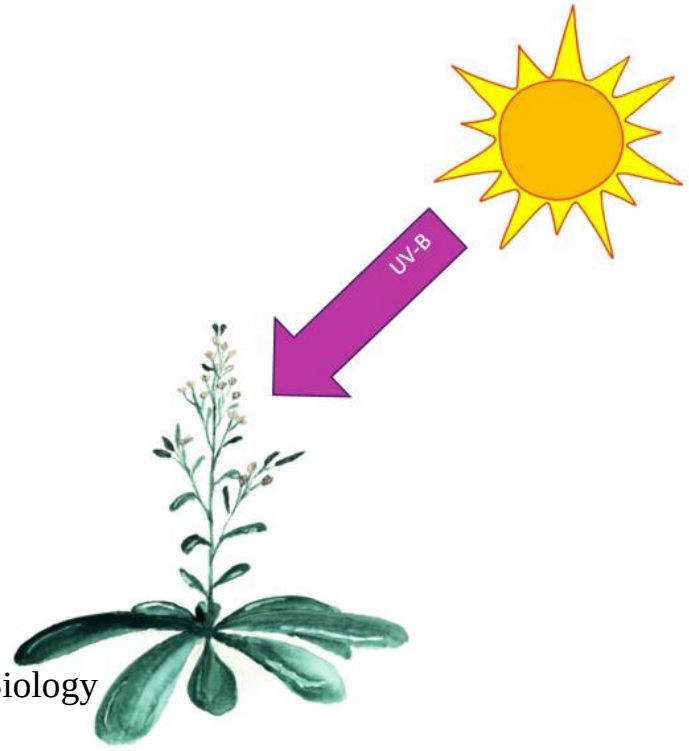
The role of UV-B in regulating flowering initiation in the model plant *Arabidopsis thaliana*



Author

Anna Zioutopoulou, BSc

Primary Supervisor

Dr. Eirini Kaiserli

Institute of Molecular Cell & Systems Biology

University of Glasgow

Secondary Supervisor

Professor John Christie

Institute of Molecular Cell & Systems Biology

University of Glasgow

Submitted in fulfilment of the requirements for the Degree of MSc by Research

College of Medical, Veterinary & Life Sciences

Institute of Molecular Cell & Systems Biology

University of Glasgow

April 2019

University of Glasgow

Abstract

UV-B irradiation ranges from 280-315 nm and is an important component of sunlight that reaches the earth. Although UV-B can be harmful in most living organisms, it can also control various photomorphogenic responses in plants. Plants perceive UV-B light through the action of UVR8, the only photoreceptor that has been discovered so far to be capable of absorbing and mediating responses to UV-B light. Additionally, UV-B has a role in modulating plant adaptive reposes, such as the inhibition of the Shade Avoidance Response, which is a number of physiological and molecular features that plants develop in order to cope with vegetative shade.

Very little is known about the role of UV-B or UVR8 in regulating the time of flowering in plants. This thesis investigates the role of UV-B and UVR8 in the initiation of photoperiodic and vegetative shade flowering in the model plant species *Arabidopsis thaliana*. For that purpose, multiple flowering experiments were conducted, under WL and WL supplemented with UV-B, as well as under low R:FR (shade) or low R:FR that was supplemented with UV-B. For the aforementioned experiments the flowering time was monitored. Moreover, gene expression analysis of key flowering regulators (*FT, CO, FLC, SOC1*), was performed on various Arabidopsis accessions and uvr8 mutant alleles and over-expressing lines, as well as mutants involved in UV-B signalling and flowering.

Our results indicate that UV-B controls photoperiodic flowering in an ecotype-specific manner, since the effect of UV-B on flowering initiation varied in Col-0, Ler and Cvi ecotypes. Overall, UV-B promoted flowering under Long Day photoperiodic conditions. UVR8 was shown to act as a negative regulator of UV-B induced early flowering, since *uvr8* mutants exhibited early flowering phenotypes, while over-expression of UVR8 led to a delay in flowering under white light supplemented with UV-B. Additionally, UV-B was found to induce early flowering in most of the UV-B signalling, light signalling and flowering mutants tested.

Vegetative shade is known to accelerate flowering initiation. The role of UV-B and UVR8 in influencing vegetative shade flowering was found to be ambiguous. Wild type, *uvr8* mutant and over-expressing lines presented a variation of phenotypes. There was a delay in flowering time observed for one of the *Arabidopsis* accessions tested (*Col-0*), while no significant change in flowering time for accessions *Ler* and *Cvi* was observed. The two *uvr8* mutants with different alleles (one of them being a null mutant and the other one expressing a truncated protein) tested depicted no change and a minor delay in flowering respectively. A significant delay in shade-

induced flowering time was observed in the UVR8 over-expressing line under UV-B conditions, compared to the *uvr8* mutant, strengthening the hypothesis that UVR8 is a negative regulator of flowering. UV-B delayed vegetative shade flowering in most signalling and flowering mutants examined, with the exception of the two late flowering mutants *zlf* and *co*. In this case the differences in flowering time, although found to be statistically significant was not as pronounced as in our WL + UV-B set of experiments.

Overall, this thesis has uncovered that UV-B can modulate flowering initiation through the action of UVR8 at the transcriptional level. Further research would be essential for further examining the role of UVR8 in mediating UV-B cross-talk with other light and temperature signalling pathways.

CONTENTS

List of Tables

List of Figures

Preface

The experimental work of the current study was presented in two occasions. A poster participation at the Young Researchers Symposium on Photobiology in Cologne (September 2018) entitled: The role of UV-B in regulating flowering initiation in the model plant *Arabidopsis thaliana*. Additionally a talk was given under the same title for the Institute of Molecular Cell and Systems Biology 2018-2019 Post-Graduate/Post-Doctoral Seminar Series (November 2018).

Acknowledgments

The current thesis presents the research of a Masters by Research program, and it was performed at the University of Glasgow, at the Institute of Molecular Cells and Systems Biology and the College of Medical Veterinary and Life Sciences, as part of the Plant Molecular Science Group.

First and foremost, I would like to thank my supervisor Dr. Eirini Kaiserli, Lecturer of Photobiology and Plant Biology at the University of Glasgow, for giving me the opportunity to work on such an interesting topic and trusting me with the realization of this project. I utterly thank her for her constant support, her truly flawless supervision, for all the knowledge she passed on to me and for all the techniques she taught me to perform during this project. I would also like to thank my second supervisor Professor John Christie, Professor of Photobiology at the University of Glasgow, for his always insightful advices and encouragement on my project.

I am extremely grateful to all the members of the Bond laboratory, past and present, for creating an ideal working environment and for developing such a unique team spirit. Especially I would like to thank Dr. Giorgio Perrella and Liz O' Donnell for their excellent training in many laboratory techniques. I am very grateful to Eirini Patitaki for her important contribution to this project. I am also very thankful to PhD candidates Elisa Vellutini, Mhairi Davidson, Weiwei Fang and MSc student Alexander Mac Leod, for our ideal collaboration, their valuable help and most importantly for their friendship inside and outside the lab. Lastly I would like to thank every other member of the Kaiserli Group and all the people in the Bower building, for creating such an intellectually stimulating environment. I am really grateful to John Panagiotou for keeping me company during the many late-hours experiments of my project.

Last but certainly not least, I would like to thank my parents, since without their selfless love and support, I would not be able to study abroad, pursue my dreams, conquer my goals and challenge myself.

Authors' Declaration

I hereby declare that the present thesis has been written entirely by myself and represents a record of my own personal research, which was performed at the University of Glasgow, at the College of Medical, Veterinary and Life Sciences and Institute of Molecular Cell and Systems Biology, under the supervision of Dr. Eirini Kaiserli and Professor John Christie and it is, entirely my own original work, unless stated otherwise in the text.

Zioutopoulou Anna

April, 2019

Abbreviations

cDNA	complementary DNA
CFP	CYAN FLUORESCENT PROTEIN
CO	CONSTANS
Col	Columbia
COP	CONSTITUTIVE PHOTOMORPHOGENIC
COR	COLD REGULATED
CRY	Cryptochrome
Cvi	Cape Verde Islands
DAPI	4’,6-Diamidino-2-phenylindole
dH_2O	distilled H_2O
DNA	Deoxyribonucleic Acid
DMSO	Dimethyl sulfoxide
EDTA	Ethylenediaminetetraacetic acid
ELF	EARLY FLOWERING
FKF1	FLAVIN-BINDING, KELCH REPEAT, F-BOX
FLC	FLOWERING LOCUS C
FR	Far Red light
FT	FLOWERING LOCUS T
GFP	GREEN FLUORESCENT PROTEIN
LB	Luria Broth medium
Ler	Landsberg erecta
LiAc	Lithium Acetate
LKP2	LOV KELCH PROTEIN2

LD	Long Days
MES	2-(N-Morpholino) ethanesulfonic acid
MOPS	3-(N-Morpholino) propanesulfonic acid
mRNA	Messenger RNA
MS	Murashige and Skoog
OD	Optical Density
O/N	Overnight
OX	Over-expression
PCR	Polymerase Chain Reaction
PEG	Polyethylene Glycol
P_{FR}	Far-red light absorbing form of Phytochrome
P_R	Red light absorbing form of Phytochrome
pH	-log10 (hydrogen ion concentration)
PHY	Phytochrome
PIF	PHYTOCHROME INTERACTING FACTOR
Pro	Promoter
qPCR	Real-time Polymerase Chain Reaction
R:FR	Red: Far Red light
RNA	Ribonucleic Acid
RT	Room Temperature
RT-PCR	Reverse Transcription Polymerase Chain Reaction
RUP	REPRESSOR OF ULTRAVIOLET-B PHOTOMORPHOGENESIS
SD	Short Days
SD L-W- media	Yeast minimal media-SD base

SDS	Sodium Dodecyl Sulfate
SOC	Suppressor of overexpression of Constans
35S	Cauliflower mosaic virus 35S promoter
TBS	Tris buffered saline
TBST	Tris buffered saline, Tween 20
TBSTT	Tris buffered saline, Tween 20, Triton
UVR8	UV-B RESISTANCE LOCUS 8
v/v	volume / volume
w/v	weight / volume
WL	Wight fluorescent Light
WL+ FR	White fluorescent light + Far Red light
WL+ FR+ UV-B	White fluorescent light + Far Red light + Ultraviolet-B irradiation
WL+ UV-B	White fluorescent Light + Ultraviolet-B irradiation
YPDA	Yeast Peptone Dextrose Adenine
ZTL	Zeitlupe
ZT	Zeitgeber Time

Chapter 1: Introduction

1.1 Light regulates plant development

Light is not only absolutely vital for plants but for all organisms that inhabit Earth. The total spectrum of solar radiation that strikes the atmosphere of the Earth ranges from 100 nm to 1,000,000 nm [1]. The band of the significant radiation power is divided into five regions that demonstrate increasing wavelengths: Ultraviolet C, ultraviolet B (280-315 nm), ultraviolet A, visible light (380-780 nm) and infrared [1] (https://www.skincancer.org/prevention/uva-and-uvb/shining-light-on-ultraviolet-radiation). At sea or ground level, sunlight comprises of visible light by 44% , 3% ultraviolet (when the Sun is at its highest point) and the remaining is infrared light [1]. This means that the atmosphere and the stratospheric ozone layer of the Earth can block 77% of the Sun's UV irradiation [1]. Of the UV irradiation that can actually reach the Earth more than 95% comprises of the longer wavelengths of UV-A with the rest being UV-B [1] (https://www.newport.com/t/introduction-to-solar-radiation). There is essentially no UV-C at ground level [1].

In humans, over-exposure to UV irradiation can cause higher risk of skin cancer, harmful effects on the eye's retina and cornea as well as compromising the immune system [2]. In plants exposure to high levels of UV irradiation (above 1 μmol m^{-2} s^{-1} of UV-B) can cause impairment in growth and general deleterious effects [3]. Smaller doses of UV radiation of course can have beneficial effects for both humans and other organisms, including of course plants. For humans UV light causes our body to produce and maintain vitamin-D which also promotes the production of serotonin [4]. In plants lower levels of UV irradiation (below 1 μmol m^{-2} s^{-1} of UV-B) lead to the induction of specific photomorphogenic responses that protect the plant from any potential damage and lead to successful adaptation and survival under stress conditions [3].

Plants are sessile organisms and therefore unable to "fly away" from any stressful and unfavourable environmental cues. Instead plants have evolved a "fight" response, governed by complex molecular mechanisms that provide a great degree of developmental plasticity in order to promote growth, but also enhance reproductive success to constantly changing of environmental conditions[5] [6]. Out of the entirety of environmental stimuli than can affect a plant's growth and development. light is without a doubt the most important one [5]. Plants have long evolved sophisticated methods of sensing and decoding every possible aspect of light stimuli (quantity, quality, direction, duration and periodicity) [7]. *Arabidopsis thaliana* is a

widely used model organism in plant biology that has provided a great system for discovering and characterising light-mediated responses [8].

Light regulates almost every aspect of a plant's life from seed germination to the ultimate life goal of a plant which is the transition to a reproductive state by floral initiation [5]. Other developmental and behavioural responses regulated by light include germination, photomorphogenesis (de-etiolation), phototropism, shade avoidance responses, stomatal opening, chloroplast movement and the entrainment of the circadian clock [5] (Figure 1.1), and, are being briefly described below.

1. Germination is the process which allows an organism to grow from seed [5]. If current environmental conditions are not in favour of the plant's growth, it is possible for the seed to stay dormant in a dry state until those conditions become more favourable once more [5]. In *Arabidopsis* as in other higher plants the germination of seeds that are in a dormant stage is controlled by light amongst other factors, thus making light a very crucial environmental stimuli for seed germination [9].
2. Photomorphogenesis can be characterised as the light-mediated development where plants develop certain growth patterns in response to the light spectrum [10]. After the stage of seed germination seedling can either follow a skotomorphogenic or a photomorphogenic developmental pattern [5]. If in darkness seedlings follow the skotomorphogenic route, thus developing longer hypocotyls and not expanded cotyledons (etiolation) [5]. Upon light perception, seedlings follow the photomorphogenic route, thus developing shorter hypocotyls and open cotyledons which are more expanded in order to allow photosynthesis to occur (de-etiolation) [5].
3. Phototropism is the growth of a plant towards a light stimulus [11]. This response happens thanks to a chemical called auxin [11]. Auxin is located in the plants cells that are farthest from the light source and induces their elongation [11].
4. The shade avoidance syndrome is a group of responses that plants employ when their light resources are compromised by neighbouring vegetation. In the natural environment it is very common for plants to grow in close proximity to each other and compete for light sources. In such cases flowers have developed mechanisms in order to avoid shade [12]. These mechanisms include the stimulation of elongation growth which is associated with reduced leaf development and leaf elevation, increased apical dominance and a reduction in branching [12]. These responses altogether can be categorised as the shade avoidance syndrome [12].

5. Stomata (mouths in Greek) are small openings or pores in plant tissue (leaves and more rarely some stems) which allow gas exchange [5]. Surrounding the stomata are located the guard cells whose function specializes in opening and closing stomatal pores [5]. It has been found that stomatal opening can be regulated by blue-light regulated components [13].
6. Chloroplasts are the plant's organelles which conduct photosynthesis [5]. When low light conditions occur chloroplasts try to maximize photosynthesis by accumulating to the upper surface of the palisade mesophyll cells [5], which are located in the leaves of the plant, right below the epidermis and cuticle [14]. On the other hand under strong light conditions chloroplasts can line up in a perpendicular way to the direction of the light source to minimize any photo damage that can be caused by excess light [13] [15]. Chloroplast relocation is primarily regulated by blue light [16].
7. The circadian clock is a time-keeping mechanism with a periodicity of 24 hr that confers diurnal patterns of gene expression in Arabidopsis. The circadian clock is triggered by the daily alternations between light and dark [17]. These transitions between light and darkness allow the plant to gather information about any fluctuations in the environmental cues it receives and plan its responses about the future changes, especially when they are repetitive like the change in day and night length that comes with the change of the seasons [17].
8. Flowering is the transition of the plant from its vegetative to reproductive state that induces inflorescence development [18]. Flowering is a complex process regulated by multiple signalling pathways and is going to be described in full detail later on as it is the focus of this thesis.

1.1.1 Plant photoreceptors

All living organisms have developed photosensory systems in order to interpret light signal. Humans and other mammals have cone cells in the retina of their eyes that include photoreceptor proteins which respond to colour and are responsible for our trichromatic vision [19].

Plants also have photoreceptor proteins that detect specific wavelengths and mediate different responses. There are five photoreceptor families in plants known up to date, each one responsible for sensing and transducing the signal from different light qualities, largely dependent on the type of chromophore or amino acid they possess (Figure 1.2).

Phytochromes

Phytochromes are the most studied photoreceptors and the first family to be identified. The phytochrome family includes five members: phyA, phyB, phyC, phyD and phyE [5]. Phytochromes absorb in the red and far-red area of the spectrum [20] but it has also been shown that phyA can be photoactivated by blue light to enhance phototropism and inhibition of hypocotyl elongation [21]. Phytochromes majorly control photomorphogenesis in plants and exist in two isoforms Pr and Pfr. Under red light illumination the biologically inactive form of the phytochrome (Pr) is converted to the biologically active form (Pfr) [5]. Both far-red light and darkness can convert the molecule back to its inactive state [5]. Phytochrome activation/inactivation basically acts as a biological light switch [22]. Plant phytochromes have an N-terminal chromophore binding module where the linear phytochromobilin chromophore associates and absorbs red and far-red light [22]. Phytochromes that are full length are located in the cytosol where they are still in their inactive form. Once they get converted in their active Pfr state they get translocated to the nucleus [23] [24] [25], where they can interact with several proteins, such as the transcription factor PHYTOCHROME INTERACTING FACTOR 3 [26] – a negative regulator of phyB signalling[27]– and mediate signal transduction that results in various photomorphogenetic and photoperiodic flowering responses [28] (Figure 1.3). Phytochromes also have the role of thermosensors in *Arabidopsis* [29]. For plants is really important to be able to sense differences in temperatures since warmer temperatures especially for *Arabidopsis thaliana* promote both flowering and elongation growth [30]. Plants like *Arabidopsis* can sense fluctuations in environmental temperature that can be as small as 1 ºC [29]. Phytochromes function as thermosensors during nigh time [29]. Specifically phyB gets proportionally inactivated to temperature when in darkness and this inactivation is important because it is involved with the phytochrome thermosensory function during the night and the integration of information on environmental temperatures, by the plant [29]. Phytochromes also have a role in germination as well as flowering [31]. Seeds are able to sense light through their phytochrome photoreceptors and trigger germination [31]. Additionally since phytochromes can measure the Pfr/Pr ratio they also trigger various physiological processes, such as flowering [31]. In LD plants like *Arabidopsis thaliana* Pfr acts by promoting flowering initiation by activation of flowering integrator gene via transportation to the nucleus by the cells of the shoot apex [31]. Under a LD photoperiod during daytime Pr can absorb red light and be converted to Pfr [31]. During nigh-time Pfr absorbs far red lights and turns back to the previous Pr state [20]. But because the say length is longer that the one of the night there is enough supply of Pfr left, which subsequently promotes flowering initiation [31].

