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Photoreceptor cross-talk in UV-B photomorphogenesis in tomato (*Solanum lycopersicum)*: Screening through phytochrome and cryptochrome mutants

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Abstract

Plant photoreceptors detect changes in the light environment and induce differential gene expression, resulting in the appropriate physiological and morphological responses. Under full sunlight, phytochromes, cryptochromes and the UV-B photoreceptor, UVR8 (UV-B RESISTANCE LOCUS 8), destabilize PHYTOCHROME INTERACTING FACTORS (PIFs) to inhibit elongation. PIFs are transcriptions factors that inhibit light-regulated genes, including auxin-related genes involved in cell elongation. In the shaded environment, the reduction in the spectral composition detected by the photoreceptors results in the activation of elongation and PIF activity. However, recent studies have shown that low levels of UV-B can still inhibit the elongation under shade.

Most photobiology studies that investigated plant responses to shade have concentrated on the model species, *Arabidopsis thaliana*. In contrast, *Solanum lycopersicum* (tomato) is another model system, but few studies have investigated plant responses to shade in tomato due to its sympodial architecture and presence of internodes which *A. thaliana* lacks. In this study, phytochrome and cryptochrome tomato mutants were exposed to low levels of UV-B under photosynthetically active radiation (PAR) as background light to investigate the possible cross-talk between these photoreceptors and the UV-B photoreceptor of tomato in regulating hypocotyl or internode elongation. Out of all the multiple phytochrome and one cryptochrome mutants, *phyAphyB2* mutant exhibited an impaired UV-B inhibition of internode elongation after three days of UV-B treatment. End-point PCR on the gene expression of PIF4 together with two UV-B responsive genes and genes involved in the catabolism of active gibberellin could not explain the impaired response of *phyAphyB2*. Nevertheless, physiological measurements indicate that phyA and phyB2 of tomato may be acting redundantly in mediating the UV-B induced inhibition of internode.

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Abbreviations

APA	Active binding domain of PHYA
APB	Active binding domain of PHYB
au	*aurea*
B	Blue light
CHS	Chalcone synthase
COP	CONSTITUTIVE PHOTOMORPHOGENIC 1
CRY	Cryptochrome protein
DET	DEETIOLATED
ein	elongated internode; *Brassica phyB* mutant
EOD-FR	End-of-day-far-red
EODT-PAR	End-of-day-treatment-PAR
EODT-UV	End-of-day-treatment-UVB
FAD	Flavin adenine dinucleotide
FHL	FAR-RED ELONGATED HYPOCOTYL LIKE
FR	Far-red light
fri	far-red insensitive; tomato *phyA* mutant
FUS	FUSCA
G	Green light
GA	Gibberellic acid
Ga2ox2	GIBBERELLIC ACID 2 OXIDASES 2
HFR1	HYPOCOTYL IN FAR RED 1
HIR	High irradiance response
HY5	LONG HYPOCOTYL 5
HYH	HY5 HOMOLOG

JA	Jasmonic acid
LeHY5	Tomato LONG HYPOCOTYL 5 gene
LFR	Low fluence response
lh	long hypocotyl
MM	Money maker
nm	Nanometer
PAR	Photosynthetically active radiation
Pfr	Active form of phytochrome capable of absorbing FR light
PHY	Phytochromes
PHYA, PHYB	Phytochrome A, phytochrome B, etc. apoprotein
PHYA, PHYB	Phytochrome A, phytochrome B, etc. gene
phyA, phyB	Phytochrome A, phytochrome B, etc. holoprotein
phyA, phyB	Phytochrome A, phytochrome B, etc. mutant
PIF	PHYTOCHROME INTERACTING FACTOR
Pr	Inactive form of phytochrome capable of absorbing R light
R	Red light
RCC1	Regulator of chromatin condensation 1
SAM	Shoot apical meristem
SAR	Shade avoidance response
sav3-2	mutant with a defect in the TAA1 pathway
FH1	FAR-RED ELONGATED HYPOCOTYL 1
SlPIF	Solanum lycopersicum PHYTOCHROME INTERACTING FACTOR
SPA	SUPRESSOR OF PHYTOCHROME A
TAA1	Tryptophan aminotransferase of *Arabidopsis* 1
tri	temporary insensitive; tomato *phyB1* mutant

Trp	Tryptophan
UV	Ultraviolet light
UVR8	UV-B RESISTANCE LOCUS 8
VLFR	Very low fluence response
WL	White light
WT	Wild-type tomato

1. Introduction

Plants are immobile organisms that use light as a source of energy and information. Unable to move, plants must synchronize their development to the changes in the light environment and at the same time increase adaptability (Novoplansky, 2009). Adaptability is driven by the demand on efficient and sufficient photosynthesis (Kharshiing & Sinha, 2015) to ensure proper growth and development (Sarlikioti et al, 2011). Plants are equipped with photoreceptors that act as light sensors capable of absorbing a specific wavelength of light from the electromagnetic spectrum. There are five different photoreceptors that can absorb light in the spectrum: phytochromes, cryptochromes, phototropins, ZEITLUPE family proteins and the UV-B photoreceptor (UV-B RESISTANCE LOCUS 8; UVR8). These photoreceptors detect the changes in the quality and quantity of light under shade, and lead to differential gene expression which, upregulate or downregulate biochemical pathways throughout the development of the plant (Roig-Villanova & Martinez-Garcia, 2016) and modify the morphology of the plant. In this literature review, the focus will be on the comparative roles of plant photoreceptors in orchestrating responses to shade avoidance. In my experimental work, the wild-type (WT), phytochromes, and cryptochromes of tomato (*Solanum lycopersicum*) photoreceptor mutants were used to investigate if a possible cross-talk between these photoreceptors, and the unidentified UV-B photoreceptor of tomato to UV-B induced hypocotyl and internode inhibition under PAR (Photosynthetically active radiation; red and blue) background.

1.1 Light detected by plants

Plants utilize light for photosynthesis but also use light to detect changes in their environment. Part of the electromagnetic spectrum (Figure 1.1) consists of the visible light, which spreads from 400 nm to 700 nm, and UV light from 100 nm to 400 nm. Visible light can be divided

into red (R), blue (B), green (G) and far-red (FR); and ultra-violet (UV) light can be divided into three: UV-A (400-320 nm), UV-B (315-280 nm) and UV-C (280-100 nm) (Hollósy, 2002). Most photobiological studies have historically focused on the visible spectra including 700-800 nm (FR), yet interest has increased in the biology of invisible (UV) wavelengths. Light absorption in the visible and UV spectrum typically controls plant growth and development. Atmospheric ozone reduces levels of UV penetrating the Earth's surface. Doses of UV-A and UV-B varies depending on the location- highest near the equator and reduced at high latitudes (Bais et al., 2015). UV-B that reaches Earth absorbed by plants can either cause damaging or non-damaging effects depending on the fluence rate. High levels of long wavelength UV-B are directly absorbed by the DNA and induce the production of highly reactive oxygen species that can damage the plant's DNA (Frohnmeyer & Staiger, 2003). In contrast, low levels of UV-B have shown to activate the synthesis of UV-B protective compounds; promote and inhibit plant growth (Ulm, 2006). Among the three types of UV, UV-C lies within the shorter wavelength and high energy per photon, which does not reach the Earth's surface (Zlatev et al., 2012). Hence, most UV studies have focused on the effect of UV-B on plant development.

1.2 *Arabidopsis*

Most physiological studies on plant photoreceptors have focused on the model species *Arabidopsis thaliana.* Three major photoreceptor families have been characterized in *A. thaliana* and regulate plant responses to light: phytochrome (PHY; detect R and FR), cryptochromes (CRY; detect blue and UV-A/B) and the UV-B photoreceptor, UVR8 (Kliebenstein et al, 2002; Rizzini et al., 2011). There are two other photoreceptors- phototropin and ZEITLIPE family of proteins that also absorb in the blue region. Phototropins are UV-A/ blue light photoreceptors involved in the phototropism, stomatal opening and migration of chloroplast (Casal, 2000; Parihar et al., 2016). ZEITLUPE also absorb B light but has its main

influence on the plants' circadian clock. Since the phototropin and ZEITLUPE photoreceptors only have minor roles in mediating elongation growth response and were not used in the experiments, these photoreceptors will not be discussed here. Nonetheless, the interactions of these photoreceptors are crucial in synchronizing the plants' development to the changing light environment.

Figure 1.1: Electromagnetic spectrum detected by plant photoreceptors. Photoreceptor proteins contain chromophores that absorb light in the electromagnetic spectrum. Tryptophan (Trp) is the chromophore of the UVR8 photoreceptor protein that perceive UV-B; Flavin Adenine Dinucleotide and methyltetrahydrofolate (MTHF) are two chromophores of crytochromes; Flavin Mononucleotide (FMN) are chromophores of phototropins and ZEITLUPES (ZTL) family proteins that also perceive blue light; phytochromobilin is the chromophore of phytochromes that perceives red and far-red light. Image adapted from Heijde and Ulm (2012).

1.2.1 Phytochromes

Phytochromes are homodimeric chromopeptides with an approximate size of 120-130 kDa and contain a tetrapyrrole chromophore called phytochromobilin (Casal, 2000, 2013; Pratt & Butler, 1970). Phytochromobillin is a tetrapyrrole chromophore that can absorb in two regions in the visible spectrum: R (600-700 nm) and FR (700-800 nm) (Smith & Whitelam, 1990) but can also absorb in the blue and UV region at low fluence rate (Pratt & Butler, 1970; Lercari et

al., 1990). Absorption changes the activity of the phytochromes from the inactive Pr to the active Pfr (Fig.1.2). The inactive Pr, which has an absorption maxima at 665 nm, absorb light

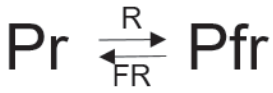

Figure 1.2: Photoequilibrium of the inactive (Pr) and active (Pfr) form of phytochromes. Absorbtion of R phototransform Pr into the Pfr which activates many biological responses. FR light absorbed by Pfr reverts it back to the inactive state, Pr.

in the R region and is converted to Pfr with an absorption maximum at 730 nm (Smith & Whitelam, 1990). Phytochromes are initially synthesized as their Pr form in the cytosol and photoconverted to the active Pfr upon absorption of R light (Klose et al, 2015). Active Pfr is translocated to the nucleus where it can directly interact with transcription factors and other growth-related genes. The phytochrome family in the model plant *A. thaliana* consists of PHYA, PHYB, PHYC, PHYD and PHYE. In tomatoes, there are five phytochromes: PHYA, PHYB1, PHYB2, PHYD and PHYE. The two phyB of tomatoes are not orthologues of *Arabidopsis* PHYB, and D. Phylogenetic analysis suggests that this was a consequence of independent duplication between *Brassicaceae* and *Solanaceae* (Pratt et al., 1997; Pratt et al., 1995). Nevertheless, responses of phytochromes between tomato and *Arabidopsis* are conserved.

1.2.1.1 Types of phytochromes

The existence of two distinct pools of phytochromes were first hypothesised by Hillman, (1967) even before it became evident that multiple phytochromes exists in the same genome (Furuya, 1993; Mathews, 2006). Biochemical, spectroscopy and immunochemical studies confirmed that two pools do exists as light labile and exists in etiolated seedlings, and one that is light stable and predominantly exists in light-grown plants (Furuya, 1993; Hillman, 1967;

Kerckhoffs, 1996). There are two types of phytochromes: TYPE I and II. This distinction allowed the identification of the functions of each photoreceptor based on their stability under light (Smith, 1995). TYPE I are light labile. The active Pfr of TYPE I phytochrome is unstable under light resulting in the reduction of the phytochrome activity. Phytochrome A has been identified as the TYPE I phytochrome since levels of PHYA in etiolated seedlings are reduced at lower steady state (Clough & Vierstra, 1997) upon transition from dark to light. TYPE II phytochromes consist of PHYB-PHYE in *Arabidopsis* and PHYB1, PHYB2, PHYD, and PHYE in tomatoes. These phytochromes are light stable and are photoreversible upon exposure to FR or end-of-day-far-red (EOD-FR) treatment. Defect on the functional form of these TYPE II photoreceptors show reduced response to red which denotes the function of PHYB family (Franklin & Quail, 2010; Sharrock, 2005).

1.2.1.2 Phytochrome response modes

Phytochromes work in three different modes: very low fluence response (VLFR), low fluence response (LFR) and high-irradiance response (HIR; Kerckhoffs, 1996; van Tuinen et al., 1995). VLFR activate biological responses upon absorption of very low fluence rate of light between 10^{-4} to 10^{-1} μmol m^{-2} (Botto et al.,1996; Shinomura et al., 1996). Seed germination responds to very low fluence rate, and PHYA mediates this developmental response. Experiments on seed exposure to pulses of FR light showed that absence of functional PHYA in *Arabidopsis*, reduced seed germination and that action spectra of VLFRs lies within monochromatic 300-780 nm to induce seed germination (Botto et al., 1996; Shinomura et al., 1996). PHYA can absorb light in the R and FR region, which results in the accumulation of active phytochrome in the nucleus. Accumulation of PHYA in the nucleus requires two chaperone proteins: FAR-RED ELONGATED HYPOCOTYL 1 (FHY1) and FAR-RED ELONGATED HYPOCOTYL-LIKE (FHL; Kaiserli and Chory, 2015). The active Pfr of PHYA must interact with FHY1/FHL

chaperone proteins to allow translocation into the nucleus. Additional absorption of FR, deactivates Pfr to Pr resulting in the detachment of the chaperone proteins. Absorption of R light is required to activate phyA Pfr and positively activate seed germination (Casal, 2013; Kaiserli & Chory, 2015; Shinomura, 1997). PhyB Pfr can also regulate seed germination at low FR light (Shinomura et al., 1996). Furthermore, PHYE can also regulate germination under continuous FR light (Hennig et al., 2002).

PhyB mediates low fluence response (LFR) and high irradiance response (HIR) since in the absence of functional *PHYB*, these responses are defective (Attridge, 1990; Mancinelli, 1994) and lose its photoreversibility (Mathews, 2010). PHYA can also respond to high fluence rate of far-red and activate far-red HIR. The inhibition of germination, de-etiolation, and stem growth are some of the developmental responses induced at low fluence rates between 1 to 1000 μmol m^{-1} (Botto et al., 1996; Shinomura et al., 1996). In contrast to PHYA translocation into the nucleus, PHYB does not require chaperone proteins to activate photomorphogenic responses (Kaiserli & Chory, 2015). LFRs are also common under canopy shade. In closed and highly dense canopy shade where levels of red are reduced by the absorption of photosynthetic pigments of upper leaves and reflect far-red, LFR commonly occurs (Mathews, 2010; Schäfer & Nagy, 2006). This response is also activated by short exposure to red light and reversed by far-red. In contrast, the HIR responses such as inhibition of hypocotyl or stem elongation are observed in plants exposed to continuous R or FR at high photon flux to photoconvert Pr to Pfr and Pfr to Pr (Fig.1.2). Since it involved the continuous irradiation of light, light-stable photoreceptors (PHYB-PHYE) control HIR (Schäfer & Nagy, 2006).

1.2.2 Cryptochromes

Cryptochromes can detect blue light and are also involved in seedling development. Two cryptochrome proteins have been characterized: CRY1 and CRY2. CRY1 regulate continuous

blue light hypocotyl inhibition and anthocyanin accumulation; CRY2 has a major role in flowering and is negatively regulated by high levels of blue light (Liu et al., 2016; Li & Yang, 2007; Wang et al., 2015). Both proteins bind to flavin adenine dinucleotide (FAD) in its reduced form (FADH) and FADH act as their chromophores to initiate photomorphogenic responses (Li & Yang, 2007). However, it is still unclear how this photoreduction mediate blue/UV-A induced photomorphogenesis due to other possible amino acid candidates involved in the photoexcitation of the FAD (Liu et al., 2010; Liu et al., 2016).

1.2.3 UVR8

UVR8 is a homodimer protein with high similarities to the human guanine nucleotide exchange factor regulator of chromatin condensation 1 (RCC1) (Christie et al., 2012; Kliebenstein et al., 2002). However, UVR8 and RCC1 have different function (Jenkins, 2009). The UVR8 homodimer dissociates into monomers upon UV-B absorption and accumulates in the nucleus and associates with chromatin through the histones (Brown et al., 2005). Two tryptophan (Trp): Trp285 and Trp233 are photoexcited by UV-B and act as the chromophore of UVR8. Absorption of UV-B by the Trp285 and Trp233 destabilizes the salt-bridges which connect the two monomers of UVR8 (Christie et al., 2012; Rizzini et al., 2011; Vanhaelewyn et al. 2016). The physiological function of UVR8 lies on the altered gene expression observed in its *uvr8* mutant form, as reviewed by Jenkins (2014). UVR8 mediate the UV-B specific induction of chalcone synthase (*CHS*), a gene which encodes the first enzyme involved in the flavonoid biosynthesis. *Arabidopsis uvr8* mutant exhibited reduced induction of CHS and displayed hypersensitivity to UV-B as shown by leaf necrosis and folding of cotyledons (Kliebenstein et al., 2002) suggesting its role in the induction of UV photoprotective compounds. UV-B can also alter plant morphology. The *uvr8* mutant exhibits long hypocotyl after irradiation of UV-B compared to short hypocotyls of the WT (Favory et al., 2009), and closed cotyledons.

1.3 Light and plant development

1.3.1 Stages of plant development and strategies under light limiting conditions

Light can influence the early stages of plant development (Schmitt et al., 1999). Plants undergo two different developmental stages: skotomorphogenesis and photomorphogenesis. Skotomorphogenesis is the development of the plant in the dark. Plants grown in the dark exhibit long pale hypocotyls, closed cotyledon and apical hook. Upon transition from seed to de-etiolated (photomorphogenic) form, the hypocotyl leaves are expanded and are subjected to light inhibition of hypocotyl elongation (Bögre & Beemster, 2008; Fernando & Schroeder, 2016; Ruberti et al., 2012). As plants continue to grow and develop, plant density can increase and eventually result in competition (Keuskamp et al., 2010). Plants have developed two strategies when subjected to shade from competition. Plants can either tolerate the shade or avoid it. Shade tolerant plants respond to the reduction of light quantity (Martínez-García et al., 2010). These plants do not invest resources towards elongation to outcompete neighbours, but through optimizing leaf photosynthesis. Instead of growth, plants focus on leaf survival through increasing its specific leaf area and increasing the photosynthetic system (Gommers et al., 2013). In contrast, shade avoiding plants exhibit longer stems or internodes, increased leaf angle, reduced branching and early flowering. Shade avoiding plants focus on growth to outgrow any neighbouring competition. Phenotypic responses of plants subjected to light competition resemble those exposed to low R:FR and reduced B light which, suggests that the phytochrome and cryptochrome photoreceptors are involved in regulating these responses (Pedmale et al., 2016).

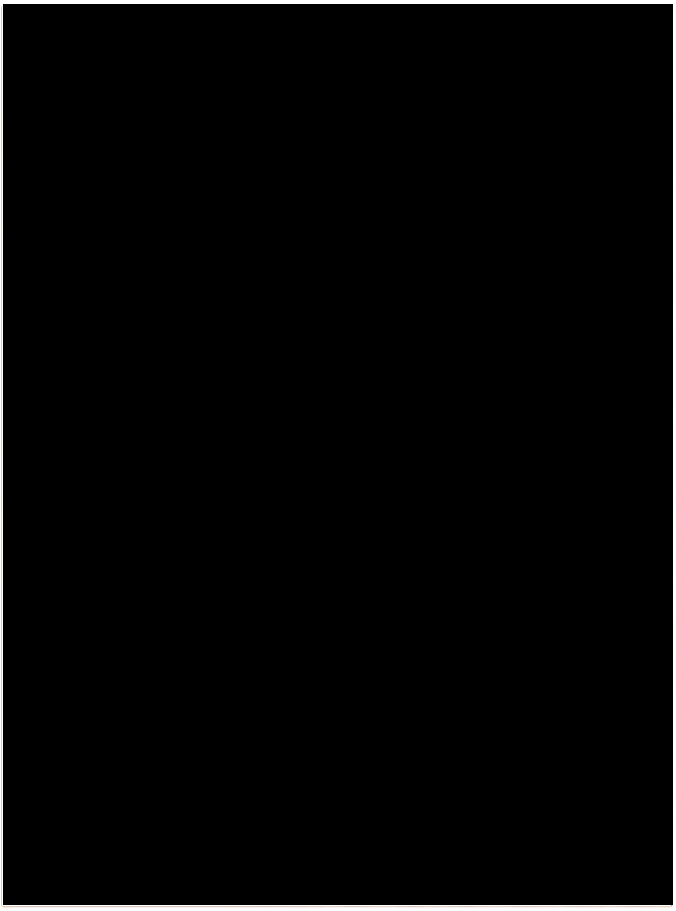

Figure 1.3. Photomorphogenic response of plants under high R:FR ratio (a.) inhibit growth and in low R:FR (b.) conditions, induce stem elongation. Under high R:FR ratio, the inactive Pr is photoconverted to the active Pfr form upon absorption of R light to positively regulate photomorphogenesis through the inhibition of stem elongation. In the shaded area, low R:FR ratio converts the active Pfr back to Pr which result in stem elongation. Image adapted from (Morelli & Ruberti, 2002)

1.3.2 Light signals that activate the Shade Avoidance Responses (SAR)

Plants that are developing in low and high-density environments detect different light quality and quantity. In a low-density environment, where plants are far-apart from each other, light is relatively constant. In environments where there is high plant density, overcrowding creates direct shade from upper leaves to the leaves below. Lower leaves detect the spectral reduction in the ratio between R:FR and the amount of photosynthetic active radiation (PAR) absorbed by the chlorophyll and carotenoids (Schmitt et al., 1999) of upper leaves. The ratio between R and FR defined as the photon irradiance between 655 and 665 nm to that of the photon

irradiance between 725 and 735 nm (Franklin & Whitelam, 2005). Under full sunlight, the levels of R:FR ratio reaching the earth is approximately 1.1 (Smith & Holmes, 1977). In shaded conditions, however, the ratio of R:FR can be reduced up to 0.71 as measured by light reflected by leaves and can be reduced up to 0.1 and 0.07 (Franklin, 2008; Martínez-García et al., 2010). The consequence of this reduction is the shade avoidance response (SAR) (Martínez-García et al., 2010; Roig-Villanova & Martinez-Garcia, 2016). Shade avoiding plants typically have phenotypes with longer hypocotyl, or internodes, increased leaf angle, reduced branching, and early flowering. (Fig. 1.3; Keuskamp et al., 2010; Pierik & de Wit, 2013). The most exaggerated and rapid plant response to shade is elongation growth (Franklin & Whitelam, 2005). Franklin and Whitelam (2006) reported that initial studies by Harry Smith and colleagues on the effect of increasing FR on the stem elongation rate of *Sinapis alba* seedlings using linear voltage displacement transducers. Their findings suggested that the elongation rate had a 10 to 15 minutes lag phase which demonstrates the rapid response of plants under conditions with high far-red (Ballaré & Pierik, 2017; Franklin, 2008).

There are two shaded conditions by which the ratio between R and FR is reduced: plant proximity and canopy shade. The effect of light signals from plant proximity was based on the analysis of plant growth in the less dense environment. Plants exhibited elongated stems even before leaves are shaded and provide information regarding possible future shading. Leaves preferentially reflect FR and this light act as a signal for proximal vegetation (Ballare et al., 1990; Casal, 2013; Gundel et al., 2014). In contrast, leaves shaded by canopies detect two types of signal: reduced R:FR ratio and PAR light. The R:FR ratio and PAR quantity under full sunlight were reduced from 1.2 to 0.1 and 1500 to 40 μmol m^{-2} s^{-1}, respectively, under two overlapping leaves (Franklin, 2008). The reduction in PAR can induce elongation responses similar to those induced by low R:FR. In the electromagnetic spectrum, the location of the PAR

absorbed by the leaves is within the R and B wavelength. The reduction of PAR can also induce hypocotyl elongation similar to low R:FR or low B light induced SAR (Hornitschek et al., 2012). The mechanism by which the plants utilize the reduction in PAR light is still not fully understood (Hersch et al., 2014), although the reduction of B light may be the main activator of this phenotypic response. Blue light is also reduced under shade and plants exposed to low B light exhibit strong hypocotyl elongation (Keller et al., 2011). The involvement of reduced B light in shade avoidance response has always been regarded to enhance the effect of low R:FR to elongation (Ballaré & Pierik, 2017) and not as the main signal that activates SAR. Multiple photoreceptors detect these light signals, and the combination of the light quality and quantity determine the plant response under canopy shade (Fankhauser & Batschauer, 2016).

