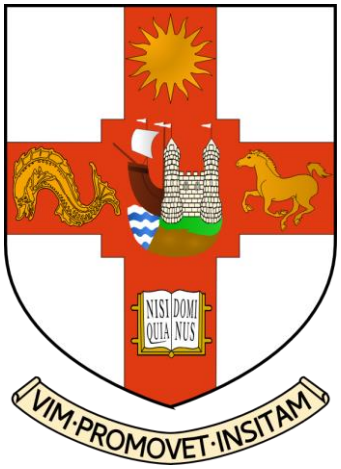
Author:
Yochikawa, Aline

Title:
Ultraviolet – B -mediated control of PHYTOCHROME INTERACTING FACTOR (PIF) transcription in Arabidopsis thaliana

Ultraviolet – B -mediated control of *PHYTOCHROME INTERACTING FACTOR* (*PIF*) transcription in *Arabidopsis thaliana*

Aline Yochikawa



School of Biological Sciences

A dissertation submitted to the University of Bristol in accordance with the requirements for award of the degree of Doctor of Philosophy in the Faculty of Life Sciences.

July 2023

Word count: 33,357

Abstract

Plants, as sessile autotrophic organisms, rely on light cues not only as a source of energy, but also to regulate developmental responses to cope with their everchanging environment. Physiological changes triggered by light vary according to the light quality that is perceived by specific specialized photoreceptors, including phytochromes, cryptochromes and UV RESISTANCE LOCUS 8 (UVR8). These photoreceptors transduce the light cues to regulate PHYTOCHROME-INTERACTING FACTORS (PIFs). PIFs are a small subset of transcription factors of the basic helix-loop-helix (bHLH) subfamily, which act as a cellular signalling hub that integrates multiple signals, including light and temperature, to regulate plant morphogenesis. The mechanisms underlying transcriptional regulation of *PIF*s are poorly understood in comparison to their posttranscriptional regulation. This thesis examines the transcriptional regulation of *PIF*s in response to low dose ULTRAVIOLET-B (UV-B) light. UV-B is shown to suppress the transcript abundance of *PIF3*, *PIF4* and *PIF5* by inhibition of promoter activity, in a UVR8- dependent manner. Evidence supporting a role for COP1 in the suppression of *PIF4* and *PIF5* transcript abundance in UV-B is also presented. Three different mechanisms controlling UV-B-mediated suppression of *PIF* transcript abundance are investigated. The first involves the plant hormones, brassinosteroids (BR). This thesis shows that BR signals are not involved in the UV-B-mediated suppression of *PIF4* transcript at high temperatures, but support a role for BR signalling in the UV-B-mediated suppression of thermomorphogenesis. The second involves a potential autoregulatory loop involving UV-B-mediated degradation of PIF protein. Data suggest that UV-B-mediated PIF4 degradation may occur via an alternative pathway to PIF5. The third investigates the role of MYB30 in regulating *PIF* transcript abundance. Data show that MYB30 is suppressed by UV-B in a UVR8-dependent manner and promotes *PIF7* transcription in white light. In addition, MYB30 regulates shade-avoidances responses to green shade.

Acknowledgements

Throughout the production of this thesis, I have received an immense deal of support of many amazing people. Firstly, I would like to thank my primary supervisor Keara Franklin for her guidance, inspiration, advice, unwavering dedication, and for being an exceptional companion in the race against time. I would like to thank my second supervisor, Andy Bailey, for our enlightening discussions. I also thank Antony Dodd for the opportunity he has given me and for believing in my potential.

I would like to thank and dedicate this thesis to my family, Akiko, Celso, Camila, Nina, and Dino. My safety net, whose unwavering support, love, belief, and encouragement propelled me forward. Whose endless efforts provided me with all the tools I needed to be where I am today.

I would like to thank all the current and former member of the Franklin and 324 lab, especially Chris Groves, Mathilda Gustavsson, and Alvaro Montiel, for creating a stimulating and fun work environment that fostered my professional growth. I would also like to acknowledge members of the Life Science Building, particularly Wei Liu and Pierre Aurelian, who have become more than my colleagues, but my family in the UK. I would also like to thank my office “bessie” Ian Prosser, for the daily doses of coffee and laughter followed by life advice.

To my dear friends Mariana Rossi, Amanda Martinelli, Max Tsunada, and Ana Laura Leme, Luisa Naves, Taynah Govea, Erico Casagrande, and Rita Vital. For the emotional support, insightful conversations, and for the enduring friendship that transcends time and distance.

This thesis was supported by the University of Bristol and BCAI studentship.

Covid-19 Statement

I have been affected by many disruptions throughout my PhD which impacted my thesis in ways I could never have predicted. I started my PhD in January 2019. I was initially researching circadian rhythms of chloroplast transcription in Arabidopsis, under the supervision of Dr. Antony Dodd. Within 8 months, Antony was hired by the John Innes Centre. I did finalize some experiments on this project for the next 4 months, but data produced during the first 12 months of my PhD could not be used in this thesis, as the project moved to the JIC. As such, it was classified as a "training year". To sum up, I lost a whole year of data collection.

I then switched supervisors and hence research project. I started a whole new PhD under the supervision of Dr. Keara Franklin in photobiology in January 2020. While I was researching literature to develop a research project for the next 3 years, the COVID-19 pandemic started spread throughout the world, and within a month the country faced the national lockdown (February 2020). Together, these factors have highly impacted my work. Firstly, because I had spent all my consumables grant on my previous research project, I had a limited amount of funding to spend on experimental materials. Because of lockdown, I was not allowed in the laboratory at all for two months. Even when lockdown rules started to ease, I was only allowed in the laboratory three times a week because of department social distancing measures. This delayed my qPCR experiments, as I had to learn new techniques with very limited support. I experienced significant delays in the delivery of lab consumables, especially for qPCR experiments, because of lockdown, Brexit, and the need for similar consumables to run COVID-19 tests across the world. Some experiments I was planning involving transgenic lines and mutants could also not be performed, because the Chinese laboratories with seed stocks were under a stricter and longer lockdown. In addition to multiple lockdowns, I have also lost approximately 45 working days in the lab due to government self-isolation guidelines, my own covid infection, and a lab flood which caused a prolonged shut down of the Life Sciences Building.

Although I received a 6-month extension on my thesis deadline due to COVID-19 disruptions, I was only granted 5 months of stipend for this period. In addition to that, as I am a foreign student under a Tier4 visa, I had to apply for a visa extension to continue my research. This cost me over £1,000, which I did not receive financial support for from the university. Therefore, while conducting my laboratory experiments and writing my thesis, I needed to work to be able to afford these expenses. I not only took on a large amount of demonstration and marking within the university, but also had to work in a part-time job to sustain myself.

The pressure and the challenges I faced in the past 4 years have affected my mental health and severely impacted this research. I would have liked to have pursued interesting results in more depth but have pushed myself to do all that was possible within the severe time constrains I faced.

Authors Declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's *Regulations and Code of Practice for Research Degree Programmes* and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED: ..... DATE:.....11/09/2023.....

Table of Contents

List of Figures

List of Tables

List of Abbreviations

16 h L/ 8 h D	16 hour light : 8 hour dark photocycle
ABA	Abscisic acid
ABI	ABA-INSENSITIVE
B	Blue
BES1	BRI1-EMS-SUPPRESSOR1
bHLH	basic Helix-Loop-Helix
BIM1	BES1-INTERACTING MYC-LIKE 1
BL	Brassinolide
BR	brassinosteroid
BRI1	BRASSINOSTEROID INSENSITIVE 1
BZR1	BRASSINAZOLE-RESISTANT 1
cDNA	complementary DNA
ChIP	Chromatin Immuno Precipitation
COP1	CONSTITUTIVELY PHOTOMORPHOGENIC 1
cry	cryptochrome
DMSO	dimethyl sulfoxide
EC	evening complex

ELF3	EARLY FLOWERING 3
ELF4	EARLY FLOWERING 4
FR	FAR-RED LIGHT
GAI	GA-INSENSITIVE 1
GUS	β-glucuronidase
HFR1	LONG HYPOCOTYL IN FAR RED 1
HLH	Helix-Loop-Helix
HY5	ELONGATED HYPOCOTYL 5
HYH	HY5 HOMOLOG
LHY	LATE ELONGATED HYPOCOTYL
LUX	LUX ARRHYTHMO
MYB30	MYB DOMAIN PROTEIN 30
PAR	Photosynthetically Active Radiation (400-700 nm)
phot	phototropin
phyA	phytochrome A
phyB	phytochrome B
PIF	PHYTOCHROME INTERACTING FACTOR
PP2A	PROTEIN PHOSPHATASE 2 ALPHA PRD PAS-related domain
qRT - PCR	quantitative reverse-transcription polymerase chain reaction
R/FR	Red : Far-Red ratio

RUP	Repressor of UV-B photomorphogenesis
RVE8	REVEILLE 8
SPA	SUPPRESSOR OF PHYA-105
TFBS	transcription factor binding site
Trp	Tryptophan - 3 letter code
UV-B	Ultraviolet-B radiation (280-315 nm)
UVR8	UV-B RESISTANCE LOCUS 8
WL	White Light
WT	Wildtype

Chapter 1

Introduction

Plants are sessile and photo-autotrophic organisms which continually adapt to the natural environment. They display complex strategies to cope with daily environmental fluctuations, as well as the biotic threats they are constantly exposed to. Light and temperature are key environmental stimuli known to trigger physiological and developmental changes in plants to optimize fitness and enhance survival. Light is not only a source of energy for plants, but also a source of information regarding the ambient environment. Plants perceive light quality (wavelength) and quantity (irradiance) through several classes of photoreceptors. These convert the physical energy of light into chemical energy, triggering signal transduction networks to initiate and regulate the appropriate developmental response (Bae & Choi, 2008). Light-regulated responses include seed germination, skotomorphogenesis (development in the dark leading to long hypocotyls and small and pale cotyledons), photomorphogenesis (development in the light, leading to short hypocotyls, large cotyledons and chlorophyll synthesis), shade avoidance (in response to shading from neighbour plants), phototropism (regulation of directional growth) and photoperiodic control of flowering, among others (Pham *et al*., 2018).

1.1 Photoreceptors

In the model plant *Arabidopsis thaliana,* multiple classes of photoreceptors have been identified and classified according to the portions of the light spectrum they maximally absorb . The phytochromes perceive red light (600-700 nm) and far-red light (700-800 nm) (Quail *et al*., 1995; Nagy & Schafer, 2002), cryptochromes, phototropins and the ZEITUPLE family perceive blue and UV-A light (315-500 nm) (Briggs & Huala, 1999; Cashmore *et al*., 1999; Briggs and Christie, 2002; Lin, 2002; Lin & Shalitin, 2003; Kaiserli *et al*., 2009; Zoltowski & Imaizumi 2014) , and UV RESISTANCE LOCUS 8 (UVR8) perceives UV-B (280-315 nm).

1.1.1 Phytochromes

Phytochromes are the mostly extensively studied photoreceptors of Arabidopsis, consisting of a small family of five members (phyA-E) that have different and overlapping functions throughout the lifecycle of plants. They are soluble homodimers formed by an apoprotein (approximately 125kDa) covalently bonded to a linear tetrapyrrole chromophore and exist in two interconvertible forms: the inactive Pr form (red light- absorbing) and the active Pfr form (far-red light- absorbing). After being exposed to red light, the inactive Pr form is converted to the active Pfr form, with the reverse process occurring under far red-light exposure (Linschitz & Kasche, 1966). Phytochromes are synthesized in the Pr form in the cytosol, but when photoactivated they undergo allosteric conformational changes and are imported to the nucleus (Nagatani, 2004). There, active Pfr interacts with other factors to orchestrate the expression of genes as well as posttranslational modulation of downstream proteins involved in light responses (Legris *et al*., 2019). The rapid light activation of phytochromes plays crucial role in enhancing the photosynthetic efficiency of emerging seedlings by switching the plant development from skotomorphogenesis to photomorphogenesis. When plants are exposed to light, Pfr initiates light signalling pathways to limit stem elongation and expand leaves for photosynthesis. A key adaptative trait resulting from phytochrome photoreversibility is the ability of plants to perceive and

respond to vegetative shade through sensing reductions in the red to far-red light ratio (R:FR). Light transmitted through and reflected from vegetation is enriched in far-red light, lowering red to far-red ratio (R:FR). Low R:FR light promotes the conversion of phyB Pfr form to its inactive Pr form, which removes Pfr-mediated suppression of stem elongation and promotes the upwards reorientation of leaves (hyponasty), in a suite of responses known as shade avoidance. Such changes in plant architecture allow the plant to increase light foraging capacity (Franklin, 2008; Casal, 2013; Buti *et al*, 2020). Similarly, thermomorphogenesis is a physiological response to an external cue, regulated, in part, by phytochromes. During thermomorphognesis phytochromes act as thermosensors, leading to remodulation of plant architecture in a similar manner to shade-avoidance (stem and petiole elongation) in response to elevated temperature (Juang *et al*., 2016 ; Legris *et al*., 2016 ; discussed in section 1.5).

Another biological process in which phytochromes perform a crucial role is entrainment of the circadian clock. Circadian rhythms allow plants to predict daily changes in their living environment due to the rotation of Earth on its axis, such as temperature and light availability oscillations, thereby facilitating an appropriate physiological response for that exact time of the day (Harmer *et al*., 2000; Dodd *et al*., 2005; Michael *et al*., 2008). In Arabidopsis, the circadian clock acts as a regulator of plant gene expression through an intrinsic network of feedback-loops in response to abiotic and biotic signals (Schaffer *et al*., 2001; Goodspeed *et al*., 2013; Liu *et al*., 2013; Kolmos *et al*., 2014). Responses controlled by the circadian clock include hypocotyl and root elongation (Nozue *et al*., 2007; Yazdanbakhsh *et al*., 2011), the timing of flowering, stomatal opening, flower and leaf movement, and photosynthetic efficiency, which collectively increase fitness (Park *et al*., 1999; Geen *et al*., 2002; Dodd *et al.,* 2004; Dodd *et al*.; 2005; Nakamichi, 2011; Dornbusch *et al*., 2014).

The regulation of phytochrome-mediated responses involves the regulation of phytochrome gene expression and transcript abundance; regulation of phytochrome protein stability; chromophore synthesis and attachment; regulation of phytochrome subcellular localization and interaction with

key partners that participate in the transduction of phytochrome activation to downstream pathways (Chen & Chory, 2011; Leviar & Monte, 2014; Pham *et al*, 2018). Although some evidence exists that phytochromes may signal in the cytoplasm (Moller *et al*., 2002), the nuclear accumulation of Pfr suggests that the majority of phytochrome-mediated activity occurs in the nucleus (Sakamoto & Nagatani 1996; Yamaguchi *et al*. 1999). PHYTOCHROME INTERACTING FACTORS (PIFs) are transcription factors that are fundamental interacting partners of PHY, and key regulators of the light-induced transitions modulated by phytochromes (Sakamoto & Nagatani 1996; Yamaguchi *et al*., 1999; Castillon *et al*., 2007; Leviar and Quail, 2011; discussed in section 1.2).

1.1.2 Cryptochromes

The Arabidopsis genome encodes three cryptochromes: cryptochrome 1 (cry1), cryptochrome 2 (cry2) and cryptochrome 3 (cry3). Both cry1 and cry2 function as photoreceptors regulating blue light-mediated developmental responses in plants (Ahmad & Cashmore 1993; Guo *et al*. 1998; El-Assal *et al*. 2001), whereas cry3 is a CRY-DASH type cry which acts to repair single stranded DNA in mitochondria and chloroplasts from UV-light damage (Kleine *et al*. 2003; Pokorny *et al*. 2008). Cryptochromes display strong homology to the DNA repair enzyme photolyase and contain a blue light-sensitive pterin or a deazaflavin (methenyltetrahydrofolate, MTHF) chromophore and a flavin adenine dinucleotide (FAD). However, they do not display DNA repair activity (Ahmad & Cashmore 1993; Lin 1998; Sancar 1994; Malhotra 1995). Processes regulated by photoactivated cryptochrome include seedling de-etiolation, cotyledon expansion, entrainment of circadian rhythms, pathogenic responses, regulation of stomatal aperture, stomatal development and leaf senescence (Ahmad & Cashmore 1993; Guo *et al*. 1998; El-Assal *et al*. 2001; Mao *et al*. 2005; Kang *et al*. 2009; Wang *et al*. 2010; Jeong *et al*. 2010; Wu & Yang 2010; Meng *et al*. 2013). The most extensively studied process regulated by crys is the promotion of de-etiolation. In a similar manner to phytochromes, cryptochromes act mainly in the nucleus and orchestrate the expression of 5-25% of genes in Arabidopsis dark-grown seedlings. These genes include transcription factors, kinases, cell cycle regulators and phytohormone signalling factors which collectively transduce blue light signals to to

drive morphological and physiological responses (Folta *et al*. 2003; Ma *et al*. 2001; Ohgishi *et al*. 2004; Sellaro *et al*. 2009).

Developmental changes in plant architecture triggered by crys mostly involve the transcriptional modulation of genes (Ma *et al*., 2001; Folta *et al*., 2003; Ohgishi *et al*., 2004; Sellaro *et al*., 2009). There are three major mechanisms described for this regulation. The first comprises the post transcriptional regulation of genes by CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1). COP1 represses photomorphogenesis in a light-dependent fashion by forming E3 ubiquitin ligase complexes with SUPRESSOR OF PHYA-105 (SPA) and SPA1 related proteins (SPA2-SPA4) to mediate proteolytic degradation of transcription factors that positively regulate plant growth (Laubinger *et al*. 2004, 2006; Lau & Deng 2012). One of the main targets of this complex is a basic-leucine zipper transcription factor LONG HYPOCOTYL 5 (HY5), which induces the expression of light regulated genes that promote de-etiolation by physically interacting with their promoters. In the dark, the COP1/SPA complexes target HY5 for ubiquitination and proteasomal degradation. Light inhibits the COP-SPA complex formation, allowing HY5 to accumulate in the nucleus and promote plant photomorphogenic development (Osterlund *et al*. 2000; Jiao *et al*. 2007; Favory *et al*. 2009). cry1 and cry2, physically bind to the SPA1 protein in blue light, suppressing COP1/SPA-mediated degradation of HY5 (Lian *et al*. 2011; Liu *et al*. 2011a, b; Zuo *et al*. 2011). The inhibition of the COP1/SPA complex ubiquitination mechanism by crys differs, however, between cry1 and cry2. Cry1 sequesters SPA1 through direct binding, inhibiting its interaction and thus complex formation with COP1 (Fankhauser & Ulm 2011; Lian *et al*. 2011; Liu *et al*. 2011a, b). In contrast, cry2 binding to SPA1 induces the interaction between cry2 and COP1 (Zuo *et al*. 2011), which is thought to inhibit the E3 ubiquitin ligase activity of COP1. Both mechanisms lead to an accumulation of HY5 in the nucleus and therefore induction of a photomorphogenic response.

The second mechanism through which crys mediate changes in plant development in response to blue light involves the interaction of cry2 with the basic helix-loop-helix transcription factor CRYPTOCHROME-INTERACTING BASIC-HELIX-LOOP-HELIX 1 (CIB) to promote flowering in inductive

photoperiods (Liu *et al*. 2008a). Cry2 regulates CIB expression and function by an, as yet, unknown mechanism. It has been shown that CIB protein is stabilized by other blue light receptors upon blue light exposure (ZEITLUPE [ZTL] and LOV KELCH PROTEIN 2 [LKP2])(Liu *et al*. 2013a).

Finally, cry1 and cry2 physically interact with PIF transcription factors inhibiting their DNA binding ability (discussed in section 1.2) to control shade avoidance, thermomorphogenesis and shoot branching, via regulation of auxin (Pedmale *et al*., 2016; Ma *et al*., 2016; Zhai *et al*., 2020).

1.1.3 Phototropins

Phototropins are receptor kinases and blue light photoreceptors present in algae and plants (Li *et al*., 2015). In Arabidopsis there are two isoforms of phototropin, phototropin 1 (phot1) and 2 (phot2). Both contain two photosensitive light, oxygen, or voltage-sensing (LOV) domains (LOV1 and LOV2 respectively) and a C-terminal serine/threonine kinase domain (Christie *et al*., 1998, 2002). Each LOV domain binds the vitamin-B derived cofactor flavin mononucleotide (FMN) as a blue light-absorbing chromophore. The photoexcitation of LOV2 promotes the unfolding of an alpha helix, driving autophosphorylation of the kinase domains, thus propagating the light signal (Christie *et al*., 1998; Harper *et al*., 2003; Jones *et al*., 2007; Zayner *et al*., 2012; Petersen *et al*., 2017). Unlike previously described photoreceptors that act mainly in the nucleus of the cell regulating gene expression, phototropins are localized on the plasma membrane and are suggested to control gene expression indirectly (Christie 2007). Phototropins likely evolved to protect plants from photodamage caused by UV radiation and to increase plant photosynthetic efficiency by triggering morphological and physiological changes in plant such as phototropism (Huala *et al*. 1997; Sakai *et al*. 2001), light-induced chloroplast movement (Jarillo *et al*. 2001; Kagawa *et al*. 2001; Sakai *et al*. 2001), stomatal opening (Kinoshita *et al*. 2001), leaf flattening (Sakai *et al*. 2001; Sakamoto & Briggs 2002) and palisade cell development (Kozuka *et al*. 2011).

1.1.4 UV RESISTANCE LOCUS 8 (UVR8)

The last higher plant photoreceptor to be discovered and characterized was UVR8, which perceives ultraviolet B (UV-B) radiation (280- 315 nm) and triggers the activation of signalling pathways regulating target genes (Kliebenstein *et al*., 2002). Unlike other photoreceptors, UVR8 does not have an external chromophore molecule as a photon sensor and perceives UV-B radiation through specific tryptophans located in its primary structure. UVR8 is a seven-bladed β-propeller protein that is found in homodimers both in the cytosol and nucleus (Brown *et al*., 2005; Kaiserli *et al*., 2007). Upon UV-B exposure, the salt bridges that hold the homodimers together are disrupted, resulting in UVR8 dissociation into active monomers (Rizzini L., 2011; Christie 2012). The monomers physically interact with COP1 and are translocated into the nucleus, where they initiate signal transduction through multiple mechanisms (Favory *et al*., 2009; Rizzini, 2011; discussed in section 1.4.3). UVR8 orchestrates expression of more than 100 genes in Arabidopsis and is involved in the regulation of metabolic and morphological responses, ranging from photomorphogenesis to stress response regulation (Jansen *et al*., 1998; Frohnmeyer & Staiger, 2003; Paul & Gwynn-Jones, 2003; Ulm & Nagy, 2005; Jenkins & Brown, 2007). Similar to phytochromes and cryptochromes, UV-B controls plant development through regulation of PHYTOCHROME INTERACTING FACTORs (PIFs) (discussed in section 1.4). To cease UV-B UVR8 mediated signals, RUP1 and RUP2 sequester UVR8 from COP1, leading to UVR8 redimerization into its inactive form.

1.2 PHYTOCHROME INTERACTING FACTORs (PIFs)

In Arabidopsis, PIFs belong to a subgroup of the basic helix-loop-helix (bHLH) transcription factor superfamily. Most PIFs contain HLH and basic domains which confer them the ability to dimerize and bind DNA, respectively (Toledo-Ortiz *et al*., 2003; Al-Sady *et al*., 2008; Shen *et al*., 2008; Bu *et al*., 2011). All contain the active phytochrome binding (APB) conserved domain, required for specific interaction with the Pfr form of phyB (Khanna *et al*., 2004). Eight out of the 15 members of the PIF

subfamily (PIF1-8) have been shown to interact with at least one of the five phytochromes in Arabidopsis and consequently have been described as the central players in transduction of light signals perceived by phytochromes. The less conserved motif APA allows physical interaction with phyA and is found in PIF1 and PIF3 (Huq *et al*., 2004; Lee & Choi, 2017).

PIFs have been shown to be master signal integrators in plants, linking information from both environmental signals (light, competitors, temperature, pathogens) with endogenous signals (circadian clock, hormones [gibberellic acid (GA), ethylene, brassinosteroids (BRs), jasmonic acid (JA), nitric oxide (NO)] and sugar metabolism) to trigger appropriate physiological responses. These include skotomorphogenesis/photomorphogenesis, diurnal and photoperiodic growth, shade-avoidance, anthocyanin biosynthesis, thermomorphogenesis, sugar-induced growth, stomata and carpel development, seed germination, photosynthesis and chloroplast development, chlorophyll and carotenoid biosynthesis, ROS-responses, cold acclimation/freezing tolerance and blue light mediated de-etiolation and phototropic responses (Khanna *et al*., 2007; Oh *et al*., 2007; Lorrain *et al*., 2008; Kim *et al*., 2008; Casson *et al*., 2009; Hornitschek *et al*., 2009; Stephenson *et al*., 2009; Gabriele *et al*., 2010; Leivar & Quail, 2011; Lee & Thomashow, 2012; Oh *et al*., 2012; Li *et al*., 2012a; Shi *et al*., 2013; Bernardo-García *et al*., 2014; Leivar & Monte, 2014; Sakuraba *et al*., 2014; Song *et al*., 2014; Lee *et al*., 2015; Liu *et al*., 2015; Zhang *et al*., 2015; Quint *et al*., 2016; Zhu *et al*., 2016a; Jeong *et al*., 2016; Campos *et al*., 2016; Shor *et al*., 2017).

In Arabidopsis, PIFs accumulate in the dark and function as repressors of photomorphogenesis. When plants are exposed to light, phytochromes in the Pfr form bind to and promote the turnover of PIFs (principally PIF1, PIF3, PIF4 and PIF5) through phosphorylation, ubiquitination and degradation via the 26S proteosome, leading to transcriptional reprogramming and conversion to the photomorphogenic state (Bauer *et al*., 2004; Monte *et al*., 2004; Shen *et al*., 2005, 2008; Al-Sady *et al*., 2006; Nozue *et al*., 2007; Lorrain *et al*., 2008; Soy *et al*., 2012; Yamashino *et al*., 2013). These transcriptional changes allow seedlings to adjust to fluctuating light conditions and maximize their fitness. Furthermore, PIFs act as

central integrators connecting light signalling to other signalling processes, such as temperature signalling (Koini *et al*., 2009; Franklin *et al*., 2011; Sun *et al*., 2012; Quint *et al*., 2016), sugar signalling (Shor *et al*., 2017) and biotic stress signalling (Windram *et al*., 2012; Yang *et al*., 2012; Campos *et al*., 2016; Gangappa *et al*., 2017). Although PIF family members show high DNA sequence similarity, share structural motifs and participate in the regulation of photomorphogenesis, they can regulate physiological responses either individually or in combination, as well as presenting overlapping and distinct functions (Jeong & Choi, 2013) (Figure 1).

1.2.1 PIF1

PIF1 is involved in suppression of light-induced seed germination by regulating the expression of abscisic acid (ABA) and GA related genes through the interaction with HFR1 (LONG HYPOCOTYL IN FAR-RED1) and LEUNIG_HOMOLOG (Oh *et al*., 2004; Shi *et al*., 2013; Lee *et al*., 2015). PIF1 also regulates hypocotyl negative gravitropism in the dark, chlorophyll biosynthesis, plastid development, inhibition of hook and cotyledon opening in the dark (Huq *et al*., 2004; Moon *et al*., 2008; Kim *et al*., 2016; Leivar *et al*., 2012; Oh *et al*., 2004; Shin *et al*., 2009).

1.2.2 PIF2

PIF2 (also known as PIL1) is stabilized in response to red light through physical interaction with phyB and is degraded via the 26S proteasome in the dark by interaction with COP1. Unlike other PIFs, PIF2 promotes seed de-etiolation in response to light (blue, red and far-red), in part, by heterodimerization with HRF1. PIF2 acts cooperatively with HFR1 to inhibit the transcription of PIF1,3,4 and 5 target genes (Luo *et al*., 2014). In addition, PIF2 binds to PIF1, PIF3, PIF4 and PIF5 to inhibit their transcriptional activities (Hornitschek *et al*., 2009; Shi *et al*., 2013; Pham *et al*., 2018).

1.2.3 PIF3

PIF3 was the first member of the PIFs to be identified. Its main role is negatively regulating seedling de-etiolation through modulating phyB accumulation (Kim *et al*., 2003; Monte *et al*., 2004; Leivar *et al*., 2008). Other functions include the suppression of chlorophyll biosynthesis and photosynthesis in etiolated seedlings (Stephenson *et al*., 2009), optimization of the temporal regulation hypocotyl growth during night cycles by interacting with TIMING OF CAB EXPRESSION 1 (TOC1) (Soy *et al*., 2016) and promotion of hypocotyl elongation in response to ethylene in light (Zhong *et al*., 2012). Recently, PIF3 has been shown to act as a negative regulator of freezing tolerance. At warm temperatures in the light, PIF3 binds to two F-box proteins (EIN3-BINDING F-BOX 1 (EBF1) and EBF2) and is degraded by the 26S proteosome. Cold temperatures promote EBF1/2 degradation, stabilising PIF3 which binds to and represses the expression of *CBF* (C-REPEAT BINDING FACTOR) promoter genes involved in cold acclimation and freezing tolerance (Jiang *et al*., 2017). Hence, PIF3 is an important integrator of light and temperature signals.

PIFs are not only circadian regulated but are also thought to regulate clock functions by directly modulating the expression of clock components. The core clock components *LATE ELONGATED HYPOCOTYL* (*LHY*) and *CIRCADIAN CLOCK-ASSOCIATED 1* (*CCA1*) have PIF binding sites (G boxes (CACGTG)) in their promoters and have been shown to be bound by PIF3 *in vitro* (Martinez-Garcia *et al*., 2000). Another component of the central oscillator loop, TOC1, directly interacts with PIF3, repressing its activity and inhibiting plant growth during the early evening (Yamashino *et al*., 2003, Soy *et al*., 2016; Martin *et al*., 2016). Yeast-2-Hybrid (Y2H) assays have further shown that all PIFs can bind to TOC1 (Yamashino *et al*., 2003). Collectively, these data suggest that PIFs are involved in both input and output pathways of the circadian clock.

1.2.4 PIF4

PIF4 regulates a variety of plant responses to light and temperature. These include skotomorphogenesis (Huq & Quail 2002; Zhu *et al*., 2016a), shade avoidance (Lorrain *et al*., 2008),

cell/organ elongation (Oh *et al*., 2012), diurnal growth patterns (Nozue *et al*., 2007; Zhu *et al*., 2016a,b), stomatal development (Casson *et al*., 2009), leaf senescence (Sakuraba *et al*., 2014; Song *et al*., 2014; Zhang *et al*., 2015), freezing tolerance (Lee & Thomashow, 2012), anthocyanin biosynthesis (Liu *et al*., 2015), flowering and thermomorphogenesis (Franklin *et al*., 2011; Stavang *et al*., 2009; Kumar *et al*., 2012). The regulation of thermomorphogenesis and shade avoidance by PIF4 is discussed in section 1.5.

PIF4 accumulates in the dark, whereas exposure to red light leads to phosphorylation, ubiquitination, and degradation of the protein in a phyB-dependent manner (Huq & Quail, 2002; Lorrain *et al*., 2008). When plants experience shade from neighbouring plants, the R:FR ratio declines, decreasing active phyB and stabilizing both PIF4 and PIF5 (Lorrain *et al*., 2008). The accumulation of both proteins induces the expression of genes that promote hypocotyl elongation, including *YUCCA* genes involved in auxin biosynthesis (Hornitshek *et al*. 2012). Other shade genes expressed in a PIF4- and PIF5-dependent manner are *LONG HYPOCOTYL IN FAR RED1* (*HFR1*) and *PHYTOCHROME REGULATED1* (*PAR1*), which encode proteins that physically interact with PIF4 and PIF5 forming inactive heterodimers to sequester them from regulating the expression of their target genes (Hao *et al*., 2012; Hornitschek *et al*., 2009). This mechanism prevents the plant over-elongating stems and lodging. In addition to low R:FR, low blue light also triggers shade avoidance in a PIF4/PIF5- dependent manner. When the plant is shaded by a canopy, reduced blue light induces shade avoidance through direct interactions between PIF4/PIF5 and cry1/cry2 to regulate stem elongation (Pedmale *et al*., 2016). Additionally, shaded environments enhance phototropism, a reorientation of growth and positioning of photosynthetic organs to further facilitate light capture (Ballaré *et al*., 1992). The inactivation and reduced activity of phyB in low R:FR boosts PIF4, PIF5 and PIF7 activity, increasing auxin, which is redistributed across the hypocotyl through perception of a blue-light gradient by phot1, resulting in an asymmetrical growth towards blue light (Liscum & Briggs, 1995; Sakai *et al*., 2001; Goyal *et al*., 2016; Boccaccini *et al*., 2020). Cry1, in turn suppresses *PIF4* expression to attenuate phototropism to modulate hypocotyl growth out of the shade (Boccaccini *et al*., 2020).

1.2.5 PIF5

PIF5 regulates plant growth and development in a similar way to PIF4, regulating many of the same pathways. Both PIFs positively regulate leaf senescence by degrading chlorophyll in the dark (Sakuraba *et al*., 2014; Song *et al*., 2014; Zhang *et al.*, 2015), negatively regulate the light-induced anthocyanin biosynthesis (Liu *et al*., 2015), and control shade-avoidance together with PIF7 (Lorrain *et al*., 2008; Hornitschek *et al*., 2009; Li *et al*., 2012). In addition, both PIFs are required to promote plant diurnal growth (Nozue *et al*., 2007).

1.2.6 PIF6

PIF6 is a regulator of seed dormancy and, like PIF2, acts as a positive regulator of photomorphogenesis (Penfield *et al*., 2010; Shimizu-Sato *et al*., 2002; Levskaya *et al*., 2009; Toettcher *et al*., 2011, 2011b, 2013).

1.2.7 PIF7

PIF7 negatively regulates seedling de- etiolation under red light (Leivar *et al*., 2008) and promotes hypocotyl elongation through the suppression of phyB levels. PIF7 is the dominant regulator of shade avoidance responses and promotes expression of auxin biosynthesis genes in shade (Li *et al*., 2012). PIF7 also acts with PIF4 to suppress freezing tolerance in long photoperiods by repressing expression of *C- REPEAT BINDING FACTOR* (*CBF*) genes involved in cold acclimation (Lee & Thomashow, 2012). Most recently, PIF7 was shown to regulate thermomorphogenesis responses together with PIF4 (Fiorucci *et al*., 2020; discussed in section 1.5).

1.2.8 PIF8

PIF8 is the least characterized member of the PIF family. PIF8 contains an APB, but not APA, motif and has been shown to interact with phyB active Pfr form, but not with phyA *in vitro* (Oh *et al*., 2020). Unlike other PIFs, PIF8 accumulates in far-red light, and is degraded in the dark. Its protein levels are modulated by phyA, phyB and COP1/SPA. In the dark COP1/SPA complex targets PIF8 for degradation. When COP1/SPA activity is inhibited by active phyA in far-red light, PIF8 proteins are stabilized. PIF8

is also degraded in red light by phyB. Together with PIF4, PIF5 and PIF7, PIF8 also represses phyA-induced seedling photomorphogenesis in far-red light but does not regulate phyB-mediated red-light responses. The mechanisms by which PIF8 modulates phyA-, but not phyB-, mediated responses have yet to be elucidated (Oh *et al*., 2020). PIF8 binds to the promoters of other PIF target genes, suggesting that PIF8 also controls the expression of photomorphogenic genes to avoid exaggerated photomorphogenic response under long far-red light exposure (Oh *et al*., 2020).

1.3 Regulation of PIF activity

It has been demonstrated through quantitative analyses using *pif* single and higher order mutants, that PIFs vary in the degree in which they regulate the transcription of shared target genes (Zhang *et al*., 2013). This could result from either the intrinsic properties of individual PIFs or different transcriptional and post transcriptional regulation. The mRNA abundance of PIFs is dynamic due to transcriptional fluctuations in response to different stimuli (circadian rhythms, abiotic signals, phytohormones), developmental stage and tissue/organ specificity. In addition, PIF activity can be regulated through a variety of post-transcriptional mechanisms. This diversity of regulation enables PIFs to act as central hubs of environmental signal integration, synchronizing environmental cues with plant development to optimize plant growth and fitness.

1.3.1 Post transcriptional regulation of PIFs

Many factors bind to PIFs and negatively regulate their activity and abundance. Within these, four groups have been characterized as suppressors of PIF- DNA binding: Helix-Loop- Helix (HLH) transcription factors, photoreceptors (phys and crys), DELLAs and evening components of the circadian clock. In contrast, binding of the E3 ubiquitin ligase COP1 has been shown to promote PIF stability, possibly through inhibiting phyB binding (Ling *et al*., 2017).

1.3.1.1 HLH proteins

HLH class proteins involved in the inhibition of PIF activity include HFR1, PAR1 (PHYTOCHROME RAPIDLY REGULATED1), PAR2 and HEC (HECATE), which will act by forming heterodimers with PIFs, preventing them from interacting with target genes (Hornitschek *et al*., 2009; Hao *et al*., 2012; Shi *et al*., 2013; Zhu *et al*., 2016b). HFR1 and HEC1/HEC2 sequester PIF1 to promote seed germination under red/far-red light (Shi *et al*., 2013; Zhu *et al*., 2016b). PIF4 and HECs form a composite feedback loop in high ambient temperatures to control thermomorphogenesis (Lee *et al*., 2021). At high ambient temperatures PIF4 promotes *HEC* and *PIF4* expression. HECs form inactive heterodimers with PIF4, preventing its occupancy at gene promoters, including its own (Lee *et al*., 2021). Interactions between HFR1, PAR1 and PAR2 with PIF4 and PIF5, lead to inhibition of shade avoidance responses (Hornitschek *et al*. 2009; Galstyan *et al*. 2011; Hao *et al*., 2012). HFR1 and PIFs also regulate each other's abundance in darkness. HFR1 interacts with PIF1 and PIF5 and promotes their degradation through heterodimer formation, while PIF1 induces HFR1 degradation by enhancing its ubiquitination by COP1. This reciprocal regulation is crucial for a fast transition from skotomorphogenesis to photomorphogenesis (Xu *et al*., 2017).

1.3.1.2 Photoreceptors

The second group of PIF regulators are photoreceptors. Light activated phytochromes can interact with PIFs inhibiting their binding DNA activity (Qiu *et al*., 2017; Park *et al*., 2012; Park *et al*., 2018). PIF1, PIF3, PIF4, PIF5, PIF6 and PIF8 interact with phyB (Ni *et al*., 1999; Huq & Quail, 2002, 2004; Khanna *et al*., 2004; Oh *et al*., 2004; Leivar *et al*., 2008a; Leivar & Quail, 2011; Ni *et al*., 2014), in addition PIF1 and PIF3 bind to phyA (Huq *et al*., 2004; Oh *et al*., 2004; Al-Sady *et al*., 2006; Shen *et al*., 2008). Phytochrome initiates PIF turnover by phosphorylation, ubiquitination, and degradation via the proteasome pathway (Pham *et al*., 2018). PIF7 is an exception, as phosphorylation results in its cytoplasmatic accumulation (Huang *et al*., 2018). Cryptochromes cry1 and cry2 bind with PIF4 and PIF5

to regulate their response under blue light to mediate hypocotyl elongation (Pedmale *et al*., 2015; Ma *et al*., 2016).

1.3.1.3 DELLA proteins

The GA-regulated DELLA family of proteins restrain plant growth by binding to and inhibiting PIFs, independently of light signals. DELLA proteins have been shown to interact with PIF1, PIF3, PIF4, and PIF6, preventing them from interacting with their target genes (de Lucas, 2008; Feng, 2008; Gallego-Bartelomé *et al*., 2010). DELLAs also degrade PIFs through the 26S proteasome pathway (Li, 2016). PIF inhibition by DELLAs results in an optimum regulation of hypocotyl elongation in response to light and GA signals (de Lucas *et al*., 2008; Feng *et al*., 2008).

1.3.1.4 Evening components of the circadian clock

The circadian clock Evening Complex (EC) consists of three components, EARLY FLOWERING3 (ELF3), EARLY FLOWERING4 (ELF4) and LUX ARRYTHMO (LUX) and suppresses *PIF4* and *PIF5* transcription under diurnal cycles (Nozue *et al*., 2007; Niwa *et al*., 2009; Lorrain *et al*., 2008). In addition, ELF3 binds to the bHLH domain of PIF4, independently from EC activity. This sequesters PIF4 and prevents it from binding to DNA to initiate the transcription of target genes (Nieto *et al*., 2015). TOC1 and the closely related PSEUDO RESPONSE REGULATOR proteins PRR5, PRR7 and PRR9 can also bind to PIFs and inhibit their transcriptional activity. In short photoperiods, TOC1 interacts with PIF3 in the nucleus, inhibiting growth during the early evening (Soy *et al*. 2016). Transcriptional waves of PRR5, 7 and 9 act similarly throughout the day and early evening to gate hypocotyl elongation to the late evening (Martin *et al*. 2018). PRR5 and 9 have also been shown to bind to and inhibit PIF4 and PIF7 to suppress shade avoidance (Zhang *et al*., 2020).

1.3.1.5 COP1

PIFs and COP1 both suppress photomorphogenesis. PIFs modulate the expression of many genes to promote skotomorphogenesis. COP1 forms complexes with SPA (SUPRESSOR OF PHYA) proteins to degrade key photomorphogenesis proteins (e.g HY5, HYH (HY5 HOMOLOG), HFR1, LAF1 (LONG AFTER

FAR-RED LIGHT 1)) via the 26S proteasome pathway. COP1 accumulates in the dark and directly targets HY5 for degradation (Osterlund *et al*. 2000; Wang & Deng, 2002). SPA1 was originally identified as a negative regulator of phyA-mediated signalling and modulates COP1 E3 ligase activity *in vitro* (Hoecker *et al*. 1999; Saijo *et al*., 2003; Seo *et al*., 2003). In dark-grown seedlings, the GSK3-like kinase BRASSINOSTEROIDINSENSITIVE (BIN2) acts as a negative regulator of PIFs, while COP1/SPA1 performs a non-canonical role to promote PIF stabilisation. BIN2 phosphorylates both PIF3 and PIF4 *in vitro*, subsequently driving their degradation (Bernardo-García *et al*., 2014; Ling *et al*., 2017). COP1 directly interacts with BIN2, while SPA1 binds to PIF3 in the BIN2-binding domain, sequestering PIF3 from phosphorylation and proteasomal degradation. The COP/SPA complex has also been shown to stabilise PIF5 (Pham *et al*. 2018; Sharma *et al*. 2019). The stabilization of PIFs in the dark by the COP1/SPA complex is important to the promotion of skotomorphogenesis.

1.3.1.5 Regulation of PIF RNA structure

Most recently, research shown that higher temperatures cause harpin formation within PIF7 5' untranslated region. This alternative conformation leads to an increase in translation, hence increasing PIF7 levels. This mechanism is an additional regulatory mechanism that integrates temperature to growth responses, as accumulation of PIF7 leads to upregulation of auxin genes (Chung *et al*., 2020).

1.3.2 Transcriptional regulation of PIFs.

PIF genes respond to environmental conditions and endogenous stimuli. The differential expression of PIFs therefore results from the precise combination of regulatory factors in different environments. Each of the PIF promoters is regulated by a particular combination of transcription factors that ultimately dictate the specificity of the response (Castillon *et al*., 2007; Jeong & Choi, 2013).

1.3.2.1 The circadian clock

In Arabidopsis, PIF4 and PIF5 proteins show rhythmic expression over a diurnal cycle and stably accumulate during the night (Nozue *et al*., 2007; Shin *et al*., 2013). Transcript levels rise throughout the night, peaking at dawn (Nozue *et al*., 2007). This rhythmic oscillation occurs due to (EC) activity. LUX binds to *PIF4/5* cis elements and subsequently recruits ELF3 and ELF4 which collectively suppress the transcription of both PIFs during the early evening (Nusinow *et al*., 2011; Herrero *et al*., 2012; Lu *et al*., 2012; Helfer *et al*., 2011). CCA1 is thought to regulate *PIF4/5* transcription through an, as yet identified, mechanism, as *CCA1*ox plants present constitutive high levels of both transcripts (Nozue *et al*., 2007). TOC1, PRR5 and PRR7 negatively regulate *PIF4/5* expression (Niwa *et al*., 2009). It is thought that PIF7 is also circadian regulated due to transcript oscillations under short-day, long-day and free-running conditions (Kidokoro *et al*., 2009; Lee & Thomashow, 2012). *PIF3* transcription is not, however, regulated by the circadian clock. PIF3 protein accumulates in the dark and is degraded in the day by photoactivated phyB (Soy *et al*., 2012).

1.3.2.2 ETHYLENE-INSENSITIVE 3 (EIN3)

Alongside circadian regulation, hormone signals also modulate PIF transcription. In the light, the ethylene signalling component ETHYLENE-INSENSITIVE3 (EIN3) directly interacts with the *PIF3* promoter, promoting its expression. This induces ethylene to switch from a hypocotyl elongation suppressor to a promoter (Solano *et al*., 1998; Kosugi & Ohashi, 2000; Zhong *et al*., 2012).

1.3.2.3 Nitric Oxide (NO)

Another factor that might regulate PIF transcription in an as-yet-uncharacterized manner is nitric oxide (NO). NO-deficient mutant plants show increased levels of *PIF1*, *PIF3* and *PIF4* transcript in addition to displaying a long hypocotyl under red light, suggesting that NO might inhibit hypocotyl elongation through the suppression of PIF transcription (Lozano-Juste *et al*., 2011).

1.3.2.5 Other PIFs

PIF4 can bind directly to its promoter G-box motif (CACGTG) and upregulate its own expression forming a self-activated transcriptional feedback loop (Zhai *et al*., 2020). PIF4 is stabilized in high ambient temperatures, and positively regulates its transcriptional expression by promoter interaction to regulate thermomorphogenesis (Lee *et al*., 2021).

1.4 Regulation of plant responses by ultraviolet-B

Ultraviolet (UV) wavelengths are situated between x-rays and visible light on the electromagnetic spectrum and are divided into three categories: UV-A (315 to 400 nm), UV-B (280 to 315 nm) and UV-C (100 to 280 nm) radiation. Most of the solar ultraviolet radiation is absorbed by Earth's stratosphere (McKenzie *et al*., 2003), however some UV-A and UV-B reach Earth's surface affecting the biosphere. UV-B corresponds to less than 0.5% of solar energy on Earth (Blumthaler, 1993). Ambient UV-B light varies according to pollution, cloud density, surface reflectance and canopy coverage (McKenzie *et al*., 2003; Paul, 2003).

In plants, UV-B is an important light signal that drives both stress and developmental responses by inducing the expression of hundreds of plant genes through nonspecific UV-B related pathways and through activation of the UVR8 photoreceptor (Casati & Walbot, 2004; Ulm *et al*., 2004; Brown *et al*., 2005; Kilian *et al*., 2007; Favory *et al*., 2009, Kilian *et al*. 2007; Jenkins 2009; Gonzalez Besteiro *et al*., 2011 Kami *et al*., 2010, Heijde & Ulm 2012). The type of downstream response is dictated by the magnitude of fluence rate, duration and wavelength of UV-B light (Brosché & Strid, 2013; Frohnmeyer & Staiger, 2003; Ulm & Nagy, 2005).

1.4.1 Stress responses

High fluence rates and short wavelengths of UV-B mediate stress responses. Plants have evolved effective photo repair and protection systems to cope with constant exposure to highly mutagenic UV-B photons irradiated from the sun (Mazza *et al*., 2000; Frohnmeyer & Staiger 2003; Ulm & Nagy 2005; Jenkins 2009). UV-B light regulates plant metabolism in order to allow plants to survive in sunlight through induction of secondary metabolites, such as flavonoids, in epidermal tissues. These act as sunscreen by absorbing the photons to avoid their penetration into the plant cell (Rizzini *et al*., 2011). Other plant protection mechanisms include hair and wax production and enhancement of cellular antioxidant systems (Steinmüller & Tevini, 1985; Li *et al*., 1993; Stapleton & Walbot, 1994; Landry *et al*., 1995; Bornman *et al*., 1997; Rozema, 1997; Jansen *et al*., 1998; Mazza *et al*., 2000; Brosché & Strid, 2003; Liakopoulos *et al*., 2006). In addition, UV-B light stimulates the expression of genes involved in DNA damage repair (e.g., DNA photolyases), and mitigation of photo oxidative damage and oxidative stress (Brown *et al*., 2005).

UV-B light also regulates biotic responses, as pathogenesis-related (PR) genes and proteinase inhibitor genes are both induced following UV-B light exposure. Moreover, UV-B treated plants have been shown to have reduced herbivory, which might be explained by both the induction and accumulation of ROS and phytohormones involved in the mediation of wounding responses, such as JA and salicylic acid (SA). Additionally, increased levels of isoflavonoids produced by plants in response to UV-B as sunscreen compound were shown to reduce herbivory (Mackerness *et al*., 1999; Izaguirre *et al*., 2003; Zavala *at al*., 2014).

1.4.2 Regulatory responses

Low photon irradiances of UV-B (< 1.0 µmol m^{-2} s^{-1}) act as an informational light cue that activates the UVR8-dependent signalling pathway, stimulating the expression of metabolic, defence and developmental genes (Kim *et al*., 1998; Jenkins *et al*., 2001; Suesslin & Frohnmeyer, 2003; Ulm & Nagy, 2005; Jenkins & Brown, 2007). Low dose UV-B regulates photomorphogenesis, as this is the threshold at which UV-B induces the UVR8- mediated expression of *HY5* and *HYH*, central players in the regulation of photomorphogenesis by indirect suppression of PIF activity (Brown & Jenkins, 2008; Hayes *et al*. 2017).