Cryptochromes

Cryptochromes are blue light/UV-A absorbing photoreceptors [32]. Arabidopsis has two cryptochromes CRY1 and CRY2 and their role mainly consists of blue light specific hypocotyl elongation inhibition and control of photoperiodic flowering initiation respectively [32]. Cryptochromes have been shown to have two domains: one at their N-terminal tail which is a Photolyase-Homologous region domain and has the ability to bind their chromophore, Flavin adenine dinucleotide, and a second domain, CRY C-terminal Extension domain, which is really important for both their regulation and function but is constitutionally unstructured [32]. In addition to these two factors there is yet another member of the CRY photoreceptor group in *Arabidopsis thaliana* CRY3. CRY3 is a CRY-DASH protein and is found in chloroplasts and mitochondria [33]. These kind of proteins can bind both DNA and RNA directly and in general have been found to demonstrate cryptochrome activity in regulation of gene transcription and development [34 35 36 37]. In *Arabidopsis* CRY3 has also the ability to catalyze *in vitro* the repair of the cyclobutane pyrimidine dimers that single-stranded DNA has [38 37 39 40]. It is possible to speculate that CRY-DASH proteins like CRY3 are triple activity factors that can photolyase single-stranded DNA, and act as photoreceptors of dual activity (photolyase and cryptochrome) [32]. Also due to its biochemical activity in *Arabidopsis*, it is possible that CRY3 participates in protecting organellar genomes against UV damage [32]. CRY1 and CRY2 accumulate in the nucleus of the cell, since they both are soluble proteins [41 42 43]. CRY1 can also be detected in the cytosol, where it is involved in root elongation and blue light stimulation of cotyledon expansion [41]. CRY1 that is located in the nucleus is actually responsible for hypocotyl inhibition under blue light and can also depolarize the cell membrane, a function that is induced by light [41]. CRY2 on the other hand remains in the nucleus even after translation [44]. CRY2 can also undergo blue light-dependent interaction with certain factors in order to directly stimulate transcription of *FT* gene, which is a flowering promoting gene [45] (Figure 1.3). Additionally CRY2 interacts with COP1-interacting proteins and suppresses COP1-dependent degradation of the CO protein, which is a positive regulator of *FT* transcription, thus positively facilitating flowering initiation [32] (Figure 1.4).

Phototropins

Phototropins absorb within the blue and UV-A area of the spectrum [13]. There are two members in the phototropin family phot1 and phot2 [13]. They are both receptor kinases and have been found to be exclusive to plants [13]. In *Arabidopsis thaliana* these two blue light activated receptor kinases present a partial overlap in their function which includes the ability to mediate

phototropism, induce chloroplast migration and stomatal opening [13]. Phot1 consists of 996 residues and two LOV (light, oxygen or voltage sensing) domains, LOV1 and LOV2 [13]. These domains can act as blue light sensing domains through the associated Flavin MonoNucleotide (FMN) chromophores [46]. phot2 consists of 915 amino acids and its two LOV domains have similar function with the ones from pho1 [46]. The kinase domains are responsible for catalysing the phosphorylation of proteins on targeted residues [13] (Figure 1.5). Phot1 is mainly responsible for low irradiance, while phot2 is responsible for higher fluence blue light responses [47]. Both phototropins are associated with the plasma membrane of the plant cell and undergo internalization upon blue light activation and autophosphorylation[5].

Zeitlupes

The Zeitlupe family of photoreceptors absorb within the blue area of the spectrum and consists of three members: ZEITLUPE (ZTL), LOV KELCH PROTEIN 2 (LKP2) and FLAVIN-BINDING KELCH REPEAT, F BOX1 (FKF1) [48] [49]. All these three proteins have important roles in blue light-dependent circadian rhythms and also use FMN as the LOV-domain associated chromophore for blue light absorption [48] [49]. ZTL triggers blue-light mediated degradation of proteins that are important clock components through the proteasome [48]. Additionally, through the interaction with another important circadian clock component protein GIGANTEA (GI), the diurnal oscillations of ZTL are maintained, thus resulting in the correct periodic expression of a core clock component TIMING OF CAB EXPRESSION 1 (TOC1) [48]. LKP2 is involved in the regulation of various circadian clock processes such as initiation of flowering time, hypocotyl elongation and leaf and cotyledon movement rhythms [50]. FKF1 has very similar functions with the other two proteins and can additionally form a complex with GI resulting in the regulation of CO expression [51] [52] [53]. From this family FKF1 is the main flowering regulator. FKF1 promotes CO protein stability by direct interaction with it or indirectly by decreasing the stability of two protein factors that repress CO expression [52] (CYCLING DOF FACTO1 and CYCLING DOF FACTOR2 [54]). For this action against CDFs FKF1 requires GI [55]. ZTL can interact with either GI or FKF1 and disrupt their interaction, thus indirectly regulate flowering in a negative way [55]. This is confirmed by the observation that ZTL over-expressing *Arabidopsis lines* are late flowering [56], while *ztl* single mutant is early flowering under SD growth conditions [57] [58]. LKP2 over-expressing plant are late flowering only under LD growth conditions [57] [59], while *lkp2* single mutant does not display any particular flowering phenotype [60] [57].

UVR8

UVR8 is the only known genetically encoded photoreceptor that can sense UV-B and mediate UV-B specific responses in plants [61]. The structure and function of UVR8 will be described in detail in the following subchapters.

Plant photoreceptors have distinct but also overlapping functions. Integration and cross-talk among different photoreceptor, clock and hormone signalling pathways ensure an optimal plant, growth development and adaptation.

1.2 Regulation of flowering initiation in Arabidopsis

Flowering is a crucial and highly regulated transition from vegetative to reproductive development, which triggers flower instead of leaf production[18]. In *Arabidopsis* there are at least 180 genes implicated in the flowering initiation process [18]. Six main flowering pathways have been identified and studied so far: the photoperiod pathway which is also connected to the circadian clock, the vernalisation pathway, the autonomous pathway, the age pathway, the gibberellin pathway and the ambient temperature pathway [18]. The photoperiodic and vernalisation pathways are responding to a variety of seasonal clues in order to control flowering, the ambient temperature pathway controls flowering in response to changing growth temperatures within a day (18 ºC – 28 ºC). The remaining three pathways mediate responses for endogenous signals (hormones, ageing) and are more detached from environmental cues [18] [62] [63].

1.3 Photoperiodic control of flowering

Determining when is the right time to flower, is absolutely essential for the reproductive success of a plant. There are three categories in which plants can be divided into according to their responses to the photoperiod (duration of light exposure within a 24-hour day): short-day plants initiate flowering when days are shorter than a critical length (usually during autumn time), long-day plants flower when days are longer than this critical length and day-neutral plants can flower independently of the length of the day [64]. *Arabidopsis thaliana* is a long-day flowering plant whose flowering initiation is promoted by long spring/summer days and repressed in the winter when the days are shorter, but will eventually flower even under short days as well [65]. Light signalling and clock pathways ensure that photoperiodic flowering initiation occurs at the right seasonal moment. In *Arabidopsis* photoperiodic signal perception and regulation occurs primarily in the rosette leaves [18].

1.3.1 Major photoperiodic flowering regulators

There are two major flowering regulators in the photoperiodic pathway and these are the proteins FLOWERING LOCUS T and CONSTANS [18]. Actually, *co* was one of the first mutants that was genetically identified and studied in Arabidopsis [66]. *Co* mutants were unable to sense and respond to the photoperiod, thus presenting a "constant" late flowering phenotype [66]. *CO* is expressed at the vascular tissue of a plant confirming that the perception of the photoperiod can take place in the leaves [65]. CO is a putative transcription factor that acts by activating the main flowering promoting gene *FT*, and does that by binding directly to its promoter [18] . A mechanism by which CO regulates *FT* transcription is by the formation of a complex with factor ASYMMETRIC LEAVES 1 (AS1), a factor that can regulated the development of the leaves by controlling the levels of growth factor gibberellin (GA) [67]. AS1 can also bind to specific region of the promoter of *FT* [67]. Another mechanism involves the activation of factor SODIUM POTASSIUM ROOT DEFECTIVE 1 (NaKR1) by CO [68]. This factor in essential for the FT transport from the leaf to the shoot apical meristem, for mediation of flowering initiation by FT [68]. CO additionally initiates the transcription of one more flowering promoter the TWIN SISTER OF FT (TSF) [18]. There have been studies where it was demonstrated that CO can also regulate flowering in more ways, by activating transcription expression of *SUPRESSOR OF OVEREXPRESSION OF CO1* (*SOC1*) [69], which is another promoter of flowering initiation. On the other hand it has been proposed that CO can also activate *TERMINAL FLOWER 1* (*TF1*) transcription [70] potentially through FT. Since TF1 is a repressor of flowering expressed in the meristem, its activation through FT could be explained as a negative feedback regulation system.

The levels of regulation of CO are both transcriptional and post-transcriptional and they ensure that *FT* transcription can only be activated under long days, when the daylength exceeds a critical threshold [65] [71]. In long days CO expression has a smaller peak towards the end of the day and a great peak about 16 hours after dawn and in the night [72] (Figure 1.6). This peak is really important for the regulation of flowering initiation and it is controlled by GIGANTEA (GI) which is an important component of the circadian clock [18]. Under long days GI interacts with a group of proteins –the F-box ubiquitin ligases– a member of which is FKF1 [73]. The interaction between GI and FKF1 stabilise the F-box proteins and they are now able to promote the degradation of the CDF factors that repress CO expression [54] [74]. In the dark CO is degraded by CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) which is an E3 ubiquitin-protein ligase [75]. Furthermore, CO protein is targeted for red-light mediated degradation through the

action of phyB during the day, whereas blue light triggers CO stabilisation by phyA and cry2 at the end of a long day. PhyA and cry2 inhibit the formation of the COP1- SUPRESSOR OF PHYTOCHROME A (SPA1) complex that targets CO for degradation [75 76 77 78 79 65].

Another really important factor that regulates CO abundance is EARLY FLOWERING 3 (ELF3) [80 81]. ELF3 is a component of the circadian clock input pathway [82] and has the ability to regulate itself in a circadian manner and can act in a zeitnehmer feedback loop [83]. ELF3 targets CO for degradation and therefore acts as a repressor of flowering [80]. The expression of the *ELF3* gene is regulated by the circadian clock and ELF3 protein is feeding back in a way that antagonizes light signalling to the clock and any possible acute induction of the circadian outputs mediated by light [84]. ELF3 expression reaches its highest accumulation at night because higher plants are able to retain light signals that cause sensitivity to the circadian clock [84]. The gating of the light input that is created to the oscillator allows in the efficient function of the circadian clock which subsequently remains unresponsive to changes in light level at night that don not originate from dawn or dusk [84]. The role of ELF3 in the circadian clock constitutes an example of how a gating mechanism works, where a circadian output feeds back in order to also regulate input pathways [84].

FT is the main protein that regulates the transition from vegetative growth to flowering in Arabidopsis [85]. As mentioned before, CO can bind to the *FT* promoter and regulate its transcription [18 65]. GI, FKF1 and CDF1 also have the ability to bind to the *FT* locus [53 52 86]. FKF1 has actually the ability to promote the accumulation of *FT* transcript through removal of CDF from the locus of the gene [52]. CRYPTOCHROME-INTERACTING BASIC-HELIX-LOOP-HELIX1 (CIB1) is yet another factor that interacts with *FT* and promotes its transcription [87]. As its name suggests this factor interacts with cry2 upon blue light exposure [87]. One more important activator of *FT* is PHYTOCHROME-INTERACTING FACTOR 4, which as well has the ability to bind to the *FT* promoter and induce flowering under higher temperatures in a CO independent manner [88 89].

On the contrary there are a number of repressors of photoperiodically regulated *FT* transcription including LIKE HETEROCHROMATIN PROTEIN 1 (LHP1) which represses *FT* directly by regulating histone H3 trimethylation on lysine 27 (H3K27me3), a mark of repression deposited by the Polycomb repressive complex 2 [90]. Lastly a group of *FT* transcript repressors includes the factors APETALA2 (AP2), TARGET OF EAT1 and 2 (TOE1 and TOE2), SCHNARCHZAPFEN (SNZ) and SCHLAFMÜTZE (SMZ) [91].

The diurnal expression of *FT* depicts two main peaks. The first peak occurs very early in the morning (ZT0.5, 0.5 hours after the light onset) and the other one occurs around dawn (ZT15; 0.5 hours after the light onset) [72] (Figure 1.6).

FT acts as a mobile signal because the protein is produced in the companion cells of the plants leaves and then gets transported to the shoot apical meristem, through the phloem sieve elements [18] [65]. In order to achieve this movement FT interacts with FT-INTERACTING PROTEIN 1 (FTIP1) [92]. Once it reaches the shoot meristem, FT interacts with the transcription factor FLOWERING LOCUS D (FLD) and together they activate the genes that will eventually lead to the formation of flowers: [65] [93] OVEREXPRESSION OF CONSTANS 1 (SOC1) is a MADS box transcription factor that interacts with AGAMOUS-LIKE 24, which is another MADS box transcription factor and together they can activate LEAFY (LFY), a factor that is ultimately responsible for the meristem floral identity [30] [94]. LFY and APETALA1 (AP1) are also activated by the SQUAMOSA BINDING PROTEIN LIKE (SPL) factors [95]. SOC1 can directly bind to the *SPL* promoters and regulate their expression [96]. *SPL*s are directly activated by the FT-FD complex formed in the shoot apical meristem, which also is able to directly activate AP1 apart from the indirect activation that was just mentioned [97]. Of course in order for the plant to maintain a balance in the expression factors that lead to floral induction there is an anti-florigen factor that acts against LFY and AP1 that is called TERMINAL FLOWER 1 and is closely related to FT [98] [99]. Flowering is a very complex process that is regulated at more than one levels by many stimuli. CO and FT factors are the key players in this multi-level regulation of floral initiation in response to the photoperiod (Figure 1.7).

1.4 Additional flowering pathways and their main flowering regulators

Apart from the photoperiodic pathway and the circadian clock there are several other factors and pathways which can regulate flowering time by interpreting and responding to different environmental stimuli, still the main regulators of all flowering pathways very often get integrated.

Gibberellin for example is a growth promoting factor which promotes the growth of higher plants' organs and subsequently promotes flowering as well [63]. An enzyme called the GIBBERELLIN 20 OXIDASE is really important because it catalyses various steps of the gibberellin biosynthesis [18]. When plants are grown under a LD photoperiod the impact of this pathway is minor despite the enhanced gibberellin biosynthesis due to the photoperiod [63]. On the other hand when plants are grown under a SD photoperiod this has more of a crucial role

in flowering regulation [63]. Gibberellin promotes floral initiation via the activation of gene transcription of genes which then encode important integrators of flowering time, such as FT, SOC1 and LFY [63].

The ambient temperature pathway controls flowering in response to the fluctuation of diurnal temperatures [18]. *Arabidopsis thaliana* species tends to grow better under higher temperatures (≥23ºC), compared to growth under lower temperatures (≤ 16 ºC) [18]. SHORT VEGETATIVE PHASE (SVP), which is one more MADS box transcription factor can repress the transcription of *FT* when the temperature is lower, but when the temperature rises, there is an upregulation of *FT* transcript levels [18]. PHYTOCHROME INTERACTING FACTOR 4 (PIF4) which is negative regulator in the phyB signalling pathway, promotes the transcription of *FT* under even warmer temperatures, especially around 27 ºC -28 ºC [100].

The vernalization pathway controls initiation of flowering time because of its response to prolonged exposure to colder temperatures [18]. The autonomous pathway mediates responses mostly to endogenous signals and accelerates flowering independently of the length of the day [18]. One of the main repressors of *FT* and *SOC1* transcript expression is FLOWERING LOCUS C (FLC), an additional member to the family of the MADS box transcription factors, and the central integrator of the autonomous and vernalization pathways [18]. FLC gets repressed via epigenetic silencing from the vernalization pathway in response to cold temperatures exposure, thus promoting flowering initiation only after the winter has ended, in spring [101] [102]. Different *Arabidopsis thaliana* ecotypes require different cooler temperatures, in order for flowering to get promoted [103]. FLC is subjected mostly to post-translational modification of its histones [104]. The autonomous pathway regulates *FLC* mainly through RNA based and chromatin modifications [104]. When plants return to warmer temperatures during spring *FLC* remains silenced in order for flowering initiation to occur [102]. This happens due to certain histone modifications of the chromatin of the *FLC* locus [105]. These modifications involve the POLYCOMB REPRESSIVE COMPLEX 2 (PRC2) [106]. PRC2 is responsible for the epigenetic silencing of *FLC* in the vernalization pathway [107] [108]. PRC2 includes four important subunits [106]. CURLY LEAF (CLF), SWINGER (SWN) and MEDEA (MEA) are the first subunit [106]. Another subunit includes the factors: EMBRYONIC FLOWER 2 (EMF2), VERNALIZATION 2 (VRN2) and FERTILIZATION INDEPENDENT SEED 2 (FIS2) [106]. MULTI-SUBUNIT SUPPRESSOR OF IRA 1‑5 (MSI1-5) [106]. The remaining two subunits are factors MULTI-SUBUNIT SUPPRESSOR OF IRA 1–5 (MSI1-5) and lastly INDEPENDENT

ENDOSPERM (FIE) [106]. PRC2 leaves a repressive mark in FLC, the trimethylation of the histone H3 on lysine 27 (H3K27me3), which maintains the chromatin to an OFF state [108].