1.3.3 Roles of phytochromes, cryptochromes and UV-B photoreceptor in Shade Avoidance

1.3.3.1 Phytochromes mediate responses to low R: FR

Plant responses to low R: FR has been strongly linked to the phytochrome activity (Ballaré & Pierik, 2017; Casal, 2013). The photoconversion of the phytochromes to inactive and active form is one of the unique features of these photoreceptors which is crucial for the detection of R:FR from plant proximity (Figure 1.2). Photoconversion occurs when R light is absorbed by the phytochrome photochromobilin which convert the inactive Pr to the active Pfr. In *Arabidopsis,* phyB is the major phytochrome that detects the reduction of R:FR and its deactivation are required to induce the SAR. The *phyB* mutant displayed elongated hypocotyl, stem, and petiole when exposed to high R:FR similar to those observed in SAR (Reed et al., 1993). Further experiments using supplemental exposure to FR (plants exposed to a few minutes of FR at night) or end-of-day far-red light (EOD-FR; plants treated with few minutes of FR at the end of the day) on *phyB* mutants suggested the roles of other phytochromes

involved in the SAR based on increased hypocotyl or internode elongation and early flowering. Similar responses were observed in the *phyAphyB, phyBphyD and phyAphyBphyE* mutants which suggest redundancy between phytochromes B, D and E photoreceptors in shade avoidance response (Aukerman et al., 1997; Devlin et al., 1996; Devlin et al., 1998). The redundancy between these photoreceptors means that plants treated with FR maintain a pool of active Pfr that are converted to the inactive Pr and hence mimics the responses observed in low R:FR conditions (Franklin & Quail, 2010).

1.3.3.2 Cryptochromes and phytochrome induce shade avoidance elongation response under low blue light

Plants exposed to low B light exhibit the same shade avoidance response as observed in low R:FR. Cryptochromes are the major photoreceptor that detects the changes in B light as displayed by the exaggerated elongation response of cryptochrome 1 (*cry1*) mutant under B light (Keller et al., 2011; Ninu et al., 1999). Phytochromes can also mediate responses to B light. The action spectra of the Pr and Pfr of phytochromes can also absorb B (Attridge, 1990). Low B light can induce a similarly exaggerated elongation as when plants are exposed to low R: FR therefore, it is possible that phytochromes also mediate such response (Xu et al., 2016). A recent study by Pedmale et al. (2016) used *Arabidopsis* plants exposed to filtered light that reduced B light, provided the evidence to support that cryptochromes together with phytochromes induce hypocotyl elongation. In this study, they found that cryptochromes (CRY1 and CRY2) and phytochromes (PHYB) bind to PHYTOCHROME INTERACTING FACTORS 4 and 5 (See 1.3.2) to induce hypocotyl elongation under the low B light. Furthermore, a comparison between the transcriptome analysis of *pif4pif*5 double mutants treated with low R:FR and low B light showed that PIFs regulate elongation under low R:FR

by promoting auxin-related genes and when under the low B light, interact with cell-wall-modifying proteins (Pedmale et al., 2016).

1.3.3.3 UV-B inhibit growth response in shade

UV-B light is also strongly reduced under canopy shade and may also provide information about competition (Fraser et al., 2016). Attenuation of UV-B does not activate elongation but inhibit it. Recent studies have shown that hypocotyl elongation induced by low R:FR and low B light are both inhibited by UV-B-dependent on the UVR8 photoreceptor. UVR8 control these responses through the regulation of multiple pathways resulting in the reduction of phytohormones involved in cell elongation (Ballaré & Pierik, 2017; Fraser et al., 2016; Scott Hayes et al., 2014; Mazza & Ballaré, 2015). See section 1.3.5 for the full molecular mechanism of UV-B signaling.

1.3.4 Cost of shade avoidance on defence

Plants subjected to shade exhibit reduced resistance against pathogen attack. This reduction in fitness has been attributed to the allocation of resources towards growth to outcompete taller neighbouring plants (Cipollini, 2004). Levels of PHYB are reduced in low R:FR and such reduction negatively regulate the jasmonic acid (JA) pathway responsible for plant immunity (Carriedo et al., 2016; Smakowska et al, 2016). *Arabidopsis* leaves exposed to supplemental FR with decreased active PHYB levels, showed increased susceptibility to *B. cinerea* compared to those exposed to high R:FR (Cerrudo et al., 2012). It has also been observed that low R:FR reduce the sensitivity of plants to JA. Defence marker genes that are usually upregulated by exogenous methyl-JA application and inoculation of the necrotrophic fungus, *Botrytis cinerea* are reduced after low R:FR exposure (Cargnel et al. 2014; Cerrudo et al., 2012). Furthermore, experiments using the mutant *sav3-2*, a mutant that has a defect in the tryptophan

aminotransferase pathway (TAA1) involved in the induction of SAR phenotype, still retained the FR down-regulation of defence. Therefore, these suggests that the downregulation of defence genes in plants is induced by the reduced activity of PHYB under low R:FR and is not due to the consequence of SAR phenotypic response (Cerrudo et al., 2012; Moreno et al., 2009)

1.3.5 Molecular mechanism, photomorphogenesis and shade avoidance responses

Shade avoidance responses are induced upon photoreceptor absorbance of reflected FR from neighbouring vegetation and reduced blue light under canopy shade. Responses activated by these light signals are inhibited by the activity of phytochrome, cryptochrome and the UVR8 (Fraser et al., 2016) under full sunlight. Downstream of the photoreceptor pathway, many genes are involved in the positive induction of photomorphogenesis. In the dark photomorphogenesis is inhibited which result in plants to display long pale hypocotyls, closed cotyledon and apical hook. Photomorphogenesis is inhibited by the CONSTITUTIVELY PHOTOMORPHOGENIC/DEETIOLATED/FUSCA (COP/DET/FUS) family of genes and PIFs (Leivar et al., 2008). The COP1 in the COP/DET/FUS proteins is an E3 ligase directly inhibit the LONG HYPOCOTYL 5 (HY5) together with the SPA protein family (Lau & Deng, 2010; Wei & Deng, 1996; Xu et al, 2015). HY5 is a bZIP transcription factor that regulates many light-inducible genes, and it is upregulated after dark to light transition (Osterlund et al, 2000). Mazza and Ballaré (2015) proposed two models which illustrate the molecular mechanisms activated when plants are under canopy shade or competition and under full sunlight (Fig. 1.4). Under limiting competition, photoreceptors are deactivated or reduced in activity resulting in the depression of PIFs (Fig. 1.4a). PIFs are bHLH transcription factors that

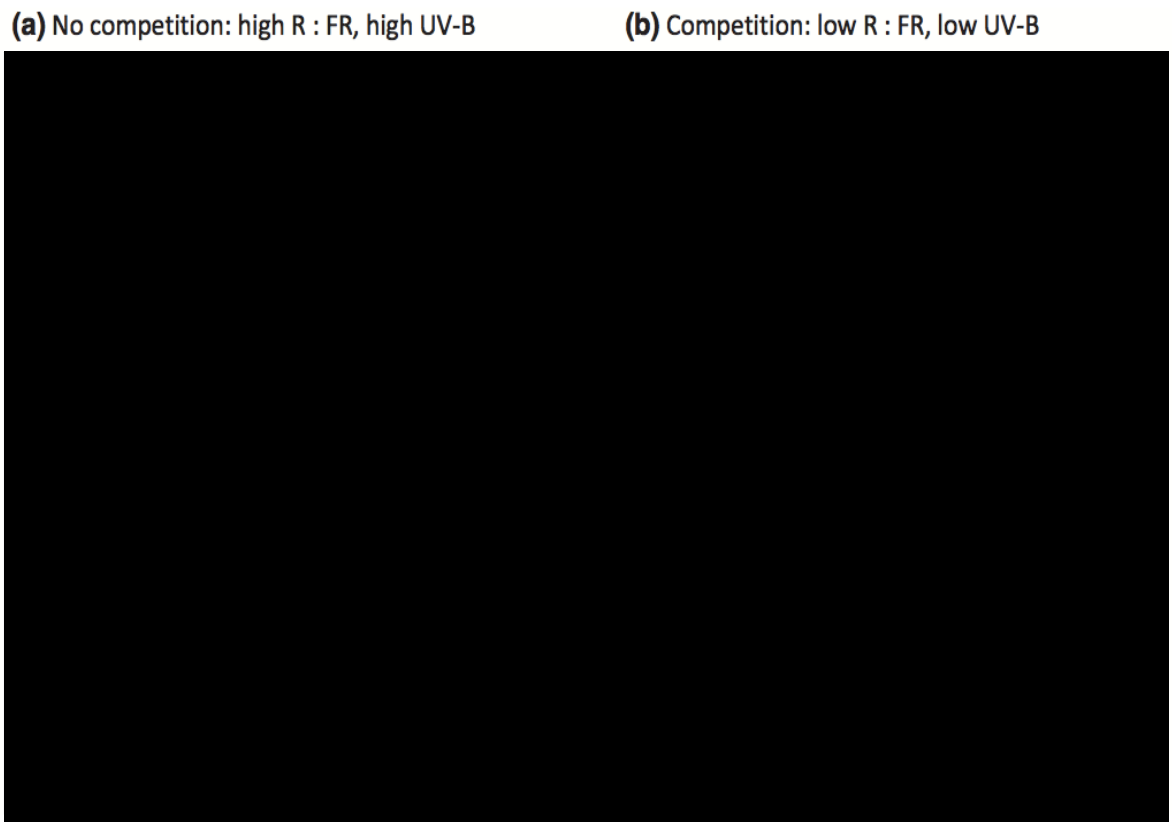

Figure 1.4 Proposed model illustrating molecular interaction between phytochromes and UVR8 in environments where there are no competitions (a.) and presence of neighbouring plant competitors (b.). Under full sunlight (a.) phytochrome directly interact with PHYTOHCROME INTERACTING FACTORS (PIFs) and inhibit its activity. UVR8 through the LONG HYPOCOTYL 5 (HY5) and Pfr decrease the active gibberellic acid (GA) and stabilize DELLA. DELLA directly interact with PIF and inhibit growth and elongation. JAZ10 is inhibited and increase transcription factors involved in defense. At the same time UV-B induced accumulation of phenolic compounds via UVR8 and HY5 to increase plant defense under full sunlight. Under competition, Pfr and UVR8 activity are reduced and PIFs are upregulated which favours growth and elongation response. Image adapted from Mazza and Ballaré (2015)

are positive regulators of SAR. Phytochromes can physically interact with PIFs to inhibit its activity. The interaction between phytochromes and PIFs occurs through PIFs Active Binding domains of phytochrome A (APA) or phytochrome B (APB) (Choi & Oh, 2016; Huq & Quail, 2002; Leivar & Monte, 2014; Leivar & Quail, 2011). The deactivation of PHYB in low R:FR reduces the PHYB-PIFs interaction, which increases the levels of PIFs that can freely activate their target DNA (Park et al., 2012). PIFs target hormone-related genes which in the case of cell elongation, auxin is upregulated by the transcription factors. Genes that were upregulated

includes the YUCCA genes which encode enzymes involved in the biosynthesis of auxin and promote the expression of IAA29, involved in the auxin signaling (Hornitschek et al., 2012). In contrast to the PHYB deactivation, levels of UV-B are also reduced under shade which reduces the synthesis of secondary metabolites resulting in the reduction of the plant defenses. However, UVR8 can still inhibit elongation under conditions that mimic canopy shading (Hayes et al., 2014; Mazza and Ballaré, 2015). UVR8 can still upregulate *Ga2ox* and induce destabilization of PIFs and inhibit upregulation of auxin-related genes (Hayes et al., 2014). In environments where there is high R:FR and UV-B (Figure 1.5b), the phytochromes, cryptochromes (not shown in the model but are involved in similar the pathway as phytochrome; Fig. 1.5) and UVR8 photoreceptor act together in inhibiting elongation growth. Phytochromes and cryptochromes act in a dual mechanism in regulating the activity of the COP1-SPA complex (Casal, 2013; Fraser et al., 2016; Xu et al., 2015). Yeast two-hybrid assay showed that CRY1 and CRY2 strongly interact with the C-terminal WD40 domain of SPA1 through the photoreceptors' C-terminal (CCT) terminal domain in a blue light-dependent manner (Lian et al., 2011). This WD40 domain is also the binding site which COP1 use to interact with SPA (Yang, Tang, & Cashmore, 2001). These results suggest that cryptochromes (CRY1 and CRY2) directly interact with the SPA resulting in the dissociation of the complex. Phytochromes also rapidly inactivate the COP1-SPA through the same interaction with SPA. SPA strongly bind to the N and C terminal of PHYA and PHYB in a FR and R light-dependent manner (Lu et al., 2015), respectively. Consequently, these interactions lead to the dissociation of the COP1-SPA1 complex and reduction on the ability of COP1 to inhibit genes that are positive activators of photomorphogenesis (Casal, 2013; de Wit et al., 2016; Fraser et al., 2016). HY5 and hypocotyl in far red 1 (HFR1) (Yang et al., 2005) are two positive regulators of photomorphogenesis that inhibit the activity of the bHLH PIFs (Figure 1.5) (de Wit et al., 2016). Under competition, the reduced activity of photoreceptors increases the COP1-SPA1

activity, and this target HY5 and HFR1 to increase PIF activity (Fig. 1.5). UVR8 can also reduce the levels of PIFs through the UVR8-dependent upregulation of HY5, and this upregulation is COP1-SPA dependent (Brown, Headland, & Jenkins, 2009). UV-B absorption induces monomerization of UVR8 dimers. The UVR8 directly interact with COP1-SPA complex which alters the complex's activity from ubiquitination to a promoter of gene expression. Upregulation of HY5 activate the synthesis of secondary metabolites involved in plant defence and act as UV-B absorbing compounds. At the same time, HY5 reduces the levels of growth promoting gibberellin (GA) phytohormone through the activation of GIBBERELLIN 2 OXIDASE 2 (*Ga2ox2*), enzymes responsible for the catabolism of active GA (Lau & Deng, 2010). Reduction of free GA increases the stabilization of DELLA proteins. DELLA proteins can directly bind to PIFs and inhibit them from binding to their DNA targets (Li et al., 2016). Since all major photoreceptor converges towards the regulation of growth promoting hormones, the possibility of photoreceptor cross-talk is highly likely. A study by Hayes et al., (2014) on UV-B treated *Arabidopsis* plants revealed attempts to show the possible cross-talks between photoreceptors using the various phytochrome mutants. *phyAphyBphyCphyDphyE* mutants exposed to low levels of UV-B exhibited UV-B inhibition of hypocotyl elongation. Inhibition of elongation suggests that phytochromes are not involved in the UV-B inhibition of hypocotyl elongation of young *Arabidopsis* plants. The study by Hayes et al., (2014) show promising findings but limits only to *Arabidopsis* thus, other model species must also be used to provide a broad knowledge of how multiple photoreceptors respond to UV-B.

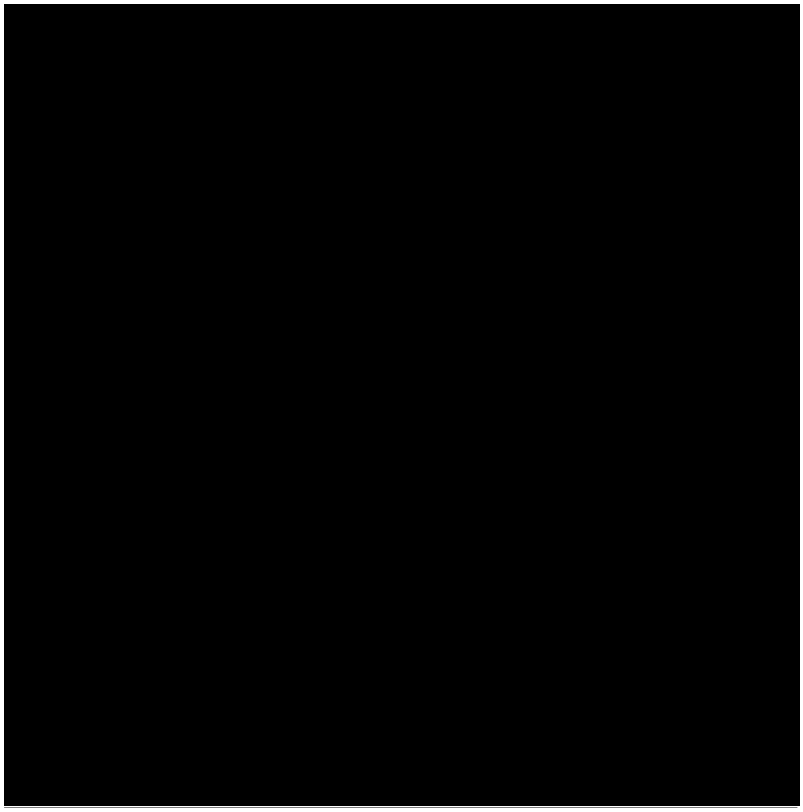

Figure 1.5 Summary of photoreceptor signaling under full sunlight. Light activation of photoreceptors collectively inhibits the PHYTOCHROME INTERACTING FACTORS (PIFS) and regulate hypocotyl elongation. Phytochromes (active Pfr) and cryptochrome (cry) inhibit the activity of CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) and SUPPRESSOR OF PHYA (SPA) E3 ligase complex increase the expression of hypocotyl in far red 1 (HFR1) and PHYTOCHROME RAPIDLY REGULATED (PAR) which negatively inhibit PIFs. PIFs are also inhibited by UVR8-COP1-SPA compels through the upregulation of LONG HYPOCOTYL 5 (HY5)/ HY5 HOMOLOG (HYH). HY5/HYH targets PIFs to inhibit its activity. The upregulation of HY5/HYH also increase stabilize DELLA to inhibit PIFs and inhibit hypocotyl elongation. Image adapted from Fraser, Hayes, and Franklin (2016)

1.4 Significance of UV-B studies

1.4.1 Ozone depletion motivated UV-B studies

The mechanistic action of visible light photoreceptors has been extensively studied for years, prior to the identification of a UV-B specific photoreceptor. The discovery of the mutant form of UVR8 in *Arabidopsis* (Kliebenstein et al., 2002) provided researchers with new tools to dissect UV-B perception and signaling transduction. UV-B studies were initially motivated by the rapid decline of ozone layer (oxygen species that absorb UV light from the sun) and

predicted increases in UV-B penetrating the Earth's atmosphere. During the early 20th century Industrial Revolution, ozone-depleting substances (ODS) such as chlorofluorocarbons or bromine-containing compounds were highly used for refrigeration and fertilizers on crops. These chemical substances absorb UV and form radicals, which can react with O_3 (ozone) in the stratosphere (Biggs & Joyner, 2013; Chipperfield et al., 2015; Kerr & McElroy, 1993). The Montreal Protocol was established in 1987 to reduce ozone depletion and ODS levels in the atmosphere to possibly slowly reduce the effect of climate change. This treaty proposed the discontinuous use of ODS and substitution with hydrofluorocarbons that are non-ozone-reactive compounds (Montzka et al., 2014). Despite the effort to reduce ozone depletion, NASA reported that the level of ultraviolet light reaching the Earth's surface has increased for that past three decades (Hille, 2010).

The most damaging part of the UV spectrum is the UV-B (280-315 nm) as it can distort the structure of DNA and can inactivate proteins (Hollósy, 2002). DNA can absorb UV-B which, can induce irreversible DNA damage (Taylor et al., 1997). High dose of UV-B (280-315nm) can disrupt cellular processes and increase oxidative stress to plants (Hollósy, 2002). These negative effects of UV on plants have been the boundary that slowed the research advancement in the area relating to UV-B. Wargent and Jordan (2013) reported that such damages are due to unrealistic UV dosage and long exposure used in the experiments from which these effects were observed. The survival of the plants under UV-B, relies on the reduction of reactive oxygen species (ROS). ROS are by-products of the electron transfer system of photosystem II, induced by absorption of high energy UV-B photons (Czégény et al., 2016). Plants must activate ROS-scavenging genes to regulate levels of ROS. At low levels of UV-B, ROS scavenging genes are not upregulated (Hectors et al., 2007) which, suggests that plant responses observed on plant irradiated to low levels of UV-B are not consequence of stress nor

DNA damage. In addition, plants have also developed protective mechanisms against the damaging effect of high UV levels (Ballare et al, 2011). Light absorbing pigments are synthesised by the plant. Compounds such as carotenoids in the chloroplast and anthocyanin act as protection from reactive oxygen species (Kovács & Keresztes, 2002). In contrast to high UV-B dose, low dose of UV-B induces DNA repair and activate non-damaging photomorphogenic responses in plants (Ballare et al, 2011). Low fluence UV-B could also regulate plant morphology through the inhibition of stem elongation (Kim et al., 1998), induce cotyledon opening (Boccalandro et al, 2001) and activate synthesis of secondary metabolites (Lee, 2016).

1.4.2 UV-B provide photoprotection and defence

Priming using low doses of UV-B, induces the synthesis of secondary metabolites that provide protection from high levels of UV (photoprotection; Singh et al., 2014) and plant defence against pathogens. Photoprotection is observed on plants grown with the presence of low dose UV-B and were less susceptible to stress under full sunlight, than those that were not (Jenkins, 2009). Priming plants with low levels of UV-B stimulate the production of flavonoids compounds that absorb light between the wavelength 280 to 340 nm (Jansen et al., 1998). These wavelengths are within the UV-B region (315-280 nm) hence, may provide UV protection. Flavonoids accumulate in the epidermal tissue. The epidermis act as a filter and accumulation of these flavonoids reduces the UV transmittance on the surface of the leaves (Barnes et al., 2015) and protect the photosynthetic machinery of the plant. Production of these compounds are usually observed through transcriptional upregulation of *CHS* regulated by the UVR8 photoreceptor through *HY5* (Kliebenstein et al., 2002; Singh et al., 2014). Priming plants with low doses of UV-B before transferring in UV-inclusive has been observed to positively increase harvestable yield, increase photosynthetic rates and increase photoprotection

(Wargent et al., 2015; Wargent et al., 2011). The synthesis of secondary metabolites has also been observed to provide defence against insect herbivores (Izaguirre et al., 2007) and fungi (Demkura & Ballaré, 2012; Kobayashi et al., 2014). Pre-exposure to UV-B before inoculation of pathogens showed increase resistance to pathogens (Ballaré, 2014). Studies have shown that UV-B induces jasmonate-dependent and independent pathways in regulating anti-herbivore defence (Demkura et al, 2010). Protection provided by UV-B through the UVR8 photoreceptor does not involve the upregulation of JA (See 1.3.3.4), instead it upregulates the synthesis of flavonoid and sinapates on the leaf tissue to induce *Arabidopsis* resistance to necrotrophic fungi (Demkura & Ballaré, 2012). Taken together, these beneficial effects of priming plants with UV-B could bring good application opportunities for horticulture and agriculture (Wargent, 2016).

1.5 Tomato as another model system

1.5.1 Why study tomato plant responses under UV-B?

1.5.1.1 Tomato: Economic importance

Tomato or *Solanum lycopersicum* (also known as *Lycopersicon esculentum* Miller) is a fruit bearing crop commonly produced worldwide due to its high economic and nutritional value. The fruit is used in different commercial product in fresh and processed form bringing in $2 billion worth of annual farm cash receipts in the United States ((USDA), 2016) alone. China (30 million tonnes) is the major producer of tomato fruit, followed by the United States of America (12.5 million tonnes), India (9.4 million tonnes), Turkey (9.2 million tonnes) and Egypt (7.2 million tonnes) in 2014 (faostat3.fao.org/browse/Q/QC/E). The popularity of tomato fruit depends on its high content of antioxidant compounds. These compounds scavenge harmful free radicals in the body and consumption have been inversely correlated to certain

types of cancer (Dorais et al., 2008; Gerszberg et al., 2015) which makes it economically important.

1.5.1.2 Tomato architecture: plant model for shade avoidance

Absorption and detection of light relies upon the architecture of the plant. *Arabidopsis* has been used extensively as a model species for studying light responses in plants however, its monopodial architecture limits it as a system (Schrager et al., 2016) to generally model light responses of plants. The monopodial growth of *Arabidopsis* has an indeterminate SAM which is continually active throughout the plant's life cycle and produces leaves and flowers (Reinhardt & Kuhlemeier, 2002) later in the development. The leaves are located at the bottom and subjected to different light quality and quantity. In comparison, tomatoes exhibit a sympodial architecture and form vegetative internodes that can overcome this limitation in the monocot system. The continuous production of lateral meristems by the plant's determinate shoot apical meristem (SAM) even after the formation of its first flower (Lozano et al., 2009) formed the sympodial growth of tomatoes (Fig. 1.6). Tomato sympodial growth enables the plant to grow towards the light bringing with it, leaves that can detect a gradient of light. Tomatoes also have long shade-avoiding vegetative internodes which Arabidopsis lack (Ninu et al., 1999). Formation of these internode and sympodial architecture thus make tomato a good model system for studying shade avoidance responses.