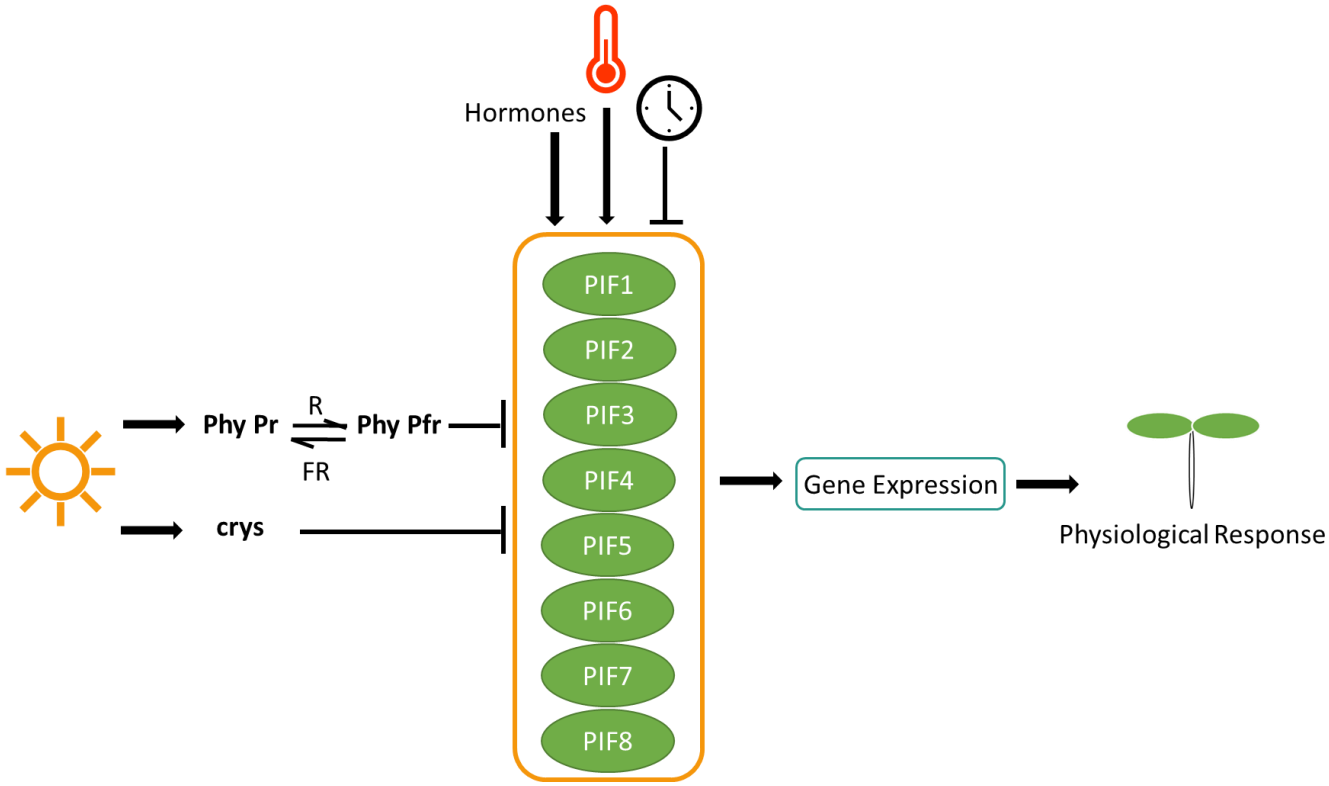


Figure 1: Simplified schematic model of PIF signal integration. Sunlight activates phytochromes and cryptochromes as it contains both red and blue light. Activated phytochromes interact with PIF1/3/4/5/6/8 and initiate their degradation by phosphorylation, ubiquitination, and degradation via the proteasome pathway. Additionally, phy-PIF interaction inhibits PIF DNA binding activity. Activated crys interact with PIF4 at increased temperatures to inhibit their DNA binding activity to suppress thermomorphogenesis. Increased temperatures boost *PIF4* transcription and PIF7 translation. In the

light, phytohormone ethylene positively regulates PIF transcription. Circadian clock evening complex negatively regulates *PIF4* and *PIF5* transcription. Ultimately, PIF regulation will cause reprogramming in gene expression that dictates the appropriate plant physiological response. Positive regulators (->). Negative regulators (-|). R (Red Light). FR (Far-red light).

1.4.3 UVR8 signalling

Environmental UV-B is perceived in higher plants by UVR8 which is present within all organs (Rizzini *et al*., 2011) and mostly located in the cell cytoplasm, but functions in the nucleus upon UV-B induced nuclear import. UVR8 exists in biologically inactive homodimers bound by salt bridges at the dimer interface. UVR8 absorbs UV-B light through conserved tryptophan residues, including tryptophan 285 (Trp-285). This causes the dimers to reversibly dissociate into active monomers, leading to a rapid accumulation in the nucleus mediated by COP1 (Christie *et al*., 2012; Wu *et al*., 2012; Rizzini *et al*., 2011; O'Hara & Jenkins 2012; Heijde & Ulm 2013; Huang *et al*., 2014; Zeng *et al*., 2015). COP1 is traditionally described as a photomorphogenesis repressor as it targets HY5 for degradation to prevent de-etiolation in dark/light grown seedlings. However, in UV-B light COP1 acts as a photomorphogenesis positive regulator. UV-B upregulates *COP1* expression by stimulating the direct binding of FAR-RED ELONGATED (FHY3) and HY5 to its promoter, the latter in a positive feedback loop as COP1 is necessary for UV-B mediated activation of *HY5* (Huang *et al*., 2012). HY5 and HYH act concomitantly and redundantly downstream of COP1 and UVR8 to orchestrate UV-B signal transduction through regulation of UV-B responsive genes (Ulm *et al*., 2004, Brown *et al*., 2005, Brown & Jenkins 2008, Stracke *et al*., 2010, Huang *et al*., 2012). COP1 is necessary for active UVR8 import to the nucleus. Nuclear activated UVR8 monomers interact with target transcription factors BRASSINAZOLE-RESISTANT2 (BES1)/BES1-INTERACTING MYC-LIKE1 (BIM1), ATWRKY36 (WRKY36), MYB DOMAIN PROTEIN 73 (MYB73)/MYB77 to reprogramme gene expression to regulate photomorphogenesis and UV-B acclimation (Figure 2). Transcriptome analysis have shown that many UV-B induced genes are transcription factors (Brown *et al*., 2005; Yin *et al*., 2015).

The homologous proteins REPRESSOR OF UV-B PHOTOMORPHOGENESIS1 (RUP1) and RUP2 are localized in the nucleus and the cytoplasm, and their expression is activated in UV-B light ,in a UVR8-dependent manner (Gruber *et al*., 2010). RUP1 and RUP2 directly interact with UVR8 forming an "off switch" mechanism, ceasing UVR8-COP1 interaction and causing UVR8 redimerization, restoring the inactive homodimer (Cloix *et al*., 2012; Heijde & Ulm 2013). Additionally with RUP proteins, SALT TOLERANCE/BBX24 (STO/BBX24) act as a negative regulator of the UV-B signalling pathway in a distinct way, by binding to COP1 and HY5, sequestering them from regulating their target genes, therefore interrupting the signal cascade (Jiang *et al*., 2012). The downregulation of UV-B responses by STO/BBX24 is possibly enhanced by RADICAL-INDUCED CELL DEATH1 (RCD1) by physical interaction (Jiang *et al*. 2009; Jiang *et al*. 2012).

Finally, UVR8 may regulate gene transcription through chromatin association in a UV-B- dependent manner (Brown *et al*., 2005, Cloix & Jenkins 2008). However, UVR8 nuclear localization occurs in the absence of UV-B radiation, and its interaction with chromatin of UVR8 target genes is constitutive and independent from UV-B signals (Brown *et al*., 2005, Kaiserli & Jenkins 2007, Cloix & Jenkins 2008). The interaction has been shown to occur with only a partial number of promoter regions of genes that are UVR8 regulated, such as At5g11260, HY5; At5g24850, cry3; At2g47460 and PFG1/MYB12 (Cloix & Jenkins 2008). More detailed investigation is required to determine the roles played by the UVR8 chromatin interaction on gene expression.

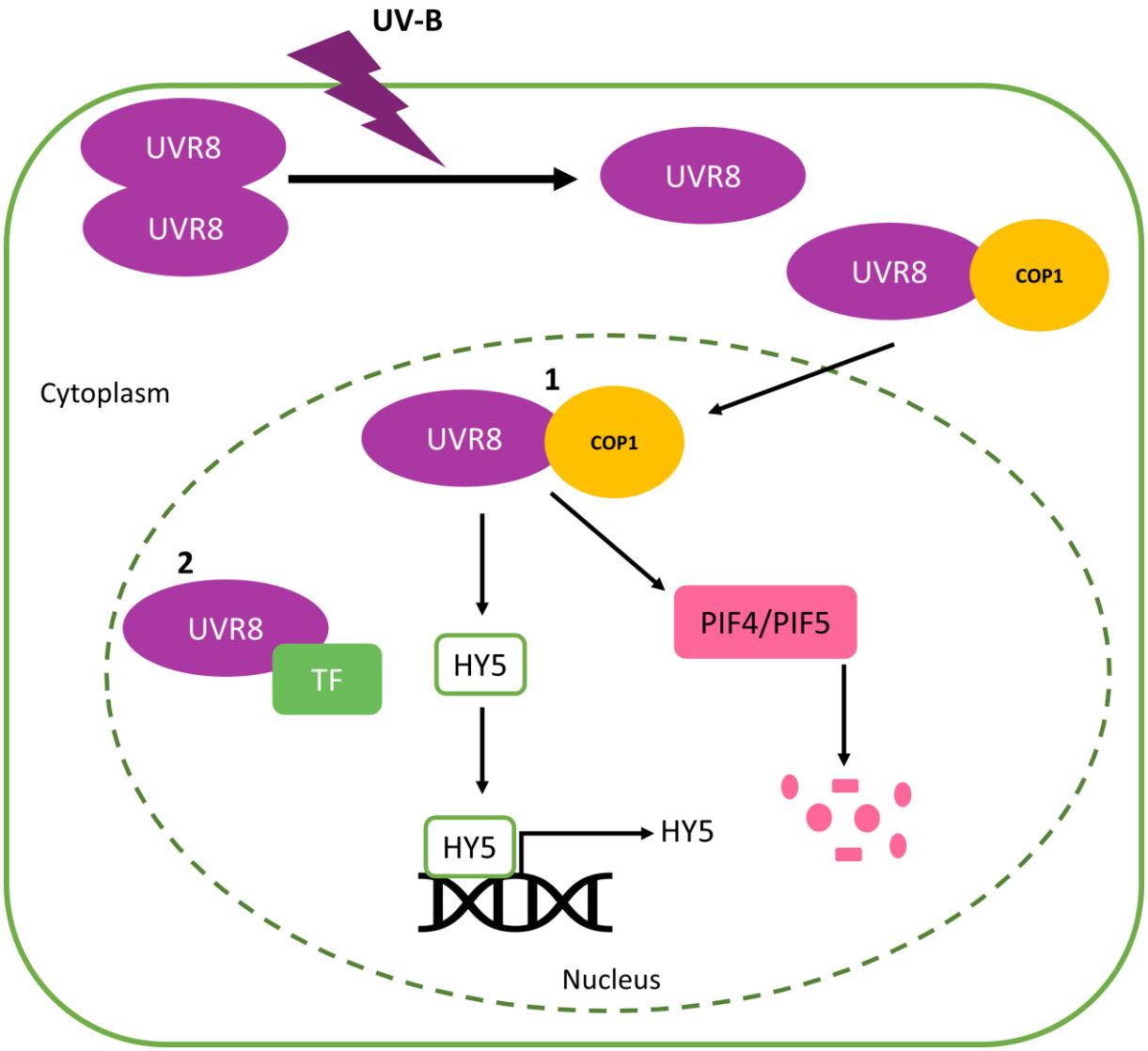


Figure 2: Model of UV-B signal transduction via UVR8. The UVR8 photoreceptor exists mostly in inactive dimers in the cytosol. Upon UV-B radiation, UVR8 dimers dissociate into active monomers that associate with COP1 to be subsequently imported to the nucleus of the cell. Nuclear UVR8 regulates gene expression by (1) inactivating COP1 activity through heterodimerization, leading to accumulation of COP1 substrates such as HY5, a positive photomorphogenesis regulator, and PIF turnover by an unknown mechanism. (2) UVR8 directly binds to transcription factors, preventing them from interacting with their target genes.

1.5 Developmental responses modulated by UVR8 and PIFs

In addition to photomorphogenesis, other plant developmental responses are modulated by UV-B in a UVR8- mediated manner through the regulation of PIFs. These include thermomorphogenesis and shade-avoidance.

1.5.1 Thermomorphogenesis

Small increases in ambient temperature can cause plants to undergo morphological and physiological changes termed thermomorphogenesis to acclimate to the ever-changing environment. In Arabidopsis, the thermomorphogenesis phenotype consists of hypocotyl and petiole elongation, early flowering, increased leaf hyponasty (Halliday *et al*., 2003; Blázquez *et al*., 2003; Balasubramanian *et al*., 2006; Hwang *et al*., 2017; Ibañez *et al*., 2017; Lau *et al*., 2018). High temperature-mediated architectural adaptations are predicted to provide a cooling mechanism by enhancing leaf evaporation, avoiding contact between photosynthetic and meristematic tissues to the hot ground, and allowing leaves to be cooled down by breeze to increase plant fitness under high temperatures (Gray *et al*., 1998; Millenaar *et al*., 2005; Koini *et al*., 2009; Van Zanten *et al*., 2009, 2010; Vasseur *et al*., 2011; Kumar & Wigge 2010; Crawford *et al*., 2012; Nomoto *et al*., 2012; Sun *et al*., 2012; Bridge *et al*., 2013; Lee *et al*., 2014; Ibañez *et al*., 2015; Sanchez-Bermejo *et al*., 2015). More recently, it has been suggested that the ecological function of thermomorphogenesis is to enhance light capture in warm canopies, where the combination of shade and high respiration make rapid escape essential for carbon acquisition (Romero-Montepaone *et al*., 2021).

Increased temperatures are sensed by phyB (Legris *et al*., 2016; Jung *et al*., 2016), an RNA thermoswitch sensor within the 5'-UTR region of PIF7 (Chung *et al*., 2020) and a prion-like domain in ELF3 (Jung *et al*., 2020). PIF4 acts downstream temperature perception, being the main signalling hub that integrates temperature cues to promote leaf and shoot elongation by accumulation and binding to promoters of auxin biosynthesis signalling genes *YUCCA8* (*YUC8*), *TRYPTOPHAN*

AMINOTRANSFERASE OF ARABIDOPSIS 1 (*TAA1*), *CYTOCHROME P450 FAMILY 79B* (*CYP79B2*) and *SMALL AUXIN UP RNA 19* (*SAUR19*) (Koini *et al*., 2009; Stavagang *et al*., 2009; Franklin *et al*., 2011; Fiorucci *et al*., 2020). Additionally, higher temperatures induce the heterodimerization of PIF4-BES1 to regulate BR levels to facilitate plant growth (Stavang *et al*., 2009; Oh *et al*., 2012; Ibanez *et al*., 2018; Martinez *et al*., 2018; Bellstaedt *et al*., 2019).

Many factors regulate thermomorphogenesis responses by modulating PIF4 abundance and/or activity. Positive regulators of PIF4 include B-box 18/23 (BBX18/23) which upregulates PIF4 activity by suppressing ELF3 function (Ding *et al*., 2018). TEOSINTE BRANCHED 1/CYCLOIDEA/PCF (TCP) transcription factors induce *PIF4* expression by direct binding to its promoter (Han *et al*., 2019). C-REPEAT BINDING FACTOR (CBF) and MYB30 also upregulate *PIF4* expression and enhance PIF4 stability by inhibiting phyB-PIF4 interaction (Dong *et al*., 2020; Yan *et al*., 2020). SHORT HYPOCOTYL UNDER BLUE1 (SHB1) interacts with CCA1/LHY to promote *PIF4* expression by directing promoter binding (Sun *et al*., 2019). The COP1- DEETIOLATED 1 (DET1)-HY5 complex regulates PIF4 transcriptionally and post-transcriptionally (Gangappa *et al*., 2017). The transcriptional regulators HEMERA (HMR) and REGULATOR OF CHLOROPLAST BIOGENESIS (RCB) act together to stabilize PIF4 and promote the expression of thermoresponsive genes (Qiu *et al*., 2019, 2021). SPA family genes regulate the kinetics of phyB-PIF4 complex (Lee *et al*., 2020). PIF4 regulates its own abundance by direct binding to its own promoter, inducing transcription (Lee *et al*., 2021). Oppositely, negative regulators of PIF4 that suppress thermomorphogenesis include cry1, TOC1, the RNA-binding protein FCA and HECATE2 (HEC2) which directly bind to PIF4 inhibiting its transcriptional activity (Lee *et al*., 2014; Zhu *et al*., 2016; Ma *et al*., 2016; Lee *et al*., 2021). In addition, multiple circadian clock components indirectly suppress PIF4 activity. ELF4 stabilizes ELF3, consequently inhibiting PIF4 function (Box *et al*., 2015; Nieto *et al*., 2015). GIGANTEA (GI) suppresses PIF4 activity by stabilizing the DELLA protein REPRESSOR OF *ga 1-3* (RGA) (Park *et al*., 2020).

In addition to PIF4, PIF7, and to some extent PIF5, have been shown to play roles in the Arabidopsis thermomophogenesis response. When plants are exposed to high temperature, *PIF4* and *PIF7* transcripts increase and decrease, respectively. The translation of PIF7 is, however, enhanced, increasing PIF7 abundance (Koini *et al*., 2009; Stavang *et al*., 2009; Franklin *et al*., 2011; Foreman *et al*., 2011; Sun *et al*., 2012; Proveniers *et al*., 2013; de Wit *et al*., 2014; Fiorucci *et al*., 2019 ; Chung *et al*., 2020). PIF4 and PIF7 proteins therefore increase rapidly following temperature elevation. These form both homodimers and heterodimers in yeast (Fiorucci *et al*., 2020) and in mesophyll protoplasts (Kidokoro *et al*., 2009). It has been suggested that PIF4 and PIF7 act as heterodimers to regulate thermoresponsive gene expression. Unlike PIF4, PIF7 cannot function as a homodimer, making PIF4 the central regulator of the thermomorphogenic response (Fiorucci *et al*., 2020). PIF4 and PIF7 drive thermomorphogenesis in an overlapping manner by enhancing the biosynthesis of the growth phytohormone auxin through positive transcriptional regulation of its precursor *YUC* (*YUCCA*) genes, ultimately driving hypocotyl elongation (Franklin *et al*., 2011; Sun *et al*., 2012; Raschke *et al*., 2015; Fiorucci *et al*., 2020).

1.5.2 UV-B suppression of thermomorphogenesis is mediated by PIFs

Low-dose UV-B antagonizes plant thermomorphogenesis by suppressing stem elongation and leaf hyponasty (Hayes *et al*., 2017). This response is a result of UV-B-mediated suppression of *PIF4* transcript by UVR8. Despite the UV-B-mediated negative regulation of *PIF4* transcript, PIF4 levels are not degraded by UV-B at 28°C, but PIF4 function is inhibited (Hayes *et al*., 2014, 2017). Since the role of UVR8 chromatin binding is uncertain, it is possible that intermediate factors regulate the UV-B-mediated suppression of *PIF4* transcript. Known negative regulators of PIF4 activity (PARs, DELLAs, HY5) have been shown not to have a major role in UV-B-mediated suppression of *PIF4* transcript abundance (Roig-Villanova *et al*., 2007; de *Lucas et al*., 2008; Feng *et al*., 2008; Nieto *et al*., 2015; Toledo-Ortiz *et al*., 2015). COP1, however, forms a complex with UVR8, and the absence of COP1 decreases *PIF4* transcript abundance and causes insensitiveness to UV-B, indicating a role of COP1 in mediating UVR8 suppression of *PIF4* transcript accumulation (Hayes *et al*., 2017). UVR8 interaction

with COP1 reduces its E3 ubiquitin ligase activity, resulting in increased abundance of PIF4 negative regulators, affecting PIF4 activity (Roig-Villanova *et al*., 2007; de Lucas *et al*., 2008; Feng *et al*., 2008; Nieto *et al*., 2015; Toledo-Ortiz *et al*., 2015). HYH may inhibit PIF4 function by competing for *PIF4* target cis elements at increased temperatures (Hayes *et al*., 2017). HFR1 is stabilized in higher temperatures and inhibits PIF4 by heterodimerization (Hornitschek *et al*., 2009).

Overall, UV-B-mediated suppression of thermomorphogenesis is regulated by UVR8-COP1 and inhibits stem elongation by repressing *PIF4* transcript abundance and activity to reduce auxin and GA signalling. Although stem elongation at higher temperatures may be an adaptative mechanism to enhance leaf cooling capacity and light foraging, over elongation can increase plant susceptibility to lodging and reduce biomass. UV-B absorption antagonizes the thermomorphogenic response to limit stem elongation, increasing plant fitness.

1.5.3 Shade avoidance

Plants grown in high density vegetation optimize their light capture by outgrowing the shade their competitors project over them. The adaptative mechanism is known as shade avoidance and it is mainly characterized by stem, petiole and internode elongation, leaf hyponasty, inhibition of branching, inhibition of seed germination and early flowering (Franklin & Whitelam 2005; Martinez-Garcia *et al.,* 2010; Casal, 2012; Ruberti *et al*. 2012, Pierik & De Wit 2013).

Sunlight contains a relatively small amount of UV-B light and roughly equivalent proportions of blue, green, red, and far-red light. Red and blue wavelengths are absorbed by photosynthetic tissue, while green and far-red wavelengths are largely transmitted. Far-red light has also been shown to be reflected from canopies to neighbouring vegetation, lowering R:FR and signalling the threat of impending shade (Ballare, 1999; Vandenbussche *et al*., 2005; Franklin, 2008). In true shade, plants are exposed to low R:FR, low blue light and little UV-B. Changes in light quality and quantity caused by shade are perceived by phytochromes, cryptochromes and UVR8, however phyB plays the major role

on shade avoidance (Lorrain *et al*., 2008). Under low R:FR phyB is shifted to its Pfr inactive form, allowing PIF accumulation. Additionally, depleted levels of phyB Pfr frees SPA to form complexes with COP1 (COP1/SPA1), that can then degrade negative regulators of PIFs (HY5, HFR1), resulting in increased PIF activity (Rolauffs *et al*., 2012; Sheerin *et al*., 2015).

Downstream of photoreceptor perception of shade, PIF4, PIF5 and PIF7 are the master regulators of shade avoidance. PIF4 and PIF5 proteins are stabilized and PIF7 activity is elevated upon dephosphorylation in low R:FR (Lorrain *et al*., 2008; Nozue *et al*., 2011; Hornitscheck *et al*., 2012; Li *et al*., 2012). PIFs enhance tryptophan-dependent auxin biosynthesis by upregulating the expression of *YUCCA* genes and other auxin signalling and cell elongation genes to ultimately promote stem elongation (Tao *et al*., 2008). In shade, PIFs upregulate the transcription of *PIL1, HFR1, PHYTOCHROME RAPIDLY REGULATED 1 (PAR1)* and *PAR 2,* which act as negative regulators of their activity. HFR1 forms heterodimers with PIF4 and PIF5, whereas PAR1 forms heterodimers with PIF4, inhibiting DNA binding capacity. (Hornitscheck *et al*., 2009; Hao *et al*., 2012). The accumulation of these proteins is predicted to prevent exaggerated shade avoidance responses.

1.5.4 UV-B suppression of shade avoidance is mediated by PIFs

Since UV-B is perceived and absorbed by plant canopy, its depletion can also be a shade cue (Favory *et al*., 2009; Hayes *et al*., 2014). Similar to other photoreceptors, UVR8 inhibits plant stem elongation by suppressing transcription factors involved in plant growth (Hayes *et al*., 2014; Liang *et al*., 2018). The absence of active UVR8 can therefore lead to enhanced activity of growth factors. In Arabidopsis, perception of low-dose UV-B in low R:FR conditions inhibits stem and petiole elongation, acting as a shade avoidance suppressor (Hayes *et al*., 2014). The UV-B suppression of shade avoidance is suggested to prevent unnecessary allocation of resources towards neighbour competition once a gap in the canopy has been reached.

UV-B activated UVR8 mediates the suppression of shade avoidance with the involvement of HYH, HY5 and COP1 (Hayes *et al*., 2014). The interaction between UVR8 and COP1 inactivates COP1´s E3-ligase function, leading to the accumulation of negative regulators of shade avoidance HY5, HYH, HFR1 and PIL1 (Kim *et al*., 2014; Podolec & Ulm 2018; Sharma *et al*., 2019; Tavridou *et al*., 2020). Higher levels of auxin in shade avoidance are GA modulated (Franklin *et al*., 2008). UV-B-mediated increases in HYH/HY5 elevates transcription of the GA catabolism gene, *GA2-oxidase* (*GA2ox1)* (Hayes *et al*., 2014). This increases DELLA stability due to enhanced GA catabolism in addition to a drop in GA biosynthesis (Hayes *et al*., 2014; Rieu *et al*., 2008). DELLAs additionally form inactive heterodimers with PIF4 (de Lucas, 2008; Feng, 2008; Gallego-Bartelomé *et al*., 2010). Concomitantly, HFR1 plays a redundant role to DELLA in inactivation of PIF4 and PIF5 through heterodimer formation (Hornitschek *et al*., 2009; Tavridou *et al*., 2020; Sharma *et al*., 2019). UV-B perceived by UVR8 additionally causes PIF4 and PIF5 protein turnover to limit shade avoidance (Hayes *et al*., 2014). PIF5 degradation is modulated by COP1-UVR8 complex formation. As PIF5 is stabilized by direct binding with COP1, sequestration of COP1 by UVR8 leads to PIF5 to degradation through the proteasome-system and suppression of auxin signalling (Pham *et al*., 2018; Sharma *et al*., 2019).

1.6 Summary

PIFs are central signalling hubs that communicate environmental signals to the plant cell and synchronize physiological responses according to external light and temperature cues. Although we know a lot about how environmental and endogenous signals interact to control PIF protein activity and abundance, less is known about the environmental control of PIF transcript abundance. In particular, little is known about how UV-B controls PIF transcription. Understanding how UV-B mediates PIF expression will provide important insights into how UV-B controls plant growth and development.

1.7 Aims

The overreaching aims of this research were (i) to establish whether UV-B perceived by UVR8 regulates the promoter activity of PIFs involved in thermomorphogenesis and shade avoidance and (ii) characterize the mechanisms that control *PIF* expression in different light and temperature treatments.

CHAPTER 2

Materials and Methods

2.1 Plant Material

All experiments were performed using the plant *Arabidopsis thaliana*. The mutants and transgenic lines used in this study are described in Table 1.

2.2 Growth Conditions

2.2.1 Seed Treatment

For sterile medium cultivation, Arabidopsis seeds were surface sterilized with gas from a solution of 3 % (v / v) HCl and 70 % (v / v) bleach for 3 hours. Seeds were then suspended in autoclaved MiliQ H_2O and placed individually on agar using sterilized cocktail sticks. For other experiments, seeds were directly sown onto compost. All seeds were stratified in darkness at 4°C for four days, then germinated in growth cabinets in white light (WL), in 16 h light/ 8 h dark cycles (long day photoperiod), at 20°C and 70 % humidity.

2.2.2 Media

Compost Media

A mixture of compost (Levington) and horticultural silver sand (3 : 1 v / v) was used for all experiments except for thermomorphogenesis assays (qPCR and petiole and hypocotyl length) and GUS staining experiments.

Agar Media

Half-strength Murashige and Skoog (MS) nutrient mix (pH 5.8; Duchefa Biochimie) with 0.8 % (w / v) agar was poured into 10 cm x 10 cm petri dishes. For chapter 4 hypocotyl, petiole and thermomorphogenesis qRT-PCR experiments, 0.5 µM brassinolide (BR) dissolved in DMSO was added to the media, and mock plates contained 0.1 % (v / v) DMSO.

Liquid Media

Half-strength Murashige and Skoog (MS) nutrient mix (pH 5.8; Duchefa Biochimie) was used with or without MG132 (50 µM).

2.2.3 Growth Chambers

Plants were grown in controlled climate chambers (Microclima 1600E, Snijder Scientific, The Netherlands). Humidity was kept constant at 70 %. White light was provided by a cool-white fluorescent tubes (400 - 700 nm). Supplementary UV-B (+ UV-B) was provided by Philips TL 100W/01 narrow band UV-B bulbs. Levels were modulated using strips of heat-proof tape wrapped around bulbs to achieve 1.0 $\mu molm^{-2}s^{-1}$. For control experiments (- UV-B), UV-B was filtered through 3-mm-thick extruded acrylic tubes that block UV-B wavelengths. Supplementary Far-Red (+ FR) LEDs were used to

modulate the Red : Far-red (R/FR) ratio as specified in experiments. Green filter (Lee Filters, number 89) was used to attenuate R/FR ratio to simulate true shade (Figure 3). Plants were germinated in WL (70 µmol $m^{-2}s^{-1}$) with a 16 h light / 8 h dark photoperiod at 20°C prior to treatment.

2.3 Light Measurements

All light measurements were performed using an Ocean Optics FLAME-S-UV-VIS spectrometer with a cosine corrector (oceanoptics.com).

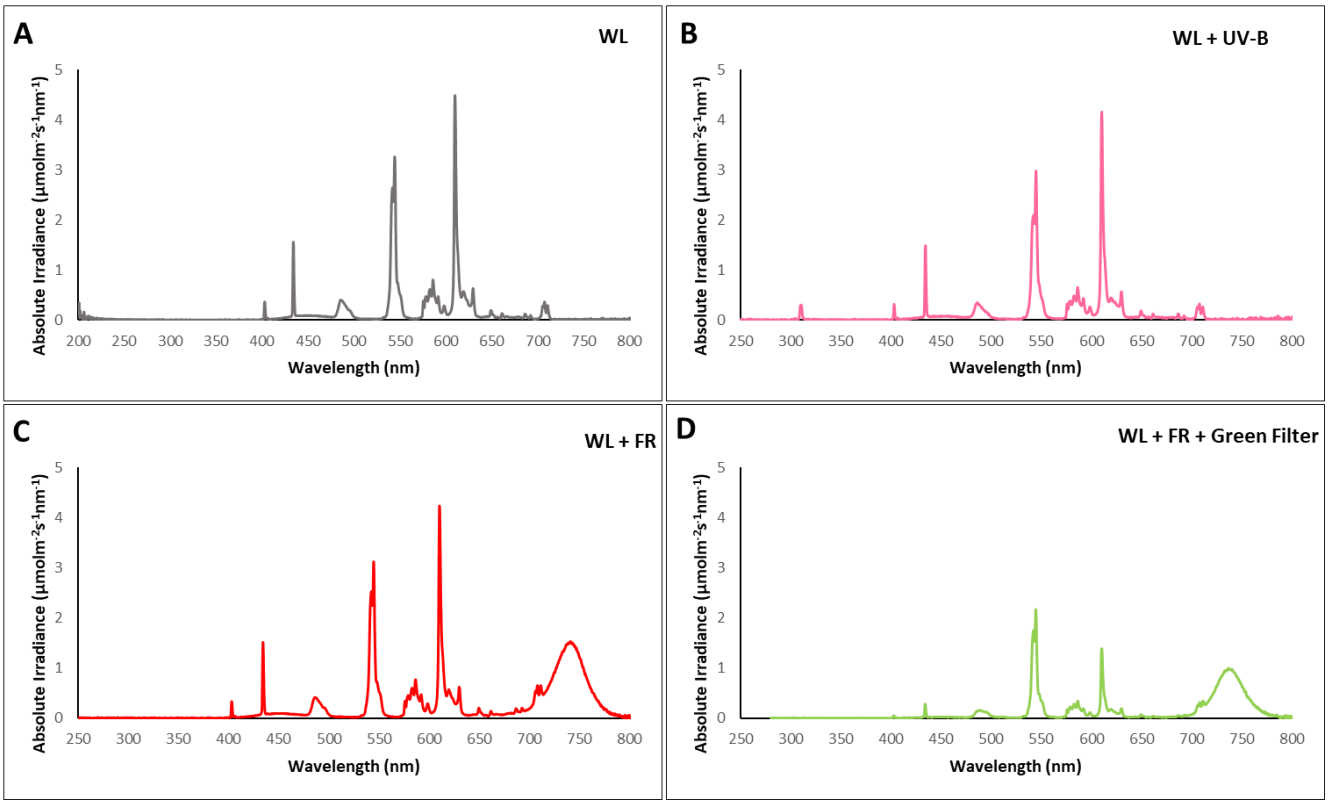


Figure 3: Light spectra from experimental conditions recorded in growth cabinets. 70 µmol $m^{-2}s^{-1}$ white light supplied by fluorescent bulbs without supplemental UV-B (A) and supplemented with UV-B at a photon irradiance of 1.0 µmol$m^{-2}s^{-1}$ using narrow band fluorescent bulbs (B), (C) supplemented with FR LEDs to produce a R/FR of 0.07, and (D) supplemented with FR LEDs and covered with a green filter (0.03 R/FR).

2.4 RNA isolation

10-day-old seedlings had approximately 50 µg of aerial tissue (~ 25/30 seedlings) harvested into 2 mL centrifugation tubes (Eppendorf UK Ltd, Stevenage, UK) containing two 3 mm steel ball bearings. These were snap frozen in liquid nitrogen using sterilized tweezers (Ethanol 70 % (v / v)). Three biological replicates of each treatment were harvested, and three biological repeats performed.

Arabidopsis seedling tissue was ground into fine powder using a vortex and total RNA extracted using the Spectrum Plant Total RNA Kit (STRN250-1KT, Sigma-Aldrich, Missouri, USA) according to the manufacturer's protocol. Genomic DNA was degraded in each RNA sample by subjection into DNAse I treatment using DNAse I Amplification Grade Kit (AMPD1, Sigma-Aldrich, Missouri, USA). RNA integrity was confirmed by agarose gel (1.0 %) electrophoresis and quantified using a Nanodrop ND 1000 spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA).

2.5 cDNA Synthesis

cDNA was synthesized from 1.0 µg of total RNA using the Applied Biosystem High-Capacity cDNA Reverse Transcription Kit, with RNase Inhibitor (Thermo Fisher Scientific, Life Technologies,) following the manufacturer's instructions. Reactions were performed using a Mastercycler Nexus Gradient (Eppendorf) using the following cycling parameters: 25 °C 10 min, 37 °C 120 min and 85 °C 5 min.

2.6 Quantitative reverse-transcription polymerase chain reaction (qRT-PCR)

qRT-PCR assays were performed using an Agilent Mx3005P Realtime PCR machine and Mx3005P System software (Agilent, USA). Reactions were carried out in a 10 µL final volume. Each reaction contained 1 x of Brilliant III ultra-fast SYBR® Green QPCR mastermix (Agilent), 200 nM of forward and reverse primers, 15 nM ROX reference dye, 2.0 µL of cDNA (1 : 100), and nuclease-free water up to 10 µL. Reactions were carried out in the following cycle conditions: 95°C 3min, 40 cycles of 95°C 20

seconds, and 60°C 20 seconds. Relative transcript abundance was normalized to the *ACTIN2* gene using Actin2F and Actin2R primers, and nuclease-free water was used as a no template control. Primer specificity and efficiency were evaluated through analysis of dissociation curves with a 1 : 10 serial dilution series. The primers used in this study are listed in Table 2. Two to three biological replicates and three technical replicates were performed and quantified for each genotype and treatment.

2.7 Determination of Relative Transcript Abundance

The data presented in this thesis are means ± SEM considering two to three biological replicates and three technical replicates. The comparative $2^{-\Delta\Delta Ct}$ method was used to calculate relative transcript abundance (Pfaffl 2000; Livak and Schmittgen, 2001) using *ACTIN2* as reference gene.

2.8 GUS Staining

pPIF3::GUS, *pPIF4::GUS* and *pPIF5::GUS* were sterilized and grown in petri dishes in 16 h light/8 h dark cycles. On day 10, plants were treated in either white light or white light supplemented with narrowband UV-B at dawn for 4 hours. Approximately 30 seedlings were then incubated in 90 % (v / v) acetone for 10 minutes. Seedling were subsequently transferred to assay buffer ((100 mM Na_2HPO_4 (pH 7.2), 100 mM NaH_2PO_4 (pH 7.2), 0.2 % (v / v) Triton-X, 2 mM 5-bromo – 4-chloro – 3-indolyl glucuronide salt (X-Gluc; Thermo Fisher Scientific), 2 mM $K_4[Fe(CN)_6]FeII$, 2 mM $K_3[Fe(CN)_6]FeIII$)) and incubated at 37°C for 12 h in the dark. Samples were washed in absolute ethanol, then washed 3 times in 70 % (v / v) ethanol and suspended in 50 % (v / v) ethanol for imaging (Keyence VHX-1000E digital microscope).

Table 1: List of mutant and transgenic lines used in this study

Genotype	Background	Reference
Columbia (Col-0)		N/A
Wassilewskija (Ws)		N/A
hy5/hyh	Ws	Holm *et al.*, 2002
uvr8-7	Ws	Favory *et al*., 2009
bri1-4	Ws	Liang *et al*., 2018
cop1-4	Col-0	Deng *et al*., 1992
uvr8-6	Col-0	Kliebenstein *et al*, 2002
*pPIF3::*GUS	Col-0	Zhang *et al*., 2013
*pPIF5::*GUS	Col-0	Zhang *et al*., 2013
pPIF4::GUS	Col-0	Galvão *et al*., 2019
35S::BES1 FLAG	Col-0	Zhang *et al*., 2018
35S::BES1 FLAG/uvr8	Col-0	Liang *et al*., 2018
bes1-D	Col-0	Ibanes *et al*., 2009
bzr1-1D	Col-0	Sun *et al*., 2010
35S::PIF4-HA	Col-0	Lorrain *et al*., 2007
pPIF4::PIF4:3xFLAG	Col-0	Kumar *et al*., 2012
myb30-1	Col-0	Zheng *et al*., 2012
myb30-2	Col-0	Zheng *et al*., 2012

Table 2: List of primers used in this study

Gene	Function	Forward 5'-3'	Reverse 5'-3-
PIF3	qPCR	GGTATGGGAATGCCTTATGCA	TGGAACTGTGGTCCGTGGTTA
PIF4	qPCR	GCCGATGGAGATGTTGAGAT	CCAACCTAGTGGTCCAAACG
PIF5	qPCR	CAGATGGCTATGCAAAGTCAGATGC	AGATTTGGTTCTGTGCTTGGAGCTG
PIF7	qPCR	ATGATGATTCCGCAACTACCTCCAC	ACGACATCTGAAACTGTTGCTGCTG
ACTIN2	qPCR	TCAGATGCCCAGAAGTGTTGTTCC	CCGTACAGATCCTTCCTGATATCC
GUS	qPCR	GAATACGGCGTGGATACGTTAG	GATCAAAGACGCGGTGATACA
BES1	qPCR	CAGCCATTCTCTGCCTCTATG	ACTCGGAGCTTTGACCAATC
BRI1	qPCR	AGCCGGGTCAGGGATAGATT	ACCCAAGGAAAATCGGACTGA
MYB30	qPCR	GGGAAACAAAGGGAGTGGTT	GCCCTTTCTTCACTCCTCCT
PIF3	genotyping	CTCCTCTGTTCTCTGCA	-
PIF4	genotyping	GATGTTTAACAAGAGAACGG	-
PIF5	genotyping	GTCCCTCCTTGCTCGATT	-
GUS	genotyping	-	ACCACCTGTTGATCCGCA
SALK_122884	genotyping	TCCTTGTTGTGACAAAGGAGG	ATGATCAGGTGAAACACCAG
SALK_122884	genotyping	TCCTTGTTGTGACAAAGGAGG	ACCCGCTAGCTGAGGAAGTAG
SALK_027644	genotyping	AAGATATGACGCAATTGCAGC	CTTTGGAGGCTTTACCTCCAC

PP2A	ChIP qPCR	CGGCTTTCATGATTCCCTCT	GCCTTAAGCTCCGTTTCCTACTT
ACT7	ChIP qPCR	CACAATGTTTGGCGGGATTGGTG	TGTACTTCCTTTCCGGTGGAGCAA
pPIF4	ChIP qPCR	GAGTCAAAGGAACATAATATCCA	GGAAGAGAGTCAAAGGAACA
MYB30	ChIP qPCR	AGGTATTTTACGCTGGAAAATGTGT	GAATCATCATAATAAGTATGGAGGTG
PRE1	ChIP qPCR	GAGGGATAATGAGGGATTTCG	CTATGTCACGTGTCACCACC ATGTC

2.9 Promoter identification and cis-element prediction of *PIF3*, *PIF4*, *PIF5* and *PIF7*

PIF genomic sequences from *Arabidopsis thaliana* were retrieved from the TAIR database, and sequences of approximately 3.0 kb base pairs upstream of the translation start codon ATG were considered promoter regions. The identification of cis elements in the promoter sequences of *PIF3*, *PIF4*, *PIF5* and *PIF7* in Arabidopsis was performed using the online prediction tool PlantPan 3.0 (Chow *et al*., 2015). Sequences within the promoter with a Hit Score ≥ 0.9 containing regulatory cis elements already described in literature were recorded. Redundancies were manually removed.

2.10 Proteasome inhibition

Col-0, *35S::PIF4-HA* and *pPIF4::PIF4 3x FLAG* plants were sown directly onto soil, stratified for 4 days in the dark at 4°C and grown in long (16 h) days at 20°C in white light. On the 9th day, plants were transferred into liquid 0.5x MS medium containing either MG132 (Thermo Fischer Scientific) (50 µM dissolved in 0.1 % (v / v) dimethyl sulfoxide (DMSO)) or 0.1 % DMSO (mock) and incubated for 16 h. At dawn, plants were transferred to white light or white light supplemented with narrowband UV-B for 4 hours. RNA extraction, DNAse I treatment, cDNA synthesis, qRT-PCR and determination of relative transcript abundance were performed as described above.

2.11 Hypocotyl and Petiole Length Measurements

For physiological assays investigating BR signalling, seeds were sterilized and plated onto 0.5 x strength MS medium (0.8 % agar (w / v)) containing 0.5 µM brassinolide (BR) dissolved in DMSO. Control plants were grown on 0.5 x MS and 0.8 % (w / v) agar containing 0.1 % (v / v) DMSO. Plants were grown vertically orientated in the growth chamber for hypocotyl elongation assays, and horizontally orientated for petiole growth measurements. For *myb30-1*/Col-0 hypocotyl elongation assays, seeds were grown directly on soil.

For hypocotyl assays, seedlings were grown in 16 h light/8 h dark photoperiods of WL at 20°C for 3 days before movement to respective treatments for 4 days. For petiole measurements, seedlings were grown in 16 h photoperiods of WL at 20°C for 10 days before movement to respective treatments for 7 days. The largest fully expanded rosette leaf on each plant was sampled for petiole measurement. Rosette leaves and hypocotyls were transferred to 1 % agar (w / v) plates supplemented with charcoal (1.5 % (w / v)) and photographed using a Nikon D80 DSLR. Hypocotyl and petiole length measurements were obtained from images using ImageJ.

2.12 Chromatin Immunoprecipitation (ChIP) qPCR

35S::BES1-Flag, 35S::BES1-Flag/uvr8 and Col-0 were grown for 10 days in WL 16 h light/ 8 h dark at 20°C. On the 10th day, plates were incubated in white light ±UV-B at ZT0 for 4 hours. ChIP was performed using the chromatin immunoprecipitation from Arabidopsis tissues protocol (Yamaguchi *et al*., 2014). Approximately 300 mg of plant tissue was collected per treatment into falcon tubes containing 1 X Phosphate-buffered saline (PBS) solution. Samples were then transferred into 1 % formaldehyde solution (v / v) in 1X PBS solution for vacuum infiltration to crosslink tissue. Once the tissue was successfully infiltrated with 1 % formaldehyde in 1X PBS, the solution was replaced by 0.125 M glycine solution and vacuum applied. Cross-linked tissue was then washed twice in 1X PBS. Plant tissues were dried on a paper towel, weighed and frozen in liquid nitrogen.

To isolate the nuclei, samples were ground into a fine powder using mortar and pestle and treated with nuclei extraction buffer (1X Protease inhibitor mini tablets (Thermo Fischer Scientific), 40 mM β-Mercaptoethanol, 100 mM MOPS pH 7.6, 10 mM Ficoll 400). Samples were filtrated twice in Miracloth and treated in nuclei lysis buffer (50 mM Tris-HCl pH 8.0, 10 mM EDTA pH 8.0, 1 % (v / v) SDS). Chromatin shearing was then performed by adding ChIP Dilution Buffer without Triton (16.7 mM Tris-HCl pH 8.0, 167 mM NaCl, 1.2 mM EDTA, 0.01 % (v / v) SDS) and sonicating each sample 5 times for 10 second each. Samples were pre-cleared by adding magnetic protein A/G beads into each sample followed by 2 hours incubation at 4°C with rotation. Tubes were then placed on a magnetic stand and cleared solution was transferred into a new tube. 2/100^{ths} of the solution was saved as a 2 % input sample. Immunoprecipitation was carried out by adding 34 µg of Monoclonal ANTI-FLAG M2 (Sigma) into each sample and incubating overnight at 4°C with rotation. On the next day, protein A/G magnetic beads were added into each sample to capture DNA/protein complexes and incubated at 4°C for 4 hours with rotation. Samples were then washed 8x (2x per wash solution) with Low Salt Wash Buffer (0.1 % (w/v) SDS, 1 % (v/v) Triton-X, 2mM EDTA, 20 mM Tris-HCl pH 8.1, 150 mM NaCl), High Salt Wash Buffer (0.1 % (w/v) SDS, 1 % (v/v) Triton-X, 2 mM EDTA, 20 mM Tris-HCl pH 8.1, 500 mM NaCl), 250 mM LiCl Wash Buffer (0.25 M LiCl, 1 % (v / v) IGEPAL-CA630, 1 % (w / v) deoxycholate, 1 mM EDTA, 10 mM Tris-HCl pH 8.1) and 0.5X TE at pH 8.0, respectively. Samples were eluted with Nuclei Lysis Buffer then incubated at 65°C in a magnetic stand to separate the sample from the beads (twice). 5 M NaCl was added in both ChIP and input samples and incubated overnight at 65°C for reverse crosslinking followed by DNA purification (Qiagen 28104) following the manufacturer instructions.

qRT-PCR assays were performed as described in section 2.6. The reactions were carried out in the following cycle conditions: 95°C 10 min, 40 cycles of 95°C 15 seconds, and 60°C 1 minute; 95°C for 15 seconds, 60°C 1 minute, 95°C 15 seconds (melting curve stage). Relative transcript abundance was normalized to the input and *ACTIN2* was used as negative control. Nuclease-free water was used as a no template control. The primers used in this study are listed in Table 2. Three independent biological replicates and two technical replicates were performed and quantified for each treatment.

2.13 Western Blotting

10-day-old Arabidopsis Col-0, *35S::PIF4-HA* and *pPIF4::PIF4-3xFLAG* plants treated with proteosome inhibitor were transferred to white light ± UV-B at ZT0 and aerial tissue was harvested and flash frozen after 4 hours. Samples were ground into fine powder then mixed with freshly made protein extraction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 % Na deoxycholate (w / v), 0.5 % (v / v) Triton X-100, 1 mM DTT, 10 µl/ml Sigma protease inhibitor cocktail, 50 µM MG132). Samples were centrifuged at 15,000 XG for 10 minutes at 4°C and supernatant was transferred to a new tube. Total protein concentration was quantified using a Bradford assay (BioRad) (Bradford, 1976). SDS-PAGE 4x loading buffer (250 mM Tris-HCL pH 6.8, 2 % SDS (w / v), 20 % β-mercaptoethanol (v / v), 40 % glycerol (v / v), 0.5 % bromophenol blue (w / v)) was added to the protein samples to a final concentration of 1 X. 50 µg of heated protein was loaded into each lane on 7.5% SDS-PAGE gels for resolving. Proteins were transferred to PVDF membrane and visualized by Ponceau staining followed by $_{d}$H2O rinsing. The membrane was blocked with 5 % skimmed milk powder in TBS-T (20 mM Tris-HCl, 150 mM NaCl, 0.1 % tween 20 (v / v) pH 7.6) for 20 minutes at room temperature. For PIF4-HA detection, the membrane was incubated in a 1:1000 dilution of anti-HA antibody conjugated to peroxidase (Roche 12013819001), and for PIF4-3xFLAG and ubiquitination the membrane was incubated in a 1:2000 dilution of anti-FLAG and anti-ubiquitin respectively, all in 3% skimmed milk in TBS-T overnight at 4°C. The following day the membranes were washed three times with TBS-T for 15 minutes each time. For PIF4-3xFLAG and ubiquitination detection, the membrane was incubated with the secondary antibody (anti-mouse and anti-rabbit conjugated to horseradish peroxidase respectively) at 1:20000 dilution in 3 % skimmed milk in TBS-T for 60 minutes at room temperature, then washed 3 times with TBS-T for 5 minutes each time. Immunoblots were visualized using the SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific) according to manufacturer's manual and chemiluminescence was detected with EvolutionCapt software. ImageJ was used to determine the protein density of bands on the blots.

2.14 Genotyping

Leaves of mutant and transgenic plants were harvested and genomic DNA (gDNA) was extracted using Edward's protocol (Edwards *et al*., 1991). Primers (Table 2) were either manually designed flaking the transgene sequence using OligoAnalyzer tool or provided by T-DNA express.