As the plant grows older and matures, the age pathway regulates the transition from a juvenile-vegetative state to an adult-reproductive one [109]. This transition is mainly controlled by the microRNA miR156 and miR172 [109]. As the plant transitions from a juvenile to an adult stage less miR156 is expressed and decreased levels of miR172 start to rise [110]. miR156 negatively regulates the group of SQUAMOSA BINDING LIKE (SPL) transcription factors [95 111]. These factors positively regulate flowering initiation by promoting the transcription of positive flowering regulators such as *SOC1*, *LFY* and *FRUITFULL* (*FUL*) [18]. Several members of the SPL protein family also promote the accumulation of miR172 via binding to its precursor genes promoters' [112]. miR172 also promotes flowering by regulating some of the *FT* transcript repressor genes that were mentioned earlier and include *AP2*, *TOE1* and *TOE2*, *SNZ* and *SMZ* [112 113].

As it was established, flowering is a very complicated process that requires the cooperation of the main flowering pathways and their flowering integrators, in order for the plant to reach its ultimate goal and avoid reproductive stress by successful flowering (Figure 1.8).

1.5 UVR8 structure and function

UVR8 is the UV-B sensing protein that mediates UV-B responses in plants [61]. *Uvr8* mutant plants cannot initiate photomorphogenic nor photo-protective responses to UV-B irradiation and are therefore extremely susceptible to UV-damage. More specifically *uvr8* mutants exhibit a loss in UV-B induced flavonoid production, which act as a natural sunscreen that protects plants from UV irradiation[114 115 116]. UVR8 is a β-propeller protein formed by 7-blade-shaped β-sheets [117 118]. Unlike other photoreceptors, UVR8 does not sense light through a prosthetic chromophore, but instead utilizes specific tryptophan amino acids which act as the chromophores used for the absorption of UV-B [117 118 4 119]. A specific cluster of tryptophans has been located at the dimer interface and they are considered really important for the reception of UV-B irradiation [4 117 118 119] . Especially there are two tryptophan residues (W233 and W285) that are essential for UV-B perception by UVR8 [117 120 121]. UVR8 shares sequence homology with mammalian proteins that are involved in the regulation of chromatin condensation, such as RCC1 which is found in humans [115]. When in darkness, UVR8 forms a homodimer and localizes in the cytosol [122]. The dimer form is maintained by salt bridge interactions between specific charged amino acids across the interface of the dimer, where

monomers come in contact with one another [117] [118] [61]. Nevertheless, upon UV-B exposure, the UVR8 dimer undergoes structural changes and dissociates from a dimeric to monomeric state [123] and translocates from the cytosol into the nucleus of the plant [124] (Figure 1.9).

1.5.1 UVR8 mediated UV-B photomorphogenesis

In order to mediate photomorphogenic responses, upon UV-B irradiation and monomerization, UVR8 interacts with COP1, an E3 ubiquitin ligase [125]. COP1 is a factor with a dual role since it acts both as a negative regulator of light signalling and a positive regulator of UV-B signalling [79] [125]. UV-B irradiation is necessary and at the same time sufficient for mediating this interaction [125]. The interaction of COP1 with UVR8 involves a 27 amino acid region close to the C-terminus of UVR8 and its β-propeller core as well as the WD40 domain of COP1 [125] [126] [127]. In studies where one of the three tryptophans considered to be responsible for the detection of UV-B was mutated, lead to UVR8 being constantly active in a monomeric state and interacted constitutively with COP1, even though the magnitude of this interaction was slightly reduced [4]. This provided the first indication that UVR8 monomerization is the molecular mechanism that activates UV-B signalling [125]. The interaction of UVR8 with COP1 leads to the induction of the expression of the basic leucine-zipper transcription factor ELONGATED HYPOCOTYL 5 (HY5) [125]. Both factors UVR8 and COP1 are necessary for the UV-B specific *HY5* expression [128] [124]. HY5 is involved in in de-etiolation and when in darkness COP1 ubiquitinates HY5, resulting in the degradation of the later by the proteasome [125]. During the day, light stabilises HY5 protein and acts as a positive regulator of photomorphogenesis [129] [130] [131]. The above events indicate the existence of a positive feedback loop of HY5 expression [132]. Another factor the HY5 HOMOLOG (HYH) has a partially redundant function with HY5 in UV-B UVR8 mediated signalling [133] [134] [135]. Upon UV-B induced expression and stabilization of HY5, photoprotective and photomorphogenic UV-B responsive genes are induced by HY5 and HYH [136]. These genes encode enzymes necessary for flavonoid biosynthesis , damage repair proteins and factors that inhibit hypocotyl elongation during photomorphogenesis and shade avoidance in the presence of UV-B [125]. UVR8 is negatively regulated by the two proteins REPRESSOR OF UV-B PHOTOTOMORPHOGENESIS 1 and 2 (RUP1 and RUP2) [137] which disrupt the binding between UVR8 and COP1 [126] and mediate the re-dimerization of the UVR8 monomers back to their inactive homodimeric state [138]. This constitutes a negative feedback loop mechanism triggered by UVR8 itself as a mean of regulating its own activity [132]

Overall the UV-B specific UVR8-mediated network leads to the biosynthesis of proteins that are really important for the plant's photoprotection and it involves specific reaction of UVR8 with COP1, the subsequent stabilization of HY5 and a negative feedback mechanism regulated by RUP and RUP2 [136] (Figure 1.10).

1.5.2 The role of UVR8 in circadian entrainment

The circadian clock plays a significant role in regulating not only flowering, but also various developmental stages of the life cycle of a plant by conferring diurnal patterns and responding to daily light fluctuations [139]. Light duration, quality as well as intensity is necessary for synchronizing, entraining, but also rhythmically attenuating the circadian clock [140]. As UV irradiation is an integral part of sunlight it has been shown that non-damaging levels of UV-B can also rhythmically entrain the circadian clock via transcriptional activation [140]. UV-B-induced clock entrainment occurs through the action of both UVR8 and COP1, whereas the UV-B signalling components HY5 and HYH are dispensable for this response[140]. The clock can on its turn rhythmically inhibit and control *UVR8* and *COP1* as well as many UV-B induced genes, apart from *HY5* [140]. Studies on the *elf3* mutant demonstrated a higher level UV-B induced gene induction that was not rhythmically attenuated by the circadian clock, however this did not lead to an increase in tolerance [140]. In theory, when a plant tries to acclimate under UV-B irradiation conditions, it has two options: The first one would be to activate its UV-B signalling network constantly in order to accumulate a significant amount of photoprotector proteins as fast as possible [140]. The energy cost for that first option though would be immense for the plant and it would prevent it from developing properly [141] [116]. The second option is to temporary restrict UV-B responsiveness by the circadian clock. The second option is what ultimately occurs in nature since it is more favourable for the plant [140].

1.6 UV-B and flowering

The potential effect of UV-B on regulating flowering initiation has not been studied extensively. A general delay in flowering time has been observed in various plant species including *Arabidopsis thaliana*, maize, *Phaseolus vulgaris* and *Vigna radiate* [142] [143] [144] [145] [146] [147] [148].

A recent study investigated the effect of UV-B irradiation on flowering initiation compared to *Arabidopsis thaliana* wild type (*Ler)* and *uvr8* mutant plants under a long day white light photoperiod in the absence and presence of UV-B [144]. UV-B irradiation lead to a significant

delay in flowering time in wild type plants [144]. Whereas *uvr8* mutants exhibited, a UV-B induced early flowering phenotype [144]. No difference was observed between flowering initiation in wild type and *uvr8* in the absence of UV-B (white light only) [144].

Furthermore, within the duration of this project two additional studies were published investigating the role of UV-B in flowering time initiation [91] [142]. The first study investigated mainly the role of the age and autonomous pathways in UV-B induced flowering [91]. A delay in flowering time was observed for the *Arabidopsis Col-0* ecotype under LD and SD photoperiodic conditions [91]. Their findings suggest that this response depends on the UVR8 signalling pathway, since analysis of flowering time of both *uvr8* and *Col-0* plants under WL ± UV-B demonstrated that *uvr8* mutant plants had a late flowering phenotype compared to the *Col-0* under WL growth conditions [91]. The described response however did not occur when both lines were grown under WL + UV-B conditions, where they did not depict any significant difference in flowering time [91]. Dotto et al. proposed a mechanism where UVR8 mediates a specific decrease in transcript abundance of two factors that are known subunits of the PRC2 complex (*CLF* and *MSI1*) [91]. The above event results in a decrease of the repressive mark of H3K27me3 on *miR156* and *FLC* genes [91]. This hypothesis was supported by an observed upregulation of *FLC* and *miR156* expression levels under WL + UV-B [91]. Moreover, downregulation of *SPL9* and *SPL10* expression was observed (SPLs are negatively regulated by miR156) [91]. This lack of a repression mark subsequently lead to an upregulation of the flowering repressor *FLC* and miR156 which prolongs the juvenile stage and inhibits transitioning to a reproductive state [91]. The above mechanism is cohesive with a downregulation in floral integrators *FT* and *SOC1* expression levels that was observed [91].

The second study demonstrated that RUP2 can repress the UVR8-mediated flowering regulation under SD photoperiod conditions in *Arabidopsis thaliana* [142]. *Rup2* and *rup1rup2* mutants were reported to have early flowering phenotypes compared to the wild type *Col-0* line, under SD conditions supplemented with UV-B [142]. Additionally the early flowering phenotype of *rup2* and *rup1rup2* under SD + UV-B was found to be UVR8-dependent because it was not observed in *rup2 uvr8* and *rup1rup2 uvr8* mutant lines, which flowered the same time as the wild type line under SD + UV-B [142]. Arongaus et al., showed no involvement of RUP2 under LD in the absence of UV-B since no difference was observed in the flowering behaviour between the wild type *Col-0* and *Arabidopsis thaliana rup1*, *rup2* and *rup1rup2* mutants [142]. They were able to demonstrate a direct interaction between RUP2 and CO via a yeast two-hybrid assay [142]. They also depicted that the early flowering phenotype of *rup2* mutant line observed under SD + UV-B conditions is in fact both CO and FT- dependent [142].

The aforementioned hypothesis was supported by the observation that the early phenotype of *rup2* mutant line (always in comparison to the wild type phenotype) under SD + UV-B was supressed in both *rup2 co* and *rup2 ft* mutants [142]. *FT* transcript levels were observed to be increased in *rup2* and *rup1rup2* mutant lines in comparison with the *Col-0* line, when grown under SD + UV-B conditions [142]. *CO* transcript levels for these mutants were not significantly altered compared to the ones of the wild type also under SD + UV-B conditions [142]. All the above data combined suggest that RUP2 can repress CO binding to the promoter of *FT*, resulting in a CO-dependent downregulation of *FT* expression and a delay in flowering time, under SD growth conditions supplemented with UV-B [142].

1.6 Shade avoidance and the role of UV-B

Shade avoidance is a light mediated response caused by the reduction of the R:FR ratio sensed by plants photoreceptors because of its neighbouring vegetation [12]. UV-B has been shown to inhibit auxin biosynthesis and hypocotyl elongation that are the two main shade avoidance responses [144]. The UV-B induced inhibition of shade avoidance depends on UVR8 as *uvr8* mutants were impaired in this response [114].

More specifically, the protein abundance of the PHYTOCHROME INTERACTING FACTORS PIF4 and PIF5 was decreased under low R:FR supplemented with UV-B and as a result auxin levels and stem elongation were also reduced [144]. UVR8 though cannot interact physically with the PIFs, leading to the conclusion that there is another pathway which links PIF4 and PIF5 UV-B induced degradation with UVR8 [144]. The repression if auxin biosynthesis by UV-B is dependent on UVR8 but not on the presence of UV-B signalling components HY5 and HYH [144]. Nevertheless, these factors can act in a redundant manner in order to also mediate partial UV-B specific inhibition of shade avoidance responses [144]. Moreover, the UVR8-COP1 interaction upregulates the expression of *HY5* and *HYH* which eventually results in the inhibition of responses such as petiole elongation and leaf elevation -two common responses of the shade avoidance syndrome in higher plants- [144] (Figure 1.11).

1.8 UV-B and vegetative shade flowering

In general shade accelerates flowering compared to high R:FR conditions, especially under a LD growth photoperiod [149]. In order for early flowering to occur under shade, a variety of factors must be coordinated [149]. First and foremost the ratio of R:FR is extremely important since it regulates the amount of the reduction in Pfr levels and therefore active phyB [149].

Additional factors which shape that response include the length of the day and the genetic background of the plant [149]. Two important events lead to the acceleration of flowering under low R:FR conditions [149]. Firstly the negative effect of *FLC* in flowering is bypassed without a corresponding decrease in *FLC* transcript levels observed [149]. The removal of the Pfr form of PHYB, PHYD and PHYE under low R:FR are the cause of the above response [149]. Secondly a CO-dependent increase in *FT* transcript abundance occurs [149]. This happens probably due to the fact that CO protein can be stabilized rapidly under shade conditions whereas its gene expression is increased under longer periods in shade [149].

The effect of UV-B in flowering time under low R:FR conditions has not been studied extensively. In a recent study the flowering phenotype of wild type *Ler* and *uvr8* mutant lines, under shade +/- UV-B suggested that low R:FR + UV-B conditions lead to a significant delay in flowering of the wild type *Ler* while the *uvr8* mutant flowering behaviour remained unaffected [144]. Therefore, more information is required to understand the mechanism of UV-B induced flowering under shade conditions.

1.9 Aims of this study

Light, temperature and circadian rhythms all have the ability to regulate flowering initiation [9] [18] [88]. Light duration, quality, photoperiod and intensity is perceived by the different families of a plant's photoreceptors [5]. Orchestration of light signals perceived by each photoreceptor family occurs primarily at the level of gene expression [65]. Currently, photoreceptors have been observed to both promote and supres the flowering initiation of the long day plant *Arabidopsis thaliana* [65]. Cryptochromes and zeitlupes for example can act as positive regulators of flowering either indirectly by promoting protein stabilisation of essential flowering-involved transcription factors or by directly inducing expression of important flowering genes [45] [55]. Phytochromes can act as positive or negative regulators of flowering [28] and so on. However, the role of UVR8 in regulating photoperiodic flowering under LD growth conditions has not been investigated yet in great extent. Plants also often have to grow to close proximity with one another and compete for light sources in order to be able to flower under vegetative shade conditions [150]. Since UV-B and its photoreceptor UVR8 have been found to inhibit shade avoidance specific developmental responses [144], it is only reasonable to wonder if UV-B and UVR8 have an effect on vegetative shade induced flowering initiation as well.

To investigate the role of UV-B and UVR8 in regulating flowering we employed both physiological, genetic and molecular approaches. More specifically the aims of the present study are the following:

1. Investigation of flowering time of specific *Arabidopsis thaliana* lines under long day photoperiodic conditions of WL ± UV-B and shade ± UV-B. These lines include different *Arabidopsis thaliana* ecotypes (*Col-0, Ler, and Cvi*) as well as *uvr8* mutant and over-expressing lines. Flowering time was investigated also for additional lines harbouring mutations in genes that were involved in UV-B and/or light signalling (*rup1rup2*, *cop1-4*, *pif4* and *pif4pif5*), and, photoperiodic flowering (*elf3-1*, *co* and *zlf*). Flowering initiation of a line over-expressing an important flowering regulator and light signalling factor (OX-PIF4) was investigated as well.
2. Gene expression analysis was performed in all of these lines, in order to monitor transcript levels of some of the most important flowering regulators (*FT*, *CO, SOC1*, *FLC*, *PIF4, and ELF3*) under WL ± UV-B conditions.

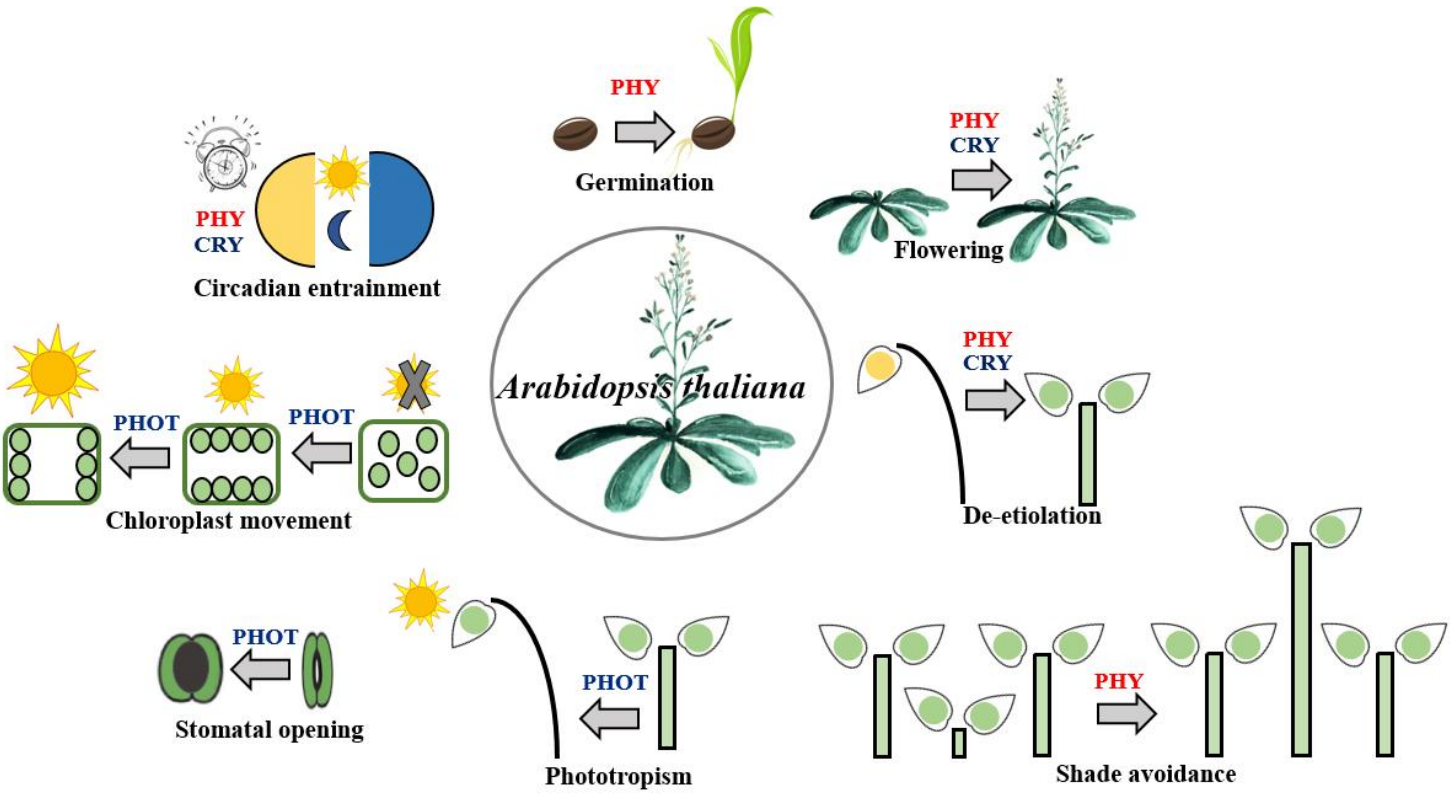


Figure 1. 1 Graphic representation of all the developmental stages of *Arabidopsis thaliana* life which are regulated by light.