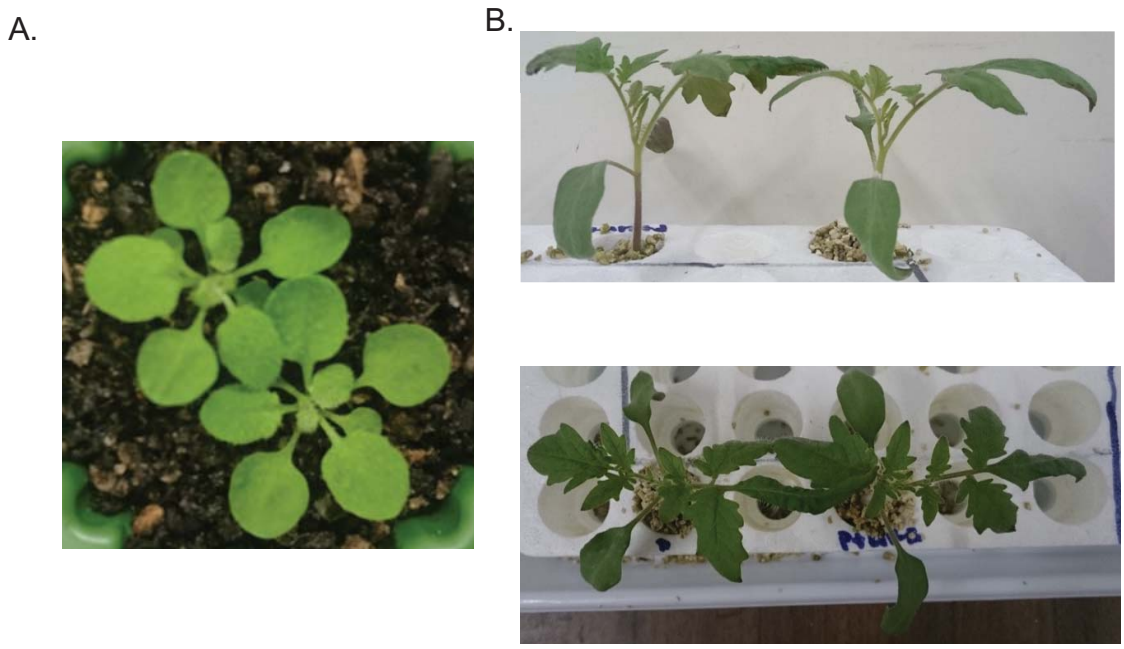

Figure 1.6: Architectural difference between two model species: wild-type Arabidopsis (A.) and tomato (B.). The image of the *Arabidopsis (Col-0)* was taken above the plant to show the leaves. Tomato images were taken above ground and on the side to show the location of the leaves, hypocotyl and internodes of tomatoes. Plants were 14 and 16 days old after sowing, respectively, at the time the images were taken. Arabidopsis images captured from Dr. Claudia Rossig's plants. Images were magnified (magnification unknown) and resized. No other alterations on the images were done.

1.5.2 Photoreceptors of Tomato

1.5.2.1 Characterization of tomato phytochromes

1.5.2.1.1 Tomato phytochrome A (far-red insensitive, *fri)*

The characterization of phytochrome A mutant of tomatoes was identified using artificial mutagenesis where wild-type tomato seeds were treated with ethyl methanesulphate (EMS) and screening using white light and monochromatic lights (R, B, FR). The mutation was a result of substitution of an adenine for a thymine at the 3' junction of one of its intron resulting in the formation of stop codons and failure to remove this intron during mRNA maturation (Lazarova et al., 1998). Immunoblot analysis of *phyA* mutant showed that the mutation reduced 99% of PHYA (Lazarova et al., 1998). Two types of phytochrome A of tomato known as far-red insensitive fri^1 and fri^2 have been characterized based on their slightly elongated hypocotyl

growth in R and B, respectively. Both mutants showed similar phenotype under continuous FR as those grown under dark which suggests that these mutants are completely blind to FR. Furthermore, these *fri* mutants exhibited normal WT phenotype under white light. These phenotypic responses are similar to *Arabidopsis phyA* mutant grown under the same conditions. Further genetic mapping showed that PHYA of tomato is also located at chromosome 10 like *Arabidopsis* (Nagatani et al., 1993; van Tuinen et al., 1995; Whitelam et al., 1993). These observations suggest that tomato PHYA is a homolog of *Arabidopsis* PHYA.

1.5.2.1.2 Tomato phytochrome B1 (temporarily red light insensitive, *tri*) and phytochrome B2

Presence of phytochrome B in tomato has been predicted before its full characterization and isolation. Sequence comparison of *Arabidopsis* PHY genes to tomatoes resulted in the identification of two *PHYB* (*PHYB1* and *PHYB2*) in tomatoes (Pratt, 1995). Since phenotypic responses of known *PHYB* mutants in different species exhibit similar long hypocotyl under R light, this enabled van Tuinen et al., (1995) to isolate *PHYB1* of tomato. Artificial mutagenesis and WL, B, R and FR light screening were used to the identify temporarily red light insensitive (*tri*) mutant or *phyB1*. The long hypocotyl, reduced anthocyanin and small cotyledon area of *tri* mutant under continuous R light have been observed in *Arabidopsis* (Reed et al., 1993), cucumber (*lh*) (López-Juez et al., 1992) and *Brassica* (*ein*) (Devlin et al., 1992) which, suggests that it is in fact a mutant form of *PHYB* gene. However, EOD-FR elongated observed from WT tomato and *Arabidopsis* was temporarily observed after two days of R light treatment suggesting that another stable phytochrome is involved and that tomato phyB1 did not have similar phenotypic response at least in the EOD-FR (van Tuinen et al., 1995). The second phytochrome B of tomato, *PHYB2,* was characterized through gamma mutagenesis of *phyAphyB1* double mutants and screened under natural daylight (Kerckhoffs et al., 1999). The

mutant exhibited long pale hypocotyl, stems, leaves and deficient in anthocyanin- phenotype exhibited by the loss of functional *PHYB2* in the *phyAphyB1phyB2* triple mutant. The role of *PHYB2* was further analysed by the isolation of monogenic *phyB2* mutant and its interaction with PHYA and PHYB1 (Weller et al, 2000).

1.5.2.1.3 Roles of phytochrome A, B1 and B2 in shade avoidance response

Mutagenized form of tomato phytochrome A, B1 and B2 enabled the discovery of the roles of each photoreceptor. Long hypocotyl, reduced anthocyanin, small cotyledon and EOD-FR are phenotypic responses of screened mutants which indicate their role throughout the early responses of young tomato plants (Appenroth et al., 2006; Kerckhoffs et al., 1999; Kerckhoffs et al., 1997; Lazarova et al., 1998; Nagatani et al., 1993; van Tuinen et al., 1995; Weller et al., 2000; Whitelam et al., 1993). Increased rate of hypocotyl is the most exaggerated phenotypic response of plants subjected to effect (Franklin & Whitelam, 2006). Physiological studies suggested that phytochrome B1 is the major regulator of R light induced hypocotyl or stem elongation, positive regulator of anthocyanin biosynthesis and cotyledon expansion under R light (Kerckhoffs et al., 1997). Residual inhibition of hypocotyl response to EOD-FR of *phyAphyB1* suggested other roles of other phytochrome. This phytochrome was phyB2 since triple mutant of *phyAphyB1phyB2* lost this residual EOD-FR response (van Tuinen et al., 1995). Further physiological analysis using multiple mutant combination of phyA, phyB1 and phyB2 indicate that phyB2 has a minor role in inhibition of hypocotyl elongation under R light and the effect of the mutation can only be observed in the absence of functional PHYB1 in the *phyB1B2* double mutants (Weller et al., 2000). Redundancy between phyB1 and phyB2 has also been observed where the effect of one of the phytochrome is only observed in the absence of the other and that this redundancy is also observed under shade (Weller et al., 2000).

1.5.2.1.4 Cryptochromes

Cryptochromes are photoreceptors that detect the blue light and UV-A in the visible spectrum. Two types of cryptochrome mutants have been isolated in Arabidopsis: *cry1* and *cry2*. Based on these mutant forms, *CRY1* has been found to be involved in repressing hypocotyl elongation, anthocyanin production, leaf and cotyledon expansion but *CRY2* is only involved during elongation, expansion and flowering (Ahmad et al., 1998). Molecular and genetic characterization of tomato cryptochrome mutants suggested that *CRY1* and *CRY2* are homologues to *Arabidopsis* cryptochromes (Perrotta et al., 2000). Cryptochromes from both species contain the conserved motifs at the C-terminal, the photolyase like domain at the N-terminal and the position of the fourth intron (Perrotta et al., 2000). Antisense construct of *CRY1* and CRY2 in tomato exhibited the same responses including internode elongation under B light (Ninu et al., 1999). These suggests that tomato *CRY1* is a positive regulator of photomorphogenesis, like phytochromes. Although the reduction in CRY1 exhibited these photomorphogenic responses, cryptochromes still require phytochrome to fully exhibit its full effect under monochromatic blue light in both *Arabidopsis* (Ahmad & Cashmore, 1997*)* and tomato (Weller et al., 2001). Experiments using double mutant form of *cry1phyA* of tomato exhibited longer hypocotyl responses under continuous blue light than the WT and other cry1 double mutant combination with phyB1 and phyB2 (Weller et al., 2001). Furthermore, the triple mutant *cry1phyAphyB1* displayed less hypocotyl elongation compared to the quadruple mutant *cry1phyAphyB1phyB2* exhibited no blue light inhibition of hypocotyl- further supports that phyB or in this case, phyB2 is required for blue light mediated inhibition of hypocotyl.

1.5.2.1.5 UV-B photoreceptor

The tomato UV-B photoreceptor has not been characterized to date but there has been studies that provided evidence that UV-B can inhibit hypocotyl and stem elongation of tomatoes

(Ballaré et al., 1995; Bertram & Lercari, 2000a, 2000b). Sequence alignment between the *Arabidopsis* UVR8 and tomato genome (www.solgenomics.net) showed 83.5% similarities with the protein Regulator of Chromosome Condensation (RCC1)-like. It may be interesting to see if this is in fact the UV-B photoreceptor in tomato.

1.6 Project Aims

The architectural form of tomato makes it a good model system for UV-B studies. Tomatoes exhibit a sympodial architecture which allows it to produce leaves above the canopy in contrast to the monopodial structure of *Arabidopsis*. Perception of UV-B light depends on the architecture of the plant and photoreceptors control the structure of the plant based on the changes in the light environment. The tomato PHYB1 mediates stem elongation, and the *phyB1* mutant has been commonly used for different photobiology studies. On the other hand, very few studies have looked at the effect of low doses of UV-B on the growth of tomatoes using *phyB2 or phyAphyB2* (Bertram & Lercari, 2000a; Kim et al., 1998) mutants. Since tomato PHYB2 (and PHYB1) is non-homologous to *Arabidopsis* PHYB and has minor roles in elongation growth under R or FR light conditions, the function of PHYB2 has not been fully understood yet under low UV-B conditions. In this thesis, tomato (*Solanum lycopersicum)* was used as a model system to investigate the possible roles of phytochromes (PHYA, PHYB1 and PHYB2) and cryptochrome (CRY1) in mediating the UV-B inhibition (under an "ultra-red" environment, i.e., no far-red light) of hypocotyl and internode elongation in young tomato plants. Mono-, double and triple mutants of phytochromes and cryptochrome mono-mutant were used to investigate cross-talk between these photoreceptors on the typical UV-B morphogenic responses in tomato, i.e., mediating the growth response of young tomato plants following exposure to low dose of UV-B. Physiological measurements and endpoint

polymerase chain reaction (PCR) were used to attempt to explain phenotypic responses of the plants after PAR + UV-B treatment.

1.6.1 The aim of the project is to answer two questions:

1. Do phytochrome and cryptochrome photoreceptors cross-talk with UV-B morphogenic responses in tomato, i.e., regulation of UV-B induced internode elongation growth?
2. Do phytochrome and cryptochrome photoreceptors control known major genes involved in the UV-B pathway?

1.6.2 Hypothesis:

UV-B light can inhibit elongation in wild type tomato and phytochrome and cryptochrome photoreceptor mutants.

2.0 Materials and Methods

2.1 Plant material and growing conditions

Tomato "Money Maker" (MM) wild type, phytochromes: *phyA* (*fri*), *phyB1* (*tri*), *phyB2*, *phyAphyB1*, *phyAphyB2*, *phyB1phyB2* and *phyAphyB1phyB2* and cryptochrome (*cry1*) mutant seeds were provided by Dr. Huub Kerckhoffs from the Institute of Agriculture and Environment, Massey University, Palmerston North. Seeds were sown into rockwool plugs and covered with vermiculite stored in growth chambers located at the Massey University Plant Growth Unit, Palmerston North. 11-13 days after sowing (DAS) young plants were randomly allocated into 30cm x 41cm Styrofoam plug holes. 13-15 DAS plants were treated with PAR (control) and PAR + UV-B separated by a mylar film which prevented UV-B from being absorbed by plants from the PAR only side. The hypocotyl and internode were measured before and after the treatment. Two growth chambers were used to grow and treat young plants. CTR8 growth chamber was equipped with fluorescent tubes (Philips 58W/ 865, Cool daylight) with white light quality as shown in Figure 2.1. An average of 100 μmol s^{-1} m^{-2} of PAR (400-700 nm) was measured for the CTR8 lighting system using the LI-250A (LI-COR) light meter. CTR3 was used as the treatment room for 13-15 DAS young tomato plant. Each chamber was equipped with micro-loggers, which measured the temperature and humidity every ten minutes. Temperature and humidity were maintained to 25°C and within 60-75 %, respectively for both chambers.

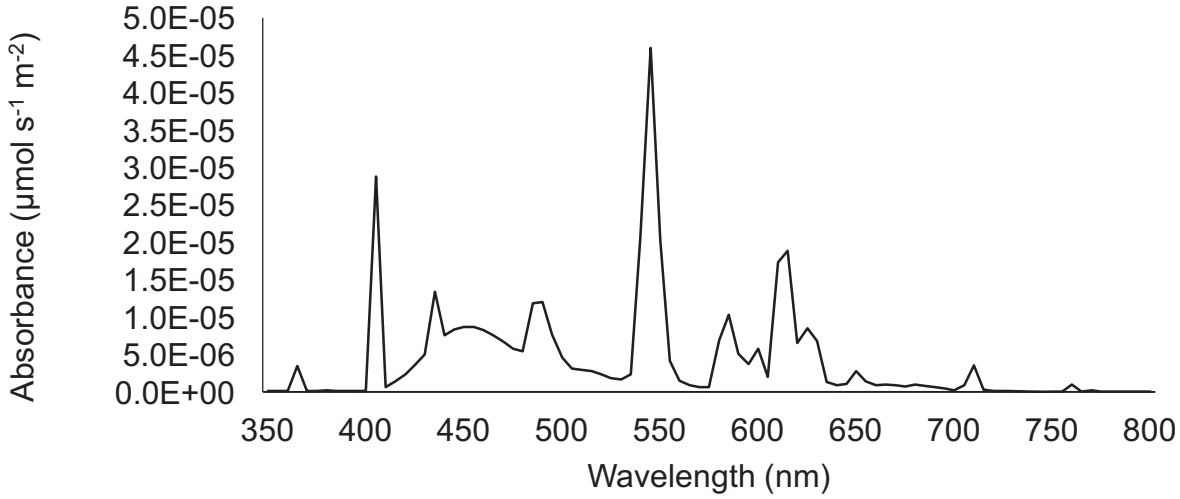

Figure 2.1: Fluorescent tubes light spectrum measured using the Optronics 756 spectroradiometer. The spectrum shows absorbance spectra of red (600-700 nm), blue/UV-A (300-500 nm), and far-red (700-750 nm) present emitted by tubes.

Table 2.0: Summary of genotypes used in the PAR and PAR+UV-B experiments.

Genotype	Genetic background	Mutant classification
Wild type (WT)	Money Maker (MM)	-
phyA (*fri*)	Money Maker (MM)	PHYA deficient
phyB1 (*tri*)	Money Maker (MM)	PHYB1 deficient
phyB2	Money Maker (MM)	PHYB2 deficient
phyAphyB1	Money Maker (MM)	PHYA and PHYB1 deficient
phyAphyB2	Money Maker (MM)	PHYA and PHYB2 deficient
phyB1B2	Money Maker (MM)	PHYB1 and PHYB2 deficient
phyAphyB1phyB2	Money Maker (MM)	PHYA, PHYB1 and PHYB2 deficient
cry1	Money Maker (MM)	CRY1 deficient

2.2 Transplanting and Allocation

WT, phytochrome and cryptochrome tomato "Money Maker" mutants at 11-13 DAS were randomly transplanted to a 30 x 40 mm. Two allocations were used: crowded and non-crowded condition. For the crowded experiment, the 30 x 40 mm Styrofoam was filled with the wild type, phytochrome and cryptochrome mutants. In the crowded condition, ten hypocotyls per genotype and five to six genotype were used per treatment (Fig. 2.2 A.) to fill the entire styrofoam. For the non-crowded experiment, plants were randomly allocated only within the outlined area on Fig. 2.2 B and alternately allocated to leave a space in between plants. All plants were watered with Peters General Purpose solution (1 gL^{-1}) throughout the experiment.

A. B.

Figure 2.2: Plants allocated in two conditions: crowded (A.; 3 days after treatment) and non-crowded condition (B; 2 days before treatment). Ten randomly chosen 11-13 DAS young tomato from each genotype were allocated randomly to both conditions two days prior to treatment.

2.3 Light Treatments

UV LED arrays were used to treat tomato WT and photoreceptor mutants. UV-B LED arrays were provided by BioLumic Ltd for the duration of the experiments performed, and were of a proprietary design. 13- 15 DAS plants were exposed to two treatment conditions: PAR only and PAR+ UV-B. Mylar film (Lee Filters, Andover, UK) was used to prevent UV-B from permeating into the PAR only treatment. The lights were positioned 30 cm above the *phyAphyB1phyB2* triple mutant (approximately 13 cm). Due to the nature of the long hypocotyl length of the phytochrome and cryptochrome mutants, the UV-B dose and PAR level detected by the plants varied. The measured UV-B dose (Optronics 756 spectroradiometer; Optronics Laboratories, Florida, USA) used in the treatments range between 0.08 and 0.09 μmol m^{-2} s^{-1}**.** Two light treatment schedules were used in the experiments Figure 2.3 and 2.4. Table 2.2 and 2.3 summarizes the experiments and treatments used, including fluences of PAR.

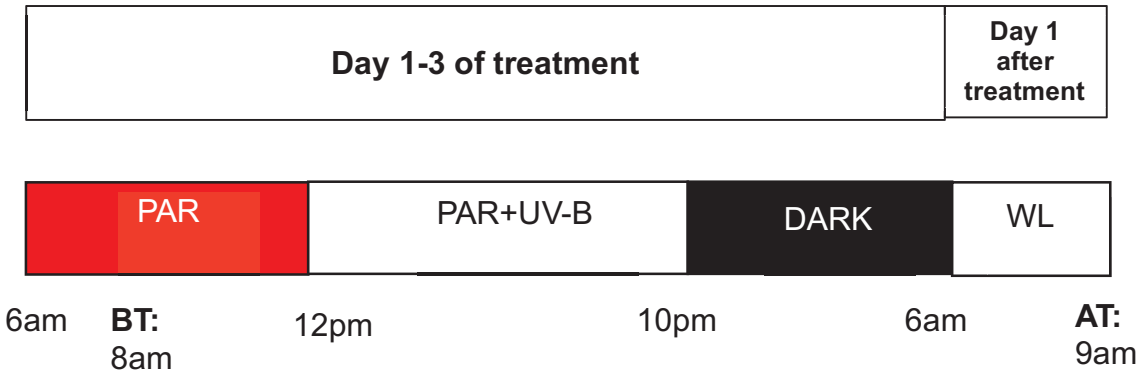

Figure 2.3: First treatment schedule. Plants were exposed to low fluence UV-B for three days. On day 1, plants were transferred from the growth room into the treatment room in the dark before 6am. PAR light was turned on at 6am and the before UV treatment (BT) measurement was done at 9am. PAR+UV-B lights were turned on at 12pm and turned off at 10pm. The plants are in the dark for 8 hours from 10pm to 6am. This treatment cycle occurs for three days. On day 1 after treatment, plants from the treatment room were transferred back to the growth room before the white light (WL) turns on at 6am. Then after UV treatment (AT) measurements were done at 9am, 3 hours after the transfer.

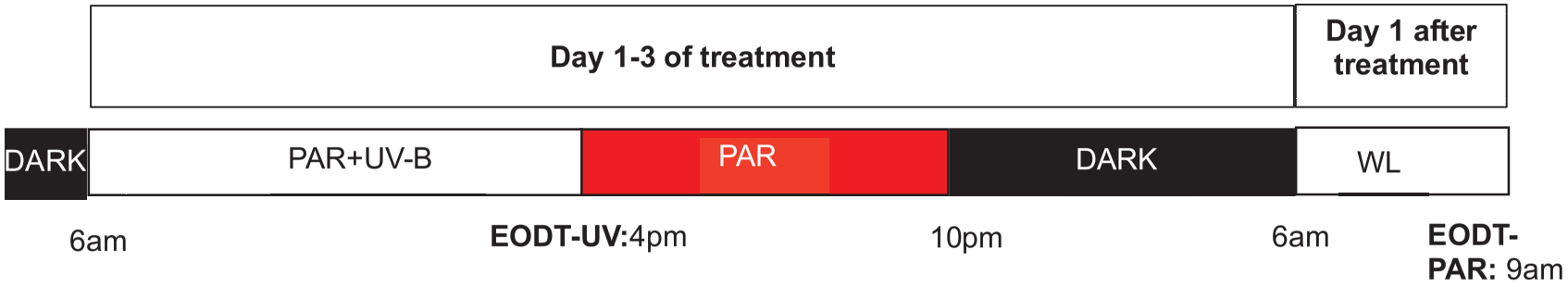

Figure 2.4: Second treatment schedule. Plants were exposed to low fluence UV-B for three days. On day 1, plants were transferred from the growth room into the treatment room in the dark before 6am. before UV treatment (BT) measurement was done 2 hours before PAR-UV-B light was turned on at 6am. PAR lights were turned on at 4pm and turned off at 10pm. The plants are in the dark for 8 hours from 10pm to 6am. This treatment cycle occurs for three days. On day 3 of the treatment, the end-of-treatment-day after UV (EODT-UV) measurements was done as soon as the UV-B was turned off at 4pm. On day 1after treatment, plants from the treatment room were transferred back to the growth room before the white light (WL) turns on at 6am. The end-of-treatment-day after UV (EODT-UV) measurements were done at 9am, 3 hours after the transfer.

2.4 Measurements

Hypocotyls and internodes of tomatoes were measured using a 0.5mm ruler before and after the UV-B treatment. The length of the hypocotyls was from the Styrofoam to just below the lowest of the two hypocotyls. The internode length was measured from the Styrofoam to just below the first true leaf (Figure 2.5). SPSS software (IBM SPSS Statistics Version 23) was used for ANOVA analysis on the differences between the hypocotyl or the internode growth rate of all genotypes treated with or without UV-B.

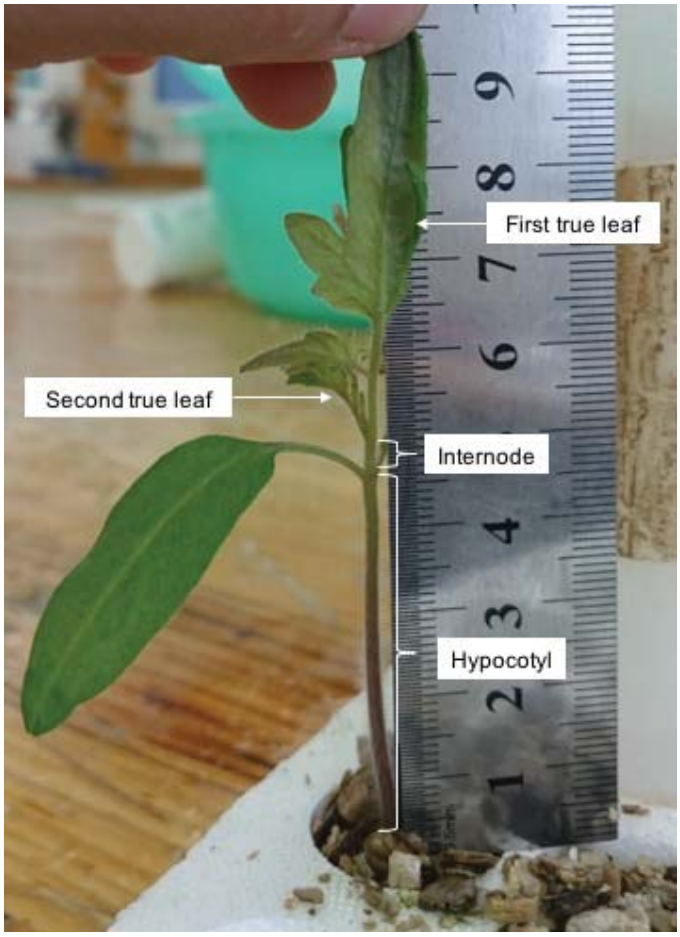

Figure 2.5: Developmental stage of WT (and other mutants) at 14 DAS on the day of treatment. Tomatoes used in the treatments were at the developmental stage where the first true leaf have a compound leaf and two leaflets and a developing second true leaf with small compound leaf and leaflets developing. Diagram shows points where hypocotyl and internode were measured.

