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

National Taiwan University

Master Thesis

在阿拉伯芥避蔭效應與茉莉酸訊息傳遞中 FIN219 與 HFR1 調控關係的功能性研究

Functional Studies of FIN219 and HFR1 Regulatory Relationship in the Integration of Shade Avoidance Syndrome and Jasmonate Signaling in *Arabidopsis*

郭蕙禎

Hui-Chen Kuo

指導教授: 謝旭亮 博士

Adviser: Hsu-Liang Hsieh, Ph.D.

中華民國 102 年 7 月

July, 2013

致謝

生命科學是一門相當奧妙又新奇的研究領域，看著植物們一天天的長大，總是讓我打從心底的讚嘆生命的偉大與複雜，但是在研究這些生理現象時，總是有許多難關要去克服。這兩年的碩士生涯對我而言相當的紮實，讓我學習到了很多，也得到了很多，這一路上有好多需要感謝的人，因為有你們，才得以讓我可以在這兩年中學習、思考、成長。首先真的很感謝謝旭亮老師願意讓我進入 919 這個大家庭之中學習，並且肯定我的努力，讓我這兩年的時光受益良多，也很感謝我的口委們：吳素幸老師、涂世隆老師、鄭萬興老師以及顏宏真老師，謝謝您們撥空幫我修改論文，並且給予我許多鼓勵與建議，讓我的論文有更多的進步。

謝謝 919 的大家，讓我可以開心的度過許多研究的時光，謝謝總是掛著微笑、永遠都很有耐心的懷如學姊，講話總是直率並且認真陪著我一起和 HFR1 奮鬥的正齡，碩二的生活中感謝有妳們的指導與陪伴，才不會感到艱辛，還有相處時間不長，但常常幫助我、指導我的孟淳，雖然常常很忙，但總是耐心教導我實驗的語安，不太常聊天，但很願意提供我實驗上協助的揚宗，常常來聊電影以及美食，讓我還有人生目標的漢威，常常說很麻煩，但帶我做實驗都很仔細的蕭蔚，講話都很簡短，但也很照顧我們的金龍以及總是帶來生氣的儷璘，謝謝有你們的關心與幫助，讓我可以快速的進入狀況，也謝謝 Swadhin 這一年來跟我一起合作，讓我學習到不少東西，也很感謝槿玟、翊郢、紫煥、思儀跟佑華，和你們這一年的相處讓我的碩二生涯不孤單，而新進來的貿允、佩吟、孟樺和彥合，雖然相處時間不長，但也很高興認識你們！最後，最感謝的是和我一起念書、做實驗、做報告、寫論文的好夥伴旻潔與建瑋，覺得能與你們一起進入這間實驗室，一起經歷許多大小事務，是我最大的福氣，謝謝你們總是挺我而且給我很大的鼓勵，沒有你們我完成不了這本論文。

很感謝九樓的學長姊與學弟妹對我的幫助，讓我能夠快速的完成實驗，謝謝葉老師家的小瑛，謝謝妳時常的鼓勵與安慰，讓我能夠有繼續奮鬥下去的勇氣，也感謝金輝，在我面臨瓶頸的時候適時的關心與伸出援手，讓我撐過難關，謝謝鄭家的各位把我當成了實驗室的一份子看待，讓我在不開心的時候可以得到許多開心的時光，感謝總是對我很好的玉梅，讓我徬徨的時候總有個好顧問可以諮詢！還有謝謝一百級的同學們，和你們一起度過了很多有趣的時光，希望大家都有美好的未來！在此特別感謝紅葳，謝謝你陪我度過許多崩潰時段，可以跟你分享許多大喜大憂，一起笑鬧真的讓我的碩士生涯有了許多值得回憶的地方！

除了植科所的大家，也謝謝一直支持我並陪伴著我的家人們，謝謝你們的關懷，讓我可以更專心的面對困難，也謝謝中研院的學長姊們，明豪、雅惠跟沈惠，謝謝你們在我想去看你們的時候都願意接見我！還有以前的同學及朋友們，謝謝你們在我傷心難過的時候給我支持與幫助，我真的很感謝你們。在此，我真誠的謝謝大家，豐富了我這兩年的碩士生涯。

蕙禎 2013.07.04

ABSTRACT

Light is one of the most effective environment factors that regulate plant growth and development. Different light sources have their own effects on seedlings. Shade-avoidance syndrome (SAS) is the phenotype that occurs at low red to far-red ratio, which will reduce the yield of crops. Although it has been noticed that jasmonates are involved in SAS, the molecular regulation between jasmonates and SAS are still unknown. *LONG HYPOCOTYL IN FAR-RED 1* (*HFR1*) is known as a negative regulator of SAS, and *FAR-RED INSENSITIVE 219* (*FIN219*) is reported to be a positive regulator in JA signaling. Both *HFR1* and *FIN219* participate in phyA signaling and they are required for SAS. Besides, COP1 has been reported to interact with FIN219 and HFR1, respectively. It is interesting to find the relationship between *HFR1* and *FIN219*, and their regulation during SAS and jasmonate (JA) signaling. Here, we found that *fin219hfr1* double mutant is insensitive to JA and has even longer hypocotyl phenotype than single mutants in shading treatment. Gene expression analyzer indicated that *HFR1* also participates in JA signaling and positively regulates *FIN219* expression, whereas *FIN219* participates in SAS signaling and negatively regulates *HFR1* expression. Protein expression studies showed the same regulatory patterns of FIN219 and HFR1. Moreover, co-immunoprecipitation studies indicated that FIN219 and HFR1 did not physically interact with each other, which implies that other regulators may be involved in this regulation mechanism. Taken together, our data indicate that FIN219 and HFR1 play vital roles in regulating seedling development in response to JA and shading conditions.

中文摘要

在植物的生長與發育過程中，光扮演著重要的調控角色，而不同波長的光有著不同的影響。在紅光遠比遠紅光低的情況之下，植物會處在類似遮蔭的環境下而產生下胚軸與節間徒長的性狀，如此的表現型被稱為避蔭效應 (Shade avoidance syndrome)。近來已經有許多植物荷爾蒙被發現參與在避蔭效應的調控，而在阿拉伯芥中茉莉酸也被證實會參與其中，但是避蔭效應與茉莉酸之間的交互作用與詳細的訊息傳遞機制卻仍然未知。*LONG HYPOCOTYL IN FAR-RED 1* (*HFR1*)已被發現在避蔭效應的訊息調控中扮演著負調控者的角色，而 *FAR-RED INSENSITIVE 219* (*FIN219*) 則在茉莉酸訊息調控中具有正調控者的功能。HFR1 和 FIN219 都已被證實參與 phyA 和避蔭效應的訊息調控之中，並且也分別被證實會與 COP1 進行交互作用；為瞭解它們在避蔭效應與茉莉酸訊息傳遞中的調控關係，我們觀察了雙突變株 *fin219hfr1* 幼苗，發現其對於茉莉酸的添加具有更不敏感的表現型，但是在低紅光/遠紅光比例下則有更顯著的長下胚軸表現型。在基因表現上顯示 *HFR1* 會參與在茉莉酸的訊息傳遞之中，並且會正調控 *FIN219* 的表現，而 *FIN219* 則會參與在避蔭效應的訊息調控中並負調控 *HFR1* 的表現。在蛋白質表現上，則可以發現 HFR1 會正調控 FIN219 而 FIN219 會負調控 HFR1 的表現，這和基因的表現趨勢相同。在共免疫沉澱檢驗中發現 FIN219 和 HFR1 不會有直接的交互作用，因此推測可能還有其他調控者參與在此調控機制之中。綜合研究的結果顯示 HFR1 以及 FIN219 在阿拉伯芥生長發育中，對於茉莉酸與遮蔭環境下有著重要的調控功能。

ABBREVIATION

Ler	Landsberg
JA	Jasmonic Acid
MeJA	Methyl Jasmonic Acid
SAS	Shade Avoidance Syndrome
FRc	Continuous Far-Red Light
Rc	Continuous Red Light
Bc	Continuous Blue Light
Wc	Continuous White Light
R: FR	Red Light: Far-Red Light Ratio
FIN219	*FAR-RED INSENSITIVE 219*
JAR1	*JASMONATE RESISTANT 1*
HFR1	*LONG HYPOCOTYL IN FAR-RED 1*
PIL1	*PIF3-LIKE 1*
PAR1	*PHYTOCHROME RAPIDLY REGULATED 1*
ATHB2	*ARABIDOPSIS THALIANA HOMEOBOX PROTEIN 2*
PIF4	*PHYTOCHROME INTERACTING FACTOR 4*
PIF5	*PHYTOCHROME INTERACTING FACTOR 5*
PIF7	*PHYTOCHROME INTERACTING FACTOR 7*
COI1	*CORONATINE INSENSITIVE 1*
JAZ1	*JASMONATE-ZIM-DOMAIN PROTEIN 1*
JIN1	*JASMONATE INSENSITIVE 1*
VSP1	*VEGETATIVE STORAGE PROTEIN 1*
LOX2	*LIPOXYGENASE 2*
AOS	*ALLENE OXIDE SYNTHASE*
OPR3	*12-OXO-PHYTODIENOIC ACID 10,11 REDUCTASE 3*
phyA	phytochrome A
phyB	phytochrome B
cry1	cryptochrome 1
cry2	cryptochrome 2

Table of Contents

INTRODUCTION 1
Light and Photoreceptors 1
Shade Avoidance Syndrome 2
Shade Avoidance Response and Photoreceptors 3
Shade Avoidance Response and Hormones 4
Jasmonates 6
JA Synthesis and Signaling Pathway 6
Negative Regulator in SAS Signaling: HFR1 8
Positive Regulator in JA Signaling: FIN219 9
The Crosstalk between SAS and JA Signaling 10
The Objective of Cross-talk Research 10
MATERIAL AND METHODS 12
Plant Materials 12
Plant Growth Conditions 12
Plasmid Construction 14
Protein Co-IP 14
Total Protein Isolation and Western Blotting 14
Total RNA Isolation and RT-PCR 15
Total Genomic DNA Isolation 15
Measurement of Hypocotyls and Roots 16
Observation of Anthers 16
Chlorophyll Extraction and Quantifications 16
Anthocyanin Extraction and Quantifications 17
RESULTS 18
FIN219 and *HFR1* synergistically regulate fertility and stamen elongation. 18

FIN219 and *HFR1* participated in JA-regulated responses of root elongation, anthocyanin synthesis and chlorophyll reduction. 18

FIN219 and *HFR1* are positive regulators of hypocotyls elongation in various light conditions except for red light. 19

FIN219 and *HFR1* are positive regulators in both FR light and JA signalings. 20

FIN219 and *HFR1* are negative regulators in low R: FR light condition. 21

HFR1 overexpression rescued the phenotypes of *hfr1* single mutant..... 22

Both *FIN219* and *HFR1* may regulate with each other in the dark or FR light..... 22

FIN219 and HFR1 affect the expression of JA biosynthesis genes in both the dark and FR light. 23

HFR1 and FIN219 proteins were induced by low R: FR treatment. 24

FIN219 and HFR1 participated in JA and SAS signaling. 25

phyA and *cry1* showed strong inhibition of hypocotyl elongation by methyl JA (MeJA). 26

FIN219 and HFR1 were regulated by photoreceptors in response to low R: FR condition. 27

HFR1 might not physically interact with FIN219 in shading condition..... 27

DISCUSSION..... 28

Phenotypic analysis showed that *fin219hfr1* was insensitive to MeJA. 28

fin219hfr1 was insensitive to MeJA and showed a longer hypocotyl than wild type under FR light and low R: FR condition..... 30

Both gene and protein expression indicated that *HFR1* induced the expression of *FIN219*, whereas *FIN219* repressed the expression of *HFR1*. 32

FIN219 and *HFR1* affect the components of JA biosynthesis and signaling. 33

PIL1, *ATHB2* and *PIF4* were induced in *hfr1-201*, but reduced in *fin219-2* under shading condition. 34

JA serves as a negative role in SAS signaling and JA signaling genes may be blocked in shading condition.. 35

Photoreceptors phyA, phyB, cry1 and cry2 all regulated the expression of *FIN219* and *HFR1*. 36

HFR1 might not physically interact with FIN219 in shading condition..... 37

FIGURES 39

Figure 1. *FIN219* and *HFR1* synergistically regulate fertility and stamen elongation.....39

Figure 2. *FIN219* and *HFR1* participated in JA-regulated responses of root elongation.....40

Figure 3. *FIN219* and *HFR1* participated in JA-regulated responses of anthocyanin synthesis. 41

Figure 4. *FIN219* and *HFR1* participated in JA-regulated responses of seedling chlorophyll reduction.....42

Figure 5. *FIN219* and *HFR1* are positive regulators of hypocotyls elongation in various light conditions except for red light.....43

Figure 6. *FIN219* and *HFR1* are positive regulators in both FR light and JA signalings.....44

Figure 7. *FIN219* and *HFR1* are negative regulators in low R: FR light condition....45

Figure 8. Both *FIN219* and *HFR1* may regulate with each other in the dark or FR light .....,,,,,,.....46

Figure 9. *HFR1* is affected by JA and impact the downstream genes.....47

Figure 10. FIN219 and HFR1 affect the expression of JA biosynthesis genes in both the dark and FR light 48

Figure 11. HFR1 and FIN219 proteins were induced by low R: FR treatment.....49

Figure 12. FIN219 modulates the expression of the shading components.....50

Figure 13. FIN219 and HFR1 participated in JA and SAS signaling.....51

Figure 14. FIN219 and HFR1 affect the expression of shading components in low R: FR condition.....52

Figure 15. The JA biosynthetic genes were still induced by MeJA under shading treatment, but the JA signaling components were decreased.....53

Figure 16. *phyA* showed strong inhibition of hypocotyl elongation by MeJA. 54

Figure 17. *cry1* showed strong inhibition of hypocotyl elongation by MeJA.....55

Figure 18. FIN219 and HFR1 were regulated by photoreceptors in response to low R: FR condition.....56

Figure 19. *HFR1* overexpression rescued the phenotypes of *hfr1*.....57

Figure 20. HFR1 might not physically interact with FIN219 in shading condition....58

Figure 21. Proposed model of the integration of JA and SAS signaling through the

regulation between FIN219 and HFR1.....59
REFERENCES 60
APPENDIX I 67
APPENDIX II..... 69
APPENDIX III 70
APPENDIX IV 71
APPENDIX V 72

INTRODUCTION

Light and Photoreceptors

Plant growth and development is regulated by many environmental factors. Among them, light is the most effective one. Light affects many aspects such as germination, de-etiolation, flowering time, and chloroplast movement (Sullivan and Deng, 2003). Plants sense light by light sensors, photoreceptors, which promote the light responses. There are at least four different types of photoreceptors found in *Arabidopsis,* including phytochromes, cryptochromes, phototropins, and zeitlupes (Lariguet and Dunand, 2005; Bae and Choi, 2008; Rizzini *et al.*, 2011). The phytochrome family of photoreceptors in *Arabidopsis* genome consists of five members, phyA to phyE. By sensing and reacting to red (R) and far-red (FR) light, they regulate a variety of developmental processes in response to light (Franklin and Quail, 2010). In particular, phytochrome A (phyA), which belongs to Type I phytochrome and is photo-labile, principally regulates far-red light signaling; however, phytochrome B (phyB), one of Type II phytochromes and relatively stable in the light, is the main photoreceptor in red light signal transduction (Chen *et al.*, 2004). In *Arabidopsis,* blue (B) light-mediated inhibition of hypocotyl growth is regulated by two cryptochromes cryptochrome 1 (cry1) and cry2, which differ in light lability and fluence rate specificity (Ahmad *et al.*, 1995; Lin *et al.*, 1998). At higher photon irradiances of blue light, cry1 function predominately in the inhibition of hypocotyl growth. Under these conditions, cry2 displays considerable light lability (Lin *et al.*, 1998). At lower photon irradiances, cry2 displays greater stability and performs a significant role in the inhibition of hypocotyl growth, thereby enhancing blue light sensitivity in light-limiting conditions (Lin *et al.*, 1998).

Shade Avoidance Syndrome

As the global population arises, how to increase the crop yields is the most important issue that we need to face. Photomorphogenesis is important in agriculture where maximizing yield is of paramount importance. However, shade-avoidance syndrome (SAS) will occur if the crops we plant are too crowded thus sacrificing the yield. Although shade avoidance responses in crops have been greatly reduced through intensive selection, they still limit the number of plants per square meter that can be sown (Devlin *et al.*, 2007). It is important to understand the regulation of SAS signaling, which may lead to an induction of economic yields.

SAS occurs as the light sources decrease and the light qualities change. R, FR and B light are the most effective light sources for plants, and sensed by phytochromes and cryptochromes, respectively. In shading condition, the relative levels of R and B light are decreased whereas FR light is increased. It is because the wavelength of FR light is lower than other visible light sources and can penetrate through the canopy more than other visible light sources. As a matter of fact, it is likely that a simultaneous occurrence of low R: FR and low B light is used to evaluate actual shade (Keuskamp *et al.*, 2010). The SAS encompasses various phenotypic traits, including elongation of internodes and hypocotyls, apical dominance, early flowering and upward leaf movement. These growth and developmental responses help plants to outgrow shade imposed by neighbors, thus allowing them to position the young leaves in the upper, better lit parts of the vegetation (Keuskamp *et al.*, 2010). As these phenotypes, many hormones have been proved that are involved in SAS, such as auxin, cytokinin, gibberellins, ethylene and brassinosteroid (Carabelli *et al.*, 2007; Pierik *et al.*, 2004; Kozuka *et al.*, 2010; Stamm *et al.*, 2010). Jasmonates (JA) are also required for SAS, it has been shown that they required phyA for full sensitivity to growth inhibition, and that phyA and

jasmonate signaling are integrated at the level of protein degradation of a target of the COI1 E3 ligase, the JAZ1 (Robson *et al.*, 2010). Even though it has been noticed that jasmonate is involved in SAS, the regulation and signaling pathway between jasmonate and SAS are still unknown.

Shade Avoidance Response and Photoreceptors

A light signal is a change in the light environment that can be perceived by plant photoreceptors. During the regulation of shade avoidance response, the regulation mechanism is a complex process that includes hormones and transcription factors. The shade avoidance response is firstly detected by the light sensors, photoreceptors, which are activated by different light sources. Photoreceptors that are involved in perceiving differences between casual light and shade light include phytochromes, cryptochromes, and phototropins. The phytochromes, including phyA and phyB, sense the R and FR; the cryptochromes, including cry1 and cry2, sense the B light; the phototropins, including phototropin 1 (phot1) and phot2, participate in phototropic responses mainly mediated by B light (Gommers *et al.*, 2013). phyA is the photoreceptor for FR, whereas phyB is the photoreceptor for R. As for cry1 and cry2, cry1 plays a major role in sensing B light. phot1 and phot2 are also involved in B light sensing.

As previously described the ratio of R and B is low in shade condition, whereas the ratio of FR is increased, which suggests the involvement of phyA, phyB, cry1, cry2, phot1 and phot2 photoreceptors. In low R: FR ratio of shade condition, the major sensor is phyB, whereas phyA and cry1 are also involved in the regulation of this process (Casal, 2012). Besides, in low B: G (green) ratio, it has been noticed that cry1 plays a major sensor in the regulation of related signaling (Sellaro *et al.*, 2010). After the perception of changes of the environments, the regulatory cascade of shade avoidance

has been initiated. PHYTOCHROME INTERACTING FACTOR (PIF) protein family plays an important role in the regulation of the SAS signal transduction. PIFs belong to a superfamily of basic helix-loop-helix (bHLH) transcription factors. It has been noticed that PIF1, PIF3, PIF4, PIF5, PIF6, PIF7 and PIF8 proteins interact physically with phyB through their conserved N-terminal domain (Leivar *et al.*, 2012). Besides, PIF1 and PIF3 also interact with phyA (Leivar and Quail, 2011). As a matter of fact, shade avoidance responses in low R: FR condition require PIF3, PIF4, PIF5 and PIF7 to regulate the expression of downstream regulators (Leivar *et al.*, 2012; Li *et al.*, 2012; Lorrain *et al.*, 2008). The PIF proteins were known to interact with DELLA proteins, which are the negative regulators of gibberellin signaling (Feng *et al.*, 2008). This also suggests that phytohormones are also involved in shade avoidance response see before. Meanwhile, shade avoidance responses require COP1, which is an E3 ligase that mediates the target proteins for degradation. Even though the regulatory mechanism of COP1 involved in shade avoidance is still unknown, an earlier study suggests that COP1 could be required for PIFs accumulation in shade avoidance response (Bauer *et al.*, 2004).

Shade Avoidance Response and Hormones

In the few decades, the regulation mechanisms of hormones and their interactions have been extensively studied. The interactions between shade avoidance and hormones have been implicated. Almost all the plant hormones have been proposed to be involved in shade avoidance responses (Casal, 2013). The interactions between auxin, gibberellins, cytokinins, ethylene and SAS signaling have been better clarified these days. It has been shown that in low R: FR condition, the auxin signaling can be regulated by two ways. First, the efflux carriers *PIN3* and *PIN7* have been shown that in

low R: FR ratio they are expressed steadily, which would enhance the hypocotyl elongation (Devlin *et al.*, 1998). On the other hand, auxin responsive gene such as *IAA19* appears in the petioles and hypocotyls. Besides, auxin can promote the degradation of DELLA proteins that are key negative regulators of gibberellin signaling. Moreover, gibberellins can regulate shade avoidance response. DELLA proteins are involved in different hormone signaling pathways and could be degraded by ubiquitins/26S proteasome-mediated protein degradation system. The involvement of gibberellins in shade avoidance response indicated that the gibberellin biosynthetic gene, *GA20ox*, in low R: FR condition was induced (Devlin *et al.*, 2003). On the other hand, RGL3 DELLA protein was shown to play a main role in shade-induced elongation growth.

Cytokinins also play a role in shade avoidance response, which breaks down the cell division to reduce the shade response. CKX6, a cytokinin oxidase, has been shown to be induced in shade condition, leading to the inhibition of the development of leaf primodia (Carabelli *et al.*, 2007). TIR1, an auxin receptor, promotes CKX6 expression, which suggests that cytokinins crosstalk with auxin. Except for these three hormones described above, ethylene is also involved in shade avoidance response. Pierik *et al.* (2009) proposed that auxin might be a downstream regulator of ethylene-induced responses. Recent data indicated that auxin signaling mutants are insensitive to ethylene. Moreover, ethylene was reported to occur in the shade avoidance response independently of gibberellins. In summary, the interactions between shade avoidance responses and hormones are complicated and involve many hormones and transcription factors, and the details of the regulation are still unclear.

Jasmonates

Jasmonates form a family of oxylipins arising from the enzymatic oxygenation of 18 and 16-carbon triunsaturated fatty acids (Wasternack and Kombrink, 2010). The best-known jasmonates are jasmonic acid (JA), methyl jasmonate (MeJA) and jasmonoyl-isoleucine (JA-Ile). Jasmonates are essential to complete the last steps of stamen development such as pollen maturation, elongation of stamen filaments and dehiscence of anther locules for pollen release. Impairment of these functions in jasmonate synthesis or perception mutants results in male sterility (Stintzi and Browse, 2000). Jasmonates also regulate the synthesis of anthocyanin (Chen *et al.*, 2007) and the reduction of chlorophyll (Okamoto *et al.*, 2009). MeJA is a fragrant volatile compound initially identified from flowers of *Jasminum grandiflorum*, and has proven to be ubiquitously present in the plant kingdom. The volatile nature of MeJA led to the discovery of its role as a signal in plant cellular responses, plant–herbivore interactions and plant–plant interactions (Cheong and Choi, 2003). Some of the SAS phenotypes are regulated by conjugated JA, so it is worthwhile to analyze the regulatory mechanisms between jasmonates and SAS signaling.