Figure modified from Sullivan and Deng, 2003 [5].

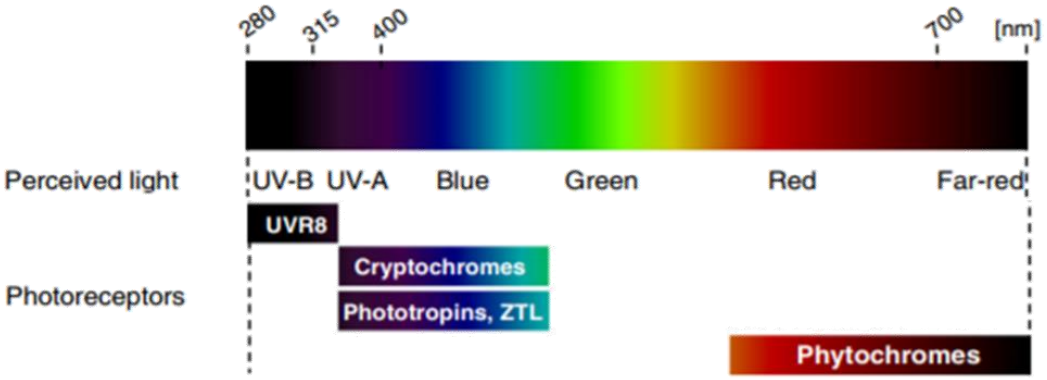


Figure 1. 2 Photoreceptor families in higher plants.

Graphical representation of all the five photoreceptor families in higher plants including *Arabidopsis thaliana*, and the area of the electromagnetic spectrum that each family can absorb.

Figure modified from Heidje and Ulm, 2012 [125].

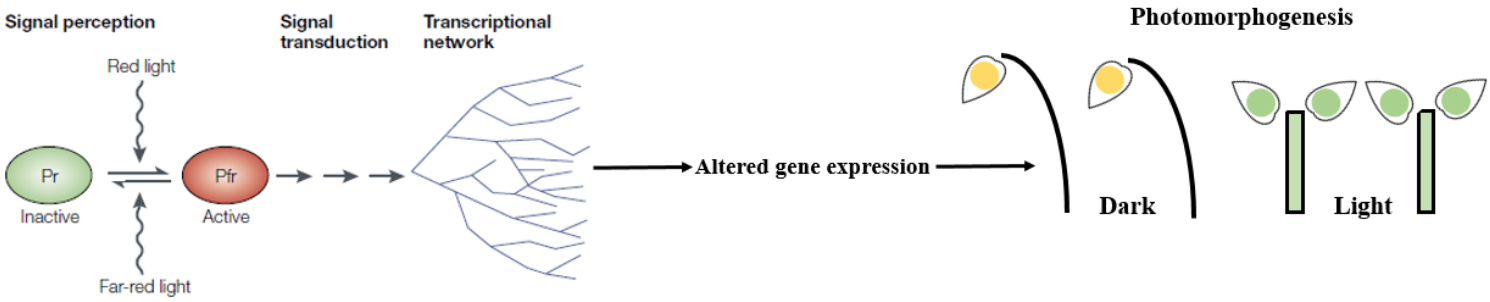


Figure 1. 3 Perception of light by phytochromes and signal transduction networks that lead to photomorphogenic responses in *Arabidopsis thaliana*.

Phytochromes exist into two forms, the biologically inactive Pr form and the active one Pfr, depending if they are under red light or far red light illumination. The active Pfr conformer has the ability to trigger signal transduction processes which through a transcriptional network can alter gene expression leading to the subsequent induction of photomorphogenic plant responses.

Figure modified from Quail, 2002 [28].

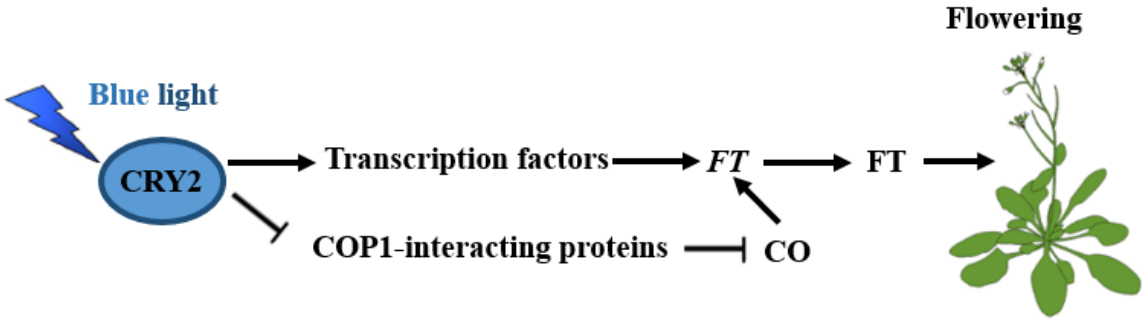


Figure 1. 4 Two alternative pathways by which CRY2 regulates gene expression and promotes flowering in *Arabidopsis thaliana*.

Under blue light CRY2 can interact with a group of transcription factors that promote the transcription of flowering inducing gene *FT*. Additionally CRY2 can also interact with COP1-interacting proteins and thus supress the degradation of CO by COP1, and promote flowering indirectly, since CO protein positively regulates the transcription of *FT*.

Figure modified from Yu et al, 2010 [32].

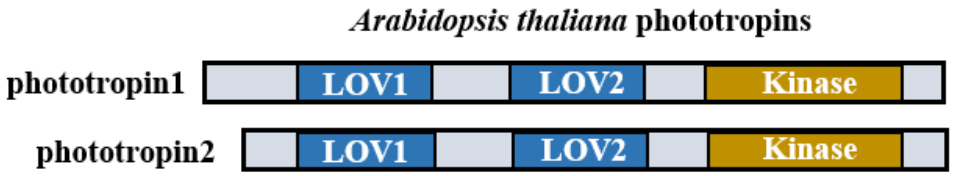


Figure 1. 5 Phototropins 1 and 2 of *Arabidopsis thaliana*.

Schematic representation of the structure of *Arabidopsis thaliana* phototropin 1 and 2 protein receptors. Both chromophore binding domains LOV1 and LOV2 as well as the catalysing kinase domains are shown.

Figure modified from Briggs and Christie, 2002 [13].

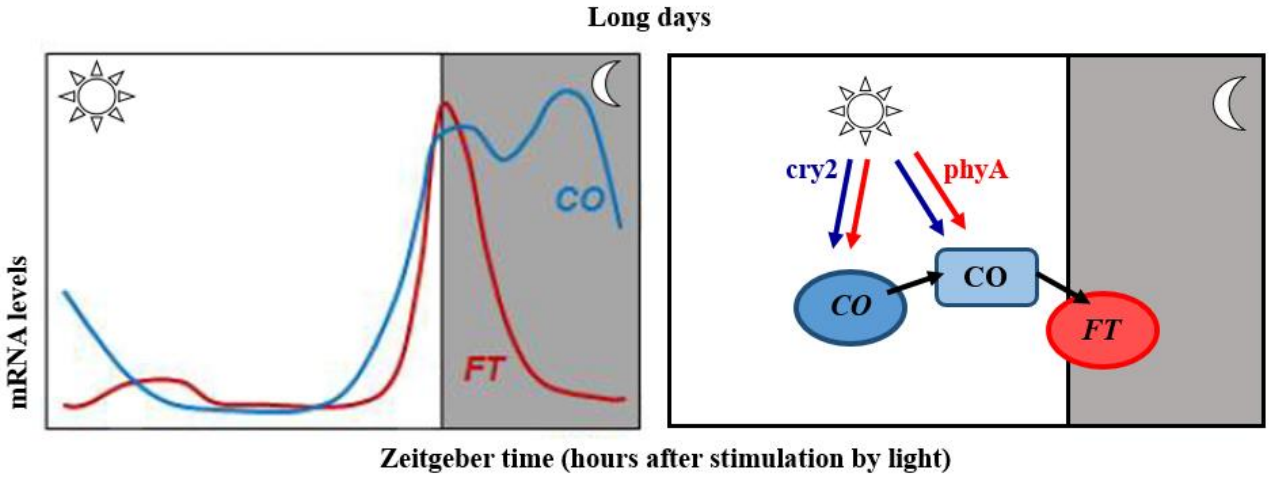


Figure 1. 6 Expression and action of transcription factor *CO* and *FT* under LD in *Arabidopsis thaliana*.

Schematic representations of the expression pattern of the *CO* and *FT* transcript during the day and their regulation in order to promote flowering. Photoreceptors cry2 and phyA promote the transcription of *CO* which then activates flowering promoter FT, in order for flowering to occur under long days.

Figure modified from Torti, 2010 [72] and Yanovsky and Kay, 2003 [71].

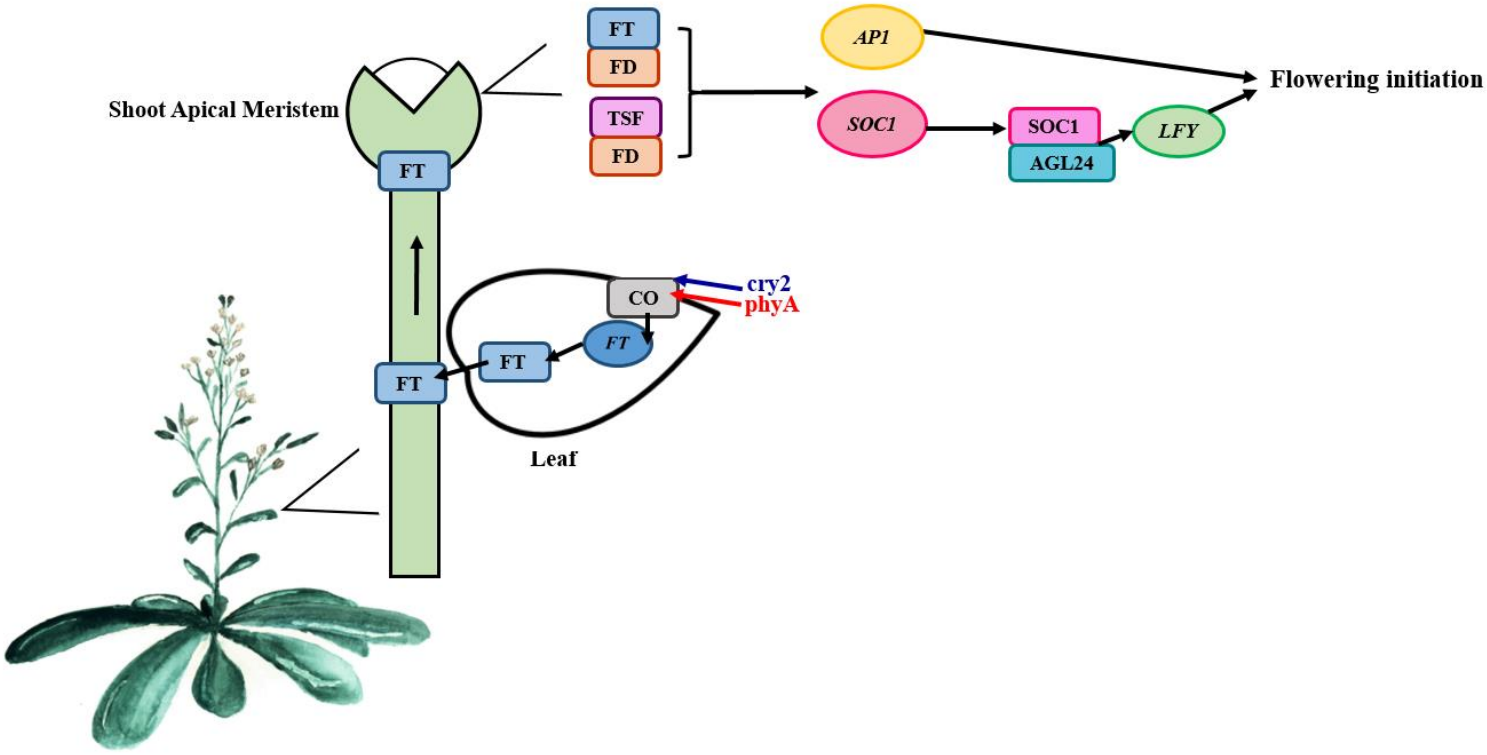


Figure 1. 7 Flowering initiation network in *Arabidopsis thaliana*.

Simplified schematic representation of the levels of control of floral induction in *Arabidopsis thaliana*. In the leaf CO promotes the transcription of *FT*. FT the travels from the leaf to the shoot apical meristem where along with its twin sister TSF, bind FD and promote the expression of *SOC1*, *AP1* and *LFY*, that eventually leads to flowering initiation.

Figure modified from Turck, Fornara and Coupland, 2008 [151].

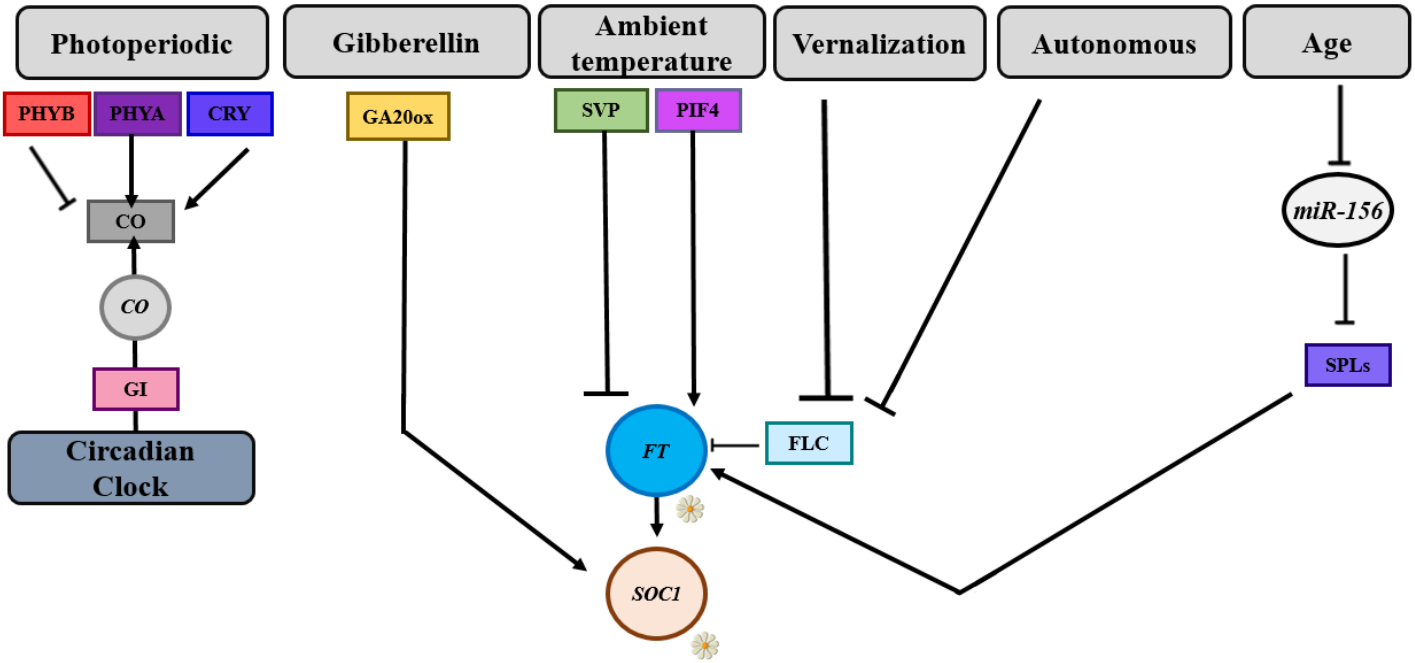


Figure 1. 8 Overview of the flowering pathways and their main regulator factors in *Arabidopsis thaliana*.

Simplified schematic representation of the six main flowering pathways in *Arabidopsis thaliana* and their main regulators. At the leaf of the plant the photoperiodic pathway in correlation with the circadian clock promote the transcript expression of *CO* factor. CO then binds to the promoter of *FT* and promotes flowering. *FT* transcript also gets promoted by the GIBBERELLIN 20 OXIDASE enzyme, thus providing control from the gibberellin pathway. In the temperature pathway SVP represses *FT* transcription, but under warmer temperatures PIF4 promotes *FT* transcript accumulation. The main repressor of *FT* is FLC, but *FLC* transcript is repressed by the vernalization and the autonomous pathways. In the shoot apical meristem the age pathway regulates flowering through the negative regulation of miR-156 which in turn regulated SPLs in a negative way. SPLs are promoters of *FT* transcript. In the shoot apical meristem FT promotes the transcription of *SOC1* which subsequently promotes the transcription of the floral meristem identity genes and leads to the initiation of flowering. Figure modified from Fornara, Montaigu and Coupland, 2010 [139].