Target sequences were amplified by PCR reaction from gDNA according to the DreamTaq Green PCR Master Mix (2X) DNA Polymerase enzyme protocol (Thermo Fischer Scientific). Reactions containing 2x DreamTaq Buffer; 0.1 µM of each forward and reverse forward and genomic DNA (approximately 240 ng) were set up to the following amplification program: (i) initial denaturation of 2 minutes at 95°C; (ii) 30 cycles of 30 seconds denaturation at 95°C, 30 seconds at 60°C of annealing temperature (primers specific Tm), 1 min/2kb extension; (iii) 10 minutes of final extension at 72°C. PCR products were verified by electrophoresis in a 1% agarose gel.

CHAPTER 3

Characterization of *PIF* transcriptional regulation in response to UV-B

3.1 Introduction

As sessile organisms, plants have evolved intricate molecular mechanisms to adapt to their everchanging environment, synchronizing their cellular responses to the external conditions. Light and temperature are the main external cues sensed by the plant cell to drive genome reprogramming. Plants perceive light photons by photoreceptors that, upon the absorption of specific wavelengths, undergo conformational changes to become active and transduce the signal to a network of transcription factors that initiate several signalling pathways to drive downstream responses (Bae & Choi, 2008). The coordination of the photoreceptor signalling allows plants to optimize their growth according to their environment, thus increasing their fitness.

Downstream photoreceptors, PIFs are the main hub of environmental signal integration. Developmental responses regulated by PIFs in response to light stimuli include seedling de-etiolation, shade avoidance and thermomorphogenesis. PIF3, PIF4, PIF5 are negative regulators of photomorphogenesis (Bauer *et al*., 2004; Monte *et al*., 2004; Shen *et al*., 2005, 2008; Al-Sady *et al*.,

2006; Nozue *et al*., 2007; Lorrain *et al*., 2008; Soy *et al*., 2012; Yamashino *et al*., 2013). PIF3, PIF4, PIF5, and PIF7 promote shade avoidance, with PIF7 performing a dominant role (Lorrain *et al*., 2008; Nozue *et al*., 2011; Hornitscheck *et al*., 2012; Leivar *et al*., 2012; Li *et al*., 2012). PIF4 and PIF7 promote thermomorphogenesis with PIF4 performing a dominant role (Koini *et al*., 2009; Stavang *et al*. 2009; Franklin *et al*., 2011; Fiorucci *et al*. 2020; Chung *et al*., 2020). PIFs control the developmental response by regulating gene expression through direct binding to DNA, and the magnitude of the response is dictated by the levels of active PIFs in the cell.

UV-B is an important portion of radiation emitted by the sun and regulates a suite of plant morphological responses via UVR8 (Rizzini *et al*., 2011; Christie *et al*., 2012), including suppression of shade avoidance and thermomorphogenesis responses. These inhibitory growth responses are mostly due to UVR8-mediated reductions of PIF stability and activity (Hayes *et al*., 2014; Hayes *et al.*, 2017). UV-B attenuates PIF4 and 5 DNA binding activity by accumulating levels of their repressors DELLA, HFR1 and HY5/HYH in an UVR8- dependent manner (Hayes *et al*., 2014; Hayes *et al*., 2017; Tavridou *et al*., 2020). Additionally, UV-B drives protein turnover of PIF4 and 5 via UVR8 (Hayes *et al*., 2014; Tavridou *et al*., 2019), causing auxin signal depletion. Some mechanisms underlying the UV-B-mediated degradation of PIFs have been characterized but not fully elucidated. For instance, this process must require intermediate regulators as UVR8 does not directly interact with PIFs (Hayes *et al*., 2014; Sharma *et al*., 2019). Recently, COP1 was identified as a component of UV-B turnover of PIF5, as COP1 facilitates PIF5 stability by direct interaction. This is reduced in UV-B through sequestration of COP1 by active UVR8, causing PIF5 to be degraded through the ubiquitin proteasome-system (Sharma *et al*., 2019). This mechanism may be similar for the UV-B-mediated degradation of PIF4, since COP1 stabilizes PIF4 in the dark (Bauer *et al*., 2004; Gangappa & Kumar, 2017; Ling *et al*., 2017; Pham *et al*., 2018), although this is currently unknown. The mechanism controlling UV-B-mediated turnover of PIF4 remains to be elucidated, but it does appear to be temperature-dependent, with no UV-B-mediated degradation observed at higher temperatures (Hayes *et al*., 2017). PIF negative regulators which accumulate upon UV-B exposure, such as DELLA, HY5/HY5 and HFR1 UV-B

do not appear to have a role in UV-B-mediated degradation of PIF4 and 5 (Hayes *et al*., 2014; Tavridou *et al*., 2020).

Despite extensive research into the posttranslational regulation of PIF levels and activity, the transcriptional regulation of PIFs is poorly understood. Understanding the upstream regulation of PIFs may be key to understanding how UV-B reduces levels of PIF4 and 5 so effectively. PIF5 is quickly degraded in UV-B, within 20 min (Sharma *et al*., 2019). Interestingly, *PIF4* and *5* show UV-B-mediated transcript suppression within 2 h in a UVR8- dependent manner (Hayes *et al*., 2017; Sharma *et al*., 2019; Travidou *et al*., 2020). Understanding the kinetics of transcript suppression could therefore provide insight into the mechanism by which UV-B downregulates PIFs. Additionally, investigating the effect of UV-B on *PIF3* and *PIF7* transcript abundance may provide wider understanding of morphological changes driven by UV-B. How UVR8 regulates gene expression has remained rather enigmatic in the literature. Direct binding of UVR8 to chromatin at target genes to regulate gene transcription has previously been suggested, but remains debated (Brown *et al*., 2005; Cloix *et al*., 2012; Binkert *et al*., 2016; Jenkins, 2017). However, direct interaction of UVR8 with transcription factors has recently been implicated in UVR8 signalling (Liang *et al*., 2018; Yang *et al*., 2018; Liang *et al*., 2019).

In this chapter, the promoter sequences of *PIFs 3, 4 , 5* and *7* are first characterized to identify regulatory motifs to guide future studies. The transcriptional regulation of these *PIFs* in response to low dose UV-B is then explored in Arabidopsis, through time course studies. These were used to establish both the kinetics of UV-B-mediated *PIF* suppression and the involvement of promoter regulation, using transgenic lines expressing *PIF* promoters fused to the *GUS* reporter gene. The potential involvement of known UV-B signalling components in the suppression of *PIF4* and *PIF5* transcript abundance were then investigated using null mutants.

3.2 Regulatory profiling of *PIF* promoters *in silico*

The promoter regions –3kb upstream the ATG codon of *PIF3*, *4*, *5* and *7* were retrieved from TAIR database and analysed in PLANTPAN 3.0 (Chow *et al*., 2015) to identify cis elements that may possibly regulate their expression. Cis elements were categorized in four groups according to their annotation in the literature: growth and development, hormone regulation, cellular architecture and light (Table 3). Cis elements that potentially regulate UV-B signalling pathways were screened and their enrichment was analysed for each *PIF* (Figure 4).

Among the cis elements found, hormone regulation- related binding motifs were enriched in all *PIF*s (Table 3). Of particular interest, Cis elements for the transcription factor BES1 were present in *PIF4*, *PIF5* and *PIF7* promoters. BES1/BZR1 are transcription factors that possess a non-canonical bHLH domain and function as key regulators of brassinosteroid (BR)- related genes. BES1 and BZR1 are both UV-B responsive and regulate *PIF* transcription. BZR1 has been shown to directly bind to the promoter of *PIF4* and induce its expression (Ibanez *et al*., 2018; Liang *et al*., 2018). In addition, active nuclear UVR8 monomers directly interact with both transcription factors, inactivating their DNA binding ability (Liang *et al*., 2018). Cis elements related to auxin signalling pathways were also of interest (NAC, ARF/B3; Table 3) since auxin signalling pathways are one of the main inducers of hypocotyl elongation and are inhibited in UV-B to promote photomorphogenesis.

Cis elements for light signalling- related transcription factors families including bHLH, bZIP and MYB were found in high abundance in the promoter regions of all *PIFs* analysed (Table 3). MYB30 is a transcription factor that physically interacts with the promoters of *PIF4* and *PIF5* under prolonged R irradiation, inducing their expression and accumulation (Yan *et al*., 2020). MYB30 has not been studied under UV-B, and it would be of interest to test whether it regulates *PIF* expression in UV-B. Furthermore, the bHLH cis elements identified in this study could indicate that PIFs can regulate each other's expression as well as their own. Evidence supporting this hypothesis is the finding of a *PIF4*

autoregulatory mechanism at higher temperatures to control thermomorphogenesis (Lee *et al*., 2021).

Finally, *in silico* analyses of *PIF3, 4, 5 and 7* promoters identified different combinations and abundance of cis elements for transcription factors related to growth and development, hormone signalling pathways and light responses, suggesting that individual *PIFs* may be differentially regulated by diverse factors including UV-B (Figure 4).

	TFBS	*pACT7*	*PIF3*	*PIF4*	*PIF5*	*PIF7*	Associated Function	Reference
	AP2	1	1	1	1	1	Regulation of reproductive and vegetative development	Okamuro, 1997
	B3	0	1	1	1	1	Plant growth and seed maturation	Swaminathan, 2008
	BBR-BPC	1	1	0	1	1	Regulation of homeotic transcription factor genes	Santi, 2003
	GRF	1	0	1	1	0	Leaf and cotyledon growth, regulation of plant longevity	Omidbakhshfard, 2015
	HD-ZIP	1	1	1	1	0	Development in response to environmental stimuli	Agalou, 2008
	NAC	1	1	1	1	1	Meristem development, hormone responses, defense	Olsen, 2005
	Storekeeper	1	1	0	1	1	Regulation of sugar-specific gene expression	Zourelidou, 2002
Growth and Development	AP2; B3; RAV	1	1	1	1	1	leaf maturation and senescence regulation	Li, 2015
	ARF	0	0	1	1	1	Promoter of primary auxin response genes	Wang, 2005
	B3; ARF;ARF	1	1	1	1	1	Auxin and abscisic acid responsiveness	Waltner, 2005
	BES1	1	0	1	1	1	Activation BR-induced gene expression	Yin, 2002
	EIN3; EIL	1	1	1	1	1	Activation of ethylene responses	Schaller, 2002
	MYB	1	1	1	1	0	Cell proliferation, secondary metabolism, defense, ABA response	Stracke, 2001
	NAC	1	1	1	1	1	Meristem development, hormone responses, defense	Olsen, 2005
Hormone Regulation	SBP	1	1	1	1	1	Control of GA level	Zhang, 2007
	ARID	1	1	1	1	1	Likely involvement in the modification of chromatin structure.	Kortschak, 2000
	ARID; Sox	1	1	1	1	1	Modification of chromatin structure	Roy, 2016
Cellullar Architecture	AT-Hook	1	1	1	1	1	Regulates chromatin dynamics	Sgarra, 2005
	bHLH	1	1	1	1	1	Light responsiveness	Duek, 2005
	bZIP	1	1	1	1	1	Light and stress signalling	Jakoby, 2002
	Dof	1	1	1	1	1	light-responsiveness, seed development, or germination	Ward, 2005
	GATA	1	1	1	1	1	Light responsiveness	Reyes, 2014
	GATA; tify	1	1	1	1	1	Light responsiveness	Reyes, 2014
	GRAS	1	0	0	0	1	Phytochrome A signal transduction	Bolle, 2000
	ZF-HD	0	1	1	1	1	Responses to environmental conditions	Henriksson, 2005
Light	MYB-related	1	1	1	1	1	Circadian rhythm control, cell proliferation	Stracke, 2001

Table 3: Transcription factor binding site (TFBS) prediction in Arabidopsis *PIF* promoter sequences. The genomic regions comprising 3.0kb upstream to ATG codon of *AtPIF 3,4,5 and 7* genes were scanned for the presence of known transcription factors binding sites (TFBS) using PLANT PAN 3.0 (CHOW *et al*., 2016). *pACT7* was used as control sequence. "0": Absence of TFBS. "1": Presence of TFBS.

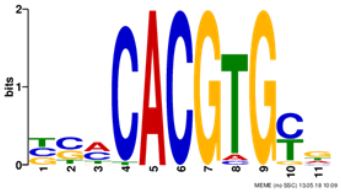
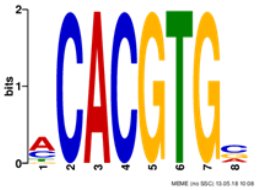
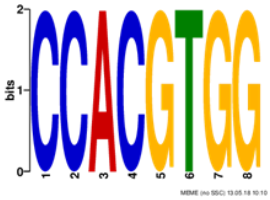
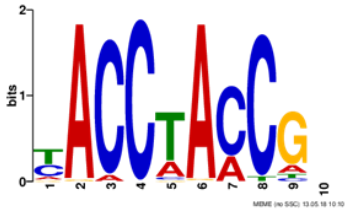
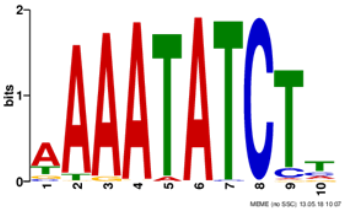
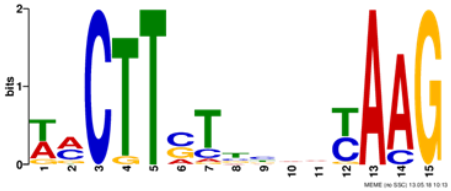
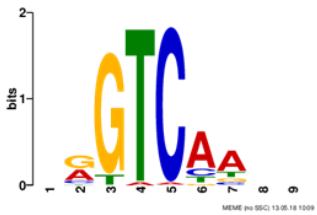
Reference Motif (PlantPan)	Transcription Factor related	*pACT7*	*pPIF3*	*pPIF4*	*pPIF5*	*pPIF7*
	B3	10	6	2	7	8
	BES1	1	0	2	2	1
	bHLH	16	9	13	13	23
	bZIP	17	1	12	8	20
	MYB	10	6	12	7	0
	MYB – related	7	4	6	5	8
	NAC	7	8	8	7	0
	NAC; NAM [66]	23	14	13	12	12

Figure 4: Identification and quantification of cis elements in the promoters of *PIF3*, *PIF4*, *PIF5* and *PIF7*. Letters on images represent the nucleotides (A, T, C and G) and the size of each base is proportional to the frequency in the position. The values next to each promoter motif indicate the number of each cis element in the isolated sequence. The transcription factor family related to the cis element is identified next to each image . Data were based on PlantPan in silico predictions (Chow *et al*., 2015).

3.3 UV-B- mediated control of *PIF* transcription

RT-qPCR assays were performed to assess the difference in transcript abundance between two light treatments: white light and white light supplemented with low dose UV-B. Wildtype Col-0 and *uvr8-6* were grown for 10 days in white light at 20°C in long days (16 h light /8 h dark) then transferred to either white light or white light supplemented with UV-B (1.0 µmol m^{-2} s^{-1}) at 20°C for four hours at ZT0 and sampled in 4 time points (pre-dawn, 1 h, 2 h and 4 h).

3.3.1 *PIF3*, *4* and *5* transcript abundance is suppressed by UV-B in a UVR8- dependent manner

Consistent with the literature, in wild type Col-0 seedlings, all PIFs tested (*PIF3*, *PIF4*, *PIF5* and *PIF7*) displayed increased transcript abundance post dawn, with an abrupt increase between time points 2 h and 4 h (Nozue *et al*., 2007; Kikodoro *et al*., 2009; Lee *et al*., 2012; Soy *et al*., 2012; Mizuno *et al*., 2014;). *PIF3*, *PIF4* and *PIF5* transcript abundance decreased in the presence of UV-B, with clear differences observed at 2 h (Figure 5). In contrast to *PIFs 3*, *4* and *5*, *PIF7* transcript abundance did not decrease following UV-B exposure, suggesting an alternative regulatory mechanism. Variation between biological repeats was, however, relatively large for this genotype, confounding interpretation. The null mutant *uvr8-6* displayed increased transcript abundance of all *PIFs* post dawn but lacked UV-B- mediated

suppression of *PIF3*, *PIF4* and *PIF5* transcript. These data therefore suggest that the UV-B-mediated suppression of *PIF3, 4* and *5* transcript abundance requires UVR8. (Figure 5).

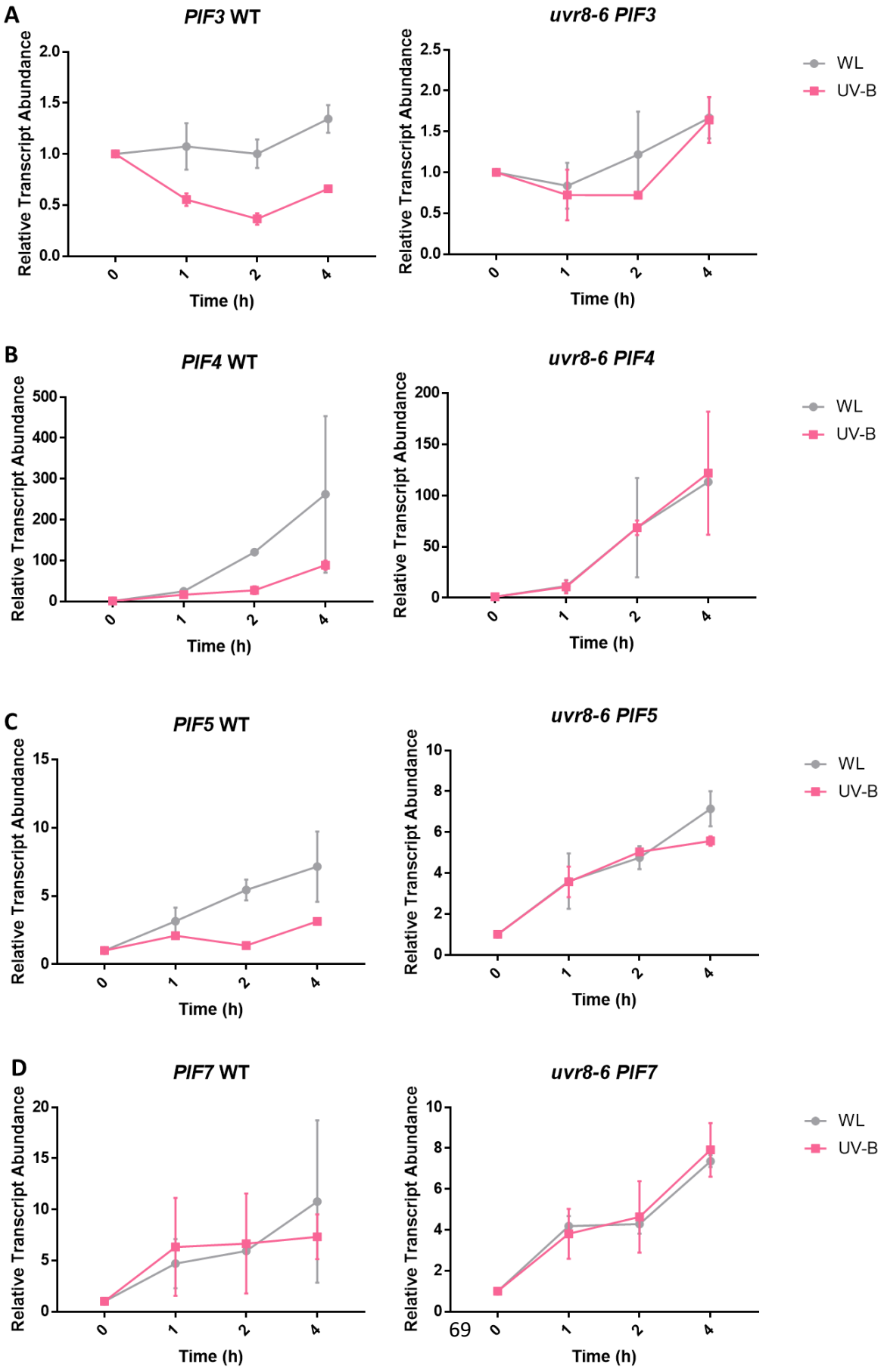

A
PIF3 WT
uvr8-6 PIF3
Relative Transcript Abundance
Time (h)
WL
UV-B
B
PIF4 WT
uvr8-6 PIF4
C
PIF5 WT
uvr8-6 PIF5
D
PIF7 WT
uvr8-6 PIF7

Figure 5: UV-B suppresses the abundance of *PIFs 3, 4* and *5* transcripts in a UVR8-dependent manner. (A-D) Relative transcript abundance of *PIF3, PIF4, PIF5 and PIF7* in WT (Col-0) and *uvr8-6* seedlings grown on soil in long days at 20°C for 10 days, then transferred to white light ±UV-B (1 $\mu molm^{-2}s^{-1}$) for 4 hours at dawn (ZT0). *PIF* transcript abundance was determined by RT-qPCR and normalized to *ACTIN2.* N = 2 ±SD.

3.3.2 UV-B-mediated suppression of *PIFs 3, 4* and *5* transcript abundance involve repression of *PIF* promoter activity

Changes in the relative transcript abundance of genes reported in qRT-PCR assays (Figure 5) can result from both changes in the promoter activity of the gene and/or post-transcriptional regulation. To assess whether the downregulation of *PIF3*, *PIF4* and *PIF5* transcripts in UV-B are a result of promoter regulation, qRT-PCR assays were performed with the genotyped (Figure 6) transgenic lines *pPIF3::GUS*, *pPIF4::GUS* and *pPIF5::GUS* (Zhang *et al*., 2013) following the same experimental design of the previous experiment, but quantifying *GUS* relative transcript abundance in place of *PIF*s.

Consistent with the UV-B-mediated transcriptional suppression of *PIF3, 4* and *5* in WT seen in Figure 5 , *GUS* transcript was negatively regulated in UV-B when driven by *PIF4* and *5* promoters, presenting similar kinetics to those observed in Figure 5 (Figure 7 A and B). Large variance in *GUS* transcript was observed between biological replicates in *pPIF3::GUS* lines, confounding interpretation (Figure 7C). Collectively, data suggest that *PIF3* transcript abundance is suppressed in UV-B but is inconclusive as to whether this occurs the level of promoter regulation or transcript turnover.

In an attempt to visualize the tissue-specific variation of *PIF3, 4* and *5* transcript abundance in different light treatments, histochemical assays were performed in the three transgenic lines in 10-day- old seedlings grown in white light at 20°C (16 h light /8 h dark photoperiods) then transferred to either white light or white light supplemented with low dose UV-B for four hours at ZT0. The seedlings showed no

significant staining differences among the light treatments. In all seedlings the most prominent areas of promoter activity were in the hypocotyl and younger tissues, and *pPIF4::GUS* showed the most intense staining pattern, possibly suggesting increased promoter activity than *PIF3* and *PIF5* (Figure 7D).

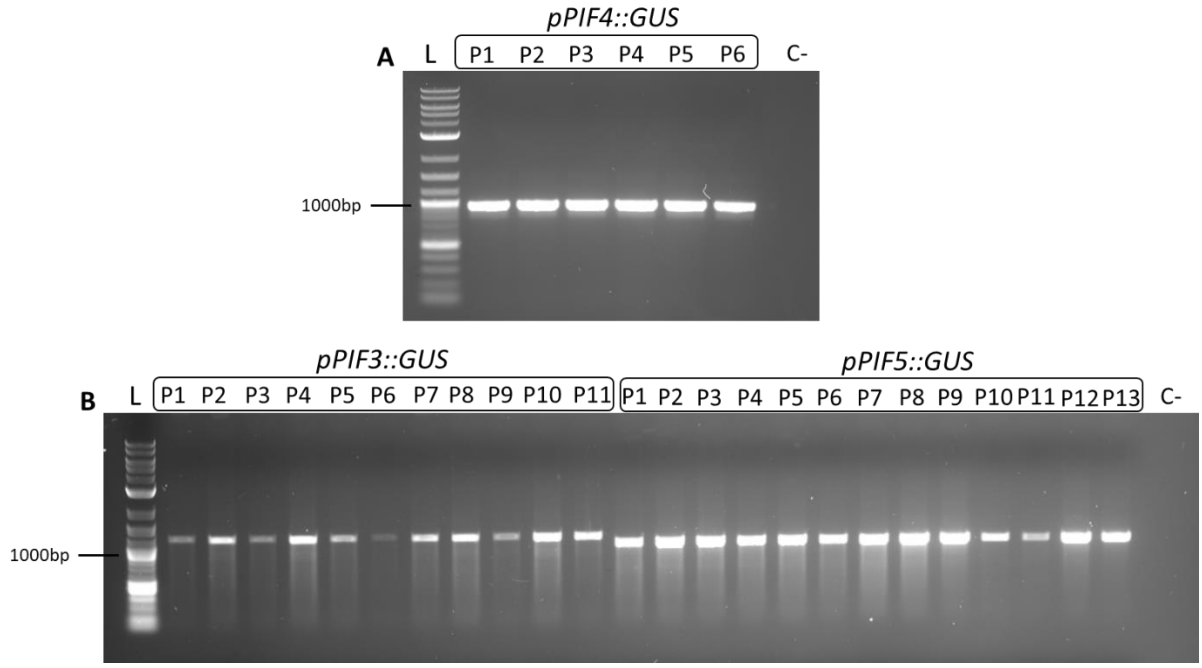


Figure 6: Genotyping of *pPIF::GUS* transgenic lines. Specific forward primers were designed for each *PIF* promoter and used with a GUS reverse primer to detect positive transgenic plants by gDNA PCR. Expected amplicon sizes: (A) *pPIF4::GUS* 921 bp, (B) *pPIF3::GUS* 1188 bp, (B) *pPIF5::GUS* 1052 bp. "P1-P13" (Plant 1-13): transgenic plants. "C-" (Negative control): water. "L" (Gene Ruler 1kb Plus Ladder).

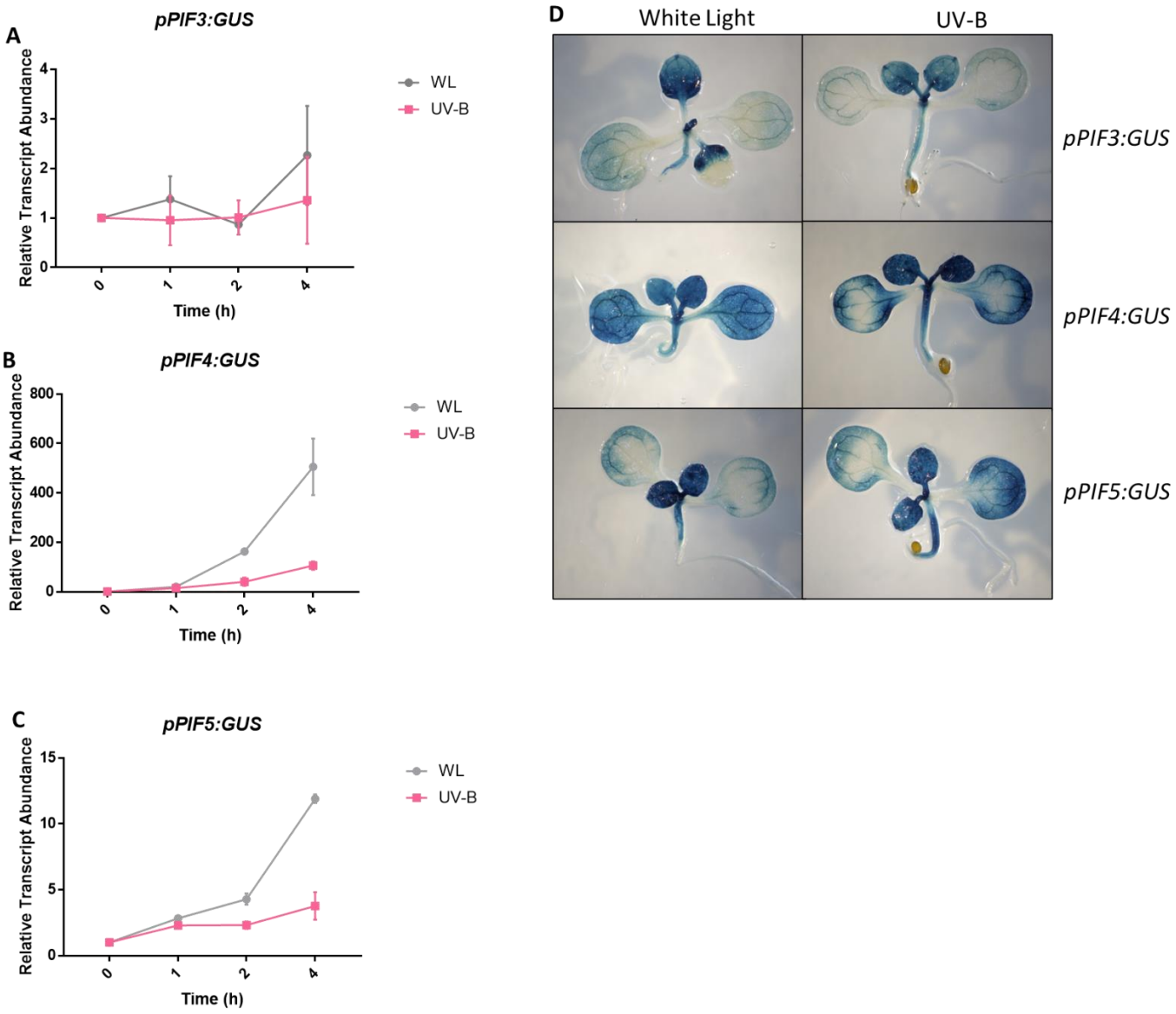


Figure 7: UV-B-mediated suppression of *PIF 4* and *5* transcript abundance involves suppression of *PIF4* and *5* promoter activity. (A-C) Relative transcript abundance of *GUS* in *pPIF3::GUS* (A), *pPIF4::GUS* (B) and (C) *pPIF5::GUS* seedlings grown on soil in long days at 20°C for 10 days, then transferred to white light ±UV-B (1 µmol $m^{-2}s^{-1}$) for 4 hours at dawn (ZT0). N = 2 ±SE. (D) Histochemical staining of GUS activity in 10-d-old *pPIF3::GUS*, *pPIF4::GUS* and *pPIF5::GUS* seedlings transferred to white light ±UV-B (1 µmol $m^{-2}s^{-1}$) at 20°C for 4 h at dawn. No difference in GUS staining was observed between light treatments in any of the transgenic lines.

3.4 Dissection of the UV-B signalling pathway involved in suppression of *PIF4* and *5* transcript abundance

UVR8 does not interact with PIFs *in vitro* or *in vivo* (Hayes *et al*., 2014; Sharma *et al*., 2019), yet it controls UV-B- mediated *PIF4 and 5* transcript abundance, PIF4 and PIF5 protein stability and PIF4 and 5 activities (Hayes *et al*., 2014; Hayes *et al*., 2017; Sharma *et al*., 2019; Tavridou *et al*,. 2020; Figures 5 and 7). It is likely that that UVR8 is mediating intermediate signalling components that repress *PIF* promoter activity in UV-B. In an attempt to identify these, mutants, deficient in the known UV-B signalling components HY5, HYH and COP1 were grown for 10 days in 16 h light/ 8 h dark cycles in white light at 20°C. On the 10th day, seedlings were transferred to either white light or white light supplemented with UV-B (1.0 µmol m^{-2} s^{-1}) for 4 hours at ZT0. qRT-PCR assays were performed to quantify the relative transcript abundance of *PIFs*.

3.4.1 UV-B-mediated suppression of *PIF4 and 5* transcript abundance does not require HY5/HYH

The photomorphogenic transcription factors HY5 and its homolog HYH, are rapidly upregulated in UV-B and accumulate in the nucleus, controlling the expression of several UV-B related genes. Upon UV-B irradiation, active UVR8 monomers interact with COP1, and the heterodimers are imported to the nucleus. Because COP1 is bound to UVR8, COP1 targets are freed from degradation, including HY5, which accumulates, and upregulates its own expression, promoting *HY5* transcription and consequently the expression of HY5-regulated genes (Brown *et al*., 2005; Brown & Jenkins 2008). Since HY5/HYH display rapid induction in UV-B and HY5 suppresses PIF activity to induce photomorphogenesis (Brown *et al*., 2005; Hayes *et al*., 2014), HY5/HYH were considered potential candidates to perform an intermediate role in the downregulation of *PIF4/5* transcription in UV-B.

To test this hypothesis, qRT-PCR assays were performed comparing *hy5/hyh* double mutants to WT controls (Ws), using the same experimental design as previous experiments. Although HY5 is a major regulator of UV-B- regulated responses, its homolog HYH can act redundantly in its absence. The *hy5/hyh* double mutant was therefore used to assess the effect of UV-B on *PIF* transcript accumulation. To confirm whether UV-B downregulates *PIF* transcript in WS, in a UVR8- dependent manner as in Col-0, we used the *uvr8-7* null-mutant line.

Consistent with the previous experiment in Col-0 (Figure 5), Ws WT plants displayed reduced transcript accumulation of *PIF3*, *PIF4* and *PIF5* when treated with UV-B. This response was lost in *uvr8-7* but not in *hy5/hyh*, suggesting it to be mediated by UVR8 but not HY5/HYH. Similar to Col-0, *PIF7* did not show any response to the UV-B treatment in any genotype (Figure 8). It should, however, be noted that low basal levels of *PIF4* and *PIF5* transcript in both *uvr8-7 and hy5/hyh* lines make the interpretation difficult.

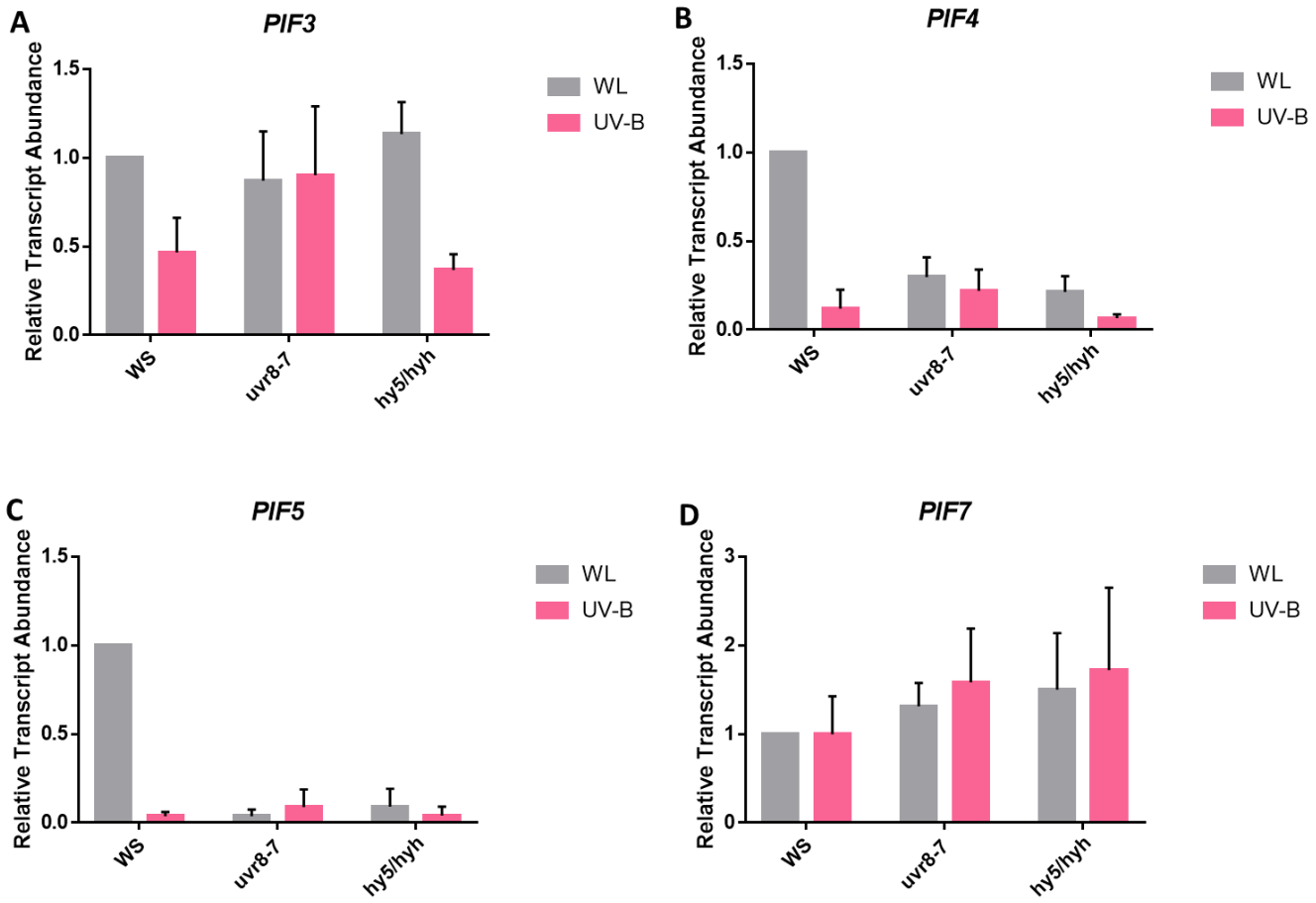


Figure 8: UV-B-mediated suppression of *PIF3*, *4* and *5* transcript abundance does not require HY5/HYH.

(A-D) Relative transcript abundance of (A) *PIF3*, (B) *PIF4*, (C) *PIF5* and (D) *PIF7* in WT (Ws), *uvr8-7* and *hy5/hyh* seedlings grown on soil in long days at 20°C for 10 days, then transferred to white light ±UV-B (1 $\mu molm^{-2}s^{-1}$) for 4 hours at dawn (ZT0). Relative transcript abundance was determined by RT-qPCR and normalized to ACTIN2. N = 2 ±SE.

3.4.2 COP1 promotes *PIF4* and *5* transcript accumulation

The photomorphogenesis suppressor COP1 is a major UV-B signalling pathway component, as it is required for active import of UVR8 monomers into the nucleus. The heterodimer formed by COP1 and

UVR8 inhibits COP1 E3 ligase activity, leading to accumulation of its target proteins in the nucleus, including HY5. Additionally, COP1 stabilizes PIF3, 4 and 5 levels in the dark (Bauer *et al*., 2004; Gangappa & Kumar 2017; Ling *et al*., 2017; Pham *et al*., 2018). Conversely, PIF5 is destabilized in UV-B by active UVR8 sequestration of COP1 from the PIF5/COP1 heterodimer (Sharma *et al*., 2019). To investigate the role of COP1 in UV-B-mediated *PIF4* and *5* suppression, qRT-PCR assays were performed using the same experimental design as for *hy5/hyh* with the nonlethal *cop1-4* mutant. *cop1-4* mutants contain a premature stop codon, which removes the C-terminal 393 amino acids. This results in a truncated protein containing only the N-terminal 282 amino acids without the 40D repeats. In *cop1-4*, *PIF3* transcript displayed a reduced response to UV-B when compared to WT controls, but large variation was observed between biological repeats (Figure 9A). *cop1-4* plants displayed low basal levels of *PIF4* and showed no additional UV-B suppression (Figure 9B). Consistent with previous reports (Hayes *et al*., 2017; Sharma *et al*. 2019), *cop1-4* plants displayed low basal levels of *PIF5* transcript, which was not further reduced by UV-B (Figure 9 B, C). Basal levels of *PIF7* transcript were lower in *cop1-4* than WT seedlings but did not decrease further upon exposure to UV-B (Figure 9D). Collectively, these data support a role for COP1 in promoting *PIF* transcript accumulation. The low basal levels of *PIF* transcript in *cop1-4* mutants make determination of the role of COP1 in UV-mediated *PIF* suppression difficult. Given the involvement of UVR8 in this response and the role of COP1 in UVR8 signalling, the involvement of COP1 in this response is, however, likely.

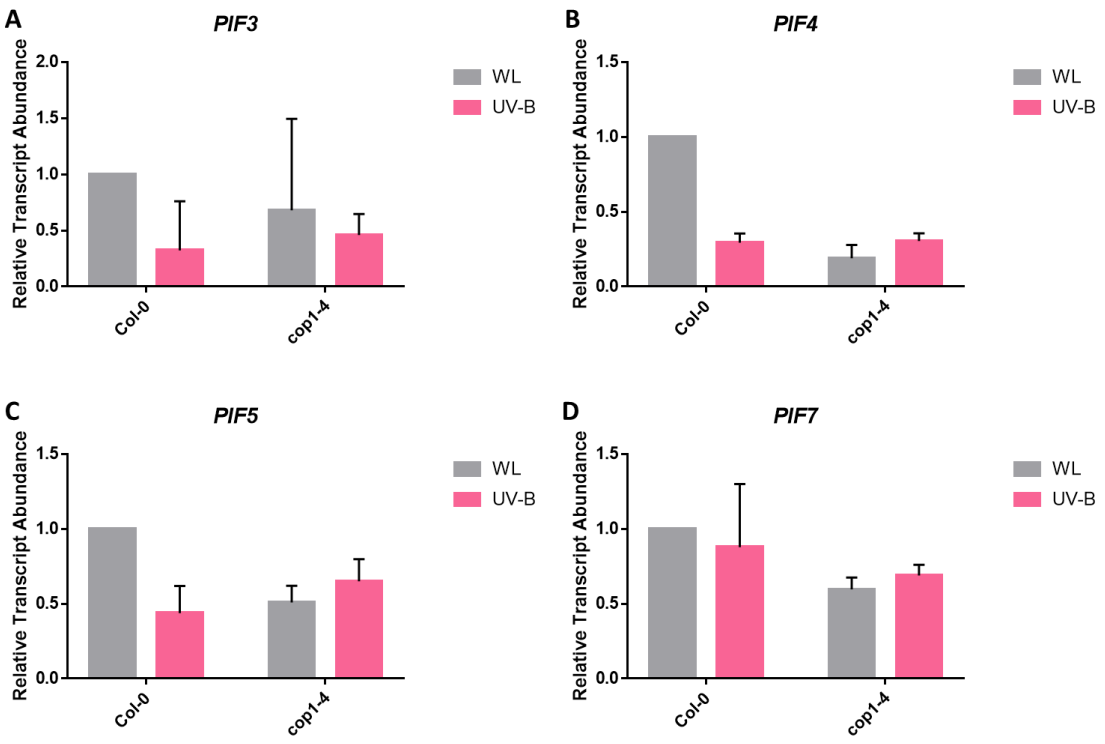


Figure 9: COP1 may promote *PIF* transcript accumulation. (A-D) Relative transcript abundance of (A) *PIF3*, (B) *PIF4*, (C) *PIF5* and (D) *PIF7* in WT (Col-0) and *cop1-4* seedlings grown on soil in long days at 20°C for 10 days, then transferred to white light ±UV-B (1 µmol $m^{-2}s^{-1}$) for 4 hours at dawn (ZT0). Relative transcript abundance was determined by RT-qPCR and normalized to ACTIN2. N = 2 ±SE.

3.5 Discussion

The results reported in this chapter demonstrate that applied low dose UV-B treatment represses the transcript accumulation of *PIF3, PIF4 and PIF5*. The lack of transcriptional regulation of *PIF7* is consistent with published reports showing it to be predominantly post transcriptionally regulated by phosphorylation/dephosphorylation and ubiquitination mechanisms (Li *et al*., 2012; Huang *et al*., 2018; Zhou *et al*., 2021; Burko *et al*., 2022). Data suggest that the UV-B-mediated suppression of *PIF3, 4* and *5*

transcript accumulation occur at the promoter level and requires the UV-B signalling components UVR8 and COP1, with no obvious requirement for HY5 and HYH.

Section 3.2 describes *in silico* regulatory profiling data for *PIF3*, *PIF4*, *PIF5* and *PIF7* promoters and suggests that all analysed sequences display binding motifs for multiple groups of transcription factors potentially involved in UV-B signalling. Cis elements for the transcription factor family BES1/BZR1 were found in the promoter regions of *PIF4*, *PIF5* and *PIF7*. BES1 and BZR1 are activated through dephosphorylation by the BR-INSENTITIVE 1 (BRI1) receptor in the presence of BR. Both BES1 and BZR1 transcription factors have been shown to be crucial for thermomorphogenesis responses. At higher temperatures, BES1 forms heterodimers with PIF4 and they act as a positive regulators of auxin biosynthesis genes (*YUCCA8* and *SAURS*) (Stavang *et al*., 2009; Franklin *et al*., 2011; Ibanez *et al*., 2018) driving hypocotyl elongation. At high ambient temperature, BZR1 binds directly to *PIF4* promoter and induces its transcription (Ibanez *et al*., 2018). Interestingly, upon UV-B irradiation, active UVR8 monomer targets BES1 and BZR1 for heterodimerization in the nucleus (Liang *et al.,* 2018). Collectively, these data raise the possibility that BES1/BZR1 act as direct regulators of *PIF4* and the sequestration of BES1 and possibly BZR1 by UVR8 inhibits the DNA interaction capacity between BES1/BZR1 and *PIF* promoters, leading to decreased transcript levels.

PIF's are transcription factors belonging to the bHLH family. Reports have demonstrated that PIF4 binds to the G-box motif within its own promoter and induces its transcription in an autoregulatory feedback loop (Zhai *et al*., 2020; Lee *et al*., 2021). The identification of cis elements for bHLH family of transcription factors in all promoters indicates the potential existence of self-regulatory mechanisms within PIF signalling, either individually or between family members. As PIF4 and PIF5 proteins are rapidly reduced in UV-B light (Hayes *et al*., 2017; Sharma *et al*., 2019), another potential mechanism that could be causing suppression of *PIF4* and *5* transcription in UV-B is reduced PIF4/5 protein abundance caused by protein turnover.

Binding motifs for MYB-related transcription factors were identified in all *PIF* promoter sequences analysed. MYB30 directly induces the expression of *PIF4* and *PIF5,* acting as a negative regulator of photomorphogenesis (Yan *et al*., 2020). It is also a direct gene target of BES1 (Liang *et al*., 2018), making MYB30 a potential candidate for a *PIF* transcriptional regulator in UV-B. Finally, several other transcription factors binding sites have been identified for a variety of transcription factor families in these sequences, providing tools to explore other mechanisms that regulate *PIF* expression.

qRT-PCR analyses in section 3.3.1 demonstrate that *PIF3*, *PIF4* and *PIF5* transcripts are negatively regulated in UV-B, displaying a greater suppression between time points 2 h and 4 h (ZT0) in a UVR8-dependent manner (Figure 5). *PIF7*, however, did not show consistent transcript suppression in UV-B in any genotype tested, supporting published findings showing that regulatory mechanisms controlling PIF7 abundance occur at the post translational level (Li *et al*., 2012; Huang *et al*., 2018; Zhou *et al*., 2021; Burko *et al*., 2022). Transcript abundance data using *pPIF::GUS* reporter lines shown in section 3.3.2 (Figure 7) provide evidence that UV-B-mediated suppression of *PIF4* and *5* occurs at the promoter level and not via transcript turnover, since the patterns of transcript decline in both light treatments were similar to those displayed in wild type Col-0. *GUS* expression driven by the *PIF3* promoter showed variation between biological replicates and minimal transcript repression in UV-B (Figure 7A), suggesting that the reduced *PIF3* transcript levels observed in UV-B (Figure 5A) are the result of transcript turnover. The GUS histochemical assay did not show any visual promoter activity differences among light treatments in any reporter line. As GUS is a stable protein and changes in *PIF* transcript abundance were reported within 2 h of UV-B treatment, it was not possible to visualise the suppression of promoter activity (Figure 7D). The highest levels of *PIF* promoter activity were observed in young leaves and hypocotyls, as shown by Zhang *et al*. (2013).

Consistent with Col-0 data, Ws seedlings also displayed UV-B-mediated suppression of *PIF3*, *PIF4* and *PIF5* transcript abundance, suggesting that the mechanism is conserved between accessions (Figure 8).

However, some results in section 3.4.1 were inconclusive. It was difficult to assess whether UVR8 and HY5/HYH act as modulators of the response, as the basal levels of *PIF4* and *PIF5* transcripts were very low in *uvr8-7* and *hy5/hyh* mutants. This could result from natural genetic variation between accessions. Nevertheless, it is clear that the responsiveness of *PIF3*, *4* and *5* transcription to UV-B is almost abolished in *uvr8-7*, yet retained in *hy5/hyh*. Together, these data suggest a role for UVR8 in the repression of *PIF* transcription in UV-B, but not HY5 nor HYH, at least in Ws (Figure 8). These findings are consistent with reported data in both high and low R:FR (Hayes *et al*., 2014). Data shown in Figure 9 may identify a new role for COP1 as promoter of *PIF* transcript accumulation in light-grown plants, as *cop1-4* plants display low basal levels of *PIF4* and *PIF5* transcript. Conversely, in dark-grown seedlings, higher levels of *PIF4* and *PIF5* transcript are observed in *cop1-4* plants (Pham *et al*., 2018). Sharma *et al*. (2019) also reported low basal levels of *PIF5* transcript in light-grown *cop1-4* plants. It is possible that COP1 controls *PIF4* and *5* regulators by positively regulating inducers and negatively regulating suppressors. This regulatory system may be dependent on the developmental stage of the plant, since 2-day old *cop1-4* seedlings display the same basal levels of *PIF5* transcript as WT (Pham *et al*., 2018b). The accumulation of *PIF4* and *5* repressors in the absence of COP1 could explain the low levels of *PIF4* and *5* in *cop1-4* plants (Sharma *et al*., 2019; Tavridou *et al*., 2020). The lack of UV-B-mediated transcriptional suppression of *PIF4* and *5* in *cop1-4* plants in addition to the proposed role of COP1 as a promoter of *PIF* transcript abundance, supports the role of COP1 in the UV-B-mediated suppression of *PIF4* and *5* (Hayes, *et al*., 2017; Sharma *et al*., 2019). Despite variation between biological repeats, some UV-B-mediated decrease in *PIF3* transcript was observed in *cop1-4*, supporting the existence of a posttranscriptional regulatory mechanism controlling *PIF3* transcript abundance. This could be investigated by identifying mechanisms that lead to transcript instability and/or degradation, including transcript silencing by regulatory RNAs, posttranscription modifications causing RNA instability and investigation of enzymes that target *PIF3* transcript for degradation.