2.5 Endpoint PCR

2.5.1 Sample preparation and RNA extraction

Samples (internode with first and second true leaves) were collected at two time points: 4 hours and 28 hours after the initial UV-B treatment and were stored in tubes immersed in liquid nitrogen. Samples were stored in -80° C freezers and were grinded into fine powder in preparation for the RNA extraction. RNA was extracted using the Quick-RNA MiniPrep (Zymo Research) and the procedures provided were followed with two modifications: extra 1 minute of centrifugation during washing using the RNA wash buffer to ensure any ethanol in the buffer has completely dried out and 50µl of DNAse/RNase-free water was added for the last extraction. The quantity of RNA per sample were measured using the Nanodrop 1000 Software (Thermo Scientific; APPENDIX A Table 1) and samples were diluted to 5 µg/µl. Table 2.1 lists the samples that were used for the gene analysis.

Table 2.1: RNA extracted from plants treated after 4 hours of initial UV-B treatment. Quantity of RNA was quantified using the Nanodrop.

Genotype	Treatment	RNA (µg/µl)
WT	PAR	617.7
phyAphyB1	PAR	906.7
phyAphyB2	PAR	326.5
cry1	PAR	685.0
WT	PAR+UV-B	741.2
phyAphyB1	PAR+UV-B	954.3
phyAphyB2	PAR+UV-B	1041.1
cry1	PAR+UV-B	447.7

2.5.2 Genomic DNA extraction

Samples of WT 14 DAS young tomato were also collected, grinded in liquid nitrogen and genomic DNA (gDNA) was extracted. Plant genomic DNA mini kit (VIOGENE) was used to extract the gDNA as instructed by the manufacturer.

2.5.3 DNAseI treatment of RNA samples and cDNA synthesis

DNAseI reaction buffer and DNAseI from the Quick-RNA MiniPrep (Zymo Research) kit was used for the DNAseI treatment and the Transcriptor First Strand cDNA Synthesis Kit (ROCHE) for cDNA synthesis. Plants treated and collected after 28 hours of UV-B treatment were not treated with DNAseI due a possible degradation of RNA during the process of treatment. cDNA synthesis of all samples was performed as instructed by the manufacturer. PCR was performed using the Mastercycler pro vapo protect PCR machine (Eppendorf) and Light Cycler 480 SYBR Green I Master kit (ROCHE). The PCR program, primers and annealing temperatures are listed in APPENDIX B. and C. Annealing temperatures for all primers were measured using Mastercycler pro vapo protect PCR machine (Eppendorf). Some of the primers were designed using the PerlPrimer (http://perlprimer.sourceforge.net/) software (APPENDIX B, Table 1). All gel images presented in the results section 3.0 were cropped and no alterations on the band intensity were applied.

Table 2.2 Summary of light treatment experiments

Experiment	Genotype	Average UV-B dose (umol/m2/s)	Average PAR (umol/m2/s)	Purpose of experiment
1	WT	0.09	81.9	Screening for mutants that could be responsive or non-responsive to UV-B induced hypocotyl or internode inhibition
	phyA			
	phyB1			
	phyB2			
	cry1			
	phyAphyB1			
	phyAphyB2			
	phyB1phyB2			
	phyAphyB1phyB2			
2	WT	0.09	81.9	To reduce canopy shading from taller phytochrome and cryptochrome mutants.
3	WT	0.09	81.9	To observe WT, phytochrome and cryptochrome mutants in less dense environment.
	phyAphyB1			
	phyAphyB2			
	cry1			

4	WT *phyAphyB1* *phyAphyB2* *cry1*	0.09	81.9	Developmental check: to examine if the hypocotyl and internode elongation response in the screening experiments were age-dependent.
5	WT	0.08	78.2	End-of-day treatment (EODT) experiments: To investigate the effect of timing of UV-B treatment and the light treatment on the last day of the experiment.

2.6 General functions of light regulated genes of interest

2.6.1 Tomato PHYTOCHROME INTERACTING FACTOR 4 (*SlPIF 4*)

Phytochrome interacting factor 4 (PIF4) is one of the bHLH transcription factor that act as a hub which, connects the environmental and hormonal signaling pathways (Leivar & Quail, 2011) in *Arabidopsis*. Tomato has eight phytochrome interacting factors (PIFs): *SlPIF1a, SlPIF1b, SlPIF3, SlPIF4, SlPIF7a, SlPIF7b, SlPIF8a* and *SlPIF8b.* Out of the eight tomato *PIF loci, SlPIF4* showed similar expression patterns as of *Arabidopsis* homologs suggesting similar functions (Rosado et al., 2016). PIF4 was first identified as *srl2* (short under red-light 2) by screening through EMS-mutagenized population under phytochrome B overexpressor line of *Arabidopsis* (Choi & Oh, 2016; Huq et al., 2000). *Pif4* or *srl2* exhibited hypersensitivity to continuous R light with short hypocotyl, expanded cotyledons and shorter hypocotyls than wild type. Similarities on the hypocotyl lengths of *srl2phyB-1* double mutants and *phyB* mutant exposed to continuous R light suggests that phyB is required to generate the hypersensitive

response of *srl2* (Huq et al., 2000). Phytochromes are positive regulator of photomorphogenesis and its interaction with PIFs partially regulate photomorphogenesis through inhibition of hypocotyl or internode elongation. PIF4 together with other PIFs directly interact with phyA and phyB. The interaction between phytochromes and PIFs occurs through PIFs APA and APB motifs. PIFs also contain a DNA binding site which allow it to bind to its target DNA with G-box motif (Choi & Oh, 2016; Huq & Quail, 2002; Leivar & Monte, 2014; Leivar & Quail, 2011). Phy-PIFs interaction disrupts the PIF from binding to its target DNA and phosphorylation of phytochrome occurs. PIF4 and its homolog PIF5 regulate hypocotyl elongation under shade conditions and elevated temperatures through regulation of auxin levels and other growth promoting hormones such as gibberellins. The role of PIFs in shaded environment relates to its direct interaction with phytochromes in the presence of high R:FR or Rc. In shaded condition, low R:FR detected by phyB under canopies favors the formation of Pr and reduces Pfr-PIF interaction resulting in the upregulation of PIF4. PIF4 in turn interact with its target promoters rich in CACGTG (Choi & Oh, 2016). Two examples are auxin biosynthesis genes such as IAA29 (Sun et al., 2013) and YUCCA genes which encodes enzymes involved in the TAA1 pathway (Tao et al., 2008). UV-B light is also reduced under canopy shade and UV-B regulates PIF4 through the HY5 upregulation of gibberellin catabolic enzymes resulting in the reduction of active gibberellin, which stabilize DELLA proteins. DELLA can interact with PIFs resulting in its inactivation and inhibition of elongation. Furthermore, PIF4 regulate hypocotyl elongation in elevated temperature (Foreman et al., 2011). Transcript abundance of PIF4 is reduced by UV-B in a UVR8-dependent manner resulting in the reduction of INDOLE ACETIC ACID 29 (IAA29) and YUCCA8 expression under high temperature environments and low dose of UV-B (Hayes et al., 2016).

2.6.2 Tomato HY5 (*LeHY5)*

Tomato HY5 (*LeHY5*) is a bZIP transcription factor, which has a predicted protein with 146 amino acids. *LeHY5* share 78% amino acid with *Arabidopsis* HY5 (Liu et al., 2004). RNA interference form of *LeHY5* showed elongated hypocotyl and low levels of chlorophyll (Liu et al., 2004). These phenotypes are consistent with the phenotype of *Arabidopsis hy5* mutants thus suggests that it is a homolog of *Arabidopsis HY5* (Liu et al., 2004). Most of what is known about the role of HY5 in photomorphogenesis is based on the studies using *Arabidopsis.* The *Arabidopsis* HY5 can inhibit hypocotyl elongation through the inhibition of auxin signaling. The transcription factor activates the expression of auxin inhibitors such as IAA14 (Cluis et al. , 2004) and repress auxin related genes involved in cell elongation such as IAA29 (Jing et al., 2013). In the UV-B signaling, HY5 and its homolog HY5 HOMOLOG (HYH) are upregulated at low fluence rate by the UVR8-COP1-SPA complex and are essential for UV-B photomorphogenesis (Brown et al., 2009; Brown & Jenkins, 2008). Hence, it was used as a UV-B or UVR8 marker gene due to the dependence of the UVR8 signaling on the presence of HY5.

2.6.3 Tomato GIBBERELLIN 2 OXIDASE 2 (*SlGa2ox2*)

Tomato GIBBERELLIN 2 OXIDASE 2 (*SlGa2ox2*) is involved in many developmental stage and ensures homeostasis of GA levels through deactivation of GA. Overexpression of Ga2ox2 gene result in dwarf phenotypes which shows the roles of active GA in elongation growth (Schomburg et al., 2003). *SlGa2ox2* is highly expressed in flowers and fruits and reduced in stems and leaves (Chen et al., 2016; Schomburg et al., 2003; Xiao et al., 2007). These enzymes' activity is to 2β hydroxylate at C19 and C20-GAs resulting on the deactivation of the active GA. The reduction of active GA increases the stabilization of DELLA proteins and activates cell elongation. Ga2oxidase enzymes can be divided into three subgroups: I, II and II (Hedden

& Thomas, 2012). *SlGa2ox2* belongs to subgroup 1 as they have the highest sequence similarities to *Arabidopsis* Ga2oxidases (*AtGA2ox1, AtGA2ox2* and *AtGA2ox3*) (Chen et al., 2016). Most studies on the *SlGa2ox2* have been focused on its function in tomato fruit development as it is highly expressed in ripening tomato fruit and less on its role in stem elongation (De Jong et al., 2009; Serrani et al., 2007). Nevertheless, *SlGa2ox2* expression levels from samples used in the PAR and PAR+UV-B experiment was used as a guide to indirectly observe the activation or deactivation of DELLA dependent internode elongation.

2.6.4 CHALCONE SYNTHASE (*CHS1*)

Chalcone synthase 1 (*CHS1*) is the first enzyme is involved in the first step of flavonoid biosynthesis (Dao et al., 2011). In tomatoes, there are two *CHS* genes: *CHS1* and *CHS2* that have been characterized using RNA interference (Schijlen et al., 2007). UV-B light positively induce the expression of *CHS* and has been observed to be UVR8 dependent (Kliebenstein et al., 2002; Vandenbussche et al., 2014). Hence, tomato *CHS1* was used as a UV-B marker gene.

3.0 Results

3.1 Seed germination

Tomato phytochrome and cryptochrome mutant plants used in the PAR+UV-B experiments germinated at a different time in relation to WT (Table 3.1). Tomato seeds from all genotypes were sown on the same day and monitored for ten days. The sowing day for each mutant was adjusted to coincide with the WT de-etiolation (hypocotyl fully expanded) day, which occurs approximately 6-7 days after sowing (DAS). The *phyA, phyB1, phyB2, cry1* and *phyAphyB2* de-etiolated earlier than WT and so were sown 24 hours later than WT; and the *phyAphyB1*, *phyB1phyB2*, and *phyAphyB1phyB2* mutants de-etiolated later than WT and so were sown 24 hours before WT seeds were sown. These adjustments ensured that all tomato genotypes were all at the same developmental stage on the first day of treatment. All plants used in the PAR and PAR+UV-B experiments were at first true leaf with three leaflets and second true leaf with on leaflet developing (Fig. 3.1). Furthermore, out of all the genotypes, *phyAphyB2* mutants had the least germination percentage compared to WT and the other mutants. The *phyAphyB2* only showed 13% germination percentage (See section 4.1 for discussion.) under white light (fluorescent tubes: Philips 58W/ 865, Cool daylight; Table 3.1).

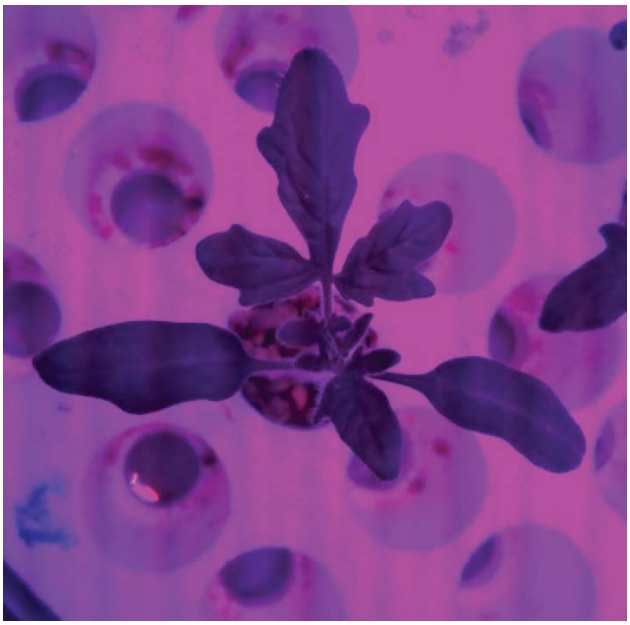

Figure 3.1: Developmental stage at which plants were treated. Plants (13-15 DAS) treated had two true leaves emerging. (Image above is 14 DAS WT under PAR light.)

Table 3.1: Tomato seeds sowing day and germination percentage.

Genotype	Sowing time of mutants with reference to WT sowing time	Germination (%)
WT (MM)	-	91
phyA (fri)	24 hours after	70
phyB1 (tri)	24 hours after	92
phyB2	24 hours after	86
cry1	24 hours after	79
phyAphyB1	Same as WT	75
phyAphyB2	24 hours after	*100
phyB1phyB2	24 hours before	100
phyAphyB1phyB2	24 hours before	69

***Germination percentage after soaking in distilled water for 24 hours and exposed to indirect light before sowing.**

3.2 Screening mutants using low fluence UV-B

Multiple mutant forms of phytochrome, and crytochrome of tomatoes (Table 2.0) were screened to identify photoreceptors that could be involved in the UV-B inhibition of hypocotyl or internode elongation. Thirteen to fifteen days-after-sowing (DAS) WT, *phyA, phyB1, phyB2, phyAphyB1, phyAphyB2, phyB1phyB2, phyAphyB1phyB2* and *cry1* were exposed just PAR light (81.9 μmol m^{-2} s^{-1}**)** only or low fluence of UV-B (~0.09 μmol m^{-2} s^{-1}) with PAR light

background (PAR+UV-B), in a crowded environment (Figure 2.2A.). The PAR+UV-B treated *phyA,* and *phyB1phyB2* mutants exhibited significantly reduced relative hypocotyl growth rate compared to PAR treated ones, while the other photoreceptor mutants showed patterns of non-significant reduction but still exhibit UV-B inhibition pattern (Figure 3.2A.). Interestingly, WT showed an unexpected increased growth rate of hypocotyl after UV-B treatment. The relative internode growth rate of WT showed the expected UV-B inhibition and *phyB1* exhibited the same significant inhibition (Figure 3.2B.). The *phyA, phyB2, cry1, phyB1phyB2* and *phyAphyB1phyB2* mutants showed non-significant UV-B growth patterns. These results suggest that the increase in hypocotyl growth rate of the WT could be due to canopy shading (reduction in R and B light) from taller mutants and that low fluence UV-B is still able to inhibit the hypocotyl and internode elongation of tomatoes despite the absence of functioning phytochrome and cryptochrome genes. Nonetheless, the *phyAphyB1* and *phyAphyB2* double mutants did not show any UV-B inhibition compared to WT. Hence, these double mutants were chosen as candidates to further investigate the roles of phyA and phyB2 in the UV-B photomorphogenesis.

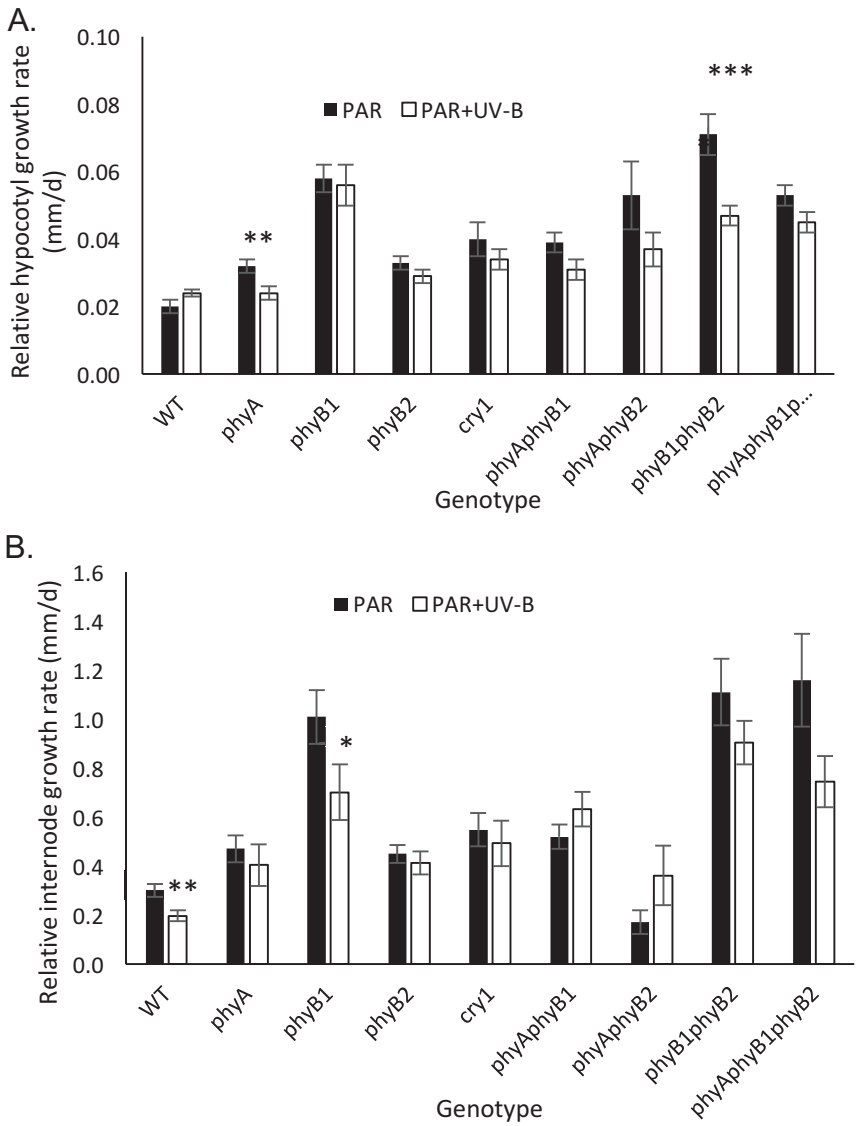

Figure 3.2: Tomato wild-type, phytochrome and cryptochrome mutants' relative hypocotyl (A.) and internode (B.) growth rate after treatment of PAR and PAR+UV-B for three days. Ten randomly chosen 11-13 DAS tomato plants from each genotype were randomly allocated in a crowded environment (Fig. 2.2A.). Plants were treated with 0.09 μmol m^{-2} s^{-1} of UV-B under 81.9 μmol m^{-2} s^{-1} of PAR background using the first treatment schedule (Fig. 2.3). PAR treated plants are the black boxes and PAR+UV-B treated are the white boxes. Asterisks suggests statistical difference between the mean of the two treatments: ANOVA *P<0.05 and **P<0.01

3.3 Increased WT hypocotyl elongation is not due to shading

A separate experiment was performed to investigate if the increased hypocotyl elongation in WT in the screening experiment (Section 3.2) was due to shading from taller mutants. Twenty randomly chosen WT plants were allocated randomly on the treatment tray (Figure 2.2B) for each treatment condition (PAR and PAR + UV-B). Figure 3.3A. shows the similar

increase in hypocotyl elongation growth rate of WT after the PAR+UV-B treatment, which suggests the hypocotyl response was not due to shading. Also, the internode elongation growth rate of WT (Figure 3.3B) exhibited a non-significant UV-B inhibition pattern, which suggests that the internode is much more sensitive to respond to a low UV-B dose. No further trials were done to further increase the confidence in the result since the initial

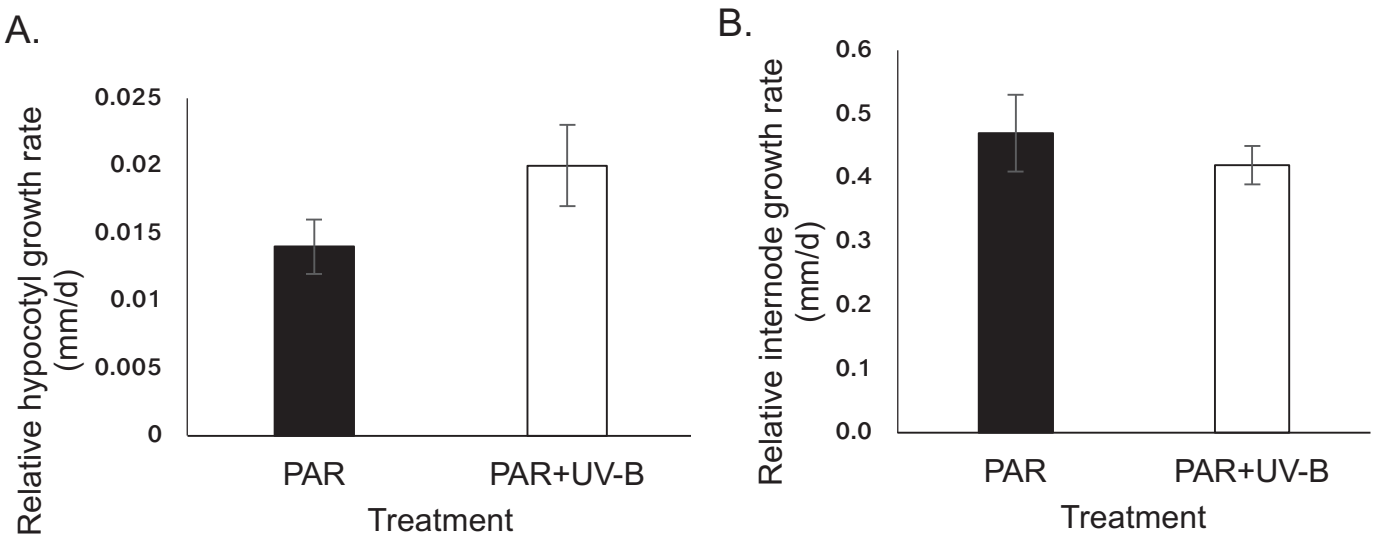

Figure 3.3 Hypocotyl and internode growth rate of WT exposed to two light conditions: PAR and PAR + UV-B for 3 days. Ten independently chosen WT plants were randomly allocated into Styrofoam for each treatment. Plants were exposed PAR (81.9 μmol m^{-2} s^{-1}) and PAR+UV-B (~0.09 μmol m^{-2} s^{-1}) for three days using the first treatment schedule (Figure 2.3) with no further repeats. PAR treated plants are the black boxes and PAR+UV-B treated are the white boxes. ANOVA P-value showed non-significant differnces for both hypocotyl and internode response.

purpose of the experiment was to investigate the elongation response of the hypocotyl and internode of WT tomato plants without any shading from taller mutant plants. Altogether, the results from this experiment suggests that the inhibition response could be dependent on the developmental stage of the plant or is age-dependent and that shading did not affect the response of the WT.

3.4 Low fluence UV-B is unable to inhibit internode of *phyAphyB2* elongation

To further investigate the roles of both phyA and phyB1 or phyB2 in regulating the UV-B induced inhibition of the hypocotyl and internode elongation, the WT, *phyAphyB1, phyAphyB2* and *cry1* were treated with the same treatment of UV-B as in the screening experiment, but in a less crowded environment (Figure 2.2B.). This new allocation set up ensured that any direct plant shading that existed in the previous treatment had been reduced. The relative hypocotyl growth rate of PAR + UV-B treated WT, *phyAphyB1* and *phyAphyB2* were much higher than those not treated with UV-B (Figure 3.4A). The hypocotyl response does not follow the response from the initial screening of mutants (Figure 3.2A.). The *cry1* mutant is the only mutant that showed a non-significant hypocotyl growth inhibition pattern in both crowded (Figure 3.2A.) and non-crowded (Figure 3.4A.) environments. The hypocotyl inhibition pattern indicates that the mutation on the UV-A/blue light photoreceptor, CRY1, may have increased the plant's sensitivity to respond to UV-B inhibition of hypocotyl elongation but may also be due to its tall phenotype- much higher UV-B detected. The relative internode elongation of the WT, *phyAphyB1,* and *cry1* showed the expected UV-B inhibition compared to those not treated with UV-B (Figure 3.4B). The growth pattern (relative growth rate) of *phyAphyB1* was evidently reduced despite it being statistically insignificant. *cry1* mutant showed the most significant inhibition out of the three genotypes. This high UV-B inhibition response of *cry1,* further supports the conclusion made in the hypocotyl response- that mutation on the functioning CRY1 induces an increased sensitivity. Interestingly, *phyAphyB2* retained the same impaired UV-B internode inhibition response (Figure. 3.4B) as when it was treated in the crowded environment (Figure 3.2B). The percentage UV-B inhibition further support that the *phyAphyB2* exhibited little-to-no UV-B inhibition compared to WT, *phyAphyB1* and *cry1* (Figure 3.5).