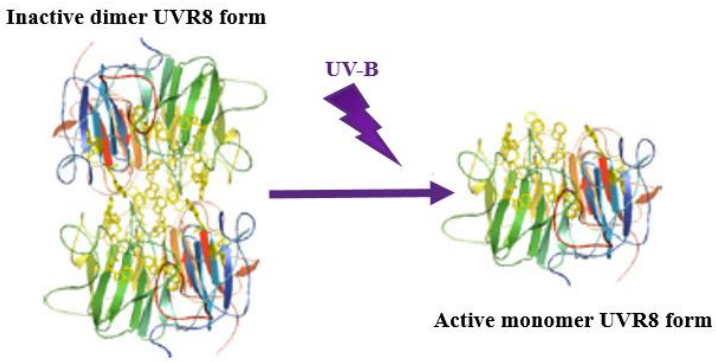


Figure 1. 9 UV-B perception by the UVR8 photoreceptor in *Arabidopsis thaliana*.
UVR8 in darkness forms a homodimer which localizes in the cytosol. When the plant is exposed to UV-B radiation, UVR8 dimer dissociates to its two monomers and goes to the nucleus, where it mediates UV-B specific photomorphogenic responses.
UVR8 structure as depicted by Heijde and Ulm, 2012 [125] and figure modified by Heijde and Ulm, 2012 [125].

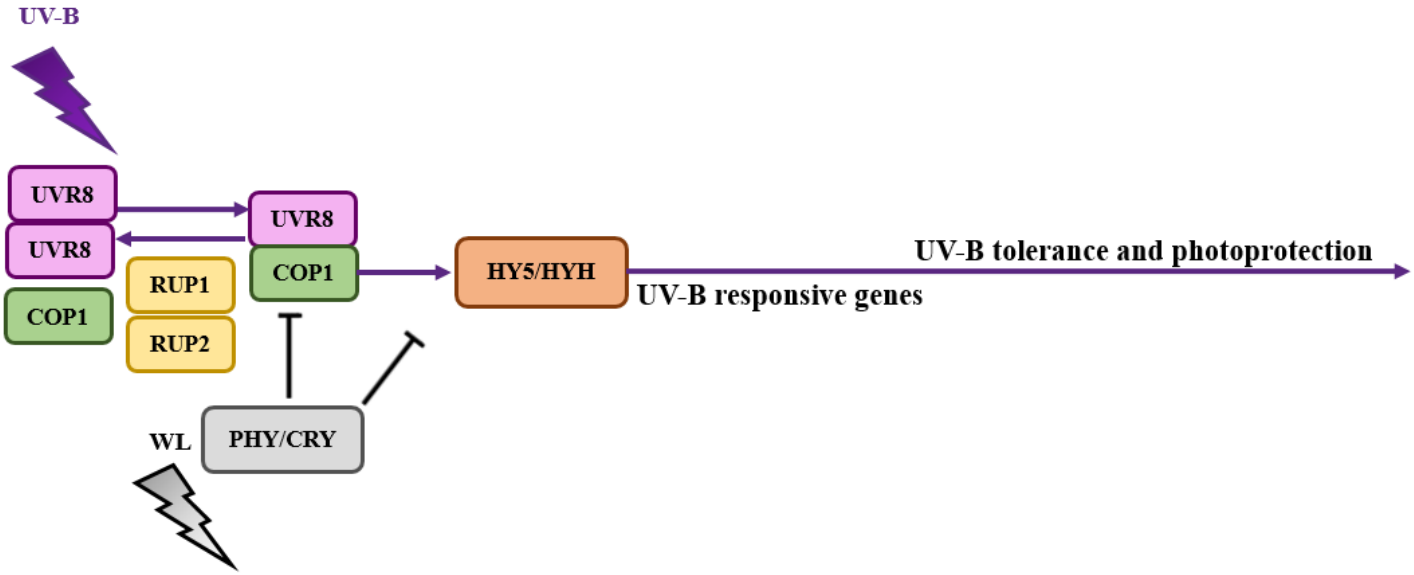


Figure 1. 10 Overview of UVR8 mediated signalling in *Arabidopsis thaliana*.
Upon UV-B irradiation the inactive homodimer of UV-B monomerizes and turns into its biologically active form. As a monomer UVR8 interacts with COP1. This interaction leads to HY5 and HYH protein stabilization and the activation of UV-B responsive genes that are important for UV-B irradiation protection and tolerance. RUP1 and RUP2 negatively regulate UVR8 by disrupting its interaction with COP1 and reverting it back to its homodimeric state.
Figure modified from Heijde and Ulm, 2012 [125].

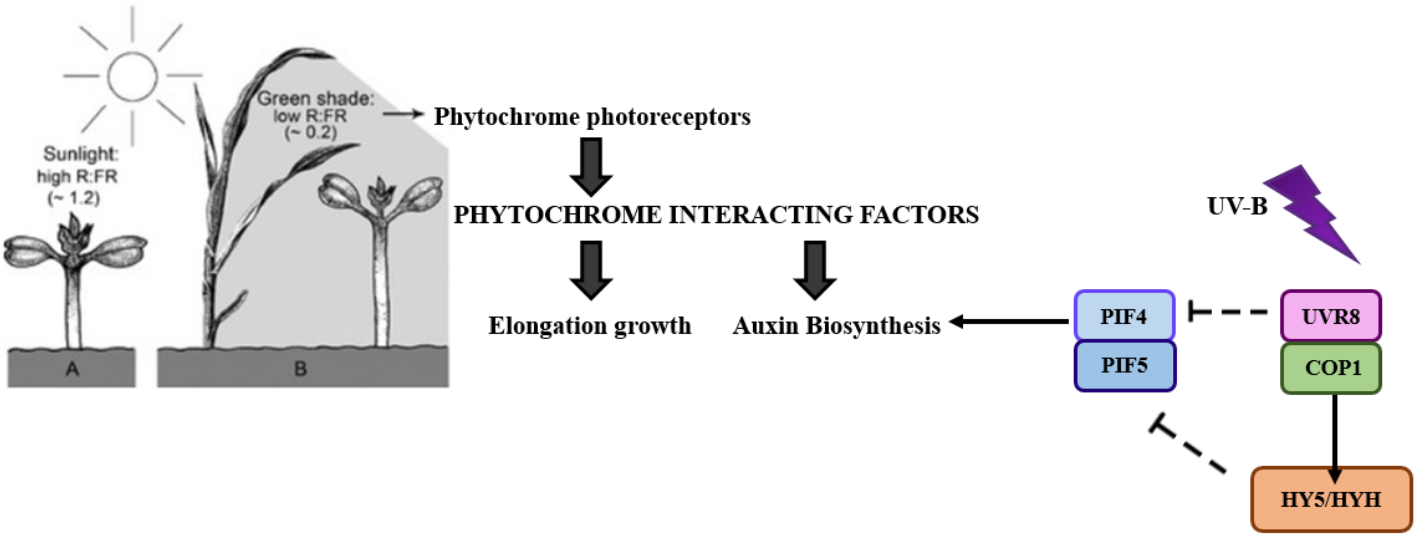


Figure 1. 11 Overview of the role of UV-B and UVR8 in shade avoidance inhibition in *Arabidopsis thaliana*.

Under vegetative shade conditions (low R:FR) the phytochrome photoreceptors of the plant mediate specific shade avoidance responses including the hypocotyl elongation growth and the biosynthesis of auxin. Under UV-B irradiation UVR8 indirectly degrades PIF4 and PIF5 leading to an inhibition resulting in a decrease in auxin biosynthesis and petiole elongation. The UV-B-specific interaction of UVR8 with COP1 upregulates the transcription of factors *HY5* and *HYH* which also contribute to the inhibition of the shade avoidance responses.

Figure modified from Hayes et al, 2014 [144] and Kutschera and Briggs, 2013 [152].

Chapter 2: Materials and Methods

2.1 Plant Material

In this study three wild type *Arabidopsis thaliana* ecotypes were used for the flowering and gene expression analysis experiments: *Columbia-0* (*Col-0*), *Landsberg erecta* (*Ler*) and *Cape Verde Islands* (*Cvi*). Mutant and transgenic lines were also used for the aforementioned experiments in either *Col-0* [*rup1rup2* [153] [154], *cop1-4* [155], *constans-10* (*co-10*) [76], *ztl lkp2 fkf1* (*zlf*) [49], *elf3-1* [156], *pif4* [157], *pif4pif5* [158], *OX-PIF4-HA* [100] and *uvr8-6* [116]] or *Ler* [*uvr8-1* [114], *uvr8-2* [115] and OX-GFPUVR8/*uvr8-1* [122]], background.

2.2 Growth conditions

Seeds used for flowering studies were stratified in sterile distilled dH_2O for 3-4 days at 4 °C and were sown on soil (Phytotron growth chambers) under long day photoperiods (16 hours light, 8 hours dark) with an illumination intensity of white fluorescent light (WL) 50 μmol $m^{-2}s^{-1}$ ± UV-B (0.2-0.3, 0.5, 1 μmol $m^{-2}s^{-1}$) for UV-B experiments and WL = 35 μmol $m^{-2}s^{-1}$, R:FR ≈ 0.14 ± UV-B (0.7 μmol $m^{-2}s^{-1}$) for experiments under shade conditions. The WL and FR light in these experiments was provided by LED lights (CEC) for UV-B experiments and by fluorescent cool-white light tubes (OSRAM) for the shade experiments. UV-B was provided by narrowband fluorescent lights in both cases (PHILIPS NARROWBAND TL 40W/0I-PS).

To test the genetic segregation of transgenic lines, seeds were surface sterilized using 50% bleach for 4-5 minutes and then washed three times with sterile distilled dH_2O. Sterilised seeds were stratified for 3-4 days at 4 °C prior to sowing on half strength Murashige and Skoog (1/2 MS) 0.8% agar medium with the corresponding amount of the selective antibiotic (kanamycin 75 μg ml^{-1}). Subsequently the selected plants were transferred to growth chambers under constant light with an illumination intensity of 100 μmol $m^{-2}s^{-1}$ of WL.

2.3 Flowering time measurements

Flowering time was monitored by either a) counting the total number of rosette leaves of each plant on the day it bolted (appearance of the first flower bud with a stem of 2 cm) or b) by calculating the number of days after germination at the time of bolting.

2.4 RNA isolation

Total RNA was extracted from plants grown for 12 days (until they reached the juvenile phase, before transition to reproductive growth) under LD conditions. The collection included two different zeitgeber times (ZT0.5 = 30 min after light onset and ZT15= 15 h after light onset). The tissue was rapidly frozen using liquid nitrogen and then stored at -80 °C. Total tissue was disrupted using a TissueLyser by Qiagen for 1 minute under the speed of 18.0 m/s. RNA was extracted using the RNeasy plant mini kit (Qiagen) according to the manufacturer's instructions. The final elution was performed in 30 µl of RNase-free water. The total amount of RNA was quantified using a spectrometer nanodrop (Implen).

2.5 Complementary DNA synthesis and Reverse Transcription Polymerase Chain Reaction

cDNA synthesis was performed on 1 µg of total RNA using the QuantiTect Reverse Transcription Kit by Qiagen according to the manufacturer's instructions. The efficiency of the synthesized cDNA was tested for each sample separately through RT-PCR using *ACTIN* primers and the GoTaq G2 DNA polymerase by Promega. A positive and negative control (no cDNA) reactions were always used for verification of the result and to eliminate the possibility of genomic DNA contamination. *ACTIN* was amplified from approximately 25 ng of cDNA using the thermal cycling profile indicated below according to the manufacturer's instructions. Each reaction was assessed by electrophoresis on a 1% agarose gel.

The sequence of the *ACTIN* primers, the components of the PCR reaction mix, and the conditions of the thermal cycling profile are described in the tables below.

Table 2. 1 ACTIN primers sequence

ACTIN Forward	CTTACAATTTCCCGCTCTGC
ACTIN Reverse	GTTGGGATGAACCAGAAGGA

Table 2. 2 RT-PCR reaction mix

Components	**µl**
Green GoTaq Buffer (5x)	5
GoTaq G2 DNA polymerase by Promega (5 u/ µl)	0.13
dNTP Mix (10 mM)	0.25
Actin Primer Forward (20mM)	0.25
Actin Primer Reverse (20mM)	0.25
cDNA (1 µg)	0.5
dH_20	up to 25

Table 2. 3 RT-PCR thermal program used for amplification

Steps	Thermal Cycling Program
1	Incubate at 95 ºC for 30 sec
2	Incubate at 95 ºC for 30 sec
3	Incubate at 55 ºC for 45 sec
4	Incubate at 68 ºC for 30 sec
5	Cycle to **Step 2** for 27 more times
6	Incubate at 68 ºC for 5 min
7	Incubate at 4 ºC forever

2.6 Real-time polymerase chain reaction (qPCR)

Real-time quantitative PCR was performed with the StepOnePlus™ Real-Time PCR System (Applied Biosystems, Life Technologies) using the Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix (Agilent Technologies). Expression of housekeeping gene *ISU* or *IPP2* was used for normalisation. The amplification efficiency for each sample was calculated by StepOne™ Software v2.2 (Life Technologies) by using the slope of the regression line in the standard curve. A series of 6 serial 4-fold standard dilutions was used in this process. The software used the standard curve in order to interpolate the target quantities of every gene and calculate the relative fold differences. The normalisation of the data is achieved by geometric averaging of *ISU* or *IPP2* as internal reference genes. [159] The sequence of the primers of the genes used in the qPCR experiments, the components of the PCR reaction mix, and the conditions of the thermal cycling profile are described in the tables below.

Table 2. 4 qPCR primers sequence

q*ISU* Forward	GCCATCGCTTCTTCATCTGTTGC
q*ISU* Reverse	TGGGAGAGAAAGATGCTTTGCG
q*IPP2* Forward	GTATGAGTTGCTTCTCCAGCAAAG
qIPP2 Reverse	GAGGATGGCTGCAACAAGTGT
q*FT* Forward	CTAGCAACCCTCACCTCCGAGAATA
q*FT* Reverse	CTGCCAAGCTGTCGAAACAATATAA
q*SOC1* Forward	TTCGCCAGCTCCAATATGCAAG
q*SOC1* Reverse	TGCTACTCGATCCTTAGTATGCC
q*CO* Forward	CAACAGCTTCACACCCAAGAACG
q*CO* Reverse	TTGCAGGGTCAGGTTGTTGCTC
q*FLC* Forward	TCTCCTCCGGCGATAACCT
q*FLC* Reverse	GCATGCTGTTTCCCATATCGAT
continued on the next page	

q*ELF3* Forward	GCACACTGATTAAGGTTCAAAAAC
q*ELF3* Reverse	CTTCACTGGATAGCTTTTAGCAG
q*PIF4* Forward	GTTGTTGACTTTGCTGTCCCGC
q*PIF4* Reverse	CGATCAGCCGATGGAGATGTT
q*HY5* Forward	GGCTGAAGAGAGGTTGTTGAGG
q*HY5* Reverse	CAGCATTAGAACCACCACCA
q*COR15* Forward	CAGCGGAGCCAAGCAGAGCAG
q*COR15* Reverse	CATCGAGGATGTTGCCGTCACC

Table 2. 5 qPCR Reaction Mix

Components	**µl**
Standards	
Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix	5.7
Dye (1mM)	0.2
Primer Forward (20mM)	0.1
Primer Reverse (20mM)	0.1
cDNA (1 µg)	2 per well
dH_20	3
DNA Samples	
Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix	12.5
Dye (1mM)	0.375
Primer Forward (20mM)	0.25
Primer Reverse (20mM)	0.25
cDNA (1 µg) diluted to a 90 fold	5 per well

Table 2. 6 qPCR thermal program used for amplification

Steps	**Thermal Cycling Program**
1	Incubate at 95 ºC for 2 min
2	Incubate at 95 ºC for 3 sec
3	Repeat step 3 for 50 times
4	Incubate at 59.5 ºC for 30 sec

2.7 Protein Extraction

Total protein extraction was achieved by resuspending ground plant tissue in 4X Laemmli Protein Sample Buffer (PSB) (Bromophenol Blue 0.1%, Tris 1 M pH 6.8, Glycerol 40%, SDS 10%, β-Mercaptoethanol 20%). The amount of PSB added to each sample was pro rata to the weight of the sample (w/v). For every 100 mg of tissue sample 100 µl of PSB were added. Afterwards the samples were boiled for 5 min at 100°C followed by centrifugation for 1 min at 14,000 g. The supernatant was transferred to a fresh tube and examined by SDS-PAGE.

2.8 Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Western Blot Transfer

Proteins were separated on a gradient 4-12% SDS polyacrylamide gel (Bis-Tris Bolt, ThermoFisher) using MOPS or MES running buffers supplied by ThermoFisher according to the manufacturer's instructions.

Western transfer of the migrated proteins was performed by using a nitrocellulose membrane (ThermoFisher) at 100V for 1h at room temperature (RT) or at 30 mA overnight at 4 °C, using Western Blot Transfer Buffer (Tris 25 mM, Glycine 190 mM, Methanol 20%), using wet-transfer and a BioRAD apparatus.

Ponceau S Staining and Blocking of Membrane

Transfer efficiency was verified by Ponceau S (0.1% Ponceau Stain, 5% Acetic Acid) staining. Non-specific binding sites of the protein transferred to the membrane were blocked for at least 30 min incubation in 4% dry milk in TBST (Tris 20 mM pH 7.5, NaCl 150 Mm, Triton 0.1%).

Primary and Secondary Antibody Incubations and development

Nitrocellular membranes carrying the protein extracts under examination were incubated with specific primary antibodies in 4% dry milk in TBST of the desirable concentration (in the current study for the anti-UVR8 [127] as well as the anti-GFP [159] primary antibody a 1:5.000 concentration was used), overnight (O/N) at 4 °C. Afterwards the membrane was washed 2 times for 5 min with TBSTT (Tris 20 mM pH 7.5, 150 Mm NaCl, Triton 0.1%, Tween 20 0.05%) and 5 min with TBST and finally incubated for 3 - 4h at RT with the secondary antibody dissolved again in 4% dry Milk in TBST in the desirable concentration. After the incubation with the primary antibody membrane was washed for 5 min for 4 times with TBST and 5 min with TBST. The membrane was then placed in TBS. Chemiluminescent development of the western blot was performed using the SuperSignal™ West Dura Extended Duration Substrate Kit (ThermoScientific) according to the manufacturer's instructions. Medical X-Ray blue films were used for visualisation of positive signal using the Xograph Compact X4 by Imaging Systems.