JA Synthesis and Signaling Pathway

JA levels rise steeply in response to insect herbivore damage and trigger the production of many proteins involved in plant defenses. In plants, JA is synthesized from linolenic acid, which is the substrate of a chloroplast-located 13-lipoxygenase (13-LOX). Among the six *LOX* genes in *Arabidopsis*, the 13-LOX encoded by *LOX2* seems to be involved in JA biosynthesis. Transgenic approaches revealed that LOX2 is responsible for at least the wound-induced JA biosynthesis (Bell *et al.*, 1995). After 13-LOX catalyzed oxygen insertion at carbon 13 of the carbon backbone, linolenic aicd

became (13S)-hydroperoxyoctadecatrienoic acid (13-HPOT). And 13-HPOT would become (9S,13S)-12-oxo-phytodienoic acid which is converted by an allene oxide synthase (AOS). The AOS contains a chloroplast target sequence and is encoded by a single copy gene in *Arabidopsis*. Therefore, AOS is regarded as a step in the regulation of JA biosynthesis (Laudert and Weiler, 1998). The highly unstable AOS product rapidly decays into ketols and racemic OPDA or is enzymatically converted by an allene oxide cyclase (AOC) to *cis*-(+)-OPDA. After cis-(+)-OPDA transfers into peroxisome, it is catalyzed by OPDA reductase (OPR) encoded by a small gene family in *Arabidopsis*. However, only OPR3 carries a peroxisomal target sequence and acts specifically with *cis*-(+)-OPDA (Strassner *et al.*, 2002). After OPR3 and three cycles of β-oxidation, JA was produced.

As the JA is formed as JA-isoleucine (JA-Ile), it triggers the downstream signaling pathway of JA responses. *CORONATINE INSENSITIVE 1* (*COI1*) interacts with SKP1 and cullin proteins to form an E3 ligase SCF^{COI1} complex, which targets JASMONATE ZIM-DOMAIN (JAZ) repressor proteins in response to JA-Ile via 26S proteasome. Without JA-Ile stimulation, JAZ proteins constitutively interact with MYC2/JIN1 (JASMONATE INSENSITIVE 1) and repress MYC2 activity (Chini *et al.*, 2007; Thines *et al.*, 2007). JAZ proteins belong to the large family of the TIFY proteins (Vanholme *et al*., 2007) and have twelve members that are induced at the transcript level by JAs. It has been noticed that each member might play a different role in the JA responses (Staswick, 2008; Shyu *et al.*, 2012). MYC2 is a Z-box and G-box binding transcription factor responsible for JA-induced gene expression such as *VEGETATIVE STORAGE PROTEIN 1* (*VSP1*) and *PLANT DEFENSIN 1.2* (*PDF1.2*) (Sheard *et al*., 2010). *VSP1* is involved in insect defence, whereas *PDF1.2* is involved in pathogen defense (Kazan and Manners, 2011). As JA-Ile triggers the JA signaling, it induces the

COI1 expression, leading to the ubiquitination and the degradation of JAZ proteins. After JAZ proteins are degraded, MYC2 would be activated and trigger the expression of downstream targets, *VSP1* and *PDF1.2*, which leads to the JA responses.

Negative Regulator in SAS Signaling: HFR1

The regulation of shade avoidance syndrome is a complex mechanism in which many regulators and hormones are involved. phyB has been shown to be the key player in shade avoidance (Reed *et al.*, 1993). On the other hand, phyA, a major factor regulating de-etiolation of seedlings in FR light, appears to also modulate the shade avoidance response in light-grown seedlings (Johnson *et al.*, 1994). When the R: FR ratio is reduced, the active phytochrome (Pfr) would change to the inactive Pr, which prevents the degradation of the bHLH transcription factors, PIFs. Shade-induced inactivation of phyB derepresses PIF-regulated gene expression (Ni *et al.*, 1999) and leads to GA-mediated DELLA degradation, which further increases the PIF protein pools and leads to the SAS phenotype (Djakovic-Petrovic *et al.*, 2007). In PIF family, PIF4 and PIF5 have been shown to be positive regulators of shade avoidance responses, participating in the regulation of some key players in these responses, including ARABIDOPSIS THALIANA HOMEOBOX PROTEIN 2 (ATHB2), a homeodomain leucine-zipper (HD-Zip) protein with a positive role, PHYTOCHROME RAPIDLY REGULATED 1 (PAR1), a basic helix–loop–helix (bHLH) transcription factor with a negative regulatory role in shade avoidance, and PIF3-LIKE1 (PIL1), another bHLH transcription factor positively regulating shade avoidance (Salter *et al.*, 2003; Roig-Villanova *et al.*, 2006; Lorrain *et al.*, 2008). More recently, PIF7 has also been reported to be involved in shade avoidance response, which plays a positive role in inducing auxin biosynthesis and subsequently an enhanced SAS (Li *et al.*, 2012).

LONG HYPOCOTYL IN FAR-RED 1 (*HFR1*) was originally identified as a positive regulator of phyA signaling, which might act downstream of COP1 in a separate pathway from HY5 to mediate photomorphogenesis in *Arabidopsis* (Kim *et al.*, 2002). It encodes an atypical bHLH transcription factor. The loss-of-function of *hfr1* mutant is defective in a subset of phyA-mediated FR light responses, including inhibition of hypocotyl elongation, and suppression of hypocotyl negative gravitropism (Fairchild *et al.*, 2000; Fankhauser and Chory, 2000; Soh *et al.*, 2000). In addition to its role in phyA signaling, HFR1 is also a component of cryptochrome 1 (cry1)-mediated blue light signaling, which indicates that HFR1 integrates light signals between both phyA and cry1 (Duek and Fankhauser, 2003). Furthermore, *HFR1* is known as a negative regulator of SAS, by negatively regulating downstream signaling components initiated by the same signal such as *PIL1*, *PAR1* and *ATHB2,* thus inhibits the SAS (Sessa *et al.*, 2005;), and it also inhibited the expression of *SAUR15* and *SAUR 68*, which would reduce the levels of auxin (Roig-Villanova *et al.*, 2007). Besides, HFR1 is phosphorylated by casein kinase II (CKII), a process leading to the reduction of HFR1 degradation (Park *et al.*, 2008).

Positive Regulator in JA Signaling: FIN219

FAR-RED INSENSITIVE 219 (*FIN219*), a positive component in phyA-mediated signaling pathway, was first discovered as a suppressor of *cop1-6* and *fin219* mutant is insensitive to FR light-mediated inhibition of hypocotyl elongation (Hsieh *et al.*, 2000). Further studies indicated that *JASMONATE RESISTANT 1* (*JAR1*) is allelic to *FIN219.* JAR1 is a jasmonate-amido synthetase responsible for conjugation of jasmonic acid to isoleucine to form JA-Ile (Staswick and Tiryaki, 2004). JA-Ile [(+)-7-iso-jasmonoyl-L-isoleucine] is currently considered to be one of the active forms

of jasmonates. The *jar1-1* and *fin219* are both insensitive to FR light and MeJA treatment (Staswick *et al.*, 2002; Chen *et al.*, 2007). These studies indicated that *FIN219/JAR1* might play a main role in the integration of light and JA signaling. Thus, it is possible that FIN219 regulates the interaction between SAS and JA signaling.

The Crosstalk between SAS and JA Signaling

It has been shown that HFR1 is ubiquitinated by COP1 E3 ligase, leading to protein degradation during photomorphogenesis, and light enhances HFR1 protein accumulation by abrogating COP1-mediated degradation of HFR1 (Yang *et al*., 2005; Jang *et al*., 2005). FIN219 was shown to interact with COP1 and regulated COP1 protein allocation between the cytoplasm and the nucleus (Wang *et al*., 2011). Besides, both HFR1 and FIN219 participate in phyA signaling and are required for SAS. Thus, it is interesting to find out the regulatory relationship between HFR1 and FIN219 during SAS and JA signaling.

The Objective of Cross-talk Research

In this work, we analyzed the regulation between HFR1 and FIN219 in both shading and JA signaling pathways. To investigate the regulation mechanism, we analyzed the phenotypic and molecular differences among *fin219-2*, *hfr1-201* and their double mutant. In phenotypic analysis, we found that the *fin219hfr1* double mutant showed an insensitive phenotype in both shading and JA treatments. Moreover, in gene expression pattern, *HFR*1 was involved in JA signaling and positively regulated *FIN219* expression, whereas *FIN219* negatively regulated *HFR1* expression. It also showed the similar patterns in protein expression analysis. Thus, FIN219 and HFR1 regulate each other to modulate seedling development in response to the integration of

SAS and JA signaling. In the mutant lines of the photoreceptors, we found that both FIN219 and HFR1 are regulated by not only phyA but also phyB and cry1, which indicate that they might be involved in the red light and blue light signaling regulations. In the co-immunoprecipitation, we found that FIN219 might not interact with HFR1 in shading condition, which suggests that there might be other regulators involved in this regulation.

MATERIAL AND METHODS

Plant Materials

The *phyA-201* (CS6219), *phyA-211* (CS6223), *phyB-1* (CS6211), *phyA-201phyB-5* (CS6224), *cry1-304* (Ahmad and Cashmore, 1993), *cry2-1* (Guo *et al.*, 1999), *cry1-304cry2-1* (Mockler *et al.*, 1999) and *fin219-2* (Salk_059774) mutants were derived from Arabidopsis Biological Research Center (ABRC). Theses T-DNA insertion mutants were generated in different ecotype, *phyA-211*, *cry1-304*, *cry2-1*, *cry1-304cry2-1* and *fin219-2* are in the Columbia-0 ecotype, *phyA-201*, *phyB-1*, *phyA-201phyB-5* are in the Lancberg ecotype. *hfr1-201* mutant was derived from Dr. Jun-Yi Yang, *hfr1* was confirmed to have a T-DNA insertion at the promoter of *HFR1* gene. *HFR1* wild-type allele was identified using the primers 5-AGAGGTCGTTGTCGTGAAG-3' and 5'-TCAGCTTTGTCCGAGAAAATG -3'; *hfr1* mutant allele was identified using the latter primer and pD991-RB86, 5'-TCGGGCCTAACTTTTGGTG -3'. The gene loci are At1g02340.1 for *HFR1* and At2g46370 for *FIN219*. In transgenic plants, *fin219hfr1* was obtained by crossing *hfr1-201* with *fin219-2*.

Plant Growth Conditions

For axenic growth, seeds were surface sterilized by incubating in 30% bleach/0.1% triton-X 100 for 7 min, followed by 4 washes with sterilized deionized water. For the measurement of primary root length, sterilized seeds were sown on 0.5% agar plates containing full-strength MS salts, 0.5 g/L MES buffer and 10 g/L sucrose. All seeds on plates were stratified in the dark at 4℃ for 3 days to synchronize germination. These seeds then were moved to continuous white light (Wc) at 22℃ and cultured for 7 days on the media without MeJA. After that, these seedlings were

transferred by tweezers to the media with 1, 5 or 10 μM MeJA and grown for 7 days before root length measurement. For the analysis of JA responses, seeds were scattered on the same media with specific amount of MeJA added to reach proper concentration when media cooled.

For the measurement of hypocotyl length, seeds sterilized as above were sown on the same media except for the amount of sucrose. The media used here contained 3 g/L sucrose. Following synchronization described before, these seeds were transferred to Wc for 10 h. Then, these seeds were exposed to continuous far red, red, or blue light for 3 days before measurement. Control plates were kept in the dark at 22℃. As shading condition, seeds were exposed to high R: FR ratio (R: FR 0.6) with W light 16 hours per day for 3 days and transferred to low R: FR ratio (R: FR 0.05) with W light 16 hours per day for 4 days at 22℃ before measurement. Control plates were kept in the high R: FR ratio with W light 16 hours per day for 7 days.

For gene expression and protein analysis, seeds were prepared as the process of hypocotyl length measurement. After specific light treatment, the seedlings were collected in dim green light and flash-frozen by liquid nitrogen.

The growth chambers were maintained at 22℃ during experiment. Single wavelength LED lights were used as light source. In our experiments, the light intensity of far-red light is 1.698 μmol m^{-2} s^{-1}; blue light is 0.947 μmol m^{-2} s^{-1}; red light is 2.757 μmol m^{-2} s^{-1}; white light is 16.641 μmol m^{-2} s^{-1}. Proper amount of 90 mm filter papers (Whatman) were used to adjust the light intensity. As shading condition, the light intensity of white light is 21.355 μmol m^{-2} s^{-1}; red light is 1.159 μmol m^{-2} s^{-1}; far-red light in high R: FR ratio is 2.01 μmol m^{-2} s^{-1}, in low R: FR ratio is 23.77 μmol m^{-2} s^{-1}.

Plasmid Construction

The CDS fragment of *HFR1* was produced by primers 5'-TCCGGATCCATGTCGAATAATCAAG-3' and 5'-GGACTCGAGTCATAGTCTTCTCATC-3' with proofreading KAPA HiFi^{TM} DNA polymerase (KAPA BIOSYSTEMS). First, the *HFR1* CDS fragment was ligated into yT&A and the products were transformed into DH5α *E. coli* strain. After sequencing, the *HFR1*/yT&A plasmid was purified and digested by *BamH*I. The CDS fragment digested with *BamH*I was further cloned into pRTL2 with 3 Flag tag. After selection of the desired *E. coli* colony, HFR1/ pRTL2 plasmid was digested by *Hind*III. The CDS fragment digested with *Hind*III was further cloned into pCAMBIA1390 plasmid, which was processed by Alkaline Phosphatase, Calf Intestinal (CIP) (NEB) to remove phosphate. The transgene was under the control of 35S promoter, and the selectable marker for plants was Hygrobacteria herbicide–resistance.

Protein Co-IP

Protein co-IP was performed as described (Staub *et al.*, 1996). Protein G Mag Sepharose Xtra(GE healthcare) was coupled to FIN219 antibody and then incubated with 2 mg protein extract from SAS-treated *fin219-2, hfr1-201,* and wild-type Arabidopsis seedlings. Pellets were analyzed on standard SDS-PAGE and subsequent western blotting.

Total Protein Isolation and Western Blotting

The collected samples were ground into fine powder before extraction. For 200 mg powder, it was suspended in 200 μl protein extraction buffer [50 mM Tris (pH 7.5); 150 mM NaCl; 10 mM $MgCl_2$; 0.1% NP-40; 1 mM PMSF; 1x Protease inhibitor] and

clarified by centrifugation. The protein concentration of the supernatant was determined by using Bradford reagent (Bio-Rad Protein Assay, Bio-Rad, Munich, Germany). 80-100 μg of total protein was separated by SDS-PAGE and transferred onto PVDF membranes. After blotting, FIN219 and HFR1 protein were first detected by specific antibodies FIN219 or HFR1with 5000 times dilution. After washing off the first detected signal, the membranes were then detected by the secondary antibodies with 5000 times dilution. Fujifilm LAS 3000 was used to visualize chemiluminescene signal.

Total RNA Isolation and RT-PCR

The RNA extraction procedures were followed according to Molecular Cloning manual (see Appendix I), and DNA contamination in samples were eliminated by using TURBO™ DNase (ABI). One microgram of RNA was converted to the first-strand cDNA pool by adding oligo-dT primer, RNasin (Promega), and reverse transcriptase (Applied Biosystems). After reverse transcription, 1μl aliquot of 2-fold diluted cDNA was used in RT-PCR for gene expression analysis. Gene specific primers used in the experiment were listed in Appendix II. The cycles and annealing temperature in RT-PCR for each gene were listed in Appendix III.

Total Genomic DNA Isolation

One true leaf about 0.5 mm diameter was collected and frozen in liquid nitrogen. After the sample was ground into powder, it was added with 200 μl DNA extraction buffer [0.2 M Tris-HCl (pH 9.0); 0.4 M LiCl; 25 mM EDTA; 1% SDS] and mixed well. Then, by centrifuging at 13000 rpm, 4℃ for 10 min, 180 μl supernatant was transferred into new eppendorf tubes and added with 180 μl isopropanol and mixed by inversion. DNA precipitation was achieved at -20℃ for more than 30 min. After that,

the sample was centrifuged at 13000 rpm, 4℃ for 10 min and the supernatant was removed completely. In the end, the DNA pellet in eppendorf was resuspended in proper amount of MQ (15 to 25 μL). For the following PCR reaction, 100 ng of total genomic DNA was taken for each PCR reaction.

Measurement of Hypocotyls and Roots

For hypocotyl measurement, seedlings were placed flat on agar plates. Seedlings and roots were taken into image and measured by using the ImageJ software (NIH).

Observation of Anthers

For anthers observation, buds corresponding to stage 12 in floral development were placed at black agar plates. The buds on 12 inflorescences were staged to the method of Smyth *et al.* (1990).

Chlorophyll Extraction and Quantifications

Seeds sterilized as above were sown on the same media as hypocotyl measurement. Following synchronization described before, these seeds were transferred to Wc for 16 h. Then, these seeds were exposed to low or high light intensity FRc light for 3 days and transferred to Wc light for 2 days before measurement. Control plates were kept in the dark for 3 days and transferred to Wc light for 2 days at 22℃. Harvested seedlings were frozen by liquid nitrogen. After grinding into powder, chlorophyll was extracted in 500 μL dimethylformadimide (DMF) at 4℃ overnight with adequate mixing. 50 μL of extracts were pipette into 450 μL absolute alcohol (Sigma), mixed and then 200 μL of 10% DMF (in EtOH) were taken for spectrophotometric analysis. Absorbance at 750, 664, and 647 nm was

measured in Tecan Infinite 200 PRO (Tecan) with plastic 96-well plate. Total chlorophyll content was determined according to the extinction coefficients calculated by Porra *et al.* (1989).

Anthocyanin Extraction and Quantifications

Seeds sterilized as above were sown on the same media as hypocotyl measurement. Following synchronization described before, these seeds were transferred to Wc for 16 h. Then, these seeds were exposed to low or high light intensity FRc light for 3 days before measurement. Control plates were kept in the dark at 22℃. Harvested seedlings were frozen by liquid nitrogen. After grinding into powder, anthocyanin was extracted in 600 μL extraction buffer (1% HCl in methanol) at 4℃ overnight with adequate mixing. After added both 400 μL RO water and chloroform, centrifuged at 13000 rpm, 4℃ for 15 mins, and then 200 μL of the mixed buffer were taken for spectrophotometric analysis. Absorbance at 530 and 657 nm was measured in Tecan Infinite 200 PRO (Tecan) with plastic 96-well plate. Total anthocyanin content was determined according to the extinction coefficients calculated by Kim *et al.* (2003).

RESULTS

FIN219 and *HFR1* synergistically regulate fertility and stamen elongation.

To elucidate the regulatory relationship between *FIN219* and *HFR1*, we generated the double mutant *fin219-2hfr1-201* line by crossing. The leaves of *fin219hfr1* showed no trichomes, which were similar to the phenotype of *hfr1-201* (Figure 1A). Previous studies indicated that both *FIN219* and *HFR1* did not show a defect in fertility. However, we found that the *fin219hfr1* double mutant plants were partially sterile (Figure 1B). To investigate the reasons for sterile phenotype, we observed the stamen of the *fin219hfr1*, and found that it was shorter than wild type (Figure 1C), which was similar to the phenotype of *coil1* (Feys *et al.*, 1994). This suggests that *HFR1* may be involved in JA signaling pathway and it may synergistically regulate the phenotype with *FIN219*. The phenotypes of fertility and stamen elongation were only observed in the double mutant, whereas both single mutants showed a normal phenotype as wild type.

FIN219 and *HFR1* participated in JA-regulated responses of root elongation, anthocyanin synthesis and chlorophyll reduction.

In *Arabidopsis*, JAs affect diverse aspects of plant biology such as the inhibition of root growth, the synthesis of anthocyanin and the reduction of chlorophyll (Staswick *et al.*, 1992; Shan *et al.*, 2009; Okamoto *et al.*, 2009). To investigate whether *HFR1* is involved in JA signaling and whether *FIN219* and *HFR1* have a synergistically effect on JA responses, we investigate the phenotype of *fin219-2*, *hfr1-201* single mutants and the *fin219hfr1* double mutant. The *hfr1-201* was insensitive to MeJA-mediated root elongation, especially at 5 μM, which suggests that *HFR1* may be involved in JA response. The *fin219hfr1* exhibited root elongation similar to *fin219-2* single mutant,

which indicates that FIN219 played a more important role in root elongation than HFR1 (Figure 2). The anthocyanin accumulation in *fin219hfr1* under low (1 $\mu molm^{-2}s^{-1}$) or high (10 $\mu molm^{-2}s^{-1}$) fluences of FR light with MeJA treatment was lower than that in wild type and respective single mutant, which implies that *FIN219* and *HFR1* have a synergistic effect on anthocyanin synthesis. Besides, the *fin219hfr1* showed lower anthocyanin level in the control than that in wild type, these results indicated that both *FIN219* and *HFR1* play key roles in the regulation of anthocyanin accumulation under MeJA and FR light treatment (Figure 3). Moreover, the chlorophyll content in *fin219-2* and *hfr1-201* single mutants was higher than that in wild type in high FR light and darkness. The chlorophyll content in *fin219hfr1* under high fluence of FR light with or without MeJA treatment was higher than those in wild type and respective single mutants, which suggests that FIN219 and HFR1 have a synergistic effect on the regulation of chlorophyll content in response to MeJA (Figure 4).

FIN219 and _HFR1_ are positive regulators of hypocotyls elongation in various light conditions except for red light.

Previous studies has showed that both *fin219-2* and *hfr1-201* were insensitive to FR light (Hsieh *et al*., 2000; Fairchild *et al*., 2000), and *hfr1-201* was also insensitive to B light (Duek and Fankhauser, 2003). Here, we found that *fin219-2* and *hfr1-201* showed longer hypocotyl phenotype in both B (0.97 $\mu molm^{-2}s^{-1}$) and FR (2.85 $\mu molm^{-2}s^{-1}$) light compared with that of wild type. The *fin219hfr1* double mutant exhibited long-hypocotyl phenotype under all light condition examined, including darkness (Figure 5A and B).

Since *fin219-2* was insensitive to MeJA (Staswick and Tiryaki, 2004), we further

examined MeJA-mediated inhibition of hypocotyl elongation of *fin219-2*, *hfr1-201* and *fin219hfr1* under all light conditions. *fin219-2* was insensitive to MeJA-mediated inhibition of hypocotyl elongation under different light conditions. In contrast, *hfr1-201* showed an insensitive phenotype to MeJA in R and FR light. The *fin219hfr1* showed an altered response of hypocotyl to MeJA under all light conditions (Figure 5C and D).

***FIN219* and *HFR1* are positive regulators in both FR light and JA signalings.**

To further understand FIN219 and HFR1 functions in FR light and JA signaling, we examined the hypocotyl lengths of *fin219-2*, *hfr1-201* single mutants and the *fin219hfr1* double mutant under the dark and the two different fluence rates of FR light. *fin219-2* single mutants exhibited a longer hypocotyl phenotype than wild-type Col-0 in low FR light (1 $\mu molm^{-2}s^{-1}$), whereas *hfr1-201* single mutants exhibited a longer hypocotyl than wild type under high FR light (10 $\mu molm^{-2}s^{-1}$). The *fin219hfr1* double mutant showed an even longer hypocotyl than wild type under both FR light and in darkness. We showed that *fin219hfr1* has long hypocotyl length in different FR light intensity (Figure 6A and B).

Since the hypocotyl elongation in *Arabidopsis* seedlings is inhibited by MeJA treatment, we examined the hypocotyl lengths. In the presence of MeJA, *fin219-2* was insensitive to MeJA-mediated inhibition of hypocotyl elongation under all light conditions. The *hfr1-201* single mutants were insensitive to MeJA treatment as well under low fluence of FR light. The *fin219hfr1* showed an even more severe response of hypocotyl elongation response to both low and high fluences of FR light with MeJA treatment (Figure 6D). To further understand the effect of different concentration of MeJA on hypocotyl elongation in low FR light, we examined the inhibition of

hypocotyl elongation of *fin219-2* and *hfr1-201* single mutants and the *fin219hfr1* double mutant (Figure 6E). The inhibition of hypocotyl elongation of *fin219-2*, *hfr1-201* single mutant and the *fin219hfr1* double mutant showed less sensitivity to MeJA-mediated inhibition of hypocotyl elongation under low FR light (Figure 6F).

