various experiments conducted in this study, including their final concentrations, pH values, and preparation details.

Protein Extraction Buffer

Tris–HCl (pH 7.5)	50 mM
NaCl	150 mM
$MgCl_2$	10 mM
NP-40	0.1%
PMSF	1 mM
1X Protease Inhibitor	

Agro-infiltration Buffer (Total 50 mL, store at -20℃)

$MgCl_2$	10 mM
MES (pH 5.6)	10 mM
AS (Acetosyringone)	200 μM
ddH_2O	add to 50 mL

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