Stripping of Membrane

After development, the nitrocellulose membrane was washed for 5 min for 3 times with sterile dH_2O. Stripping Buffer was applied to the membrane (Bioland Sciences) for 15 min followed

by 3 washes for 5 min with sterile dH_2O. Blocking and incubation with specific primary and secondary antibodies was performed as described above.

2.9 Isolation of plasmid DNA (small scale)

Plasmid DNA was isolated using the QIAprep Spin Miniprep Kit (Qiagen) according to the manufacturer's instructions. In the last step the final elution of the DNA was performed in 30 μl of sterile distilled dH_2O. The concentration of the purified DNA was quantified using a spectrometer nanodrop (Implen).

2.10 Transformation of *E.coli* DH5a cells

Competent *E.coli* DH5a cells (50 μl) (Life Technologies) were incubated with approximately 100 ng of the desirable plasmid according to manufacturer's instructions, and transformed by both heat shock and electroporation of the competent cells. Transformed cells were spread by glass beads on LB agar plates containing the appropriate antibiotic for selection and incubated at 37 ^oC O/N.

2.11 Transformation of *A. tumefaciens* cells

Homemade competent *A. tumefaciens* cells were incubated with 80 ng of the desirable plasmid on ice. An electro-pulse was performed by a GenePulser Xcell (240V, 25 μF, 200 Ω). After the electroporation LB growth medium was immediately added to the cells, which were transferred to grow at a 37 °C shaking incubator for approximately 3h. The cells were peletted by centrifugation (16,000 g, 1 min) and the pellet was resuspended in 100 μl of LB and spread on LB agar plates containing the appropriate antibiotics for selection (gentamycin 30 μg ml^{-1} and kanamycin 50 μg ml^{-1}). The plates were incubated at least for 2-3 days at 28-30 ^oC.

2.13 Floral Dipping

Floral dipping of *Arabidopsis* plants of the desired background with *A. tumefaciens* cells carrying a plasmid construct that contained the gene of interest was conducted as described previously (Clough and Bent, 1998). O/N liquid cultures originating from the colonies of the successful *A. tumefaciens* transformation were centrifuged at 4,000 g and the pellet was resuspended in the Floral Dipping Medium **(**5% Sucrose, 0.05% SILWET) (FDM) until the OD of the resuspended cultures was adjusted to 0.8 – 1. *Arabidopsis thaliana* flowers were

submerged in the Agrobacteria-containing FDM. Plants were placed under high humidity and moderate light to recover for 2 days prior to repeating the floral dip.

2.14 Transgenic *Arabidopsis thaliana* lines Generation Selection

T1 selection (acquirement of transformed heterozygous plants)

T1 transformed seeds were selected by sowing the entire population of seeds collected from transformed plants on ½ MS containing the appropriate antibiotic. The plates were incubated in constant WL for approximately 2 weeks. The seedlings that were resistant to the antibiotic were transferred to soil in individual pots, in order to grow and give the next generation of seeds.

T2 selection / 3:1 resistance (acquirement of homozygous plants)

T2 generation of seeds were screened for 3:1 segregation (resistant: sensitive) to the appropriate antibiotic (kanamycin 75 μg ml^{-1}) using the Chi-square test. Approximately 40-50 seeds were sown on ½ MS containing the desired antibiotic and grown for approximately 2 weeks under constant WL. This method is used to determine if an observed group of data is compatible with a set null hypothesis, which in this case is to confirm which plants show 3:1 resistance ratio to the antibiotic, which means that they have only one copy of the transgene. For that matter Observed and Expected values are compared. The sum of $(O-E)^2/E$ for all classes is compared to a table of values. Values less than 5% ($p = 0.05$) indicates that the null hypothesis is rejected and the alternative one is accepted. Finally the seedlings that showed 3:1 resistance were transferred to soil in individual pots in order to grow and give the next generation of seeds.

T3 selection / 100% resistance (acquirement of homozygous plants)

T3 generation of seeds were selected for 100% resistance. The seedlings derived from a plant which is homozygous will all show resistance to the antibiotic. These seedlings were transferred separately to soil in order to grow and give the next generation of seeds which will be the generation of transgenic plants that is suitable to be used in experiments.

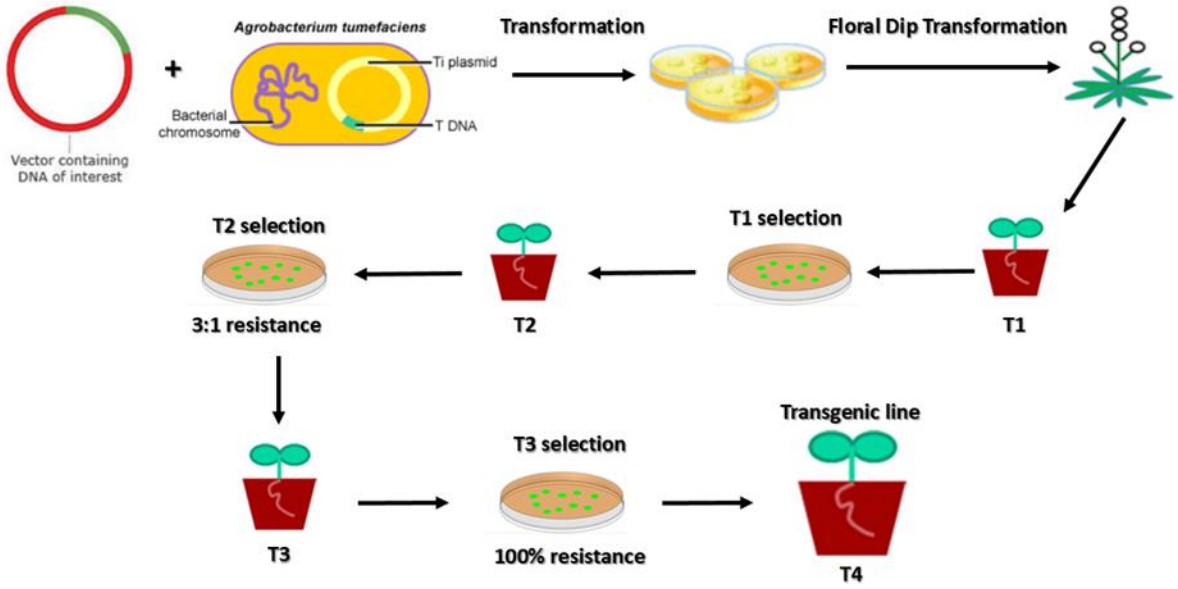


Figure 2. 1 Overview of the procedure required for generating homozygous transgenic *Arabidopsis thaliana* lines.

2.15 Isolation of genomic DNA from *Arabidopsis thaliana* plants

Crude genomic DNA isolation was performed by grinding the plant tissue (100 mg) in liquid nitrogen using a mortar and pestle followed by the addition of 400 µl extraction buffer (200 mM Tris-HCl pH 7.5, 250 mM NaCl, 25 mM EDTA, 0.5% SDS) and vortexing of the tissue for 5 sec. The samples were then centrifuged at 10,000 g and the supernatant was transferred to a new tube. The samples were then incubated at 65°C for 2 min and then 300 µl of cold isopropanol was added to the supernatant. The samples were mixed and left to incubate at RT for 10 minutes followed by centrifugation at 10,000 g. The supernatant was removed and the pellet was washed using cold ethanol. After the ethanol was removed, the pellets were dried using a speed vacuum and the DNA was resuspended by adding 30 µl of sterile dH_2O.

2.16 Genotyping of mutant *uvr8-6* (*Col-0*) *Arabidopsis thaliana* lines

PCR was performed on 3 µl of genomic DNA for each individual sample using 2 different sets of primers: the *UVR8* gene specific primers were used in combination with the left border primer LBb1.3. Primers for genotyping were designed using the T-DNA primer design software (http://signal.salk.edu/tdnaprimers.2.html). GoTaq G2 DNA polymerase by Promega was used according to the manufacturer's instructions. Positive and negative control reactions were used for verification of the result. The PCR was then amplified using a thermal cycling profile shown in Table 2.8. Each reaction was assessed by electrophoresis on a 1% agarose gel.

The sequence of the *UVR8* primers and the conditions of the thermal cycling profile are described in the tables below. The Reaction Mix is indicated in table 2.2.

Table 2. 7 UVR8 and LBb1.3 primers sequence

UVR8 Forward	AATGGCATTGACTTCAGATGG
UVR8 Reverse	TTTGCTTGAACCATCCGTTAG
LBb1.3	ATTTTGCCGATTTCGGAAC

Table 2. 8 PCR Reaction Mix and thermal program used for amplification

Steps	**Thermal Cycling Program**
1	Incubate at 95 ºC for 1 min
2	Incubate at 95 ºC for 30 sec
3	Incubate at 55 ºC for 30 sec
4	Incubate at 72 ºC for 1 min
5	Cycle to **Step 2** for 35 more times
6	Incubate at 72 ºC for 5 min
7	Incubate at 4 ºC forever

2.17 Competent yeast cell preparation

An O/N pre-culture of 25 µl of frozen MaV203 yeast cells in 5 ml of YPDA was set in a shaking incubator at 30 ºC. The next day 1 ml of the O/N culture was added to 50 ml of YPDA and incubated again O/N at 30 ºC. On the third day the whole culture was inoculated in 400 ml of YPDA (Yeast extract 1%, Peptone 2%, Glucose (+)D 2%, Adenine Hemisulphate 0.004% pH 7.5) and incubated at 30 ºC once again until it reached an OD of 0.7 – 0.9. Afterwards the culture was centrifuged at 1000 g for 5 min at 4 ºC and the pellet was resuspended in 50 ml of sterile dH_2O. The culture was centrifuged once more in the same conditions and the pellet was washed with 25 ml of TE/LiAc (Tris 10mM pH 7.5, EDTA 1mM, Lithium Acetate 100 mM pH 7.5) and centrifuged once more. Finally, the pellet was resuspended using the minimum possible amount of TE/LiAc and mixed with freshly boiled salmon sperm (100 µl of salmon sperm for 1 ml of competent yeast cells).

2.18 Transformation of competent yeast cells

Yeast transformation was performed the same day as the preparation of the competent yeast cells in order to increase the efficiency of the transformation. Approximately 100 ng of each plasmid DNA (pDEST22, pDEST 32) was added to 50 µl of competent yeast cells and 200 µl of TE/LiAC/PEG (Tris 10mM pH 7.5, EDTA 1mM, Lithium Acetate 100mM pH 7.5, PEG

50%) and mixed by vortexing. Then the reactions were incubated for 30 min at 30 ^{o}C and during that incubation they were vortexed every 5 min. Afterwards 20 μl of DMSO were added to each reaction followed by a heat shock at 42 ^{o}C for 20 min. Each reaction was then centrifuged at 1000 g for 5 min and the supernatant was removed. The pellet was subsequently washed 2 times with 200 μl of sterile dH_2O. The pellet was then resuspended in 100 μl of 0,8% NaCl and spread on plates containing SD L-W- media (Glucose(+)D 2%, Bacto Agar 2% pH 5.7). The plates were incubated for 2-3 days at 30°C.

2.19 Yeast Two-Hybrid Assay

The transformation of MaV203 yeast cells and the analysis of protein interactions on minimal (SD L- W-) and selective media (SD L-, W-, H- 100mM 50mM 25mM 10mM 5mM 3-Amino-1,2,4-Triazole) was performed according to the manufacturer's instructions by ProQuest Two-Hybrid System from Invitrogen. In order to confirm the protein expression levels of the interactions, a Western Blot was performed for all the yeast colonies. For that about 2.3 mg of the yeast was resuspended in 100 μl of sterile dH2O and then 100μl 0.2M NaOH were added to them. The samples were incubated at RT for 5 min and then they were centrifuged for 1 min at the maximum speed of the centrifuge.

2.20 Statistical Analysis

The statistical analysis of the results acquired in the current research was performed using the Student's t-test and the single factor ANOVA Analysis Tools provided from Excel. Post-Hoc test Holm-Bonferroni was conducted for results required by single factor ANOVA analysis.

Chapter 3: The role of UV-B in regulating photoperiodic flowering

3.1 Introduction

UV-B is an integral part of sunlight as it penetrates the atmosphere causing various photomorphogenic or stress responses in plants, depending on its intensity. Very little is known about the role of UV-B in regulating flowering initiation in plants and in particular in *Arabidopsis* ecotypes. There is a general UV-B dependent delay in flowering time observed in Arabidopsis but also maize and *Vigna radiate* [144] [143] [145] [146] [148], but there is minimal information on the cause or the molecular mechanism behind this response.

For this reason, one of the main aims of this study is to investigate if UV-B radiation has different effects on flowering initiation in selected *Arabidopsis thaliana* ecotypes, mutants and overexpressing lines of light signalling and flowering components. To achieve this, we conducted flowering experiments where plants were grown either solely under a long day photoperiod of white light (50 μmol $m^{-2}s^{-1}$) (WL) or WL that was supplemented with UV-B (0.5 μmol $m^{-2}s^{-1}$). The UV-B intensity was calculated based on measuring the intensity of UV-B on a sunny day in Glasgow (0.5 – 1.2 μmol $m^{-2}s^{-1}$). Separate measurements were conducted in the months of March, April, May, June, July and September and a mean of total UV-B intensity at floral level was calculated based on the aforementioned measurements. Three possible UV-B intensities were initially tested (0.3, 0.5 and 1 μmol $m^{-2}s^{-1}$) and one of them was chosen (0.5 μmol $m^{-2}s^{-1}$) based on its efficiency in mediating UV-B depended photomorphogenesis (Figure 3.1), but not too high to cause damage to the plants beyond the point where they could survive the constant radiation. The treatment of UV-B irradiation was chosen to be continuous, even though in previous studies similar experiments were conducted using shorter intervals of stronger UV-B radiation [91] [144]. The reason behind the choice of a constant UV-B irradiation was that when growing in the natural environment plants receive radiation from the sun in a constant basis during the day. Of course the level of radiation fluctuates during the day and for that reason we conducted measurement in different times during the day and on different days throughout the months of spring, summer and autumn in order to generate a general mean of the average UV-B radiation plants would naturally receive growing in a climate similar to the one of an average north European city like Glasgow.

UV-B is known to induce the expression of the *HY5* transcription factor through the action of the UV-B photoreceptor UVR8 [115]. *HY5* transcript levels were examined using quantitative

RT-PCR analysis (qRT-PCR) in wild type *Ler* and *uvr8* mutant plants. Our results show that there is a clear induction of *HY5* gene expression in wild type plants exposed to WL supplemented with UV-B, but not in *uvr8* mutants (Figure 3.1.A), as expected based on previous observations [115]. Furthermore, we examined the hypocotyl and petiole phenotype of young seedlings exposed to WL +/- UV-B (Figure 3.1.B). Wild type plants exhibited inhibition of hypocotyl and petiole elongation in response to WL supplemented with UV-B, while this was not the case for *uvr8* mutant plants, a result that agrees with published findings [116].

Moreover we assessed if the UV-B intensity used for these experiments was causing non-specific stress responses to the plants, since high doses of UV-B can cause damage to the plant's cellular components and also provoke oxidative stress which results in activating stress pathways [160]. Transcript levels of *COR15* gene, whose encoded protein COLD – REGULATED 15A is a marker for stress-induced responses (including flowering) [161], were monitored using qRT-PCR. Our data indicate that there was no increase in *COR15* transcript levels and we therefore concluded that the plants growing under WL supplemented with UV-B (0.5 μmol $m^{-2}s^{-1}$) did not demonstrate stress-related phenotypes (Figure 3.2).

In order to assess flowering time two variables were taken into consideration: a) the number of rosette leaves each plant had on the day the bolt reached approximately 2 cm in height and b) the number of days after germination when the first bud emerged. From these two parameters the number of rosette leaves was chosen as the most reliable assay in order to investigate changes regarding flowering initiation in response to UV-B. This decision was based mainly in two factors. First of all flowering experiments in published literature use primarily the number of rosette leaves at the time of bolting to avoid any growth rate defects presented in many mutant genotypes [144 149]. Furthermore, it is well-established that UV-B inhibits hypocotyl elongation [154] but also delays plant growth altogether which can result in an increase in the number of days that have passed before bolting, which is not directly related to flowering initiation but to growth rate.

3.2 The role of UV-B in regulating flowering initiation in three *Arabidopsis* ecotypes

The following *Arabidopsis* natural ecotypes were selected for detailed examination of flowering initiation in response to UV-B: *Landsberg erecta* (*Ler*), *Columbia – 0* (*Col-0*) and *Cape Verde islands* (*Cvi*). *Ler* and *Col-0* are the most common Arabidopsis backgrounds used for genetic screens, therefore a plethora of information is available for these ecotypes. *Ler* and

Col-0 were originally collected from Europe and USA [162] whereas *Cvi* originates from a very different ecological landscape, as it originates from Africa [163]. More specifically *Cvi* comes from a complex of volcanic islands, the Cape Verde islands which have a latitude of about 14° and 18°N and have a geography that includes an active volcano as well as plutonic rocks [164]. All the above along with the fact that this specific ecotype comes from islands and is therefore isolated from the mainland leads to the probability that this ecotype could present variations in developmental, physiological and biochemical traits [163], compared to the other *Arabidopsis* ecotypes. Variations like this have been described before for *Arabidopsis* species and are most likely caused by the broadness of their geographic distribution [163].

Our flowering experiments on the afore-mentioned genotypes indicate that UV-B induces early flowering in *Col*-0 and *Ler* ecotypes (Figure 3.3), but delays flowering initiation under long photoperiods in the *Cvi* ecotype (Figure 3.4).

Also, since all the *Arabidopsis* mutant lines examined are in either *Col*-0 or *Ler* backgrounds, it is worth noting that for every replicate of a flowering experiment that was conducted, all the flowering data collected including the wild type data, were collected simultaneously before being split into different figures.