***FIN219* and *HFR1* are negative regulators in low R: FR light condition.**

Hypocotyl elongation in *Arabidopsis* seedling is increased in low R: FR condition. Previous studies have shown that both *fin219* and *hfr1* have a longer hypocotyl phenotype than wild type under low R: FR condition (Robson *et al.*, 2010; Sessa *et al.*, 2005). We further examined their regulatory relationship by using *fin219-2*, *hfr1-201* single mutants and the *fin219hfr1* double mutant grown under low and high R: FR conditions (Figure 7A). Both *fin219-2* and *hfr1-201* exhibited consistent phenotypic response as previous studies under low R: FR condition. However, the *fin219hfr1* double mutant exhibited even longer hypocotyl phenotype than wild type and the respective single mutants, which implies that FIN219 and HFR1 may have a synergistic effect under low R: FR condition (Figure 7B).

To further understand the effect of MeJA on shading responses, we examined the hypocotyl lengths of *fin219-2* and *hfr1-201* single mutants and the *fin219hfr1* double mutant in low R: FR condition with MeJA treatment (Figure 7C). The inhibition of hypocotyl length of *fin219-2* and the *fin219hfr1* showed the MeJA-mediated inhibition of hypocotyl elongation under shading condition similar to wild type, in contrast, *hfr1-201* exhibited a more sensitive phenotype than wild type under the same condition. Taken together, these data indicate that HFR1 may play a role in MeJA-mediated response under SAS signaling pathway (Figure 7D).

HFR1 overexpression rescued the phenotypes of _hfr1_ single mutant.

In order to verify whether *HFR1* over expression can rescue *hfr1-201*, we introduced *35S::HFR1* in the *hfr1-201* mutant. The T_2 seedlings of *HFR1ox/hfr1-201* transgenic plants showed that three-quarters of the seedlings had the hypocotyl lengths similar to wild type in low and high FR light (Figure 8A), suggesting that *HFR1* over expression can rescue the defects of *hfr1* mutant (Figure 8B). Moreover, three-quarters of the *HFR1ox/hfr1-201* transgenic T_2 seedlings showed responses to MeJA effect similar to wild type in low FR light condition (Figure 8C and D). In shading condition, the three-quarters of the seedlings showed the hypocotyl lengths similar to wild type, which confirmed the previous studies (Figure 7A and B).

Both _FIN219_ and _HFR1_ may regulate with each other in the dark or FR light.

Previous studies showed that both FIN219 and HFR1 were positive regulators in phyA-mediated FR light signaling (Fairchild *et al.*, 2000; Hsieh *et al.*, 2000). We examined the levels of *FIN219* and *HFR1* gene expression by RT-PCR and protein gel blot analyze in Col-0, *fin219-2*, *hfr1-201* single mutants and the *fin219hfr1*double mutant. The level of *FIN219* transcript in *hfr1-201* was lower in the dark; but largely the same as wild type in FR light. In contrast, the level of HFR1 transcript in *fin219-2* increased in the dark and the FR light conditions (Figure 9A). Taken together these data indicate FIN219 negatively regulate HFR1 transcript in both dark and FR conditions, however, HFR1 positively regulate FIN219 transcript in the dark.

Further protein gel blot analysis revealed that the levels of FIN219 protein remained largely the same as the wild type under the dark and the FR light condition. But HFR1 levels in *fin219-2* were slightly decrease in the dark, and increase in the FR light (Figure 9B). Thus, these data indicated that FIN219 negatively regulate HFR1 at

the transcript and protein levels, especially in FR light.

To further understand the effect of MeJA on FIN219 and HFR1 gene expression under FR light, we analyzed the levels of *FIN219* and *HFR1* transcript and protein levels. RT-PCR analysis reviewed that *FIN219* transcript levels in wild type were reduced by MeJA in FR light, but not in the dark. *HFR1* transcript levels were reduced in the dark and FR light. Interestingly, *FIN219* transcript in *hfr1-201* were increased by MeJA under both dark and FR light. In contract, *HFR1* transcript levels in *fin219-2* were decreased by MeJA in the dark but not in FR light (Figure 10A). In addition, FIN219 protein levels in wild type were increased by MeJA under the dark and FR light. FIN219 levels in *hfr1-201* were also increased by MeJA. HFR1 protein levels in wild type were decreased significantly by MeJA in both dark and FR light. In contrast, HFR1 protein levels in *fin219-2* were also decreased by MeJA, similar to those in wild type. Thus the results indicated that MeJA and FIN219 may affect specific form of HFR1 protein in terms of phosphorylation and dephosphorylation (Figure 10B). It supposed that *HFR1* is affected by JA and impact the downstream genes.

FIN219 and HFR1 affect the expression of JA biosynthesis genes in both the dark and FR light.

Since the *fin219hfr1* result in partial sterility, we further examined whether both genes affect JA biosynthesis, including *LOX2*, *AOS* and *OPR3*, or signaling component, such as *JAZ1*, *MYC2* and *VSP1* transcript levels in Col-0, *fin219-2*, *hfr1-201* and the *fin219hfr1* by MeJA treatment in the dark and FR light. The results indicate that *fin219-2* with MeJA treatment resulted in reduction of *AOS* transcript in the dark and FR light, but the *AOS* transcript level in *hfr1-201* did not show significant changes by MeJA. Both *fin219-2* and *hfr1-201* resulted in altered levels of *OPR3* in the presence

or absence of MeJA under the dark and FR light conditions (Figure 11A). Besides, JA signaling components in wild type were induced by MeJA in the dark and FR light. Among these signaling components, *MYC2* was most affected by *fin219-2* and *hfr1-201* single mutants with MeJA treatment under the dark and FR light conditions (Figure 11B).

HFR1 and FIN219 proteins were induced by low R: FR treatment.

To understand how shading treatment affects FIN219 and HFR1 expression, we further examined the *FIN219* and *HFR1* transcript by RT-PCR and protein levels by gel blot analysis, the levels of *FIN219* and *HFR1* expression in wild type were increased in low R: FR condition. Interestingly, we found that *FIN219* expression was slightly decreased in *hfr1-201*, but *HFR1* gene levels in *fin219-2*were increased by shading (Figure 12A).

FIN219 protein level in wild type was significantly increased by shading treatment (low R: FR ratio). However, this increase of FIN219 level by shading was decreased in *hfr1-201*. HFR1 was substantially increased in *fin219-2* in low R: FR condition (Figure 12B). Taken together, these data indicate that HFR1 may positively regulate FIN219 levels and FIN219 negatively regulate HFR1.

Besides, shading signaling components, including *PAR1*, *PIL1* and *ATHB2* were up-regulated by shading treatment. *fin219-2* showed reduction of *PIL1*, *PAR1* and *ATHB2* compared to wild type, whereas *hfr1-201* showed an increase of these genes (Figure 13A). The gene expressions of *PIF4* and *PIF5* in *hfr1-201* were increased, which confirmed the previous data; but *PIF7* was decreased in *hfr1-201*. *fin219-2* showed reduction of *PIF4* and *PIF7* but increase of *PIF5*. Thus, these data indicate that FIN219 modulates the expression of these *PIF* components (Figure 13B).

FIN219 and HFR1 participated in JA and SAS signaling.

To understand the effect of MeJA on *FIN219* and *HFR1* expression in response to shading condition, we examined the transcripts and protein levels of FIN219 and HFR1 in the presence or absence of MeJA under shading conditions. The expression levels of *FIN219* and *HFR1* in wild type under shading treatment were increased, which is consistent with previous results. Under shading condition, *FIN219* was increased in JA treatment whereas *HFR1* was decreased in JA treatment (Figure 14A).

In addition, FIN219 protein levels were increased by MeJA treatment under high R: FR condition, but slightly reduced by MeJA under low R: FR condition. In contrast, HFR1 levels in wild type were drastically reduced by MeJA under high R: FR ratio and remained unchanged by MeJA under low R: FR condition. FIN219 reduction in *hfr1-201* under low R: FR ratio was increased by MeJA treatment. However, HFR1 level increased in *fin219-2* under low R: FR condition was significantly reduced by MeJA. HFR1 also showed two bands which suggested that HFR1 expression was more complex than synthesized its expression only (Figure 14B). Therefore, MeJA reduces FIN219 effect by shading.

Furthermore, we examined the effect of MeJA on SAS components, including *PIL1*, *PAR1*, *ATHB2*, *PIF4*, *PIF5* and *PIF7*. *PIL1*, *ATHB2*, *PIF4*, *PIF5* and *PIF7* were increased in low R: FR condition. Interestingly, *PIL1*, *PAR1* and *PIF5* in Col-0 were decreased in JA treatment under low R: FR condition. Besides, *PIF4* and *ATHB2* were also decreased in *fin219-2* and *hfr1-201* mutants by JA treatment (Figure 15). In addition, increased levels of JA biosynthetic genes by shading were still induced by MeJA treatment, but the JA signaling components, especially the *MYC2*, was decreased by MeJA treatment (Figure 16).

***phyA* and *cry1* showed strong inhibition of hypocotyl elongation by methyl JA (MeJA).**

Previous studies indicated that *phyA, phyB, cry1* and *cry2* were involved in shading responses (review by Franklin, 2008). It is interesting to see how these photoreceptors regulate FIN219 and HFR1 in shading condition. We examined the hypocotyl lengths of *fin219-2* single mutant, and *fin219-2* and photoreceptors double or triple mutants in low R: FR condition. Surprisingly, the *fin219phyA* double mutant showed longer hypocotyl phenotype than wild type and respective single mutants under shading condition. As well, the *fin219phyB* showed a phenotype similar to *phyB* mutant under both high and low R: FR conditions (Figure17A). However, the *fin219cry1* double mutant showed an increase hypocotyl phenotype compared to wild type and respective single mutants in high R: FR condition but it showed a phenotype similar to *cry1* mutant in low R: FR condition, which suggests that FIN219 and cry1 may play a negatively role in low R: FR (Figure 18A).

Moreover, *fin219-2* exhibited less sensitivity to MeJA-mediated inhibition of hypocotyl elongation under shading condition, however, *phyA-211*showed a more sensitive response to MeJA than wild type under shading condition. The *fin219phyA* showed a response to MeJA-mediated inhibition of hypocotyl elongation, similar to the *fin219-2* under shading condition. In contrast, in *phyB* mutant showed a slightly increased sensitivity to MeJA effect under shading. The *fin219phyB* double mutant showed less sensitivity to MeJA effect compared to respective single mutants (Figure17B). Besides, the *cry1-304* showed a substantially increased sensitivity to MeJA under shading condition. The *fin219cry1* double mutant showed sensitivity to MeJA similar to *fin219-2* under shading (Figure 18B).

FIN219 and HFR1 were regulated by photoreceptors in response to low R: FR condition.

To clarify the regulation among FIN219, HFR1 and photoreceptors, we investigate the levels of FIN219 and HFR1 proteins in different photoreceptor mutants under shading condition. FIN219 was increased in *phyA-211* and *phyB-1* single mutant and *phyAphyB* double mutant compare to wild type under shading condition, whereas HFR1 showed decreased levels in both *phyA-211* and *phyB-1* as well as *phyAphyB* under shading (Figure 19A). FIN219 was increased in *cry1-304* mutant, but decreased in *cry2-1* mutant under shading. HFR1 showed opposite patterns as FIN219 under the same condition. In *cry1cry2* double mutant, FIN219 slightly decreased under shading compared with wild type and respective single mutants, whereas HFR1 was significantly decreased (Figure 19B).

HFR1 might not physically interact with FIN219 in shading condition.

To understand whether FIN219 interacts with HFR1 in shading condition, we performed co-immunoprecipitaiton (co-IP) studies under shading condition. Unfortunately, we did not detect any signal of HFR1 in the precipitated product by FIN219 monoclonal antibodies, suggesting that FIN219 may not interact with HFR1 in shading condition (Figure 20).

DISCUSSION

Shade avoidance has been noticed for a long time since people started growing crops, but the physiological regulation of shade avoidance started at the middle of the twenty century, which indicates that hormones are involved in the regulation of shade avoidance response (Selman and Ahmed, 1962). The molecular regulation mechanism of shade avoidance was not identified until the last two decades. It is worthwhile to figure out the details of the regulatory mechanism in shade avoidance syndrome, which decreases the efficiency of cultivation. Even though many hormones have been pointed out to be involved in the regulation of shade responses (Carabelli *et al*., 2007; Pierik *et al*., 2004; Kozuka *et al*., 2010; Stamm *et al*., 2010), the details of the regulation are still unknown. Jasmonates, the well-known plant defense hormone, have been proposed to participate in shade avoidance responses. Besides, *phyB* mutant showed increased plant susceptibility to *Botrytis cinerea* in shade condition (Cerrudo *et al*., 2012). It has been noticed that *phyA* is insensitive to MeJA treatment whereas *coi1* shows an elongated hypocotyl phenotype in low R: FR condition (Robson *et al*. 2010). But the crosstalk mechanism between MeJA and SAS signaling is still not clear.

FIN219, the key enzyme for the conjugation of jasmonic acid with isoleucine, triggers downstream JA responses (Staswick and Tiryaki, 2004). *FIN219* has also been noticed to be involved in FR signaling as a positive regulator (Hsieh *et al*., 2000), and its mutant showed an elongated hypocotyl phenotype in low R: FR condition (Robson *et al*. 2010). It is interesting to find out the roles of *FIN219* in both SAS and JA signaling and the regulation between these two pathways.

Phenotypic analysis showed that *fin219hfr1* was insensitive to MeJA.

In this study, we included *HFR1*, a SAS mediated marker gene that plays a

negative role in shade response, and discover that both *FIN219* and *HFR1* participate in SAS and JA signaling. We found that *fin219hfr1* shows partial sterility and a shorter anther phenotype which were not observed in wild type and respective single mutants (Figure 1B). It has been noticed that JA induces the elongation of anthers. Moreover, JA biosynthetic mutants, *dad1* and *opr3*, show shorter anthers and sterile phenotypes (Stintzi and Browse, 2000; Ishiguro *et al*., 2001). It is possible that *fin219hfr1* might be defective in JA biosynthesis that causes the fertility phenotype (Figure 1C). The *fin219hfr1* also shows no trichomes (Figure 1A), which is consistent with the fact that JA regulates the formation of trichomes (Traw and Bergelson, 2003).

MeJA treatment can inhibit the root elongation (Staswick *et al*., 1992). The *fin219hfr1* showed insensitivity to MeJA-mediated inhibition of root elongation, which confirmed our hypothesis (Figure 2A). But surprisingly, *hfr1-201* was also insensitive to MeJA-mediated inhibition of root elongation, which implies that *HFR1* may be involved in JA signaling (Figure 2B). Besides, JA regulates anthocyanin synthesis by increasing the regulated gene expression, and induced the accumulation of anthocyanin (Chen *et al*., 2007; Shan *et al*., 2009). The anthocyanin level in the *fin219hfr1* was lower than that in wild type, which indicates that the double mutant is insensitive to MeJA induced anthocyanin accumulation (Figure 3A). Moreover, previous studies indicated that FR induced the synthesis of anthocyanin (Lange *et al*. 1971). Our data pointed out in high fluence of FR, the amount of anthocyanin in wild-type seedlings was increased, whereas the *fin219hfr1* showed lower anthocyanin accumulation than wild type (Figure 3B).

JA can inhibit the synthesis of chlorophylls (Okamoto *et al.*, 2009). Nevertheless, it has been noticed that FR light would block the synthesis of chlorophylls, which is so-called FR blockage of greening (Barnes *et al*., 1996). The chlorophyll content of the

fin219hfr1 with MeJA treatment was higher than that in wild type, which suggests that FIN219 and HFR1 play a role in MeJA regulated chlorophyll levels under FR light (Figure 4A) and *hfr1-201* also showed a high content of chlorophylls. It is interesting to find out that after high fluences of FR light condition, seedlings show a faster greening than the treatment with low fluences of FR light. This suggests that different fluences of FR light will have different effects on the regulation of chlorophyll synthesis.

***fin219hfr1* was insensitive to MeJA and showed a longer hypocotyl than wild type under FR light and low R: FR condition.**

The previous data showed that the *fin219hfr1* was insensitive to MeJA and *HFR1* might be involved in JA signaling. *HFR1* is involved in FR and B light signaling, whereas *FIN219* is involved in FR light signaling (Fairchild *et al*., 2000; Duek and Fankhauser, 2003; Hsieh *et al*., 2000). In B and FR light conditions, the *fin219hfr1* showed significant differences from wild type and respective single mutants, and both single mutants also showed significant differences from wild type (Figure 5A). These data confirmed the previous studies and also indicated that *FIN219* may play a role in B light signaling. On the other hand, in R light condition, only *fin219-2* and the *fin219hfr1* showed significant differences from wild type, which suggests that *FIN219* may regulate R light signaling (Figure 5B). Compared with wild type, *fin219-2* and the *fin219hfr1* showed significant phenotype in MeJA-mediated inhibition of hypocotyl elongation under all light conditions, including the darkness. However, *hfr1-201* only showed a phenotype under R and FR light conditions (Figure 5C). In conclusions, *FIN219* is a major regulator in JA signaling to modulate MeJA-mediated inhibition of hypocotyl elongation under all conditions, whereas *HFR1* might be a minor regulator

that only affects some aspects of MeJA-mediated responses under some conditions (Figure 5D).

In addition, *fin219-2* showed an insensitive phenotype under low fluences of FR light, whereas *hfr1-201* shows a defect under high fluences of FR light. The *fin219hfr1* showed an even severe phenotype under all light conditions (Figure 6A), which indicates that FIN219 and HFR1 act in a parallel manner to modulate hypocotyl elongation under FR light (Figure 6B). With MeJA treatment, the *hfr1-201* single mutant showed less sensitivity to MeJA-mediated inhibition of hypocotyl elongation under low intensity of FR light, but exhibited hypersensitivity under high FR light, which suggests that *HFR1* may have differential activities in JA signaling in response to various fluences of FR light (Figure 6D), which is supported by the fact that *hfr1* mutant shows less sensitivity to low FR light in response to different concentrations of MeJA (Figure 6F). Further, the double mutant *fin219hfr1* was insensitive to the MeJA-mediated inhibition of hypocotyl elongation under different fluences of light (Figure 6D) as well as under various concentrations of MeJA (Figure 6F). Thus, FIN219 and HFR1 are involved in the integration of FR light and JA signaling to regulation hypocotyl elongation.

Both *FIN219* and *HFR1* are involved in SAS signaling under low R: FR condition (Robson *et al*. 2010; Sessa *et al*., 2005). Here, we found that the *fin219hfr1* showed an even longer phenotype than respective single mutants under low R: FR condition (Figure 7A), which indicates that *FIN219* and *HFR1* have synergistic effects under shade signaling (Figure 7B). Intriguingly, *hfr1* mutant showed hypersensitivity to MeJA-mediated inhibition of hypocotyl elongation under shade condition. However, *fin219hfr1* and *fin219-2* exhibited sensitivity to MeJA similar to wild type under shade (Figure 7C and D), which implies that FIN219 may act in the downstream of shade

signaling in response to JA.

Both gene and protein expression indicated that *HFR1* induced the expression of *FIN219*, whereas *FIN219* repressed the expression of *HFR1*.

Both *FIN219* and *HFR1* transcripts were induced under FR light compared to those in the dark (Figure 9A), whereas both FIN219 and HFR1 protein levels remained largely the same in FR light and in the dark (Figure 9B), which confirms the previous research showing that *FIN219* and *HFR1* both are positive regulators of phyA-mediated FR light signaling, (Fairchild *et al*., 2000; Hsieh *et al*., 2000). Compared with wild-type transcript levels, *HFR1* was induced in *fin219-2* under both FR light and the darkness conditions, whereas *FIN219* was reduced in *hfr1-201*under the darkness, but showed no differences under FR light condition. In addition, the expression of FIN219 protein levels in *hfr1-201* were largely the same as the wild type, but the levels of HFR1 in *fin219-2* were increased under FR light condition. Taken together, these data indicate that FIN219 negatively regulates the levels of HFR1 transcripts and proteins in both the dark and FR conditions; however, HFR1 positively regulates FIN219 transcripts in the dark.

FIN219 and HFR1 still regulated with each other under FR light with MeJA treatment. In wild type, *FIN21*9 showed reduction in FR light condition with MeJA treatment, whereas in darkness with MeJA treatment, FIN219 transcripts remained largely the same with the control. However, *HFR1* showed decreased expression under both FR light and the darkness with MeJA treatment. *HFR1* was induced in *fin219-2* under the darkness, and decreased in the presence of MeJA; in contrast, *FIN219* was induced in *hfr1-201* under both FR light and dark conditions with MeJA treatment (Figure 10A). These data revealed that *HFR1* might be repressed by MeJA and

negatively regulated by *FIN219*; *FIN219* might be positively regulated by *HFR1*. By contrast, FIN219 protein levels were increased under both the darkness and FR light conditions with MeJA treatment, whereas HFR1 showed reduction under the same conditions. Intriguingly, HFR1 showed two bands, likely phosphorylated and unphosphorylated ones (Figure 10B), which may be caused by CKII (Park *et al*., 2008).

FIN219 **and** ***HFR1*** **affect the components of JA biosynthesis and signaling.**

LOX2, AOS and OPR3 are involved in JA biosynthesis and their gene expressions are upregulated by MeJA (Wallis and Browse, 2002). Besides, *MYC2* and *VSP1* are upregulated by MeJA as well; whereas the repressors, JAZ proteins, are degraded during the signaling, but the *JAZ*s genes can be activated by JA treatment and MYC2 (Memelink, 2009).

Both JA synthesis and signaling genes in wild type with MeJA treatment were all induced under both the darkness and FR light conditions. The expression of *OPR3* in *fin219-2* and *hfr1-201* showed altered levels compared with those in wild type under darkness and FR light conditions with or without MeJA treatment. It is not surprising that *AOS* was reduced in *fin219-2* compared with wild type, but the expression of *AOS* in *hfr1-201* showed no significant difference from wild type (Figure 11A), which suggests that *HFR1* may play a minor role in JA biosynthesis, as supported by the *hfr1* mutant. In contrast, all signaling genes in wild type were induced under the dark and FR light conditions with MeJA treatment. As compared with wild type, *MYC2* was the most effective genes and decreased in both *fin219-2* and *hfr1-201* (Figure 11B). Even though the *fin219hfr1* showed a partial sterility at the adult stage (Figure 1), the transcripts and protein levels of JA biosynthetic and JA signaling components in

seedlings were not significantly altered (Figure 11). It is possible that these genes are regulated by both FIN219 and HFR1 at the adult stage rather than the seedling stage.

PIL1, *ATHB2* and *PIF4* were induced in *hfr1-201*, but reduced in *fin219-2* under shading condition.

HFR1 is a negative regulator in SAS signaling and induced by low R: FR light to repress the expression of downstream regulators (Sessa *et al*., 2005). Both *FIN219* and *HFR1* were induced in shading condition and their proteins showed similar patterns. In shading condition, *HFR1* was induced in *fin219-2*, whereas *FIN219* was slightly decreased in *hfr1-201* (Figure 12A). In contrast, FIN219 protein levels were decreased in *hfr1-201*, but HFR1 was increased in *fin219-2* in shading condition, which is consistent with their transcript level (Figure 12B). Moreover, the regulation between *FIN219* and *HFR1* in shading condition is similar to that in FR light condition. We conclude that *HFR1* is repressed by MeJA and negatively regulated by *FIN219*; *FIN219* is positively modulated by *HFR1*. The regulation of SAS signaling involves a complex mechanism. *PIL1*, *PAR1* and *ATHB2* are the key regulators that are involved in SAS signaling (Martinez-Garcia *et al*., 2010). Besides, PIF4, PIF5 and PIF7 as well as HFR1 are bHLH transcription factors and have been shown are involved in SAS signaling (Lorrain *et al*., 2008). In shading condition, all genes were induced, and *PIL1* and *ATHB2* are significantly induced. Here, we found that the expression of *PIL1* and *ATHB2* in *hfr1-201* was increased, whereas *PAR1*, a negative regulator, showed no differences from wild type. However, all of these genes in *fin219-2* were reduced compared with those in wild type, which is likely caused by substantial increase of HFR1 proteins, leading to suppression of these genes under shade condition (Figures 12B and 13A). In addition, both *fin219-2* and *hfr1-201* enhanced the expression of

PIF5, but repressed the expression of *PIF7*. Moreover, *PIF4* was increased in *hfr1-201*, but reduced in *fin219-2* (Figure 13B). It is possible that the regulation of PIF protein family is more complex during SAS signaling. It is worthy to pursue their regulation at protein levels under shading.