In conclusion, this chapter indicates the existence of UV-B-mediated regulatory pathways negatively regulating *PIF3, 4* and *5* transcript accumulation, through regulation of *PIF4* and *5* promoter activity and *PIF3* transcript stability. All appear to require UVR8 and COP1. UV-B is a strong suppressor of hypocotyl elongation, and inhibitor of developmental responses that induce hypocotyl growth, such as thermomorphogenesis. Thermomorphogenesis is characterized by hypocotyl elongation and leaf hyponasty when plants experience elevated temperatures. These responses are highly dependent on PIF4 abundance, as it induces the expression of auxin biosynthesis genes (eg. *YUCCA8*) to promote hypocotyl elongation. UV-B has been shown to cause PIF4 protein turnover at 20°C but not 28°C (Hayes *et al*., 2017). The mechanisms by which PIF4 levels are reduced in UV-B at high temperature have been poorly characterised, but predominantly involve suppression of *PIF4* transcript accumulation, independently of the known thermomorphogenesis regulator, ELF3. Characterizing *PIF4* transcript regulation in thermomorphogenesis could therefore allow the testing of a possible novel regulatory pathway suggested in this chapter. In particular, I hypothesize that UV-B-mediated suppression of *PIF4* via active UVR8 during thermomorphogenesis involves the BR-related transcription factors BES1 and BZR1.

CHAPTER 4

The role of brassinosteroid signalling in UV-B-mediated *PIF4* suppression during thermomorphogenesis

4.1 Introduction

High ambient temperatures cause plants to alter their architecture and development in order to acclimate. In Arabidopsis, these morphological changes are characterized by hypocotyl and petiole elongation, leaf hyponasty, reduction in leaf area and root elongation (Koini *et al*., 2009; Franklin *et al*., 2011; Lee *et al*., 2014). Collectively, these temperature adaptations are termed thermomorphogenesis, and are suggested to provide the plant with a cooling mechanism via better access to cooling moving air to protect photosynthetic and meristematic tissues from heat damage from the heat-absorbing soil (Gray *et al*., 1998; Crawford *et al*., 2012; Bridge *et al*., 2013). More recently, it has been suggested that the primary ecological function of thermomorphogenesis is to enhance light capture in warm shaded

conditions, where high respiration rates require increased photosynthesis to compensate carbon balance (Romero-Montepaone *et al*., 2021). Plants sense high temperatures through inactivation of phyB (Jung *et al*., 2016; Legris *et al*., 2016), the thermoswitch 5'UTR region of PIF7 mRNA (Chung *et al*., 2020), and through the prion-like domain in ELF3 (Jung *et al*., 2020).

Along with auxin, BR is a crucial regulator of thermomorphogenesis. The mechanisms by which BR regulates thermomorphogenesis remain largely unknown, but defects in BR signalling pathway result in impaired thermomorphogenesis (Gray *et al*., 1998; Ibanez *et al*., 2018). BR are a class of steroid hormones that integrate external cues using kinases at the cell surface that activate signalling pathways resulting in changes in gene expression and growth (Wang *et al*., 2001). The perception of BR occurs through the recognition of the biologically active BR, brassinolide (BL), by the hydrophobic portion of the leucine-rich repeat (LLR) ectodomain of the membrane receptor kinase (RK) BRASSINOSTEROID INSENSITIVE 1 (BRI1), which contains a 70 amino acid island domain (Kinoshita *et al*, 2005; She *et al*., 2011). BL binding causes BRI1 to heterodimerize with BRI1-associated kinase 1 (BAK1, or SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE 3, SERK3), another RK, which leads to the intracellular dissociation of BRI1 KINASE INHIBITOR 1 (BKI1) from the BRI1 endodomain. These initiates signal transduction through a phosphorylation cascade and activates BRI1 SUPPRESSOR 1 (BSU1) (Nam & Li 2002; MoraGarcia *et al*., 2004; Wang *et al*., 2008; Kim *et al*., 2011; Santiago *et al*., 2013; Sun *et al*., 2013). Activated BSU1 dephosphorylates Y200 of-BR-INSENSITIVE 2 (BIN2), a GSK3-like kinase, causing inactivation of its negative phosphorylation signalling. This leads to accumulation of non-phosphorylated BES1 and BZR1 transcription factors, activating them for BR-regulated gene expression (He *et al.*, 2005; Yin *et al*., 2005; Sun *et al*., 2010; Yu *et al*., 2011; Kim *et al*., 2011). BES1 and BZR1 are usually described as transcriptional repressors, however they have been reported to upregulate genes through dimerization with other factors (Yin *et al*., 2005; Sun *et al*., 2010). BES1 heterodimerizes with PIF4 through interaction between the BES1 C-terminal region and the PIF4 N-terminal DNA-binding HLH domain, hindering the BRRE-element recognition region to suppress BES1

recruitment of its co-repressor TOPLESS (TPL). This modifies the DNA binding specificity of BES1, allowing the recognition of 5'-CATGTG-3' element, an upregulated PIF-binding element (PBE), switching BES1 from a repressor to an activator factor (Zhang *et al*., 2013; Pfeiffer *et al*., 2014; Martínez *et al*., 2018).

Both BR and PIF's are key factors required for hypocotyl elongation at elevated temperatures, since Arabidopsis seedlings fail to display hypocotyl elongation at 28°C in the absence of BL, and *pifq* mutants have impaired growth at 28°C even with BL (Ibanez *et al*., 2018; Martínez *et al*., 2018). Increased temperatures induce upregulation of BR biosynthetic genes, increased levels of dephosphorylated BES1 and accumulation of PIF4. Collectively, these boost levels of PIF4-BES1 complex and enhance the expression of auxin-related genes and cell wall-modifying genes, leading to cell growth and hypocotyl elongation (Matínez *et al*., 2018). Additionally, high ambient temperatures cause BZR1 to be translocated to the nucleus, where it directly interacts with the *PIF4* promoter, inducing its expression and amplifying the feedforward loop driving growth (Ibañez *et al*., 2018).

Thermomorphogenic responses are antagonized by low fluence rates of UV-B. Arabidopsis seedlings grown at 28°C in white light supplemented with low doses of UV-B display significantly shorter hypocotyls than white light controls (Hayes *et al*., 2017). Additionally, UV-B significantly suppresses high-temperature- induced leaf hyponasty and petiole length (Hayes *et al*., 2017). The mechanisms by which UV-B inhibits thermomorphogenesis are not completely understood, but unlike at 20°C, PIF4 and PIF5 protein levels are not decreased in UV-B at higher temperatures (Hayes *et al*., 2014,2017; Sharma *et al*., 2019). These observations suggest that UV-B-mediated suppression of *PIF4* transcript accumulation is central to this response. Intriguingly, active nuclear UVR8 monomers interact with BR signalling components BES1 and BZR1, inhibiting their DNA-binding capacity (Liang *et al.*, 2018). As BZR1 has been shown to positively regulate *PIF4* expression (Ibanez *et al*., 2018), it is possible that UVR8 binding to BZR1 and BES1 contributes to *PIF4* suppression during the UV-B-mediated inhibition of thermomorphogenesis (Figure 10).

To further investigate the mechanisms through which UV-B suppresses *PIF* transcript accumulation, this chapter explores the role of brassinosteroid signalling in the suppression of *PIF4* transcription during thermomorphogenesis. In particular, it investigates whether enhancing BR responses in UV-B at 28°C can overcome UV-B-mediated suppression of thermomorphogenesis through the upregulation of *PIF4* transcript abundance (Figure 10).

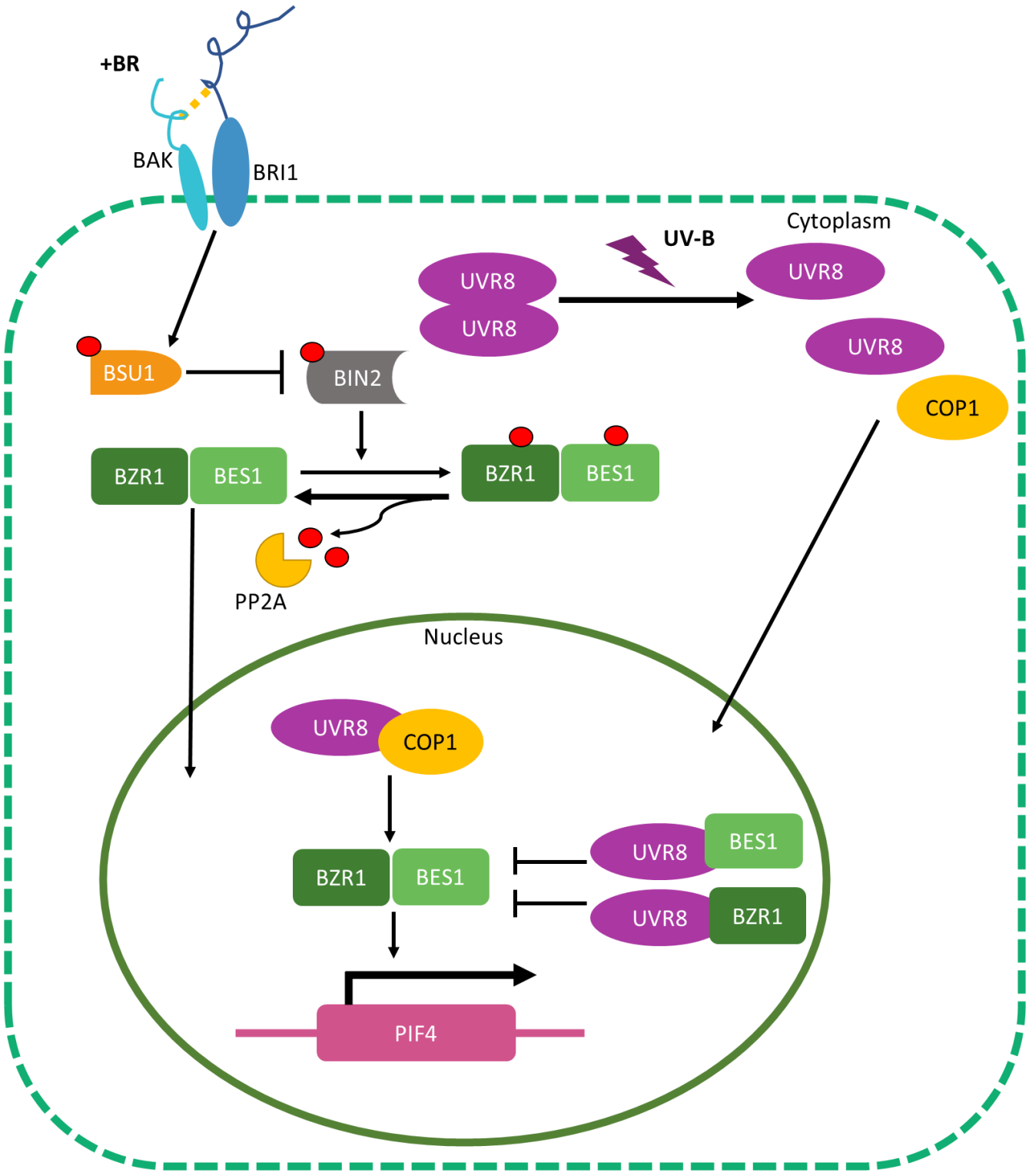


Figure 10: Proposed model showing the roles of BES1 and BZR1 in the UV-B- mediated suppression of *PIF4* transcription. BR is perceived by the BRI-1 ectodomain, resulting in heterodimerization of BAK1 with

BRI1 and phosphatase activation of BSU1. Activated BSU1 dephosphorylates BIN2, inactivating its negative phosphorylation signalling. PROTEIN PHOSPHATASE 2A (PP2A) phosphatase dephosphorylates BZR1 and BES1 leading to accumulated active BES1 and BZR1 in the nucleus. In the absence of UV-B, UVR8 exists in inactive dimers in the cytosol of the plant cell and upon UV-B radiation is converted into active monomers. The free cytosolic active UVR8 monomers physically interact with CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1), which facilitates importation of the UVR8-COP1 complex into the nucleus of the cell. In the nucleus of the cell, UVR8 binds to brassinosteroid signalling components, BRI1-EMS-SUPPRESSOR1 (BES1) and BRASSINOZOLE RESISTANT 1 (BZR1). BZR1 (and potentially BES1) promote *PIF4* transcription by directly binding to its promoter. Sequestration of BES1/BZR1 by UVR8 in UV-B may therefore suppress *PIF4* transcript abundance.

4.2 Enhanced BR signals partially overcome UV-B suppression of thermomorphogenesis

Seedlings were sterilized with hydrochloric acid and plated on 0.5x strength MS media supplemented with Brassinolide (0.5 µM) or DMSO (mock control). For hypocotyl elongation assays, plants were grown vertically for 3 days in white light at 20°C then transferred to white light ± UV-B (1.0 µmol m^{-2} s^{-1}) at 20°C or 28°C for four days in long days photoperiod (16 h light/8 h dark). For petiole elongation assay plants were grown horizontally at 20°C for 10 days in white light, then transferred to either 20°C or 28°C ±UV-B for 7 days. Hypocotyls and petioles were photographed then measured with ImageJ. Two-way ANOVA tests were performed to detect significant differences between treatments.

4.2.1. Optimization of experimental BL concentration

Three different concentrations of BL (0.1 µM, 0.5 µM and 1.0 µM) were tested to assess the effects on hypocotyl elongation. A t-test was performed to determine significant differences compared to the

untreated controls. All concentrations tested showed significant differences ($p < 0.05$), therefore the 0.5 µM concentration was selected as working concentration as it is the lowest concentration that displayed the most prominent hypocotyl elongation response (Figure 11).

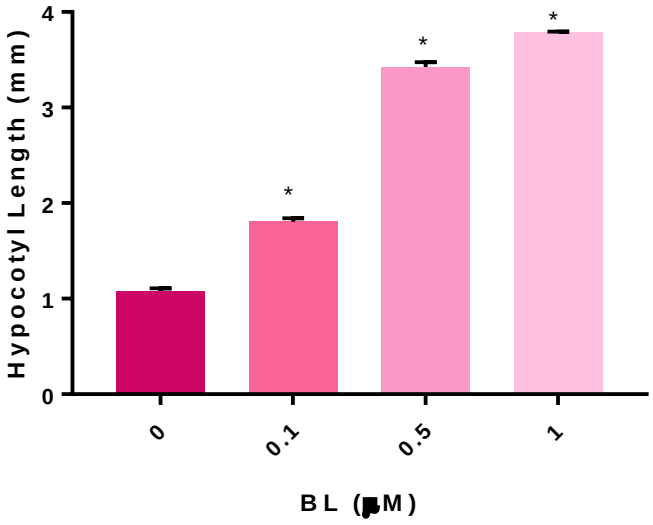


Figure 11: Concentration assay to stablish optimum concentration of brassinolide supplementation treatment. Col-0 seedlings were vertically grown on 0.5 x strength MS media supplemented with different concentrations of BL at 20°C for 7 days and measured. Data represent mean hypocotyl length ($n \geq 15$) ±SE. Asterisks represent statistically significant means ($p < 0.05$, t-test).

4.2.2 UV-B-mediated suppression of hypocotyl elongation at 28°C requires UVR8 and is antagonized by BL supplementation

Having established the optimum concentration of BL for promoting hypocotyl elongation, Col-0 and *uvr8-6* seedlings were grown ±BL (0.5 µM) at either 20°C or 28°C ±UV-B to test the effectiveness of BL at antagonizing UV-B-mediated suppression of thermomorphogenesis. In the absence of BL, Col-0 displayed

short hypocotyls in WL at 20°C , WL+UV-B at 20°C and WL+ UV-B at 28°C. Significantly longer hypocotyls were recorded in WL at 28°C, consistent with published observations (Hayes *et al*., 2017; Figures 12A and 10B). When supplemented with BL, increased hypocotyl elongation was observed in all conditions, with an exaggerated response at 28°C in both light treatments. Seedlings treated with BL in WL+UV-B at 28°C were significantly longer than mock controls, suggesting that increased BR signalling can overcome UV-B-mediated inhibition of thermomorphogenesis (Figures 12 A and B).

Uvr8-6 plants did not respond to UV-B either in the presence or absence of BL, consistent with the requirement of UVR8 in UV-B-mediated thermomorphogenesis suppression (Hayes *et al*., 2017; Figures 12A and C).

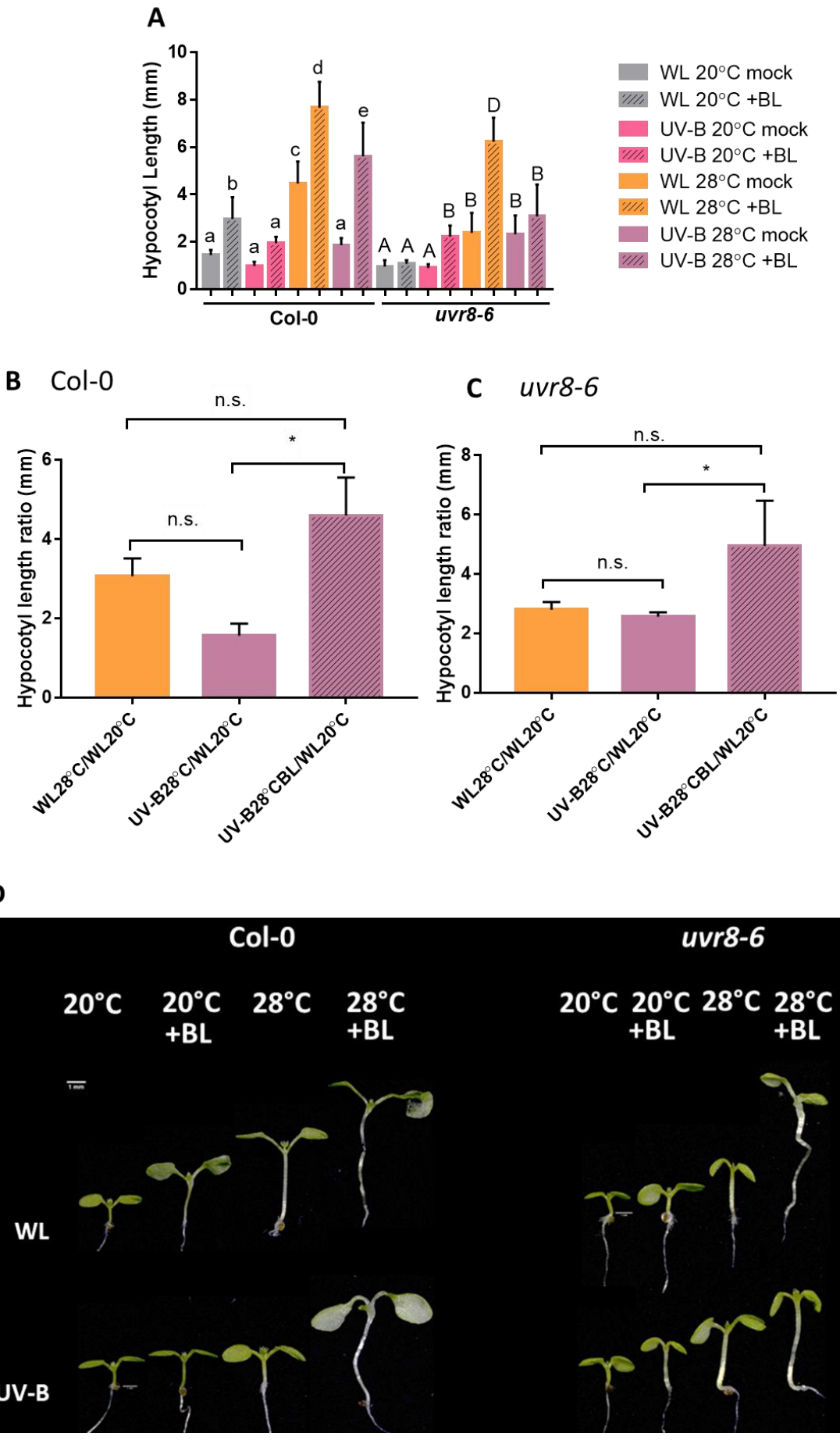

A
Hypocotyl Length (mm)
Col-0
uvr8-6
WL 20°C mock
WL 20°C +BL
UV-B 20°C mock
UV-B 20°C +BL
WL 28°C mock
WL 28°C +BL
UV-B 28°C mock
UV-B 28°C +BL
B Col-0
Hypocotyl length ratio (mm)
n.s.
*
WL28°C/WL20°C
UV-B28°C/WL20°C
UV-B28°CBL/WL20°C
C uvr8-6
D
Col-0
uvr8-6
20°C
20°C +BL
28°C
28°C +BL
WL
UV-B

Figure 12: BL supplementation overcomes UV-B-mediated suppression of thermomorphogenesis in Col-0. Seedling were grown vertically on 0.5 x strength MS media ± BL at 20°C for 3 days, then transferred to either 20°C or 28°C ± UV-B for 4 days before hypocotyls were measured (A) High temperature treatments normalized with the control treatment (B and C). Arabidopsis hypocotyl lengths as grown in 10A (D). BL = Brassinolide (0.5 µM) supplementation. Mock = DMSO. Data represent mean hypocotyl length ($n \geq 15$) ±SE. Different letters and asterisks represent statistically significant differences by Dunnet's test ($p < 0.05$, one-way ANOVA). Scale bar = 1 mm.

4.2.3 BR may be involved in the UV-B-mediated suppression of hypocotyl elongation in thermomorphogenesis

To test whether UV-B-mediated inhibition of thermomorphogenesis involves BZR1 and BES1, the same hypocotyl elongation assay was performed using the gain of function lines *bzr1-1d* and *bes1-d*. Single and double loss of function mutants in these genes fail to show BR-deficient phenotypes, probably due to redundancy with other family members (Chen *et al.* 2019). The intragenic dominant mutant *bzr1-1D* (brassinazole-resistant 1-1D) contains a proline mutation (Pro234 to Leu) that alters the PEST (putative proline-, glutamic acid-, serine- and threonine- rich domain) binding domain recognized by PROTEIN PHOSPHATASE 2A (PP2A), enhancing BZR1 dephosphorylation and activation by PP2A (Tang *et al*., 2011). Light grown *bzr1-1D* plants display a semi-dwarf phenotype compared to WT due to activation of a BR feedback inhibition pathway that suppresses BR synthesis (Wang *et al*., 2002; Tang *et al*., 2011). *bzr1-1d* is, however, hypersensitive to BL (Wang *et al*., 2002). The gain of function *bes1-D* (bri1-EMS-suppressor 1) is due to a monogenic semidominant mutation and displays a hypersensitive BL phenotype and constitutive BR responsiveness (Yin *et al*., 2005; Martinez *et al*., 2018). It was therefore hypothesized that

BR supplementation would be more effective at overcoming the UV-B-mediated suppression of thermomorphogenesis in these lines.

Bzr1-1d plants presented similar phenotypes to WT in WL ± UV-B. However, *bzr1-1d* plants showed increased sensitivity to BL especially in UV-B at 28°C, significantly antagonizing UV-B inhibition of hypocotyl elongation (Figure 13) and providing circumstantial evidence for a role for BR signalling in the UV-B response. *bes1-d* plants, however, did not display the over elongated hypocotyl as described in the literature (Yin *et al*., 2002; Tang *et al.*, 2011; Martinez *et al*., 2018). Instead, they behaved similar to Col-0 in both temperatures and light treatments with and without BL application (Figure 14). Therefore, no conclusions could be drawn on the effects of BES1 hypersensitivity on the BR-mediated antagonism of UV-B-mediated thermomorphogenesis suppression.

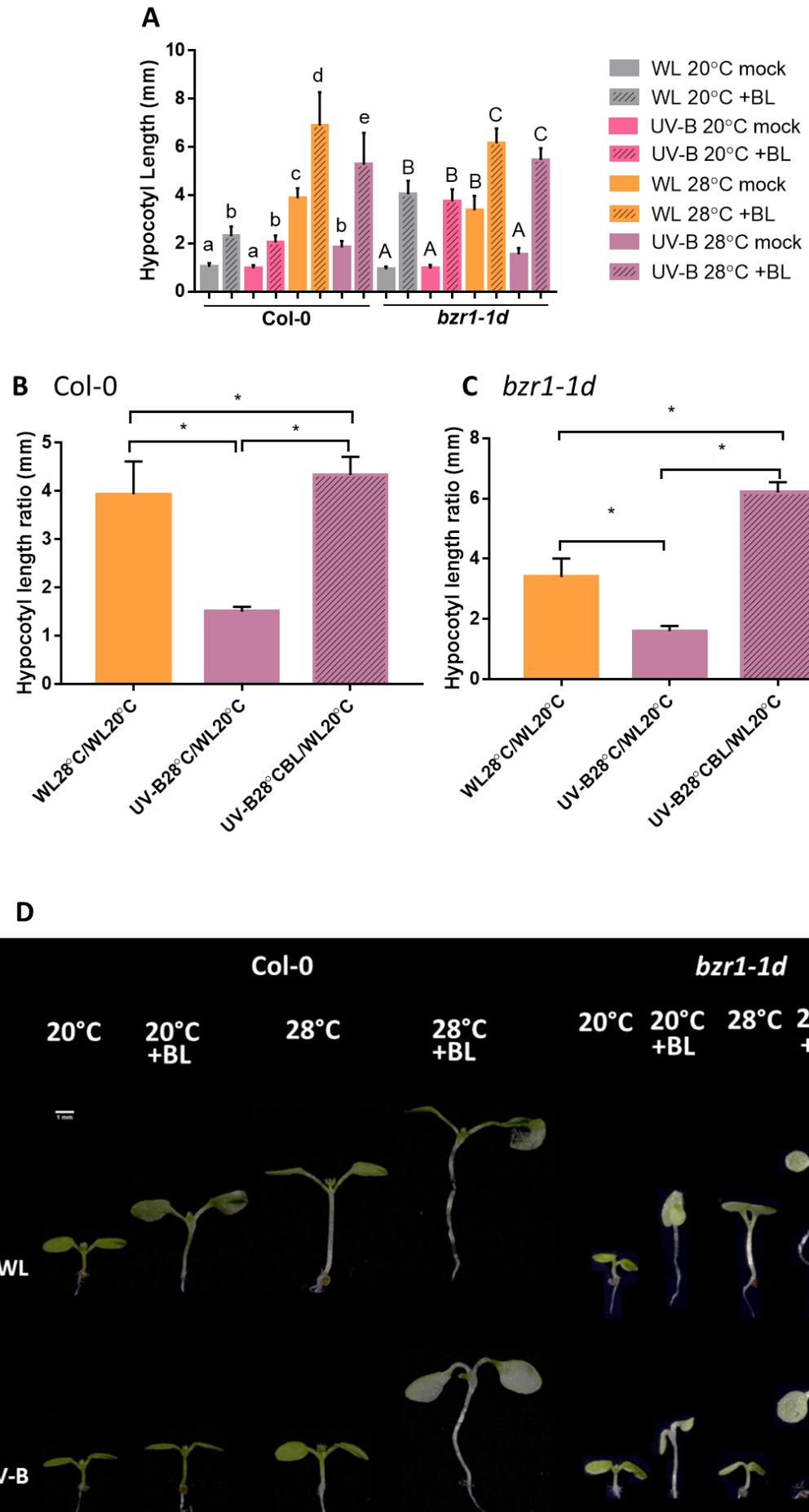
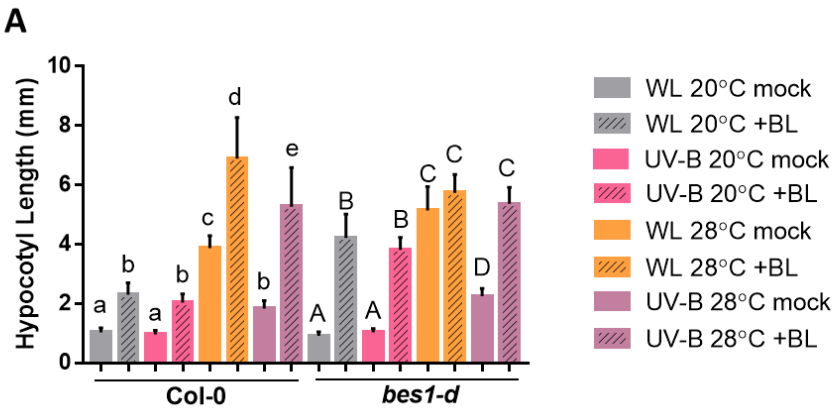
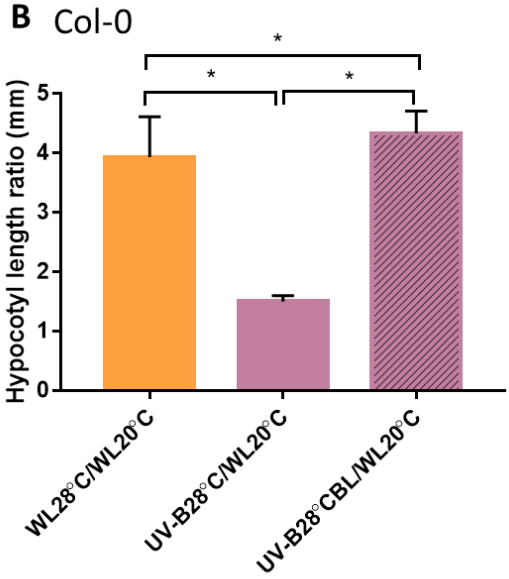
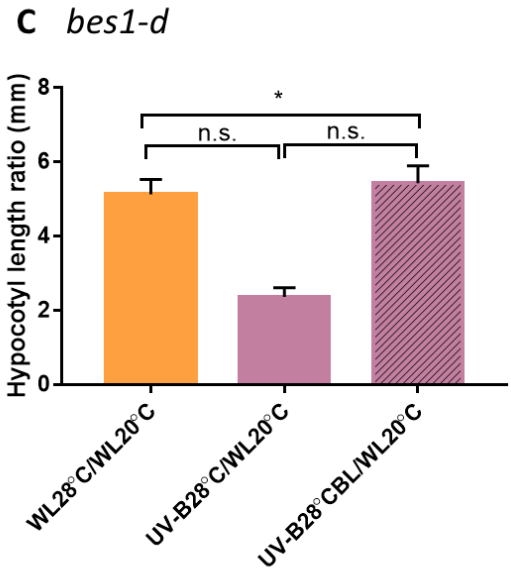

A
Hypocotyl Length (mm)
WL 20°C mock
WL 20°C +BL
UV-B 20°C mock
UV-B 20°C +BL
WL 28°C mock
WL 28°C +BL
UV-B 28°C mock
UV-B 28°C +BL
Col-0
bzr1-1d
B Col-0
Hypocotyl length ratio (mm)
WL28°C/WL20°C
UV-B28°C/WL20°C
UV-B28°CBL/WL20°C
C bzr1-1d
Hypocotyl length ratio (mm)
WL28°C/WL20°C
UV-B28°C/WL20°C
UV-B28°CBL/WL20°C
D
Col-0
bzr1-1d
20°C
20°C +BL
28°C
28°C +BL
20°C
20°C +BL
28°C
28°C +BL
WL
UV-B

Figure 13: BR is more effective at antagonizing UV-B-mediated suppression of thermomorphogenesis in *bzr1-1d*. Seedling were grown vertically on 0.5 x strength MS media ± BL at 20°C for 3 days, then transferred to either 20°C or 28°C ± UV-B for 4 days before hypocotyls were measured (A). High temperature treatments normalized with the control treatment (B and C). Arabidopsis hypocotyl lengths as grown in 11A (D). BL = Brassinolide (0.5 µM) supplementation. Mock = DMSO. Data represent mean hypocotyl length ($n \geq 15$) ±SE. Different letters and asterisks represent statistically significant differences by Dunnet's test ($p < 0.05$, one-way ANOVA). Scale bar = 1 mm.


A
Hypocotyl Length (mm)
10
8
6
4
2
0
a
b
a
b
c
d
b
e
A
B
A
B
C
C
D
C
Col-0
bes1-d
WL 20°C mock
WL 20°C +BL
UV-B 20°C mock
UV-B 20°C +BL
WL 28°C mock
WL 28°C +BL
UV-B 28°C mock
UV-B 28°C +BL


B Col-0
Hypocotyl length ratio (mm)
5
4
3
2
1
0
*
*
*
WL28°C/WL20°C
UV-B28°C/WL20°C
UV-B28°CBL/WL20°C


C bes1-d
Hypocotyl length ratio (mm)
8
6
4
2
0
*
n.s.
n.s.
WL28°C/WL20°C
UV-B28°C/WL20°C
UV-B28°CBL/WL20°C

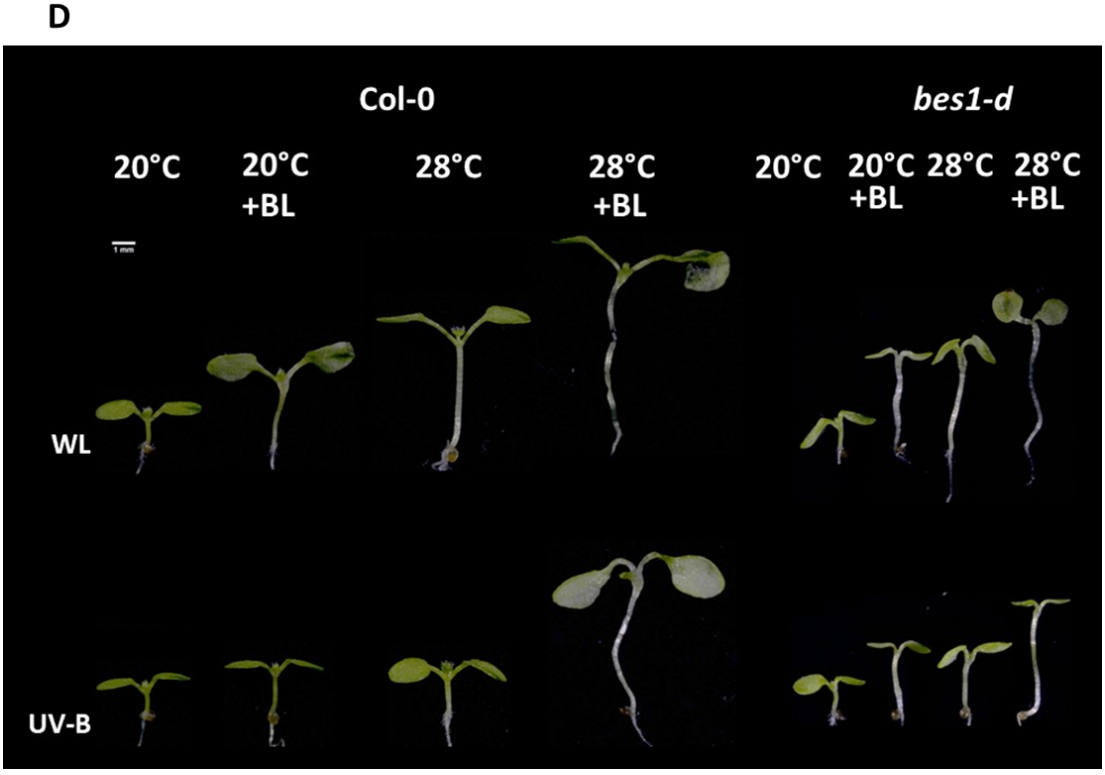

D
Col-0
bes1-d
20°C
20°C +BL
28°C
28°C +BL
20°C
20°C +BL
28°C
28°C +BL
WL
UV-B

Figure 14: BR antagonizes the UV-B-mediated suppression of thermomorphogenesis similarly in WT and *bes1-d*. Seedling were grown vertically on 0.5 x strength MS media ± BL at 20°C for 3 days, then transferred to either 20°C or 28°C ± UV-B for 4 days before hypocotyls were measured (A). High temperature treatments normalized with the control treatment (B and C). Arabidopsis hypocotyl lengths as grown in 12A (D). BL = Brassinolide (0.5 μM) supplementation. Mock = DMSO. Data represent mean hypocotyl length (n ≥ 15) ±SE. Different letters and asterisks represent statistically significant differences by Dunnet's test ($p < 0.05$, one-way ANOVA). Scale bar = 1 mm.

4.2.4 UV-B repression of hypocotyl elongation is regulated via BR- dependent and -independent pathways

bri1-4 is a T-DNA mutant in the Ws background containing a deletion in the coding sequence that causes a frame shift and the introduction of a premature stop codon into *BRI1*, producing truncated forms of BRI1 (Noguchi *et al*., 1999). Although *bri1-4* can respond to other phytohormones, it is insensitive to BR, generating dwarf plants. The *bri1-4* line was used to test whether the absence of BR signalling pathway plays affects UV-B-mediated inhibition of thermomorphogenesis. Consistent with published literature (Noguchi *et al*., 1999), this line was largely insensitive to BL application (Figure 15). Because *bri1-4* is a dwarf line with a limited hypocotyl elongation response to high temperature, it was difficult to assess the difference in the hypocotyl lengths among treatments. Although differences were subtle and not statistically significant, UV-B treatment inhibited hypocotyl elongation at both temperatures.

These data suggest that UV-B-mediated suppression of hypocotyl elongation during thermomorphogenesis potentially occurs through BR- dependent and -independent mechanisms.

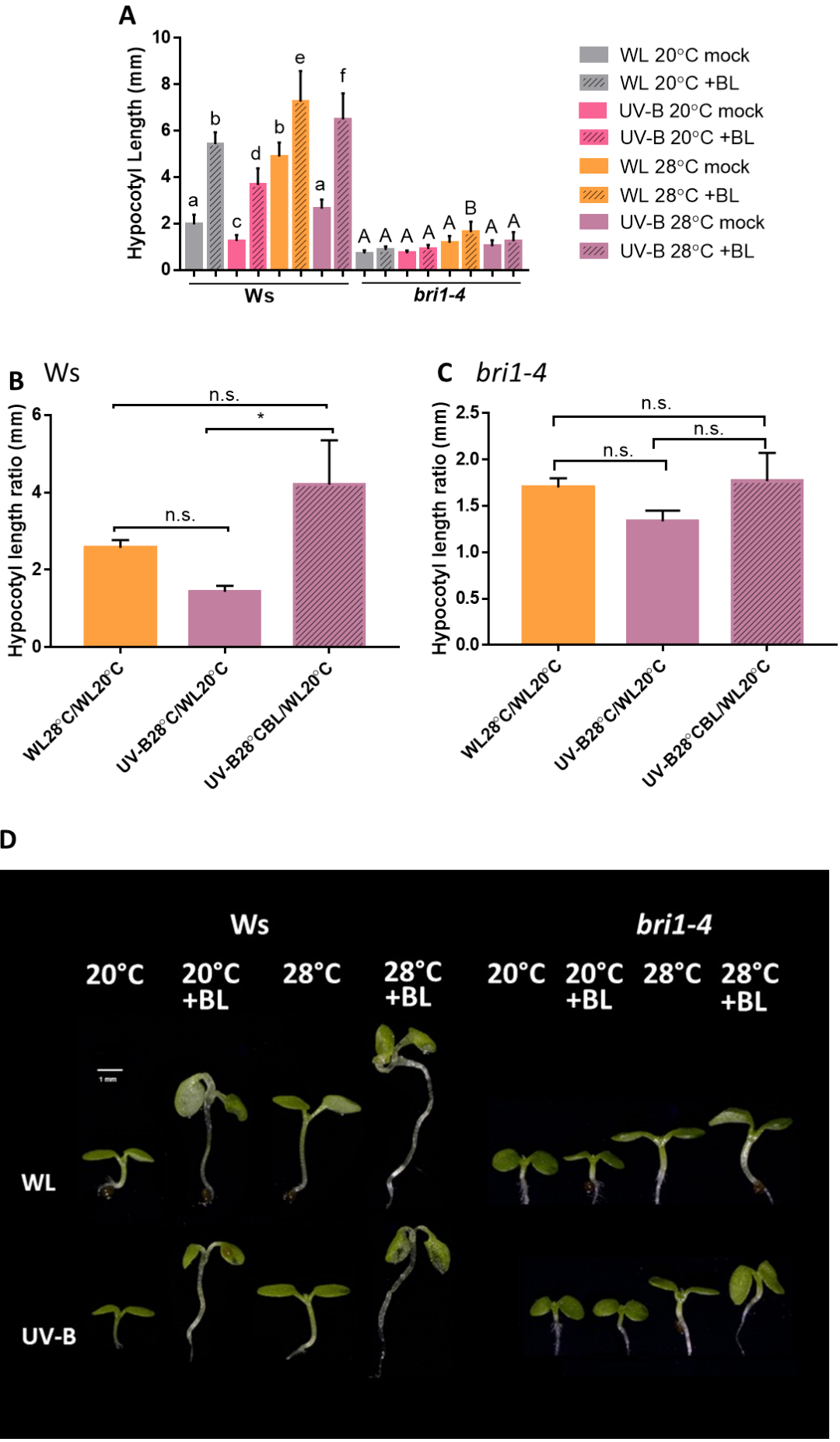

A
Hypocotyl Length (mm)
10
8
6
4
2
0
a
b
c
d
b
e
a
f
A
A
A
A
A
B
A
A
Ws
bri1-4
WL 20°C mock
WL 20°C +BL
UV-B 20°C mock
UV-B 20°C +BL
WL 28°C mock
WL 28°C +BL
UV-B 28°C mock
UV-B 28°C +BL
B
Ws
Hypocotyl length ratio (mm)
6
4
2
0
n.s.
*
n.s.
WL28°C/WL20°C
UV-B28°C/WL20°C
UV-B28°CBL/WL20°C
C
bri1-4
Hypocotyl length ratio (mm)
2.5
2.0
1.5
1.0
0.5
0.0
n.s.
n.s.
n.s.
WL28°C/WL20°C
UV-B28°C/WL20°C
UV-B28°CBL/WL20°C
D
Ws
bri1-4
20°C
20°C +BL
28°C
28°C +BL
20°C
20°C +BL
28°C
28°C +BL
WL
UV-B

Figure 15: UV-B-mediated repression of hypocotyl elongation is regulated via brassinosteroid-dependent and -independent pathways. Seedling were grown vertically on 0.5 x strength MS media ± BL at 20°C for 3 days, then transferred to either 20°C or 28°C ± UV-B for 4 days before hypocotyls were measured (A). High temperature treatments normalized with the control treatment (B and C). Arabidopsis hypocotyl lengths as grown in 13A (D). BL = Brassinolide (0.5 μM) supplementation. Mock = DMSO. Data represent mean hypocotyl length (n ≥ 15) ±SE. Different letters and asterisks represent statistically significant differences by Dunnet's test ($p < 0.05$, one-way ANOVA). Scale bar = 1 mm.

4.2.5 BL supplementation has no effect on petiole elongation

Petiole elongation at increased temperatures is an architectural modification that characterises thermomorphogenesis in adult plants. To test whether BL supplementation could antagonise UV-B inhibition of thermomorphogenesis, we supplemented plates with BL and measured the petiole elongation of Col-0 plants grown in WL ± UV-B at 20°C and WL ± UV-B at 28°C. In contrast to data from hypocotyl elongation assays, petioles did not elongate in response to BL treatment but instead displayed a slightly decreased length compared to the mock treated controls (Figure 16). These results suggests that the hormonal control of stem elongation differs between seedlings and adult plants. Mock treated plants displayed longer petioles at 28°C compared to 20°C, and reduced length when exposed to UV-B, consistent with previous work reporting UV-B-mediated repression of thermomorphogenesis (Hayes *et al*., 2017).

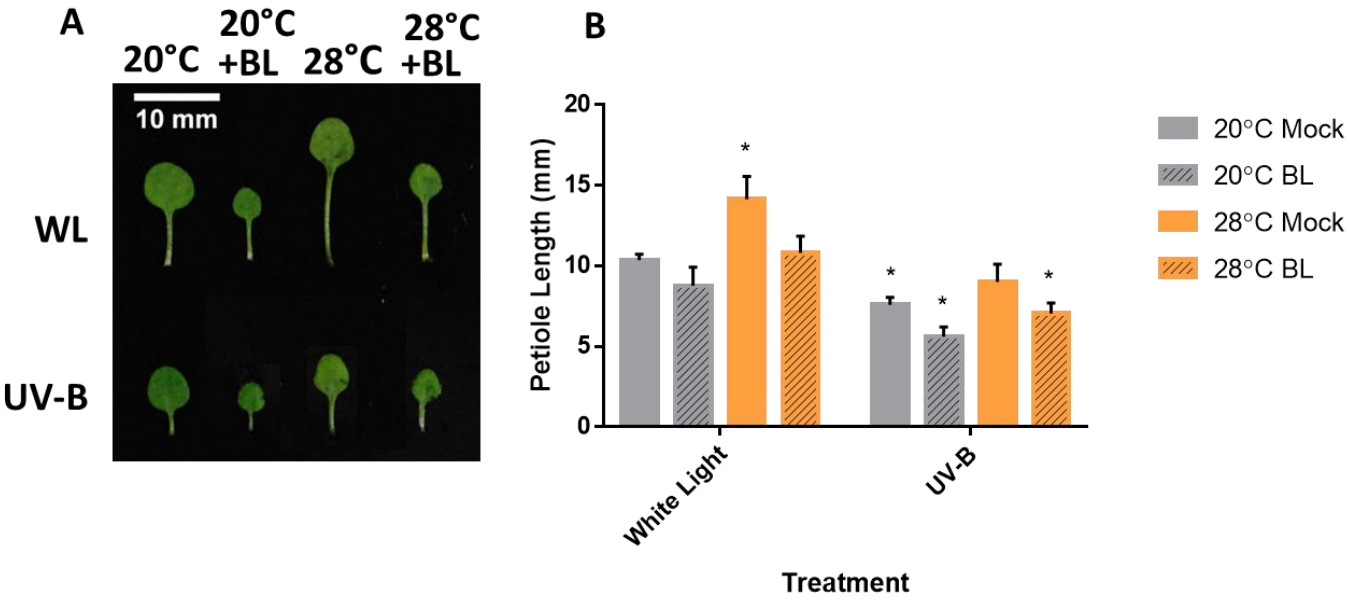


Figure 16: Col-0 plants did not display enhanced petiole elongation when supplemented with BL in UV-B light at 20°C or 28°C. Col-0 plants were grown on 0.5 x strength MS media with or without BL supplementation (0.5 µM) at 20°C for 10 days, then transferred to either 20°C or 28°C ±UV-B for 7 days before petiole lengths were measured. Data show mean lengths and are representative from three independent replicates ± SE. n ≥ 15. Asterisks represent statistically significant means ($p < 0.05$, Two-way ANOVA).

4.3 BL signalling does not appear to perform a significant role in the UV-B-mediated suppression of *PIF4* transcription during thermomorphogenesis

4.3.1 Testing the effects of the *bri1* mutation and BL supplementation on UV-B-mediated inhibition of *PIF4* transcript accumulation

Downregulation of *PIF4* transcript abundance in UV-B at 28°C is the main mechanism for UV-B-suppression of thermomorphogenesis. Because UV-B inhibition of hypocotyl elongation at 28°C was abolished when plants were exposed to exogenous application of BL in section 4.2.2 (Figure 12) and BZR1 promotes *PIF4* transcription at 28°C (Ibanez *et al*., 2018), it has been hypothesized that the inhibition of hypocotyl elongation by UV-B at high temperature may involve UVR8-mediated suppression of BZR1/BES1-mediated *PIF4* transcription. To test this hypothesis, qRT-PCR assays were performed to determine relative transcript abundance of *PIF4* in 10-day-old seedlings grown on 0.5x strength MS media supplemented with ±BL and treated with ±UV-B at 20°C and 28°C for 4 hours. Since *PIF4* transcription is circadian regulated, we transferred seedlings to light and temperature treatments at ZT3, when *PIF4* transcript levels peak in long photoperiods (16 h light/ 8 h dark) (Nomoto *et al*., 2013). This would therefore allow us to observe maximum effects of UV-B-mediated *PIF4* suppression.

To investigate UV-B suppression of *PIF4* transcript mechanism in higher temperatures, WT Col-0 and *uvr8-6* mutant were first used. Consistent with data shown in Chapter 3 (Figure 5B), *PIF4* transcript was suppressed in UV-B in an UVR8-dependent manner at 20°C (Figure 17A) and at 28°C, consistent with the literature (Hayes *et al*., 2017). BL supplementation showed minimal effects on *PIF4* transcript abundance. Next, the effects of BR signalling pathway on UV-B-suppression of *PIF4* were assessed using Ws and *bri1-4* plants. Similar to Col-0, *PIF4* transcript was suppressed in UV-B in both temperatures in Ws (Figure 17B). Interestingly, clear UV-B-mediated suppression of *PIF4* transcript accumulation was recorded in *bri1-4* mutants (Figure 17B), suggesting that BR signalling does not perform a major role in this response. *PIF4* transcript abundance was also more responsive to high temperature in Col-0, than Ws, which may represent differences in the kinetics of this response between different accessions. Again, BL supplementation showed minimal effects on *PIF4* transcript abundance in Ws and *bri1-4* (Figure 17B).

Collectively, these data suggest that brassinosteroid signalling does not perform a major regulatory role in UV-B-mediated suppression of *PIF4* transcript abundance.