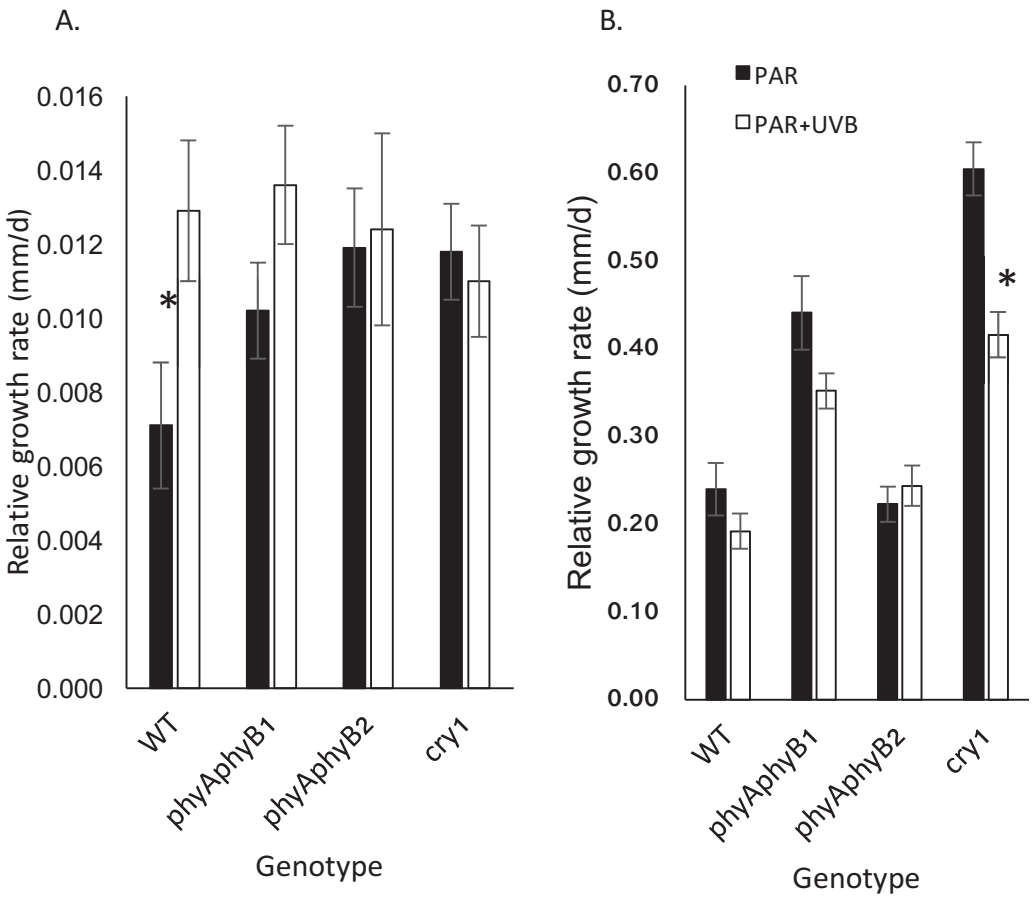

Figure 3.4: Relative hypocotyl (A.) and internode (B.) growth rate of phytochrome and cryptochrome mutants after exposure to low dose of UV-B for three days. Ten plants for each genotpes were randomly chosen and allocated to a non-crowded environment using the first treatment schedule. Plants were exposed PAR (81.9 μmol m^{-2} s^{-1}) and PAR+UV-B (~0.09 μmol m^{-2} s^{-1}). PAR treated plants are the black boxes and PAR+UV-B treated are the white boxes. ANOVA *P-value = <0.05

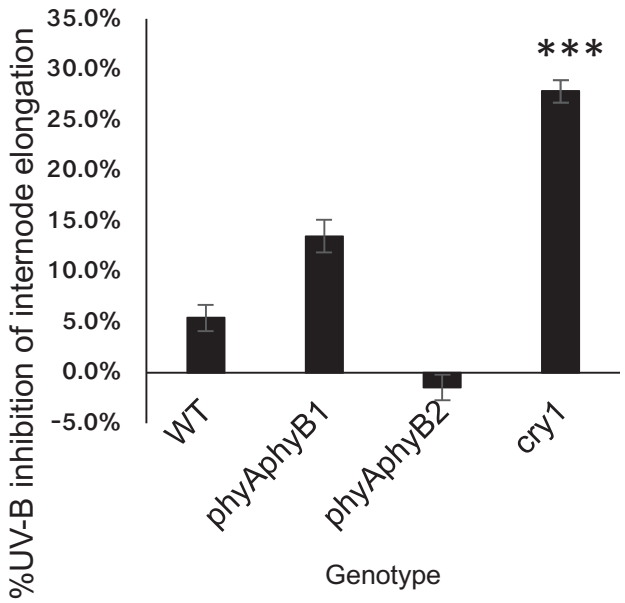

Figure 3.5: Percentage UV-B inhibtion of internode elongation after 3 days of PAR + UV-B treatment. Calculated percentage inhibition between PAR and PAR+UV-B treatments. ANOVA ***P-value = <0.001

3.4.1 UV-B inhibition responses of the hypocotyl and internode may be age-dependent

Also, a developmental check was done to examine if the elongation responses of the hypocotyl and internode observed in the treatment experiments was age-dependent. The elongation growth of the WT, *phyAphyB1*, *phyAphyB2* and *cry1* hypocotyl and internode was monitored from the day of sowing (D0) until 16 days after sowing (DAS). Here, it is important to note that the actual PAR+UV-B treatment was conducted between 14-16 DAS. Figure 3.6 shows that the hypocotyls of all genotypes were still elongating at a slower rate compared to the internode. The internode of all genotypes was still rapidly extending from 12 DAS, which may explain the sensitivity of the internodes to respond to the low dose of UV-B. These results suggest that the UV-B inhibition responses of the hypocotyl and internode may be age-dependent.

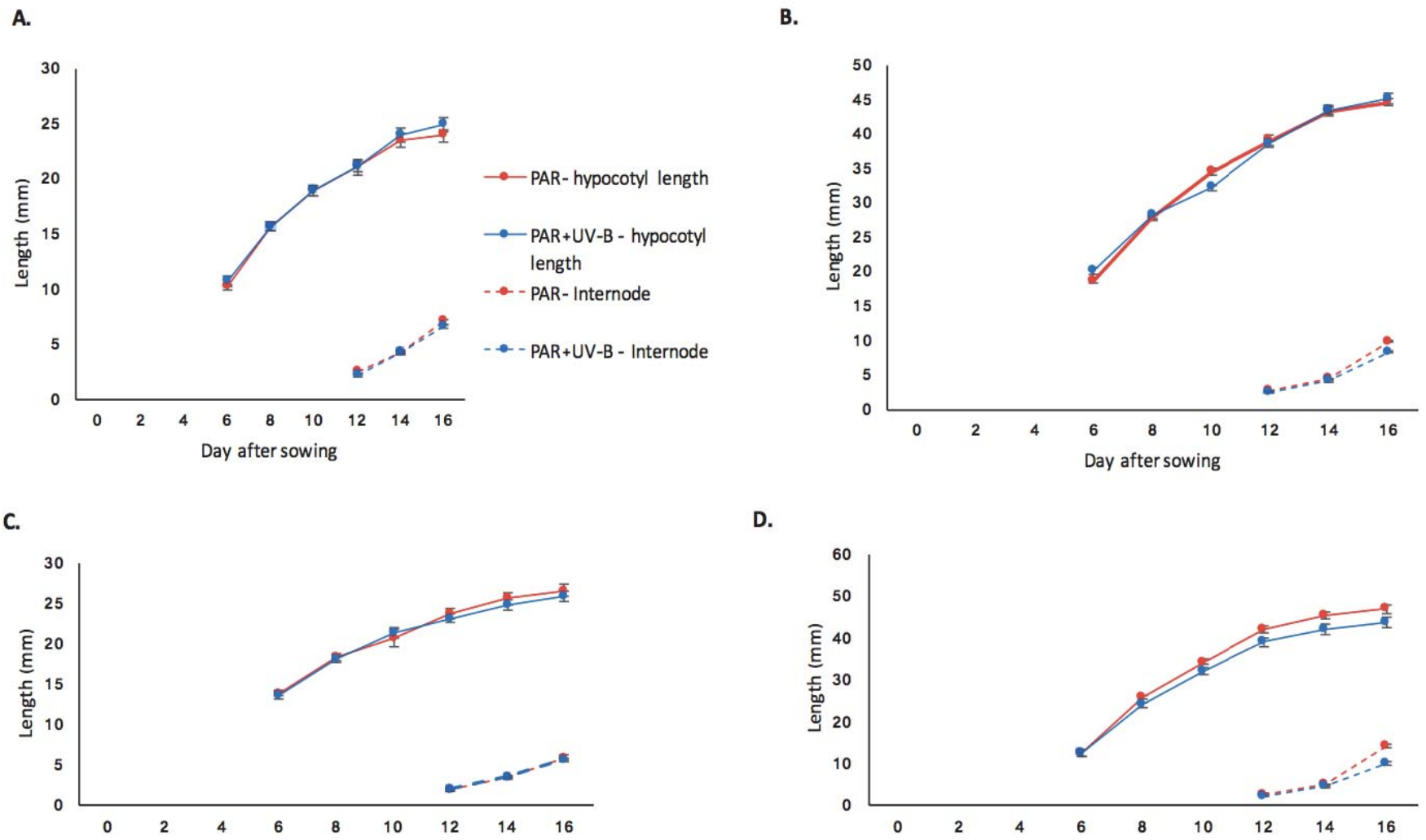

Figure 3.6: Developmental check of hypocotyl and internode growth of WT (A.), *phyAphyB1* (B.), *phyAphyB2* (C.) and *cry1* (D.) of tomatoes throughout the experimental period. The hypocotyl and internode length were measured from day the hypocotyl emerged from the vermiculite (6 DAS (Day after sowing)) until after the treatment. PAR and PAR+UV-B treatments were done from 14-16 DAS. Plants were exposed white light (measured PAR: ~100 µmol m^{-2} s^{-1}) then transferred PAR (80 µmol m^{-2} s^{-1}) and PAR+UV-B (~0.09 µmol m^{-2} s^{-1}) on the day of the treatment.

3.5 UV-B treatment is much more effective in inhibiting the hypocotyl when applied in the morning

The end-of-the-day treatment (EODT) experiment was used to check if the UV-B inhibition response of WT in the screening experiment (Section 3.2) was dependent on the timing of the treatment and the light treatment on the last day of the experiment. In the first treatment schedule (Figure 2.3), PAR was introduced in the morning (6 am- 12 pm) and UV-B was introduced in the afternoon (12 pm – 10- pm). A new treatment schedule was used where the light treatments were switched - the UV-B treatment starts from 6 am – 4 pm and PAR start from 4 pm-10 pm as shown in Figure 2.4. The hypocotyl and internode length were measured on the third day of treatment at two EODT: EODT-PAR (end-of-day-treatment with PAR) and EODT-UVB (end-of-day-treatment with UV-B). Figure 3.7 illustrates that the hypocotyl exhibited a non-significant UV-B inhibition pattern even after the EODT-PAR (circled) and that the internodes were unresponsive to UV-B. The inhibition on the WT hypocotyl suggests that in this new treatment schedule, the hypocotyls were much more sensitive in responding to UV-B when the UV-B treatment was done early in the morning (Figure 3.7 A.) compared to late in the afternoon (Figures 3.2 A. and 3.3 A.).

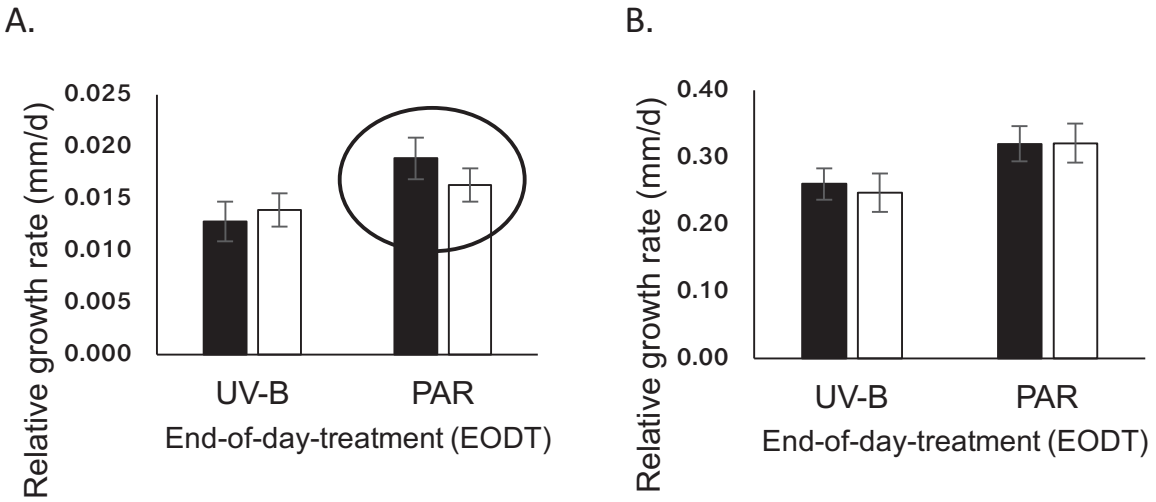

Figure 3.7 End-of-day-treatment (EODT) experiment on wild-type tomatoes. Hypocotyls (A.) and internodes (B.) were measured at two EODT: UV-B and PAR. EODT-UVB meant that measurements were done on the last day of treatment as soon as the UV-B treatment finished. EODT-PAR means that the measurements were done after the last PAR treatment plus 8 hours of dark period. Black bars represent PAR treated WT plants and white bars represent PAR+UV-B treated WT plants. Encircled bars show non-significant UV-B inhibition after the EODT-PAR treatment.

3.6 PCR troubleshooting using housekeeping genes: TUBULIN and ACTIN

3.6.1 TUBULIN primers are not annealing to tomato tubulin

TUBULIN was used as the housekeeping gene to optimize and normalize PCR products from the tomato RNA samples. RNA was extracted from a total of 16 samples (8 from after 4 hours of initial UV-B treatment; 8 from 28 hours after UV-B treatment). The quantity of the RNA was checked using Nanodrop (APPENDIX A, Table 1) and the RNA quality using the smear test (Figure 3.8). The smear test clearly illustrates that there were pipetting errors (samples escaping the well) or problems on the agarose gel itself since, all the smears that were not seen in the first trial (Figure 3.8A.) were observed in the second trial (Figure 3.8B.). Since the RNA showed good quality and quantity, the samples were treated with DNAseI and a block PCR was done using TUBULIN primers (APPENDIX B, Table 1). Block PCR was used to check for the presence of DNA. The DNAseI treated samples (Figure 3.9A.) showed presence of DNA since 8 out of the 18 samples showed PCR products using the TUBULIN primers. New

sets of RNA samples were treated with 2µl of DNAseI and gel electrophoresis showed no signs of TUBULIN apart from the positive control (Figure 3.9B.). cDNAs was then synthesized (Figure 3.9C.). Another block PCR using TUBULIN primers was performed and PCR products were passed through 2% agarose for 30 minutes to allow separation of samples. Figure 3.9C showed no trace of PCR product. These results present three possible explanations to why the products were not observed: (1.) RNA may have been degraded during the DNAseI treatment due to high temperatures used during the treatment; (2.) primers self-annealed; (3.) the TUBULIN primers were not working. The TUBULIN primers were blasted in the NCBI database, and results showed that these primers do not and cannot adhere to tomato TUBULIN. Hence, no dilutions or further troubleshooting were performed using the TUBULIN primers.

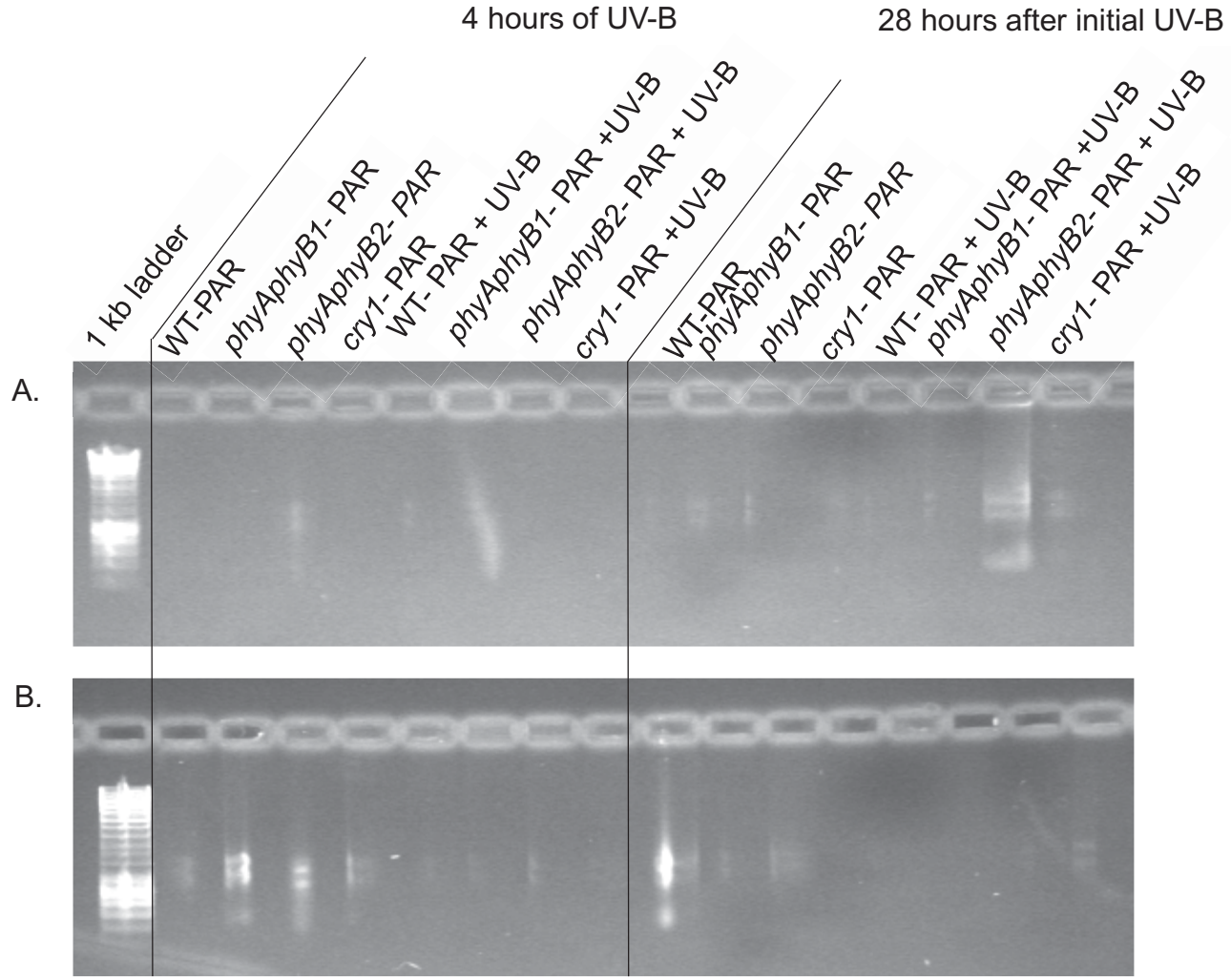

Figure 3.8 RNA quality smear test. A total of 16 RNA samples loaded into each well that were extracted with the Quick RNA Miniprep kit. Two independent sets: trial 1 (A.) and trial 2 (B.) were passed through 1% agarose gel due to pipetting error and possibly problems with the gel itself. Gel images were cropped and resized. No alterations were applied on the intensity of the bands.

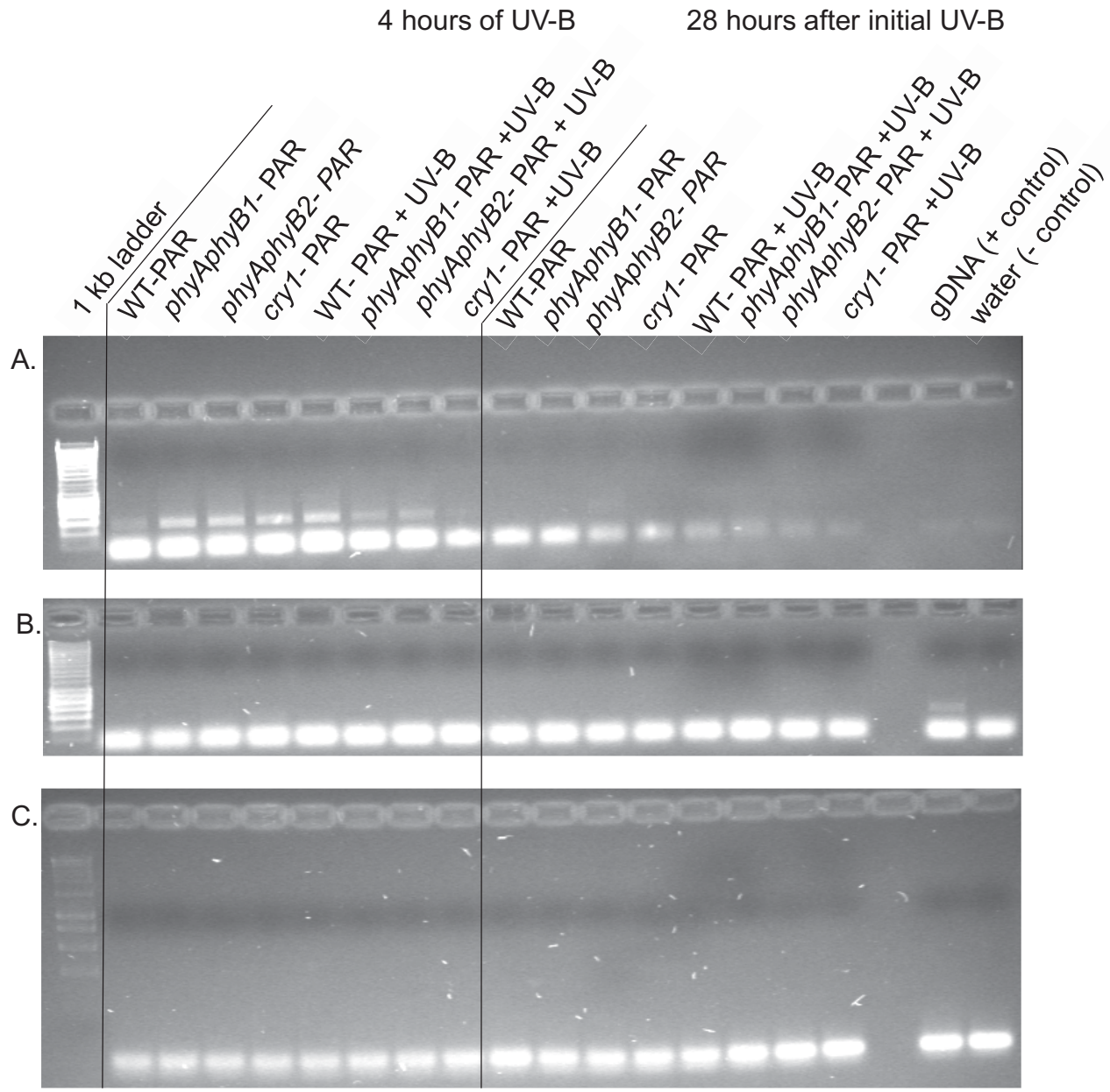

Figure 3.9 Block PCR products of DNAse treated (A. and B.) and synthesized cDNA (C.) from all 16 samples together with gDNA of WT (grown under white light) as positive control and water as negative control. TUBULIN primers were used. A. and B. were pass through 1% agarose gel for 15 minutes and C. was pass through 2% through 2% agarose. Genomic DNA from non-treated WT was used as positive control and water was used as negative control. Gel images were cropped and resized to fit the format here. No alterations were applied on the intensity of the bands.

3.6.2 ACTIN primers are more consistent in amplifying tomato *ACTIN*

Newly designed primers of ACTIN (APPENDIX B, Table 1) and PP2Acs were used as new housekeeping genes. Figure 3.10 shows that ACTIN (A.) has the most consistent band intensity throughout the 7 samples compared to PP2Ac (B.) and faint band in the positive control (band is not visible when printed on paper). Samples 8-16 did not produce any PCR products. New cDNA samples were synthesized for samples 8-16 and PCR products using ACTIN primers did not show any bands (data not shown). These results suggest cDNA was not synthesized in these samples. ACTIN products from samples 1-7 were used to normalize the other PCR products and the rest were not used. APPENDIX D Fig. 1 shows the summary of optimization steps used throughout the end-point PCR experiment.