JA serves as a negative role in SAS signaling and JA signaling genes may be blocked in shading condition..

As the expressions of *FIN219* and *HFR1* under shading condition with MeJA treatment are altered and their regulation with each other remains consistent. The expression of *FIN219* under low R: FR with MeJA treatment was induced, whereas *HFR1* was reduced. It is interesting that the expression of *FIN219* under both shading and MeJA treatments showed higher than respective single treatment, whereas the expression of *HFR1* was induced by shade and reduced by both shade and MeJA treatments (Figure 14A). In contrast, their protein levels were increased by shade and showed opposite levels with MeJA treatment (Figure 14B). In conclusions, the shade can increase the levels of FIN219 and HFR1, but MeJA may reduce the levels of both proteins and the effects of shade responses.

In SAS related genes, *PIL1*, *PAR1* and *ATHB2* were induced in shading condition, whereas when treated with MeJA, they all showed a decreased expression level. In both *fin219-2* and *hfr1-201* mutants, *PAR1* was induced but *ATHB2* was depressed, and the expression of *PIL1* showed no significant differences from wild type (Figure 15A). *PIF4*, *PIF5* and *PIF7* all showed no significant differences from wild type under shading condition with MeJA treatment. However, *PIF4* in both *fin219-2* and *hfr1-201* single mutant was induced, and *PIF7* was depressed, and *PIF5* showed no significant differences from wild type (Figure 15B). Besides, *LOX2*, *AOS* and *OPR3* genes were

induced under shade condition with MeJA treatment (Figure 16A). The JA synthesis might be induced in shading condition, leading to increased amount of JA. On the other hand, the expression of JA signaling genes, *JAZ1*, *MYC2* and *VSP1*, was slightly decreased under shade condition with MeJA treatment (Figure 16B), which implies that the JA signaling might be blocked in shading, leading to the long hypocotyl phenotype under shading (Figure 7A). Thus, the expression of SAS related genes will be reduced by JA treatment, whereas JA synthesis genes will be induced and JA signaling genes reduce in shading condition.

Photoreceptors phyA, phyB, cry1 and cry2 all regulated the expression of *FIN219* and *HFR1.*

phyA, phyB, cry1 and cry2 are all involved in SAS regulation (Smith *et al*., 1997; Lin *et al*., 1998). Here, we found that both *FIN219* and *HFR1* have roles in both JA and SAS signaling. It is worthwhile to investigate the effects of these photoreceptors on FIN219 and HFR1 expression in shading condition and MeJA treatment. In *phyA*, *phyB* and the double mutant, both *phyA-211* and *fin219-2* showed an elongated phenotype under low R: FR condition, whereas *phyB-1* and *phyAphyB* showed no differences from the controls. These data confirm the previous results showing that phyB is the major regulator in SAS signaling (Casal, 2012). Interestingly, we found that the *fin219phyA* double mutant showed a more elongated phenotype in shading condition, whereas *fin219phyB* showed a phenotype similar to the *phyB* single mutant, which suggests that FIN219 may be involved in both phyA and phyB signaling pathways and is regulated by both photoreceptors (Figure 17A). In addition, both *fin219-2* and *phyA-211* are insensitive to MeJA under high R: FR condition, whereas under low R: FR condition they both are reversed to the same level of MeJA-mediated

inhibition of hypocotyl elongation compared with wild type (Figure 17B). This indicates that JA plays a negative role in SAS signaling. Both *cry2-1* and *fin219-2* showed an elongated phenotype under low R: FR condition, whereas *cry1-304* and the *cry1cry2* showed a similar phenotype between low R: FR and the control (Figure 18A), which is consistent with previous data (Sellaro *et al*., 2010). Fascinatingly, both the *fin219cry1* and the *fin219cry2* showed a phenotype similar to their single mutants, *cry1-304* or *cry2-1*, which suggests that *FIN219* is regulated by cryptochromes, and the functions of cry1 and cry2 in shading condition are not the same. When treated with MeJA, *cry2-1* and *fin219-2* showed a phenotype similar to wild type under low R: FR condition, whereas *cry1-304* and *cry1cry2* double mutant showed enhanced sensitivity to MeJA treatment under both low and high R: FR condition (Figure 18B).

Both FIN219 and HFR1 were induced in low R: FR condition. Interestingly, FIN219 showed an increased level in both *phyA-201* and *phyB-1*, and showed even higher levels in *phyAphyB* double mutant. By contrast, FR1 showed a decreased level in both *phyA-201* and *phyB-1*, as well as in *phyAphyB* (Figure 19A). Besides, FIN219 level was induced in *cry1-304* but reduced in *cry2-1*, whereas HFR1 level was reduced in *cry1-304* but induced in *cry2-1* (Figure 19B). These data confirmed that HFR1 increased the level of FIN219, whereas FIN219 decreased the level of HFR1. Moreover, photoreceptors phyA, phyB and cry1 negatively regulate FIN219 and cry2 positively modulates FIN219 levels under shade condition, but HFR1 is regulated by these photoreceptors in an opposite manner with FIN219.

HFR1 might not physically interact with FIN219 in shading condition.

Co-IP studies indicated that FIN219 did not interact with HFR1 under low R:FR ratio condition (Figure 20), however, plant extracts immunoprecipitated with FIN219

monoclonal antibodies gave rise to the facts that FIN219 levels were increased under low R: FR, and positively regulated by HFR1 under shade condition. Whether HFR1 is the direct target of FIN219 remains to be elucidated.

In conclusions, we proposed a model to illustrate the regulatory relationship of FIN219 and HFR1 in the cross-talk between shade and JA signaling (Figure 21). FIN219 negatively regulated by various photoreceptors, including phyB, phyA and CRY1, functions as a negative regulator in response to shade condition and antagonize HFR1 functions to regulate the components such as ATHB2, PIL1 and PAR1 in the downstream of shade signaling, leading to shade responses. Besides, HFR1 as a negative regulator for shade responses can positively regulate FIN219 levels, which forms a feedback loop to suppress negative effects, results in the expression of positive regulators and subsequent shade responses (Figure 21). It is worthwhile to investigate further to clarify the effects of jasmonates on the regulation mechanism.

FIGURES

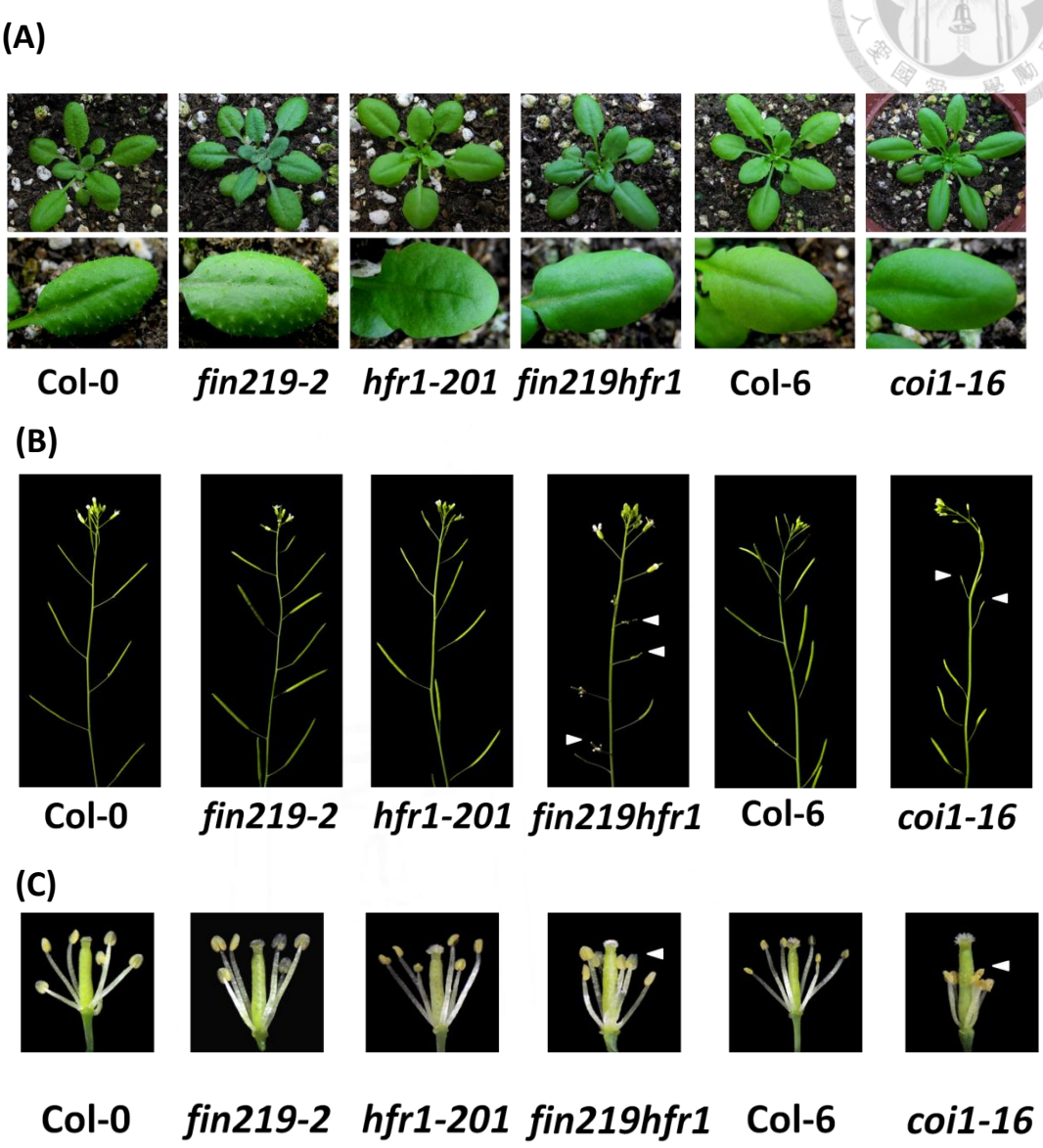

Figure 1. *FIN219* and *HFR1* synergistically regulate sterility and stamen elongation.

(A) Phenotype of *fin219-2, hfr1-201* and *fin219hfr1* plants and leaves. Plants were planted in 25 ℃ and 16 hr light condition for 3 weeks.

(B) Phenotype of *fin219hfr1* and *coi1-16*. Pods were taken from five-week-old plants, which were planted in the same condition with (A).

(C) Phenotype of *fin219-2, hfr1-201* and *fin219hfr1* flowers. Flowers were taken at stage 12, and the petals were eliminated.

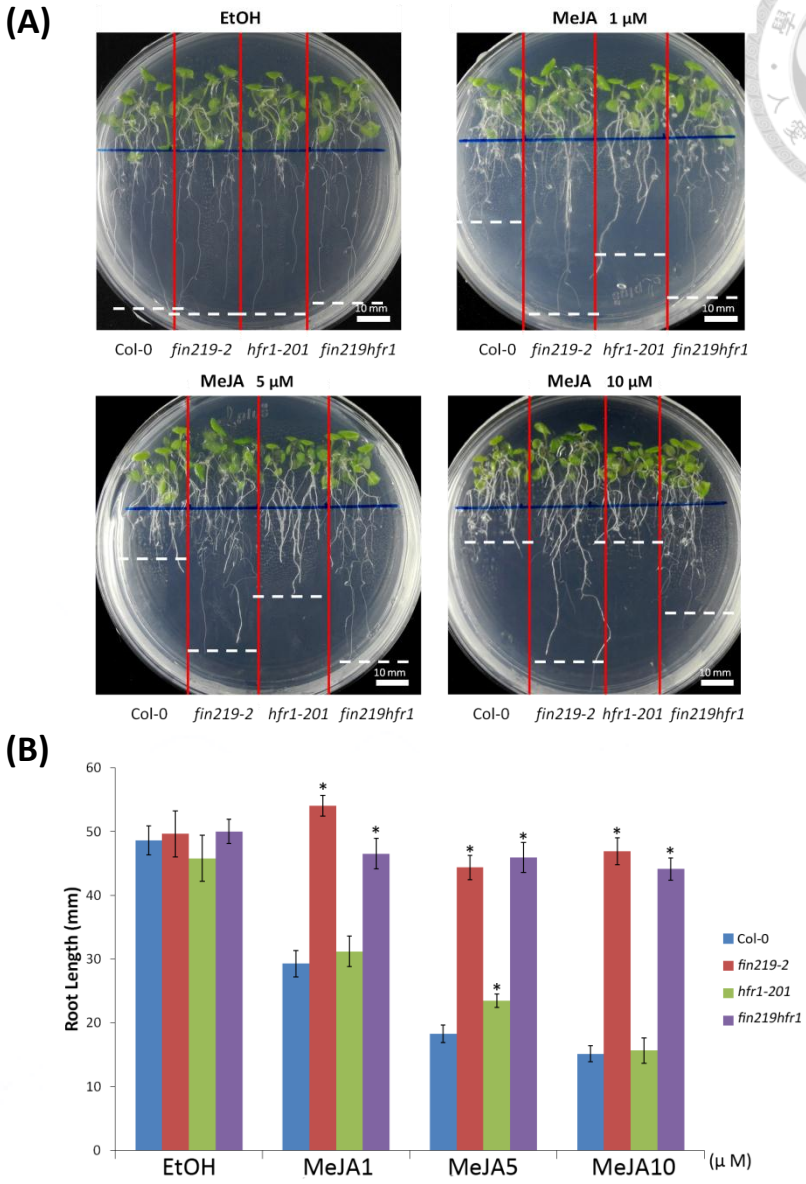

Figure 2. *FIN219* and *HFR1* participated in JA-regulated responses of root elongation.

(A) Phenotype of seedling root growth on media containing 1, 5 or 10 μM MeJA.

(B) Quantification of root lengths of seedlings grown on media containing 1, 5 or 10 μM MeJA. In (A) and (B), seedlings were grown first in continuous white light for 7 days on the media without MeJA, and then transferred to the medium without or with different concentrations of MeJA. After 7 days, new growth of root lengths of seedlings were measured. The *error bars* denote standard errors of the mean (n=12). Significant difference between samples was achieved by ANOVA and Duncan's HSD tests with $P<0.05$. The error bars denote standard error of the mean (n=12).

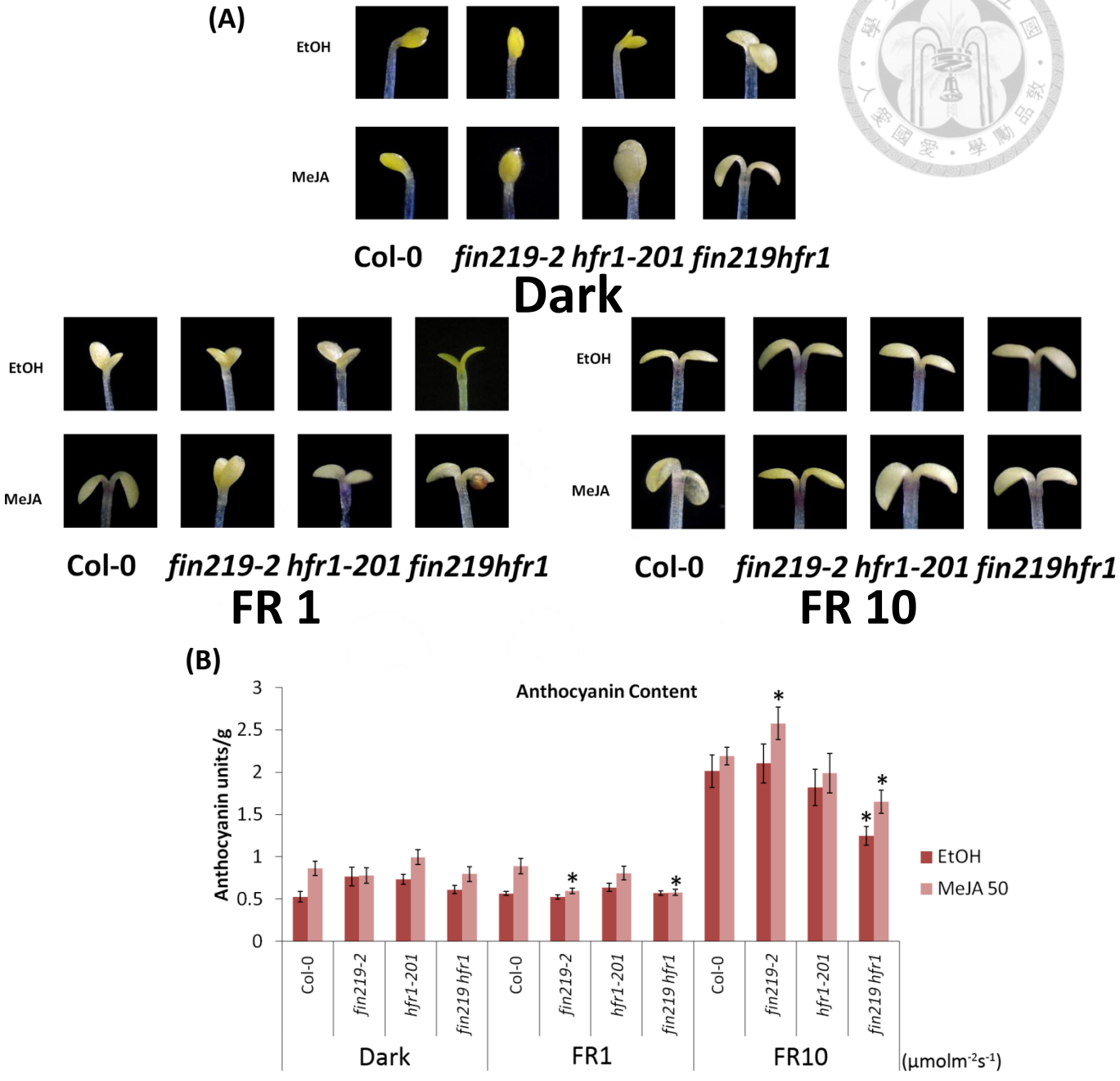

Figure 3. ***FIN219*** **and** ***HFR1*** **participated in JA-regulated responses of anthocyanin synthesis.**

(A) Phenotype of top portions of *fin219*, *hfr1* and *fin219hfr1* seedlings grown on media containing 50 μM MeJA in the dark (top), low far-red light (bottom left) or high far-red light (bottom right) condition for 3 days.

(B) Quantification of anthocyanin content of seedlings grown on media containing 50 μM MeJA. In (A) and (B), the seedlings were analyzed after 3 days treatment. The far-red light intensity was 1 $\mu molm^{-2}s^{-1}$ in low light condition and was 10 $\mu molm^{-2}s^{-1}$ in high light condition. The error bars denote standard error of the mean (n=3).

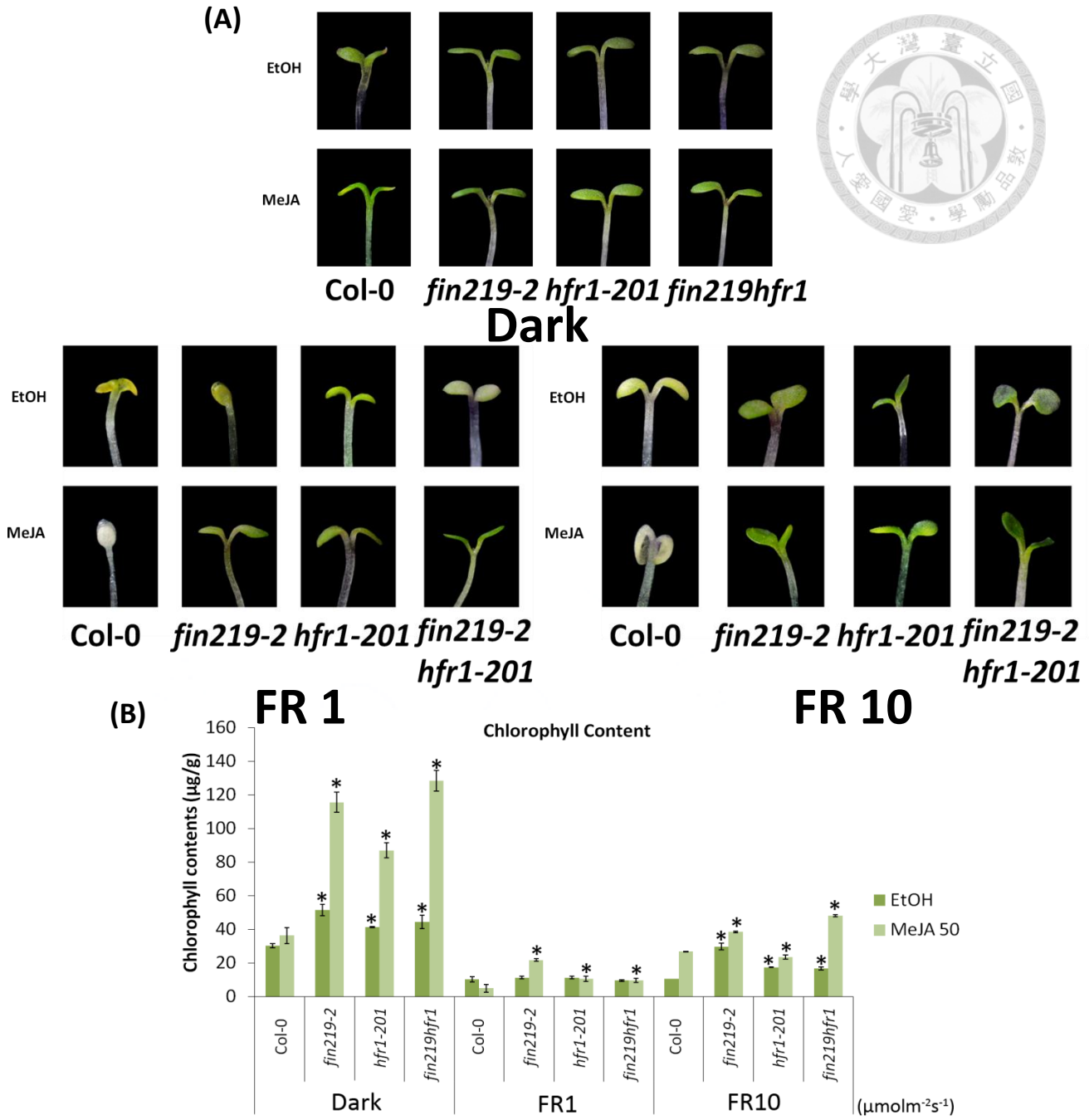

Figure 4. *FIN219* and *HFR1* participated in JA-regulated responses of chlorophyll reduction.

(A) Phenotype of *fin219*, *hfr1* and *fin219hfr1* cotyledons grown on media containing 50 μM MeJA in dark (top), low far-red light (bottom left) or high far-red light (bottom right) condition for 3 days and then were transferred into white light for 2 days.

(B) Quantification of chlorophyll content of seedlings grown on media containing 50 μM MeJA. In (A) and (B), the seedlings were analyzed after 5 days treatment. The far-red light intensity was 1 $\mu molm^{-2}s^{-1}$ in low light condition and was 10 $\mu molm^{-2}s^{-1}$ in high light condition. The white light intensity was 5.87 $\mu molm^{-2}s^{-1}$. The error bars denote standard error of the mean (n=3).