As demonstrated in Figure 3.3, UV-B induces an early flowering phenotype in *Col-0* and *Ler* ecotypes. In order to investigate this response at the molecular level, we examined the transcript levels of selected genes encoding key flowering regulators in *Arabidopsis thaliana*. Floral pathway integrators of two main flowering pathways -the photoperiodic pathway, which is controlled by the day length and the circadian clock, and the autonomous/vernalization- were primarily investigated [18]. Two zeitgeber time points ZT 0.5 and ZT 15 were primarily chosen based on the expression patterns of the chosen genes but ZT 0.5 was eventually selected because as it was observed in our experiments and also on previous experiments of our labs, it was the time point were expression pattern differences were more distinct. FT (FLOWERING LOCUS T) and CO (CONSTANS) are the main pathway integrators of the photoperiod pathway. [18] CO promotes flowering by activating the transcription of *FT* gene and FT initiates the transition from vegetative growth to flowering. [18] Our results indicate that *FT* and *CO* transcript levels are upregulated in *Col-0* and *Ler* plants grown under WL supplemented with UV-B compared to the ones grown solely under WL (Figure 3.5). As demonstrated in the indicated figure, it is evident that there is a greater induction of *FT* over *CO* on both *Col-0* and *Ler* ecotypes. Also the upregulation of both genes in *Ler* ecotype is higher under the WL supplemented with UV-B tested conditions.

SOC1 (SUPPRESSOR OF OVEREXPRESSION OF CO 1) is a secondary transcription factor that promotes flowering regulated primarily by the autonomous and the vernalisation pathways [30]. *SOC1* is regulated antagonistically by CO and *FLC* [30]. CO activates *SOC1* through *FT* in order to promote flowering in *Arabidopsis* species. *FT* and *SOC1* act downstream of *CO*. More specifically *FT* regulates *SOC1* in a positive way in order to promote flowering. Moreover it has been found that in plants that *FT* is not active, there is a down regulation of *SOC1*, thus making *FT* necessary for *SOC1* activation through *CO* [165].

On the other hand *FLC* which encodes a transcription factor that represses flowering and is also a major component of the vernalization and autonomous pathways, represses *SOC1* expression [18 30]. The results acquired from our experiments indicate that *SOC1* transcript levels were reduced in *Col-0* and remain almost unchanged in *Ler* plants grown in WL supplemented with UV-B compared to the ones grown solely in WL (Figure 3.6.A). *FLC* transcript levels were also affected on plants grown under WL that was supplemented with UV-B compared to the ones grown only under WL, but less significantly than the other genes tested. *Ler* showed and *Col-0* showed upregulation of *FLC* transcript levels when grown under WL that was supplemented with UV-B (Figure 3.6.B).

Moreover we investigated the transcript levels of *FT* and *FLC* on *Cvi* ecotype. Our results indicate that there is downregulation of *FT* gene in this ecotype (Figure 3.7.A), which is consistent with the delay in flowering initiation. On the other hand, *FLC* levels remain almost unchanged in Cvi (Figure 3.7.B).

In conclusion, based on the flowering time experiments and gene expression analysis of plants exposed to WL and or WL supplemented with UV-B, it is evident that UV-B induces early flowering in *Col-0* and *Ler* ecotypes by inducing *FT* and *CO* gene expression. On the other hand UV-B delays flowering in the *Cvi* ecotype, probably through the downregulation of its *FT* transcript levels.

3.3 The role of UVR8 in UV-B dependent flowering initiation

UVR8 photoreceptor is the UV-B sensing protein that mediates UV-B mediated responses in plants. UVR8 is the only known UV-B photoreceptor up to date [61] ,therefore, we wanted to investigate further the role of this photoreceptor in photoperiodic-controlled flowering initiation in *Arabidopsis thaliana* by examining *uvr8* mutant and over-expressing lines.

We conducted flowering experiments using the same UV-B intensity as described previously on *Ler* wild-type plants and selected *uvr8* mutant and over-expressing lines. More specifically we used one UVR8 over-expressing line in an *uvr8* mutant background (OXUVR8 = *35S*$_{pro}$*GFP-UVR8*/*uvr8-1*). This line is considered as an over-expressing line since it expresses higher levels of UVR8 than the Ler wild type (Figure 3.11). We also used two different *uvr8* mutant lines: *uvr8-1* and *uvr8-2*. *uvr8-1* mutants have a single recessive mutation leading to a deletion of 15 nucleotides [114]. This deletion results in absence of UVR8 protein production (null allele) [122]. *uvr8-2* mutants results in a premature stop codon on the 400th amino acid [115]. These mutants are still able to produce truncated but non-functional UVR8 protein [126] [127].

In order to access UVR8 protein expression, the aforementioned lines were examined by western blot analysis with a UVR8 specific antibody [127] (Figure 3.11).

Flowering experiments on the afore-mentioned lines indicate that UV-B induces early flowering in *uvr8-1* and *uvr8-2* mutant lines, but delays flowering in the OXUVR8 line (Figure 3.8.A).

As demonstrated in Figure 3.8.A, UV-B induces an early flowering phenotype in *uvr8-1* and *uvr8-2*, but delays flowering in OXUVR8, although this last change was not statistically significant. Additionally it is demonstrated that under WL *uvr8-1* mutants flower earlier than wild type plants, while *uvr8-2* mutants and OXUVR8 *Arabidopsis* flower approximately the same time as the wild type. When *uvr8-1* and *uvr8-2* mutants are exposed to WL + UV-B, they flower earlier compared to wild type plants, while the OXUVR8 line shows a late flowering phenotype. A flowering experiment was also conducted investigating flowering time of three additional *uvr8* mutant lines only this time the lines had a different wild-type background (*Col-0* instead of *Ler*). Under WL that was supplemented with UV-B, two of these lines were observed to flower significantly earlier than their corresponding ones that were grown solely under WL (Figure 3.8.A).

In order to investigate these responses at the molecular level, we examined once more the transcript levels of selected genes encoding key flowering regulators in *Arabidopsis.* Firstly we monitored *FT* and *CO* transcript levels in WT, *uvr8-1* and OXUVR8 lines. Our results indicate that *FT* and *CO* genes are upregulated in *uvr8-1* mutants but downregulated in the OXUVR8 line, in plants grown under WL supplemented with UV-B compared to the ones grown solely under WL (Figure 3.8.B). As demonstrated in the indicated figure, it is evident that there is a greater induction of *FT* in *uvr8-1* compared to the wild type. Also overall there is a greater induction of *FT* over *CO* in both wild type and mutant plants. It is also worth noting that the

downregulation of the *FT* gene is greater than *CO* in OXUVR8 plants grown under supplementary UV-B compared to the ones grown only under WL.

We also examined *SOC1* and *FLC* transcript levels. Our results as depicted in Figure 3.8.B suggest that there is a slight downregulation of *SOC1* transcript levels in wild type and *uvr8* mutant plants when they are grown under UV-B radiation, but it is not one of great significance. The same observation was made for OXUVR8, where a minor upregulation of *SOC1* is observed under UV-B, but it is not significant. On the other hand *FLC* transcript levels are upregulated in wild type, *uvr8-1* and OXUVR8 when grown under UV-B. This upregulation is greater in the *uvr8-1* mutant and the UVR8 over-expressing line compared to the wild type plants, as depicted in the same Figure (3.8.B).

Apart from monitoring the transcript levels of the four aforementioned genes, we also wanted to investigate the transcript abundance of other genes that affect flowering in a way. EARLY–FLOWERING 3 (ELF3) is a protein that in long days it has been found to repress the initiation of flowering [80]. Additionally *ELF3* transcript is regulated by the circadian clock and it has been shown that *ELF3* transcripts accumulate in a circadian way and the fact that this accumulation is dependent of the functional ELF3 in conditions of constant WL, is a further proof that the protein functions within a ZT loop, making *ELF3* a circadian clock input pathway component [80] [166]. Also ELF3 and the circadian clock are important in helping *Arabidopsis thaliana* acclimation under UV-B [167] [140]. Our results are depicted in Figure 3.9 indicate that *ELF3* transcript levels are downregulated in wild type and *uvr8* mutant plants that are grown under UV-B light compared to the ones that were grown solely under WL. This downregulation is greater in the wild type plants and correlates with their early flowering phenotype. Also in the OXUVR8 line there is an upregulation of the *ELF3* transcript which may be connected to their late flowering phenotype, but for this particular line the transcript monitoring should be repeated because of the presence of the larger error bar in the transcript level of plants grown under WL.

Another gene we proceeded on monitoring was *PIF4* which encodes the transcription factor PHYTOCHROME–INTERACTING FACTOR 4. PIF4 protein abundance and function is regulated by light and it is known for promoting hypocotyl elongation [168] [169]. PIF4 has also been shown to promote flowering initiation at high ambient temperature (28 ^{o}C) by up-regulating *FT* in a temperature dependent manner by direct binding of PIF4 to the *FT* promoter [169]. In response to UV-B PIF4 protein abundance is decreased by proteasomal degradation [144]. Our results depicted in Figure 3.10, show a downregulation in *PIF4* mRNA in both wild type

as well as OXUVR8 plants grown under WL that was supplemented with UV-B. On the contrary, *uvr8-1* mutants show an upregulation of the transcript levels of *PIF4*.

3.4 Investigation of the role of UV-B in flowering initiation by examining mutants of key light signalling and photoperiodic components

As a next step we tested if UV-B radiation causes any changes in flowering time in *Arabidopsis thaliana* lines that were either mutant or over-expressed genes that are involved in flowering and/ or UVR8 function.

3.4.1 The effect of UV-B on mutants lacking photoperiodic flowering components

To assess the effect of UV-B in photoperiodic flowering initiation, we examined the phenotype of two mutant lines lacking essential flowering-inducing components, *co* and *zlf*. CO as described previously is one of the main transcription factors and signal integrators regulating photoperiod flowering by directly inducing *FT* expression [18]. *Zlf* [5] is a triple mutant line for ZEITLUPE/LKP2/FKF1 proteins, which are blue light sensing clock components acting positively in flowering initiation under LDs [48]. ZTL is necessary for the maintenance of a normal circadian period [48]. More specifically ZTL and another protein GIGANTEA (GI) interact through a BL enhanced interaction that leads in sustaining ZTL's diurnal oscillations and results in the correct period of a core clock component TIMING OF CAB EXPRESSION 1 (TOC1) that ultimately maintains a circadian oscillations. [48 170 171]. *Zlf* mutants have a very late flowering phenotype under LD [5].

To assess the role of UV-B in the flowering initiation of these mutants, we conducted flowering experiments similar to the ones that have been described above, as well as monitoring the transcript levels of *FT, CO, SOC1* and *FLC* genes. Our results are depicted in Figure 3.12. From the flowering experiment results (Figure 3.12.A), we can conclude that *co* and *zlf* mutants show an early flowering phenotype, similar to the wild type one, when grown under WL that was supplemented with UV-B compared to the ones grown solely under WL.

Transcript expression levels are presented in Figure 3.12.B. *FT* transcript was found to be upregulated in plants grown under UV-B conditions for both *co* and *zlf* mutants, with the induction being greater in *zlf* mutants. Still the difference of the *FT* induction for these mutants under WL + UV-B compared to the ones grown under WL only, is less dramatic than the one of the wild type line *Col-0*. Of course both of these lines present lower levels of *FT* transcript

than the wild type *Col-0* since the transcription of this gene is not promoted [18] [49]. On the other hand, *CO* transcript levels seem to be downregulated in *zlf* mutants grown under WL + UV-B compared to the ones grown only under WL. This response is contradictory to the one that was observed in wild type plants were there was an upregulation of the transcript levels of *CO* under WL + UV-B conditions. *co* mutant has a T-DNA insertion after the start codon [49]. Nevertheless there is presence of *CO* transcript in this mutant, though significantly reduced compared to the wild type *Col-0*. This is a common phenomenon because the effect of the insertion on the expression of the mutated gene depends on factors like the position of the insertion in the gene [172]. It also has been observed that a T-DNA insertion in the coding region of the protein generates a knockout in about 90% of all cases [172].

As depicted also in Figure 3.12.B, *SOC1* transcript abundance is induced in *co* and *zlf* mutants grown under WL + UV-B compared to the ones grown under WL only. This response also contradicts the one observed in wild type plants in which under the same conditions *SOC1* was monitored to be downregulated. Finally we monitored *FLC* transcript levels and an upregulation of *FLC* transcript in all lines under WL + UV-B conditions was observed. This upregulation is greater in the *co* mutant grown under WL + UV-B compared to their corresponding ones that were grown solely under WL.

Additionally *elf3-1* mutant line was investigated, in order to determine potential changes in flowering time and flowering regulator genes transcript abundance. ELF3 as mentioned above is a really important factor in controlling photoperiodic flowering pathway – by targeting CO for degradation and therefore acting as a repressor of flowering [80]. For this reason *elf3-1* mutant line has an early flowering phenotype compared to the wild type plants [80]. Moreover in a published study it was investigated if lower photomorphogenic UV-B radiation has a part in the circadian clock entrainment [140]. UV-B was found to be able to partly entrain the circadian clock via the activation of responsive clock genes [140]. Additionally the role of the clock was investigated using *elf3* mutant plants which are arrhythmic [140]. *elf3* mutant plants demonstrated the same tolerance of UV-B as wild type plants, suggesting that their incapability to control low UV-B photomorphogenic responses (because they are arrhythmic), does not help increasing tolerance under UV-B [140]. Figure 3.13.A shows that *elf3-1* mutants have a late flowering UV-B induced phenotype compared to the wild type early flowering phenotype that was observed under the same conditions.

Transcript expression levels are presented in Figure 3.13.B. *FT* transcript levels of wild type and *elf3-1* mutant plants both under WL and WL supplemented with UV-B conditions are

presented. There is a clear upregulation of *FT* in plants that have been grown under WL + UV-B compared to the ones grown only under WL. This induction is even greater in *elf3-1* mutants compared to the wild type UV-B initiated *FT* upregulation. *CO* is also upregulated in both wild type and *elf3-1* mutant *Arabidopsis thaliana* that were grown under WL + UV-B and there is a similar induction pattern with the difference being greater between non-UV-B and UV-B-treated *elf3-1* plants. The upregulation of *FT* transcript levels though is more dramatic than the one of *CO*, as presented in Figure 3.13B.

SOC1 transcript abundance is induced in *elf3-1* mutants grown under WL+ UV-B compared to the ones that were grown under WL only (Figure 3.13.B). This response is opposing to the one observed in wild type plants for whom a downregulation of this gene is observed. Lastly *FLC* transcript levels were monitored. While in wild type plants that were grown under WL + UV-B an upregulation of *FLC* transcript is observed, in *elf3-1* mutants there is a mere downregulation, as presented in Figure 3.13.B.

3.4.2 The effect of UV-B on the flowering initiation of light signalling mutants and OXPIF4

Mutant lines compromised in the expression of genes that are involved in regulating light-induced development were also investigated with regards to UV-B induced flowering initiation. In particular, the double mutant, *pif4pif5* and the OXPIF4 over-expressing lines were tested for flowering time differences and transcript level abundance in the presence of supplementary UV-B.

PHYTOCHROME–INTERACTING FACTOR 4 (PIF4) and PIF5 are both transcription factors acting positively in mediating hypocotyl elongation of *Arabidopsis thaliana* in darkness, low-intensity light [144]. PIFs are negatively regulated by phytochromes and promote the shade avoidance syndrome [173 174 175]. The aforementioned responses are antagonized under UV-B where PIF4 and PIF5 both undergo degradation [144].

A flowering experiment conducted on *pif4pif5* as described before under WL and WL supplemented with UV-B is shown in Figure 3.14.A. UV-B was observed to induce an early flowering phenotype in *pif4pif5*, which is a comparable response to wild type plants.

Gene expression analysis of *FT*, *CO*, *SOC1* and *FLC* transcript levels for *Col-0* and *pif4pif5* is shown in Figure 3.14.B. UV-B leads to an upregulation of *FT* transcript levels in *pif4pif5* lines in WL + UV-B compared to WL. The same response is also observed in wild type plants, where

the *FT* induction under UV-B is even greater. There is an upregulation of *CO* as well in *pif4pif5* plants grown under WL + UV-B compared to the ones gown under WL only. This reaction is also observed in wild type plants but the induction is greater for *pif4pif5*.

Figure 3.14.B demonstrates the expression analysis of *SOC1* transcripts levels in *pif4pif5* mutant line showing no significant difference between plants grown under WL and WL supplemented with UV-B. This response differentiates from wild type plants in which there is a downregulation of this gene under WL + UV-B.

On the contrary, *FLC* transcript levels are induced by UV-B both in the wild type plants and the *pif4pif5* ones. This upregulation is greater in *pif4pif5*, as presented in Figure 3.14.B.

In addition to *pif4pif5*, we were interested in investigating the UV-B induced flowering phenotypes of the PIF4 over-expressing line (OXPIF4-HA) [100], which has an early flowering phenotype compared to wild type [169]. This response is consistent with our data shown in Figure 3.15.A when comparing the flowering time of OXPIF4 plants grown under WL compared to the wild type ones grown under WL, but also when comparing OXPIF4 plants that were grown under WL + UV-B irradiation compared to the wild type plants grown also under UV-B. On the contrary, OX-PIF4 grown under WL and WL supplemented with UV-B shows a reversion of the early flowering phenotype induced by UV-B that is observed in the wild type *Col-0*. In other words UV-B seems to delay flowering initiation in OXPIF4. These responses indicate that OX-PIF4 line induces early flowering under WL + UV-B, however, flowering is delayed under WL + UV-B compared to solely WL growth conditions (Fig. 3.15.A).

Figure 3.15.B presents the gene expression analysis of *FT*, *CO*, *SOC1* and *FLC* mRNA levels in OXPIF4 +/- UV-B. It is observed that UV-B causes a downregulation of *FT* gene expression in the *PIF4* overexpression line, a response that contradicts the one that UV-B causes in wild type plants where *FT* is upregulated. This downregulation of *FT* in OXPIF4 correlates with the delayed flowering phenotype shown in Fig 3.15.A. Moreover, there is also a downregulation of *CO* in OXPIF4 under UV-B compared to the ones grown only under WL. This downregulation is even greater than the one observed in the *FT* gene. This response also contradicts the one of the wild type, in which *CO* is induced under WL+UV-B.