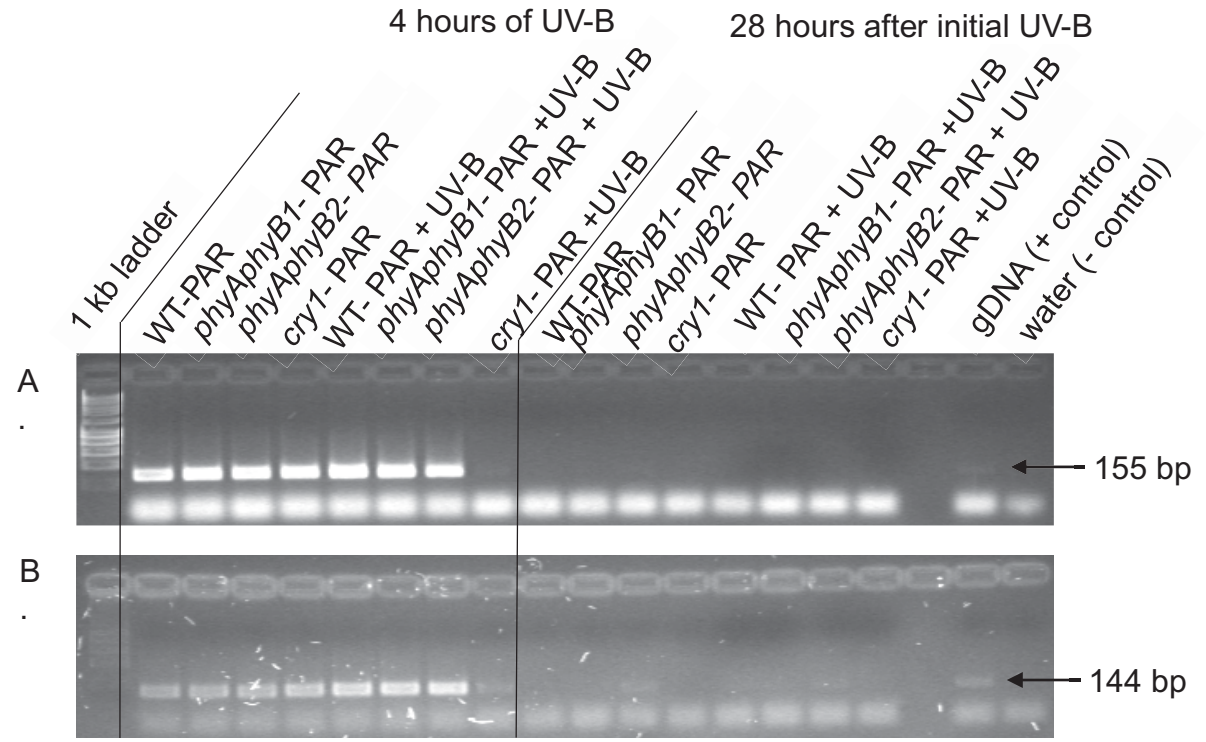

Figure 3.10 PCR products using ACTIN primers (A.) and PP2Acs primers (B.). Samples were passed through 1% agarose gel for 15 minutes. gDNA WT grown under white light was used as positive control. Genomic DNA from non-treated WT was used as positive control and water was used as negative control. Gel images were cropped and resized to fit the format here. No alterations were applied on the intensity of the bands.

3.7 UV-B increases expression of light regulated genes

The expression of four known light regulated genes were qualitatively quantified using endpoint PCR. Samples used for the PCR were from WT, *phyAphyB1* and *phyAphyB2* PAR and PAR+UV-B treatments, collected after the first four hours of initial UV-B treatment. *SlPIF4*, *LeHY5*, *CHS1* and *Ga2ox2* (See Table 3.2 for summary of these genes' functions) genes were amplified using primers shown in APPENDIX B, Table 1 and normalized using ACTIN. Here, it is important to note that the PCR products from the *cry1* mutant were not fully optimized using the ACTIN primers and therefore, were not presented in Fig. 3.11. The expressions of all four genes was all upregulated in the WT PAR+UV-B treated plants compared to WT that were only treated with PAR. *SlPIF4* in *phyAphyB1* and *phyAphyB2* showed similar intensity bands but were slightly downregulated after the PAR+UV-B treatment compared to WT. This suggests a possible redundancy between phyB1 and phyB2 in regulating the expression of *SlPIF4* when exposed to UV-B. Similar band intensity from the WT, *phyAphyB1* and *phyAphyB2* of PAR+UV-B suggests similar upregulation of *LeHY5* expression (Fig. 3.11) and that absence of phytochromes does not affect the regulation of *LeHY5*. The *Ga2ox2* showed an upregulation in all genotypes treated with PAR+UV-B compared to those that were treated with PAR only. There is a slight reduction of *Ga2ox2* expression in *phyAphyB1* and *phyAphyB2* compared to WT PAR+UV-B plants but this could be a result of pipetting errors. *CHS1* in all genotypes was slightly upregulated after PAR+UV-B compared to PAR only. Altogether, these results suggest that the upregulation of *SlPIF4, LeHY5, CHS1* and *Ga2ox2* in WT PAR+UV-B treated plants, may be dependent on UV-B light or the UV-B photoreceptor of tomato and partially regulated by phyA and phyB1 or phyB2.

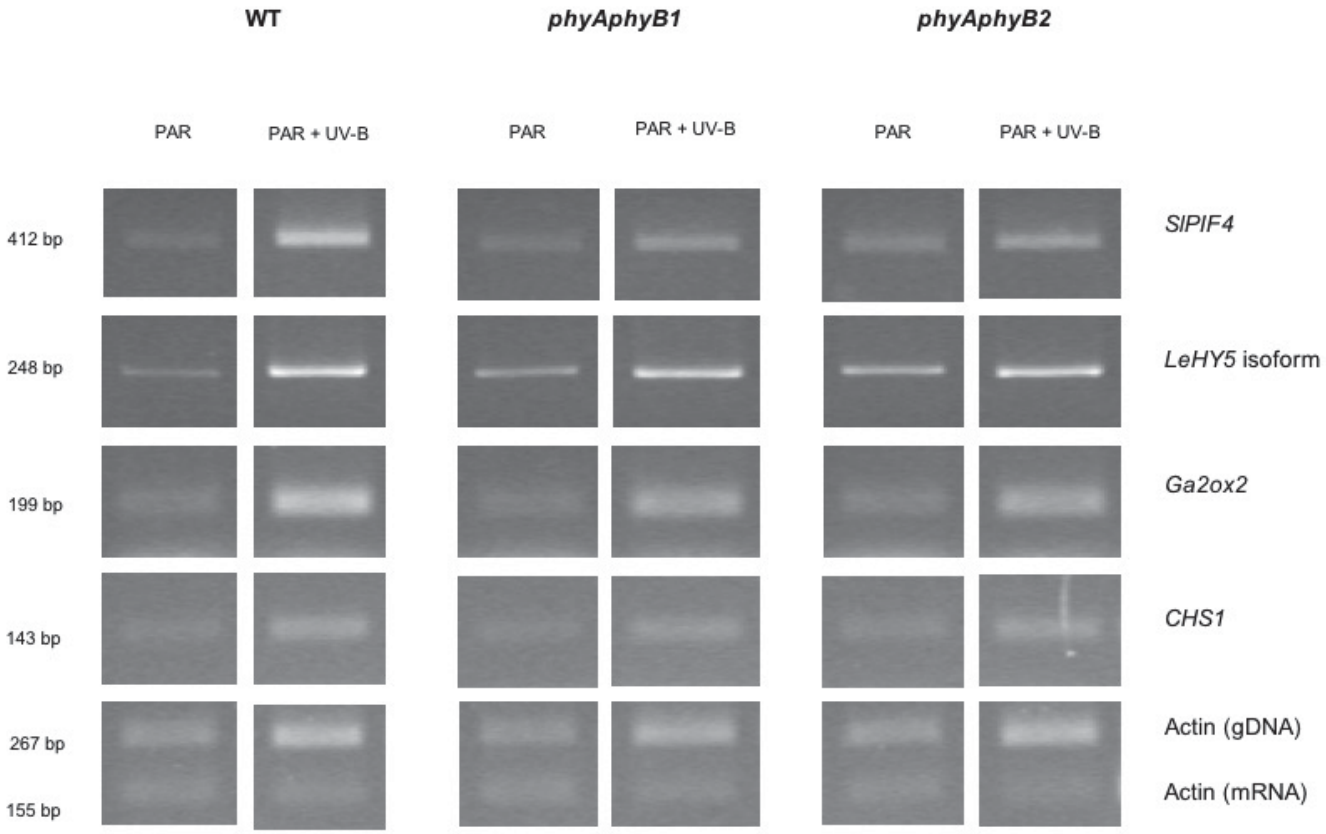

Figure 3.11: Expression levels of light regulated genes in tomato. WT, *phyAphyB1* and *phyAphyB2* plant samples were collected after 4 hours of initial PAR+UV-B treatment. PCR was used to amplify the tomato PHYTOCHROME INTERACTING FACTOR 4 (*SlPIF4;* 412 base pairs, bp), LONG HYPOCOTYL 5 (*LeHY5;* 248 bp) isoform, GIBERELLIC 2 OXIDASE 2 (*Ga2ox2;* 199 bp) and CHALCONE SYNTHASE 1 *(CHS1;* 143 bp). All PCR products were normalized using ACTIN mRNA (155 bp). Gel images were magnified and cropped. No alterations were applied on the intensity of the bands.

Table 3.2: Light regulated genes used in gene expression analysis.

Genes	Gene size (base pairs, bp)	Functions
SlPIF4	412	• Activate elongation response under shade. • See section 2.6.1
LeHY5	248	• UV-B responsive gene. • See section 2.6.2
Ga2ox2	199	• Reduce levels of active GA, hormone involved in cell elongation. • See section 2.6.3
CHS1	143	• UV-B responsive gene. • Catalyzes first step of flavonoid synthesis. • See section 2.6.4

4.0 Discussion

Phytochromes can absorb in the UV region (Pratt & Butler, 1970) and may have a role in the UV-B signaling. Studies have shown that phytochromes and the UVR8 photoreceptors act together in the induction of UV-B induced photomorphogenesis (Boccalandro et al., 2001; Kim et al., 1998; Wade et al., 2001). Here, physiological measurements of internode elongation in the PAR and PAR + UV-B experiments revealed that phyA and phyB2 of young tomatoes might be involved in the UV-B inhibition of internode elongation (Fig. 3.2 B., 3.4 B., 3.5). UV-B inhibition of elongation response is a consequence of differential gene expression; hence known UV-B-induced genes were analyzed to explain the phenotypic response of *phyAphyB2* after the UV-B treatment. WT *SlPIF4, LeHY5, Ga2ox2* and *CHS1* (slightly) were upregulated after four hours of UV-B (Fig. 3.11), suggesting a dose-dependent response and possible adaptive and long-term response, to induce the UV-B inhibition of internode in young tomatoes. Thus, experiments here demonstrate the potential crosstalk between phytochrome and the UV-B photoreceptor in regulating inhibition response under low fluence UV-B light and attempt to explain the cross-talk using known UV-B induced genes.

4.1 Germination of tomato phytochrome and cryptochrome mutants

Tomato is one of the plant species that only requires imbibition to germinate in total darkness (Shinomura, 1997) and takes about 6-7 days after sowing to de-etiolate (hypocotyl fully expanded). The Pfr active form of phytochrome activates the germination in plants (Shinomura, 1997) and the effect of mutations on phytochrome genes could affect the timing of germination, hence could have an impact on the timing of de-etiolation. Indeed, seedlings of phytochrome and cryptochrome mutants de-etiolated earlier or later than WT (Table 3.1). In tomatoes, germination has been found to be inhibited by phytochrome A after FR pulses and induced by phytochrome B2 after R pulses (Appenroth et al., 2006). In the PAR and PAR+UV-B

experiments, the *phyAphyB2* mutants had the lowest germination percentage compared to WT and the other mutants. The *phyAphyB2* only showed 13% germination rate under white light and showed no signs of root growth; perhaps showing an inability for plants to push through the seed coat (data not shown). Since it seems that the roots were unable to escape the seed coat, *phyAphyB2* seeds were placed on a filter paper with 15 mL of distilled water in a petri dish covered with a transparent lid for 24 hours to soften the seed coat. In the attempt to further increase the rate of seed germination, the soaked seeds were placed under indirect white light to reduce exposure to FR. The levels of FR and R light were not measured. The pre-soaked seeds were sown into Rockwool Plugs and vermiculite on the same day as WT. Consequently, the percentage germination of *phyAphyB2* increased almost to 100% compared to seeds that were not pre-soaked in water and directly sown into the moist Rockwool Plugs.

4.2 Increase in WT hypocotyl elongation may be due to UV-B entrainment on the plants' circadian clock

The circadian clock controls UV-B signaling pathways at different times of the day in response to various fluence rates of UV-B light (Jenkins, 2017). At low fluence rate, UV-B through UVR8 can entrain circadian clock genes and regulate genes involved in the UV-B signaling (Jenkins, 2017). Takeuchi et al., (2014), as reported by Jenkins (2017), observed that WT *Arabidopsis* in Columbia ecotype exhibited stronger UV-B inhibition at night than day. In the experiments in section 3.2 and 3.3, tomato plants were treated in the morning; the WT hypocotyl treated with PAR+UV-B exhibited increased hypocotyl growth rate and UV-B inhibition (Figures 3.2 and 3.3). Since it has been observed that UV-B inhibition increased when introduced at night (Takeuchi et al., 2014), WT tomato treated with PAR+UV-B in the afternoon, the hypocotyl displayed a non-significant inhibition (encircled) and the internode was not responsive to UV-B in the afternoon after the EODT-PAR (Fig. 3.7). These results

suggest that low fluence UV-B may entrain the hypocotyl and internode elongation response and that sensitivity of hypocotyl and internode depends on the timing of UV-B treatment.

4.3 Phytochrome A and B2 activity may be involved interacting with the UV-B photoreceptor of tomatoes in regulating internode elongation under UV-B

In highly dense canopies, the reduction of PAR light activates elongation growth on plants subjected to canopy shade (Franklin, 2008). This elongation response may attribute to the decrease in both R and B light. A similar phenotypic response observed in conditions where the ratio of R:FR light were low and a consequence of phytochrome deactivation and cryptochromes (reduced activity in low blue light condition; Sharrock (2005)). At the same time, low levels of UV-B are also detected by the plant under dense canopy and activate photomorphogenic responses (Ballaré et al., 1991; Heijde & Ulm, 2012; Lee, 2016; Vanhaelewyn et al., 2016). UV-B light through the UVR8 photoreceptor has been observed to inhibit elongation induced by low R:FR, and under low B light (Hayes et al., 2014). Since UV-B light can induce phytochrome photoconversion (Pratt & Butler, 1970), it is possible that phytochromes or cryptochromes could be involved in the UV-B signaling pathway. The initial study by Lercari et al. (1990) exposed the tomato *aurea* (*au*) mutant (mutant defective in chromophore biosynthesis) low fluence of UV-B. Their results suggest that active phytochromes are not involved in the UV-B induced hypocotyl inhibition. A similar inhibition response was also observed in the *long hypocotyl* (*lh*) mutant of cucumber deficient in phytochrome B (Ballaré et al., 1991) and *Arabidopsis phyA* and *phyB* (Kim et al., 1998). Collectively, these results suggest that phytochromes are not involved in the UV-B inhibition of hypocotyl elongation in these species. However, UV-B experiments by Kim et al. (1998) proposed that the PHYA and PHYB of *Arabidopsis* act redundantly in mediating the UV-B inhibition of hypocotyl elongation. *phyAphyB* exhibited an impaired UV-B inhibition of

internode response compared to the WT, *phyA,* and *phyB.* Furthermore, they suggested that the inhibition response to UV-B may be due to residual phytochrome activity cross-talk with the UVR8 photoreceptor in the *phyA* and *phyB* together with the tomato *aurea* mutant (Kim et al., 1998). Since tomato phyB1 and phyB2 are not orthologous of *Arabidopsis* phyB (Pratt et al., 1995), it is not possible to expect that the observations by Kim et al. (1998) may apply to those of tomatoes.

Bertram and Lercari (2000a), investigated the UV-B response of stem growth using *phyA, phyB1,* high-pigment-1 (*hp1;* display exaggerated phytochrome response) and *au* of tomatoes and found the same UV-B inhibition response. They did not use *phyB2, phyAphyB2,* and *phyB1phyB2* in their experiment. In the PAR and PAR+UV-B experiments, *phyB2, phyAphyB2,* and *phyB1phyB2* together with the other mutants listed in Table 2.0 were exposed to low levels of UV-B under PAR light background to screen which mutants will or will not respond to the UV-B inhibition of hypocotyl or internode elongation. Comparison between the PAR and PAR+UV-B treated WT and photoreceptor mutants, the *phyAphyB1* and *phyAphyB2* did not show any internode inhibition (Fig. 3.2) which is consistent with the previous study by Kim et al. (1998). The limitation of this screening, however, is that the plants subjected to shading from taller mutants and the unexpected increase in hypocotyl growth rate of WT may have been induced through this shading (Fig. 3.2 A.). To reduce shading from taller plants, WT, *phyAphyB1, phyAphyB2* and *cry1* mutants were allocated to a non-crowded environment as shown figure 2.2 B. These plants were treated with the same low fluence UV-B as in the screening experiment. The WT and *phyAphyB1* exhibited non-significant inhibition of internode elongation and *cry1* showed a significant UV-B induced internode inhibition response (Fig. 3.4). Interestingly, *phyAphyB2* did not exhibit UV-B inhibition compared to the other genotypes (Fig. 3.4 and 3.5). These results suggest that phyA and phyB2 redundantly act in mediating the UV-B inhibition of internode elongation (Kim et al., 1998).

The activity of phyA and phyB and their interaction (Sharrock, 2005) may be required to induce the UV-B inhibition of internode elongation. Lercari et al. (1990) investigated the fluence response rate of *au* mutant (unable to synthesize active phytochromobilin) and showed that UV-B at a low fluence of <4 μmol m^{-2} s^{-1}, UV-B was ineffective in inhibiting the hypocotyl elongation of the *au* mutant. Furthermore, their results also showed that at the UV-B fluence around <0.1 μmol m^{-2} s^{-1}, which is the same as the treatment used in the PAR+UV-B experiment, has little to no effect on the *au* mutant (Lercari et al., 1990). The interaction between phyA and phyB may be required in the UV-B signaling involved in the inhibition response of internodes. PhyA can both act as an inhibitor of hypocotyl elongation in VLFR under continuous R light (Rc) or R light pulses treatments. PhyA antagonizes phyB in *Arabidopsis* (Mazzella et al., 1997) under these treatments. A similar antagonistic action of PHYA to the deactivation of PHYB under low R:FR has also been observed (Martínez-García et al., 2014). Although UV-B was not present in these experiments, the antagonistic interaction between the two phytochromes may be significant in the inhibition response of WT tomato since red light is still present during the PAR and PAR+UV-B treatments. In the screening experiment, it is evident that the *phyAphyB2* impaired UV-B internode inhibition is absent in the triple mutant *phyAphyB1phyB2*. This response may be explained by the antagonistic interaction of phyA and phyB as an essential part of the UV-B signaling pathway together with PHYB2 activity under continuous red light or background PAR. It would be interesting to see the phenotypic response of these mutants to the combination of low R:FR, to naturally activate PHYA, and deactivate PHYB1 and PHYB2 activity, together with the same PAR background and low fluence UV-B used in the PAR and PAR+UV-B treatments.

4.4 Tomato LONG HYPOCOTYL 5 (*LeHY5*) and Chalcone Synthase 1 (*CHS*1) as UV-B-responsive marker genes

Photoreceptors modulate photomorphogenic responses through converging signaling pathways. Upstream of the pathways, the photoreceptors all converge towards the regulation of the COP1-SPA1 E3 ligase activity. In the UV-B signaling, the active monomer of the UVR8 photoreceptor of *Arabidopsis* interacts with the COP1-SPA1 complex, which results in the abolishment of its enzyme activity. Consequently, genes targeted by COP1are upregulated. *HY5* and *CHS* are two genes that have been observed to be upregulated in the presence of UV-B. UV-B can upregulate the expression of *HY5*. Hence, it is a good UV-B-responsive gene (Ulm et al., 2004). The significant role of HY5 on the UVR8-dependent photomorphogenesis was observed as the dependence of UV-B signaling on the presence of HY5/HYH (See section 1.7.1; Brown et al. (2009); Brown and Jenkins (2008)). In the PAR and PAR +UV-B experiments, the same upregulation of tomato *LeHY5* was observed after only four hours of UV-B treatment on all genotypes. Furthermore, *CHS1* was also used as a UV-B marker gene, and upregulation is also dependent on UVR8 (Kliebenstein et al., 2002; Vandenbussche et al., 2014). The upregulation of *CHS1* was relatively small after the first four hours of UV-B (Fig. 3.11). It has been observed that the upregulation of *CHS* is slower than *HY5* (Brown et al., 2009) and that longer UV-B exposure is still required to see the upregulation response. The upregulation of these UV-B marker genes also indicates that a possible existence of a similar UVR8 dependent upregulation of *LeHY5* and *CHS1* is present in tomato. However, the UV-B photoreceptor of tomato must first be characterized to support this response confidently. Based on sequence alignment, there is 83.5% sequence similarity between *Arabidopsis* UVR8 and the protein Regulator of Chromosome Condensation (RCC1)-like protein in the tomato (Solyc05g018615.1.1) genome (www.solgenomic.net). This uncharacterized UV-B photoreceptor of tomato may be a candidate gene, which can be specifically targeted.

4.5 Dose-dependent upregulation of tomato *SlPIF4* after four hours of UV-B.

In the presence of light, photoreceptors act collectively to downregulate the expression of PIFs (Fig. 1.5; Fraser et al. (2016); Zhao et al., (2007)). PIFs are transcription factors that negatively control photomorphogenesis by binding to promoters of DNA involved in the synthesis of hormones involved in cell elongation and activate their transcription. Levels of auxin biosynthesis genes INDOLE-3-ACETIC ACID INDUCIBLE 29 (IAA29) and IAA19 together with YUCCA genes involved in the TAA1 pathway are target genes of PIF4 (Hornitschek et al., 2012). The similar expression pattern of *SlPIF4* to the Arabidopsis *PIF4*, suggests similarities in functions (Rosado et al., 2016). In the PAR and PAR+UV-B experiments, *SlPIF4* was used to visualize the first effects of the low dose of UV-B in the first 4 hours and 28 hours after initial UV-B treatment (Fig. 3.11) and correlate it with levels of auxin. The cDNA for PCR was harvested at the 28 hours of UV-B did not work despite multiple troubleshooting. Nevertheless, samples from 4 hours of UV-B presented an upregulation of *SlPIF4* in the WT and slight upregulation in the *phyAphyB1* and *phyAphyB2* (Figure 3.11), which indicate redundancy of phyB1 and phyB2. It is evident that PIFs are downregulated by PAR alone as a result of R light induced degradation of PIFs (Li et al., 2016). Strong band intensity of *SlPIF4* in the WT after the UV-B treatment was not typical since *Arabidopsis PIF4* and *PIF5* exposed to 2 hours of ~1.0 μmol m^{-2} s^{-1} of UV-B can downregulate the expression of these PIFs (Hayes et al., 2014). These results partially suggest that upregulation of *SlPIF4* exhibited in the PAR+UV-B experiment may be a dose-dependent response and may be dependent on the UV-B photoreceptor of tomato. More experiments using higher UV-B dose should be done to confirm this dose-dependency fully. In addition to this, the reduction of PIFs may be associated with the downregulation of auxin resulting in the inhibition of elongation. Since plant samples were only harvested on plants exposed to only four hours of UV-B, the

expression of *SlPIF4* shown in figure 3.11 cannot be used to explain the lack of UV-B internode inhibition of *phyAphyB2.*

4.6 Gibberellic 2 oxidase2 (Ga2ox2) upregulation typical response to UV-B inhibition of cell elongation

Gibberellin (GA) is another growth promoting hormone involved in cell elongation (Olszewski et al., 2002). In low R:FR condition, bioactive GA increases through the upregulation of GA biosynthesis genes *Ga20ox1* and *Ga20ox2*. GA control elongation through destabilizing DELLA and target it for proteasomal degradation. (Djakovic-Petrovic et al., 2007; Hisamatsu et al., 2005) Under UV-B, the UVR8 photoreceptor interacts with COP1 and upregulates the HY5/HYH bZIP transcription factors (Brown et al., 2009; Brown & Jenkins, 2008). HY5/HYH target promoters of enzymes such as Ga2-oxidases (Ga2ox) involved in the catabolism of active GA (Hayes et al., 2014). In the PAR+UV-B experiments, the *SlGa2ox2* gene of tomato was up-regulated in the WT after 4 hours of UV-B exposure and slightly reduced in the double mutants. The upregulation is UV-B light dependent since the intensity of the band increased when UV-B (plus background PAR lights) was introduced compared to just PAR lights. The reduction in the *phyAphyB1* and *phyAphyB2* may be a result of pipetting error, and it is hard to tell if there is a downregulation of the *SlGa2ox2* expression after the four hours of UV-B. The expression of *Ga2ox2* was used to correlate it with the levels of GA after initial UV-B exposure. GA levels may be reduced because of the upregulation of *Ga2ox2* and consequently stabilized DELLA proteins. DELLA proteins can directly interact with PIF3 and PIF4 to inhibit PIFs from binding to their DNA targets (Li et al., 2016). The reduction of PIFs downregulates auxin-related genes or YUCCA genes which encode enzymes involved in the biosynthesis of auxin (Li et al., 2016). The inhibition of internode elongation observed in WT and *phyAphyB1 (*possibly in *cry1*) may be the result of the reduction of active GA and auxin. The

downregulation of PIF was not observed in the WT *SlPIF4* (Fig. 3.11) and this further support the idea that the upregulation of *SlPIF4* may be dose-dependent. Given that the inhibition still occurred in the WT internode, it is possible that this is a short-term response and the UV-B induced degradation of PIF may have taken place after 28 or 56 hours after initial UV-B treatment. The UV-B dose used by Hayes et al. (2014a) that degraded the overexpressed 35S:: PIF4-HA in *Arabidopsis* was ten times higher (~1μmol $m^{-2}s^{-1}$) than the dose used in the PAR+UV-B experiments here (~0.09μmol $m^{-2}s^{-1}$). It would have been interesting to observe if *SlPIF4* is still upregulated after 28 or 56 hours of UV-B. Furthermore, it is possible that the upregulation of *SlGa2ox2* by UV-B does not reduce the active GA levels *in planta* since it cannot inactivate GA through hydroxylation to the C19 or C20 of bioactive GAs (Chen et al., 2016; Serrani et al., 2007). It is possible that the other remaining *SlGa2ox* genes, especially *Ga2ox1* (which could have been used as another candidate gene instead of the *Ga2ox2*) may have acted as a long-term response that may have reduced the expression of *PIF4* and result in the inhibition of internode elongation in WT after the last day of treatment.