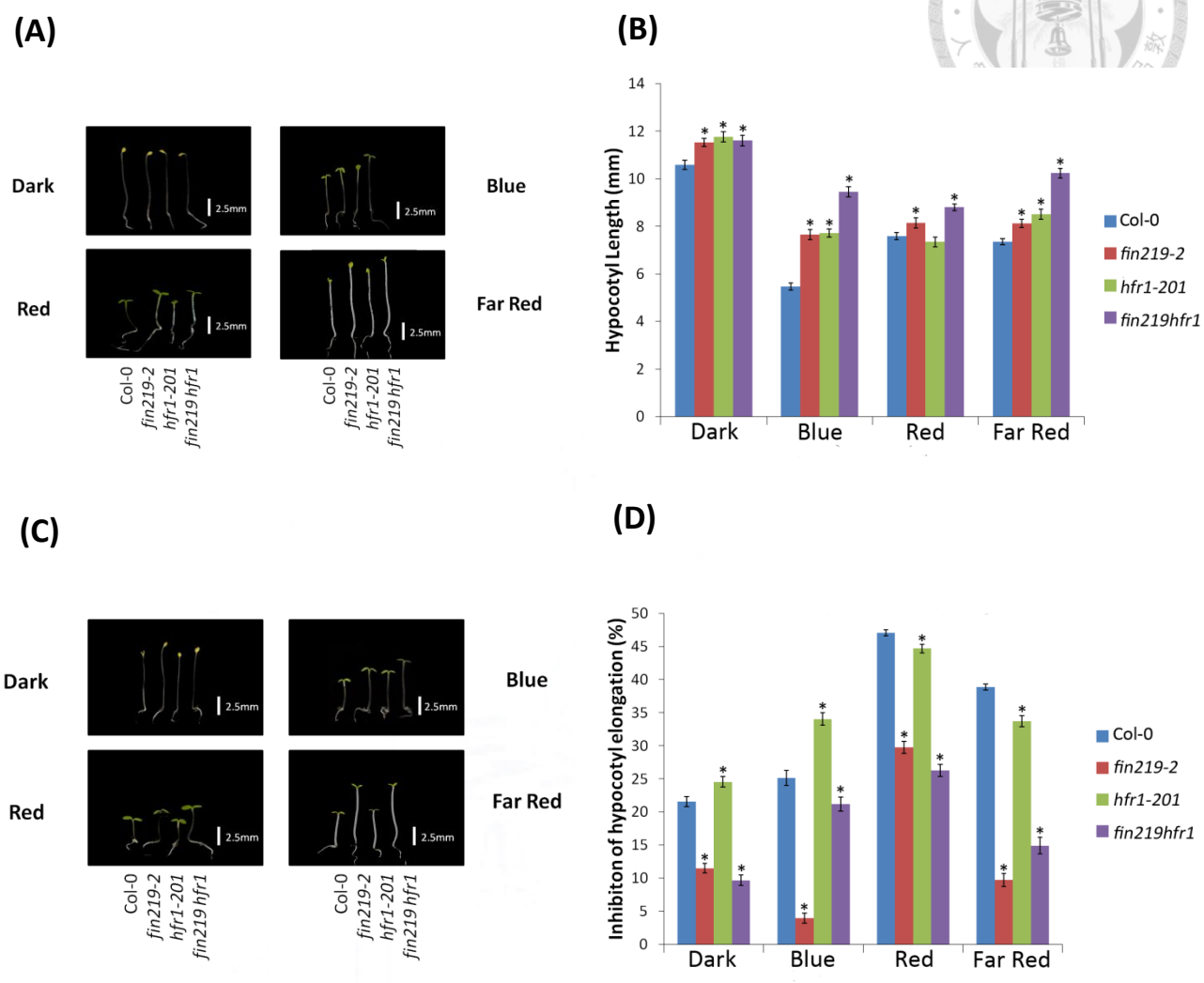

Figure 5. *FIN219* and *HFR1* are positive regulators of hypocotyls elongation in various light conditions.

(A) Phenotype of *fin219*, *hfr1* and *fin219hfr1* seedlings grown in dark, blue, red or far-red light for 3 days.

(B) Quantification of hypocotyl lengths of *fin219*, *hfr1* and *fin219hfr1* seedlings grown in dark, blue, red or far-red light for 3 days.

(C) Phenotype of *fin219*, *hfr1* and *fin219hfr1* seedlings grown on media containing 50 μM MeJA in the dark, blue, red or far-red light condition for 3 days.

(D) Quantification of hypocotyl inhibition of seedlings grown on media containing 50 μM MeJA in the dark, blue, red or far-red light for 3 days.

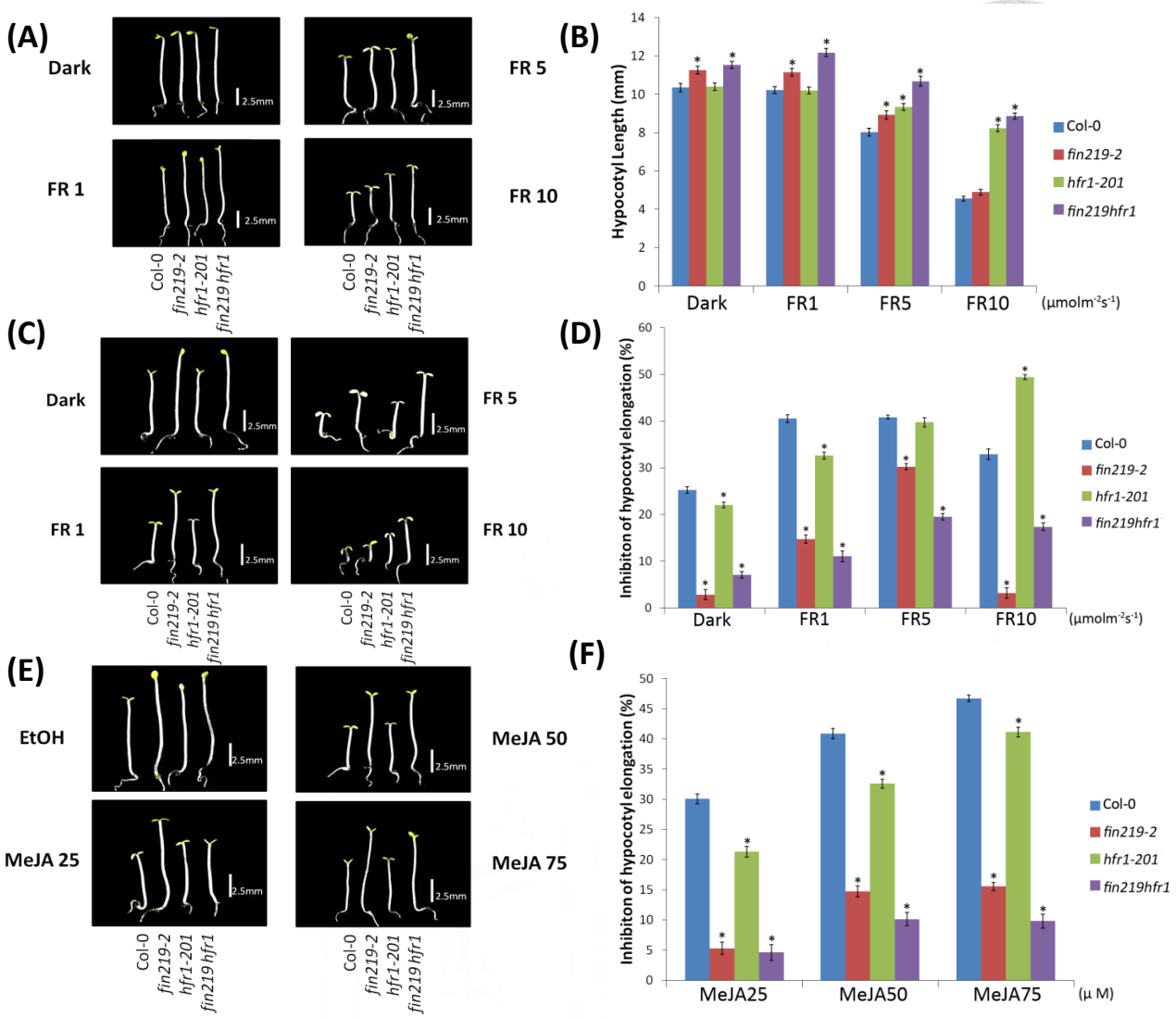

Figure 6. *FIN219* and *HFR1* are positive regulators in both FR light and JA signalings.

(A) Phenotype of *fin219*, *hfr1* and *fin219hfr1* seedlings grown in the dark, 1, 5 or 10 μmolm^{-2}s^{-1} of far-red light for 3 days.

(B) Quantification of hypocotyl length of *fin219*, *hfr1* and *fin219hfr1* seedlings grown in dark, 1, 5 or 10 μmolm^{-2}s^{-1} of far-red light for 3 days.

(C) Visual phenotype of *fin219*, *hfr1* and *fin219hfr1* seedlings were grown on media containing 50 μM MeJA in dark, 1, 5 or 10 μmolm^{-2}s^{-1} of far-red light condition for 3 days.

(D) Quantification of hypocotyl inhibition of seedlings grown on media containing 50 μM MeJA in dark, 1, 5 or 10 μmolm^{-2}s^{-1} of far-red light for 3 days.

(E) Visual phenotype of *fin219*, *hfr1* and *fin219hfr1* seedlings were grown on media containing 25, 50 or 75 μM MeJA in 1 μmolm^{-2}s^{-1} of far-red light condition for 3 days.

(F) Quantification of hypocotyl inhibition of seedlings grown on media containing 25, 50 or 75 μM MeJA in 1 μmolm^{-2}s^{-1} of far-red light for 3 days.

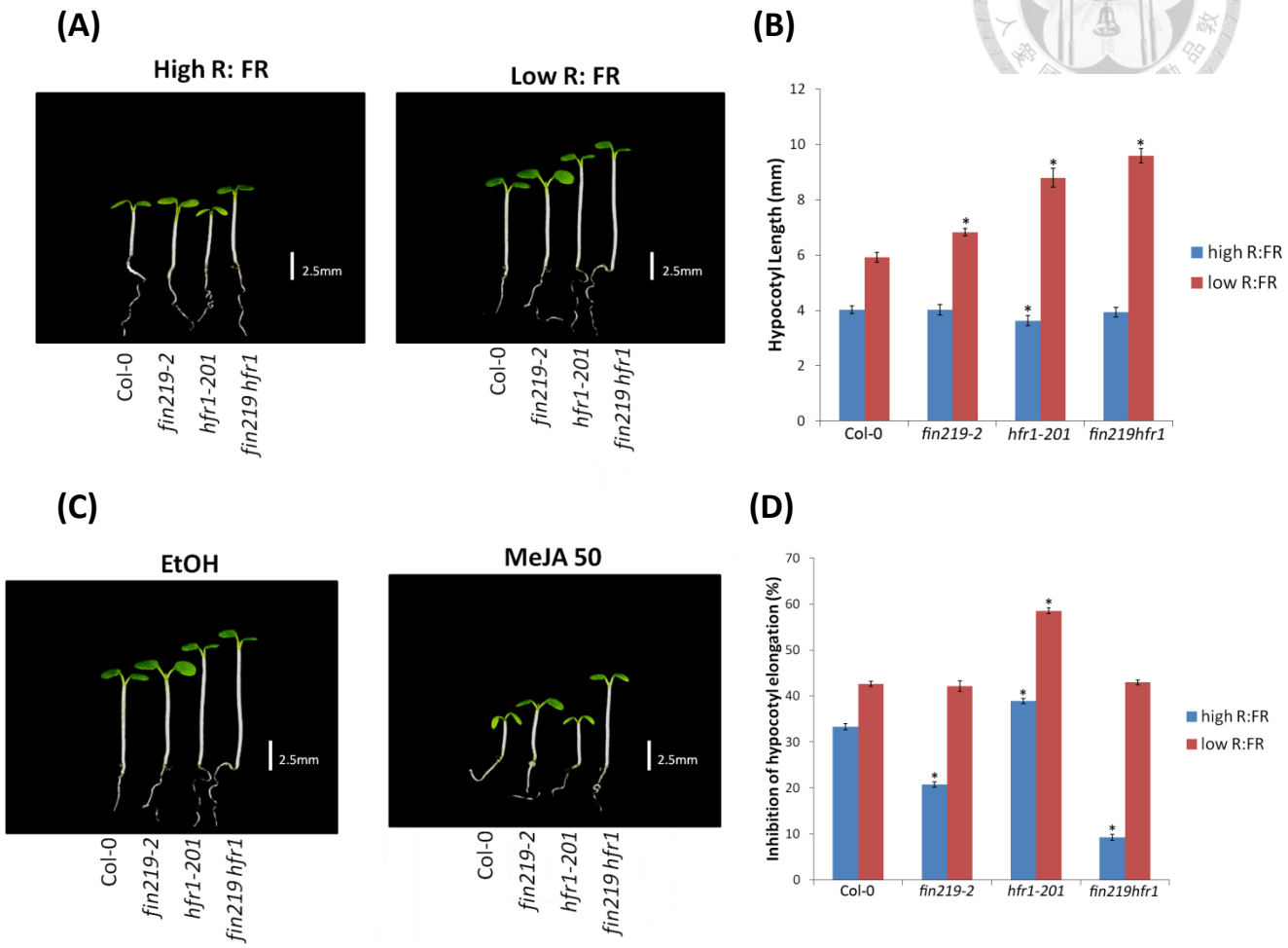

Figure 7. *FIN219* and *HFR1* are negative regulators in low R: FR light condition.

(A) Phenotype of *fin219*, *hfr1* and *fin219hfr1* seedlings grown in high R: FR or low R: FR condition for 7 days.

(B) Quantification of hypocotyl lengths of *fin219*, *hfr1* and *fin219hfr1* seedlings grown in high R: FR or low R: FR condition for 7 days.

(C) Phenotype of *fin219*, *hfr1* and *fin219hfr1* seedling grown on media containing 50 μM MeJA in low R: FR condition for 7 days.

(D) Quantification of hypocotyl lengths of *fin219*, *hfr1* and *fin219hfr1* seedlings grown on media containing 50 μM MeJA in low R: FR condition for 7 days. In (A) to (D), all seedlings were first grown in high R: FR condition for 2 days, in which the red light intensity was 1.159 $\mu molm^{-2}s^{-1}$, the far-red light intensity was 2.01 $\mu molm^{-2}s^{-1}$, and the white light intensity was 21.355 $\mu molm^{-2}s^{-1}$. After 2 days, low R: FR treatment was changed with far-red light 23.77 $\mu molm^{-2}s^{-1}$ intensity, whereas the red and white light condition was still the same. The low R: FR condition was treated for 4 days.

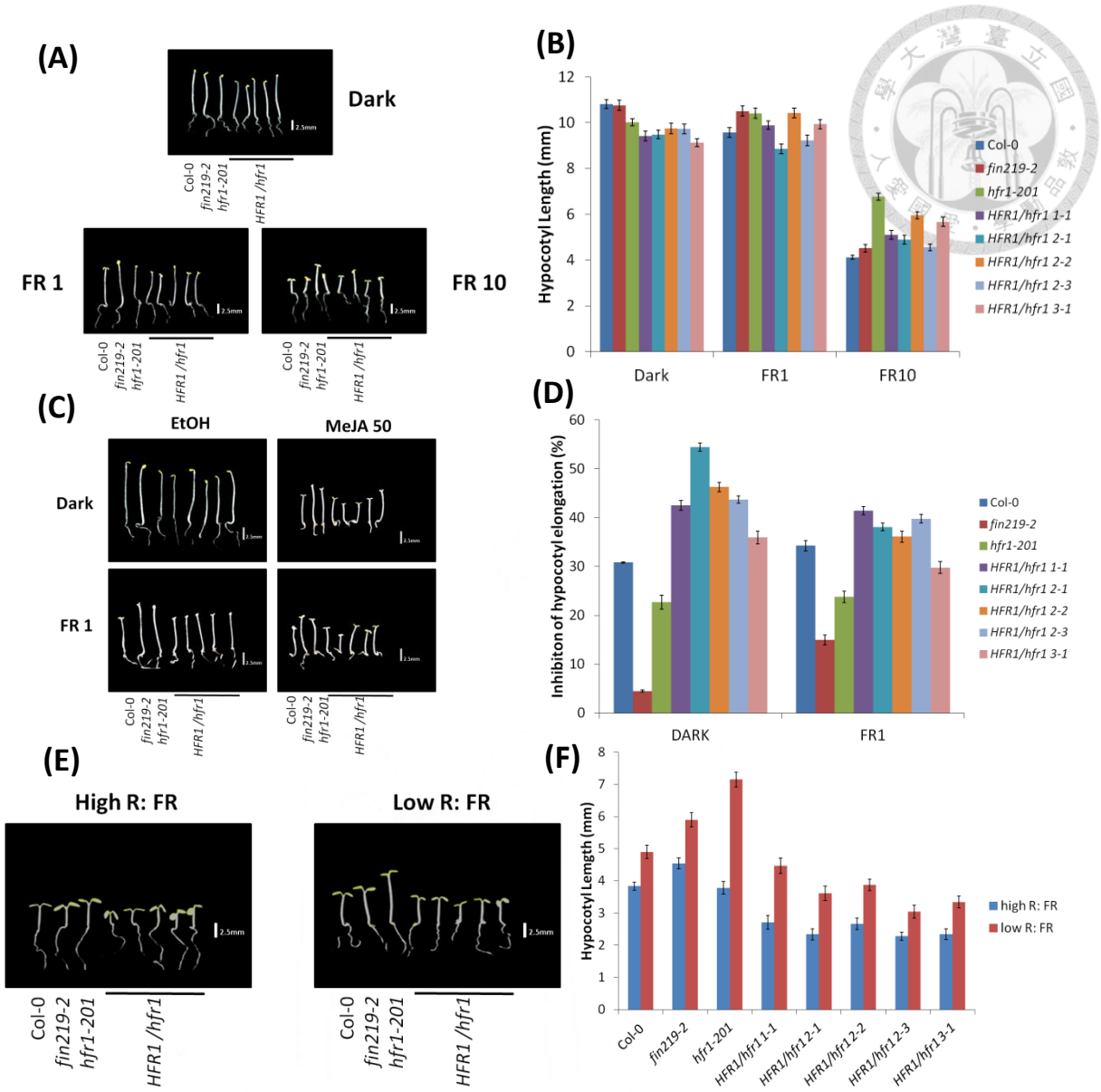

Figure 8. *HFR1* overexpression rescued the phenotypes of *hfr1*.

(A) Phenotype of hypocotyls of the 3-day-old seedlings grown in darkness, low far-red light (1 μmolm^{-2}s^{-1}) and high far-red light (10 μmolm^{-2}s^{-1}).

(B) Quantification of hypocotyl lengths of seedlings shown in (A).

(C) Phenotype of hypocotyls of the 3-day-old seedlings grown on media containing 50 μM MeJA in darkness or low far-red light (1 μmolm^{-2}s^{-1}).

(D) Quantification of the inhibition of elongation hypocotyls of seedlings shown in (C).

(E) Phenotype of hypocotyls of the 7-day-old seedlings grown under high R: FR or low R: FR condition for 7 days.

(F) Quantification of hypocotyl lengths of seedlings shown in (E). The *error bars* denote standard error of the mean (n=30). Significant difference between samples was achieved by ANOVA and Duncan's HSD tests with $P<0.05$. The error bars denote standard error of the mean (n=30).

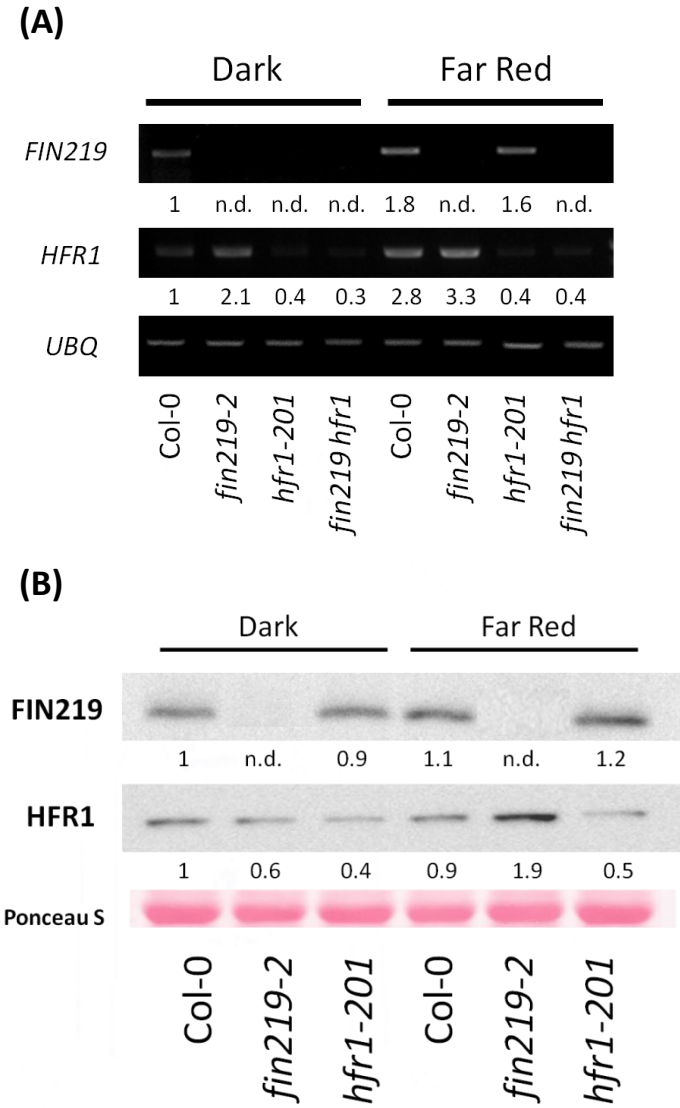

Figure 9. Both *FIN219* and *HFR1* may regulate with each other in the dark or FR light.

(A) Analysis of *FIN219* and *HFR1* expression by RT-PCR in *fin219-2*, *hfr1-201* and *fin219hfr1*. Samples were treated with far-red light for 3 days before collection. *FIN219* and *HFR1* gene specific primers were used for amplification. *UBQ10* was included as an internal control.

(B) Analysis of FIN219 and HFR1 expression by Western blots in *fin219-2* and *hfr1-201*. Samples were treated with far-red light for 3 days before collection. FIN219 and HFR1 protein specific antibodies were used for detection. Ponceau S staining is shown as loading control.

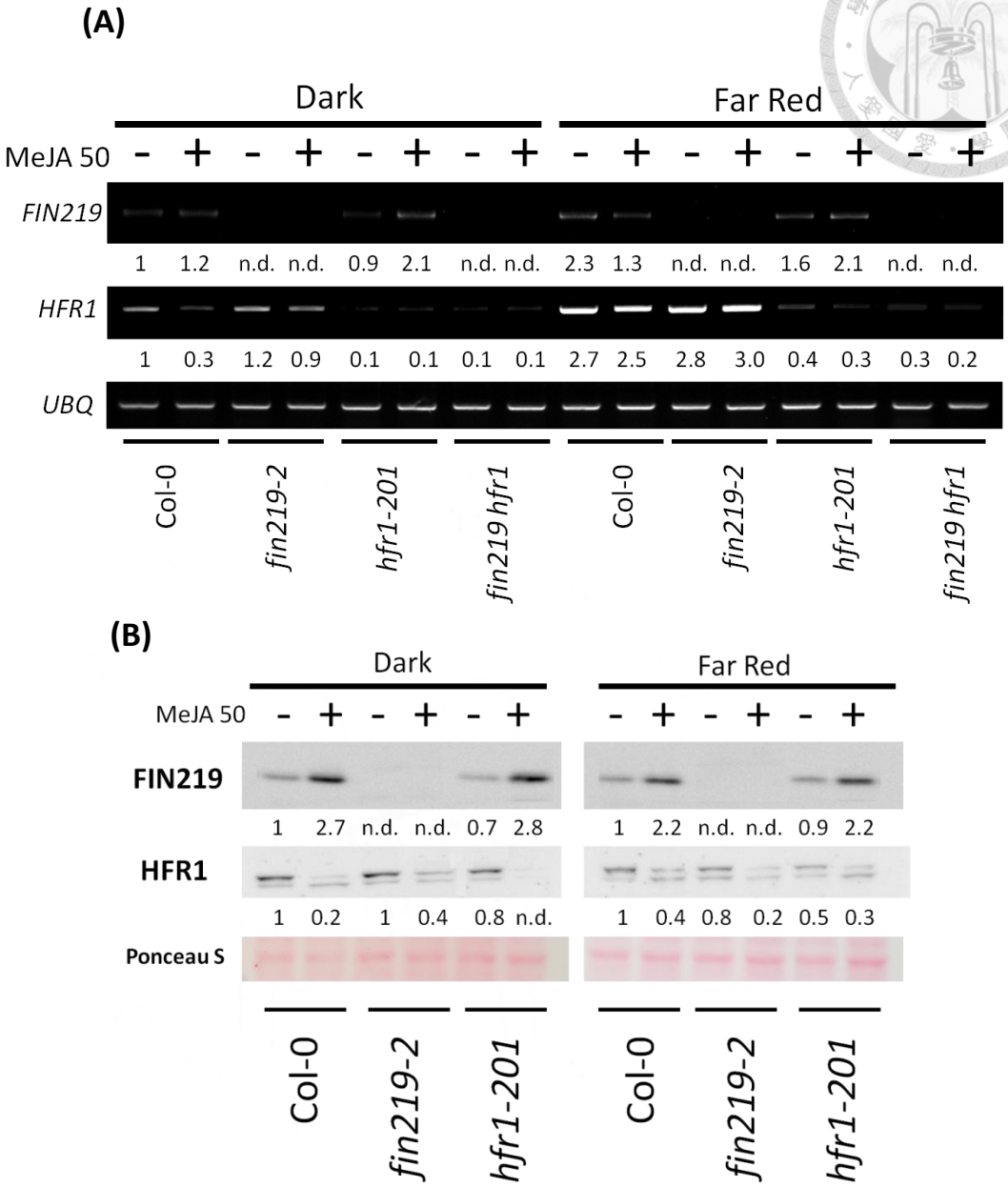

Figure 10. *HFR1* is affected by JA and affects the downstream genes.