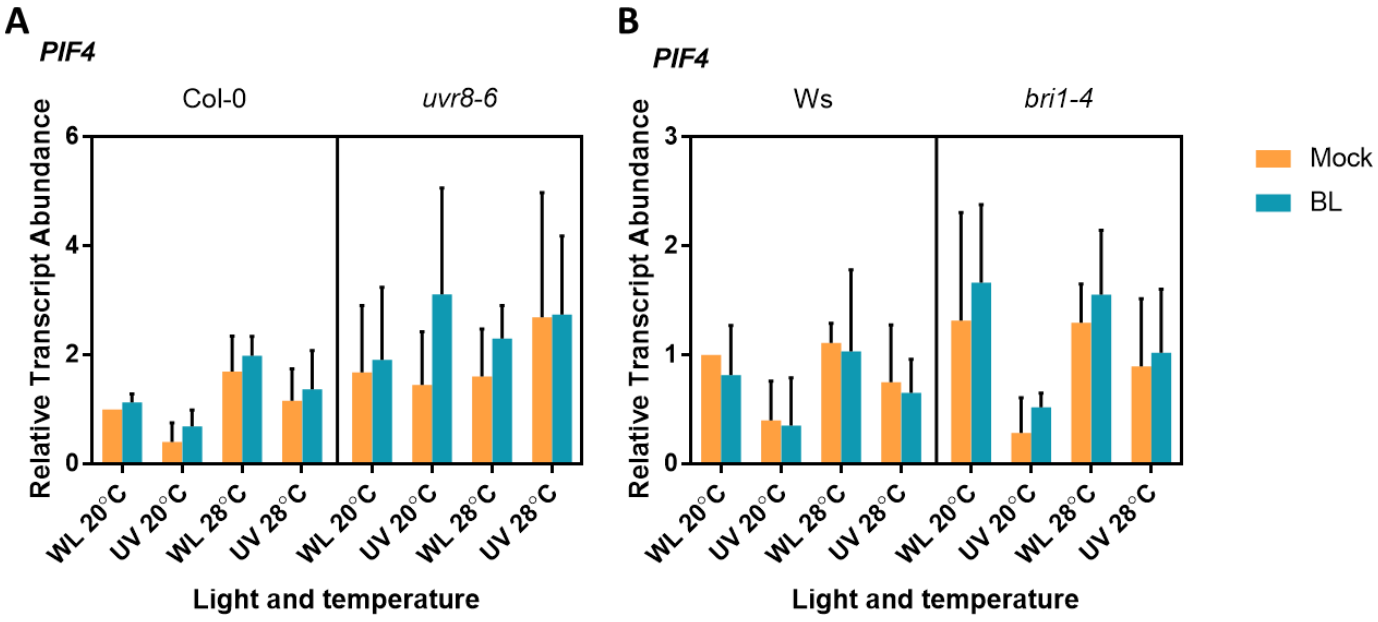


Figure 17: BL supplementation cannot effectively reverse UV-B-mediated suppression of *PIF4* transcript abundance. Col-0, Ws, *uvr8-6* and *bri1-4* seedlings were grown on 0.5 x strength MS media ±BL for 10 days at 20°C then transferred to either 20°C or 28°C ± UV-B for 4 h at ZT3. (A and B). Data represent mean relative *PIF4* transcript abundance ± SE. Values were determined by qRT-PCR and normalized to *ACTIN2*. Data are from three independent biological replicates. Statistically significant differences were calculated using a two-way ANOVA and Tukey's post hoc test ($p < 0.05$).

4.3.2 Characterisation of BES1 binding to *PIF4* in the presence of UV-B

In addition to analysing the effects of BL feeding on *PIF4* transcript accumulation, a parallel experiment analysing the effect of UV-B on BES1 binding to the *PIF4* promoter was attempted simultaneously.

A Chromatin Immunoprecipitation (ChIP)- qPCR experiment was attempted using the lines *35S::BES1-Flag*/Col-0 and *35S::BES1uvr8-Flag*/Col-0 (Liang *et al*. 2018). The aim was to quantify *PIF4* promoter enrichment following the immunoprecipitation of BES1. Using the *in silico* analysis from chapter 2, two pairs of primers were designed flanking the regions for BES1-binding cis elements in the *PIF4* promoter (-2337bp in the positive strand) (Table 2). Primers for previously established BES1 targets were also designed (Yin *et al*., 2002; Li *et al*., 2009; Bai *et al*., 2012; Liang *et al*., 2018). As BES1 interacts with *MYB30* and *PRE1* promoters, but not with *ACTIN7* (*ACT7*) nor *PP2A* promoters, these served as positive and negative controls respectively (Yin *et al*., 2002; Li *et al*., 2009; Bai *et al*., 2012; Liang *et al*., 2018).

Seedlings of *35S::BES1-Flag*, *35::BES1uvr8-Flag* and Col-0 were grown in soil for 10 days (16 h light/8 h dark), then moved to ± UV-B at 20°C for 4 hours (ZT0) and sampled. The assay was performed following the chromatin immunoprecipitation from Arabidopsis tissue protocol by Yamaguchi *et al*., 2014 and qRT-PCR was carried out following manufacturer's instructions (Brilliant III ultra-fast SYBR® Green QPCR mastermix (Agilent)).

The first two ChIP- qPCR assays generated low signals for the positive controls *MYB30* and *PRE1* and strong signals for the negative control primers *PP2A* and *ACT7*, as well for the negative control plant Col-0. Thus, some method optimization to improve qRT-PCR results was required. Because the ChIP protocol contains extensive steps, as well the qPCR, it was difficult to address the specific steps that needed improving. Low ChIP- qPCR signals can be related to low abundance of transcription factor. Hence, the first step of troubleshooting was to increase the amount of starting tissue harvested. Instead of using 300-600 mg of plant tissue following Yamaguchi *et al*., 2014, 2-3 g of seedlings were harvested as described in Liang *et al*., 2018. In addition to increasing the amount of starting tissue, optimization in the tissue infiltration step was attempted. Two different vacuum pumps were tested and chosen according to the most effective result in the shorter amount of time. Then, to improve the nuclei isolation and shearing of chromatin steps, different amounts of sonication with varied weight of plant tissue were tested to generate the

optimal average DNA fragment sizes, which is a DNA smear in the 200-1000 bp size range with a peak around 500 bp (Figure 18).

Although such optimization steps were carried out, the negative control Col-0 was still generating signals in the qPCR results, while the negative and positive control primers for the transgenic lines were still presenting contrary signals. Unfortunately, due to severe time constraints, it was not possible to perfect this experiment. Future work would include optimization of antibody concentration per amount of plant tissue and the use of adult plants to increase the amount of tissue available as well as using less seeds.

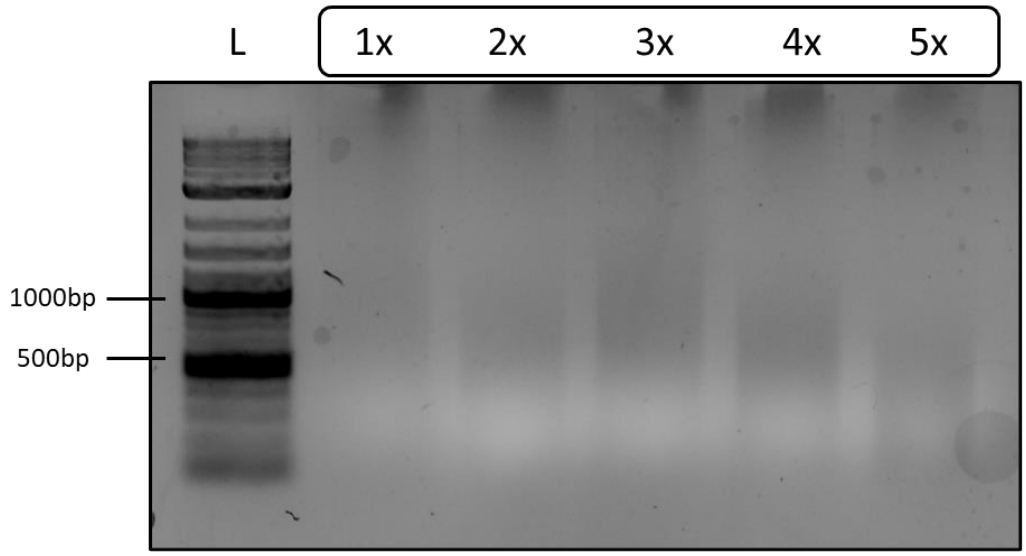


Figure 18: Testing sonication efficiency for BES1 ChIP. Col-0 gDNA samples containing different amounts of tissue were used for optimization of sonication efficiency. The amounts of tissue from 1 x – 5 x are 0.506 mg, 0.343 mg, 0.637 mg, 0.700 mg and 0.415 mg respectively "1 x – 5 x" (times of sonication with a 10 seconds pulse and 50 seconds interval within sonication at 70 % amplitude). "L" (Gene ruler): 1 kb Plus Ladder.

4.5 Discussion

Thermomorphogenesis is a plant acclimatory response to elevated temperatures characterized mainly by stem and petiole elongation predominantly driven by PIF4. These responses are antagonized by low doses of UV-B through downregulation of *PIF4* transcript (Hayes *et al*., 2017). BR also regulates thermomorphogenesis and participates in UV-B mediated responses. In this chapter, the mechanisms by which UV-B mediates BR signals to potentially suppress *PIF4* transcript accumulation were explored.

Data shown in Figure 12A provides evidence UVR8-mediated suppression of hypocotyl growth at 28°C in UV-B in Col-0 plants, consistent with the literature (Hayes *et al*., 2017). Figure 15A shows the same UV-B-mediated suppression of hypocotyl elongation in Col-0 and Ws, evidencing that these mechanisms may be conserved between assessions. To test whether BR- mediated targets are involved in UV-B-mediated *PIF4* transcript suppression resulting in shorter stems, a number of hypocotyl and petiole elongation assays were performed. Firstly, plants were tested for increased BR signalling following feeding with BL. Then, mutants with altered activity of BR signalling components known to physically interact with active UVR8 and bind the *PIF4* promoter were selected for hypocotyl elongation assays with and without BL feeding at different temperatures.

Research has shown that active UVR8 targets BES1 and BZR1 in the nucleus (Liang *et al*., 2018), and BZR1 upregulates *PIF4* in higher temperatures (Ibanez *et al*., 2018). Additionally, *in silico* data shown in Chapter 3 (Table 3 and Figure 4) identified e cis elements for BES1 in the *PIF4* promoter. Hence, a possible mechanism for UV-B-mediated suppression of *PIF4* is UVR8 sequestration of the closely related BES1 and BZR1 at higher temperatures (Figure 10). To test this hypothesis, *bes1-d* and *bzr1-1d* gain of function mutants were grown for hypocotyl measurement assays and treated with WL ± UV-B ± BL at either 20°C or 28°C (Section 4.2). WT plants treated with BL expressed a significantly taller hypocotyls compared to the untreated controls and overcame the UV-B-mediated suppression of hypocotyl growth at 28°C. (Figure

12B, 13B, 14B and 15B). This suggests a role of BR in the UV-B-mediated inhibition of thermomorphogenesis. Although *bes1-d* plants did not display the expected exaggerated long hypocotyls as described in the literature, they displayed increased sensitivity to BL (Yin *et al*., 2002). *bes1-d* plants were observed to antagonize UV-B-mediated suppression of thermomorphogenesis similarly to WT. This suggests that BES1 may not perform a major role in this response. However, since *bes1-d* plants displayed mild phenotypes compared to the literature, the conclusions cannot be fully addressed. Further work involving this line would require sequencing of the mutated region to check for alterations in the locus. With more time, a fully functional line displaying constitutively active BES1 could be obtained and genotyped. qRT-PCR assays could then be performed to test the role of BES1 in the UV-B-mediated suppression of *PIF4*. Observing whether these lines show altered UV-B-mediated suppression of hypocotyl elongation at 28°C in the presence and absence of supplementary BL would help establish whether BES1 performs a role in the UV-B-mediated suppression of thermomorphogenesis. In contrast to *bes1-D* plants, *bzr1-1d* plants are shorter than WT due to activation of an inhibitory feed-back loop that suppresses BR signals (Wang *et al*., 2002; Tang *et al*., 2011). They are, however, hypersensitive to BL (Wang *et al*., 2002). Data presented in section 4.2.3 show that BL can antagonize UV-B-mediated hypocotyl inhibition at 28°C in WT plants and this response is enhanced in *bzr1-1d* mutants (Figure 13), potentially evidencing a role for BZR1 in the UV-B-mediated inhibition of thermomorphogenesis.

In addition to experiments using BR hypersensitive plants, a mutant showing complete disruption of the BR signalling pathway (*bri1-4*) was also analysed in the same hypocotyl assays. Because *bri1-4* is a dwarf line, significant changes in hypocotyl elongation between treatments were difficult to determine. There was, however, a small decrease in hypocotyl length when *bri1-4* was treated at 28°C+UV-B compared to 28°C+WL, suggesting that UV-B inhibition of hypocotyl at 28°C can occur independently of an active BR signalling pathway (Figure 15).

To further examine the role of BR signalling during UV-B-mediated suppression of thermomorphogenesis, petiole elongation assays were performed as this is the second mostly characterized phenotype mediated by *PIF4* (Koini *et al*., 2009). Whilst there was a significant UV-B suppression of hypocotyl elongation in Col-0 seedlings ± BL (Figure 12), BL supplementation did not show any effects of counteracting suppression of petiole elongation in UV-B treated plants (Figure 16). This suggests that BR plays a role in thermomorphogenesis in an age/or tissue-specific manner.

Collectively, the results gathered from the experiments in this chapter support dependency of UVR8 in the UV-B-mediated suppression of hypocotyl growth at 28°C and a potential role for BR signalling. Since UV-B-mediated suppression of thermomorphogenesis is mainly mediated by suppression of *PIF4* transcript accumulation (Hayes *et al*., 2017), qRT-PCR was performed to assess the roles of BR signalling and UV-B on *PIF4* abundance using the lines *bri1-4*, *uvr8-6*, Ws and Col-0 in the presence and absence of UV-B and BL at 20°C and 28°C (Figure 14). Consistent with data in Chapter 3 (Figure 5B), *PIF4* transcript is suppressed in UV-B at 20°C in both WT assessions, whereas it is upregulated at 28°C+WL and downregulated at 28°C+UV-B, consistent with previous reports (Hayes *et al*., 2017). UV-B-mediated suppression of *PIF4* transcript is mediated by UVR8 at both temperatures, supported by findings in Chapter 3 (Figure 5B) and Hayes *et al*. (2017). *Bri1-4* behaved similarly to the Ws control in all conditions. Finally, BL supplementation did not affect *PIF4* transcript abundance of any of the lines assessed in all light and temperature treatments, suggesting that BR may be involved in the UV-B-mediated suppression of thermomorphogenesis, but not by downregulating *PIF4*.

It is not known whether BES1 binds to the *PIF4* promoter, however, *in silico* analysis in Chapter 3 (Table 3 and Figure 4) showed two cis elements for the BES1 TFBS in the *PIF4* promoter. Additionally, BES1 is highly similar to BZR1, which was shown to directly upregulate *PIF4* at elevated temperatures (Ibanez *et al*., 2018). Thus, BES1 binding to the *PIF4* promoter was investigated in parallel. To test the direct interaction between BES1 and *PIF4,* a (ChIP) qPCR was attempted (Section 4.3.2). Due to lack of time for protocol

optimization, this experimental assay was not concluded. Testing whether BES1 binds to *PIF4* would provide insights into how BR signalling is integrated with UV-B- mediated developmental responses. If successful, this assay would allow us to detect TF/DNA-interactions in addition to the specific sites where it occurs. In addition to a ChIP- qPCR, it would be insightful to perform a Y1H assay using *PIF4* as bait to search for transcription factors that bind to it in WL±UV-B at 20°C and 28°C. The most relevant transcription factors could then be tested *in planta* through a transient assay, followed by mutant analysis under thermomorphogenic conditions.

In conclusion, the data reported in this chapter suggest the existence of BR-dependent and -independent mechanisms during UVR8-mediated inhibition of thermomorphogenesis. It is unlikely, however, that UV-B-mediated suppression of *PIF4* transcript accumulation at 28°C is BR mediated. It is possible that BR-mediated genes involved in hypocotyl elongation are downregulated in the presence of UV-B, while an alternative pathway mediated by UVR8 suppresses *PIF4* transcript levels, possibly through the same mechanism as at 20°C. Chapters 3 and 4 have therefore established UVR8 and COP1 as regulators of *PIF4* transcript accumulation in UV-B and discarded a potential role for BZR1 and BES1. Chapters 5 and 6 will explore other known transcription factors that directly regulate *PIF4* and investigate whether they act in the downregulation of *PIF4* transcript in UV-B.

Chapter 5

The role of UV-B in the regulation of PIF4 positive feedback loop

5.1 Introduction

PIF abundance and activity are dynamically regulated according to the light quality available and dictate the nature of the plant developmental response through regulation of their target genes. UV-B limits hypocotyl elongation in Arabidopsis seedlings, in part due to turnover of PIF4 and PIF5 proteins (Hayes *et al*., 2014,2017; Pham *et al*., 2018; Sharma *et al*., 2019). While the molecular mechanisms controlling UV-B-mediated PIF5 turnover have been described in the literature (Sharma *et al*., 2019), the mechanisms controlling UV-B-mediated PIF4 turnover remain to be elucidated, despite the importance of PIF4 for plant growth. In addition to degrading PIF4 protein, data from Chapter 3 (Figure 5B) and 4 (Figure 17) provide evidence of the UV-B-mediated downregulation of *PIF4* transcript abundance. More recently, PIF4 was shown to physically interact with the G-box domain within its promoter to self-activate transcription (Huq

& Quail, 2002; Zhai *et al*., 2020; Lee *et al*., 2021). These findings are supported by *in silico* analysis data reported in chapter 3 (Table 3 and Figure 4) showing predicted cis elements for bHLH family of transcription factors in the promoter of *PIF4*, moreover G-box motifs (CACGTG). It is possible that UV-B-mediated depletion of PIF4 levels decrease *PIF4* transcript promotion, hence resulting in the UV-B suppression of *PIF4* transcript observed in chapters 3 and 4.

In general, PIFs are stable in the dark and are degraded upon light exposure. Light activated photoreceptors phys and CRYs physically interact with PIFs to regulate their levels and activity (Ni *et al*., 1999; Huq & Quail, 2002; Huq *et al*., 2004; Khanna *et al*., 2004; Oh *et al*., 2004; Leivar *et al*., 2008a; Leivar & Quail, 2011; Pedmale *et al*., 2015; Ma *et al*., 2016). PIFs are phosphorylated and ubiquitinated following phy interaction and degraded via the 26S proteasome (Bauer *et al*., 2004; Monte *et al*., 2004; Shen *et al*., 2005, 2008; Al-Sady *et al*., 2006; Nozue *et al*., 2007; Lorrain *et al*., 2008; Soy *et al*., 2012; Yamashino *et al*., 2013; Ni *et al*., 2014). PIF1 and PIF 3/4 light-mediated phosphorylation events are mediated by CASEIN KINASE II (CK2) (Bu *et al*., 2011) and BIN2 (Bernardo-Garcia *et al*., 2014; Ling *et al*., 2017) respectively. PIF3 phosphorylation additionally involves PHOTOREGULATORY PROTEIN KINASES 1-4 (PPK1-PPK4) (Ni *et al*. 2017). PIFs are ubiquitinated by CULLIN (CUL) RING UBIQUITIN LIGASEs and their substrate recognition components varies. EIN3 BINDING F-BOX (EBF1) and LIGHT-RESPONSE BRIC-A-BRACK/TRAMTRACK/BROAD (LBR) act as substrate recognition components for PIF3 (Dong *et al*., 2017), BLADE ON PETIOLE (BOP1/2) for PIF4 (Zhang *et al*., 2017) and COP/SPA for PIFs 1 and 5 (Zhu *et al*., 2015; Pham *et al*., 2018). Studies have shown that UV-B supplementation enhances PIF4 and PIF5 degradation within 2 hours of treatment (Hayes *et al*., 2014; Sharma *et al*., 2019; Tavridou *et al*., 2019). Kinetic analyses of the PIF5 response have revealed rapid protein degradation within 20 minutes of UV-B exposure and involvement of the 26S proteosome. COP1 has been shown to perform a non-canonical role to stabilise PIFs (Smirnova *et al*., 2012; Sharma *et al*., 2019). Active UVR8 competes with PIF5 for COP1, reducing the abundance of COP1-PIF5 complexes (Sharma *et al.*, 2019). The requirement for the phytochrome-binding

APB domain of PIF5 for this response, suggests that UVR8 sequestration of COP1 leads to PIF5 to being targeted by phyB and degraded via the 26S proteosome (Sharma *et al*., 2019).

Due to functional similarities between PIF4 and 5, it is plausible to postulate that they are degraded by UV-B in a similar fashion, via the ubiquitin/26 proteasome system. Kinetic data of *PIF4* transcript abundance in UV-B presented in Chapter 3 (Figures 5B and 7B) show strong UV-B-mediated suppression of transcript between timepoints 2 h and 4 h (UV-B treatment started at ZT0). Since UV-B mediated turnover of PIF4 is observed within 2 hours treatment, it was hypothesized that rapid PIF4 turnover in UV-B mediated by the 26S proteasome reduces *PIF4* transcription as a result of depletion of PIF4 levels, hence breaking a self-activation system.

To further understand UV-B-mediated suppression of *PIF4* transcript accumulation, this chapter focussed on investigating the correlation between rapid UV-B degradation of PIF4 with its subsequent transcript suppression. More specifically, this chapter tested whether inhibition of the 26S proteasome by MG132 to prevent UV-B-mediated degradation of PIF4 inhibits UV-B-mediated *PIF4* transcript suppression.

5.2 UV-B-mediated suppression of *PIF4* transcript accumulation occurs independently of the 26S proteasome

qRT-PCR assays were performed to assess the difference in transcript abundance between two light treatments, with and without the proteasome inhibitor MG132 (dissolved in DMSO): white light and white light supplemented with low dose UV-B. Wildtype Col-0 was grown for 9 days in white light at 20°C in long days (16 h light /8 h dark) then treated with and without MG132 (50 µM) for 16 h. Controls plants were treated in parallel with DMSO. On the 10th day at ZT0, plants were transferred to either white light or white light supplemented with UV-B (1.0 µmol m^{-2} s^{-1}) at 20°C and harvested for RNA extraction after 2 h.

5.2.1 UV-B-mediated suppression of *PIF4* but not *PIF5* transcript abundance is observed in the presence and absence of MG132

To investigate the possibility that UV-B-mediated *PIF4* and *5* transcript suppression results directly from UV-B-mediated PIF protein turnover, plants were treated with the protease inhibitor MG132 to prevent PIF degradation by the 26S proteosome. MG132 (carbobenzoxyl-L-leucyl-L-leucyl-L-leucinal) is a peptide-aldehyde ubiquitin-proteasome pathway inhibitor. It directly targets and inhibits the activity of the beta subunits of the 20S proteasome, the core of the proteolysis system, blocking the proteolytic activity of the 26S proteosome (Lee *et al*., 1998). Since PIF4 and 5 are degraded in the light via the ubiquitin 26S proteasome pathway, inhibition of the machinery would potentially enable PIF4/5 accumulation and facilitate a feed forward autoactivation system in UV-B.

Consistent with results reported in Chapters 3 (Figures 5B and 7B) and 4 (Figure 17), *PIF4* transcript abundance was suppressed by UV-B in the DMSO mock treatment. Interestingly, UV-B-mediated suppression of *PIF4* transcript was also observed in MG132 treated plants (Figure 19A). This could suggest that PIF4 protein levels are being degraded independently from the 26S proteasome, highlighting the existence of an alternative protein degradation system for PIF4 in UV-B. Alternatively, this result may suggest insufficient uptake of the proteasome inhibitor by the plant tissue or a *PIF4* transcript suppression mechanism which operates independently from PIF4 protein. In contrast, *PIF5* transcript abundance showed no UV-B-mediated suppression in UV-B when MG132 was present (Figure 19B). It should, however, be noted that a smaller UV-B response was observed in the mock treatment when compared to data reported in chapter 3 (Figure 5C and 7C). There was also large variation between biological repeats, making interpretation difficult (Figure 19B). Due to the surprising nature of the result, future work in this chapter focussed on PIF4.

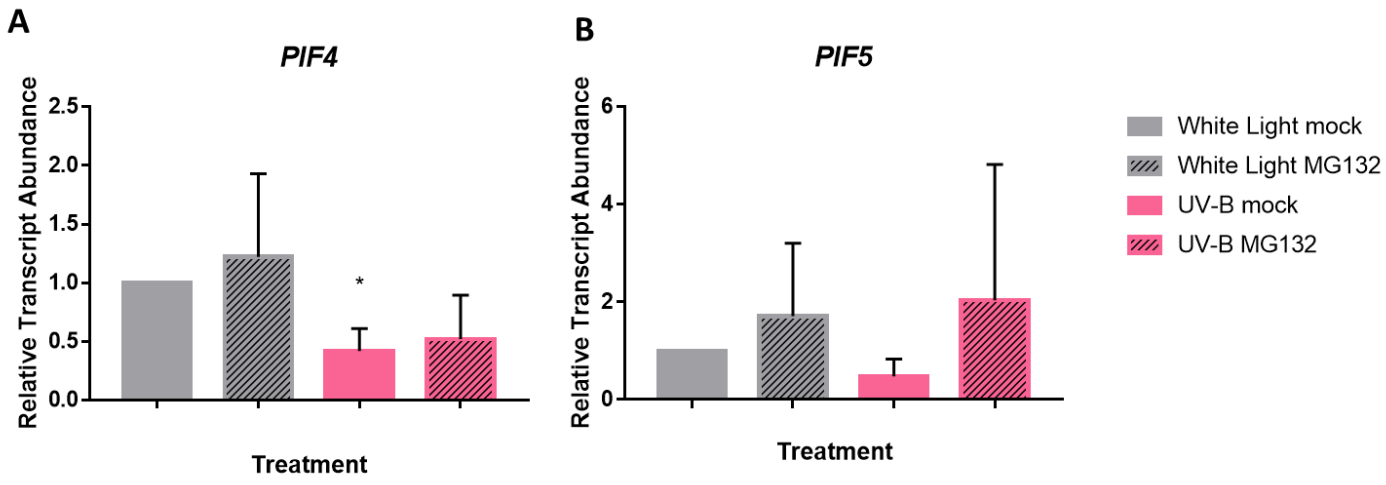


Figure 19: UV-B-mediated suppression of *PIF5* but not *PIF4* transcript abundance was inhibited in the presence of MG132. Col-0 seedlings were grown on 0.5 x strength MS in white light for 9 days, then were treated in MG132 (proteosome inhibitor) (50 µM) for 16 h. On the 10th day, plants were transferred to white light ±UV-B at dawn for 2 h. Data represent mean relative *PIF4* and *PIF5* transcript abundance. Asterisks represent statistically significant means when compared to untreated control ($p < 0.05$, two-way ANOVA). Expression was determined by qRT-PCR and normalized to *ACTIN2*. Data are from three independent biological replicates ±SE. "mock"= DMSO.

5.3 UV-B-mediated degradation of PIF4 may not require the 26S proteasome

To investigate whether UV-B-mediated suppression of *PIF4* transcript abundance is correlated with low PIF4 protein levels in UV-B, western blots were performed to assess PIF4 stability in WL±MG132 and WL±UV-B±MG132. Wildtype Col-0 and *35S::PIF4-HA* plants were grown for 9 days in white light at 20°C in long days (16 h light /8 h dark) then treated with and without MG132 (50 µM) for 16 h. At ZT0, plants were transferred to either white light or white light supplemented with UV-B (1.0 µmol m^{-2} s^{-1}) at 20°C and sampled after 2 h.

5.3.1 MG132 treated plants display high levels of ubiquitinated proteins

For western blotting, plants expressing HA epitope-tagged PIF4 under the control of the constitutive CaMV 35S promoter (*35S::PIF4-HA/*Col-0; Lorrain *et al*., 2007) were used to evaluate protein levels, and Col-0 was used as negative control. To confirm plant assimilation of MG132, western blotting was performed on plants treated with WL±UV-B±MG132, and blots were incubated with anti-ubiquitin (anti-Ubi) antibody. The level of ubiquitinated protein in each lane is proportional to the intensity of the smear on the membrane. Proteosome inhibitor treated samples displayed an enhanced intensity smear of ubiquitinated proteins, confirming the imbibement of MG132 into the plant tissue (Figure 20).

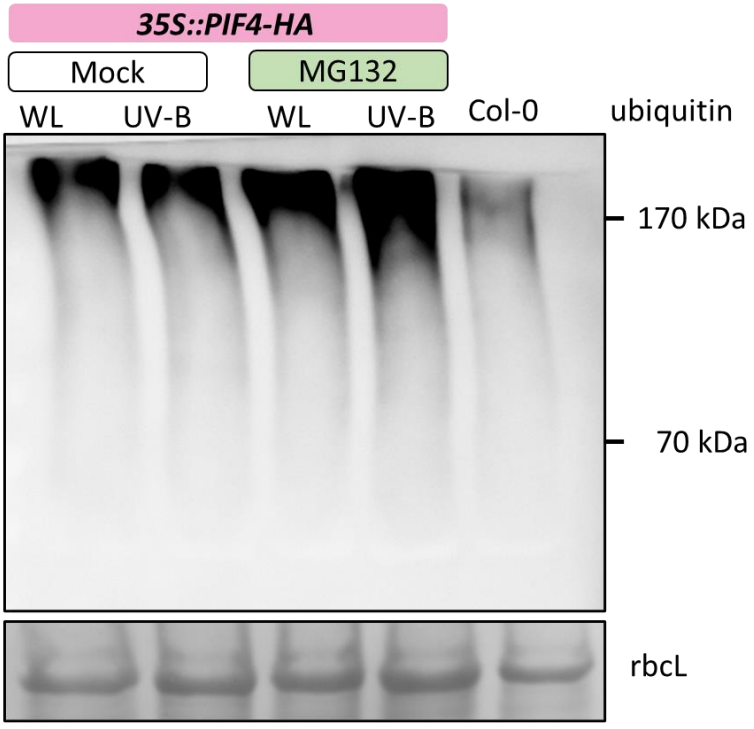


Figure 20: MG132 treated plants accumulate ubiquitinated proteins. Representative western blot incubated with anti-ubi and anti-mouse antibodies from 4 different plant samples showing efficacy of

proteosome inhibition ± MG132 in *35S::PIF4-HA* plants following 16 h of MG132 treatment and 2 h white light ± UV-B treatment. Equal total protein extracts loaded per lane. “M” (mock): DMSO. “T” (MG132): 50 µM. “WT” (wild type Col-0). Ponceau stained Rubisco large subunit (rbcL) is shown as a loading control below.

5.3.2 MG132 does not inhibit UV-B-mediated degradation of PIF4

PIF4 and 5 levels are regulated by phytochromes. Exposure to high R:FR switches phyB into its photoactive form Pfr, that tags PIF4 and 5 for phosphorylation and ubiquitination via the 26S proteosome (Bauer *et al*., 2004; Monte *et al*., 2004; Shen *et al*., 2005, 2008; Al-Sady *et al*., 2006; Nozue *et al*., 2007; Lorrain *et al*., 2008; Soy *et al*., 2012; Yamashino *et al*., 2013). A similar system appears to be involved in the UV-B -mediated degradation of PIF5 by UVR8 (Sharma *et al*., 2019). MG132 inhibition of proteosome activity inhibits UV-B-mediated degradation of PIF5. Because PIF4 and 5 are functionally similar and both proteins show significant UVR8-mediated degradation within 2 hours of UV-B treatment (Hayes *et al*., 2014, 2017; Sharma *et al*., 2019; Tavridou *et al*., 2019), it is reasonable to predict that their UV-B degradation is mediated by a similar mechanism.

To address whether the proteasome system is involved in the UV-B degradation of PIF4, western blots were performed to compare PIF4 stability in plants treated in WL±UV-B±MG132. Consistent with previous studies, PIF4 levels were significantly reduced in UV-B (Figure 21) (Hayes *et al*., 2014, 2017; Tavridou *et al*., 2019). Intriguingly, MG132 did not suppress UV-B-mediated degradation of PIF4 similarly to PIF5 (Sharma *et al*., 2019). Instead, MG132 treated plants displayed the same magnitude of UV-B- mediated PIF4 degradation as the mock treatment (Figure 21). Given that MG132 caused the accumulation of

ubiquitinated proteins in this experiment (Figure 20), this could indicate that UV-B degradation of PIF4 occurs independently of the 26S proteasome.

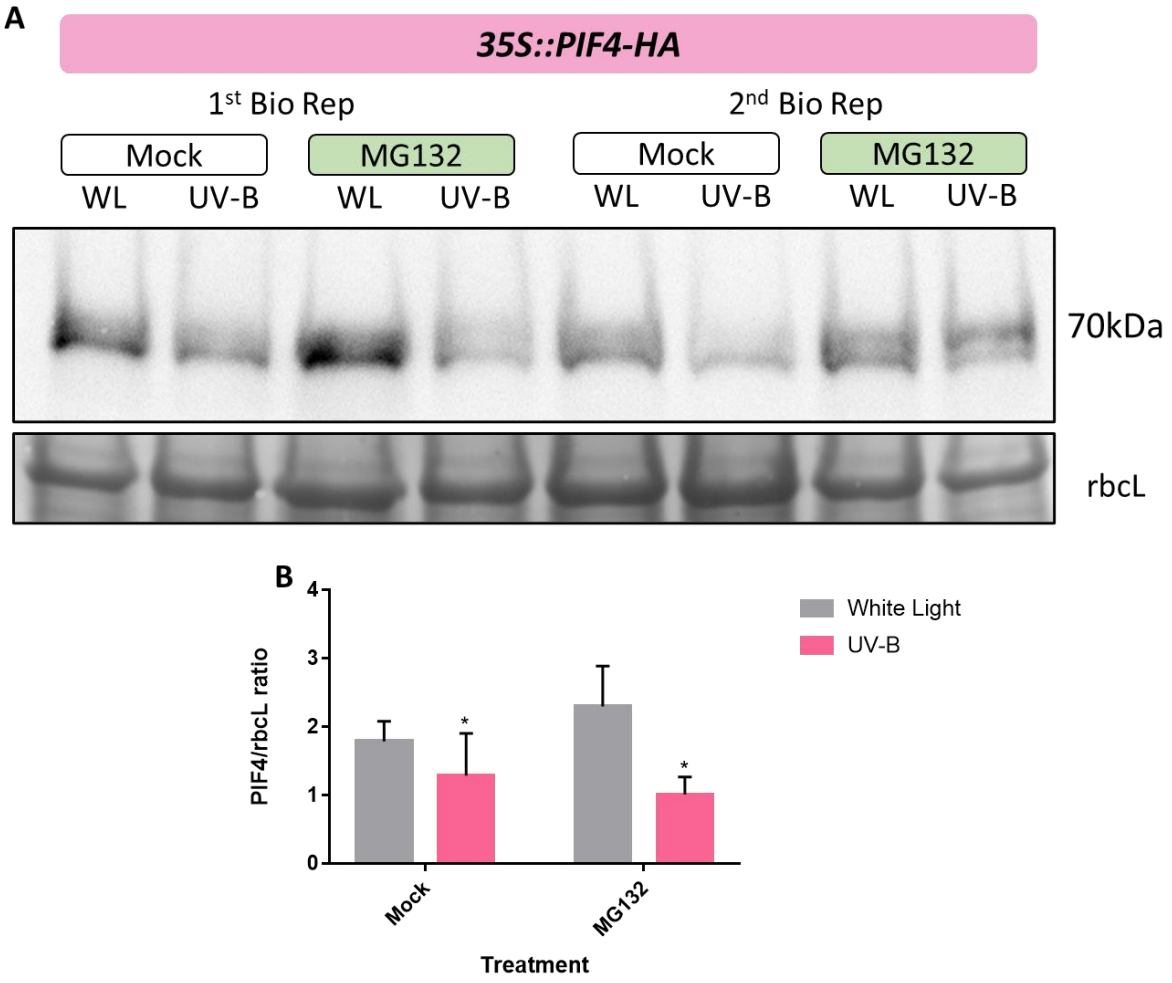


Figure 21: UV-B degradation of PIF4 may occur independently of the 26S proteasome. (A) Western blots of PIF4 in *35S::PIF4-HA* plants from 2 biological repeats. 9-day-old seedlings grown in long days (16 h light/ 8 h dark) were transferred to 0.5 x strength MS liquid medium ± MG132 for 16 h. Plants were transferred to WL±UV-B at ZT0 for 2 h. PIF4 was detected using anti-HA antibody. Col-0 was used as negative control and ponceau straining of the Rubisco large subunit (rbcL) was used as loading control. Equal total protein extracts loaded per lane. (B) Quantification of PIF4/rbcL ratio from 3 independent biological replicates (n = 3) ±SE. Asterisks represent statistically significant means ($p < 0.05$, two-way ANOVA). "Mock" = DMSO.

5.4 Significant UV-B-mediated suppression of *PIF4* and PIF4 abundance are observed in MG132 treated *pPIF4::PIF4:3xFLAG* seedlings

To explore the correlation between UV-B-mediated control of PIF4 protein abundance and transcript regulation in the same plant tissue (without the potential artefacts induced by constitutive gene expression), qRT-PCR and western blotting analyses of *PIF4* transcript and protein abundance were performed in the transgenic line *pPIF4::PIF4:3xFLAG*/Col-0 (Kumar *et al.,* 2012). The experimental design for this experiment followed the qRT-PCR and western blotting designs performed in sections 5.2 and 5.3.

Consistent with data shown in section 5.3.2 and published research (Hayes *et al*., 2014, 2017; Tavridou *et al*. 2019), a 2 h UV-B treatment significantly reduced levels of PIF4 (Figure 22A). Consistent with results in section 5.3, UV-B+MG132 treated plants still displayed a strong reduction in PIF4 levels (Figure 22A), despite the tissue absorption of the proteasome inhibitor (Figure 22B). Finally, *PIF4* transcript abundance was strongly suppressed in UV-B both in the presence and absence of MG132, consistent with section 5.2 (Figure 19A). Taken together, these data suggest UV-B-mediated PIF4 degradation may occur via different mechanisms to PIF5 (Sharma *et al.* 2019). Without an effective treatment to suppress UV-B-mediated PIF4 abundance, the hypothesis that UV-B inhibits PIF4 transcription via rapid turnover of PIF4 protein cannot be effectively tested.

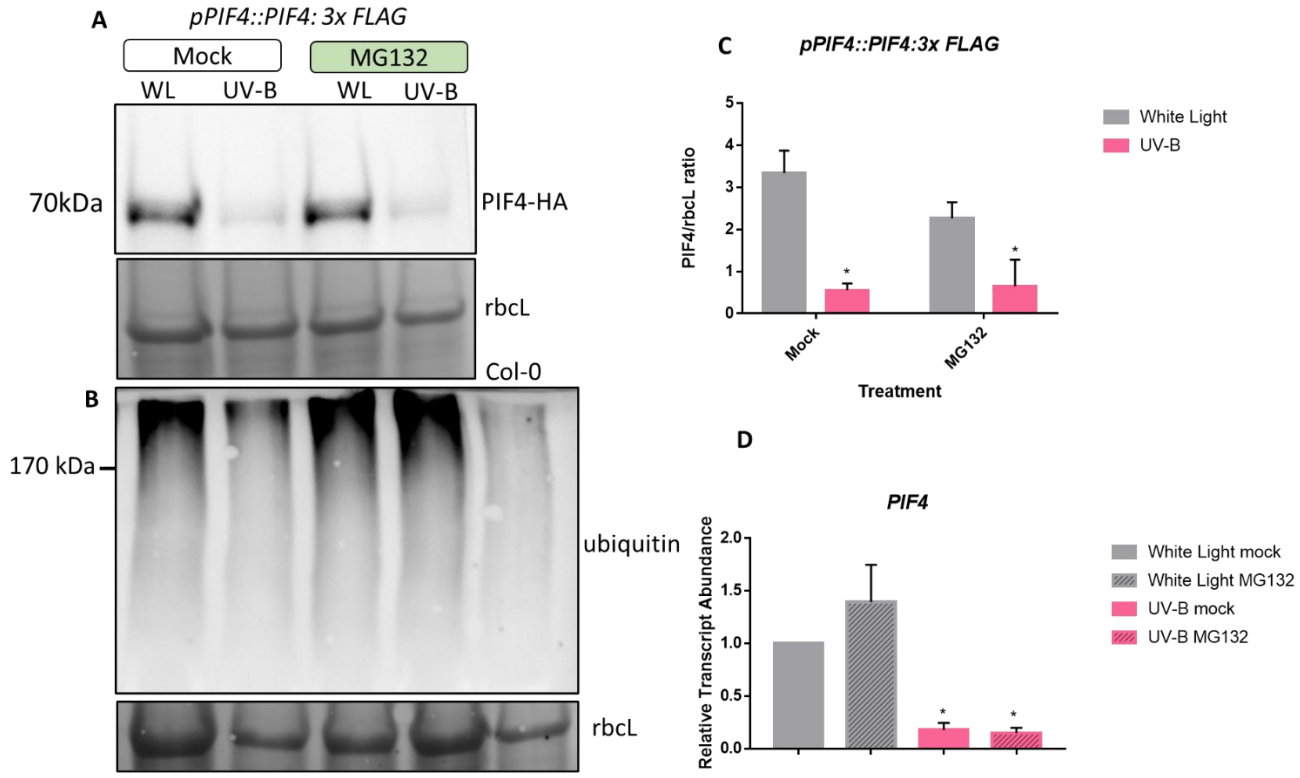


Figure 22: MG132 incubation does not inhibit UV-B-mediated PIF4 degradation in pPIF4::PIF4:3xFLAG seedlings. 9-day-old seedlings grown in long days (16 h light/ 8 h dark) at 20°C were transferred to 0.5 x MS liquid medium ± MG132 for 16 h. Plants were transferred at dawn to white light ± UV-B for 2 h. Col-0 was used as negative control. (A) Western blot of PIF4 in *pPIF4::PIF4:3x FLAG* plants. PIF4 was detected using an anti-HA antibody and ponceau straining of the Rubisco large subunit (rbcL) was used as loading control. (B) Representative Western blot with anti-ubiquitin and anti-mouse antibodies showing efficacy of proteosome inhibition ± MG132 in *pPIF4::PIF4:3x FLAG* plants in white light ± UV-B. Equal total protein extracts loaded per lane. (C) Quantification of PIF4/rbcL ratio of three independent biological replicates (n = 3) ±SE. Asterisks represent statistically significant means ($p < 0.05$, two-way ANOVA). (D) Relative *PIF4* transcript abundance in *pPIF4::PIF4:3x FLAG* plants in WL±UV-B±MG132. Data represent mean relative *PIF4* transcript abundance. Asterisks represent statistically significant means ($p < 0.05$, two-way ANOVA). Expression was determined by qRT-PCR and normalized to *ACTIN2*. Data are from three independent

biological replicates ±SE. “M” (mock): DMSO. “T” (treatment with MG132): 50 µM. “WT” (Wild type Col-0).

5.5 Discussion

This chapter presents data obtained from investigating a potential mechanism underlying UV-B-mediated suppression of *PIF4* transcript abundance. The investigation specifically focused on elucidating the role of PIF4 degradation via the 26S proteasome pathway, which could suppress *PIF4* transcription via suppression of an auto activation system. The kinetics of UV-B-mediated suppression of *PIF4* in chapter 3 (Figures 5B, 7B) showed strong suppression of transcript abundance between time points 2 h and 4 h after dawn. Coincidently, PIF4 levels exhibit a rapid decline in UV-B, showing a significant turnover as early as 2 h post-dawn (Hayes *et al*., 2014, 2017). Although earlier timepoints were not recorded in these studies, PIF5 protein levels have been observed to decrease rapidly, within 20 min of UV-B exposure, suggesting that protein turnover may precede transcript suppression (Sharma *et al.,* 2019). Recently, PIF4 has been shown to auto-activate its own transcription by directly binding to its own promoter (Huq & Quail, 2002; Zhai *et al*., 2020; Lee *et al*., 2021). The mechanism by which UV-B degrades PIF4 protein has not been characterized yet, but published work on UV-B turnover of PIF5 shows the involvement of the 26S proteasome (Sharma *et al*., 2019). Because PIF4 and PIF5 are homologous (Lorrain *et al*., 2008) and display similar degradation kinetics in UV-B, it was hypothesized that UV-B turnover of PIF4 is also mediated by the 26S proteasome system.

Consistent with data shown in chapters 3 and 4 *PIF4* displayed strong transcript suppression within 2 h UV-B exposure (Figure 19A). This response was still observed in MG132 treated plants, suggesting that UV-B-mediated degradation of PIF4 may either not involve the 26S proteasome or insufficient uptake of MG132 by the plant tissue. In contrast, *PIF5* transcript levels showed no UV-B-mediated suppression when

MG132 treated, despite large variation in the data (Figure 19B). MG132 impedes protein degradation by the 26S proteosome via inhibition of the 20S proteasome, the core of the proteolysis system (Lee *et al*., 1998). Proteins that are regulated via the proteasome system are still modified by phosphorylation and ubiquitination, but if the 20S proteasome is inhibited, their degradation through the 26S proteasome pathway is prevented. Therefore, when plants are treated with MG132, it is expected that they accumulate ubiquitinated proteins. Transgenic lines overexpressing PIF4-HA (*35S::PIF4-HA*) were treated as in Figure 19, then western blots were performed to assess the levels of ubiquitinated proteins in the plant tissue. Blots incubated with anti-ubi antibody showed an intense smear for MG132 treated plants, indicating high levels of ubiquitinated proteins (Figure 20). The effects of MG132 on PIF4 stability were assessed in parallel. Consistent with previous studies, PIF4 showed significant degradation in UV-B (Hayes *et al*., 2014, 2017; Tavridou *et al*., 2019). However, contrary to what has been reported for PIF5 (Sharma *et al*., 2019), MG132 incubation did not have any effect on the UV-B-mediated turnover of PIF4 (Figure 21). These data could suggest the existence of an alternative degradation pathway for PIF4 in UV-B conditions that is not affected by MG132. Additionally, MG132 may have a non-specific effect on other cellular processes that could indirectly affect PIF4 stability in UV-B. Although MG132 treatment has been used successfully to promote PIF4 stability via inhibition of the 26S proteosome in the absence of UV-B (de Lucas *et al*., 2008; Kudo *et al*., 2016; Li *et al*., 2016), additional approaches may be needed to prevent the UV-B-mediated degradation of PIF4. With more time, other types of proteasome inhibitors could have been tested. Bortezomib is another proteasome inhibitor previously used to stabilize PIF4 in Arabidopsis (Pham *et al*., 2018; Lee *et al*., 2021). Bortezomib is an irreversible inhibitor that selectively targets and specifically binds to the catalytic site of the 26S proteasome, inhibiting chymotrypsin-like activity. Other proteasome inhibitors used in Arabidopsis that could be tested for their ability to inhibit UV-B-mediated PIF4 degradation are MG115, lactacystin, and epoxomicin. Similar to MG132, MG115 belongs to the peptide aldehyde class of proteasome inhibitors and differs in its potency and specificity. While MG132 is

a potent inhibitor that irreversibly inhibits chymotrypsin-like activity of the 20S and 26S proteasome, MG115 is a reversible inhibitor that preferentially inhibits the chymotrypsin-like activity of the 20S proteasome but has less effect on the 26S proteasome. Lactacystin and epoxomicin are natural proteasome inhibitors that irreversibly bind to the active site of the 20S proteasome and inhibit its activity. Testing the effects of different proteasome inhibitors on PIF4 in UV-B would allow greater understanding of the nature of UV-B-mediated PIF4 turnover. Most recently, the UV-B-inducible proteins RUP1/RUP2 were shown to serve as a substrate receptor of CULLIN 4-DAMAGED DNA BINDINGPROTEIN 1 (CUL4-DDB1) E3 apparatus (Ren *et al*., 2019). The CUL4-DDB1-RUP1/RUP2 complex formation negatively regulates HYH levels in UV-B via ubiquitination/degradation through the proteasome pathway (Ren *et al*., 2019). It is possible that UV-B turnover of PIF4 may also be mediated via this pathway. Again, to explore this mechanism, optimization of proteasome inhibitor usage is needed. Alternatively, UV-B turnover of PIF4 could be achieved via lysosome/vacuole and autophagic degradation pathways independent of the proteosome system (Piper *et al*., 2007; Gao *et al*., 2015).

Finally, experiments were repeated in *pPIF4::PIF4:3xFLAG* lines to enable the effects of UV-B on PIF4 protein and transcript to be analysed simultaneously in the same plants. Consistent with data in section 5.3 and published data (Hayes *et al*., 2014), PIF4 protein levels were reduced in UV-B both in MG132 treated and mock treated plants. Although, qRT-PCR analyses showed strong UV-B-mediated suppression of *PIF4* transcript in mock and MG132 treated plants, the ineffectiveness of MG132 in inhibiting PIF4 protein degradation in UV-B means that the role of UV-B in regulating a PIF4 auto-activating system on transcriptional control cannot be evaluated. Our analyses of PIF5 provide circumstantial evidence that PIF5 may act to self-regulate its own transcription , although further validation is required to confirm this hypothesis. Future experiments could assess the ability of PIF5 to bind to its own promoter and activate transcription using Y1H, transactivation assays and ChIP qPCR.