5.0 Conclusion

Plants use photoreceptors to synchronize their development to the changing light environment. Photoreceptors of plants detect light changes and cross-talk in regulating plant development. In the PAR and PAR+UV-B experiments here, one of the aims is to provide some evidence of cross-talk between the phytochromes, cryptochrome and UV-B photoreceptor of tomato in regulating the UV-B induced inhibition of internode elongation at low doses of UV-B. Indeed, the exposure of multiple mutants of phytochromes and cryptochromes to low fluence UV-B in crowded and non-crowded experiments presented that phytochromes may be involved in the inhibition. The low fluence of UV-B inhibited the hypocotyl (Figure 3.2A.) and internode extension (Figures 3.3B, 3.4B, and 3.5) in WT, phytochromes and cryptochrome mutants

except for *phyAphyB2*. The impaired UV-B inhibition response to the internode of *phyAphyB2* suggests that there is a cross-talk between the active form of these photoreceptors and the UV-B photoreceptor. Further experiments using younger plants should be used to observe if phyA and phyB2 of tomato plants may also regulate the hypocotyl elongation response of tomato to UV-B since the hypocotyls observed in the PAR and PAR+UV-B experiments were not as responsive as the internodes. Also, higher UV-B dose >0.1 μmol m^{-2} s^{-1} should be used to produce more significant UV-B inhibition result.

Gene expression analysis on the major UV-B related genes were used to explain the impaired UV-B inhibition response by the *phyAphyB2* mutant plants. Endpoint PCR failed to explain the response exhibited by the *phyAphyB2*. UV-B failed to reduce the expression of *SlPIF4*, but upregulated the *LeHY5* and *CHS1* UV-B responsive genes. Future experiments should analyze samples from tomato plants exposed to 28 or 56 hours after initial UV-B irradiation to visualize the long-term response of hormone-related genes. Also, levels of auxin and active GA could be measured together with the *SlPIF4* and *Ga2ox2* (or other Ga2ox e.g. *Ga2ox1*) gene expression analysis. These measurements will directly explain the inhibition or elongation response of internode after UV-B exposure. To further strengthen the results presented here, the tomato UV-B photoreceptor must be characterized and isolated. If ever isolated, the mutant form together with the *phyAphyB2* mutants should be investigated to confirm that these photoreceptors do act redundantly in mediating UV-B internode inhibition. Nevertheless, the results presented here, provide some evidence of tomato phytochromes and UV-B photoreceptor cross-talk in regulating the UV-B inhibition of elongation.

APPENDICES

APPENDIX A

Table 1: RNA concentrations of 16 samples measured using Nanodrop.

Sample No.	Genotype	Hours after initial UV-B treatment	Light treatment	RNA concentration based on absorbance peaks between 260-280nm (μg/μL)
1	WT	4	PAR	617.7
2	*phyAphyB1*	4	PAR	906.7
3	*phyAphyB2*	4	PAR	326.5
4	*cry1*	4	PAR	685.0
5	WT	4	PAR+UVB	741.2
6	*phyAphyB1*	4	PAR+UVB	952.3
7	*phyAphyB2*	4	PAR+UVB	1041.1
8	*cry1*	4	PAR+UVB	447.7
9	WT	28	PAR	728.0
10	*phyAphyB1*	28	PAR	722.8
11	*phyAphyB2*	28	PAR	1105.7
12	*cry1*	28	PAR	917.8
13	WT	28	PAR+UVB	1253.6
14	*phyAphyB1*	28	PAR+UVB	903.1
15	*phyAphyB2*	28	PAR+UVB	411.1
16	*cry1*	28	PAR+UVB	540.8

APPENDIX B

Table 1: Primers used in endpoint PCR.

Primer name	Sequence	References
PIF4-4F	TAAGTGAAACAACGGCCACA	PerlPrimer (http://perlprimer.sourceforge.net/)
PIF4-4R	AGTTTAGCCACGCGACAGTT	
Ga2ox2-F	ATAGCGACTCCGTTTTCAGG	(Livne et al., 2015)
Ga2ox2-R	TTTTCATCAGGTGGGACAGA	
LeHY5-F	AAGCAAGGGTGAAGGAATTG	(Calvenzani et al., 2010)
LeHY5-R	ACAATCCACCCGAAACTAGC	
CHS1-F	AAACTCTTGTCCCCGATAGC	Giuntini et al. (2008)
CHS1-R	CCCTAGAGGTTGAAATGCTTC	
Actin1_F	CTTCCCTCAGCACCTTCCAG	PerlPrimer (http://perlprimer.sourceforge.net/)
Actin1_R	GCATCTCTGGTCCAGTAGGAA	
TUBULIN_F	CACATTGGTCAGGCCGGTAT	(Livne et al., 2015)
TUBULIN_R	CGCGAGATGAGATAAACCA	
PP2Ac_F	TGTAACCCGAAGAACACCCG	PerlPrimer (http://perlprimer.sourceforge.net/)
PP2Ac_R	CCGCTCACACCATTGTAGCA	

Table 2: Annealing temperatures used for amplification of target genes during PCR.

Primer name	Annealing Temperatures (°C)
PIF4-F	53.4
PIF4-R	
Ga2ox2-F	53.4
Ga2ox2-R	
LeHY5-F	58.1
LeHY5-R	
CHS1-F	53.4
CHS1-R	
Actin1_F	56.7
Actin1_R	
TUBULIN_F	50.2
TUBULIN_R	
PP2Ac_F	50.2
PP2Ac_R	

APPENDIX C

Table 1: PCR program used to amplify target genes.

Step	Temperature (°C)	Time (minutes)	Cycle number
Initial Denaturation	95	2	1
Denaturation	95	1	
Annealing	see Appendix B Table 2	1	30
Extension	72	1	
Final Extension	72	5	1
Soaking	4		Indefinitely

APPENDIX D

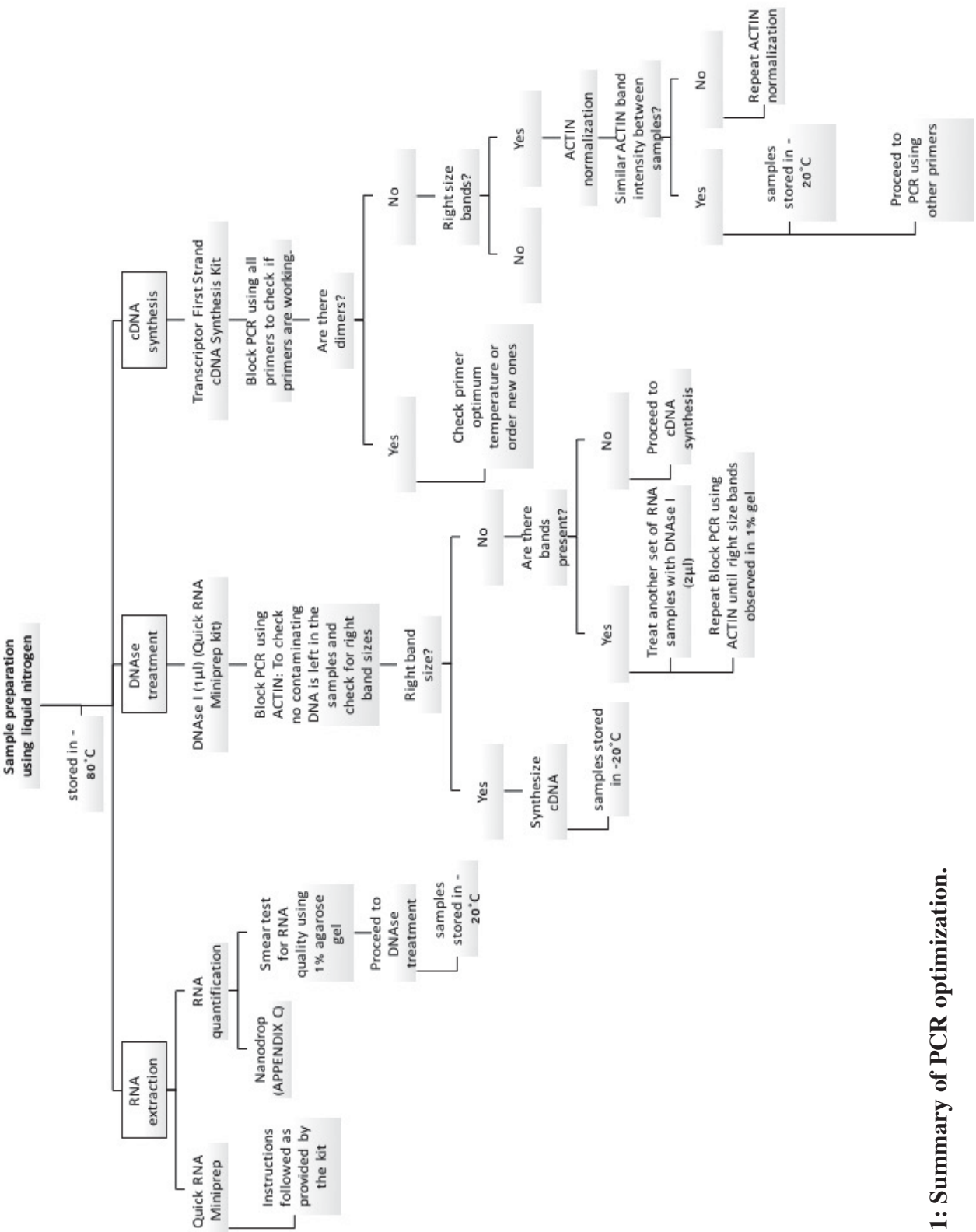

Figure 1: Summary of PCR optimization.

REFERENCES

(USDA), U. S. D. o. A. E. R. S. (2016, 3 February, 2016). Tomatoes. Retrieved from http://www.ers.usda.gov/topics/crops/vegetables-pulses/tomatoes.aspx

Ahmad, M., & Cashmore, A. R. (1997). The blue-light receptor cryptochrome 1 shows functional dependence on phytochrome A or phytochrome B in Arabidopsis thaliana. *The Plant Journal, 11*(3), 421-427.

Ahmad, M., Jarillo, J. A., & Cashmore, A. R. (1998). Chimeric proteins between cry1 and cry2 Arabidopsis blue light photoreceptors indicate overlapping functions and varying protein stability. *The Plant Cell Online, 10*(2), 197-207.

APPENROTH, K. J., Lenk, G., Goldau, L., & Sharma, R. (2006). Tomato seed germination: regulation of different response modes by phytochrome B2 and phytochrome A. *Plant, Cell & Environment, 29*(4), 701-709.

Attridge, T. H. (1990). *Light and plant responses: a study of plant photophysiology and the natural environment*: Cambridge University Press.

Aukerman, M. J., Hirschfeld, M., Wester, L., Weaver, M., Clack, T., Amasino, R. M., & Sharrock, R. A. (1997). A deletion in the PHYD gene of the Arabidopsis Wassilewskija ecotype defines a role for phytochrome D in red/far-red light sensing. *The Plant Cell, 9*(8), 1317-1326.

Bais, A., McKenzie, R., Bernhard, G., Aucamp, P., Ilyas, M., Madronich, S., & Tourpali, K. (2015). Ozone depletion and climate change: impacts on UV radiation. *Photochemical & Photobiological Sciences, 14*(1), 19-52.

Ballaré, C. L. (2014). Light regulation of plant defense. *Annual review of plant biology, 65*, 335-363.

Ballaré, C. L., Barnes, P. W., & Flint, S. D. (1995). Inhibition of hypocotyl elongation by ultraviolet-B radiation in de-etiolating tomato seedlings. I. The photoreceptor. *Physiologia Plantarum, 93*(4), 584-592.

Ballaré, C. L., Barnes, P. W., & Kendrick, R. E. (1991). Photomorphogenic effects of UV-B radiation on hypocotyl elongation in wild type and stable-phytochrome-deficient mutant seedlings of cucumber. *Physiologia Plantarum, 83*(4), 652-658.

Ballaré, C. L., Caldwell, M. M., Flint, S. D., Robinson, S. A., & Bornman, J. F. (2011). Effects of solar ultraviolet radiation on terrestrial ecosystems. Patterns, mechanisms, and interactions with climate change. *Photochemical & Photobiological Sciences, 10*(2), 226-241.

Ballaré, C. L., & Pierik, R. (2017). The shade avoidance syndrome: Multiple signals and ecological consequences. *Plant, Cell & Environment*. *In print.*

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

Barnes, P. W., Flint, S. D., Ryel, R. J., Tobler, M. A., Barkley, A. E., & Wargent, J. J. (2015). Rediscovering leaf optical properties: New insights into plant acclimation to solar UV radiation. *Plant Physiology and Biochemistry, 93*, 94-100.

Bertram, L., & Lercari, B. (2000a). Evidence against the involvement of phytochrome in UVB-induced inhibition of stem growth in green tomato plants. *Photosynthesis research, 64*(2), 107-117.

Bertram, L., & Lercari, B. (2000b). *Time-dependent effectiveness of UV-treatment on the control of the elongation growth of tomato transplants.* Paper presented at the IV International ISHS Symposium on Artificial Lighting 580.

Biggs, R. H., & Joyner, M. E. (2013). *Stratospheric ozone depletion/UV-B radiation in the biosphere* (Vol. 18): Springer Science & Business Media.

Boccalandro, H. E., Mazza, C. A., Mazzella, M. A., Casal, J. J., & Ballaré, C. L. (2001). Ultraviolet B radiation enhances a phytochrome-B-mediated photomorphogenic response in Arabidopsis. *Plant Physiology, 126*(2), 780-788.

Bögre, L., & Beemster, G. (2008). *Plant growth signaling* (Vol. 10): Springer Science & Business Media.

Botto, J. F., Sanchez, R. A., Whitelam, G. C., & Casal, J. J. (1996). Phytochrome A mediates the promotion of seed germination by very low fluences of light and canopy shade light in Arabidopsis. *Plant Physiology, 110*(2), 439-444.

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 of the United States of America, 102*(50), 18225-18230.

Brown, B. A., Headland, L. R., & Jenkins, G. I. (2009). UV-B action spectrum for UVR8-mediated HY5 transcript accumulation in Arabidopsis. *Photochemistry and photobiology, 85*(5), 1147-1155.

Brown, B. A., & Jenkins, G. I. (2008). UV-B signaling pathways with different fluence-rate response profiles are distinguished in mature Arabidopsis leaf tissue by requirement for UVR8, HY5, and HYH. *Plant Physiology, 146*(2), 576-588.

Calvenzani, V., Martinelli, M., Lazzeri, V., Giuntini, D., Dall'Asta, C., Galaverna, G., Tonelli, C., Ranieri, A. & Petroni, K. (2010). Response of wild-type and high pigment-1 tomato fruit to UV-B depletion: flavonoid profiling and gene expression. *Planta, 231*(3), 755-765.

Cargnel, M. D., Demkura, P. V., & Ballaré, C. L. (2014). Linking phytochrome to plant immunity: low red: far-red ratios increase Arabidopsis susceptibility to Botrytis cinerea by reducing the biosynthesis of indolic glucosinolates and camalexin. *New Phytologist, 204*(2), 342-354.

Carriedo, L. G., Maloof, J. N., & Brady, S. M. (2016). Molecular control of crop shade avoidance. *Current Opinion in Plant Biology, 30*, 151-158.

Casal, J. J. (2000). Phytochromes, cryptochromes, phototropin: photoreceptor interactions in plants. *Photochemistry and photobiology, 71*(1), 1-11.

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

Cerrudo, I., Keller, M. M., Cargnel, M. D., Demkura, P. V., de Wit, M., Patitucci, M. S., Pierik, R., Pieterse, C.M.J. & Ballaré, C. L. (2012). Low red/far-red ratios reduce Arabidopsis resistance to Botrytis cinerea and jasmonate responses via a COI1-JAZ10-dependent, salicylic acid-independent mechanism. *Plant Physiology, 158*(4), 2042-2052.

Chen, S., Wang, X., Zhang, L., Lin, S., Liu, D., Wang, Q., . . . Wu, H. (2016). Identification and characterization of tomato gibberellin 2-oxidases (GA2oxs) and effects of fruit-specific SlGA2ox1 overexpression on fruit and seed growth and development. *Horticulture Research, 3*, 16059.

Chipperfield, M., Dhomse, S., Feng, W., McKenzie, R., Velders, G., & Pyle, J. (2015). Quantifying the ozone and ultraviolet benefits already achieved by the Montreal Protocol. *Nature Communications, 6*.

Choi, H., & Oh, E. (2016). PIF4 Integrates Multiple Environmental and Hormonal Signals for Plant Growth Regulation in Arabidopsis. *Molecules and Cells, 39*(8), 587.

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

Cipollini, D. (2004). Stretching the limits of plasticity: can a plant defend against both competitors and herbivores? *Ecology, 85*(1), 28-37.
Clough, R., & Vierstra, R. (1997). Phytochrome degradation. *Plant, Cell & Environment, 20*(6), 713-721.
Cluis, C. P., Mouchel, C. F., & Hardtke, C. S. (2004). The Arabidopsis transcription factor HY5 integrates light and hormone signaling pathways. *The Plant Journal, 38*(2), 332-347.
Czégény, G., Mátai, A., & Hideg, É. (2016). UV-B effects on leaves—Oxidative stress and acclimation in controlled environments. *Plant Science, 248*, 57-63.
Dao, T., Linthorst, H., & Verpoorte, R. (2011). Chalcone synthase and its functions in plant resistance. *Phytochemistry Reviews, 10*(3), 397.
De Jong, M., Mariani, C., & Vriezen, W. H. (2009). The role of auxin and gibberellin in tomato fruit set. *Journal of Experimental Botany*, erp094.
de Wit, M., Galvão, V. C., & Fankhauser, C. (2016). Light-Mediated Hormonal Regulation of Plant Growth and Development. *Annual review of plant biology, 67*, 513-537.
Demkura, P. V., Abdala, G., Baldwin, I. T., & Ballaré, C. L. (2010). Jasmonate-dependent and-independent pathways mediate specific effects of solar ultraviolet B radiation on leaf phenolics and antiherbivore defense. *Plant Physiology, 152*(2), 1084-1095.
Demkura, P. V., & Ballaré, C. L. (2012). UVR8 mediates UV-B-induced Arabidopsis defense responses against Botrytis cinerea by controlling sinapate accumulation. *Molecular plant, 5*(3), 642-652.
Devlin, P. F., Halliday, K. J., Harberd, N. P., & Whitelam, G. C. (1996). The rosette habit of Arabidopsis thaliana is dependent upon phytochrome action: novel phytochromes control internode elongation and flowering time. *The Plant Journal, 10*(6), 1127-1134.
Devlin, P. F., Patel, S. R., & Whitelam, G. C. (1998). Phytochrome E influences internode elongation and flowering time in Arabidopsis. *The Plant Cell, 10*(9), 1479-1487.
Devlin, P. F., Rood, S. B., Somers, D. E., Quail, P. H., & Whitelam, G. C. (1992). Photophysiology of the elongated internode (ein) mutant of Brassica rapa ein mutant lacks a detectable phytochrome B-like polypeptide. *Plant Physiology, 100*(3), 1442-1447.
Djakovic-Petrovic, T., Wit, M. d., Voesenek, L. A., & Pierik, R. (2007). DELLA protein function in growth responses to canopy signals. *The Plant Journal, 51*(1), 117-126.
Dorais, M., Ehret, D. L., & Papadopoulos, A. P. (2008). Tomato (Solanum lycopersicum) health components: from the seed to the consumer. *Phytochemistry Reviews, 7*(2), 231-250.
Fankhauser, C., & Batschauer, A. (2016). Shadow on the Plant: A Strategy to Exit. *Cell, 164*(1), 15-17.
Favory, J.-J., Stec, A., Gruber, H., Rizzini, L., Oravecz, A., Funk, M., . . . Ulm, R. (2009). Interaction of COP1 and UVR8 regulates UV-B-induced photomorphogenesis and stress acclimation in Arabidopsis. *Embo Journal, 28*(5), 591-601. doi:10.1038/emboj.2009.4
Fernando, V., & Schroeder, D. (2016). Shedding light on plant development: light signalling in the model plant Arabidopsis thaliana. *Ceylon Journal of Science, 45*(1).
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.
Franklin, K. A. (2008). Shade avoidance. *New Phytologist, 179*(4), 930-944.
Franklin, K. A., & Quail, P. H. (2010). Phytochrome functions in Arabidopsis development. *Journal of Experimental Botany, 61*(1), 11-24.

Franklin, K. A., & Whitelam, G. C. (2005). Phytochromes and shade-avoidance responses in plants. *Annals of Botany, 96*(2), 169-175.
Franklin, K. A., & Whitelam, G. C. (2006). The roles of phytochromes in adult plants. In E. Schäfer & F. Nagy (Eds.), *Photomorphogenesis in plants and bacteria: function and signal transduction mechanisms* (pp. 475-497): Springer Science & Business Media.
Fraser, D. P., Hayes, S., & Franklin, K. A. (2016). Photoreceptor crosstalk in shade avoidance. *Current Opinion in Plant Biology, 33*, 1-7.
Frohnmeyer, H., & Staiger, D. (2003). Ultraviolet-B radiation-mediated responses in plants. Balancing damage and protection. *Plant Physiology, 133*(4), 1420-1428.
Furuya, M. (1993). Phytochromes: their molecular species, gene families, and functions. *Annual review of plant biology, 44*(1), 617-645.
Gerszberg, A., Hnatuszko-Konka, K., Kowalczyk, T., & Kononowicz, A. K. (2015). Tomato (Solanum lycopersicum L.) in the service of biotechnology. *Plant Cell, Tissue and Organ Culture (PCTOC), 120*(3), 881-902.
Giuntini, D., Lazzeri, V., Calvenzani, V., Dall'Asta, C., Galaverna, G., Tonelli, C., Petroni, K. & Ranieri, A. (2008). Flavonoid profiling and biosynthetic gene expression in flesh and peel of two tomato genotypes grown under UV-B-depleted conditions during ripening. *Journal of agricultural and food chemistry, 56*(14), 5905-5915.
Gommers, C. M., Visser, E. J., St Onge, K. R., Voesenek, L. A., & Pierik, R. (2013). Shade tolerance: when growing tall is not an option. *Trends in plant science, 18*(2), 65-71.
Gundel, P. E., Pierik, R., Mommer, L., & Ballaré, C. L. (2014). Competing neighbors: light perception and root function. *Oecologia, 176*(1), 1-10.
Hayes, S., Sharma, A., Fraser, D. P., Trevisan, M., Cragg-Barber, C. K., Tavridou, E., Fankhauser, C., Jenkins, G.I. & Franklin, K. A. (2016). UV-B perceived by the UVR8 photoreceptor inhibits plant thermomorphogenesis. *Current Biology, 127(1)*. 120-127, ISSN 0960-9822, https://doi.org/10.1016/j.cub.2016.11.004.
Hayes, S., Velanis, C. N., Jenkins, G. I., & Franklin, K. A. (2014a). UV-B detected by the UVR8 photoreceptor antagonizes auxin signaling and plant shade avoidance. *Proceedings of the National Academy of Sciences of the United States*(32), 11894. doi:10.1073/pnas.1403052111
Hectors, K., Prinsen, E., De Coen, W., Jansen, M. A., & Guisez, Y. (2007). Arabidopsis thaliana plants acclimated to low dose rates of ultraviolet B radiation show specific changes in morphology and gene expression in the absence of stress symptoms. *New Phytologist, 175*(2), 255-270.
Hedden, P., & Thomas, S. G. (2012). Gibberellin biosynthesis and its regulation. *Biochemical Journal, 444*(1), 11-25.
Heijde, M., & Ulm, R. (2012). UV-B photoreceptor-mediated signaling in plants. *Trends in plant science, 17*(4), 230-237.
Hennig, L., Stoddart, W. M., Dieterle, M., Whitelam, G. C., & Schäfer, E. (2002). Phytochrome E controls light-induced germination of Arabidopsis. *Plant Physiology, 128*(1), 194-200.
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), 6515-6520.
Hille, K. (2010). UV Exposure Has Increased Over the Last 30 Years, but Stabilized Since the Mid-1990s. Retrieved from http://www.nasa.gov/topics/solarsystem/features/uv-exposure.html
Hillman, W. S. (1967). The physiology of phytochrome. *Annual Review of Plant Physiology, 18*(1), 301-324.