(A) Analysis of *FIN219* and *HFR1* expression by RT-PCR in *fin219-2*, *hfr1-201* and *fin219hfr1*. Samples were grown on media containing 50 μM MeJA and treated with far-red light for 3 days before collection. *FIN219* and *HFR1* gene specific primers were used for amplification. *UBQ10* was included as an internal control.

(B) Analysis of FIN219 and HFR1 expression by Western blots in *fin219-2* and *hfr1-201*. Samples were grown on media containing 50 μM MeJA and treated with far-red light for 3 days before collection. FIN219 and HFR1 protein specific antibodies were used for detection. Ponceau S staining is shown as loading control.

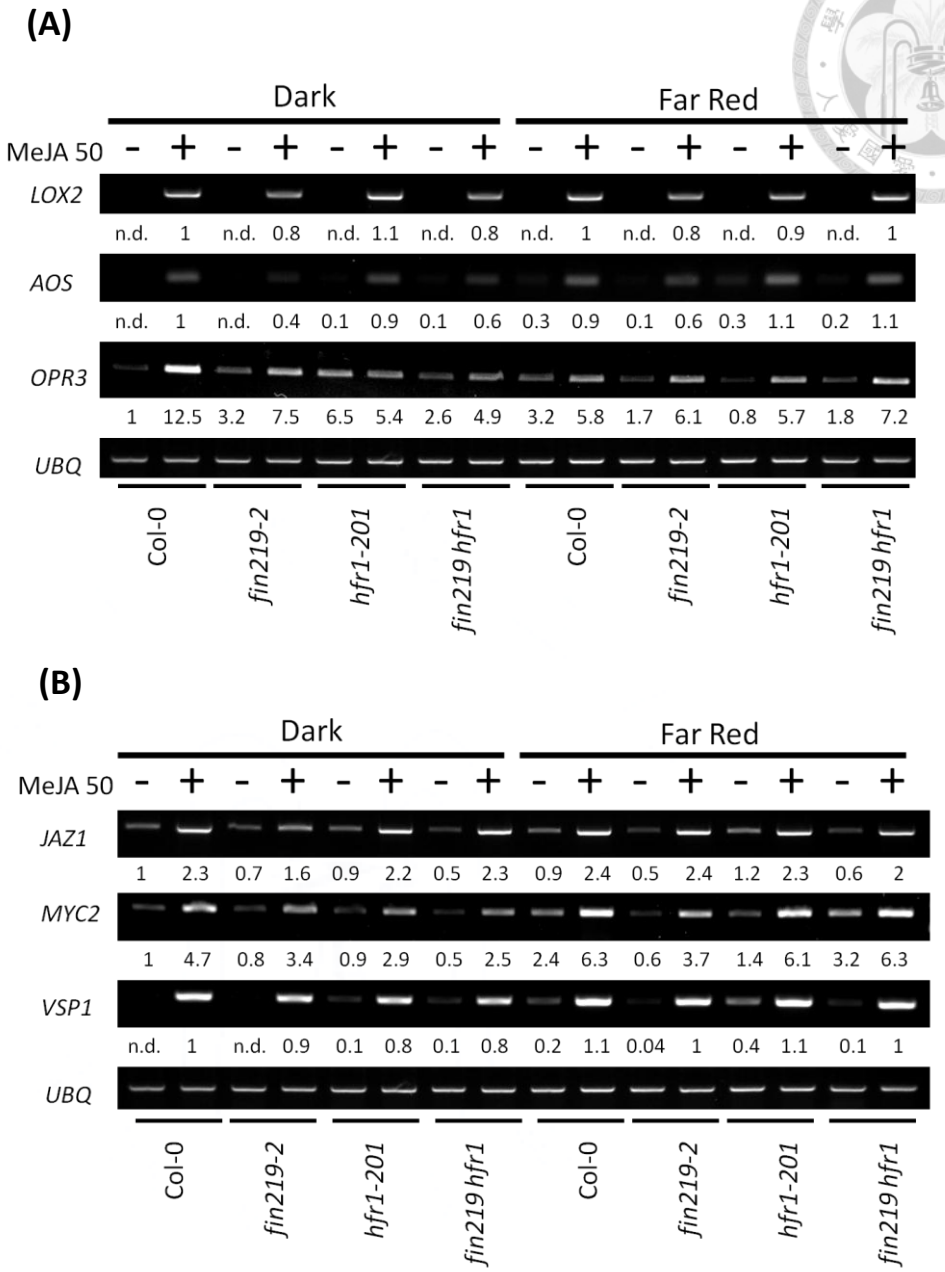

Figure 11. FIN219 and HFR1 affect the expression of JA biosynthesis genes in both the dark and FR light.

(A) Analysis of JA synthesis genes *LOX2*, *AOS* and *OPR3* by RT-PCR in *fin219-2*, *hfr1-201* and *fin219hfr1*.

(B) Analysis of JA signaling genes *JAZ1*, *MYC2* and *VSP1* by RT-PCR in *fin219-2*, *hfr1-201* and *fin219hfr1*. In (A) and (B), Samples were grown on media containing 50 μM MeJA and treated with far-red light for 3 days before collection. *FIN219* and *HFR1* gene specific primers were used for amplification. *UBQ10* was included as an internal control.

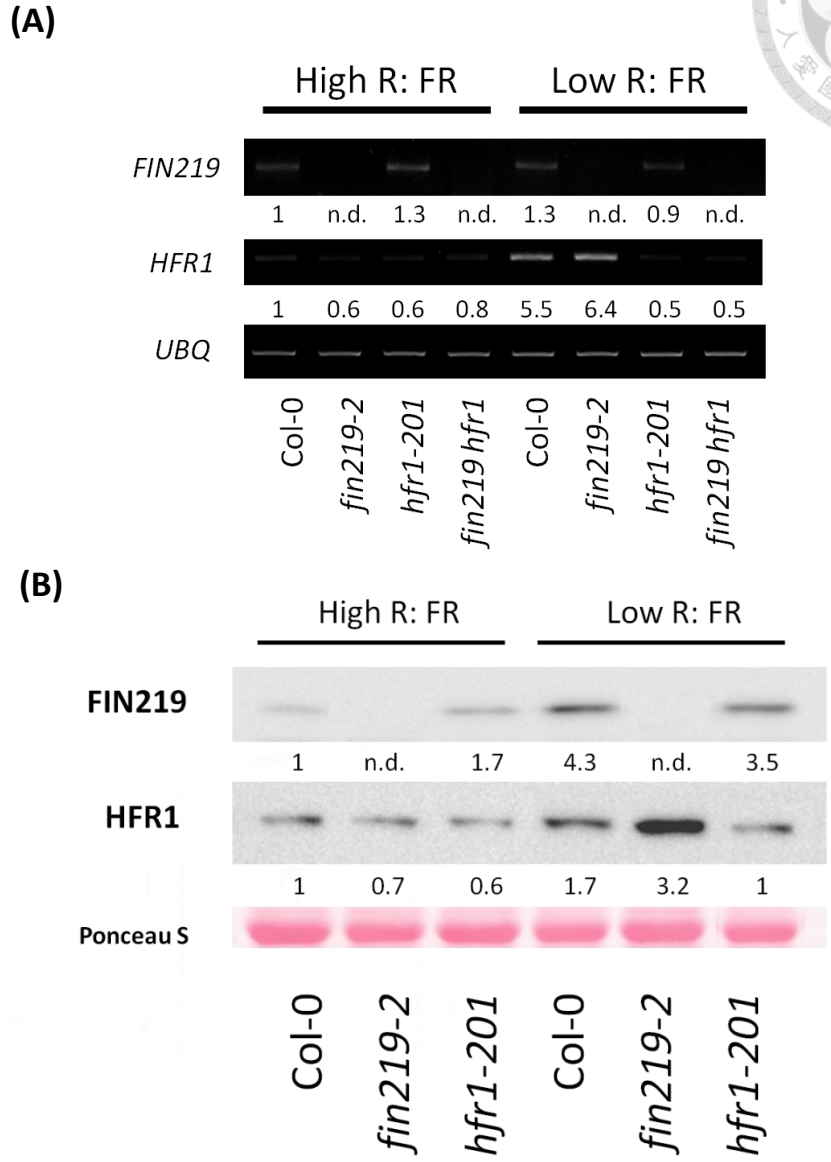

Figure 12. HFR1 and FIN219 proteins were induced by low R: FR treatment.

(A) Analysis of *FIN219* and *HFR1* expression by RT-PCR in *fin219-2*, *hfr1-201* and *fin219hfr1*. Samples were first treated in high R: FR condition for 2 days and then transferred to low R: FR condition for 4 days before collection. *FIN219* and *HFR1* gene specific primers were used for amplification. *UBQ10* was included as an internal control.

(B) Analysis of FIN219 and HFR1 expression by Western blots in *fin219-2* and *hfr1-201*. Samples were first treated in high R: FR condition for 2 days and then transferred to low R: FR condition for 4 days before collection. FIN219 and HFR1 protein specific antibodies were used for detection. Ponceau S staining is shown as loading control.

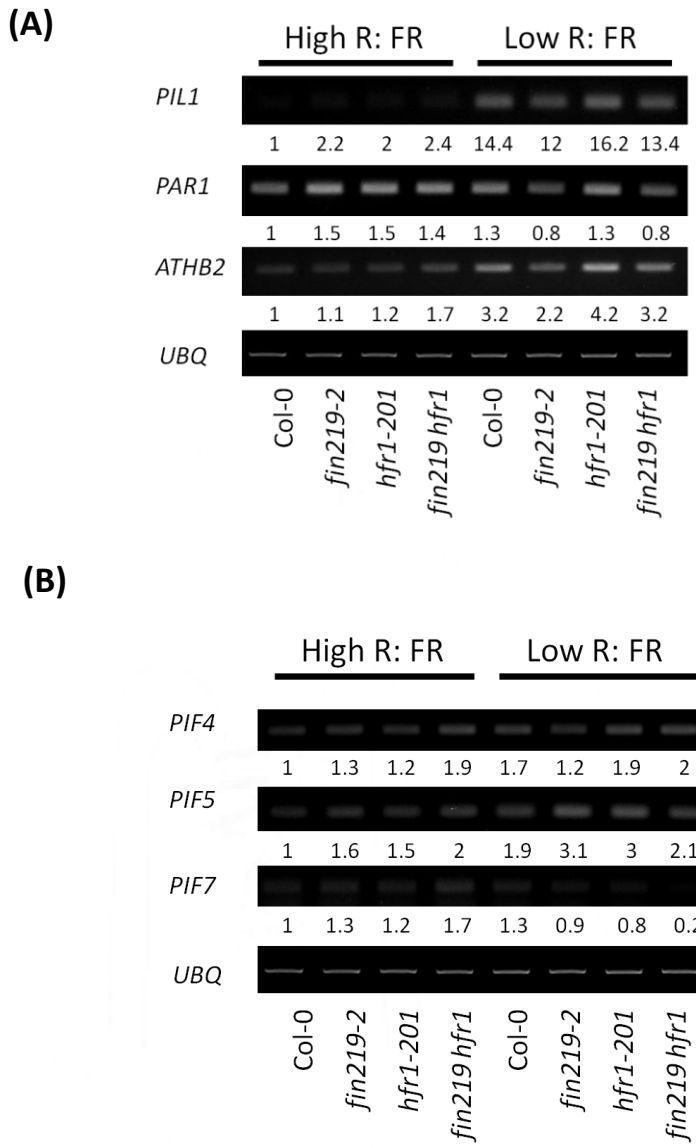

Figure 13. Both HFR1 and FIN219 modulate the expression of the shading components.

(A) Analysis of SAS signaling genes *PIL1*, *PAR1* and *ATHB2* by RT-PCR in *fin219-2*, *hfr1-201* and *fin219hfr1*.

(B) Analysis of SAS related genes *PIF4*, *PIF5* and *PIF7* by RT-PCR in *fin219-2*, *hfr1-201* and *fin219hfr1*. In A and B, Samples were first treated in high R: FR condition for 2 days and then transferred to low R: FR condition for 4 days before collection. *FIN219* and *HFR1* gene specific primers were used for amplification. *UBQ10* was included as an internal control.

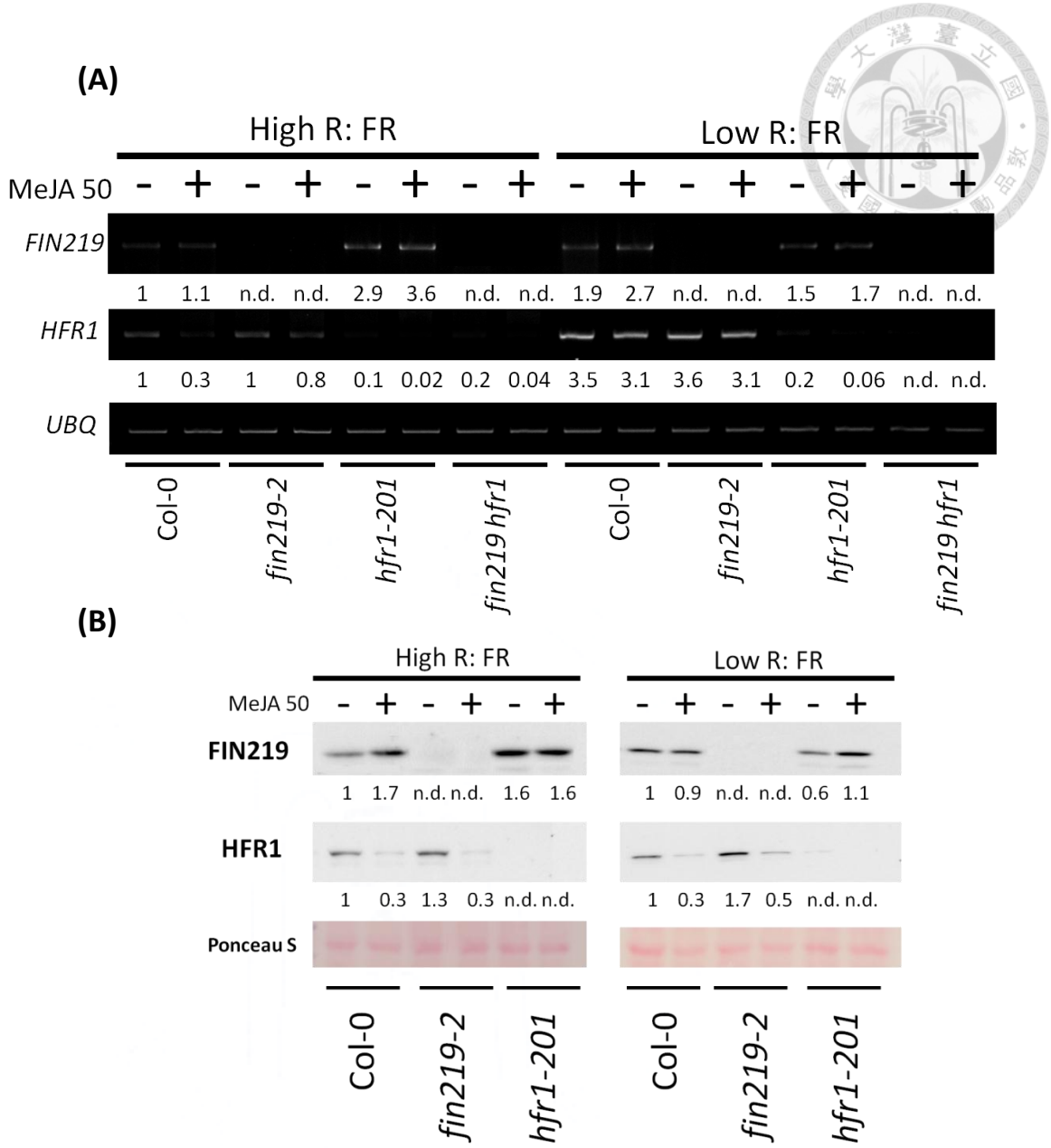

Figure 14. FIN219 and HFR1 participated in JA and SAS signaling.

(A) Analysis of *FIN219* and *HFR1* expression by RT-PCR in *fin219-2*, *hfr1-201* and *fin219hfr1*. Samples were grown on media containing 50 μM MeJA and first treated in high R: FR condition for 2 days and then transferred to low R: FR condition for 4 days before collection. *FIN219* and *HFR1* gene specific primers were used for amplification. *UBQ10* was included as an internal control.

(B) Analysis of FIN219 and HFR1 expression by Western blots in *fin219-2* and *hfr1-201*. Samples were grown on media containing 50 μM MeJA and first treated in high R: FR condition for 2 days and then transferred to low R: FR condition for 4 days before collection. FIN219 and HFR1 protein specific antibodies were used for detection. Ponceau S staining is shown as loading control.

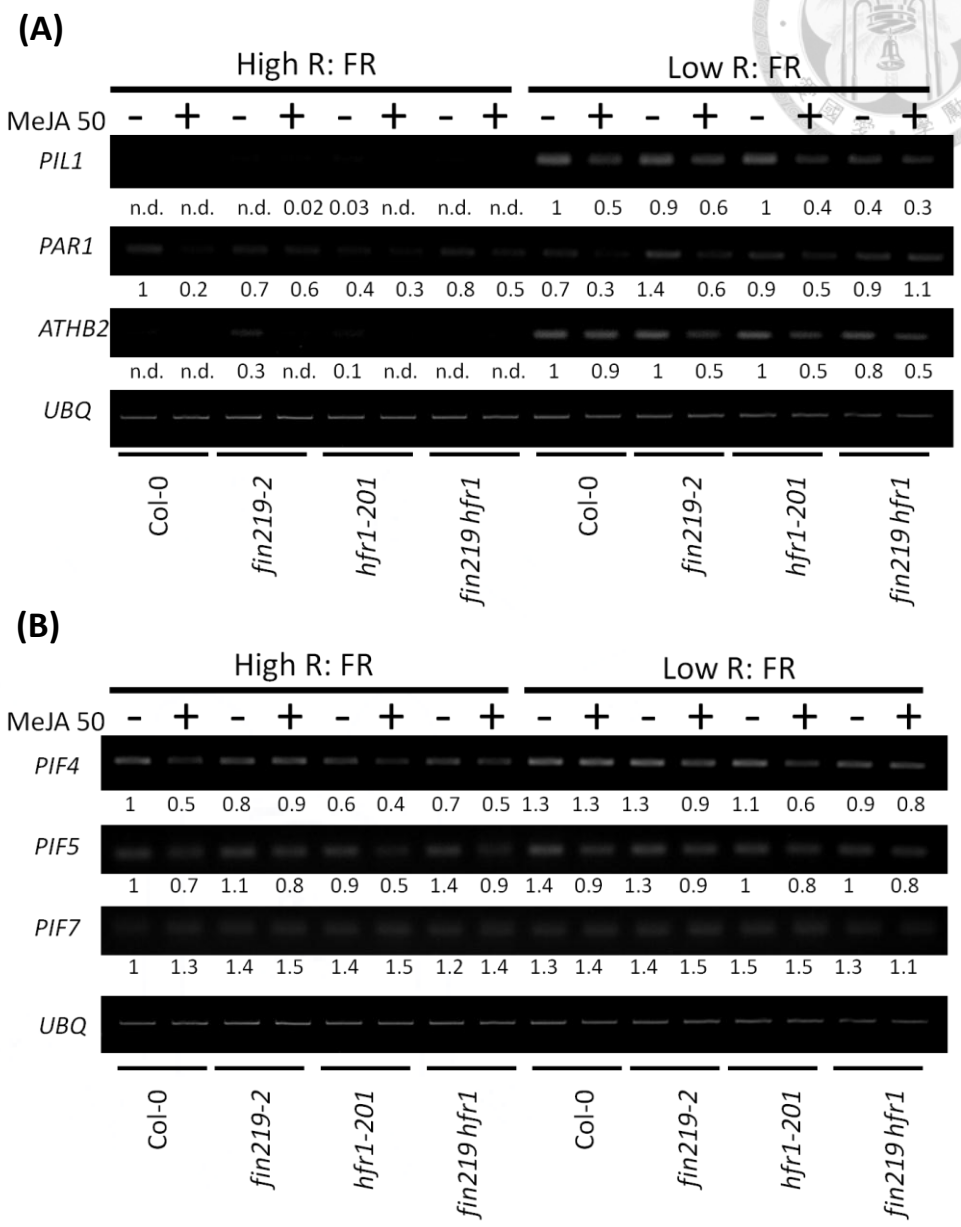

Figure 15. FIN219 and HFR1 affect the expression of shading components with MeJA treatment under low R: FR condition.

(A) Analysis of SAS signaling genes *PIL1*, *PAR1* and *ATHB2* by RT-PCR in *fin219-2*, *hfr1-201* and *fin219hfr1*.

(B) Analysis of SAS related genes *PIF4*, *PIF5* and *PIF7* by RT-PCR in *fin219-2*, *hfr1-201* and *fin219hfr1*. In (A) and (B), Samples were grown on media containing 50 μM MeJA and first treated in high R: FR condition for 2 days and then transferred to low R: FR condition for 4 days before collection. *FIN219* and *HFR1* gene specific primers were used for amplification. *UBQ10* was included as an internal control.