Chapter 6

Investigating the role of MYB30 as an upstream regulator of *PIF* abundance and activity

6.1 Introduction

Inhibition of *PIF4* transcript accumulation is the main mechanism underlying the suppression of thermomorphogenesis in UV-B (Hayes *et al*., 2017). The mechanisms by which UV-B suppresses *PIF* transcript abundance are partially understood. UV-B-mediated suppression of *PIF4* and *5* transcript accumulation is dependent on UVR8 and COP1 (Hayes et al., 2014, 2017; Sharma *et al*., 2019; Figures 5, 7, 9), however intermediate players are yet to be characterized as COP1 is not a chromatin binder and the significance of UVR8-chromatin association remains uncertain (Cloix *et al*., 2008; Binkert *et al.* 2016).

Recent studies have reported MYB30 to act as a direct positive regulator of *PIF4* and *5* in prolonged R (Yan *et al*., 2020), consistent with *in silico* data reported in chapter 3 (Table 3 and Figure 4). MYB30 belongs to the MYB family of transcription factors, which are characterized by the conserved DNA-binding domain MYB (Ogata *et al*., 1996). The MYB domain is composed of one to four imperfect amino acid sequence repeats (R) of about 52 amino acids (Ogata *et al*., 1996). The MYB-family regulates plant development, metabolism, cell cycle and responses to abiotic and biotic stresses (Dubos *et al*., 2010). MYB30 is an R2R3-MYB, the largest family of MYB in plants, and the most well characterized (Rosinski *et al*., 1998; Jin *et al*., 1999). MYB30 has been reported to play role in programmed cell death related to hypersensitive responses to bacterial pathogens (Daniel *et al*., 1999; Vailleau *et al*., 2002; Raffaele *et al*., 2006, 2008). MYB30 is also involved in BR-regulated gene expression (Li *et al*., 2009; Dubos *et al*., 2010). Research has shown that MYB30 is induced and stabilized by phyA and phyB, and rapidly accumulates in prolonged R (Yan *et al*., 2020). Conversely, MYB30 is degraded in the dark, but MG132 treatment inhibits its dark degradation, suggesting that MYB30 is degraded via the 26S proteasome (Yan *et al*., 2020). MYB30 promotes PIF4 and 5 protein accumulation by direct binding to their promoters to induce transcription in prolonged R, and by sequestering phyB to prevent phyB-PIF binding (Yan *et al*., 2020). The PIF4/5 accumulation mediated by MYB30 in prolonged R is thought to prevent exaggerated photomorphogenic responses (Yan *et al*., 2020).

Since MYB30 levels are light regulated and the MYB30 protein has been shown to directly regulate *PIF4* and *5* transcript abundance, this chapter tested whether MYB30 is involved in UV-B-mediated transcript suppression of *PIF4* and *PIF5* via qPCR assays. MYB30 effects on developmental responses orchestrated by PIFs were also investigated using null mutant line for *MYB30*.

6.2 *MYB30* transcript abundance is regulated by UV-B

6.2.1 UV-B-mediated suppression of *MYB30* transcript abundance requires UVR8

To test whether *MYB30* transcript abundance is UV-B regulated, qRT-PCR assays were performed to assess variations in transcript abundance between two light treatments: white light and white light supplemented with low doses of UV-B. Wildtype Col-0 and *uvr8-6* were grown on soil for 10 days in white light at 20°C in long days (16 h light /8 h dark) then transferred to either white light or white light supplemented with UV-B (1.0 µmol m^{-2} s^{-1}) at 20°C for four hours at ZT0.

MYB30 transcript abundance was significantly reduced in UV-B (Figure 23). This response was abolished in the *uvr8-6* genotype (Figure 23). These results suggest that *MYB30* transcript is suppressed by UV-B in a response mediated by UVR8. Low levels of MYB30 in UV-B could therefore contribute to UV-B-mediated suppression of *PIF4* and *PIF5* transcript and consequently the low protein levels in these conditions.

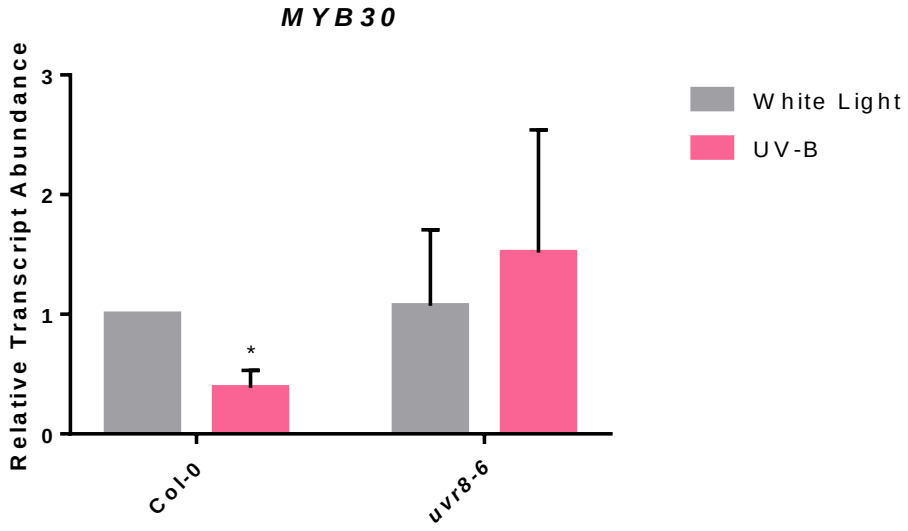


Figure 23: UV-B-mediated suppression of *MYB30* transcript abundance requires UVR8. Col-0 and *uvr8-6* seedling were grown on soil at 20°C for 10 days, then transferred to white light ±UV-B at dawn for 4 h. Data represent mean relative *MYB30* transcript abundance. N = 3 ± SE. Asterisks represent statistically

significant means ($p < 0.05$, t-test). Expression was determined by qRT-PCR and normalized to ACTIN2. Data are from three independent biological replicates.

6.2.2 UV-B rapidly suppresses *MYB30* transcript abundance

To investigate whether UV-B-mediated suppression of *MYB30* transcript abundance could be contributing to the UV-B-mediated suppression of *PIF4/5* transcripts, the kinetics of *MYB30* suppression in UV-B were analysed to compare with those of *PIF4/5*. Fast UV-B-mediated suppression of *MYB30* transcript levels would suggest transcription factor depletion for *PIF4/5* transcriptional activation, resulting in the observed *PIF4/5* transcript reduction within 2 h (chapters 3 and 4). To investigate this hypothesis, time course qRT-PCR assays were conducted to characterize the kinetics of the UV-B-mediated downregulation of *MYB30* transcript. Col-0 plants were grown following the same experimental design as in 6.2.1, but samples were harvested at 6 different time points after dawn (0 h, 0.5 h, 1 h, 1.5 h, 2 h, 2.5 h and 4 h).

Interestingly, *MYB30* displayed rapid UV-B-mediated suppression of transcript accumulation at 0.5 h of treatment, although statistically significant differences were not observed until 4 h (Figure 24). Data from chapter 3 show UV-B-mediated suppression of *PIF4/5* transcript abundance to occur at 2 h (Figures 5B,C, 7B, C). As UV-B-mediated suppression of *MYB30* transcript occurs prior to UV-B suppression of *PIF4/5* transcript, it is possible that the earlier reduction of *MYB30* transcript could result in lower *PIF4/5* transcriptional activation.

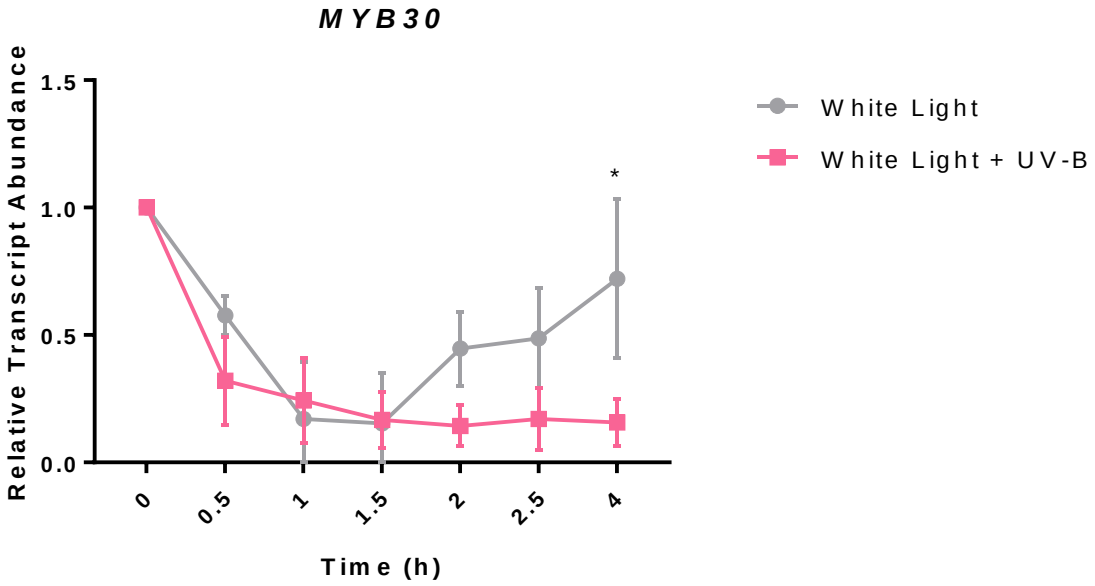


Figure 24: UV-B suppresses *MYB30* transcript abundance within 0.5 h treatment. Relative transcript abundance of *MYB30* in WT (Col-0). Seedlings were grown on soil at 20°C for 10 days, then transferred to white light ± UV-B at ZT0 and sampled at time points 0 h, 0.5 h, 1 h, 1.5 h, 2 h, 2.5 h and 4 h. Transcript abundance was determined by qRT-PCR and normalized to *ACTIN2*. N = 3 ± SE. Asterisks represent statistically significant means ($p < 0.05$, two-way ANOVA and Tukey's post hoc). Data are from three independent experiments.

6.3 Characterization of MYB30 as a transcriptional regulator of *PIF*s in different light conditions

6.3.1 MYB30 suppresses *PIF4* and *5* transcript abundance in WL

To investigate the role of MYB30 as an upstream regulator of *PIFs*, qRT-PCR assays were performed using the mutant line *myb30-1*/Col-0. *myb30-1* is a knockout allelic mutant which contains a T-DNA insertion in the beginning of the third exon of *MYB30*, resulting in abolishment of MYB30 (Zheng *et al.*, 2012). The mutant *myb30-1* obtained from the *Arabidopsis* Biological Resources Center (ABRC) was genotyped for

the presence of the exogenous T-DNA within *MYB30*, and plants homozygous for the transgene were selected and used for qRT-PCR experiments (Figure 25).

Time course qRT-PCR assays were performed following the same experimental design as described in 6.2 and 6.3, using Col-0 and *myb30-1* genotypes and sampling plant tissue at six different time points post dawn (0 h, 0.5 h, 1 h, 1.5 h, 2 h, 2.5 h and 4 h). Consistent with results reported in Chapter 3 (Figure 5B, 7B), UV-B-mediated suppression of *PIF4* transcript is observed between time points 2 h and 4 h in Col-0 (Figure 26A). UV-B-mediated suppression of *PIF5* transcript was observed slightly earlier than for *PIF4*, at time point 1.5 h in Col-0 (Figure 26C). *Myb30-1* plants showed very similar UV-B-mediated suppression of *PIF4/5* transcript abundance to Col-0, yet displayed increased transcript levels in WL, especially for *PIF4*. However, because the genotypes were harvested on different days, the comparison among transcript abundance between genotypes cannot be fully addressed. It is possible that MYB30 positively regulates a negative regulator for *PIF4*. Alternatively, MYB30 could be acting as a direct suppressor of *PIF4* transcription under these experimental conditions.

Collectively, these data suggest that MYB30 does not perform a significant role in the UV-B-mediated suppression of *PIF4/5* transcript abundance. Nevertheless, these data suggest a new mechanism for transcriptional regulation of *PIF4/5*, highlighting MYB30 as a possible negative regulator of *PIF4/5* transcript abundance in WL.

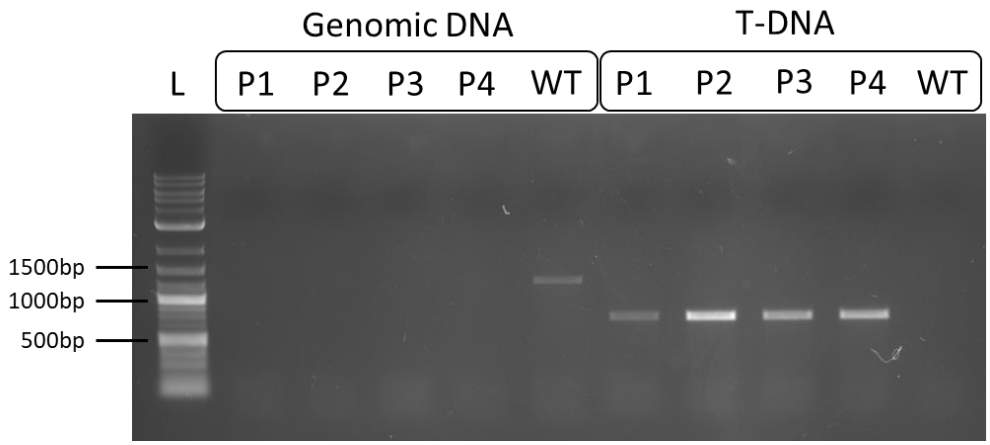


Figure 25: : Genotyping of the *myb30-1 SALK* line showed all seed stocks to be homozygous. Specific T-DNA primers were used to detect the presence of the T-DNA transgene by gDNA PCR (T-DNA). Expected amplicon size for the T-DNA is 465-765 bp. Amplification of *MYB30* (1096 bp amplicon) indicates the absence of the T-DNA insertion (Genomic DNA). WT = genomic DNA of Arabidopsis Col-0. "P1 - P4" (Mutant plants 1 - 4). L = Gene ruler (1 kb Plus Ladder). bp= Base pairs.

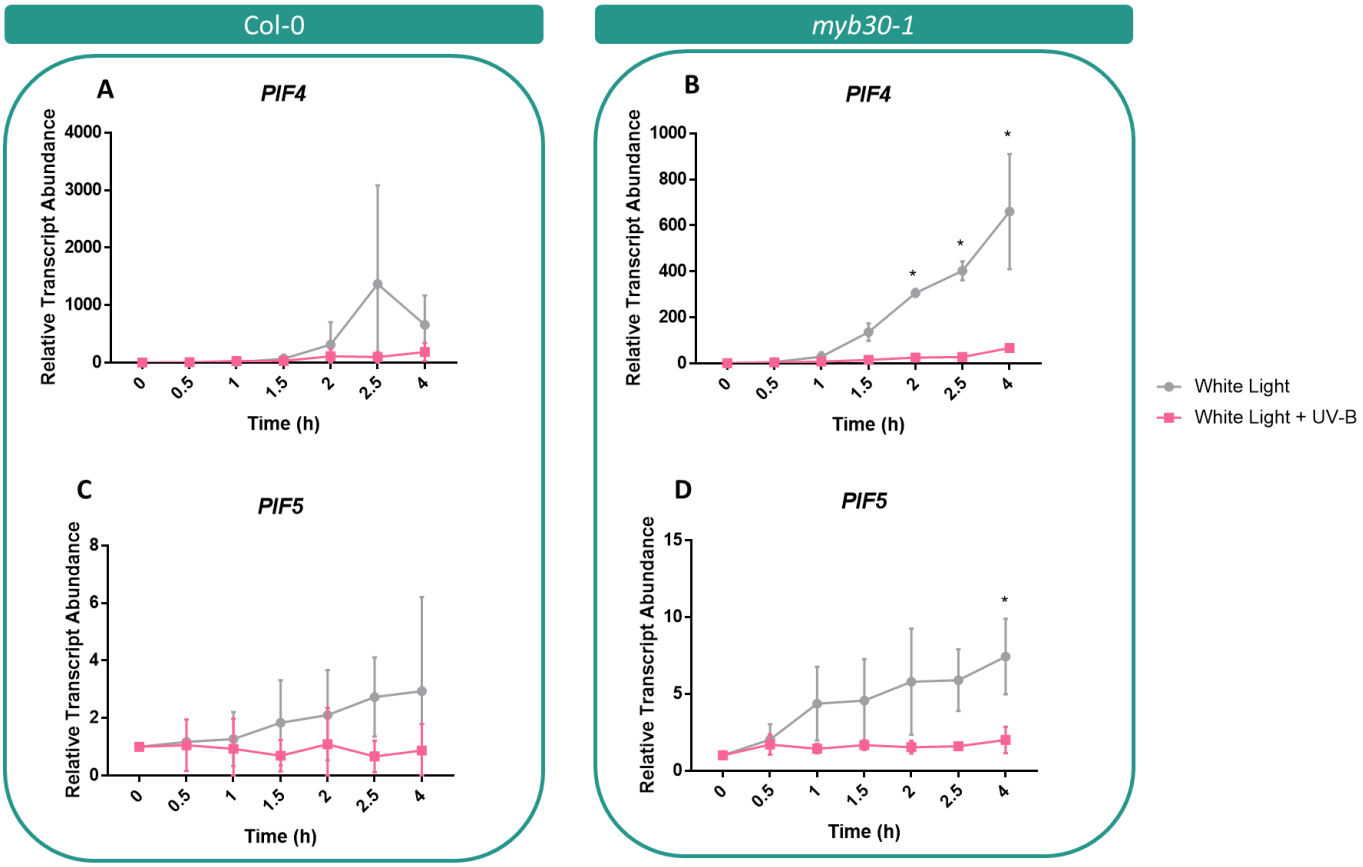


Figure 26: MYB30 may suppress *PIF4* and *PIF5* transcript abundance in WL. Relative transcript abundance of *PIF4* and *PIF5* in WT (Col-0; A and C) and *myb30-1* (B and D). Seedlings were grown on soil at 20°C for 10 days, then transferred to white light ±UV-B for 4 hours at ZT0. Plants were sampled at time points 0 h, 0.5 h, 1 h, 1.5 h, 2 h, 2.5 h and 4 h. Transcript abundance was determined by qRT-PCR and normalized to *ACTIN2*. N = 3 ±SE. Asterisks represent statistically significant means ($p < 0.05$, two-way ANOVA and Tukey's post hoc). Data are from three independent experiments.

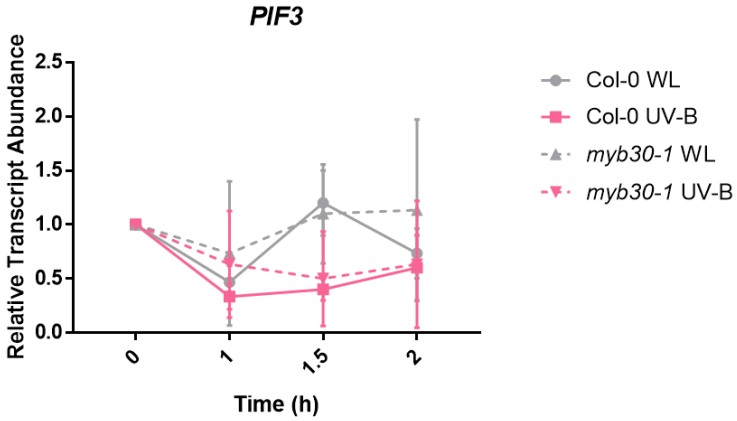
6.3.2 MYB30 positively regulates *PIF7* transcript abundance in WL

Since MYB30 showed an inhibitory effect on *PIF4/5* transcript abundance in WL and cis elements for MYB and MYB-related transcription factor were found within the *PIF3* and *7* promoters (Table 3 and Figure 4), qPCR assays to assess effects of MYB30 on *PIF3* and *7* transcript abundance in WL ± UV-B were conducted.

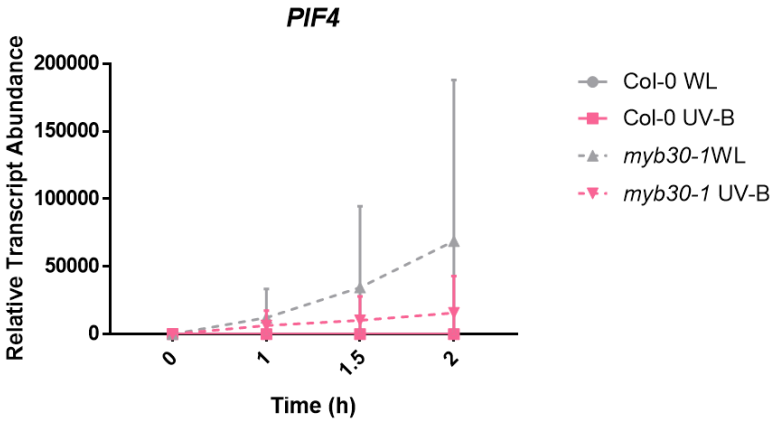
PIF4 and *PIF5* were additionally quantified to further investigate results obtained in Figure 23, with both genotypes grown in parallel. The assays were performed following the same experimental design as in section 6.3.1 and sampled at timepoints 0 h, 1 h, 1.5 h, and 2 h. Harvesting time points were selected according to the most significant changes in *PIF* transcript abundance within light treatments in *myb30-1* plants (Figure 26B, D).

Consistent with Chapter 3 (Figure 5A), *PIF3* transcript abundance was suppressed by UV-B treatment (Figure 27A). *PIF3* transcript abundance was similar to *myb30-1* plants in both light treatments, in accordance with published data (Yan *et al*., 2020). Consistent with reported data in this thesis and the literature, *PIF4/5* transcript abundance was suppressed in UV-B in a MYB30-independent manner (Figure 5B,C, 7B,C, 26A, C; Hayes *et al*., 2014, 2017; Sharma *et a*l., 2019). Again, *myb30-1* plants displayed increased *PIF4/5* transcript abundance in WL but variation between biological repeats was large (Figure 24B, C). Surprisingly and in contrast to results in Figure 5D, *PIF7* transcript displayed UV-B-mediated suppression in Col-0 (Figure 27D). This discrepancy can potentially be explained by reduced variation between experimental repeats in these experiments. As with *PIF3/4*/*5*, *PIF7* transcript abundance was similarly supressed by UV-B in Col-0 and *myb30-1* plants. However, *PIF7* transcript abundance was strongly reduced in WL in *myb30-1* plants. These data therefore suggest that MYB30 acts as a positive transcriptional regulator of *PIF7* in WL.

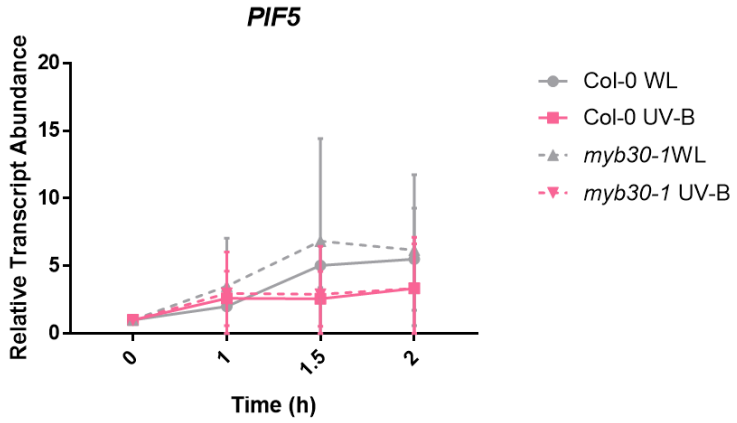
A


PIF3
Relative Transcript Abundance
2.5
2.0
1.5
1.0
0.5
0.0
0
1
1.5
2
Time (h)
Col-0 WL
Col-0 UV-B
myb30-1 WL
myb30-1 UV-B

B


PIF4
Relative Transcript Abundance
200000
150000
100000
50000
0
0
1
1.5
2
Time (h)
Col-0 WL
Col-0 UV-B
myb30-1WL
myb30-1 UV-B

C


PIF5
Relative Transcript Abundance
20
15
10
5
0
0
1
1.5
2
Time (h)
Col-0 WL
Col-0 UV-B
myb30-1WL
myb30-1 UV-B

D

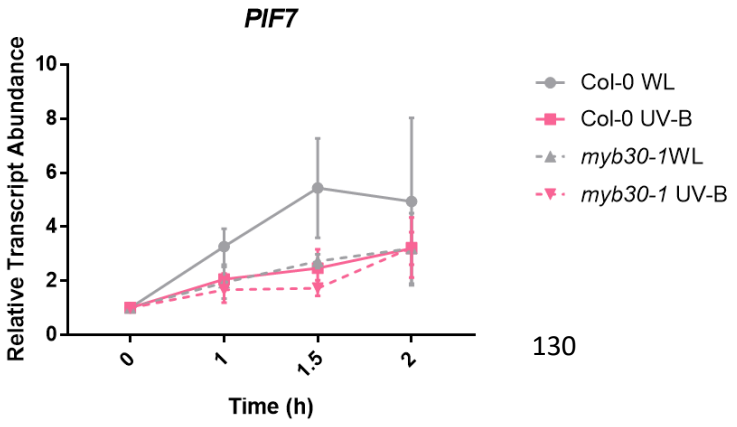

PIF7
Relative Transcript Abundance
10
8
6
4
2
0
0
1
1.5
2
Time (h)
Col-0 WL
Col-0 UV-B
myb30-1WL
myb30-1 UV-B

Figure 27: MYB30 promotes *PIF7* transcript abundance in WL. Relative transcript abundance of (A) *PIF3,* (B) *PIF4*, (C) *PIF5* and (D) *PIF7* in Col-0 and *myb30-1* genotypes. Seedlings were grown on soil at 20°C for 10 days, then transferred to white light ± UV-B for 2 h at ZT0. Plants were sampled at time points 0 h, 0.5 h, 1 h, 1.5 h, 2 h. Expression was determined by RT-qPCR and normalized to *ACTIN2*. N = 3 ± SE. ($p < 0.05$, two-way ANOVA and Tukey's post hoc). Data are from three independent experiments.

6.4 The role of MYB30 in shade and high temperature responses

Plant organs will alter their size according to the external cues they are exposed to. In low R/FR ratio (shade) and increased temperatures, hypocotyls elongate to outcompete neighbours for light or facilitate cooling, respectively (Franklin & Whitelam 2005, Franklin *et al*., 2008; Hwang *et al*., 2017; Ibañez *et al*., 2017; Martinez-Garcia *et al*. 2010; Lau *et al*., 2018). These responses are termed shade-avoidance and thermomorphogenesis respectively. Both responses are regulated by PIF4- and PIF7-mediated promotion of auxin biosynthesis. Shade-avoidance responses are predominantly regulated by PIF7, whereas PIF4 is the main regulator of thermomorphogenesis (Lorrain *et al*., 2008; Koini *et al*., 2009; Franklin *et al*., 2011; Nozue *et al*., 2011; Hornitscheck *et al*., 2012; Li *et al*., 2012; Nomoto *et al*., 2012; Yamashino *et al*., 2013; Fiorucci *et al*. 2021). More recently, research has been conducted in plants experiencing both shade and warm temperatures in combination (Burko *et al*., 2022). Results have shown synergistic hypocotyl elongation in shade and high temperatures combined when compared to these treatments alone (Burko *et al*., 2022). PIF7 was identified as the master regulator of thermomorphogenesis in the shade (Burko *et al*., 2022). Active (de-phosphorylated) PIF7 accumulates in the nucleus in response to shade, and in response to high temperatures due to increased translation via alternative harpin formation in its mRNA 5'UTR region (Nagatani *et al*., 1991; Somers *et al*., 1991; Yanovski *et al*., 1995; Leivar *et al*., 2008; Li *et al*., 2012; Huang *et al*., 2018; Chung *et al*., 2020). The mechanisms underlying *PIF7* transcriptional regulation,

however, remain unknown. Results in section 6.3.2 (Figure 24D) indicate a novel MYB30-mediated regulatory mechanism controlling *PIF7* transcript accumulation in WL. To assess the physiological consequences of MYB30-mediated *PIF7* regulation, hypocotyl elongation assays were performed comparing Col-0 and *myb30-1* plants in environments where PIF7 performs a key regulatory role.

6.4.1 *Myb30-1* plants display short hypocotyls but still respond to low R:FR and high temperature

Col-0 and *myb30-1* were grown on soil for 3 days in white light (R/FR = 6.7) at 20°C then transferred to high (6.7), or low (0.07) R/FR at 20°C or 28°C for four days in long day photoperiods (16 h light/8 h dark). Hypocotyls were photographed then measured with ImageJ. One-way ANOVA tests were performed to detect significant differences between treatments.

Consistent with the literature, Col-0 plants displayed elongated hypocotyls in both low R/FR ratio and high temperature conditions (Figure 25; Leivar *et al*., 2008; Koini *et al*., 2009; Franklin *et al*., 2011; Lee *et al*., 2014). However, the combined treatment of low R/FR and high temperature did not result in the synergistic phenotype reported in Burko *et al.* (2022). *myb30-1* plants showed short hypocotyls consistent with Yan *et al.* (2020) but displayed similar responses to low R/FR and high temperature (Figure 28). *myb30-1* plants displayed shorter hypocotyls compared to Col-0 among all treatments (Figure 28). This contrasts with the elevated levels of *PIF4* and *PIF5* observed in this line (Figure 26). Because hypocotyl elongation in low R/FR was subtle compared to the literature (Cole *et al*., 2010; Burko *et al*., 2022), growth conditions were optimized to promote more prominent hypocotyl elongation phenotype.

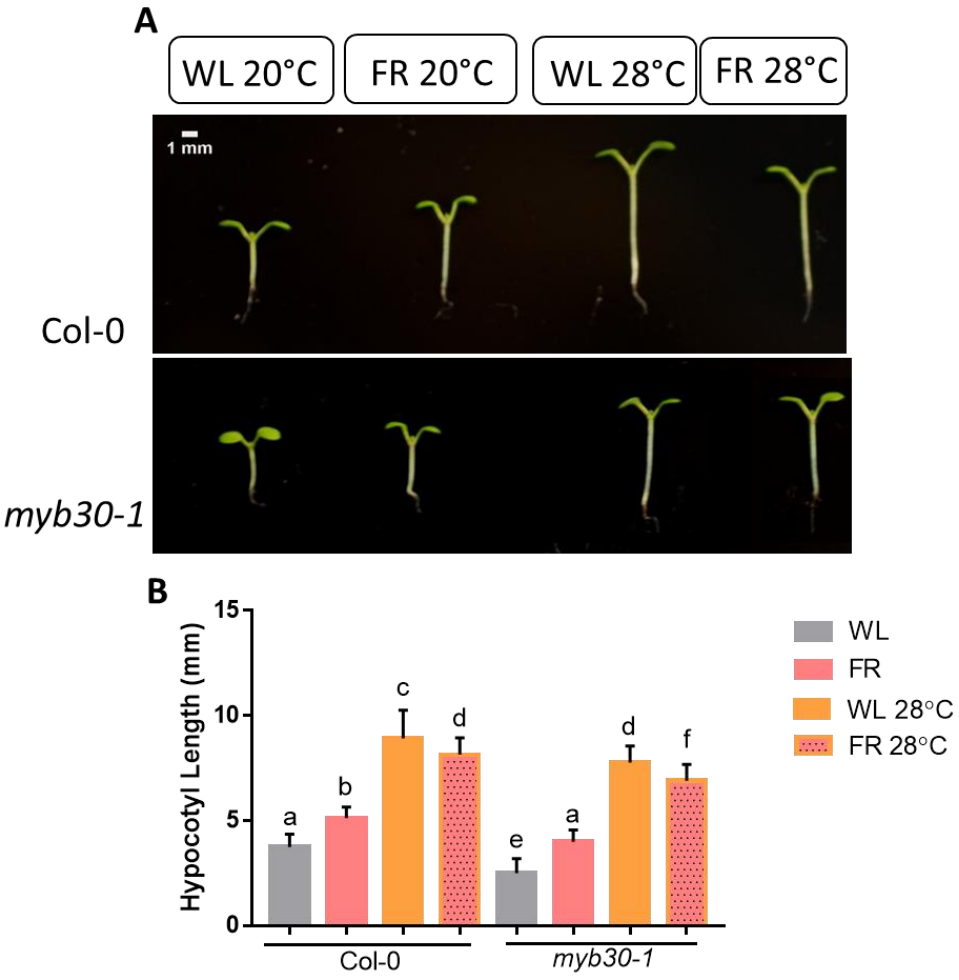
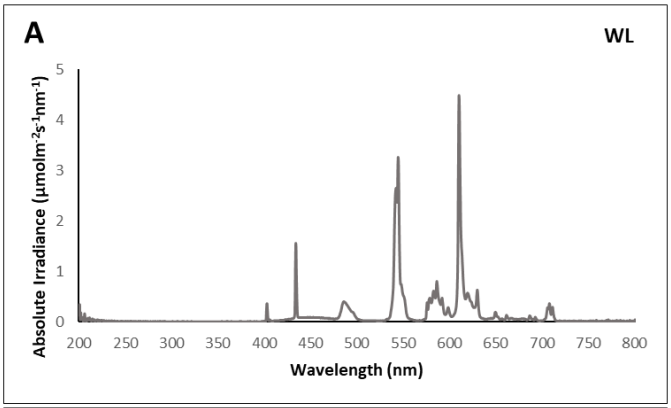


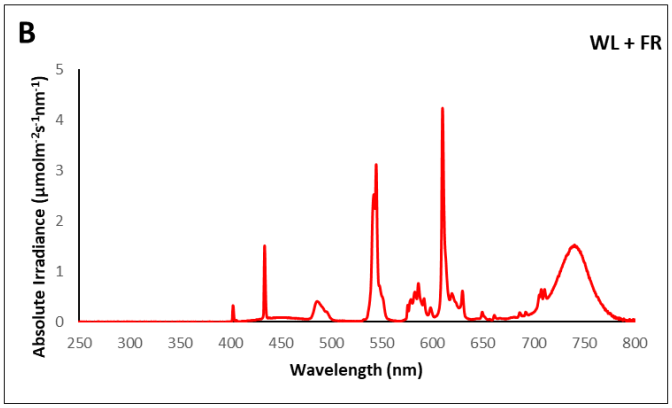
Figure 28: Hypocotyl elongation responses to low R/FR and high temperature are unaffected in *myb30-1* plants. (A – B) Seedlings were grown for 3 days in long-days (16 h light/ 8 h dark) at 20°C in WL (R/FR = 6.7), then transferred to high (6.7) and low (0.07) R/FR at 20°C and 28°C for 4 days. (B) Data represent mean hypocotyl length (n ≥ 20) ±SE. Letters (a – f) represent statistically significant means ($p < 0.05$, one-way ANOVA and Tukey's post hoc). WL = white light. FR = low R/FR.

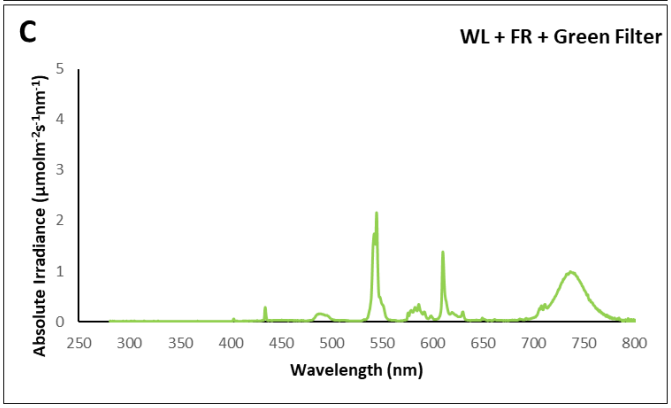
6.4.2 MYB30 promotes hypocotyl elongation responses to prolonged green shade

Since PIF7 is a master regulator of shade avoidance responses (Li *et al*., 2012) and MYB30 regulates *PIF7* abundance (Figure 27), the role of MYB30 in shade avoidance was further investigated in conditions producing a more exaggerated shade avoidance phenotype. Seeds were grown on soil in constant white light (as in Burko *et al*., 2022) at PAR 75.8 µmol $m^{-2}s^{-1}$ and 20°C for three days then transferred to continuous high (6.7) R/FR and low (0.07) R/FR ± green filter (0.03 R/FR, PAR 31.9 µmol $m^{-2}s^{-1}$) for four days. Green filter blocks red and blue light, lowering both PAR and R/FR (Figure 29). Hypocotyls were photographed then measured with ImageJ. One-way ANOVA tests were performed to detect significant differences between treatments.

Col-0 plants displayed a stronger hypocotyl elongation response when treated with low R/FR under green filter (Figure 30) than low R/FR alone, consistent with the lower PAR and R/FR in this condition (Hersch *et al*. 2014). In contrast to previous experiments, *myb30-1* mutants did not display short hypocotyls in continuous white light which may reflect the already short hypocotyls in these conditions. Consistent with previous data (Figure 28), *myb30-1* plants displayed the same magnitude of hypocotyl elongation in low R/FR as Col-0 (Figure 30). This could be due to higher levels of PIF4 compensating for reduced levels of PIF7. Interestingly, *myb30-1* plants showed an impaired shade-avoidance response under the green filter. This result could represent the very low R/FR in this condition or the combination of low R/FR and low PAR in green shade. PIF4 may be insufficient to compensate reduced levels of PIF7 in this condition, resulting in the partial elongation phenotype. Collectively, these results suggest that MYB30 may perform a role in plant responses to canopy shade through positive regulation of *PIF7* transcript abundance.







D

Light treatment	PAR ($\mu molm^{-2}s^{-1}$)	R/FR ratio
WL	75.8	6.7
WL + FR	73.1	0.07
WL + FR + G	31.9	0.03

Figure 29: Light spectra from experimental conditions recorded in growth cabinets. 70 µmol $m^{-2}s^{-1}$ white light supplied with fluorescent bulbs without supplemental FR LEDs (A) and supplemented with FR LEDs at a R/FR of 0.07 (B) and supplemented with FR LEDs and covered with a green filter (0.03 R/FR) (C). (D) PAR and R/FR ratio values for each light condition. WL = white light. FR = low R/FR. G = green filter.

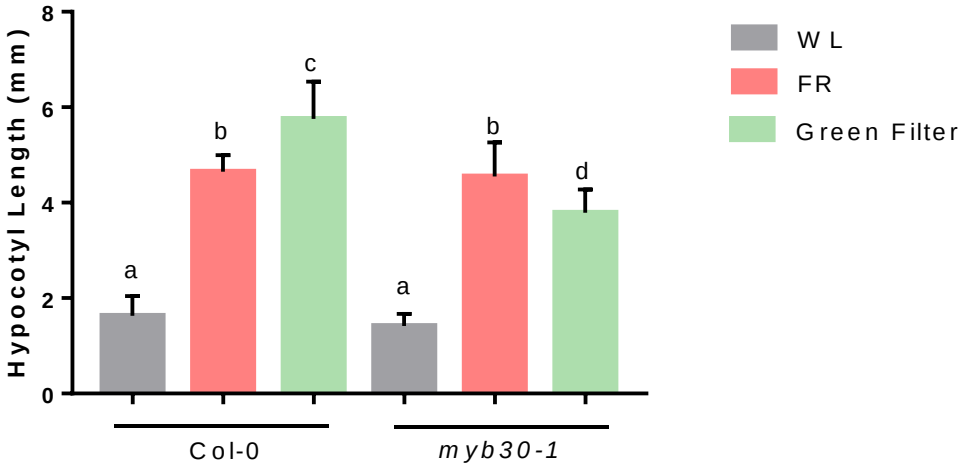


Figure 30: *myb30-1* shows impaired shade avoidance responses to green shade. Seedlings were grown on soil for 3 days at 20°C in continuous white light at PAR 75.8 µmol$m^{-2}s^{-1}$, then transferred to continuous high (6.7) R/FR (PAR 75.8) and low (0.07) R/FR (PAR 76.8) ± green filter (R/FR = 0.03, PAR 31.9 µmol$m^{-2}s^{-1}$). Data represent mean hypocotyl lengths (n ≥ 20) ±SE. Letters (a – d) represent statistically significant means ($p < 0.05$, one-way ANOVA and Tukey's post hoc). WL = white light. FR = low R/FR. G = Green filter.

6.5 Discussion

This chapter investigated the possible role of MYB30 as a regulator of *PIF* transcript abundance in different environmental conditions. First, the role of MYB30 in mediating the UV-B suppression of *PIF4* and *PIF5* transcript abundance was investigated. Next, the role of MYB30 in the regulation of other *PIF* transcript abundances was tested under WL±UV-B. Finally, *myb30-1* plants were analysed in conditions where PIF7 drives developmental responses.

Section 6.2 focused on investigating the role of MYB30 as a possible regulator of UV-B-*PIF* signalling, since MYB30 was recently reported to directly upregulate *PIF4* and *5* transcription under prolonged R light (Yan *et al*., 2020). qPCR analyses in section 6.2.1 showed that *MYB30* transcript abundance is suppressed in

UV-B in a UVR8- dependent manner (Figure 23). These results provided circumstantial evidence that *MYB30* transcript abundance could be involved in the UVR8-mediated suppression of *PIF4/5* transcripts. To investigate this mechanism, the kinetics of UV-B-mediated *MYB30* and *PIF4/5* transcript suppression were assessed by qPCR. Interestingly, *MYB30* showed rapid transcript suppression within 30 minutes of UV-B exposure, 1.5 hours earlier than *PIF4/5* (Figure 5B,C). However, the possibility of MYB30 regulating UV-B-mediated *PIF4/5* suppression was excluded since *myb30-1* plants displayed similar responses to Col-0 (Figure 26B,D, 27B,C). Nevertheless, *myb30-1* plants displayed strong enhancement of *PIF4* (and to a lesser extent, *PIF5*) transcript abundance in WL (Figures 26, 27) suggesting that MYB30 represses *PIF4/5*. This is in contrast Yan *et al* (2020), where MYB30 has been observed to act oppositely in both WL and R. This antagonistic result could be due to differences between experimental conditions. Plants in this study were soil grown in WL for 10 days in 8 h D/ 16 h L then transferred to respective light treatments before harvesting for qPCR. In Yang *et al.* (2020), plants were grown in the dark for 4 days then treated in different light conditions for 1 h. As such, the seedlings were undergoing de-etiolation and were not light acclimated. Additionally, experimental WL used in this chapter was of > 70 µmol $m^{-2}s^{-1}$, while Yang (2020) applied nearly 2-fold lower WL irradiance (40 µmol $m^{-2}s^{-1}$). Differences in the developmental stage of plants, as well as growth conditions could significantly affect transcriptional responses, especially as *MYB30* and *PIF4/5* are genes involved in the regulation of development. Since seedlings in Yang *et al.* (2020) were dark grown experiencing light only for 1 h during treatment, regulatory factors that potentially downregulate *MYB30* and/or *PIF4/5* in light conditions could have been masked. The low light irradiance used in Yang *et al*. (2020) may also have masked regulatory factors that require higher irradiance to be expressed.

In section 6.3.2, qPCR assays were performed using Col-0 and *myb30-1* plants to investigate whether MYB30 could also regulate the transcript abundance of other *PIFs* described in Chapter 3 (Table 3 and Figure 4). In contrast to *PIF4* and *5*, *PIF3* did not show any transcript variation in *myb30-1* plants in any

light condition, suggesting that *PIF3* transcript is not regulated by MYB30 (Figure 27A). Interestingly, *PIF7* displayed a strong transcript suppression under WL in *myb30-1* plants. This suggests that MYB30 acts as a positive regulator of *PIF7* transcript abundance in WL, providing novel regulatory mechanism controlling *PIF7* abundance. To test the role of MYB30 on PIF7 activity, hypocotyl elongation was compared in Col-0 and *myb30-1* in conditions where PIF7 activity has been demonstrated. Shade avoidance is mainly regulated by PIF7, with additional roles for PIF4 and PIF5 (Lorrain *et al*., 2008; Li *et al*., 2012), whereas thermomorphogenesis is mainly regulated by PIF4 with some role of PIF7 (Koini *et al*., 2009; Franklin *et al*., 2011; Nomoto *et al*., 2012; Yamashino *et al*., 2013; Fiorucci *et al*. 2021). Because recent research showed that PIF7 promotes over elongated hypocotyls when shade-avoidance and thermomorphogenesis signals are combined, this treatment was also investigated (Burko *et al*., 2022). In section 6.4.1, *myb30-1* plants showed the same elongation pattern as Col-0 in all treatments. This observation could be explained by elevated levels of PIF4 compensating for low levels of PIF7. However, due to the weak shade-avoidance response observed in WT plants, experimental conditions were optimised to promote low R/FR-induced hypocotyl elongation. Firstly, long day photoperiods were changed to continuous light, as in the methods of Burko *et al.* (2022). Secondly, a green filter treatment was also added to further decrease the R/FR ratio and reduce PAR. Continuous shade caused a strong hypocotyl elongation response in Col-0 (Figure 30). In continuous low R/FR, Col-0 plants displayed two-fold longer hypocotyls then in WL (Figure 30). When treated under the green filter, hypocotyls were longer than low R/FR treatment alone, consistent with the lower R/FR and low PAR in these conditions (Cole *et al*., 2010; Hersch *et al*. 2014). *myb30-1* plants behaved similarly to Col-0 in low R/FR, which could be also explained by PIF4 compensatory role (Figure 30). However, *myb30-1* plants grown under the green filter displayed impaired hypocotyl elongation when compared to Col-0. These plants displayed longer hypocotyls than WL-grown plants, but shorter hypocotyls than low R/FR-grown plants. These results suggest that MYB30 may induce *PIF7* transcript abundance in green 'true' shade to upregulate auxin biosynthesis genes (Li *et al*. 2012). The partially

elongated hypocotyl in green shade could result from residual levels of PIF7, together with PIF5 and elevated levels of PIF4.

With more time, it would be interesting to test the effect of combined continuous green shade and increased temperature on hypocotyl elongation in *myb30-1* plants. Further work could include crossing *myb30-1* with *pif4*, *pif7* and *pif4/7* to exclude redundancy and investigate the effects of MYB30 on PIF abundance and activity in different environmental conditions. Measuring PIF7 levels in *myb30-1* would additionally correlate the transcriptional regulation of *PIF7* by MYB30 with protein levels.

The research presented in this chapter initially identified downregulation of *MYB30* transcript in UV-B, mediated by UVR8. This was rapidly induced within 30 minutes of treatment, preceding UVR8-mediated suppression of *PIF4* and *PIF5* transcript abundance. Despite these temporal kinetics and the ability of MYB30 to directly promote *PIF4* and *5* transcript accumulation in R, MYB30 did not appear to be involved in UV-B-mediated suppression of *PIF4/5* transcript abundance. Instead, MYB30 was shown to be a *PIF4/5* transcript suppressor in WL. One of the key findings from this chapter is that MYB30 also upregulates *PIF7* transcript abundance, which coincides with an impairment of hypocotyl elongation of *myb30-1* under continuous green shade (Figure 30). *In silico* analysis in chapter 3 (Table 3 and Figure 4) support the possibility that MYB30 could be directly binding to the promoter of *PIF7* to regulate transcription. Further work is required to elucidate the mechanisms by which MYB30 regulates PIF7 transcript abundance and the physiological significance of this response.

Chapter 7

General Discussion

Plants as sessile autotrophic organisms have developed internal signalling mechanisms to cope with the daily changes in their environment. Light and temperature are pivotal cues sensed by plants to predict environmental stress and promote the initiation of appropriate developmental responses. Some of these responses include the regulation of stem elongation and petiole length. These are adaptative responses which act, in part, to optimize light photon capture to improve plant fitness. Changes in light quality and temperature are sensed by photoreceptors that communicate external signals to the plant cell via PIFs. PIFs are a major light signalling hub in Arabidopsis, whose activity and regulation have been extensively investigated to explain developmental responses in different environmental conditions. Most recently, PIFs 4 and 5 were shown to be regulated by the UV-B photoreceptor, UVR8. UVR8 is activated upon UV-B exposure and induces gene reprogramming to trigger a wide variety of protective and developmental responses, including suppression of stem and petiole elongation. These responses are suggested to be an adaptive mechanism to stop plants from lodging due to over elongation caused by shade and high temperature. Recent research shown that these UV-B-mediated developmental responses are regulated by UVR8 through suppression and/or degradation of PIF4 and PIF5 (Hayes *et al*., 2014, 2017; Sharma *et al*., 2019). Collectively, these mechanisms stop auxin biosynthesis and limit stem elongation.

The research in this thesis was focused on characterizing the transcriptional regulation of PIFs, focusing on UV-B treatments and investigating four potential transcriptional regulatory mechanisms. Understanding the mechanisms that regulate *PIF* transcription is key to fully characterizing the full range of developmental responses orchestrated by this group of transcription factors. Additionally, these finding could be translated into commercial crops grown in controlled environments, where light regimes could be modulated to improve crop yield.

PIF promoter characterization

Preliminary in silico analyses were performed to predict cis elements in the promoters of *PIF 3/4/5/7* to identify transcription factors that possibly directly regulate *PIF* transcription in response to UV-B. The regulatory mechanisms tested in this thesis were chosen based on the enrichment of cis elements on each promoter and their likely involvement in the UV-B signalling pathway (Table 3, Figure 4).

UV-B-mediated regulation of *PIF* transcription

Chapter 3 investigates UV-B-mediated transcriptional regulation of *PIF*s and supports published findings that applied low dose UV-B suppresses *PIF 4/5* transcript abundance in a UVR8- dependent manner (Hayes *et al*., 2014, 2017). It additionally provides evidence that *PIF3* transcript abundance is suppressed in UV-B via UVR8 (Figure 5A), in contrast to PIF7, whose transcript did not respond to UV-B in these experiments (section 3.3.1). This result is consistent with published research showing that *PIF7* is predominantly post transcriptionally regulated via phosphorylation of PIF7 protein and modification of RNA structure (Li *et al.,* 2012; Chung *et al.*, 2020). The chapter shows that UV-B-mediated suppression of *PIF4/5* transcript abundance occurs at the level of promoter activity through experiments with *PIFpromoter::GUS*

transgenic lines. These experiments suggest an alternative regulatory mechanism controlling UV-B-mediated suppression of *PIF3* transcript, as it is not controlled at the level of promoter activity (Figure 7C).