Hisamatsu, T., King, R. W., Helliwell, C. A., & Koshioka, M. (2005). The involvement of gibberellin 20-oxidase genes in phytochrome-regulated petiole elongation of Arabidopsis. *Plant Physiology, 138*(2), 1106-1116.

Hollósy, F. (2002). Effects of ultraviolet radiation on plant cells. *Micron, 33*(2), 179-197.

Hornitschek, P., Kohnen, M. V., Lorrain, S., Rougemont, J., Ljung, K., López-Vidriero, I., Franco-Zorilla, J.M., Solano, R., Trevisan, M. & Pradervand, S. (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.

Huq, E., Kang, Y., Halliday, K. J., Qin, M., & Quail, P. H. (2000). SRL1: a new locus specific to the phyB-signaling pathway in Arabidopsis. *The Plant Journal, 23*(4), 461-470.

Huq, E., & Quail, P. H. (2002). PIF4, a phytochrome-interacting bHLH factor, functions as a negative regulator of phytochrome B signaling in Arabidopsis. *The EMBO journal, 21*(10), 2441-2450.

Izaguirre, M. M., Mazza, C. A., Svatos, A., Baldwin, I. T., & Ballare, C. L. (2007). Solar ultraviolet-B radiation and insect herbivory trigger partially overlapping phenolic responses in Nicotiana attenuata and Nicotiana longiflora. *Annals of Botany, 99*(1), 103-109.

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.

Jenkins, G. I. (2009). Signal transduction in responses to UV-B radiation. *Annual review of plant biology, 60*, 407-431.

Jenkins, G. I. (2014). The UV-B photoreceptor UVR8: from structure to physiology. *The Plant Cell, 26*(1), 21-37.

Jenkins, G. I. (2017). Photomorphogenic Responses to Ultraviolet-B light. *Plant, Cell & Environment*.

Jing, Y., Zhang, D., Wang, X., Tang, W., Wang, W., Huai, J., Xu, G. Chen, D., Li, Y. & Lin, R. (2013). Arabidopsis chromatin remodeling factor PICKLE interacts with transcription factor HY5 to regulate hypocotyl cell elongation. *The Plant Cell, 25*(1), 242-256.

Kaiserli, E., & Chory, J. (2015). The Role of Phytochromes in Triggering Plant Developmental Transitions. *eLS*.

Keller, M. M., Jaillais, Y., Pedmale, U. V., Moreno, J. E., Chory, J., & Ballaré, C. L. (2011). Cryptochrome 1 and phytochrome B control shade-avoidance responses in Arabidopsis via partially independent hormonal cascades. *The Plant Journal, 67*(2), 195-207.

Kerckhoffs, L., Kelmenson, P., Schreuder, M., Kendrick, C., Kendrick, R., Hanhart, C.J, Koorneef, M., Pratt, L.H. & Cordonnier-Pratt, M.-M. (1999). Characterization of the gene encoding the apoprotein of phytochrome B2 in tomato, and identification of molecular lesions in two mutant alleles. *Molecular and General Genetics MGG, 261*(6), 901-907.

Kerckhoffs, L., Sengers, M., & Kendrick, R. (1997). Growth analysis of wild-type and photomorphogenic-mutant tomato plants. *Physiologia Plantarum, 99*(2), 309-315.

Kerckhoffs, L. H. J. (1996). Physiological functions of phytochromes in tomato: a study using photomorphogenic mutants *Physiological functions of phytochromes in tomato: a study using photomorphogenic mutants* (pp. 1-19): Kerkchoffs.

Kerr, J., & McElroy, C. (1993). Evidence for large upward trends of ultraviolet-B radiation linked to ozone depletion. *Science, 262*(5136), 1032-1034.

Keuskamp, D. H., Sasidharan, R., & Pierik, R. (2010). Physiological regulation and functional significance of shade avoidance responses to neighbors. *Plant signaling & behavior, 5*(6), 655-662.
Kharshiing, E., & Sinha, S. P. (2015). Plant Productivity: Can Photoreceptors Light the Way? *Journal of Plant Growth Regulation, 34*(1), 206-214.
Kim, B. C., Tennessen, D. J., & Last, R. L. (1998). UV-B-induced photomorphogenesis in Arabidopsis thaliana. *The Plant Journal, 15*(5), 667-674.
Kliebenstein, D. J., Jackie, E. L., Landry, L. G., & Last, R. L. (2002). Arabidopsis UVR8 Regulates Ultraviolet-B Signal Transduction and Tolerance and Contains Sequence Similarity to Human Regulator of Chromatin Condensation 1, 234.
Klose, C., Viczián, A., Kircher, S., Schäfer, E., & Nagy, F. (2015). Molecular mechanisms for mediating light-dependent nucleo/cytoplasmic partitioning of phytochrome photoreceptors. *New Phytologist, 206*(3), 965-971.
Kobayashi, M., Kanto, T., Fujikawa, T., Yamada, M., Ishiwata, M., Satou, M., & Hisamatsu, T. (2014). Supplemental UV radiation controls rose powdery mildew disease under the greenhouse conditions. *Environmental Control in Biology, 51*(4), 157-163.
Kovács, E., & Keresztes, Á. (2002). Effect of gamma and UV-B/C radiation on plant cells. *Micron, 33*(2), 199-210. doi:http://dx.doi.org/10.1016/S0968-4328(01)00012-9
Lau, O. S., & Deng, X. W. (2010). Plant hormone signaling lightens up: integrators of light and hormones. *Current Opinion in Plant Biology, 13*(5), 571-577.
Lazarova, G. I., Kerckhoffs, L. H. J., Brandstädter, J., Matsui, M., Kendrick, R. E., Cordonnier-Pratt, M. M., & Pratt, L. H. (1998). Molecular analysis of PHYA in wild-type and phytochrome A-deficient mutants of tomato. *The Plant Journal, 14*(6), 653-662.
Lee, J.-H. (2016). UV-B signal transduction pathway in arabidopsis. *Journal of Plant Biology, 59*(3), 223-230.
Leivar, P., & Monte, E. (2014). PIFs: systems integrators in plant development. *The Plant Cell, 26*(1), 56-78.
Leivar, P., Monte, E., Oka, Y., Liu, T., Carle, C., Castillon, A., . . . Quail, P. H. (2008). Multiple phytochrome-interacting bHLH transcription factors repress premature seedling photomorphogenesis in darkness. *Current Biology, 18*(23), 1815-1823.
Leivar, P., & Quail, P. H. (2011). PIFs: pivotal components in a cellular signaling hub. *Trends in plant science, 16*(1), 19-28.
Lercari, B., Sodi, F., & Di Paola, M. L. (1990). Photomorphogenic responses to UV radiation: Involvement of phytochrome and UV photoreceptors in the control of hypocotyl elongation in Lycopersicon esculentum. *Physiologia Plantarum, 79*(4), 668-672.
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*.
Li, Q. H., & Yang, H. Q. (2007). Cryptochrome signaling in plants. *Photochemistry and photobiology, 83*(1), 94-101.
Lian, H.-L., He, S.-B., Zhang, Y.-C., Zhu, D.-M., Zhang, J.-Y., Jia, K.-P., Sun, S-X., Li, L. & Yang, H.-Q. (2011). Blue-light-dependent interaction of cryptochrome 1 with SPA1 defines a dynamic signaling mechanism. *Genes & Development, 25*(10), 1023-1028.
Liu, B., Liu, H., Zhong, D., & Lin, C. (2010). Searching for a photocycle of the cryptochrome photoreceptors. *Current Opinion in Plant Biology, 13*(5), 578-586.
Liu, B., Yang, Z., Gomez, A., Liu, B., Lin, C., & Oka, Y. (2016). Signaling mechanisms of plant cryptochromes in Arabidopsis thaliana. *Journal of Plant Research, 129*(2), 137-148.

Liu, Y., Roof, S., Ye, Z., Barry, C., van Tuinen, A., Vrebalov, J., . . . Giovannoni, J. (2004). Manipulation of light signal transduction as a means of modifying fruit nutritional quality in tomato. *Proceedings of the National Academy of Sciences of the United States of America, 101*(26), 9897-9902.

Livne, S., Lor, V. S., Nir, I., Eliaz, N., Aharoni, A., Olszewski, N. E., Eshed, Y. & Weiss, D. (2015). Uncovering DELLA-independent gibberellin responses by characterizing new tomato procera mutants. *The Plant Cell, 27*(6), 1579-1594.

López-Juez, E., Nagatani, A., Tomizawa, K.-l., Deak, M., Kern, R., Kendrick, R. E., & Furuya, M. (1992). The cucumber long hypocotyl mutant lacks a light-stable PHYB-like phytochrome. *The Plant Cell, 4*(3), 241-251.

Lozano, R., Giménez, E., Cara, B., Capel, J., & Angosto, T. (2009). Genetic analysis of reproductive development in tomato. *International Journal of Developmental Biology, 53*(8-9-10), 1635-1648.

Lu, X. D., Zhou, C. M., Xu, P. B., Luo, Q., Lian, H. L., & Yang, H. Q. (2015). Red-light-dependent interaction of phyB with SPA1 promotes COP1-SPA1 dissociation and photomorphogenic development in arabidopsis. *Molecular plant, 8*(3), 467-478. doi:10.1016/j.molp.2014.11.025

Mancinelli, A. L. (1994). The physiology of phytochrome action. In R. E. Kendrick & G. H. Kronenberg (Eds.), *Photomorphogenesis in plants* (pp. 211-269): Springer Science & Business Media.

Martínez-García, J. F., Gallemí, M., Molina-Contreras, M. J., Llorente, B., Bevilaqua, M. R. R., & Quail, P. H. (2014). The Shade Avoidance Syndrome in Arabidopsis: The Antagonistic Role of Phytochrome A and B Differentiates Vegetation Proximity and Canopy Shade. *PLoS ONE, 9*(10), 1-11. doi:10.1371/journal.pone.0109275

Martínez-García, J. F., Galstyan, A., Salla-Martret, M., Cifuentes-Esquivel, N., Gallemí, M., & Bou-Torrent, J. (2010). Regulatory components of shade avoidance syndrome. *Advances in botanical research, 53*, 65-116.

Mathews, S. (2006). Phytochrome-mediated development in land plants: red light sensing evolves to meet the challenges of changing light environments. *Molecular Ecology, 15*(12), 3483-3503.

Mathews, S. (2010). Evolutionary studies illuminate the structural-functional model of plant phytochromes. *The Plant Cell, 22*(1), 4-16.

Mazza, C. A., & Ballaré, C. L. (2015). Photoreceptors UVR8 and phytochrome B cooperate to optimize plant growth and defense in patchy canopies. *New Phytologist*.

Mazzella, M., Alconada Magliano, T., & Casal, J. (1997). Dual effect of phytochrome A on hypocotyl growth under continuous red light. *Plant, Cell & Environment, 20*(2), 261-267.

Montzka, S. A., McFarland, M., Andersen, S. O., Miller, B. R., Fahey, D. W., Hall, B. D., Hu, L., Siso, C. & Elkins, J. W. (2014). Recent trends in global emissions of hydrochlorofluorocarbons and hydrofluorocarbons: Reflecting on the 2007 adjustments to the Montreal Protocol. *The Journal of Physical Chemistry A*.

Morelli, G., & Ruberti, I. (2002). Light and shade in the photocontrol of Arabidopsis growth. *Trends in plant science, 7*(9), 399-404.

Moreno, J. E., Tao, Y., Chory, J., & Ballaré, C. L. (2009). Ecological modulation of plant defense via phytochrome control of jasmonate sensitivity. *Proceedings of the National Academy of Sciences, 106*(12), 4935-4940.

Nagatani, A., Reed, J. W., & Chory, J. (1993). Isolation and initial characterization of Arabidopsis mutants that are deficient in phytochrome A. *Plant Physiology, 102*(1), 269-277.

Ninu, L., Ahmad, M., Miarelli, C., Cashmore, A. R., & Giuliano, G. (1999). Cryptochrome 1 controls tomato development in response to blue light. *The Plant Journal, 18*(5), 551-556.

Olszewski, N., Sun, T.-p., & Gubler, F. (2002). Gibberellin signaling biosynthesis, catabolism, and response pathways. *The Plant Cell, 14*(suppl 1), S61-S80.

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.

Parihar, P., Singh, R., Singh, S., Tripathi, D. K., Chauhan, D. K., Singh, V. P., & Prasad, S. M. (2016). Photoreceptors mapping from past history till date. *Journal of Photochemistry and Photobiology B: Biology, 162*, 223-231.

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.

Pedmale, U. V., Huang, S.-s. C., Zander, M., Cole, B. J., Hetzel, J., Ljung, K., Reis, P. A.B., Sridevi, P., Nito, K. & Nery, J. R. (2016). Cryptochromes interact directly with PIFs to control plant growth in limiting blue light. *Cell, 164*(1), 233-245.

Perrotta, G., Ninu, L., Flamma, F., Weller, J. L., Kendrick, R. E., Nebuloso, E., & Giuliano, G. (2000). Tomato contains homologues of Arabidopsis cryptochromes 1 and 2. *Plant molecular biology, 42*(5), 765-773.

Pierik, R., & de Wit, M. (2013). Shade avoidance: phytochrome signalling and other aboveground neighbour detection cues. *Journal of Experimental Botany*, ert389.

Pratt, L., & Butler, W. (1970). Phytochrome conversion by ultraviolet light. *Photochemistry and photobiology, 11*(6), 503-509.

Pratt, L., CORDONNIER-PRATT, M. M., Kelmenson, P., Lazarova, G., Kubota, T., & Alba, R. (1997). The phytochrome gene family in tomato (Solarium lycopersicum L.). *Plant, Cell & Environment, 20*(6), 672-677.

Pratt, L. H. (1995). Phytochromes: differential properties, expression patterns and molecular evolution. *Photochemistry and photobiology, 61*(1), 10-21.

Pratt, L. H., Cordonnier-Pratt, M.-M., Hauser, B., & Caboche, M. (1995). Tomato contains two differentially expressed genes encoding B-type phytochromes, neither of which can be considered an ortholog of Arabidopsis phytochrome B. *Planta, 197*(1), 203-206.

Reed, J. W., Nagpal, P., Poole, D. S., Furuya, M., & Chory, J. (1993). Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout Arabidopsis development. *The Plant Cell, 5*(2), 147-157.

Reinhardt, D., & Kuhlemeier, C. (2002). Plant architecture. *EMBO reports, 3*(9), 846-851.

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

Roig-Villanova, I., & Martinez-Garcia, J. F. (2016). Plant Responses to Vegetation Proximity: A Whole Life Avoiding Shade. *Frontiers in Plant Science, 7*, 10. doi:10.3389/fpls.2016.00236

Rosado, D., Gramegna, G., Cruz, A., Lira, B. S., Freschi, L., de Setta, N., & Rossi, M. (2016). Phytochrome Interacting Factors (PIFs) in Solanum lycopersicum: Diversity, Evolutionary History and Expression Profiling during Different Developmental Processes. *PLoS ONE, 11*(11), e0165929.

Ruberti, I., Sessa, G., Ciolfi, A., Possenti, M., Carabelli, M., & Morelli, G. (2012). Plant adaptation to dynamically changing environment: the shade avoidance response. *Biotechnology advances, 30*(5), 1047-1058.

Sarlikioti, V., De Visser, P. H., Buck-Sorlin, G., & Marcelis, L. (2011). How plant architecture affects light absorption and photosynthesis in tomato: towards an ideotype for plant architecture using a functional–structural plant model. *Annals of Botany*, mcr221.

Schäfer, E., & Nagy, F. (2006). Physiological basis of photomorphogenesis *Photomorphogenesis in plants and bacteria: function and signal transduction mechanisms* (pp. 13-23): Springer Science & Business Media.

Schijlen, E. G., de Vos, C. R., Martens, S., Jonker, H. H., Rosin, F. M., Molthoff, J. W., Tikuniv, Y.M., Angenent, G.C., van Tunen, A.J. & Bovy, A. G. (2007). RNA interference silencing of chalcone synthase, the first step in the flavonoid biosynthesis pathway, leads to parthenocarpic tomato fruits. *Plant Physiology, 144*(3), 1520-1530.

Schmitt, J., Dudley, S. A., & Pigliucci, M. (1999). Manipulative approaches to testing adaptive plasticity: phytochrome-mediated shade-avoidance responses in plants. *the american naturalist, 154*(S1), S43-S54.

Schomburg, F. M., Bizzell, C. M., Lee, D. J., Zeevaart, J. A., & Amasino, R. M. (2003). Overexpression of a novel class of gibberellin 2-oxidases decreases gibberellin levels and creates dwarf plants. *The Plant Cell, 15*(1), 151-163.

Schrager Lavelle, A., Herrera, L. A., & Maloof, J. N. (2016). Tomato phyE is Required for Shade Avoidance in the Absence of phyB1 and phyB2. *Frontiers in Plant Science, 7*, 1275.

Serrani, J. C., Sanjuán, R., Ruiz-Rivero, O., Fos, M., & García-Martínez, J. L. (2007). Gibberellin regulation of fruit set and growth in tomato. *Plant Physiology, 145*(1), 246-257.

Sharrock, R. A. (2005). Interactions of the Arabidopsis Type II phytochromes. In M. Wada, K.-i. Shimazaki, & M. Iino (Eds.), *Light sensing in plants*: Springer.

Shinomura, T. (1997). Phytochrome regulation of seed germination. *Journal of Plant Research, 110*(1), 151-161.

Shinomura, T., Nagatani, A., Hanzawa, H., Kubota, M., Watanabe, M., & Furuya, M. (1996). Action spectra for phytochrome A-and B-specific photoinduction of seed germination in Arabidopsis thaliana. *Proceedings of the National Academy of Sciences, 93*(15), 8129-8133.

Singh, S., Agrawal, S., & Agrawal, M. (2014). UVR8 mediated plant protective responses under low UV-B radiation leading to photosynthetic acclimation. *Journal of Photochemistry and Photobiology B: Biology, 137*, 67-76.

Smakowska, E., Kong, J., Busch, W., & Belkhadir, Y. (2016). Organ-specific regulation of growth-defense tradeoffs by plants. *Current Opinion in Plant Biology, 29*, 129-137.

Smith, H. (1995). Physiological and ecological function within the phytochrome family. *Annual review of plant biology, 46*(1), 289-315.

Smith, H., & Holmes, M. (1977). The function of phytochrome in the natural environment—III. Measurement and calculation of phytochrome photoequilibria. *Photochemistry and photobiology, 25*(6), 547-550.

Smith, H., & Whitelam, G. (1990). Phytochrome, a family of photoreceptors with multiple physiological roles. *Plant, Cell & Environment, 13*(7), 695-707.

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. *The Plant Cell, 25*(6), 2102-2114.

Takeuchi, T., Newton, L., Burkhardt, A., Mason, S., & Farré, E. M. (2014). Light and the circadian clock mediate time-specific changes in sensitivity to UV-B stress under light/dark cycles. *Journal of Experimental Botany*, eru339.

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

Taylor, R., Tobin, A., & Bray, C. (1997). *DNA damage and repair in plants.* Paper presented at the SEMINAR SERIES-SOCIETY FOR EXPERIMENTAL BIOLOGY.

Ulm, R. (2006). UV-B perception and signalling in higher plants *Photomorphogenesis in Plants and Bacteria* (pp. 279-304): Springer.

Ulm, R., Baumann, A., Oravecz, A., Máté, Z., Ádám, É., Oakeley, E. J., Schafer, E. & Nagy, F. (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 of the United States of America, 101*(5), 1397-1402.

van Tuinen, A., Kerckhoffs, L. H. J., Nagatani, A., Kendrick, R. E., & Koornneef, M. (1995). Far-red light-insensitive, phytochrome A-deficient mutants of tomato. *Molecular and General Genetics MGG, 246*(2), 133-141. doi:10.1007/BF00294675

van Tuinen, A., Kerckhoffs, L. H. J., Nagatani, A., Kendrick, R. E., & Koornneef, M. (1995). A temporarily red light-insensitive mutant of tomato lacks a light-stable, B-like phytochrome. *Plant Physiology, 108*(3), 939-947.

Vandenbussche, F., Tilbrook, K., Fierro, A. C., Marchal, K., Poelman, D., Van Der Straeten, D., & Ulm, R. (2014). Photoreceptor-mediated bending towards UV-B in Arabidopsis. *Molecular plant, 7*(6), 1041-1052.

Vanhaelewyn, L., Prinsen, E., Van Der Straeten, D., & Vandenbussche, F. (2016). Hormone-controlled UV-B responses in plants. *Journal of Experimental Botany, 67*(15), 4469-4482.

Wade, H. K., Bibikova, T. N., Valentine, W. J., & Jenkins, G. I. (2001). Interactions within a network of phytochrome, cryptochrome and UV-B phototransduction pathways regulate chalcone synthase gene expression in Arabidopsis leaf tissue. *Plant Journal, 25*(6), 675-685. doi:10.1046/j.1365-313X.2001.01001.x

Wang, J., Du, X., Pan, W., Wang, X., & Wu, W. (2015). Photoactivation of the cryptochrome/photolyase superfamily. *Journal of Photochemistry and Photobiology C: Photochemistry Reviews, 22*, 84-102.

Wargent, J. (2016). *UV LEDs in horticulture: from biology to application.* Paper presented at the VIII International Symposium on Light in Horticulture 1134.

Wargent, J., Nelson, B., McGhie, T., & Barnes, P. (2015). Acclimation to UV-B radiation and visible light in Lactuca sativa involves up-regulation of photosynthetic performance and orchestration of metabolome-wide responses. *Plant, Cell & Environment, 38*(5), 929-940.

Wargent, J. J., Elfadly, E. M., Moore, J. P., & Paul, N. D. (2011). Increased exposure to UV-B radiation during early development leads to enhanced photoprotection and improved long-term performance in Lactuca sativa. *Plant, Cell & Environment, 34*(8), 1401-1413.

Wargent, J. J., & Jordan, B. R. (2013). From ozone depletion to agriculture: understanding the role of UV radiation in sustainable crop production. *New Phytologist, 197*(4), 1058-1076.

Wei, N., & Deng, X.-W. (1996). The role of the COP/DET/FUS genes in light control of Arabidopsis seedling development. *Plant Physiology, 112*(3), 871.

Weller, J. L., Perrotta, G., Schreuder, M. E., Van Tuinen, A., Koornneef, M., Giuliano, G., & Kendrick, R. E. (2001). Genetic dissection of blue-light sensing in tomato using mutants deficient in cryptochrome 1 and phytochromes A, B1 and B2. *The Plant Journal, 25*(4), 427-440.

Weller, J. L., Schreuder, M. E., Smith, H., Koornneef, M., & Kendrick, R. E. (2000). Physiological interactions of phytochromes A, B1 and B2 in the control of development in tomato. *The Plant Journal, 24*(3), 345-356.

Whitelam, G. C., Johnson, E., Peng, J., Carol, P., Anderson, M. L., Cowl, J. S., & Harberd, N. P. (1993). Phytochrome A null mutants of Arabidopsis display a wild-type phenotype in white light. *The Plant Cell, 5*(7), 757-768.

Xiao, J., Zhang, J., Zhang, Y., Wang, T., Chen, R., Li, H., & Ye, Z. (2007). Isolation and expression of GA 2-oxidase2 in tomato: Full Length Research Paper. *DNA Sequence, 18*(6), 474-479.

Xu, P. B., Lian, H. L., Wang, W. X., Xu, F., & Yang, H. Q. (2016). Pivotal Roles of the Phytochrome-Interacting Factors in Cryptochrome Signaling. *Mol Plant, 9*(4), 496-497. doi:10.1016/j.molp.2016.02.007

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.

Yang, H.-Q., Tang, R.-H., & Cashmore, A. R. (2001). The signaling mechanism of Arabidopsis CRY1 involves direct interaction with COP1. *The Plant Cell, 13*(12), 2573-2587.

Yang, J., Lin, R., Sullivan, J., Hoecker, U., Liu, B., Xu, L. & Wang, H. (2005). Light regulates COP1-mediated degradation of HFR1, a transcription factor essential for light signaling in Arabidopsis. *The Plant Cell, 17*(3), 804-821.

Zhao, X. Y., Yu, X. H., Liu, X. M., & Lin, C. T. (2007). Light regulation of gibberellins metabolism in seedling development. *Journal of integrative plant biology, 49*(1), 21-27.

Zlatev, Z. S., Lidon, F. J., & Kaimakanova, M. (2012). Plant physiological responses to UV-B radiation. *Emir J Food Agric, 24*(6), 481-501.