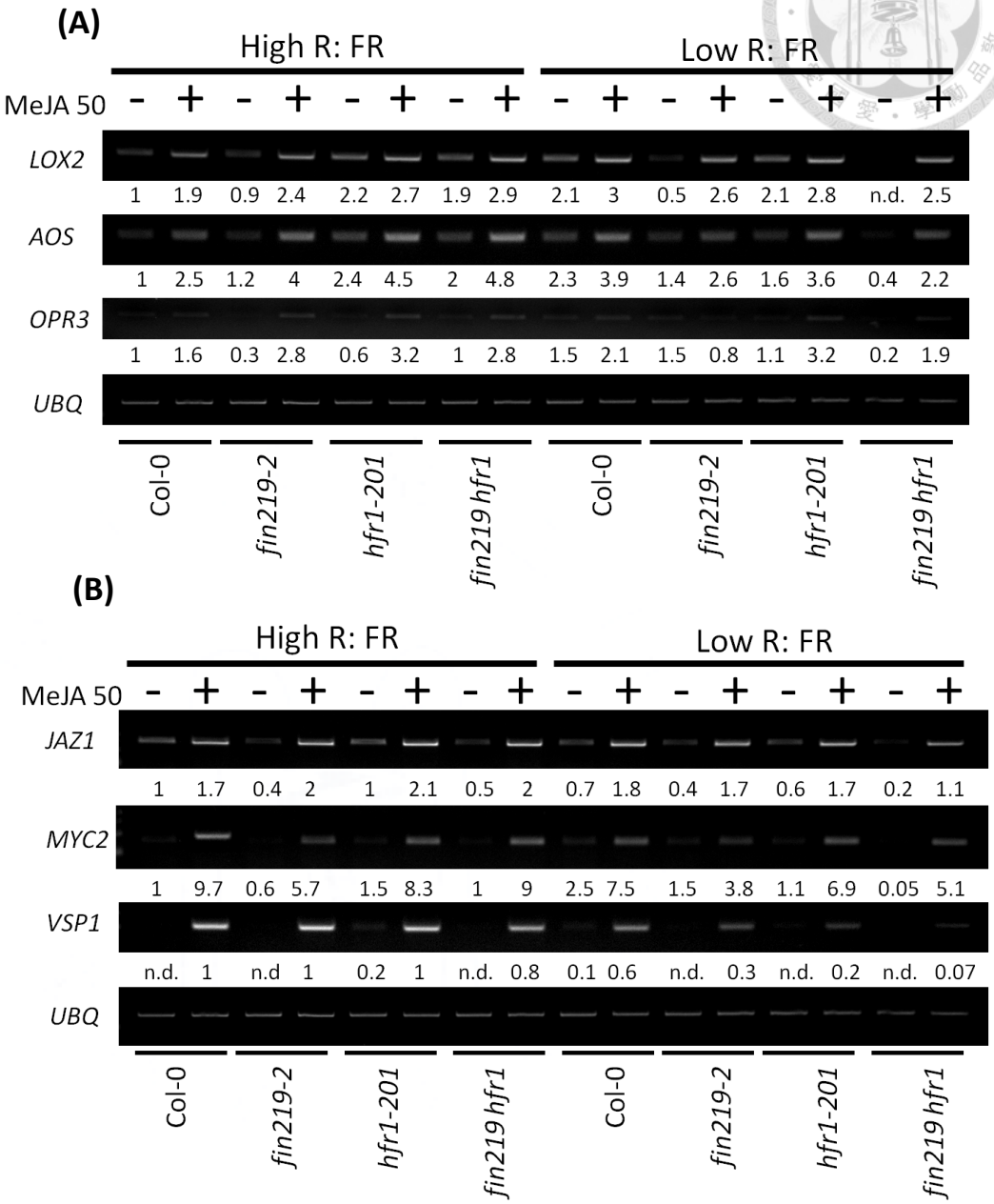

Figure 16. The JA biosynthetic genes were still induced by MeJA under shading treatment, but the JA signaling components were decreased.

(A) Analysis of JA synthesis genes *LOX2*, *AOS* and *OPR3* by RT-PCR in *fin219-2*, *hfr1-201* and *fin219hfr1*.

(B) Analysis of JA synthesis genes *LOX2*, *AOS* and *OPR3* by RT-PCR in *fin219-2*, *hfr1-201* and *fin219hfr1*. In (A) and (B), Samples were grown on media containing 50 μM MeJA and first treated in high R: FR condition for 2 days and then transferred to low R: FR condition for 4 days before collection. *FIN219* and *HFR1* gene specific primers were used for amplification. *UBQ10* was included as an internal control.

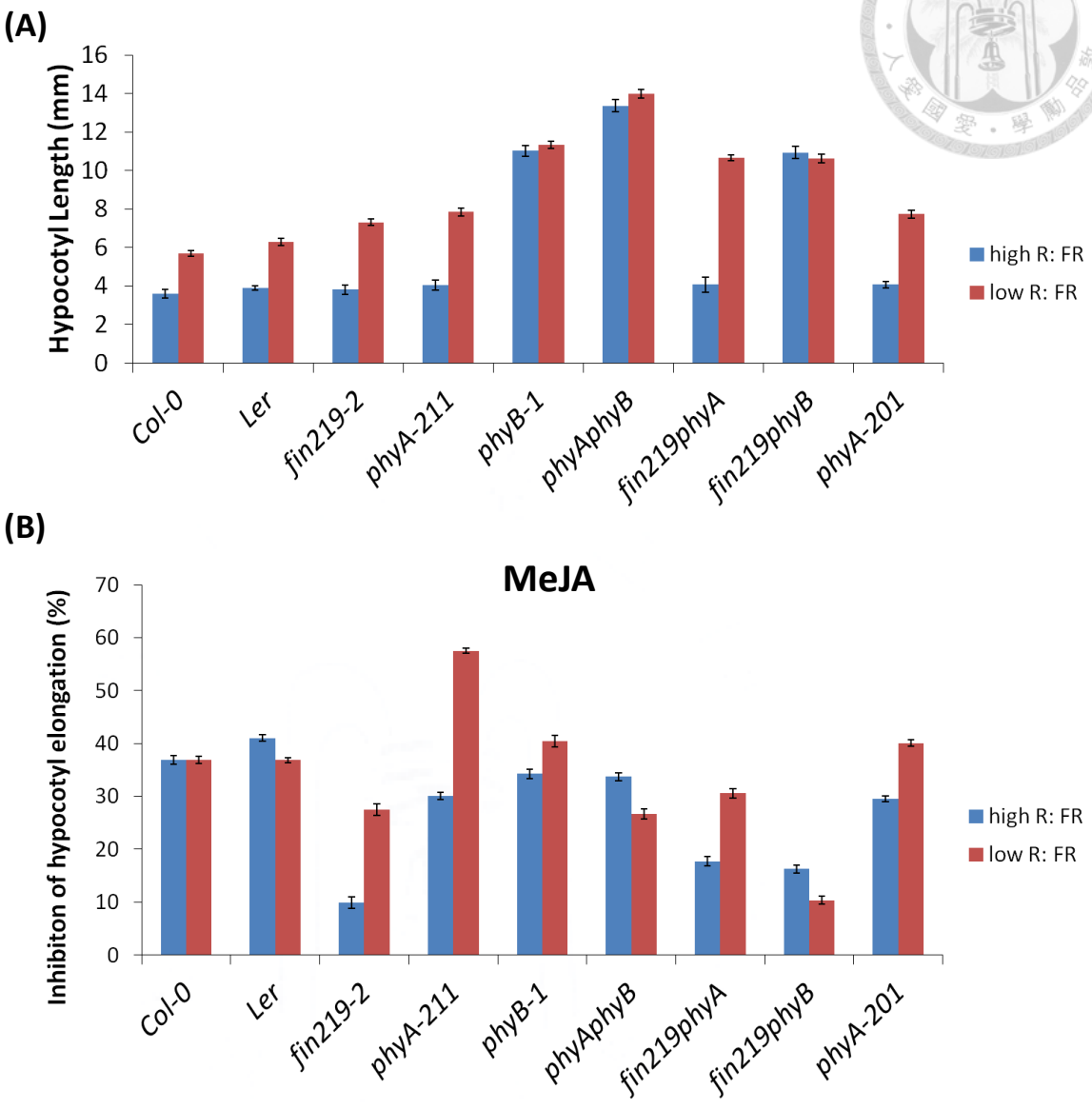

Figure 17. phyA and phyB interact with FIN2*19* genetically under shading condition.

(A) Quantification of hypocotyl lengths of *fin219*, *phyA-211*, *phyB-1* and their double mutant seedlings grown in high R: FR or low R: FR condition for 7 days.

(B) Quantification of hypocotyl lengths of *fin219*, *phyA-211*, *phyB-1* and their double mutant seedlings grown on media containing 50 μM MeJA in low R: FR condition for 7 days. In (A) and (B), all seedlings were first grown in high R: FR condition for 2 days, in which the red light intensity was 1.159 $\mu molm^{-2}s^{-1}$, the far-red light intensity was 2.01 $\mu molm^{-2}s^{-1}$, and the white light intensity was 21.355 $\mu molm^{-2}s^{-1}$. After 2 days, low R: FR treatment was changed with far-red light 23.77 $\mu molm^{-2}s^{-1}$ intensity, whereas the red and white light condition was still the same. The low R: FR condition was treated for 4 days.

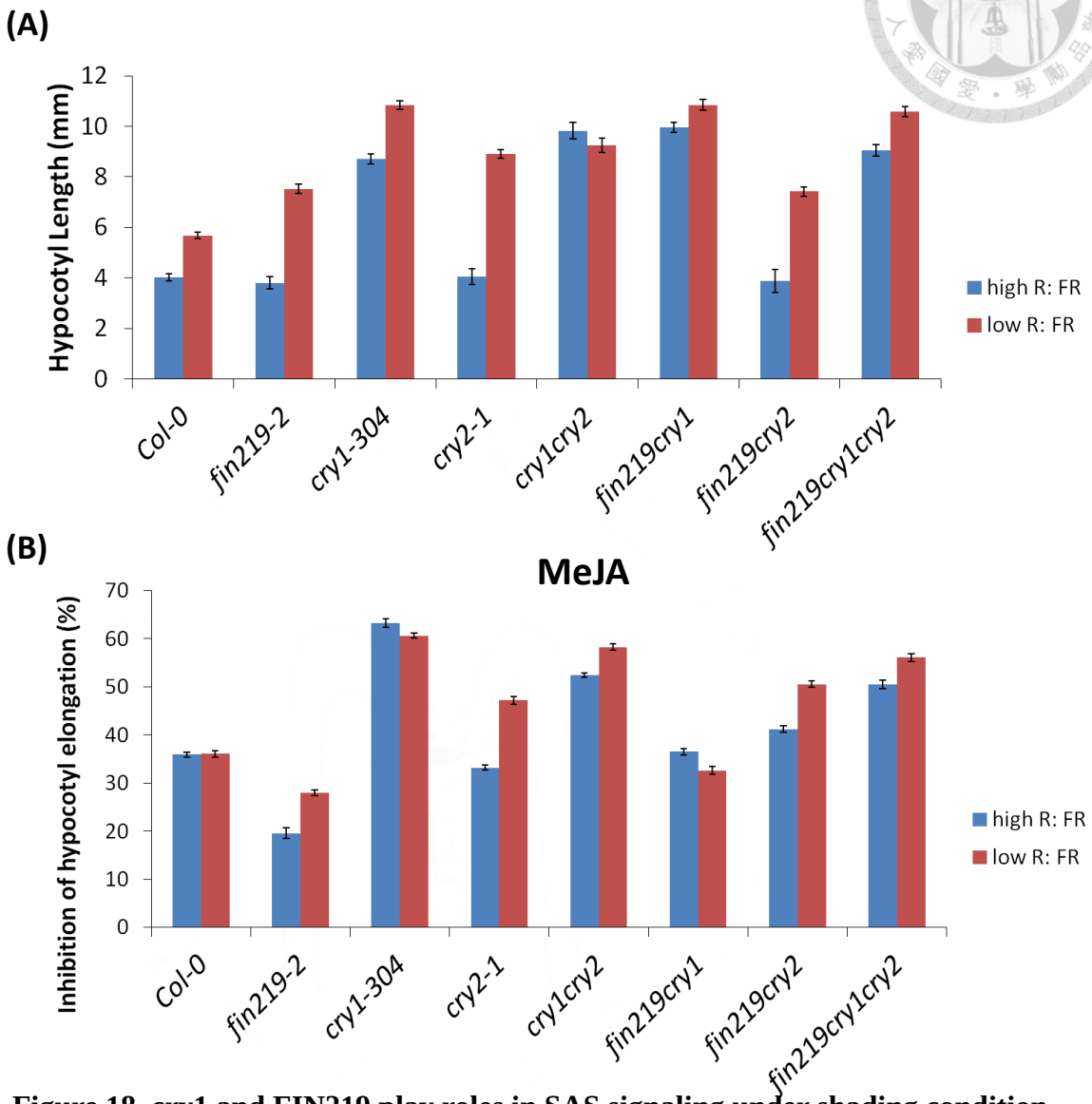

Figure 18. cry1 and FIN219 play roles in SAS signaling under shading condition.

(A) Quantification of hypocotyl lengths of *fin219*, *cry1-304*, *cry2-1* and their double mutant seedlings grown in high R: FR or low R: FR condition for 7 days.

(B) Quantification of hypocotyl lengths of *fin219*, *cry1-304*, *cry2-1* and their double mutant seedlings grown on media containing 50 μM MeJA in low R: FR condition for 7 days. In (A) and (B), all seedlings were first grown in high R: FR condition for 2 days, in which the red light intensity was 1.159 $\mu molm^{-2}s^{-1}$, the far-red light intensity was 2.01 $\mu molm^{-2}s^{-1}$, and the white light intensity was 21.355 $\mu molm^{-2}s^{-1}$. After 2 days, low R: FR treatment was changed with far-red light 23.77 $\mu molm^{-2}s^{-1}$ intensity, whereas the red and white light condition was still the same. The low R: FR condition was treated for 4 days.

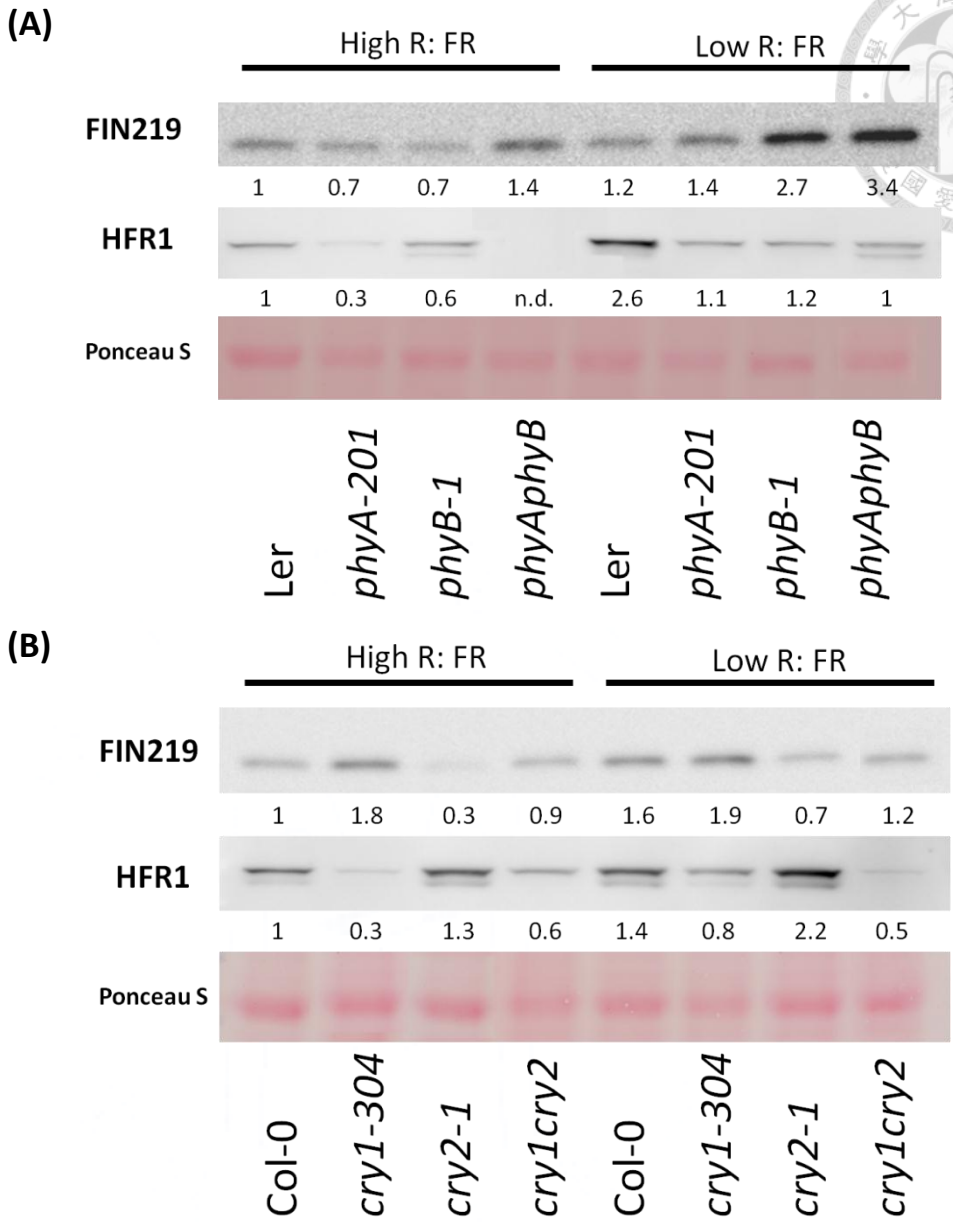

Figure 19. FIN219 and HFR1 were regulated by photoreceptors in response to low R: FR condition.

(A) Analysis of FIN219 and HFR1 expression by western blots in *phyA-211*, *phyB-1* and their double mutant seedlings grown in high R: FR or low R: FR condition for 7 days.

(B) Analysis of FIN219 and HFR1 expression by Western blots in *cry1-304*, *cry2-1* and their double mutant seedlings grown in high R: FR or low R: FR condition for 7 days. In (A) and (B), Samples were first treated in high R: FR condition for 2 days and then transferred to low R: FR condition for 4 days before collection. FIN219 and HFR1 protein specific antibodies were used for detection. Ponceau S staining is shown as loading control.

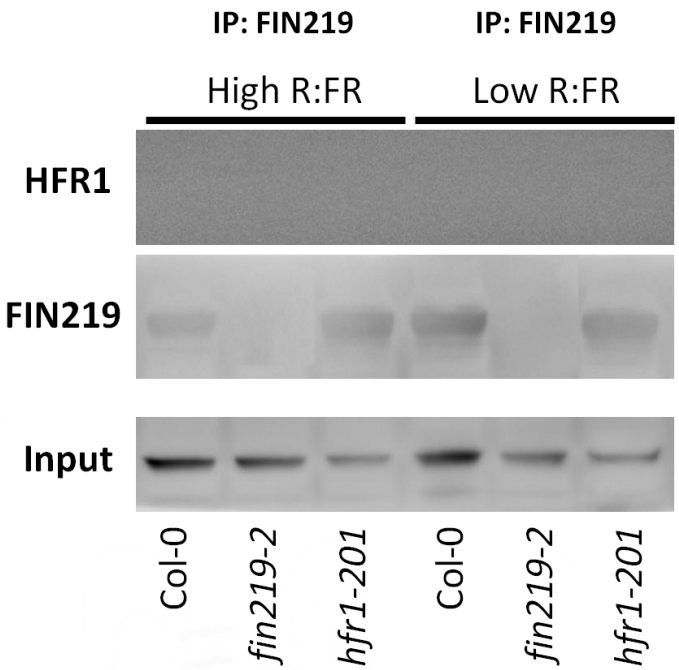

Figure 20. HFR1 did not physically interact with FIN219 in shading condition.

Analysis of FIN219 and HFR1 expression by Western blots in co-immunoprecipitation. The *fin219-2*, *hfr1-201* and wild-type seedlings were grown in high R: FR or low R: FR condition for 7 days. The analysis of HFR1 expression by Western blots was detected as input control. IP: immunoprecipitation.

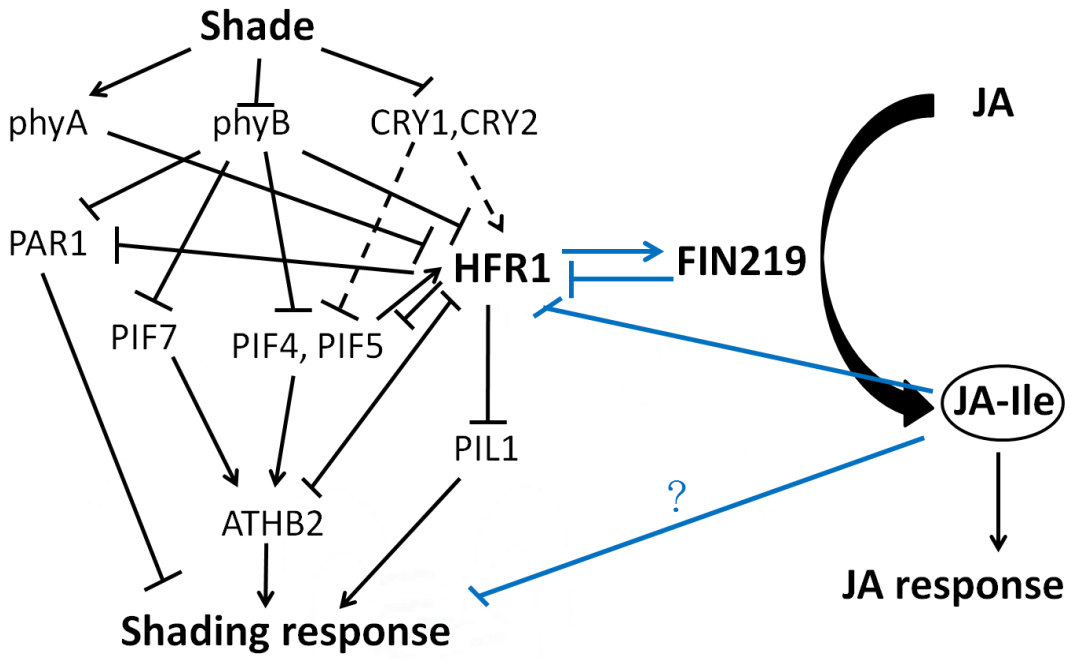

Figure 21. A model to illustrate the integration of JA and SAS signalings through the regulation between FIN219 and HFR1.

REFERENCES

Ahmad, M., and Cashmore, A.R. (1993). *HY4* gene of *A. thaliana* encodes a protein with characteristics of a blue light photoreceptor. Nature **366:** 162-166.

Ahmad, M., Lin, C., and Cashmore, A.R. (1995). Mutations throughout an *Arabidopsis* blue-light photoreceptor impair blue-light-responsive anthocyanin accumulation and inhibition of hypocotyl elongation. Plant J. **8:** 653-658.

Bae, G., and Choi, G. (2008). Decoding of light signals by plant phytochromes and their interacting proteins. Annu. Rev. Plant Biol. **59:** 281-311.

Barnes, S.A., Nishizawa, N.K., Quaggio, R.B., Whitelam, G.C., and Chua, N.H. (1996). Far-red light blocks greening of *Arabidopsis* a phytochmme A-mediated change in plastid seedlings via development. Plant Cell **8:** 601-615.

Bauer, D., Viczian, A., Kircher, S., Nobis, T., Nitschke, R., Kunkel, T., Panigrahi, K.C.S., Adam, E., Fejes, E., Schafer, E., and Nagy, F. (2004). Constitutive photomorphogenesis 1 and multiple photoreceptors control degradation of phytochrome interacting factor 3, a transcription factor required for light signaling in *Arabidopsis*. Plant Cell **16:** 1433–1445.

Bell, E., Creelman, R.A., and Mullet, J.E. (1995). A chloroplast lipoxygenase is required for wound-induced jasmonic acid accumulation in *Arabidopsis*. Proc. Natl. Acad. Sci. USA **92:** 8675–8679.

Carabelli, M., Possenti, M., Sessa, G., Ciolfi, A., Sassi, M., Morelli, G., and Ruberti, I. (2007). Canopy shade causes a rapid and transient arrest in leaf development through auxin-induced cytokinin oxidase activity. Gene Dev. **21:** 1863-1868.

Casal, J.J. (2012). Shade avoidance. *Arabidopsis* Book **10**

Casal, J.J. (2013). Photoreceptor signaling networks in plant responses to shade. Annu. Rev. Plant Biol. **64:** 403-427.

Cerrudo, I., Keller, M.M., Cargnel, M.D., Demkura, P.V., de Wit, M., Patitucci, M.S., Pierik, R., Pieterse, C.M.J., and Ballare, C.L. (2012). Low red/far-red ratios reduce *Arabidopsis* resistance to *Botrytis cinerea* and jasmonate responses via a COI1-JAZ10-dependent, salicylic acid-independent mechanism. Plnat Physiol. **158:** 2042-2052.

Chen, I.C., Huang, I.C., Liu, M.J., Wang, Z.G., Chung, S.S., and Hsieh, H.L. (2007). Glutathione S-transferase interacting with Far-Red Insensitive 219 is involved in Phytochrome A-mediated signaling in *Arabidopsis*. Plant Physiol. **143:** 1189-1202.

Chen, M., Chory, J., and Fankhauser, C. (2004). Light signal transduction in higher

plants. Annu. Rev. Genet. **38:** 87-117.

Chen, Q.F., Dai, L.Y., Xiao, S., Wang, Y.S., Liu, X.L., and Wang, G.L. (2007). The *COI1* and *DFR* genes are essential for regulation of jasmonate-induced anthocyanin accumulation in *Arabidopsis*. J. Integr. Plant Bio. **49:** 1370-1377.