Since the role of UVR8 chromatin-binding remains unclear (Cloix *et al*., 2008; Binkert *et al.* 2016), it is reasonable to hypothesize that UVR8 may regulate *PIF* transcription via association with other regulators. Hence, experiments using mutants deficient in known components of the UV-B signalling pathway, COP1 and HYH/HY5, were performed to test their role in the UV-B-mediated suppression of *PIF* transcript. Despite low basal levels of *PIF* transcript in *cop1-4* plants, data produced in this thesis suggest COP1 involvement in the UV-B-mediated suppression of *PIF4* and *PIF5* transcript abundance. These findings support published findings suggesting a role for COP1 in UV-B-mediated suppression of *PIF5* abundance (Sharma *et al.,* 2019). COP1 may potentially positively regulate inducers or negatively regulate suppressors of *PIF 4/5*. The apparent lack of requirement for COP1 in UV-B-mediated suppression of *PIF3* transcript abundance is indicative of an alternative regulatory mechanism controlling *PIF3* transcript suppression or the existence of a posttranscriptional regulatory mechanism in UV-B. Conversely, it is likely that HY5/HYH do not participate in the UV-B-mediated suppression of *PIF 3, 4* and *5* transcript accumulation. Because starting levels of *PIF 4/5* in *hyh/hy5* plants were low, assessing their role in the UV-B response was unclear. Nevertheless, *hyh/hy5* plants still respond to UV-B, consistent with the *PIF4* findings of Hayes *et al.* (2017). Taken together, Chapter 3 has identified negative regulation of *PIF 3/4/5* transcript abundance in UV-B. This occurs via promoter regulation of *PIF4* and *PIF5*, and possibly via transcript stability of *PIF3.* All UV-B responses are mediated by UVR8, with a role for COP1 in *PIF 4/5* suppression.

If further time was available, it would be informative to identify the regions of different *PIF* promoters responsive to UV-B signals through the creation of promoter truncated versions fused to a reporter gene. These could be further mutated to identify the specific sites required for transcription factor binding in UV-B that may be target for intermediate regulators. A Y1H (Yeast-one-hybrid) assay could then be

performed, creating a WL library and a UV-B library using *PIF4* and *PIF5* promoter regions as baits. This approach would establish which transcription factors bind to the promoters of different *PIFs* in UV-B and WL. These experiments could be finalized by testing the most relevant promoter-transcription factor interactions *in planta* via transactivation assays.

Investigating UV-B-mediated suppression of *PIF4* transcript at high temperatures

Since chapter 3 provided evidence of promoter regulation of *PIF4* in UV-B, Chapter 4 focused on understanding the mechanism in which *PIF4* transcript is UV-B suppressed at high temperatures. Recent reports have shown that the main mechanism underlying UV-B-mediated inhibition of thermomorphogenesis is suppression of *PIF4* transcript abundance (Hayes *et al*., 2017). *In silico* analysis in chapter 3 identified cis elements for the BR-regulated transcription factor BES1 in the *PIF4* promoter (Table 3). This, in addition to reports showing that BES1/BZR1 are direct targets of active UVR8 (Liang *et al*., 2018), and that BZR1 directly upregulates *PIF4* at high temperatures (Ibanez *et al*., 2018) opened a new mechanistic avenue for investigating UV-B-mediated regulation of *PIF4* transcript abundance. It was hypothesized that active UVR8 sequesters BES1/BZR1 from promoting *PIF4* transcription, resulting in thermomorphogenesis antagonism (Figure 8). Data provided in chapter 4 support published studies showing that UV-B-mediated suppression of thermomorphogensis requires UVR8 (Hayes *et al*., 2017). It also supports a partial role for BR in the UV-B-mediated suppression of thermomorphogenesis, since elevated BR signalling can overcome UV-B-mediated suppression of hypocotyl growth. However, measurements of *PIF4* transcript abundance suggest that *PIF4* transcript suppression is not regulated through inhibition of BR signalling in UV-B. Overall, although Chapter 4 presents evidence of a partial involvement of BR in the UV-B-mediated suppression of thermomorphogenesis, it does not support a role for UVR8-mediated sequestration of BZR1/BES1 in *PIF4* transcriptional suppression.

The previously suggested Y1H experiment could also facilitate identification of regulatory factors controlling UV-B-mediated suppression of *PIF4* transcript abundance at high temperature. Data from the UV-B library screen with the *PIF4* promoter could be screened to identify transcription factors responsive to UV-B and heat. Mutants for these genes could then be obtained or generated for further analysis of *PIF4* transcript abundance under these conditions. Selected transcription factors could be used in Yeast-2-hybrid assays to test for UVR8 interaction, providing additional downstream signalling components in the UV-B signalling pathway.

Investigating the existence of a PIF4 auto-activation system in UV-B

Chapter 5 focused on investigating the role of a PIF4 auto-activation system on the UV-B-mediated suppression of *PIF4* transcript abundance. The rapid UV-B-mediated turnover of PIF protein (Sharma *et al*., 2019), combined with slower UV-B-suppression of *PIF* transcript observed in Chapter 3 and published evidence of PIF4 binding to its own promoter (Zhai *et al.,* 2020; Lee *et al.,* 2021) prompted investigation into the existence of an auto-activation system could mediate this response. It was postulated that the rapid UV-B turnover of PIF4 could reduce its own transcript promotion. To test this hypothesis, this study first aimed to stabilize PIF4 in UV-B. While PIF5 levels are stabilized in UV-B when MG132 treated (Sharma *et al*., 2019), PIF4 turnover in UV-B was still observed in both overexpression and native promoter lines, despite the presence of MG132 and accumulation of ubiquitinated proteins. These results suggest that PIF4 may be degraded in UV-B by an alternative pathway to PIF5, despite their high similarity. Alternatively, it is possible that MG132 was not effective in stabilizing PIF4 in UV-B in these experiments, requiring optimization of proteosome inhibitor levels or type. Future experiments could use test MG115 or Bortezomib which have been successfully shown to prevent proteosome-mediated degradation of PIFs in Arabidopsis (Shen *et al.,* 2005; Xu *et al.*, 2017). Additionally, Chapter 5 shows that UV-B-mediated

suppression of *PIF4* transcript abundance is unaffected following incubation in MG132. This result is in contrast to *PIF5*, where UV-B-mediated transcript suppression is abolished in the presence of proteosome inhibitor. Collectively, this chapter provides circumstantial evidence that the UV-B-mediated turnover of PIF4 and PIF5 occur via different mechanisms. The role of a PIF4 auto-activation mechanism in the UV-B-mediated suppression of its own transcript remains to be elucidated.

MYB30 as a novel regulator of PIF transcript abundance

Chapter 6 aimed to investigate the role of MYB30 as a transcriptional regulator of *PIF*s, as recent research has shown that MYB30 directly promotes *PIF4* and *PIF5* transcription (Yan *et al*., 2020), in addition to the presence of MYB and MYB-related cis elements in the promoters of *PIF* (Chapter 3). Firstly, *MYB30* was shown to be transcriptionally repressed by UV-B in a UVR8-dependent manner. It was hypothesized that rapid repression of *MYB30* transcript abundance in UV-B could result in the decreased transcription of *PIF 4/5* in UV-B observed in chapter 3. However, qRT-PCR experiments using the null mutant *myb30-1* suggested that MYB30 does not have a role in the UV-B suppression of *PIF 3/4/5* transcript abundance. Instead, MYB30 seems to act as a *PIF4/5* transcriptional repressor in WL, oppositely to results observed by Yan *et al.* (2020). The role for MYB30 in regulating *PIF3/7* transcript abundance was additionally investigated. While MYB30 presented no regulatory role for *PIF3*, *PIF7* transcript abundance was strongly reduced in *myb30*-1 in WL, suggesting the existence of a potentially novel regulatory mechanism promoting *PIF7* transcript abundance. This finding is supported by observations showing a partial inhibition of hypocotyl elongation under true shade in *myb30-1* plants. Intriguingly, *PIF7* transcript was supressed by UV-B in these experiments, independently of MYB30, suggesting that the interpretation of previous data may have been negatively affected by reduced numbers of biological replicates, or highly variable suppression of *PIF7* transcript regulation in UV-B. Moreover, chapter 6 data presents novel roles

for MYB30 in supressing the transcript abundance of *PIF*s *4* and (to a lesser extent) *PIF5* in WL. Further work elucidating the mechanisms by which MYB30 regulates *PIF7* transcript abundance using transgenic lines expressing promoter-reporter constructs would be key to identifying whether regulation occurs at the promoter level. Production of double and triple mutant lines of *myb30pif4pif7*, *myb30pif4* and *myb30pif7* would remove the confounding effects of MYB30 on *PIF4* transcript accumulation and help elucidate the effect of MYB30 on *PIF7*-mediated responses to true shade more clearly.

6.1 Conclusions

This thesis set out to identify and characterize the transcriptional regulatory mechanisms controlling *PIF*s in response to different external cues, but mainly in low dose UV-B. Evidence for UVR8-mediated suppression of *PIF 3/4/5* transcript abundance in UV-B is presented, with conflicting results obtained for *PIF7* across multiple experiments. Data show that UV-B-mediated suppression of *PIF 4/5* occurs at the promoter level and requires COP1, suggesting a new role for COP1 in the UV-B signalling pathway. In contrast, UV-B-mediated suppression of *PIF3* does not require COP1 or occur at the promoter level, suggesting an alternative regulatory pathway, possibly at the posttranscriptional level. Despite clear links between UV-B signalling, BR signalling and *PIF4* regulation, no evidence was obtained to link BZR1/BES1 sequestration by UVR8 to *PIF* transcriptional suppression in UV-B. Data did, however, suggest a partial role for BR signalling in the UV-B-mediated suppression of thermomorphogenesis. This research suggests alternative mechanisms exist for PIF4 and PIF5 turnover in UV-B, with PIF4 degradation possibly mediated by a pathway other than the 26-proteasome system. Furthermore, this chapter identifies novel roles for MYB30 as a repressor of *PIF 4/5* transcript abundance and a positive regulator of *PIF7* in WL, possibly mediating shade-avoidance responses to true shade by upregulating *PIF7* transcription. Collectively, this thesis has identified a number of novel PIF regulatory mechanisms, laying the foundation for further

research. Severe time limitations prevented deeper mechanistic dissection. Understanding the transcriptional regulation of different PIF family members will be central to understanding the regulatory processes orchestrated by each and translating these findings into commercial crops.

References

A.-H.-Mackerness, S., Surplus, S. L., Blake, P., John, C. F., Buchanan-Wollaston, V., Jordan, B. R., & Thomas, B. (1999). Ultraviolet-B-induced stress and changes in gene expression in Arabidopsis thaliana: role of signalling pathways controlled by jasmonic acid, ethylene and reactive oxygen species. Plant, Cell & Environment, *22*(11), 1413-1423

Agalou, A., S. Purwantomo, E. Övernäs, H. Johannesson, X. Zhu, A. Estiati, R. J. de Kam, P. Engström, I. H. Slamet-Loedin & Z. Zhu (2008). "A genome-wide survey of HD-Zip genes in rice and analysis of drought-responsive family members." Plant Molecular Biology, 66: 87-103.

Ahmad, M., & Cashmore, A. R. (1993). HY4 gene of A. thaliana encodes a protein with characteristics of a blue-light photoreceptor. Nature, *366*(6451), 162-166.

Al-Sady, B., Ni, W., Kircher, S., Schäfer, E., & Quail, P. H. (2006). Photoactivated phytochrome induces rapid PIF3 phosphorylation prior to proteasome-mediated degradation. Molecular Cell, *23*(3), 439-446.

Al-Sady, B., Kikis, E. A., Monte, E., & Quail, P. H. (2008). Mechanistic duality of transcription factor function in phytochrome signaling. *Proceedings of the* National Academy of Sciences, *105*(6), 2232-2237.

Bae, G., & Choi, G. (2008). Decoding of light signals by plant phytochromes and their interacting proteins. Annual Review of Plant Biology, *59*, 281-311.

Bai, M.-Y., J.-X. Shang, E. Oh, M. Fan, Y. Bai, R. Zentella, T.-p. Sun & Z.-Y. Wang (2012). "Brassinosteroid, gibberellin and phytochrome impinge on a common transcription module in Arabidopsis." Nature Cell Biology, 14(8): 810-817.

Balasubramanian, S., Sureshkumar, S., Lempe, J., & Weigel, D. (2006). Potent induction of Arabidopsis thaliana flowering by elevated growth temperature. PLoS Genetics, *2*(7), e106.

Balcerowicz, M. (2020). Phytochrome-interacting factors at the interface of light and temperature signalling. Physiologia Plantarum, *169*(3), 347-356.

Ballaré, C. L., Scopel, A. L., & Sánchez, R. A. (1990). Far-red radiation reflected from adjacent leaves: an early signal of competition in plant canopies. Science, *247*(4940), 329-332.

Bauer, D., A. Vicziaén, S. Kircher, T. Nobis, R. Nitschke, T. Kunkel, K. C. Panigrahi, E. Aédaém, E. Fejes & E. Schafer (2004). "Constitutive photomorphogenesis 1 and multiple photoreceptors control degradation of phytochrome interacting factor 3, a transcription factor required for light signaling in Arabidopsis." The Plant Cell, 16(6): 1433-1445.

Bernardo-García, S., de Lucas, M., Martínez, C., Espinosa-Ruiz, A., Daviere, J. M., & Prat, S. (2014). BR-dependent phosphorylation modulates PIF4 transcriptional activity and shapes diurnal hypocotyl growth. Genes & Development, *28*(15), 1681-1694.

Bellstaedt, J., J. Trenner, R. Lippmann, Y. Poeschl, X. Zhang, J. Friml, M. Quint & C. Delker (2019). "A mobile auxin signal connects temperature sensing in cotyledons with growth responses in hypocotyls." Plant Physiology, 180(2): 757-766.

Binkert, M., C. D. Crocco, B. Ekundayo, K. Lau, S. Raffelberg, K. Tilbrook, R. Yin, R. Chappuis, T. Schalch & R. Ulm (2016). "Revisiting chromatin binding of the Arabidopsis UV-B photoreceptor UVR8." BMC Plant Biology, 16: 1-11.

Blázquez, M. A., Ahn, J. H., & Weigel, D. (2003). A thermosensory pathway controlling flowering time in Arabidopsis thaliana. Nature Genetics, *33*(2), 168-171.

Blumthaler, M., Huber, M., & Ambach, W. (1993, November). Measurements of direct and global UV spectra under varying turbidity. In Atmospheric Radiation, (Vol. 2049, pp. 195-198). SPIE.

Boccaccini, A., M. Legris, J. Krahmer, L. Allenbach-Petrolati, A. Goyal, C. Galvan-Ampudia, T. Vernoux, E. Karayekov, J. J. Casal & C. Fankhauser (2020). "Low blue light enhances phototropism by releasing cryptochrome1-mediated inhibition of PIF4 expression." Plant Physiology, 183(4): 1780-1793.

Bolle, C., Koncz, C., & Chua, N. H. (2000). PAT1, a new member of the GRAS family, is involved in phytochrome A signal transduction. Genes & Development, 14(10), 1269-1278.

Bornman, J. F., Reuber, S., Cen, Y. P., & Weissenböck, G. (1997, January). Ultraviolet radiation as a stress factor and the role of protective pigments. In Seminar Series-Society for Experimental Biology, (Vol. 64, pp. 157-170). Cambridge University Press.

Box, M. S., B. E. Huang, M. Domijan, K. E. Jaeger, A. K. Khattak, S. J. Yoo, E. L. Sedivy, D. M. Jones, T. J. Hearn & A. A. Webb (2015). "ELF3 controls thermoresponsive growth in Arabidopsis." Current Biology, 25(2): 194-199.

Bridge, L. J., Franklin, K. A., & Homer, M. E. (2013). Impact of plant shoot architecture on leaf cooling: a coupled heat and mass transfer model. Journal of The Royal Society Interface, *10*(85), 20130326.

Brosché, M., & Strid, Å. (2003). Molecular events following perception of ultraviolet-B radiation by plants. Physiologia Plantarum, *117*(1), 1-10.

Brown, B. A., Cloix, C., Jiang, G. H., Kaiserli, E., Herzyk, P., Kliebenstein, D. J., & Jenkins, G. I. (2005). A UV-B-specific signaling component orchestrates plant UV protection. Proceedings of the National Academy of Sciences, *102*(50), 18225-18230.

Bu, Q., Castillon, A., Chen, F., Zhu, L., & Huq, E. (2011a). Dimerization and blue light regulation of PIF1 interacting bHLH proteins in Arabidopsis. Plant Molecular Biology, 77: 501–511.

Buti, S., Hayes, S., & Pierik, R. (2020). The bHLH network underlying plant shade-avoidance. Physiologia Plantarum, *169*(3), 312-324.

Burko, Y., Willige, B. C., Seluzicki, A., Novák, O., Ljung, K., & Chory, J. (2022). PIF7 is a master regulator of thermomorphogenesis in shade. Nature Communications, *13*(1), 4942.

Campos, M. L., Y. Yoshida, I. T. Major, D. de Oliveira Ferreira, S. M. Weraduwage, J. E. Froehlich, B. F. Johnson, D. M. Kramer, G. Jander & T. D. Sharkey (2016). "Rewiring of jasmonate and phytochrome B signalling uncouples plant growth-defense tradeoffs." Nature Communications, 7(1): 12570.

Casal, J. J. (2013). Photoreceptor signaling networks in plant responses to shade. Annual Review of Plant Biology, *64*, 403-427.

Casal, J. J., & Balasubramanian, S. (2019). Thermomorphogenesis. Annual Review of Plant Biology, *70*, 321-346.

Casson, S.A., Franklin, K.A., Gray, J.E., Grierson, C.S., Whitelam, G.C., & Hetherington, A.M. (2009). phytochrome B and PIF4 regulate stomatal development in response to light quantity. Current Biology, 19: 229–234.

Castillon, A., Shen, H., & Huq, E. (2007). Phytochrome interacting factors: central players in phytochrome-mediated light signaling networks. Trends in Plant Science, *12*(11), 514-521.

Chaves, I., R. Pokorny, M. Byrdin, N. Hoang, T. Ritz, K. Brettel, L.-O. Essen, G. T. van der Horst, A. Batschauer & M. Ahmad (2011). "The cryptochromes: blue light photoreceptors in plants and animals." Annual Review of Plant Biology, 62: 335-364.

Chen, M., & Chory, J. (2011). Phytochrome signaling mechanisms and the control of plant development. Trends in Cell Biology, *21*(11), 664-671.

Chen, L.-G., Z. Gao, Z. Zhao, X. Liu, Y. Li, Y. Zhang, X. Liu, Y. Sun & W. Tang (2019). "BZR1 family transcription factors function redundantly and indispensably in BR signaling but exhibit BRI1-independent function in regulating anther development in Arabidopsis." Molecular Plant, 12(10): 1408-1415.

Christie, J. M., Reymond, P., Powell, G. K., Bernasconi, P., Raibekas, A. A., Liscum, E., & Briggs, W. R. (1998). Arabidopsis NPH1: a flavoprotein with the properties of a photoreceptor for phototropism. Science, 282(5394), 1698-1701.

Christie, J. M., Swartz, T. E., Bogomolni, R. A., & Briggs, W. R. (2002). Phototropin LOV domains exhibit distinct roles in regulating photoreceptor function. The Plant Journal, 32(2), 205-219.

Christie, J. M., Corchnoy, S. B., Swartz, T. E., Hokenson, M., Han, I. S., Briggs, W. R., & Bogomolni, R. A. (2007). Steric interactions stabilize the signaling state of the LOV2 domain of phototropin 1. Biochemistry, 46(32), 9310-9319.

Christie, J. M., A. S. Arvai, K. J. Baxter, M. Heilmann, A. J. Pratt, A. O'Hara, S. M. Kelly, M. Hothorn, B. O. Smith & K. Hitomi (2012). "Plant UVR8 photoreceptor senses UV-B by tryptophan-mediated disruption of cross-dimer salt bridges." Science, 335(6075): 1492-1496.

Chung, B. Y., M. Balcerowicz, M. Di Antonio, K. E. Jaeger, F. Geng, K. Franaszek, P. Marriott, I. Brierley, A. E. Firth & P. A. Wigge (2020). "An RNA thermoswitch regulates daytime growth in Arabidopsis." Nature Plants, 6(5): 522-532.

Cloix, C., E. Kaiserli, M. Heilmann, K. J. Baxter, B. A. Brown, A. O'Hara, B. O. Smith, J. M. Christie & G. I. Jenkins (2012). "C-terminal region of the UV-B photoreceptor UVR8 initiates signaling through interaction with the COP1 protein." Proceedings of the National Academy of Sciences, 109(40): 16366-16370.

Cole, B., Kay, S. A., & Chory, J. (2011). Automated analysis of hypocotyl growth dynamics during shade avoidance in Arabidopsis. The Plant Journal, *65*(6), 991-1000.

Crawford, A. J., McLachlan, D. H., Hetherington, A. M., & Franklin, K. A. (2012). High temperature exposure increases plant cooling capacity. Current Biology, *22*(10), R396-R397.

Daniel, X., Lacomme, C., Morel, J. B., & Roby, D. (1999). A novel myb oncogene homologue in Arabidopsis thaliana related to hypersensitive cell death. The Plant Journal, *20*(1), 57-66.

De Lucas, M., J.-M. Daviere, M. Rodríguez-Falcón, M. Pontin, J. M. Iglesias-Pedraz, S. Lorrain, C. Fankhauser, M. A. Blázquez, E. Titarenko & S. Prat (2008). "A molecular framework for light and gibberellin control of cell elongation." Nature, 451(7177): 480-484.

Delker, C., L. Sonntag, G. V. James, P. Janitza, C. Ibanez, H. Ziermann, T. Peterson, K. Denk, S. Mull & J. Ziegler (2014). "The DET1-COP1-HY5 pathway constitutes a multipurpose signaling module regulating plant photomorphogenesis and thermomorphogenesis." Cell Reports, 9(6): 1983-1989.

Ding, L., S. Wang, Z.-T. Song, Y. Jiang, J.-J. Han, S.-J. Lu, L. Li & J.-X. Liu (2018). "Two B-box domain proteins, BBX18 and BBX23, interact with ELF3 and regulate thermomorphogenesis in Arabidopsis." Cell Reports, 25(7): 1718-1728. e1714.

Dong, H., J. Dumenil, F.-H. Lu, L. Na, H. Vanhaeren, C. Naumann, M. Klecker, R. Prior, C. Smith & N. McKenzie (2017). "Ubiquitylation activates a peptidase that promotes cleavage and destabilization of its activating E3 ligases and diverse growth regulatory proteins to limit cell proliferation in Arabidopsis." Genes & Development, 31(2): 197-208.

Dong, X., Y. Yan, B. Jiang, Y. Shi, Y. Jia, J. Cheng, Y. Shi, J. Kang, H. Li & D. Zhang (2020). "The cold response regulator CBF1 promotes Arabidopsis hypocotyl growth at ambient temperatures." The EMBO Journal, 39(13): e103630.

Dubos, C., Stracke, R., Grotewold, E., Weisshaar, B., Martin, C., & Lepiniec, L. (2010). MYB transcription factors in Arabidopsis. Trends in Plant Science, *15*(10), 573-581.

Duek, P. D., & Fankhauser, C. (2005). bHLH class transcription factors take centre stage in phytochrome signalling. Trends in Plant Science, 10(2), 51-54.

El-Din El-Assal, S., Alonso-Blanco, C., Peeters, A. J., Raz, V., & Koornneef, M. (2001). A QTL for flowering time in Arabidopsis reveals a novel allele of CRY2. Nature Genetics, *29*(4), 435-440.

Fairchild, C.D., Schumaker, M.A., & Quail, P.H. (2000). HFR1 encodes an atypical bHLH protein that acts in phytochrome A signal transduction. Genes Development, 14: 2377–2391.

Favory, J. J., A. Stec, H. Gruber, L. Rizzini, A. Oravecz, M. Funk, A. Albert, C. Cloix, G. I. Jenkins & E. J. Oakeley (2009). "Interaction of COP1 and UVR8 regulates UV-B-induced photomorphogenesis and stress acclimation in Arabidopsis." The EMBO Journal, 28(5): 591-601.

Feng, S., C. Martinez, G. Gusmaroli, Y. Wang, J. Zhou, F. Wang, L. Chen, L. Yu, J. M. Iglesias-Pedraz & S. Kircher (2008). "Coordinated regulation of Arabidopsis thaliana development by light and gibberellins." Nature, 451(7177): 475-479.

Findlay, K. M., & Jenkins, G. I. (2016). Regulation of UVR8 photoreceptor dimer/monomer photo-equilibrium in Arabidopsis plants grown under photoperiodic conditions. Plant, Cell & Environment, *39*(8), 1706-1714.

Fiorucci, A.-S., V. C. Galvão, Y. Ç. Ince, A. Boccaccini, A. Goyal, L. Allenbach Petrolati, M. Trevisan & C. Fankhauser (2020). "PHYTOCHROME INTERACTING FACTOR 7 is important for early responses to elevated temperature in Arabidopsis seedlings." New Phytologist, 226(1): 50-58.

Folta, K. M., Pontin, M. A., Karlin-Neumann, G., Bottini, R., & Spalding, E. P. (2003). Genomic and physiological studies of early cryptochrome 1 action demonstrate roles for auxin and gibberellin in the control of hypocotyl growth by blue light. The Plant Journal, *36*(2), 203-214.

Fankhauser, C., & Ulm, R. (2011). Light-regulated interactions with SPA proteins underlie cryptochrome-mediated gene expression. Genes & Development, *25*(10), 1004-1009.

Franklin, K. A., & Whitelam, G. C. (2005). Phytochromes and shade-avoidance responses in plants. Annals of Botany, *96*(2), 169-175.

Franklin, K. A. (2008). Shade avoidance. New Phytologist, *179*(4), 930-944.

Franklin, K. A. (2009). Light and temperature signal crosstalk in plant development. Current opinion in Plant Biology,, *12*(1), 63-68.

Franklin, K. A., S. H. Lee, D. Patel, S. V. Kumar, A. K. Spartz, C. Gu, S. Ye, P. Yu, G. Breen & J. D. Cohen (2011). "Phytochrome-interacting factor 4 (PIF4) regulates auxin biosynthesis at high temperature." Proceedings of the National Academy of Sciences, 108(50): 20231-20235.

Foreman, J., Johansson, H., Hornitschek, P., Josse, E. M., Fankhauser, C., & Halliday, K. J. (2011). Light receptor action is critical for maintaining plant biomass at warm ambient temperatures. The Plant Journal, *65*(3), 441-452.

Frohnmeyer, H., & Staiger, D. (2003). Ultraviolet-B radiation-mediated responses in plants. Balancing damage and protection. Plant Physiology, *133*(4), 1420-1428.

Gabriele, S., Rizza, A., Martone, J., Circelli, P., Costantino, P., & Vittorioso, P. (2010). The Dof protein DAG1 mediates PIL5 activity on seed germination by negatively regulating GA biosynthetic gene AtGA3ox1. The Plant Journal, *61*(2), 312-323.

Gallego-Bartolomé, J., E. G. Minguet, J. A. Marín, S. Prat, M. A. Blázquez & D. Alabadí (2010). "Transcriptional diversification and functional conservation between DELLA proteins in Arabidopsis." Molecular Biology and Evolution, 27(6): 1247-1256.

Gangappa, S. N., Berriri, S., & Kumar, S. V. (2017). PIF4 coordinates thermosensory growth and immunity in Arabidopsis. Current Biology, *27*(2), 243-249.

Gangappa, S. N., & Kumar, S. V. (2017). DET1 and HY5 control PIF4-mediated thermosensory elongation growth through distinct mechanisms. Cell Reports, *18*(2), 344-351.

Gray, W. M., Östin, A., Sandberg, G., Romano, C. P., & Estelle, M. (1998). High temperature promotes auxin-mediated hypocotyl elongation in Arabidopsis. Proceedings of the National Academy of Sciences, *95*(12), 7197-7202.

Goyal, A., Karayekov, E., Galvão, V. C., Ren, H., Casal, J. J., & Fankhauser, C. (2016). Shade promotes phototropism through phytochrome B-controlled auxin production. Current Biology, *26*(24), 3280-3287.

Guo, H., Yang, H., Mockler, T. C., & Lin, C. (1998). Regulation of flowering time by Arabidopsis photoreceptors. Science, *279*(5355), 1360-1363.

Halliday, K. J., Salter, M. G., Thingnaes, E., & Whitelam, G. C. (2003). Phytochrome control of flowering is temperature sensitive and correlates with expression of the floral integrator FT. The Plant Journal, *33*(5), 875-885.

Han, X., Yu, H., Yuan, R., Yang, Y., An, F., & Qin, G. (2019). Arabidopsis transcription factor TCP5 controls plant thermomorphogenesis by positively regulating PIF4 activity. Iscience, *15*, 611-622.

Harper, S. M., Neil, L. C., & Gardner, K. H. (2003). Structural basis of a phototropin light switch. Science, 301(5639), 1541-1544.

Hao, Y., Oh, E., Choi, G., Liang, Z., and Wang, Z.Y. (2012). Interactions between HLH and bHLH factors modulate light-regulated plant development. Molecular Plant, 5: 688–697.

Hayes, S., Velanis, C. N., Jenkins, G. I., & Franklin, K. A. (2014). UV-B detected by the UVR8 photoreceptor antagonizes auxin signaling and plant shade avoidance. Proceedings of the National Academy of Sciences, *111*(32), 11894-11899.

Hayes, S., A. Sharma, D. P. Fraser, M. Trevisan, C. K. Cragg-Barber, E. Tavridou, C. Fankhauser, G. I. Jenkins & K. A. Franklin (2017). "UV-B perceived by the UVR8 photoreceptor inhibits plant thermomorphogenesis." Current Biology, 27(1): 120-127.

He, J. X., Gendron, J. M., Sun, Y., Gampala, S. S., Gendron, N., Sun, C. Q., & Wang, Z. Y. (2005). BZR1 is a transcriptional repressor with dual roles in brassinosteroid homeostasis and growth responses. Science, *307*(5715), 1634-1638.

Heijde, M., & Ulm, R. (2012). UV-B photoreceptor-mediated signalling in plants. Trends in Plant Science, *17*(4), 230-237.

Heijde, M., & Ulm, R. (2013). Reversion of the Arabidopsis UV-B photoreceptor UVR8 to the homodimeric ground state. Proceedings of the National Academy of Sciences, *110*(3), 1113-1118.

Helfer, A., Nusinow, D. A., Chow, B. Y., Gehrke, A. R., Bulyk, M. L., & Kay, S. A. (2011). LUX ARRHYTHMO encodes a nighttime repressor of circadian gene expression in the Arabidopsis core clock. Current Biology, *21*(2), 126-133.

Henriksson, E., Olsson, A. S., Johannesson, H., Johansson, H., Hanson, J., Engström, P., & Söderman, E. (2005). Homeodomain leucine zipper class I genes in Arabidopsis. Expression patterns and phylogenetic relationships. Plant Physiology, 139(1), 509-518.

Herrero, E., E. Kolmos, N. Bujdoso, Y. Yuan, M. Wang, M. C. Berns, H. Uhlworm, G. Coupland, R. Saini & M. Jaskolski (2012). "EARLY FLOWERING4 recruitment of EARLY FLOWERING3 in the nucleus sustains the Arabidopsis circadian clock." The Plant Cell, 24(2): 428-443.

Hersch, M., Lorrain, S., de Wit, M., Trevisan, M., Ljung, K., Bergmann, S. & Fankhauser, C. (2014). Light intensity modulates the regulatory network of the shade avoidance response in Arabidopsis. *Proceedings of the* National Academy of Sciences, *111*(17), pp.6515-6520.

Hoecker, U., Tepperman, J.M., & Quail, P.H. (1999). SPA1: A WD-repeat protein specific to phytochrome A signal transduction. Science, 284: 496–499.

Hornitschek, P., Lorrain, S., Zoete, V., Michielin, O., & Fankhauser, C. (2009). Inhibition of the shade avoidance response by formation of non-DNA binding bHLH heterodimers. The EMBO Journal, *28*(24), 3893-3902.

Hornitschek, P., M. V. Kohnen, S. Lorrain, J. Rougemont, K. Ljung, I. López-Vidriero, J. M. Franco-Zorrilla, R. Solano, M. Trevisan and S. Pradervand (2012). "Phytochrome interacting factors 4 and 5 control seedling growth in changing light conditions by directly controlling auxin signaling." The Plant Journal, 71(5): 699-711.

Huala, E., Oeller, P. W., Liscum, E., Han, I. S., Larsen, E., & Briggs, W. R. (1997). Arabidopsis NPH1: a protein kinase with a putative redox-sensing domain. Science, *278*(5346), 2120-2123.

Huang, X., Yang, P., Ouyang, X., Chen, L., & Deng, X. W. (2014). Photoactivated UVR8-COP1 module determines photomorphogenic UV-B signaling output in Arabidopsis. PLoS Genetics, *10*(3), e1004218.

Huang, X., X. Ouyang, P. Yang, O. S. Lau, G. Li, J. Li, H. Chen & X. W. Deng (2012). "Arabidopsis FHY3 and HY5 positively mediate induction of COP1 transcription in response to photomorphogenic UV-B light." The Plant Cell, 24(11): 4590-4606.

Huang, X., Zhang, Q., Jiang, Y., Yang, C., Wang, Q., & Li, L. (2018). Shade-induced nuclear localization of PIF7 is regulated by phosphorylation and 14-3-3 proteins in Arabidopsis. Elife, *7*, e31636.

Huq, E., & Quail, P.H. (2002). PIF4, a phytochrome-interacting bHLH factor, functions as a negative regulator of phytochrome B signaling in Arabidopsis. EMBO Journal, 21: 2441–2450.

Huq, E., Al-Sady, B., Hudson, M., Kim, C., Apel, K., & Quail, P.H. (2004). Phytochrome-interacting factor 1 is a critical bHLH regulator of chlorophyll biosynthesis. Science, 305: 1937–1941.

Huq, E., & Quail, P. H. (2005). Phytochrome signaling. Handbook of Photosensory Receptors, 151-170.

Hwang, G., Zhu, J. Y., Lee, Y. K., Kim, S., Nguyen, T. T., Kim, J., & Oh, E. (2017). PIF4 promotes expression of LNG1 and LNG2 to induce thermomorphogenic growth in Arabidopsis. Frontiers in Plant Science, 8, 1320.

Ibañes, M., Fàbregas, N., Chory, J., & Caño-Delgado, A. I. (2009). Brassinosteroid signaling and auxin transport are required to establish the periodic pattern of Arabidopsis shoot vascular bundles. Proceedings of the National Academy of Sciences, *106*(32), 13630-13635.

Ibañez, C., Y. Poeschl, T. Peterson, J. Bellstädt, K. Denk, A. Gogol-Döering, M. Quint & C. Delker (2015). "Developmental and phenotypic plasticity of Arabidopsis thaliana accessions across an ambient temperature range." BioRxiv: 017285.

Ibañez, C., Y. Poeschl, T. Peterson, J. Bellstädt, K. Denk, A. Gogol-Döring, M. Quint & C. Delker (2017). "Ambient temperature and genotype differentially affect developmental and phenotypic plasticity in Arabidopsis thaliana." BMC Plant Biology, 17(1): 1-14.

Ibañez, C., C. Delker, C. Martinez, K. Bürstenbinder, P. Janitza, R. Lippmann, W. Ludwig, H. Sun, G. V. James & M. Klecker (2018). "Brassinosteroids dominate hormonal regulation of plant thermomorphogenesis via BZR1." Current Biology, 28(2): 303-310. e303.

Izaguirre, M. M., Scopel, A. L., Baldwin, I. T., & Ballaré, C. L. (2003). Convergent responses to stress. Solar ultraviolet-B radiation and Manduca sexta herbivory elicit overlapping transcriptional responses in field-grown plants of Nicotiana longiflora. Plant Physiology, *132*(4), 1755-1767.

Jakoby, M., Weisshaar, B., Dröge-Laser, W., Vicente-Carbajosa, J., Tiedemann, J., Kroj, T., & Parcy, F. (2002). bZIP transcription factors in Arabidopsis. Trends in Plant Science, 7(3), 106-111.

Jang, K., Gil Lee, H., Jung, S. J., Paek, N. C., & Joon Seo, P. (2015). The E3 ubiquitin ligase COP1 regulates thermosensory flowering by triggering GI degradation in Arabidopsis. Scientific Reports, *5*(1), 12071.

Jansen, M. A., Gaba, V., & Greenberg, B. M. (1998). Higher plants and UV-B radiation: balancing damage, repair and acclimation. Trends in Plant Science, *3*(4), 131-135.

Jarillo, J. A., Gabrys, H., Capel, J., Alonso, J. M., Ecker, J. R., & Cashmore, A. R. (2001). Phototropin-related NPL1 controls chloroplast relocation induced by blue light. Nature, *410*(6831), 952-954.

Jenkins, G. I., & Brown, B. A. (2007). UV-B perception and signal transduction. Light and Plant Development, *30*, 155-182.

Jenkins, G. I. (2009). Signal transduction in responses to UV-B radiation. Annual Review of Plant Biology, *60*, 407-431.

Jeong, R. D., Chandra-Shekara, A. C., Barman, S. R., Navarre, D., Klessig, D. F., Kachroo, A., & Kachroo, P. (2010). Cryptochrome 2 and phototropin 2 regulate resistance protein-mediated viral defense by negatively regulating an E3 ubiquitin ligase. Proceedings of the National Academy of Sciences, *107*(30), 13538-13543.

Jeong, J., & Choi, G. (2013). Phytochrome-interacting factors have both shared and distinct biological roles. Molecules and Cells, *35*(5), 371-380.

Jiang, L., Wang, Y., Björn, L. O., & Li, S. (2009). Arabidopsis radical-induced cell death1 is involved in UV-B signaling. Photochemical & Photobiological Sciences, *8*(6), 838-846.

Jiang, L., Wang, Y., Li, Q. F., Björn, L. O., He, J. X., & Li, S. S. (2012). Arabidopsis STO/BBX24 negatively regulates UV-B signaling by interacting with COP1 and repressing HY5 transcriptional activity. Cell Research, *22*(6), 1046-1057.

Jiao, Y., Lau, O.S., & Deng, X.W. (2007). Light-regulated transcriptional networks in higher plants. Nature Reviews Genetics, 8: 217–230.

Jin, H., E. Cominelli, P. Bailey, A. Parr, F. Mehrtens, J. Jones, C. Tonelli, B. Weisshaar & C. Martin (2000). "Transcriptional repression by AtMYB4 controls production of UV-protecting sunscreens in Arabidopsis." The EMBO Journal, 19(22): 6150-6161.

Jones, M. A., Feeney, K. A., Kelly, S. M., & Christie, J. M. (2007). Mutational analysis of phototropin 1 provides insights into the mechanism underlying LOV2 signal transmission. Journal of Biological Chemistry, 282(9), 6405-6414.

Jung, J.-H., M. Domijan, C. Klose, S. Biswas, D. Ezer, M. Gao, A. K. Khattak, M. S. Box, V. Charoensawan & S. Cortijo (2016). "Phytochromes function as thermosensors in Arabidopsis." Science, 354(6314): 886-889.

Jung, J.-H., A. D. Barbosa, S. Hutin, J. R. Kumita, M. Gao, D. Derwort, C. S. Silva, X. Lai, E. Pierre & F. Geng (2020). "A prion-like domain in ELF3 functions as a thermosensor in Arabidopsis." Nature, 585(7824): 256-260.

Kaiserli, E., & Jenkins, G. I. (2007). UV-B promotes rapid nuclear translocation of the Arabidopsis UV-B–specific signaling component UVR8 and activates its function in the nucleus. The Plant Cell, *19*(8), 2662-2673.

Kami, C., Lorrain, S., Hornitschek, P., & Fankhauser, C. (2010). Light-regulated plant growth and development. Current Topics in Developmental Biology, *91*, 29-66.

Kang, C. Y., Lian, H. L., Wang, F. F., Huang, J. R., & Yang, H. Q. (2009). Cryptochromes, phytochromes, and COP1 regulate light-controlled stomatal development in Arabidopsis. The Plant Cell, *21*(9), 2624-2641.

Kagawa, T., T. Sakai, N. Suetsugu, K. Oikawa, S. Ishiguro, T. Kato, S. Tabata, K. Okada & M. Wada (2001). "Arabidopsis NPL1: a phototropin homolog controlling the chloroplast high-light avoidance response." Science, 291(5511): 2138-2141.

Kaiserli, E., Sullivan, S., Jones, M. A., Feeney, K. A., & Christie, J. M. (2009). Domain swapping to assess the mechanistic basis of Arabidopsis phototropin 1 receptor kinase activation and endocytosis by blue light. The Plant Cell, *21*(10), 3226-3244.

Khanna, R., Huq, E., Kikis, E.A., Al-Sady, B., Lanzatella, C., & Quail, P.H. (2004). A novel molecular recognition motif necessary for targeting photoactivated phytochrome signaling to specific basic helix-loop-helix transcription factors. Plant Cell, 16: 3033–3044.

Khanna, R., Shen, Y., Marion, C. M., Tsuchisaka, A., Theologis, A., Schäfer, E., & Quail, P. H. (2007). The basic helix-loop-helix transcription factor PIF5 acts on ethylene biosynthesis and phytochrome signaling by distinct mechanisms. The Plant Cell, *19*(12), 3915-3929.

Kidokoro, S., K. Maruyama, K. Nakashima, Y. Imura, Y. Narusaka, Z. K. Shinwari, Y. Osakabe, Y. Fujita, J. Mizoi & K. Shinozaki (2009). "The phytochrome-interacting factor PIF7 negatively regulates DREB1 expression under circadian control in Arabidopsis." Plant Physiology, 151(4): 2046-2057.

Kim, J., Yi, H., Choi, G., Shin, B. ,Song, P.S., & Choi, G. (2003). Functional characterization of phytochrome interacting factor 3 in phytochrome mediated light signal transduction. Plant Cell, 15: 2399–2407.

Kim, T. W., Guan, S., Burlingame, A. L., & Wang, Z. Y. (2011). The CDG1 kinase mediates brassinosteroid signal transduction from BRI1 receptor kinase to BSU1 phosphatase and GSK3-like kinase BIN2. Molecular Cell, *43*(4), 561-571.

Kinoshita, T., Doi, M., Suetsugu, N., Kagawa, T., Wada, M., & Shimazaki, K. I. (2001). Phot1 and phot2 mediate blue light regulation of stomatal opening. Nature, *414*(6864), 656-660.

Kinoshita, T., Caño-Delgado, A., Seto, H., Hiranuma, S., Fujioka, S., Yoshida, S., & Chory, J. (2005). Binding of brassinosteroids to the extracellular domain of plant receptor kinase BRI1. Nature, *433*(7022), 167-171.

Kleine, T., Lockhart, P., & Batschauer, A. (2003). An Arabidopsis protein closely related to Synechocystis cryptochrome is targeted to organelles. The Plant Journal, *35*(1), 93-103.

Koini, M. A., Alvey, L., Allen, T., Tilley, C. A., Harberd, N. P., Whitelam, G. C., & Franklin, K. A. (2009). High temperature-mediated adaptations in plant architecture require the bHLH transcription factor PIF4. Current Biology, *19*(5), 408-413.

Kortschak, R. D., Tucker, P. W., & Saint, R. (2000). ARID proteins come in from the desert. Trends in Biochemical Sciences, 25(6), 294-299.

Kozuka, T., Kong, S. G., Doi, M., Shimazaki, K. I., & Nagatani, A. (2011). Tissue-autonomous promotion of palisade cell development by phototropin 2 in Arabidopsis. The Plant Cell, *23*(10), 3684-3695.

Kumar, S. V., & Wigge, P. A. (2010). H2A. Z-containing nucleosomes mediate the thermosensory response in Arabidopsis. Cell, *140*(1), 136-147.

Kumar, S.V., Lucyshyn, D., Jaeger, K.E., Alós, E., Alvey, E., Harberd, N.P., & Wigge, P.A. (2012). Transcription factor PIF4 controls the thermosensory activation of flowering. Nature, 484: 242–245.

Kosugi, S., & Ohashi, Y. (2000). Cloning and DNA-binding properties of a tobacco Ethylene-Insensitive3 (EIN3) homolog. Nucleic Acids Research, 28: 960–967.

Landry, L. G., Chapple, C. C., & Last, R. L. (1995). Arabidopsis mutants lacking phenolic sunscreens exhibit enhanced ultraviolet-B injury and oxidative damage. Plant Physiology, *109*(4), 1159-1166.

Lau, O. S., Z. Song, Z. Zhou, K. A. Davies, J. Chang, X. Yang, S. Wang, D. Lucyshyn, I. H. Z. Tay & P. A. Wigge (2018). "Direct control of SPEECHLESS by PIF4 in the high-temperature response of stomatal development." Current Biology, 28(8): 1273-1280. e1273.

Lau, O. S., & Deng, X. W. (2012). The photomorphogenic repressors COP1 and DET1: 20 years later. Trends in Plant Science, *17*(10), 584-593.

Laubinger, S., Fittinghoff, K., & Hoecker, U. (2004). The SPA quartet: a family of WD-repeat proteins with a central role in suppression of photomorphogenesis in Arabidopsis. The Plant Cell, *16*(9), 2293-2306.

Lee, D. H., & Goldberg, A. L. (1998). Proteasome inhibitors cause induction of heat shock proteins and trehalose, which together confer thermotolerance in Saccharomyces cerevisiae. Molecular and Cellular Biology, *18*(1), 30-38.

Lee, H. J., Jung, J. H., Llorca, L. C., Kim, S. G., Lee, S., Baldwin, I. T., & Park, C. M. (2014). FCA mediates thermal adaptation of stem growth by attenuating auxin action in Arabidopsis. Nature Communications, *5*, 5473.

Lee, N., Park, J., Kim, K., & Choi, G. (2015). The transcriptional coregulator LEUNIG_HOMOLOG inhibits light-dependent seed germination in Arabidopsis. The Plant Cell, *27*(8), 2301-2313.

Lee, N., & Choi, G. (2017). Phytochrome-interacting factor from Arabidopsis to liverwort. Current Opinion in Plant Biology, *35*, 54-60.

Lee, C.M., & Thomashow, M.F. (2012). Photoperiodic regulation of the C-repeat binding factor (CBF) cold acclimation pathway and freezing tolerance in Arabidopsis thaliana. Proceedings of National Academy of Sciences of the United Stated of America, 109: 15054–15059.

Lee, S., Paik, I., & Huq, E. (2020). SPAs promote thermomorphogenesis by regulating the phyB-PIF4 module in Arabidopsis. Development, *147*(19), dev189233.

Lee, S., Zhu, L., & Huq, E. (2021). An autoregulatory negative feedback loop controls thermomorphogenesis in Arabidopsis. PLoS Genetics, *17*(6), e1009595.

Legris, M., C. Klose, E. S. Burgie, C. C. R. Rojas, M. Neme, A. Hiltbrunner, P. A. Wigge, E. Schäfer, R. D. Vierstra & J. J. Casal (2016). "Phytochrome B integrates light and temperature signals in Arabidopsis." Science, 354(6314): 897-900.

Legris, M., Ince, Y. Ç., & Fankhauser, C. (2019). Molecular mechanisms underlying phytochrome-controlled morphogenesis in plants. Nature Communications, *10*(1), 5219.

Leivar, P., Monte, E., Al-Sady, B., Carle, C., Storer, A., Alonso, J. M., Ecker, J.R., & Quail, P.H. (2008a). The Arabidopsis phytochrome-interacting factor PIF7, together with PIF3 and PIF4, regulates responses to prolonged red light by modulating phyB levels. Plant Cell, 20: 337–352.

Leivar, P., & Quail, P. H. (2011). PIFs: pivotal components in a cellular signaling hub. Trends in Plant Science, *16*(1), 19-28.

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

Leivar, P., & Monte, E. (2014). PIFs: systems integrators in plant development. The Plant Cell, *26*(1), 56-78.

Levskaya, A., Weiner, O. D., Lim, W. A., & Voigt, C. A. (2009). Spatiotemporal control of cell signalling using a light-switchable protein interaction. Nature, *461*(7266), 997-1001.

Li, F.-W., C. J. Rothfels, M. Melkonian, J. C. Villarreal, D. W. Stevenson, S. W. Graham, G. K.-S. Wong, S. Mathews & K. M. Pryer (2015). "The origin and evolution of phototropins." Frontiers in Plant Science, 6: 637.

Li, L., X. Yu, A. Thompson, M. Guo, S. Yoshida, T. Asami, J. Chory & Y. Yin (2009). "Arabidopsis MYB30 is a direct target of BES1 and cooperates with BES1 to regulate brassinosteroid-induced gene expression." The Plant Journal, 58(2): 275-286.

Li, K., Yu, R., Fan, L. M., Wei, N., Chen, H., & Deng, X. W. (2016). DELLA-mediated PIF degradation contributes to coordination of light and gibberellin signalling in Arabidopsis. Nature Communications, *7*(1), 11868.

Li, L., K. Ljung, G. Breton, R. J. Schmitz, J. Pruneda-Paz, C. Cowing-Zitron, B. J. Cole, L. J. Ivans, U. V. Pedmale & H.-S. Jung (2012a). "Linking photoreceptor excitation to changes in plant architecture." Genes & Development, 26(8): 785-790.