Cheong, J.J., and Choi, Y.D. (2003). Methyl jasmonate as a vital substance in plants. Trends Genet. **19:** 409-413.

Chini, A., Fonseca, S., Fernandez, G., Adie, B., Chico, J.M., Lorenzo, O., Garcia-Casado, G., Lopez-Vidriero, I., Lozano, F.M., Ponce, M.R., Micol, J.L., and Solano, R. (2007). The JAZ family of repressors is the missing link in jasmonate signalling. Nature **448:** 666-671.

Devlin, P.F., Christie, J.M., and Terry, M.J. (2007). Many hands make light work. J. Exp. Bot. **58:** 3071-3077.

Devlin, P.F., Patel, S.R., and Whitelam, G.C. (1998). Phytochrome E influences internode elongation and flowering time in *Arabidopsis*. Plant Cell **10:** 1479–1487.

Devlin, P.F., Yanovsky, M.J., and Kay, S.A. (2003). A genomic analysis of the shade avoidance response in *Arabidopsis*. Plant Physiol. **133:** 1617–1629.

Djakovic-Petrovic, T., Wit, M., Voesenek, L.A.C.J., and Pierik, R. (2007). DELLA protein function in growth responses to canopy signals. Plant J. **51:** 117-126.

Duek, P.D., and Fankhauser, C. (2003). HFR1, a putative bHLH transcription factor, mediates both phytochrome A and cryptochrome signaling. Plant J. **34:** 827-836.

Fairchild, C.D., Schumaker, M.A., and Quail, P.H. (2000). HFR1 encodes an atypical bHLH protein that acts in phytochrome A signal transduction. Gene Dev. **14:** 2377-2391.

Fankhauser, C., and Chory, J. (2000). RSF1, an *Arabidopsis* locus implicated in phytochrome A signaling. Plant Physiol. **124:** 39-45.

Feng, S., Martinez, C., Gusmaroli, G., Wang, Y., Zhou, J., Wang, F., Chen, L., Yu, L., Iglesias-Pedraz, J.M., Kircher, S., Schäfer, E., Fu, X., Fan, L.M., and Deng, X.W. (2008).Coordinated regulation of *Arabidopsis thaliana* development by light and gibberellins. Nature **451:** 475–479.

Feys, B.J.F., Benedetti, C.E., Penfold, C.N., and Turner, J.G. (1994). *Arabidopsis* mutants selected for resistance to the phytotoxin coronatine are male sterile, insensitive to methyl jasmonate, and resistant to a bacterial pathogen. Plant Cell **6:** 751-759.

Franklin, K.A. (2008). Shade avoidance. New Phytol. **179:** 930-944.

Franklin, K.A., and Quail, P.H. (2010). Phytochrome functions in *Arabidopsis* development. J. Exp. Bot. **61:** 11-24.

Gommers, C.M.M., Visser, E.J.W., Onge, K.R.S., Voesenek, L.A.C.J., and Pierik,

R. (2013). Shade tolerance: when growing tall is not an option. Trends Plant Sci. **18:** 65-71.

Guo, H., Duong, H., Ma, N., and Lin, C. (1999). The *Arabidopsis* blue light receptor cryptochrome 2 is a nuclear protein regulated by a blue light dependent post transcriptional mechanism. Plant J. **19:** 279-287.

Hornitschek, P., Lorrain, S., Zoete, V., Michielin, O., and Fankhauser, C. (2009). Inhibition of the shade avoidance response by formation of non-DNA binding bHLH heterodimers. EMBO J. **28:** 3893-3902.

Hsieh, H.L., Okamoto, H., Wang, M., Ang, L.H., Matsui, M., Goodman, H., and Deng, X.W. (2000). FIN219, an auxin-regulated gene, defines a link between phytochrome A and the downstream regulator COP1 in light control of *Arabidopsis* development. Genes Dev. **14:** 1958-1970.

Ishiguro,S., Kawai-Oda, A., Ueda, J., Nishida, I., and Okada, K. (2001). The *DEFECTIVE IN ANTHER DEHISCENCE1* Gene Encodes a Novel Phospholipase A1 Catalyzing the Initial Step of Jasmonic Acid Biosynthesis, Which Synchronizes Pollen Maturation, Anther Dehiscence, and Flower Opening in *Arabidopsis*. Plant Cell **13:** 2191-2209.

Jang, I.C., Yang, J.Y., Seo, H.S., and Chua, N.H. (2005). HFR1 is targeted by COP1 E3 ligase for post-translational proteolysis during phytochrome A signaling. Gene Dev. **19:** 593-602.

Johnson, E., Bradley, M., Harberd, N.P., and Whitelam, C.C. (1994). Photoresponses of light-crown *phyA* mutants of *Arabidopsis*. Plant Physiol. **105:** 141-149.

Kazan, K., and Manners, J.M. (2011). The interplay between light and jasmonate signalling during defence and development. J. Exp. Bot. **62:** 4087-4100.

Keuskamp, D.H., Sasidharan, R., and Pierik, R. (2010). Physiological regulation and functional significance of shade avoidance responses to neighbors. Plant Signal. Behav. **5:** 655–662.

Kim, J., Yi, H., Choi, G., Shin, B., Song, P.S., and Choi, G. (2003). Functional characterization of Phytochrome Interacting Factor 3 in phytochrome-mediated light signal transduction. Plant Cell **15:** 2399-2407.

Kim, Y.M., Woo, J.C., Song, P.S., and Soh, M.S. (2002). HFR1, a phytochrome A-signalling component, acts in a separate pathway from HY5, downstream of COP1 in *Arabidopsis thaliana*. Plant J. **30:** 711-719.

Kozuka, T., Kobayashi, J., Horiguchi, G., Demura, T., Sakakibara, H., Tsukaya, H., and Nagatani, A. (2010). Involvement of auxin and brassinosteroid in the regulation of petiole elongation under the shade. Plant Physiol. **153:** 1608-1618.

Lange, H., Shropshire, W., and Mohr, H. (1971). An analysis of phytochrome-

mediated anthocyanin synthesis. Plant Physiol. **47:** 649-655.

Lariguet, P., and Dunand, C. (2005). Plant photoreceptors: phylogenetic overview. J. Mol. Evol. **61:** 559-569.

Laudert, D., and Weiler, E.W. (1998). Allene oxide synthase: a major control point in *Arabidopsis thaliana* octadecanoid signalling. Plant J. **15:** 675–684.

Leivar, P., Monte, E., Cohn, M.M., and Quail, P.H. (2012). Phytochrome signaling in green *Arabidopsis* seedlings: impact assessment of a mutually negative phyB–PIF feedback loop. Mol. Plant **5:** 734–749.

Leivar, P., and Quail, P.H. (2011). PIFs: pivotal components in a cellular signaling hub. Trends Plant Sci. **16:** 19–28.

Li, L., Ljung, K., Breton, G., Schmitz, R.J., Pruneda-Paz, J., Cowing-Zitron, C., Cole, B.J., Ivans, L.J., Pedmale, U.V., Jung, H.S., Ecker, J.R., Kay, S.A., and Chory, J. (2012). Linking photoreceptor excitation to changes in plant architecture. Genes Dev. **26:** 785–790.

Lin, C., Yang, H., Guo, H., Mockler, T., Chen, J., and Cashmore, A.R. (1998). Enhancement of blue-light sensitivity of *Arabidopsis* seedlings by a blue light receptor cryptochrome 2. Proc. Natl. Acad. Sci. USA **95:** 2686-2690.

Lorrain, S., Allen, T., Duek, P.D., Whitelam, G.C., and Fankhauser, C. (2008). Phytochrome-mediated inhibition of shade avoidance involves degradation of growth-promoting bHLH transcription factors. Plant J. **53:** 312–323.

Martinez-Garcia, J.F., Galstyan, A., Salla-Martret, M., Cifuentes-Esquivel, N., Gallemi, M., and Bou-Torrent, J. (2010). Regulatory components of shade avoidance syndrome. Adv. Bot. Res. **53:** 66-116.

Memelink, J. (2009). Regulation of gene expression by jasmonate hormones. Phytochemistry **70:** 1560-1570.

Mockler, T.C., Guo, H., Yang, H., Duong, H., and Lin, C. (1999). Antagonistic action of *Arabidopsis* cryptochromes and phytochrome B in the regulation of floral induction. Dev.**126:** 2073-2082.

Moreno, J.E., Tao, Y., Chory, J., and Ballare, C.L. (2009). Ecological modulation of plant defense via phytochrome control of jasmonate sensitivity. Proc. Natl. Acad. Sci. U S A **106:** 4935-4940.

Ni, M., Tepperman, J.M., and Quail, P.H. (1999). Binding of phytochrome B to its nuclear signalling partner PIF3 is reversibly induced by light. Nature **400:** 781-784.

Okamoto, H., Gobel, C., Capper, R.G., Saunders, N., Feussner, I., and Knight, M.R. (2009). The a-subunit of the heterotrimeric G-protein affects jasmonate responses in *Arabidopsis thaliana*. J. Exp. Bot. **60:** 1991-2003.

Park, H.J., Ding, L., Dai, M., Lin, R., and Wang, H. (2008). Multisite

phosphorylation of *Arabidopsis* HFR1 by casein kinase II and a plausible role in regulating its degradation rate. J. Biol. Chem. **283:** 23264-23273.

Pierik, R., Cuppens, M.L.C., Voesenek, L.A.C.J., and Visser, E.J.W. (2004). Interactions between ethylene and gibberellins in phytochrome-mediated shade avoidance responses in Tobacco. Plant Physiol. **136:** 2928-2936.

Pierik, R., Djakovic-Petrovic, T., Keuskamp, D.H., Wit, M., and Voesenek, L.A.C.J. (2009). Auxin and ethylene regulate elongation responses to neighbor proximity signals independent of gibberellin and DELLA proteins in *Arabidopsis*. Plant Physiol. **149:** 1701-1712.

Porra, R.J., Thompson, W.A., and Kriedemann, P.E. (1989). Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochim. Biophys. Acta **975:** 384-394.

Reed, J.W., Nagpal, P., Poole, D.S., Furuya, M., and Chory, J. (1993). Mutations in the gene for the red/far red light receptor phytochrome B alter cell elongation and physiological responses through *Arabidopsis* development. Plant Cell **5:** 147-157.

Rizzini, L., Favory, J.J., Cloix, C., Faggionato, D., O'Hara, A., Kaiserli, E., Baumeister, R., Schafer, E., Nagy, F., Jenkins, G.I., and Ulm, R. (2011). Perception of UV-B by the *Arabidopsis* UVR8 protein. Science **332:** 103-106.

Robson, F., Okamoto, H., Patrick, E., Harris, S.R., Wasternack,C., Brearley, C., and Turner, J.G. (2010). Jasmonate and phytochrome A signaling in *Arabidopsis* wound and shade responses are integrated through JAZ1 stability. Plant Cell **22:** 1143-1160.

Roig-Villanova, I., Bou, J., Galstyan, A., Carretero-Paulet, L., Portoles, S., Rodriguez-Concepcion, M., and Martinez-Garcia, J.F. (2007). Interaction of shade avoidance and auxin responses: a role for two novel atypical bHLH proteins. EMBO J. **26:** 4756–4767.

Roig-Villanova, I., Bou, J., Sorin, C., Devlin, P.F., and Martinez-Garcia, J.F. (2006). Identification of primary target genes of phytochrome signaling. Early transcriptional control during shade avoidance responses in *Arabidopsis*. Plant Physiol. **141:** 85–96.

Salter, M.G., Franklin, K.A., and Whitelam, G.C. (2003). Gating of the rapid shade-avoidance response by the circadian clock in plants. Nature **426:** 680–683.

Sellaro, R., Crepy, M., Trupkin, S.A., Karayekov, E., Buchovsky, A.S., Rossi, C., and Casal, J.J. (2010). Cryptochrome as a sensor of the blue/green ratio of natural radiation in *Arabidopsis*. Plant Physiol. **154:** 401–409.

Selman, I.W., and Ahmed, E.O.S. (1962). Some effects of far-red irradiation and gibberellic acid on the growth of tomato plants. Ann. Appl. Biol. **50:** 479-485.

Sessa, G., Carabelli, M., Sassi, M., Ciolfi, A., Possenti, M., Mittempergher, F., Becker, J., Morelli, G., and Ruberti1, I. (2005). A dynamic balance between gene activation and repression regulates the shade avoidance response in *Arabidopsis*. Gene Dev. **19:** 2811-2815.

Shan, X.Y., Zhang, Y.S., Peng, W., Wang, Z.L., and Xie, D.X. (2009). Molecular mechanism for jasmonate-induction of anthocyanin accumulation in *Arabidopsis*. J. Exp. Bot. **60:** 3849-3860.

Sheard, L.B., Tan, X., Mao, H., Withers, J., Ben-Nissan, G., Hinds, T.R., Kobayashi, Y., Hsu, F.F., Sharon, M., Browse, J., He, S.Y., Rizo, J., Howe, G.A., and Zheng, N. (2010). Jasmonate perception by inositol-phosphate-potentiated COI1-JAZ co-receptor. Nature **468:** 400-405.

Shyu, C., Figueroa, P., DePew, C.L., Cooke, T.F., Sheard, L.B., Moreno, J.E., Katsir, L., Zheng, N., Browse, J., and Howe, G.A. (2012). JAZ8 lacks a canonical degron and has an EAR motif that mediates transcriptional repression of jasmonate responses in *Arabidopsis*. Plant Cell **24:** 536-550.

Smith, H., Xu, Y., and Quail, P.H. (1997). Antagonistic but complementary actions of phytochromes A and B allow optimum seedling de-etiolation. Plant Physiol. **114:** 637-641.

Smyth, D.R., Bowman, J.L., and Meyerowitz, E.M. (1990). Early flower development in *Arabidopsis*. Plant Cell **2:** 755-767.

Soh, M.S., Kim, Y.M., Han, S.J., and Song, P.S. (2000). REP1, a basic helix-loop-helix protein, is required for a branch pathway of phytochrome A signaling in *Arabidopsis*. Plant Cell **12:** 2061-2073.

Stamm, P., and Kumar, P.P. (2010). The phytohormone signal network regulating elongation growth during shade avoidance. J. Exp. Bot. **61:** 2889-2903.

Staswick, P.E. (2008). JAZing up jasmonate signaling. Trends Plant Sci. **13:** 66-71.

Staswick, P.E., and Tiryaki, I. (2004). The oxylipin signal jasmonic acid is activated by an enzyme that conjugates it to isoleucine in *Arabidopsis*. Plant Cell **16:** 2117-2127.

Staswick, P.E., Su, W., and Howell, S.H. (1992). Methyl jasmonate inhibition of root growth and induction of a leaf protein are decreased in an *Arabidopsis thaliana* mutant. Proc. Natl. Acad. Sci. U S A **89:** 6837-6840.

Staswick, P.E., Tiryaki, I., and Rowe, M.L. (2002). Jasmonate response locus *JAR1* and several related *Arabidopsis* genes encode enzymes of the firefly luciferase superfamily that show activity on jasmonic, salicylic, and indole-3-acetic acids in an assay for adenylation. Plant Cell **14:** 1405-1415.

Staub, J.M., Wei, N., and Deng, X.W. (1996). Evidence for FUSG as a component of the nuclear-localized COP9 complex in *Arabidopsis*. Plant Cell **14:** 1405-1415.

Stintzi, A., and Browse, J. (2000). The *Arabidopsis* male-sterile mutant, *opr3*, lacks the 12-oxophytodienoic acid reductase required for jasmonate synthesis. Proc. Natl. Acad. Sci. USA **97:** 10625-10630.

Strassner, J., Schaller, F., Frick, U.B., Howe, G.A., Weiler, E.W., Amrhein, N., Macheroux, P., and Schaller, A. (2002). Characterization and cDNA-microarray expression analysis of 12-oxophytodienoate reductases reveals differential roles for octadecanoid biosynthesis in the local versus the systemic wound response. Plant J. **32:** 585–601.

Sullivan, J.A., and Deng, X.W. (2003). From seed to seed: the role of photoreceptors in *Arabidopsis* development. Dev. Biol. **260:** 289-297.

Thines, B., Katsir, L., Melotto, M., Niu, Y., Mandaokar, A., Liu, G., Nomura, K., He, S.Y., Howe, G.A., and Browse, J. (2007). JAZ repressor proteins are targets of the SCF(COI1) complex during jasmonate signalling. Nature **448:** 661-665.

Traw, M.B. and Bergelson, J. (2003). Interactive effects of jasmonic acid, salicylic acid, and gibberellin on induction of trichomes in *Arabidopsis*. Plant Physiol. **133:** 1367-1375.

Vanholme, B., Grunewald,W., Bateman, A., Kohchi, T., and Gheysen, G. (2007). The tify family previously known as ZIM. Trends Plant Sci. **12:** 239-244.

Wallis, J.G., and Browse, J. (2002). Mutants of *Arabidopsis* reveal many roles for membrane lipids. Prog. Lipid Res. **41:** 254–278.

Wang, J.G., Chen, C.H., Chien, C.T., and Hsieh, H.L. (2011). FAR-RED INSENSITIVE219 modulates CONSTITUTIVE PHOTOMORPHOGENIC1 activity via physical interaction to regulate hypocotyl elongation in *Arabidopsis*. Plant Physiol. **156:** 631-646.

Wasternack, C., and Kombrink, E. (2010). Jasmonates: structural requirements for lipid-derived signals active in plant stress responses and development. ACS Chem. Biol. **5:** 63-77.

Yang, J.P., Lin, R.C., Sullivan, J., Hoecker, U., Liu, B.L., Xu, L., Deng, X.W., and Wang, H.Y. (2005). Light regulates COP1-mediated degradation of HFR1, a transcription factor essential for light signaling in *Arabidopsis*. Plant Cell **17:** 804-821.

APPENDIX I

Procedures of RNA Extraction:

- Sample preparation:
  (1) After specific treatment, 50 mg to 100 mg of samples are collected in dark room and frozen in liquid nitrogen immediately.
  (2) Samples are grinded into fine powder and remained at very low temperature all the time.

- 1st Day RNA Extraction: (The following procedures should be in chemical hood)
  (1) Add 500 µl Buffer A into grinded samples and vortex for 30 sec.
  (2) Add 500 µl phenol (pH 4.3), vortex for 30 sec, and centrifuge at 13000 rpm, 4℃ for 20 min.
  (3) Transfer supernatant into new eppendorf and add equal volume of phenol (pH 4.3); vortex for 30 sec, and centrifuge at 13000 rpm, 4℃ for 20 min.
  (4) Transfer supernatant into new eppendorf and add equal volume of Chloroform (to eliminate the phenol); vortex for 30 sec, and centrifuge at 13000 rpm, 4℃ for 20 min.
  (5) Transfer supernatant into new eppendorf, add equal volume of 6 M LiCl and invert the tubes for several times, and then store at -20℃ overnight (for RNA participation).

- 2nd Day RNA Extraction:
  (1) After precipitation, centrifuge at 13000 rpm, 4℃ for 20 min and remove supernatant.
  (2) Add 150 μl 3 M LiCl to wash the pellet and centrifuge at 13000 rpm, RT for 10 min; remove supernatant as clean as possible (repeat again).
  (3) Suspend the pellet in 150 μl 2% KOAc and incubate in 55℃ for 15 min; during the incubation, tap the eppendorf occasionally.
  (4) Centrifuge at 13000 rpm, RT for 10 min; remove the supernatant.
  (5) Add 2.5X volume of cold 100% EtOH and incubate in 4℃ for 30 min.
  (6) Centrifuge at 13000 rpm, 4℃ for 15 min; remove the supernatant.
  (7) Dry the pellet and suspend in DEPC-MQ.

Buffer A (4℃ store)

1 M	Tris-Cl	pH 7.3
5 mM	EDTA	pH 8.0
1%	SDS	

APPENDIX II

Primers used in RT-PCR are listed below.

Gene	Primer No.	Primer Name	Sequence (5' to 3')
FIN219	823	FIN219-F	GCAGTCGACATGTTGGAGAAGGTTGAAACTTTC
	834	FIN219-R	GGCGGATCCAAAACGCTGTGCTGAAGTAGCT
HFR1	1567	HFR1-BamHI-Fw	TCCGGATCCATGTCGAATAATCAAG
	1568	HFR1-XhoI-Rv	GGACTCGAGTCATAGTCTTCTCATC
LOX2	843	LOX2-fw-1815	TGCACGCCAAAGTCTTGTCA
	844	LOX2-rv-2195	GGCCACCAAGGTTCGTCTTT
AOS	1569	AOS Fw	TGGTTATCGAAAGCCACGAC
	1570	AOS Rv	AATCTCTCCGGCACAAACTC
OPR3	845	OPR3-fw-182	CTTCTCCTTCTTCCAGATCGGC
	846	OPR3-rv-664	TTCGGGTACTTCACGTGGGA
JAZ1	853	JAZ1-fw-989	CACCCTCAAGCCGTACCAAG
	854	JAZ1-rv-1203	GCAAGGGGATTTAGACAGGCG
MYC2	847	MYC2-fw-510	GATCTCCGGTGGTGTTGCTC
	848	MYC2-rv-908	GACGGGTCGTTCTCACCTTG
VSP1	849	VSP1-fw-692	GCATCTCATACTCAAGCCAAACG
	850	VSP1-rv-969	GTGGTGCCAAAACGGCTACA
PIL1	1571	PIL1 Fw	GTGTTTCTCAGACTCAGGCTACTTC
	1572	PIL1 Rv	CGGACGCAGACTTTGGGAATTG

Gene	Primer No.	Primer Name	Sequence (5' to 3')
PAR1	1573	PAR1 Fw	TCATGCTCAGCCACCGTGAAATC
	1574	PAR1 Rv	CCTTGACCTCATCTTCTTCTTCTTC
ATHB2	1575	ATHB2 Fw	TGAGCCCACCCACTACTTTGAC
	1576	ATHB2 Rv	AGGAGCCCACGCATTGACC
PIF4	1561	PIF4 Fw	ATCATCTCCGACCGGTTTGC
	1562	PIF4 Rv	AGTGGCTCACCAACCTAGTG
PIF5	1563	PIF5 Fw	GCGGGAAATCAGACCGTGCAACAA
	1564	PIF5 Rv	CGCCGGAGATCCAAATCCCAACAT
PIF7	1565	PIF7 Fw	AGTTTCAGCTGCAGAGTCCG
	1566	PIF7 Rv	CCACTCGCACTTGCAGTG
UBQ10	77	UBQ primer 1	GATCTTTGCCGGAAAAGAATTGGAGGATGGT
	78	UBQ primer 2	CGACTTGTCATTAGAAAGAAAGAGATAACAGG

APPENDIX III

Cycles and annealing temperature in RT-PCR are listed below.

Gene	Cycles	Temperature (℃)	Gene	Cycles	Temperature (℃)
FIN219	35	57	*PIL1*	29	57
HFR1	30	52	*PAR1*	29	57
LOX2	29	55	*ATHB2*	29	57
AOS	27	53	*PIF4*	29	55
OPR3	29	55	*PIF5*	30	60
JAZ1	29	55	*PIF7*	29	55
MYC2	27	53	*UBQ10*	26	57
VSP1	27	53			

APPENDIX IV

PCR Reaction: (Total: 10 µl)

Template	x
10 µM Fw primer	0.1
10 µM Rv primer	0.1
10 mM dNTP	0.2
10X buffer	1
Taq	0.1
MQ	10-x

Reverse Transcription (RT) Reaction: (Total: 20 µl)

Template (1 µg RNA)	x
100 µM oligo-dT primer	1.5
100 mM dNTP	0.8
10X RT buffer	2
RNasin	0.5
RTase	1
DEPC-MQ	20-x

APPENDIX V

Clone List

Clone No.	Clone Name	Strain	Resistance
HHL1018	HFR1-CDS/yT&A	DH5α	Amp
HHL1020	3xFlag HFR1-CDS/pRTL2	DH5α	Amp
HHL1021	3xFlag-HFR1-CDS /pCAMBIA1390	DH5α	Kana
HHL1022	3xFlag-HFR1-CDS /pCAMBIA1390	GV3101	Kana