Li, L. I., Peng, W. F., Liu, Q. Q., Zhou, J. J., Liang, W. H., & Xie, X. Z. (2012b). Expression patterns of OsPIL11, a phytochrome-interacting factor in rice, and preliminary analysis of its roles in light signal transduction. Rice Science, *19*(4), 263-268.

Li, X. J., Li, M., Zhou, Y., Hu, S., Hu, R., Chen, Y., & Li, X. B. (2015). Overexpression of cotton RAV1 gene in Arabidopsis confers transgenic plants high salinity and drought sensitivity. PLoS One, 10(2), e0118056.

Liakopoulos, G., Stavrianakou, S., & Karabourniotis, G. (2006). Trichome layers versus dehaired lamina of Olea europaea leaves: differences in flavonoid distribution, UV-absorbing capacity, and wax yield. Environmental and Experimental Botany, *55*(3), 294-304.

Lian, H.L., S.-B. He, Y.C. Zhang, D.M. Zhu, J.Y. Zhang, K.P. Jia, S.X. Sun, L. Li & H.Q. Yang (2011). "Blue-light-dependent interaction of cryptochrome 1 with SPA1 defines a dynamic signaling mechanism." Genes & Development, 25(10): 1023-1028.

Liang, T., S. Mei, C. Shi, Y. Yang, Y. Peng, L. Ma, F. Wang, X. Li, X. Huang & Y. Yin (2018). "UVR8 interacts with BES1 and BIM1 to regulate transcription and photomorphogenesis in Arabidopsis." Developmental Cell, 44(4): 512-523. e515.

Liang, T., C. Shi, Y. Peng, H. Tan, P. Xin, Y. Yang, F. Wang, X. Li, J. Chu & J. Huang (2020). "Brassinosteroid-activated BRI1-EMS-SUPPRESSOR 1 inhibits flavonoid biosynthesis and coordinates growth and UV-B stress responses in plants." Plant Cell, 32(10): 3224-3239.

Ling, J. J., Li, J., Zhu, D., & Deng, X. W. (2017). Noncanonical role of Arabidopsis COP1/SPA complex in repressing BIN2-mediated PIF3 phosphorylation and degradation in darkness. Proceedings of the National Academy of Sciences, *114*(13), 3539-3544.

Liscum, E., & Briggs, W. R. (1995). Mutations in the NPH1 locus of Arabidopsis disrupt the perception of phototropic stimuli. The Plant Cell, *7*(4), 473-485.

Linschitz, H., Kasche, V., Butler, W. L., & Siegelman, H. W. (1966). The kinetics of phytochrome conversion. Journal of Biological Chemistry, *241*(14), 3395-3403.

Liu, B., Zuo, Z., Liu, H., Liu, X., & Lin, C. (2011a). Arabidopsis cryptochrome 1 interacts with SPA1 to suppress COP1 activity in response to blue light. Genes & Development, *25*(10), 1029-1034.

Liu, L.-J., Y.-C. Zhang, Q.-H. Li, Y. Sang, J. Mao, H.-L. Lian, L. Wang & H.-Q. Yang (2008). "COP1-mediated ubiquitination of CONSTANS is implicated in cryptochrome regulation of flowering in Arabidopsis." The Plant Cell, 20(2): 292-306.

Liu, H., Yu, X., Li, K., Klejnot, J., Yang, H., Lisiero, D., & Lin, C. (2008). Photoexcited CRY2 interacts with CIB1 to regulate transcription and floral initiation in Arabidopsis. Science, *322*(5907), 1535-1539.

Liu, H., Liu, B., Zhao, C., Pepper, M., & Lin, C. (2011). The action mechanisms of plant cryptochromes. Trends in Plant Science, *16*(12), 684-691.

Liu, H., Q. Wang, Y. Liu, X. Zhao, T. Imaizumi, D. E. Somers, E. M. Tobin & C. Lin (2013a). "Arabidopsis CRY2 and ZTL mediate blue-light regulation of the transcription factor CIB1 by distinct mechanisms." Proceedings of the National Academy of Sciences, 110(43): 17582-17587.

Liu, Y., Li, X., Li, K., Liu, H., & Lin, C. (2013b). Multiple bHLH proteins form heterodimers to mediate CRY2-dependent regulation of flowering-time in Arabidopsis. PLoS Genetics, *9*(10), e1003861.

Liu, Z., Zhang, Y., Wang, J., Li, P., Zhao, C., Chen, Y., & Bi, Y. (2015). Phytochrome-interacting factors PIF4 and PIF5 negatively regulate anthocyanin biosynthesis under red light in Arabidopsis seedlings. Plant Science, *238*, 64-72.

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

Lozano-Juste, Jorge, & José León. "Nitric oxide regulates DELLA content and PIF expression to promote photomorphogenesis in Arabidopsis." Plant Physiology, 156.3 (2011): 1410-1423.

Lu, S.X., Webb, C.J., Knowles, S.M., Kim, S.H., Wang, Z., & Tobin, E.M. (2012). CCA1 and ELF3 Interact in the control of hypocotyl length and flowering time in Arabidopsis. Plant Physiology, 158: 1079–1088.

Luo, Q., Lian, H. L., He, S. B., Li, L., Jia, K. P., & Yang, H. Q. (2014). COP1 and phyB physically interact with PIL1 to regulate its stability and photomorphogenic development in Arabidopsis. The Plant Cell, *26*(6), 2441-2456.

Ma, L., Li, J., Qu, L., Hager, J., Chen, Z., Zhao, H., & Deng, X. W. (2001). Light control of Arabidopsis development entails coordinated regulation of genome expression and cellular pathways. The Plant Cell, *13*(12), 2589-2607.

Ma, D., Li, X., Guo, Y., Chu, J., Fang, S., Yan, C., Noel, J.P., & Liu, H. (2016). Cryptochrome 1 interacts with PIF4 to regulate high temperature-mediated hypocotyl elongation in response to blue light. Proceedings of the National Academy of Sciences of the United States of America, 113, 224-229

Mao, J., Zhang, Y. C., Sang, Y., Li, Q. H., & Yang, H. Q. (2005). A role for Arabidopsis cryptochromes and COP1 in the regulation of stomatal opening. Proceedings of the National Academy of Sciences, *102*(34), 12270-12275.

Martín, G., A. Rovira, N. Veciana, J. Soy, G. Toledo-Ortiz, C. M. Gommers, M. Boix, R. Henriques, E. G. Minguet & D. Alabadí (2018). "Circadian waves of transcriptional repression shape PIF-regulated photoperiod-responsive growth in Arabidopsis." Current Biology, 28(2): 311-318. e315.

Martinez-Garcia, J.F., Galstyan, A., Salla-Martret, M., Cifuentes-Esquivel, N., Gallemi, M. & Bou-Torrent, J., (2010). Regulatory components of shade avoidance syndrome. In Advances in Botanical Research (Vol. 53, pp. 65-116). Academic Press.

Martínez, C., Espinosa‐Ruíz, A., de Lucas, M., Bernardo‐García, S., Franco‐Zorrilla, J. M., & Prat, S. (2018). PIF 4-induced BR synthesis is critical to diurnal and thermomorphogenic growth. The EMBO Journal, *37*(23), e99552.

Mazza, C. A., Boccalandro, H. E., Giordano, C. V., Battista, D., Scopel, A. L., & Ballaré, C. L. (2000). Functional significance and induction by solar radiation of ultraviolet-absorbing sunscreens in field-grown soybean crops. Plant Physiology, *122*(1), 117-126.

McKenzie, R. L., Aucamp, P. J., Bais, A. F., Björn, L. O., & Ilyas, M. (2007). Changes in biologically-active ultraviolet radiation reaching the Earth's surface. Photochemical & Photobiological Sciences, *6*(3), 218-231.

Meng, Y., Li, H., Wang, Q., Liu, B., & Lin, C. (2013). Blue light–dependent interaction between cryptochrome2 and CIB1 regulates transcription and leaf senescence in soybean. The Plant Cell, *25*(11), 4405-4420.

Millenaar, F. F., Cox, M. C., van Berkel, Y. E. D. J., Welschen, R. A., Pierik, R., Voesenek, L. A., & Peeters, A. J. (2005). Ethylene-induced differential growth of petioles in Arabidopsis. Analyzing natural variation, response kinetics, and regulation. Plant Physiology, *137*(3), 998-1008.

Møller, S. G., Ingles, P. J., & Whitelam, G. C. (2002). The cell biology of phytochrome signalling. New Phytologist, *154*(3), 553-590.

Monte, E., Tepperman, J.M., Al-Sady, B., Kaczorowski, K.A., Alonso, J.M., Ecker, J.R., Li, X., Zhang, Y., & Quail, P.H. (2004). The phytochrome-interacting transcription factor, PIF3, acts early, selectively, and positively in light-induced chloroplast development. Proceedings of the National Academy of Sciences of the United States of America, 101: 16091–16098.

Moon, J., Zhu, L., Shen, H., & Huq, E. (2008). PIF1 directly and indirectly regulates chlorophyll biosynthesis to optimize the greening process in Arabidopsis. Proceedings of the National Academy of Sciences of the United States of Ametica, 105: 9433–9438.

Mora-García, S., Vert, G., Yin, Y., Caño-Delgado, A., Cheong, H., & Chory, J. (2004). Nuclear protein phosphatases with Kelch-repeat domains modulate the response to brassinosteroids in Arabidopsis. Genes & Development, *18*(4), 448-460.

Nagatani, A. (2004). Light-regulated nuclear localization of phytochromes. Current Opinion in Plant Biology, *7*(6), 708-711.

Nam, K. H., & Li, J. (2002). BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. Cell, *110*(2), 203-212.

Ni, M., Tepperman, J. M., & Quail, P. H. (1999). Binding of phytochrome B to its nuclear signalling partner PIF3 is reversibly induced by light. Nature, *400*(6746), 781-784.

Ni, W., Xu, S. L., Tepperman, J. M., Stanley, D. J., Maltby, D. A., Gross, J. D., Burlingame, A.L, Wang, Z.Y. & Quail, P. H. (2014). A mutually assured destruction mechanism attenuates light signaling in Arabidopsis. Science, *344*(6188), 1160-1164.

Nieto, C., López-Salmerón, V., Davière, J. M., & Prat, S. (2015). ELF3-PIF4 interaction regulates plant growth independently of the evening complex. Current Biology, *25*(2), 187-193.

Niwa, Y., Yamashino, T., & Mizuno, T. (2009). The circadian clock regulates the photoperiodic response of hypocotyl elongation through a coincidence mechanism in Arabidopsis thaliana. Plant and Cell Physiology, *50*(4), 838-854.

Noguchi, T., S. Fujioka, S. Choe, S. Takatsuto, S. Yoshida, H. Yuan, K. A. Feldmann & F. E. Tax (1999). "Brassinosteroid-insensitive dwarf mutants of Arabidopsis accumulate brassinosteroids." Plant Physiology, 121(3): 743-752.

Nomoto, Y., Kubozono, S., Miyachi, M., Yamashino, T., Nakamichi, N., & Mizuno, T. (2012). A circadian clock-and PIF4-mediated double coincidence mechanism is implicated in the thermosensitive photoperiodic control of plant architectures in Arabidopsis thaliana. Plant and Cell Physiology, *53*(11), 1965-1973.

Nomoto, Y., Kubozono, S., Yamashino, T., Nakamichi, N., & Mizuno, T. (2012). Circadian clock-and PIF4-controlled plant growth: a coincidence mechanism directly integrates a hormone signaling network into the photoperiodic control of plant architectures in Arabidopsis thaliana. Plant and Cell Physiology, *53*(11), 1950-1964.

Nozue, K., Covington, M.F., Duek, P.D., Lorrain, S., Fankhauser, C., Harmer, S.L., & Maloof, J.N. (2007). Rhythmic growth explained by coincidence between internal and external cues. Nature, 448: 358–361.

Nusinow, D.A., Helfer, A., Hamilton, E.E., King, J.J., Imaizumi, T., Schultz, T.F., Farré, E.M., & Kay, S.A. (2011). The ELF4-ELF3LUX complex links the circadian clock to diurnal control of hypocotyl growth. Nature, 475: 398–402.

Ogata, K., C. Kanei-Ishii, M. Sasaki, H. Hatanaka, A. Nagadoi, M. Enari, H. Nakamura, Y. Nishimura, S. Ishii & A. Sarai (1996). "The cavity in the hydrophobic core of Myb DNA-binding domain is reserved for DNA recognition and trans-activation." Nature Structural Biology, 3(2): 178-187.

Oh, E., Kim, J., Park, E., Kim, J. I., Kang, C., & Choi, G. (2004). PIL5, a phytochrome-interacting basic helix-loop-helix protein, is a key negative regulator of seed germination in Arabidopsis thaliana. The Plant Cell, *16*(11), 3045-3058.

Oh, E., S. Yamaguchi, J. Hu, J. Yusuke, B. Jung, I. Paik, H.-S. Lee, T.-p. Sun, Y. Kamiya & G. Choi (2007). "PIL5, a phytochrome-interacting bHLH protein, regulates gibberellin responsiveness by binding directly to the GAI and RGA promoters in Arabidopsis seeds." The Plant Cell, 19(4): 1192-1208.

Oh, E., Zhu, J.Y., & Wang, Z.Y. (2012). Interaction between BZR1 and PIF4 integrates brassinosteroid and environmental responses. Nature Cell Biology, 14, 802-809.

Oh, J., Park, E., Song, K., Bae, G., & Choi, G. (2020). PHYTOCHROME INTERACTING FACTOR8 inhibits phytochrome A-mediated far-red light responses in Arabidopsis. The Plant Cell, *32*(1), 186-205.

O'Hara, A., & Jenkins, G. I. (2012). In vivo function of tryptophans in the Arabidopsis UV-B photoreceptor UVR8. The Plant Cell, *24*(9), 3755-3766.

Ohgishi, M., Saji, K., Okada, K., & Sakai, T. (2004). Functional analysis of each blue light receptor, cry1, cry2, phot1, and phot2, by using combinatorial multiple mutants in Arabidopsis. Proceedings of the National Academy of Sciences, *101*(8), 2223-2228.

Okamuro, J. K., Caster, B., Villarroel, R., Van Montagu, M., & Jofuku, K. D. (1997). The AP2 domain of APETALA2 defines a large new family of DNA binding proteins in Arabidopsis. Proceedings of the National Academy of Sciences, 94(13), 7076-7081.

Olsen, A. N., Ernst, H. A., Leggio, L. L., & Skriver, K. (2005). NAC transcription factors: structurally distinct, functionally diverse. Trends in Plant Science, 10(2), 79-87.

Omidbakhshfard, M. A., Proost, S., Fujikura, U., & Mueller-Roeber, B. (2015). Growth-regulating factors (GRFs): a small transcription factor family with important functions in plant biology. Molecular Plant, 8(7), 998-1010.

Oravecz, A., A. Baumann, Z. Máté, A. Brzezinska, J. Molinier, E. J. Oakeley, E. Adam, E. Schafer, F. Nagy & R. Ulm (2006). "CONSTITUTIVELY PHOTOMORPHOGENIC1 is required for the UV-B response in Arabidopsis." The Plant Cell, 18(8): 1975-1990.

Osterlund, M. T., Hardtke, C. S., Wei, N., & Deng, X. W. (2000). Targeted destabilization of HY5 during light-regulated development of Arabidopsis. Nature, *405*(6785), 462-466.

Paik, I., Kathare, P. K., Kim, J. I., & Huq, E. (2017). Expanding roles of PIFs in signal integration from multiple processes. Molecular Plant, *10*(8), 1035-1046.

Park, E., Park, J., Kim, J., Nagatani, A., Lagarias, J. C., & Choi, G. (2012). Phytochrome B inhibits binding of phytochrome-interacting factors to their target promoters. The Plant Journal, *72*(4), 537-546.

Park, Y. J., Kim, J. Y., Lee, J. H., Lee, B. D., Paek, N. C., & Park, C. M. (2020). GIGANTEA shapes the photoperiodic rhythms of thermomorphogenic growth in Arabidopsis. Molecular Plant, *13*(3), 459-470.

Park, Y. J., Lee, H. J., Ha, J. H., Kim, J. Y., & Park, C. M. (2017). COP 1 conveys warm temperature information to hypocotyl thermomorphogenesis. New Phytologist, *215*(1), 269-280.

Paul, N. D., & Gwynn-Jones, D. (2003). Ecological roles of solar UV radiation: towards an integrated approach. Trends in Ecology & Evolution, *18*(1), 48-55.

Pedmale, U.V., Huang, S.S., Zander, M., Cole, B.J., Hetzel, J., Ljung, K., Reis, P.A., Sridevi, P., Nito, K., Nery, J.R., et al. (2016). Cryptochromes interact directly with PIFs to control plant growth in limiting blue light. Cell, 164, 233-245.

Penfield, S., Josse, E. M., & Halliday, K. J. (2010). A role for an alternative splice variant of PIF6 in the control of Arabidopsis primary seed dormancy. Plant Molecular Biology, *73*, 89-95.

Petersen, J., Inoue, S. I., Kelly, S. M., Sullivan, S., Kinoshita, T., & Christie, J. M. (2017). Functional characterization of a constitutively active kinase variant of Arabidopsis phototropin 1. Journal of Biological Chemistry, 292(33), 13843-13852.

Pfeiffer, A., Shi, H., Tepperman, J. M., Zhang, Y., & Quail, P. H. (2014). Combinatorial complexity in a transcriptionally centered signaling hub in Arabidopsis. Molecular Plant, *7*(11), 1598-1618.

Pham, V. N., Kathare, P. K., & Huq, E. (2018a). Phytochromes and phytochrome interacting factors. Plant Physiology, *176*(2), 1025-1038.

Pham, V. N., Kathare, P. K. & Huq, E. (2018b). Dynamic regulation of PIF5 by COP-SPA complex to optimise photomorphogenesis in Arabidopsis. Plant Journal, 96, 260–273

Pham, V. N., Xu, X., & Huq, E. (2018c). Molecular bases for the constitutive photomorphogenic phenotypes in Arabidopsis. Development, *145*(23), dev169870.

Piper, R. C., & Luzio, J. P. (2007). Ubiquitin-dependent sorting of integral membrane proteins for degradation in lysosomes. Current Opinion in Cell Biology, *19*(4), 459-465.

Pokorny, R., Klar, T., Hennecke, U., Carell, T., Batschauer, A., & Essen, L. O. (2008). Recognition and repair of UV lesions in loop structures of duplex DNA by DASH-type cryptochrome. Proceedings of the National Academy of Sciences, *105*(52), 21023-21027.

Proveniers, M. C., & van Zanten, M. (2013). High temperature acclimation through PIF4 signaling. Trends in Plant Science, *18*(2), 59-64.

Qiu, Y., Pasoreck, E. K., Reddy, A. K., Nagatani, A., Ma, W., Chory, J., & Chen, M. (2017). Mechanism of early light signaling by the carboxy-terminal output module of Arabidopsis phytochrome B. Nature Communications, *8*(1), 1905.

Qiu, Y., Li, M., Kim, R. J. A., Moore, C. M., & Chen, M. (2019). Daytime temperature is sensed by phytochrome B in Arabidopsis through a transcriptional activator HEMERA. Nature Communications, *10*(1), 140.

Qiu, Y., E. K. Pasoreck, C. Y. Yoo, J. He, H. Wang, A. Bajracharya, M. Li, H. D. Larsen, S. Cheung & M. Chen (2021). "RCB initiates Arabidopsis thermomorphogenesis by stabilizing the thermoregulator PIF4 in the daytime." Nature Communications, 12(1): 2042.

Quint, M., Delker, C., Franklin, K. A., Wigge, P. A., Halliday, K. J., & van Zanten, M. (2016). Molecular and genetic control of plant thermomorphogenesis. Nature Plants, *2*(1), 15190.

Raschke, A., C. Ibañez, K. K. Ullrich, M. U. Anwer, S. Becker, A. Glöckner, J. Trenner, K. Denk, B. Saal & X. Sun (2015). "Natural variants of ELF3 affect thermomorphogenesis by transcriptionally modulating PIF4-dependent auxin response genes." BMC Plant Biology, 15(1): 1-10.

Raffaele, S., Rivas, S., & Roby, D. (2006). An essential role for salicylic acid in AtMYB30-mediated control of the hypersensitive cell death program in Arabidopsis. FEBS Letters, *580*(14), 3498-3504.

Raffaele, S., F. Vailleau, A. Leger, J. Joubès, O. Miersch, C. Huard, E. Blée, S. Mongrand, F. Domergue & D. Roby (2008). "A MYB transcription factor regulates very-long-chain fatty acid biosynthesis for activation of the hypersensitive cell death response in Arabidopsis." The Plant Cell, 20(3): 752-767.

Ren, H., J. Han, P. Yang, W. Mao, X. Liu, L. Qiu, C. Qian, Y. Liu, Z. Chen & X. Ouyang (2019). "Two E3 ligases antagonistically regulate the UV-B response in Arabidopsis." Proceedings of the National Academy of Sciences, 116(10): 4722-4731.

Reyes, J. C., Muro-Pastor, M. I., & Florencio, F. J. (2004). The GATA family of transcription factors in Arabidopsis and rice. Plant Physiology, 134(4), 1718-1732.

Rizzini, L., J.-J. Favory, C. Cloix, D. Faggionato, A. O'Hara, E. Kaiserli, R. Baumeister, E. Schäfer, F. Nagy & G. I. Jenkins (2011). "Perception of UV-B by the Arabidopsis UVR8 protein." Science, 332(6025): 103-106.

Rieu, I., S. Eriksson, S. J. Powers, F. Gong, J. Griffiths, L. Woolley, R. Benlloch, O. Nilsson, S. G. Thomas & P. Hedden (2008). "Genetic analysis reveals that C19-GA 2-oxidation is a major gibberellin inactivation pathway in Arabidopsis." The Plant Cell, 20(9): 2420-2436.

Roig-Villanova, I., Bou-Torrent, J., Galstyan, A., Carretero-Paulet, L., Portoles, S., Rodríguez-Concepción, M., & Martínez-García, J. F. (2007). Interaction of shade avoidance and auxin responses: a role for two novel atypical bHLH proteins. The EMBO Journal, *26*(22), 4756-4767.

Rolauffs, S., Fackendahl, P., Sahm, J., Fiene, G., & Hoecker, U. (2012). Arabidopsis COP1 and SPA genes are essential for plant elongation but not for acceleration of flowering time in response to a low red light to far-red light ratio. Plant Physiology, *160*(4), 2015-2027.

Romero-Montepaone, S., R. Sellaro, C. Esteban Hernando, C. Costigliolo-Rojas, L. Bianchimano, E. L. Ploschuk, M. J. Yanovsky & J. J. Casal (2021). "Functional convergence of growth responses to shade and warmth in Arabidopsis." New Phytologist, 231(5): 1890-1905.

Rosinski, J. A., & Atchley, W. R. (1998). Molecular evolution of the Myb family of transcription factors: evidence for polyphyletic origin. Journal of Molecular Evolution, *46*, 74-83.

Roy, A., A. Dutta, D. Roy, P. Ganguly, R. Ghosh, R. K. Kar, A. Bhunia, J. Mukhobadhyay & S. Chaudhuri (2016). "Deciphering the role of the AT-rich interaction domain and the HMG-box domain of ARID-HMG proteins of Arabidopsis thaliana." Plant Molecular Biology, 92: 371-388.

Rozema, J., van de Staaij, J., Björn, L. O., & Caldwell, M. (1997). UV-B as an environmental factor in plant life: stress and regulation. Trends in Ecology & Evolution, *12*(1), 22-28.

Saijo, Y., Sullivan, J.A., Wang, H., Yang, J., Shen, Y., Rubio, V., Ma, L., Hoecker, U., & Deng, X.W. (2003). The COP1–SPA1 interaction defines a critical step in phytochrome A-mediated regulation of HY5 activity. Genes & Development, 17: 2642–2647

Sakai, T., T. Kagawa, M. Kasahara, T. E. Swartz, J. M. Christie, W. R. Briggs, M. Wada & K. Okada (2001). "Arabidopsis nph1 and npl1: blue light receptors that mediate both phototropism and chloroplast relocation." Proceedings of the National Academy of Sciences, 98(12): 6969-6974.

Sakamoto, K., & Nagatani, A. (1996). Nuclear localization activity of phytochrome B. *The Plant Journal*, *10*(5), 859-868.Sakamoto K, Briggs WR (2002) Cellular and subcellular localization of phototropin 1. Plant Cell, 14(8):1723–1735

Sakuraba, Y., Jeong, J., Kang, M. Y., Kim, J., Paek, N. C., & Choi, G. (2014). Phytochrome-interacting transcription factors PIF4 and PIF5 induce leaf senescence in Arabidopsis. Nature Communications, *5*(1), 1-13.

Sanchez-Bermejo, E., W. Zhu, C. Tasset, H. Eimer, S. Sureshkumar, R. Singh, V. Sundaramoorthi, L. Colling & S. Balasubramanian (2015). "Genetic architecture of natural variation in thermal responses of Arabidopsis." Plant Physiology, 169(1): 647-659.

Santi, L., Y. Wang, M. R. Stile, K. Berendzen, D. Wanke, C. Roig, C. Pozzi, K. Müller, J. Müller & W. Rohde (2003). "The GA octodinucleotide repeat binding factor BBR participates in the transcriptional regulation of the homeobox gene Bkn3." The Plant Journal, 34(6): 813-826.

Santiago, J., Henzler, C., & Hothorn, M. (2013). Molecular mechanism for plant steroid receptor activation by somatic embryogenesis co-receptor kinases. Science, *341*(6148), 889-892.

Schaller, G. E., & Kieber, J. J. (2002). Ethylene. The Arabidopsis book/American Society of Plant Biologists, 1.

Sgarra, R., J. Lee, M. A. Tessari, S. Altamura, B. Spolaore, V. Giancotti, M. T. Bedford & G. Manfioletti (2006). "The AT-hook of the chromatin architectural transcription factor high mobility group A1a is arginine-methylated by protein arginine methyltransferase 6." Journal of Biological Chemistry, 281(7): 3764-3772.

Sellaro, R., Hoecker, U., Yanovsky, M., Chory, J., & Casal, J. J. (2009). Synergism of red and blue light in the control of Arabidopsis gene expression and development. Current Biology, *19*(14), 1216-1220.

Seo, H. S., Yang, J. Y., Ishikawa, M., Bolle, C., Ballesteros, M. L., & Chua, N. H. (2003). LAF1 ubiquitination by COP1 controls photomorphogenesis and is stimulated by SPA1. Nature, *423*(6943), 995-999.

Sharma, A., Sharma, B., Hayes, S., Kerner, K., Hoecker, U., Jenkins, G. I., & Franklin, K. A. (2019). UVR8 disrupts stabilisation of PIF5 by COP1 to inhibit plant stem elongation in sunlight. Nature Communications, *10*(1), 4417.

She, J., Z. Han, T.-W. Kim, J. Wang, W. Cheng, J. Chang, S. Shi, J. Wang, M. Yang and Z.-Y. Wang (2011). "Structural insight into brassinosteroid perception by BRI1." Nature, 474(7352): 472-476.

Shen, H., Moon, J., & Huq, E. (2005). PIF1 is regulated by light-mediated degradation through the ubiquitin-26S proteasome pathway to optimize photomorphogenesis of seedlings in Arabidopsis. The Plant Journal, *44*(6), 1023-1035.

Sheerin, D. J., C. Menon, S. zur Oven-Krockhaus, B. Enderle, L. Zhu, P. Johnen, F. Schleifenbaum, Y.-D. Stierhof, E. Huq & A. Hiltbrunner (2015). "Light-activated phytochrome A and B interact with members of the SPA family to promote photomorphogenesis in Arabidopsis by reorganizing the COP1/SPA complex." The Plant Cell, 27(1): 189-201.

Shen, H., Moon, J., & Huq, E. (2005). PIF1 is regulated by light-mediated degradation through the ubiquitin-26S proteasome pathway to optimize photomorphogenesis of seedlings in Arabidopsis. The Plant Journal, *44*(6), 1023-1035.

Shen, H., Zhu, L., Castillon, A., Majee, M., Downie, B., & Huq, E. (2008). Light-induced phosphorylation and degradation of the negative regulator PHYTOCHROME-INTERACTING FACTOR1 from Arabidopsis depend upon its direct physical interactions with photoactivated phytochromes. Plant Cell, 20: 1586–1602.

Shi, H., Zhong, S., Mo, X., Liu, N., Nezames, C.D., & Deng, X.W. (2013). HFR1 sequesters PIF1 to govern the transcriptional network underlying light-initiated seed germination in Arabidopsis. Plant Cell, 25: 3770–3784.

Shimizu-Sato, S., Huq, E., Tepperman, J. M., & Quail, P. H. (2002). A light-switchable gene promoter system. Nature Biotechnology, *20*(10), 1041-1044.

Shin, J., Kim, K., Kang, H., Zulfugarov, I.S., Bae, G., Lee, C.H., Lee, D., & Choi, G. (2009). Phytochromes promote seedling light responses by inhibiting four negatively-acting phytochrome interacting factors. Proceedings of the National Academy of Sciences of the United States of America, 106: 7660–7665.

Shor, E., Paik, I., Kangisser, S., Green, R., & Huq, E. (2017). PHYTOCHROME INTERACTING FACTORS mediate metabolic control of the circadian system in Arabidopsis. New Phytologist, *215*(1), 217-228.

Smirnova, O. G., Stepanenko, I. L., & Shumny, V. K. (2012). Mechanism of action and activity regulation of COP1, a constitutive repressor of photomorphogenesis. Russian Journal of Plant Physiology, *59*, 155-166.

Solano, R., Stepanova, A., Chao, Q., & Ecker, J.R. (1998). Nuclear events in ethylene signaling: A transcriptional cascade mediated by ETHYLENE-INSENSITIVE3 and ETHYLENE-RESPONSE-FACTOR1. Genes & Development, 12: 3703–3714.

Somers, D. E., Devlin, P. F., & Kay, S. A. (1998). Phytochromes and cryptochromes in the entrainment of the Arabidopsis circadian clock. Science, *282*(5393), 1488-1490.

Song, Y., Yang, C., Gao, S., Zhang, W., Li, L., & Kuai, B. (2014). Age-triggered and dark-induced leaf senescence require the bHLH transcription factors PIF3, 4, and 5. Molecular Plant, *7*(12), 1776-1787.

Soy, J., Leivar, P., González-Schain, N., Sentandreu, M., Prat, S., Quail, P. H., & Monte, E. (2012). Phytochrome-imposed oscillations in PIF3 protein abundance regulate hypocotyl growth under diurnal light/dark conditions in Arabidopsis. The Plant Journal, *71*(3), 390-401.

Soy, J., P. Leivar, N. González-Schain, G. Martín, C. Diaz, M. Sentandreu, B. Al-Sady, P. H. Quail & E. Monte (2016). "Molecular convergence of clock and photosensory pathways through PIF3–TOC1 interaction and co-occupancy of target promoters." Proceedings of the National Academy of Sciences, 113(17): 4870-4875.

Stapleton, A. E., & Walbot, V. (1994). Flavonoids can protect maize DNA from the induction of ultraviolet radiation damage. Plant Physiology, *105*(3), 881-889.

Stavang, J. A., J. Gallego-Bartolomé, M. D. Gómez, S. Yoshida, T. Asami, J. E. Olsen, J. L. García-Martínez, D. Alabadí & M. A. Blázquez (2009). "Hormonal regulation of temperature-induced growth in Arabidopsis." The Plant Journal, 60(4): 589-601.

Steinmüller, D., & Tevini, M. (1985). Action of ultraviolet radiation (UV-B) upon cuticular waxes in some crop plants. Planta, *164*(4), 557-564.

Stephenson, P. G., Fankhauser, C., & Terry, M. J. (2009). PIF3 is a repressor of chloroplast development. Proceedings of the National Academy of Sciences, *106*(18), 7654-7659.

Stracke, R., Werber, M., & Weisshaar, B. (2001). The R2R3-MYB gene family in Arabidopsis thaliana. Current Opinion in Plant Biology, 4(5), 447-456.

Stracke, R., J. J. Favory, H. Gruber, L. Bartelniewoehner, S. Bartels, M. Binkert, M. Funk, B. Weisshaar & R. Ulm (2010). "The Arabidopsis bZIP transcription factor HY5 regulates expression of the PFG1/MYB12 gene in response to light and ultraviolet-B radiation." Plant, Cell & Environment, 33(1): 88-103.

Sun, Y., X.-Y. Fan, D.-M. Cao, W. Tang, K. He, J.-Y. Zhu, J.-X. He, M.-Y. Bai, S. Zhu & E. Oh (2010). "Integration of brassinosteroid signal transduction with the transcription network for plant growth regulation in Arabidopsis." Developmental Cell, 19(5): 765-777.

Sun, J., Qi, L., Li, Y., Chu, J., & Li, C. (2012). PIF4–mediated activation of YUCCA8 expression integrates temperature into the auxin pathway in regulating Arabidopsis hypocotyl growth. PLoS Genetics, *8*(3), e1002594.

Sun, J., Qi, L., Li, Y., Zhai, Q., & Li, C. (2013). PIF4 and PIF5 transcription factors link blue light and auxin to regulate the phototropic response in Arabidopsis. Plant Cell, 25, 2102-2114.

Sun, Y., Han, Z., Tang, J., Hu, Z., Chai, C., Zhou, B., & Chai, J. (2013). Structure reveals that BAK1 as a co-receptor recognizes the BRI1-bound brassinolide. Cell Research, *23*(11), 1326-1329.

Sun, Q., Wang, S., Xu, G., Kang, X., Zhang, M., & Ni, M. (2019). SHB1 and CCA1 interaction desensitizes light responses and enhances thermomorphogenesis. Nature Communications, *10*(1), 3110.

Swaminathan, K., Peterson, K., & Jack, T. (2008). The plant B3 superfamily. Trends in Plant Science, 13(12), 647-655.

Tang, W., M. Yuan, R. Wang, Y. Yang, C. Wang, J. A. Oses-Prieto, T.-W. Kim, H.-W. Zhou, Z. Deng & S. S. Gampala (2011). "PP2A activates brassinosteroid-responsive gene expression and plant growth by dephosphorylating BZR1." Nature Cell Biology, 13(2): 124-131.

Tao, Y., J.-L. Ferrer, K. Ljung, F. Pojer, F. Hong, J. A. Long, L. Li, J. E. Moreno, M. E. Bowman & L. J. Ivans (2008). "Rapid synthesis of auxin via a new tryptophan-dependent pathway is required for shade avoidance in plants." Cell, 133(1): 164-176.

Tavridou, E., Pireyre, M., & Ulm, R. (2020). Degradation of the transcription factors PIF4 and PIF5 under UV-B promotes UVR8-mediated inhibition of hypocotyl growth in Arabidopsis. The Plant Journal, *101*(3), 507-517.

Toettcher, J. E., Gong, D., Lim, W. A., & Weiner, O. D. (2011a). Light control of plasma membrane recruitment using the Phy–PIF system. In *Methods in enzymology* (Vol. 497, pp. 409-423). Academic Press,

Toettcher, J. E., Gong, D., Lim, W. A., & Weiner, O. D. (2011b). Light-based feedback for controlling intracellular signaling dynamics. Nature Methods, *8*(10), 837-839.

Toettcher, J. E., Weiner, O. D., & Lim, W. A. (2013). Using optogenetics to interrogate the dynamic control of signal transmission by the Ras/Erk module. Cell, *155*(6), 1422-1434.

Toledo-Ortiz, G., Huq, E., & Quail, P. H. (2003). The Arabidopsis basic/helix-loop-helix transcription factor family. The Plant Cell, *15*(8), 1749-1770.

Toledo-Ortiz, G., H. Johansson, K. P. Lee, J. Bou-Torrent, K. Stewart, G. Steel, M. Rodriguez-Concepcion & K. J. Halliday (2014). "The HY5-PIF regulatory module coordinates light and temperature control of photosynthetic gene transcription." PLoS Genetics, 10(6): e1004416.

Ulm, R., A. Baumann, A. Oravecz, Z. Máté, É. Ádám, E. J. Oakeley, E. Schäfer & F. Nagy (2004). "Genome-wide analysis of gene expression reveals function of the bZIP transcription factor HY5 in the UV-B response of Arabidopsis." Proceedings of the National Academy of Sciences, 101(5): 1397-1402.

Ulm, R., & Nagy, F. (2005). Signalling and gene regulation in response to ultraviolet light. Current Opinion in Plant Biology, *8*(5), 477-482.

Vailleau, F., Daniel, X., Tronchet, M., Montillet, J. L., Triantaphylidès, C., & Roby, D. (2002). A R2R3-MYB gene, AtMYB30, acts as a positive regulator of the hypersensitive cell death program in plants in response to pathogen attack. Proceedings of the National Academy of Sciences, *99*(15), 10179-10184.

Van Zanten, M., Voesenek, L. A., Peeters, A. J., & Millenaar, F. F. (2009). Hormone-and light-mediated regulation of heat-induced differential petiole growth in Arabidopsis. Plant Physiology, *151*(3), 1446-1458.

Van Zanten, M., Pons, T. L., Janssen, J. A. M., Voesenek, L. A. C. J., & Peeters, A. J. M. (2010). On the relevance and control of leaf angle. Critical Reviews in Plant Science, *29*(5), 300-316.

Vasseur, F., Pantin, F., & Vile, D. (2011). Changes in light intensity reveal a major role for carbon balance in Arabidopsis responses to high temperature. Plant, Cell & Environment, *34*(9), 1563-1576.

Vile, D., M. Pervent, M. Belluau, F. Vasseur, J. Bresson, B. Muller, C. Granier & T. Simonneau (2012). "Arabidopsis growth under prolonged high temperature and water deficit: independent or interactive effects?" Plant, Cell & Environment, 35(4): 702-718.

Xu, X., Paik, I., Zhu, L., & Huq, E. (2015). Illuminating progress in phytochrome-mediated light signaling pathways. Trends in Plant Science, *20*(10), 641-650.

Xu, X., Kathare, P. K., Pham, V. N., Bu, Q., Nguyen, A., & Huq, E. (2017). Reciprocal proteasome-mediated degradation of PIFs and HFR1 underlies photomorphogenic development in Arabidopsis. Development, *144*(10), 1831-1840.

Waltner, J. K., Peterson, F. C., Lytle, B. L., & Volkman, B. F. (2005). Structure of the B3 domain from Arabidopsis thaliana protein At1g16640. Protein Science, 14(9), 2478-2483.

Wang, S., Tiwari, S. B., Hagen, G., & Guilfoyle, T. J. (2005). AUXIN RESPONSE FACTOR7 restores the expression of auxin-responsive genes in mutant Arabidopsis leaf mesophyll protoplasts. The Plant Cell, 17(7), 1979-1993.

Wang, Z. Y., Seto, H., Fujioka, S., Yoshida, S., & Chory, J. (2001). BRI1 is a critical component of a plasma-membrane receptor for plant steroids. Nature, *410*(6826), 380-383.

Wang, Z.-Y., T. Nakano, J. Gendron, J. He, M. Chen, D. Vafeados, Y. Yang, S. Fujioka, S. Yoshida & T. Asami (2002). "Nuclear-localized BZR1 mediates brassinosteroid-induced growth and feedback suppression of brassinosteroid biosynthesis." Developmental Cell, 2(4): 505-513.

Wang, X., U. Kota, K. He, K. Blackburn, J. Li, M. B. Goshe, S. C. Huber & S. D. Clouse (2008). "Sequential transphosphorylation of the BRI1/BAK1 receptor kinase complex impacts early events in brassinosteroid signaling." Developmental Cell, 15(2): 220-235.

Wang, F. F., Lian, H. L., Kang, C. Y., & Yang, H. Q. (2010). Phytochrome B is involved in mediating red light-induced stomatal opening in Arabidopsis thaliana. Molecular Plant, *3*(1), 246-259.

Wang, H., & Deng, X. W. (2004). Phytochrome signaling mechanism. The Arabidopsis Book/American Society of Plant Biologists, *3*.

Ward, J. M., Cufr, C. A., Denzel, M. A., & Neff, M. M. (2005). The Dof transcription factor OBP3 modulates phytochrome and cryptochrome signaling in Arabidopsis. The Plant Cell, 17(2), 475-485.

Windram, O., P. Madhou, S. McHattie, C. Hill, R. Hickman, E. Cooke, D. J. Jenkins, C. A. Penfold, L. Baxter & E. Breeze (2012). "Arabidopsis defense against Botrytis cinerea: chronology and regulation deciphered by high-resolution temporal transcriptomic analysis." The Plant Cell, 24(9): 3530-3557.

de Wit, M., Lorrain, S., & Fankhauser, C. (2014). Auxin-mediated plant architectural changes in response to shade and high temperature. Physiologia Plantarum, *151*(1), 13-24.

Wu, D., Q. Hu, Z. Yan, W. Chen, C. Yan, X. Huang, J. Zhang, P. Yang, H. Deng & J. Wang (2012). "Structural basis of ultraviolet-B perception by UVR8." Nature, 484(7393): 214-219.

Wu, L., & Yang, H. Q. (2010). CRYPTOCHROME 1 is implicated in promoting R protein-mediated plant resistance to Pseudomonas syringae in Arabidopsis. Molecular Plant, *3*(3), 539-548.

Yamaguchi, R., Nakamura, M., Mochizuki, N., Kay, S. A., & Nagatani, A. (1999). Light-dependent translocation of a phytochrome B-GFP fusion protein to the nucleus in transgenic Arabidopsis. The Journal of Cell Biology, *145*(3), 437-445.

Yamaguchi, N., Winter, C. M., Wu, M. F., Kwon, C. S., William, D. A., & Wagner, D. (2014). PROTOCOLS: Chromatin immunoprecipitation from Arabidopsis tissues. The Arabidopsis book/American Society of Plant Biologists, *12*.

Yamashino, T. (2013). From a repressilator-based circadian clock mechanism to an external coincidence model responsible for photoperiod and temperature control of plant architecture in Arabodopsis thaliana. Bioscience, Biotechnology, and Biochemistry, *77*(1), 10-16.

Yamashino, T., Matsushika, A., Fujimori, T., Sato, S., Kato, T., Tabata, S., & Mizuno, T. (2003). A link between circadian-controlled bHLH factors and the APRR1/TOC1 quintet in Arabidopsis thaliana. Plant Cell Physiology, 44: 619–629.

Yamashino, T., Nomoto, Y., Lorrain, S., Miyachi, M., Ito, S., Nakamichi, N., Fankhauser, C., & Mizuno, T.(2013).Verification at the protein level of the PIF4-mediated external coincidence model for the temperature-adaptive photoperiodic control of plant growth in Arabidopsis thaliana. Plant Signaling & Behavior,8: e23390.

Yan, Y., C. Li, X. Dong, H. Li, D. Zhang, Y. Zhou, B. Jiang, J. Peng, X. Qin & J. Cheng (2020). "MYB30 is a key negative regulator of Arabidopsis photomorphogenic development that promotes PIF4 and PIF5 protein accumulation in the light." The Plant Cell, 32(7): 2196-2215.

Yang, D.-L., J. Yao, C.-S. Mei, X.-H. Tong, L.-J. Zeng, Q. Li, L.-T. Xiao, T.-p. Sun, J. Li & X.-W. Deng (2012). "Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade." Proceedings of the National Academy of Sciences, 109(19): E1192-E1200.

Yin, R., Arongaus, A. B., Binkert, M., & Ulm, R. (2015). Two distinct domains of the UVR8 photoreceptor interact with COP1 to initiate UV-B signaling in Arabidopsis. The Plant Cell, *27*(1), 202-213.

Yin, Y., Wang, Z. Y., Mora-Garcia, S., Li, J., Yoshida, S., Asami, T., & Chory, J. (2002). BES1 accumulates in the nucleus in response to brassinosteroids to regulate gene expression and promote stem elongation. Cell, *109*(2), 181-191.

Yin, R., Arongaus, A. B., Binkert, M., & Ulm, R. (2015). Two distinct domains of the UVR8 photoreceptor interact with COP1 to initiate UV-B signaling in Arabidopsis. *The Plant Cell*, *27*(1), 202-213.

Yin, Y., Vafeados, D., Tao, Y., Yoshida, S., Asami, T., & Chory, J. (2005). A new class of transcription factors mediates brassinosteroid-regulated gene expression in Arabidopsis. Cell, *120*(2), 249-259.

Yu, X., L. Li, J. Zola, M. Aluru, H. Ye, A. Foudree, H. Guo, S. Anderson, S. Aluru & P. Liu (2011). "A brassinosteroid transcriptional network revealed by genome-wide identification of BESI target genes in Arabidopsis thaliana." The Plant Journal, 65(4): 634-646.

Zayner, J. P., Antoniou, C., & Sosnick, T. R. (2012). The amino-terminal helix modulates light-activated conformational changes in AsLOV2. Journal of Molecular Biology, 419(1-2), 61-74.

Zavala, J. A., Mazza, C. A., Dillon, F. M., Chludil, H. D., & Ballare, C. L. (2015). Soybean resistance to stink bugs (N ezara viridula and P iezodorus guildinii) increases with exposure to solar UV-B radiation and correlates with isoflavonoid content in pods under field conditions. Plant, Cell & Environment, *38*(5), 920-928.

Zeng, Q., Q. Wang, X. Chen, K. Xia, J. Tang, X. Zhou, Y. Cheng, Y. Chen, L. Huang & H. Xiang (2016). "Analysis of lncRNAs expression in UVB-induced stress responses of melanocytes." Journal of Dermatological Science, 81(1): 53-60.

Zhai, H., L. Xiong, H. Li, X. Lyu, G. Yang, T. Zhao, J. Liu & B. Liu (2020). "Cryptochrome 1 inhibits shoot branching by repressing the self-activated transciption loop of PIF4 in Arabidopsis." Plant Communications, 1(3): 100042.

Zhang, Y., Schwarz, S., Saedler, H., & Huijser, P. (2007). SPL8, a local regulator in a subset of gibberellin-mediated developmental processes in Arabidopsis. Plant Molecular Biology, 63, 429-439.

Zhang, Y., Mayba, O., Pfeiffer, A., Shi, H., Tepperman, J.M., Speed, T.P., & Quail, P.H. (2013). A quartet of PIF bHLH factors provides a transcriptionally centered signaling hub that regulates seedling morphogenesis through differential expression-patterning of shared target genes in Arabidopsis. PLoS Genetics, 9: e1003244.

Zhang, Y., Liu, Z., Chen, Y., He, J. X., & Bi, Y. (2015). PHYTOCHROME-INTERACTING FACTOR 5 (PIF5) positively regulates dark-induced senescence and chlorophyll degradation in Arabidopsis. Plant Science, *237*, 57-68.

Zhang, B., Holmlund, M., Lorrain, S., Norberg, M., Bako, L., Fankhauser, C., & Nilsson, O. (2017). BLADE-ON-PETIOLE proteins act in an E3 ubiquitin ligase complex to regulate PHYTOCHROME INTERACTING FACTOR 4 abundance. Elife, *6*, e26759.15

Zhang, Y., Pfeiffer, A., Tepperman, J. M., Dalton-Roesler, J., Leivar, P., Grandio, E. G., & Quail, P. H. (2020). Central clock components modulate plant shade avoidance by directly repressing transcriptional activation activity of PIF proteins. Proceedings of the National Academy of Sciences, *117*(6), 3261-3269.

Zheng, Y., Schumaker, K. S., & Guo, Y. (2012). Sumoylation of transcription factor MYB30 by the small ubiquitin-like modifier E3 ligase SIZ1 mediates abscisic acid response in Arabidopsis thaliana. Proceedings of the National Academy of Sciences, *109*(31), 12822-12827.

Zhong, S., Shi, H., Xue, C., Wang, L., Xi, Y., Li, J., Quail, P.H., Deng, X.W., & Guo, H. (2012). A molecular framework of light-controlled phytohormone action in Arabidopsis. Current Biology, 22: 1530–1535.

Zhou, Y., Xun, Q., Zhang, D., Lv, M., Ou, Y., & Li, J. (2019). TCP transcription factors associate with PHYTOCHROME INTERACTING FACTOR 4 and CRYPTOCHROME 1 to regulate thermomorphogenesis in Arabidopsis thaliana. Iscience, *15*, 600-610.

Zhu, J. Y., Oh, E., Wang, T., & Wang, Z. Y. (2016a). TOC1–PIF4 interaction mediates the circadian gating of thermoresponsive growth in Arabidopsis. Nature Communications, *7*(1), 13692.

Zhu, L., Xin, R., Bu, Q., Shen, H., Dang, J., & Huq, E. (2016b). A negative feedback loop between PHYTOCHROME INTERACTING FACTORs and HECATE proteins fine-tunes photomorphogenesis in Arabidopsis. The Plant Cell, *28*(4), 855-874.

Zhu, L., Q. Bu, X. Xu, I. Paik, X. Huang, U. Hoecker, X. W. Deng & E. Huq (2015). "CUL4 forms an E3 ligase with COP1 and SPA to promote light-induced degradation of PIF1." Nature Communications, 6(1): 7245.

Zoltowski, B. D., & Imaizumi, T. (2014). Structure and function of the ZTL/FKF1/LKP2 group proteins in Arabidopsis. The Enzymes, *35*, 213-239.

Zourelidou, M., De Torres‐Zabala, M., Smith, C., & Bevan, M. W. (2002). Storekeeper defines a new class of plant-specific DNA-binding proteins and is a putative regulator of patatin expression. The Plant Journal, 30(4), 489-497.

Zuo, Z., Liu, H., Liu, B., Liu, X., & Lin, C. (2011). Blue light-dependent interaction of CRY2 with SPA1 regulates COP1 activity and floral initiation in Arabidopsis. Current Biology, *21*(10), 841-847.