UV-B leads to an upregulation of *SOC1* under WL+ UV-B and this response is contradictory to the one of the wild type plants where there is a downregulation of the SOC1 (Fig. 3.15.B). However, UV-B initiates an upregulation of *FLC* in both the wild type and OXPIF4.

3.4.3 The role of UV-B in regulating the flowering initiation of UV-B signalling mutants

Lastly the effect of UV-B on flowering initiation was assessed in mutants of key UV-B signalling components, *cop1-4* and *rup1rup2* mutants. COP1, RUP1 and RUP2 are all important for the proper function and regulation of UVR8.

CONSTITUTIVE PHOTOMORPHOGENIC 1 is a positive regulator of UV-B signalling [136]. Specifically COP1 is required for UV-B induced gene expression and physiological responses [176] . UVR8 as currently known is a homo-dimer when in an inactive state but monomerizes when it senses UV-B radiation [125]. Upon UV-B irradiation, COP1 interacts with UVR8 resulting in the activation of *HY5* gene expression, which encodes the ELONGATED HYPOCOTYL 5 protein which is subsequently stabilized and ultimately leads to the activation of a couple UV-B responsive genes [115] [124]. However, under WL COP1 is a well-established negative regulator of light signalling and photomorphogenesis because as it targets CO and HY5 proteins for degradation [18] [177]. REPRESSOR OF UV-B PHOTOMORPHOGENESIS 1 (RUP1) and RUP2 proteins act as a negative feedback for regulating the action of UVR8 [154].

Figure 3.16.A depicts the differences in flowering time between lines grown under WL and WL supplemented with UV-B in wild type *Col-0*, *cop1-4* and *rup1rup2* mutants. It is evident that UV-B induces early flowering in all of the afore-mentioned lines.

Figure 3.16.B presents the transcript levels of the flowering inducers *FT, CO, SOC1* and the repressor *FLC* in wild type *Col-0*, *cop1-4* and *rup1rup2* mutants grown under either WL or WL that was supplemented with UV-B. As depicted in the first chart in all three lines there is an upregulation of *FT* transcript levels under WL supplemented with UV-B. However, *rup1rup2* shows a dramatic increase in the transcript levels of *FT* in both WL and WL with UV-B, thus the gene expression analysis for this line should be repeated in the future. The second chart presents the transcript levels of *CO*. While there is an upregulation of *CO* in wild type plants under WL + UV-B, *cop1-4* mutants show unchanged *CO* transcript levels and *rup1rup2* mutants show a downregulation of *CO* in response to UV-B (Fig. 3.16.B).

In wild type plants and *cop1-4* mutants there is a downregulation of *SOC1* caused by UV-B, which is greater in the wild type. This response is contradictory to the one of *rup1rup2* mutants in which there is an induction of *CO* in response to UV-B. Lastly as depicted in the fourth chart (Fig. 3.16.B) UV-B induces the transcript abundance of *FLC* in wild type and *rup1rup2*

Arabidopsis, while *cop1-4* exhibits no change in *FLC* transcript levels under both WL and WL supplemented with UV-B.

3.5 Discussion

The current chapter is focused on the investigation of the potential changes that UV-B radiation can cause in the initiation of flowering in *Arabidopsis thaliana* and also in the gene expression levels of well-established flowering regulators. Multiple flowering experiments were conducted in order to determine differences in the flowering time of *Arabidopsis* genotypes grown under WL or WL supplemented with UV-B. Furthermore, gene expression analysis enabled monitoring of the transcript abundance of important flowering regulators such as *FT, CO, SOC1* and *FLC*.

Overall, the results obtained from this study suggest that UV-B appears to induce early flowering initiation in response to long photoperiods mainly through the induction of *FT* (Figures 3.3.A and 3.5.A). Moreover UVR8 appears to act as a negative regulator of flowering (Figure 3.8). However, well-characterised early flowering mutants demonstrated a UV-B induced delay in flowering (Figures 3.13.A and 3.15.A). Also the transcript abundance of the flowering repressor *FLC* was found to be increased under UV-B radiation in two of the three ecotypes investigated (Figure 3.6.B) and in almost all of the mutant categories monitored (Figures 3.12.B, 3.14.B, 3.15.B and 3.16.B). However the UV-B-induced *FLC* levels did not correlate with the flowering time under UV-B. This observation suggests that UV-B may affect the autonomous and vernalisation pathways in a different manner by upregulating *FLC* expression.

3.5.1 UV-B regulates photoperiodic flowering in an ecotype-specific manner

Currently there is very limited information on how UV-B affects flowering in plants. Published studies describing flowering experiments that have been conducted under moderate UV-B irradiation conditions reported a UV-B induced delay in flowering initiation in both *Columbia-0* [143] and *Landsberg erecta* [144] ecotypes. Our study examines the effect of UV-B on both *Col-0*, *Ler* along with an additional ecotype, *Cvi*. The results from our flowering experiments indicate that UV-B induces an early flowering phenotype in *Col-0* and *Ler* ecotypes (Figure 3.3) and a delay in *Cvi* flowering initiation (Figure 3.4). Moreover, *Col-0* and *Ler* exhibit a UV-B induced upregulation in the transcript levels of *FT* and *CO* (Figure 3.5), both of which encode proteins that are positive flowering regulators of the photoperiod pathway [18]. Such

increase in *FT* and *CO* levels support the UV-B induced early flowering phenotype of *Col-0* and *Ler* observed in the current study. A UV-B induced upregulation of the transcript abundance of *FLC* (Figure 3.6.B) was also observed in *Col-0* and *Ler*, which encodes a protein that is a known repressor of flowering[18]. *SOC1* transcript levels, which also encodes a protein that induces flowering [30], were also investigated and found downregulated in *Col-0* and *Ler* (Figure 3.6A), excluding this factor for having a part in the early phenotype of this lines. Lastly *FT* transcript levels in *Cvi* ecotype were downregulated by UV-B correlating with the observed late flowering phenotype, while *FLC* levels remained unchanged (Figure 3.7). A possible explanation for the differences between *Cvi* and the other ecotypes that were tested may come from the different environment that these ecotypes originate from. As it was mentioned before *Cvi* comes from a group of islands in West Africa with a particular geography (volcanic and plutonic rocks [164]) which makes its growth environment particularly different. Moreover, in Cape Verde islands the photoperiod length is 12h of light and 12 h of darkness all year long (timeanddate website), a factor that is important for the potential differences of this ecotype in flowering initiation. Additionally, this ecotype is isolated from the mainland as it is an islandic population and for this reason it is quite possible that *Cvi* ecotype developed a variation in certain characteristics. This phenomenon is quite common amongst *Arabidopsis thaliana* species [163]. More specifically it has been observed that *Arabidopsis* wide distribution leads to phenotypic and genetic variation that is necessary in order to adapt in different environmental conditions [163]. In general *Cvi* has been observed to need more days in order to flower than *Ler* [163], an observation that was made in this study as well (Figures 3.3.B and 3.4). In another study where flowering time was investigated in different *Arabidopsis* ecotypes it was observed that *Cvi* flowers later than both *Col-0* and *Ler* ecotypes [178]. The difference in flowering time was greater between *Cvi* and *Ler*, since the latest was observed to be the earliest flowering out of all three, and less significant between *Cvi* and *Col-0* [178]. The above observation derived from the number of total leaves counted on the day of bolting for each ecotype [178]. Additionally it was demonstrated that *Cvi* has significantly higher transcript levels of *FLC* than both *Cvi* and *Ler* [178], an observation that was confirmed in the current study as well (Figure S3.1). Lastly besides the aforementioned study were *Ler* was found to be earliest flowering than *Col-0* based on the number of leaves counted [178] it has also been observed that *Col-0* has been observed to need more days in order to flower than *Ler* [179]. These results also correlate with the current study (Figure 3.3).

3.5.2 UVR8 is a negative regulator of flowering

UVR8 is a unique genetically-encoded UV-B photoreceptor [122]. This study investigated if UVR8 plays a role in the regulation of flowering initiation in response to UV-B, using *Arabidopsis* lines that either lack or over-express UVR8 protein. In our case results demonstrated that UV-B induces an early flowering phenotype in *uvr8* mutants grown under WL that was supplemented with UV-B compared to growing only under WL independently of their wild type background ecotype (Figure 3.8.A). Furthermore, OXUVR8 demonstrated a UV-B induced delay in flowering. However, this response requires further investigation to assess its statistical significance (Figure 3.8.A). The above data combined lead to the conclusion that the UVR8 photoreceptor acts as a negative regulator of flowering initiation. Some of these observations correlate with previous data from our lab. Previous flowering experiments conducted in our lab under WL (50 μmol m^{-2} s^{-1}) and WL (50 μmol m^{-2} s^{-1}) that was supplemented with UV-B (1 μmol m^{-2} s^{-1}) in LD photoperiod for *Ler* and *uvr8-1* *Arabidopsis* lines showed that *uvr8-1* mutants demonstrated an early flowering phenotype compared to the wild type *Ler* both under WL and WL that was supplemented with UV-B conditions (Kaiserli lab, unpublished data, 2016). This observation correlates with the current study.

Overall the gene expression analysis results indicate that the *FT* transcript abundance is increased in *uvr8* mutants grown under WL + UV-B compared to the ones grown under WL but also compared to the wild type grown under WL + UV-B (Figure 3.8.B). Accordingly, over-expression of UVR8 resulted in a lower accumulation of *FT* transcript under WL+UV-B compared to WL, which is the opposite phenotype to *uvr8-1*. Furthermore, OXUVR8 showed a general lower expression of *FT* under both WL and WL + UV-B compared to wild type (Figure 3.8.B). These results consistently support the flowering initiation experiments clearly supporting the hypothesis that UVR8 acts as a repressor of flowering.

In addition to *FT*, *CO* gene expression is also increased in *uvr8-1* grown under WL +/- UV-B, however, this induction is smaller compared to the wild type (Figure 3.8.B). These observations correlate with previous data from experiments conducted in our lab. In previous transcript expression analysis experiments monitoring *FT* and *CO* transcript levels of *Ler* and *uvr8-1* lines that were grown under WL (50 μmol m^{-2} s^{-1}) in LD photoperiod, it was observed that there is an upregulation of *FT* and *CO* transcript levels in *uvr8-1* compared to *Ler* (Kaiserli lab, unpublished data, 2015). In an additional experiment monitoring *FT* transcript levels of *Ler* and *uvr8-1* grown under WL (50 μmol m^{-2} s^{-1}) + UV-B (1 μmol m^{-2} s^{-1}) in LD photoperiod

conditions, it was observed that *FT* is upregulated in *uvr8-1* compared to wild type (Kaiserli lab, unpublished data, 2016). These data further support the hypothesis that UVR8 acts as a repressor of flowering.

SOC1 transcript levels remained almost unaffected in all lines in the presence or absence of UV-B indicating that neither UVR8 nor UV-B regulate *SOC1*, which is primarily involved in flowering induction through the autonomous and hormone pathways [18]. *FLC* transcript abundance was induced in a similar manner in WT, *uvr8* and OXUVR8 lines exposed to UV-B suggesting that UV-B may control the autonomous/vernalisation pathway independent of UVR8 (Figure 3.8.B).

In a recently published study the role of UV-B and UVR8 in regulating flowering time was also tested [143]. The lab conducted flowering experiments under WL and WL that was supplemented with UV-B as well as expression analysis experiments testing various factors involved in flowering in *Col-0* and *uvr8* mutant *Arabidopsis* lines [143]. Dotto et al. observed a delay in flowering time of wild type plants under WL + UV-B in LD photoperiod growth conditions (counting both the number of rosette leaves at bolting and the number of days before flowering) [143]. Moreover, the afore-mentioned study showed a downregulation of *FT* transcript abundance in wild type *Arabidopsis* under WL + UV-B compared to WL growth conditions [143]. These results are contradictory to our current findings (Figures 3.3 and 3.5) but it is important to consider the very different UV-B radiation conditions used in each study. In our study we used WL (50 μmol m^{-2} s^{-1}) supplemented with continuous UV-B radiation (0.5 μmol m^{-2} s^{-1}) which started from the day of germination in LD photoperiods. Whereas Dotto et al., used WL supplemented with 9.14 μmol m^{-2} s^{-1} of UV-B only for one hour per day at ZT 4 of LD photoperiods, starting 9 days after germination [143]. Interestingly, despite these different conditions they also found a downregulation of *SOC1* transcript levels in *Col-0* plants grown under WL + UV-B compared to the ones grown only under WL [143], an observation that correlates with our study (Figure 3.6.A). Finally, Dotto et al., observed an upregulation of *FT* and *FLC* transcript levels and no significant change in *SOC1* transcript levels of *uvr8* mutants grown under WL + UV-B compared to the ones grown only under WL [143], observations which also correlate with our findings (Figure 3.8).

Transcript levels of *ELF3* which encodes flowering repressor ELF3 [80], were monitored as well in *Ler*, *uvr8* and OXUVR8 lines. A decrease in *ELF3* transcript abundance was observed in wild type plants exposed to UV-B, which correlates with the UV-B induced early flowering phenotype (Figures 3.9 and 3.3.A). Interestingly, the UV-B regulated repression of *ELF3* gene

expression was absent in *uvr8* mutants (Figure 3.9). However, *uvr8* showed an overall reduction of *ELF3* in WL that resembled *ELF3* levels of wild type in UV-B. Overexpression of UVR8 demonstrated an even greater reduction in *ELF3* transcripts under WL +/- UV-B compared to the wild type and the *uvr8* mutant line, even though there seems to be partial UV-B specific induction of *ELF3* retained (Figure 3.9). This data suggests that the UV-B specific repression of *ELF3* is at least partially regulated by UVR8.

Finally, OXUVR8 showed a general decrease in *PIF4* transcript levels in WL +/- UV-B compared to WT and *uvr8*. The effect of UV-B on *PIF4* is not very significant (Figure 3.10). It has been reported that PIF4 protein undergoes degradation in response to UV-B [144], so perhaps PIF4 is regulated primarily at the protein level, however, there is a possibility that a reduction of PIF4 protein levels could also lead to lower *PIF4* transcripts due to a PIF4-dependent feedback loop.

3.5.3 UV-B induces early flowering in *co*, *zlf*, *pif4pif5*, *cop1-4* and *rup1rup2* mutants

Apart from *UVR8* mutants and overexpressors, we also tested flowering time and performed gene expression analysis in various *Arabidopsis* lines that carry mutations in gene loci encoding protein components of light signalling and/or photoperiodic flowering. Our results demonstrate that UV-B induces an early flowering phenotype in most mutant lines examined, *co*, *zlf*, *pif4pif5*, *cop1-4* and *rup1rup2* mutants (Figures 3.12.A, 3.14.A and 3.16.4) suggesting that these components do not play an essential role in UV-B induced early flowering. Furthermore, *co* and *zlf* retain an induction of *FT* and *SOC1* transcript abundance under UV-B consistent with their flowering phenotype (Figure 3.12.B), whereas *CO* transcript abundance was reduced under UV-B in *zlf* mutant line. (Figure 3.12.B). In *co* mutant line we can still observe low levels of *CO* transcript, since perhaps the point of the mutation still allows some *CO* transcript to be produced. This data indicates that perhaps UV-B regulates flowering independent of CO or through an additional pathway (autonomous pathway may be considered).

The light signalling mutants *pif4pif5* showed elevated transcript levels of *FT* and *CO* under WL+UV-B conditions similar to WT, supporting their early flowering phenotype. However, *SOC1* transcript abundance remained unchanged in *pif4pif5* under UV-B (Figure 3.14.B). *FLC* transcript abundance was upregulated under WL + UV-B conditions (Figure 3.14.B), a response similar to most of the mutants tested in our study, suggesting that this might be a more general reaction under UV-B conditions. *pif4pif5* double mutant has been shown to have

delayed flowering under a 12 hour light and 12 hour dark photoperiod [180], but in our case of the LD photoperiods *pif4pif5* mutant flowered later than WT, though this difference was not found to be statistically significant. PIF4 is involved in flowering induction in a temperature dependent manner by binding to the *FT* promoter [100 74]. In our experiments PIF4 does not seem to play an essential role in UV-B induced early flowering.

Lastly, examination of mutants in UV-B light signalling components, *cop1-4* and *rup1rup2*, showed an upregulation of *FT* transcripts under WL + UV-B compared to WL, correlating with their early flowering phenotype (Figure 3.16.B). *CO* and *FLC* abundance under UV-B remain unchanged in *cop1-4* mutants, whereas *SOC1* was downregulated (Figure 3.16.B). The above observations exclude *CO* and *FLC* factors from having a role in the early induced flowering under UV-B conditions *in cop1-4*. Published studies have shown that *cop1* mutants exhibit late flowering under WL in SD and LD [75], although there is a study that argues their late flowering phenotype to be SD specific [155]. In our experiments *cop1-4* mutant line was observed to flower later than WT under WL but the difference was not calculated as statistically significant. It is worth noting though that the number of plants counted in population that is being statistically tested is very important. A very large or an equally smaller number can lead to variable results. *cop1-4* mutants were observed to be harder to germinate and grow due to their constitutively photomorphogenic phenotype, thus making it more difficult to examine a larger number of plants. Therefore, more experimental repeats need to be performed to make any conclusions. *rup1rup2* mutants present a downregulation of *CO* but an upregulation of *SOC1* and *FLC* under UV-B radiation (Figure 3.16.B). The role of RUP1 and RUP2 proteins in flowering has also been investigated previously. One of the reports suggests that RUP1 and RUP2 play a role in flowering as it was observed that the overexpression of *RUP1* (indicated as *EFO1-EARLY FLOWERING BY OVEREXPRESSION 1* in the study) and *RUP2* (indicated as *EF02*), promote floral transition [137]. When the corresponding mutants were tested it was concluded that although RUP2 seems to be indeed a floral repressor, RUP1 is not actually involved in flowering time [137]. In the same study it was observed that *rup1rup2* mutants flower approximately the same time as wild type a result that correlates with our study [137]. Also it was shown that in the *rup1rup2* mutants there is a diurnal *FT* upregulation under WL compared to the wild type in SD, which was associated specifically with the *rup2* mutant [137]. Our results suggest that there is an upregulation of this *FT* in *rup1rup2* mutants compared to WL also in LD, suggesting that RUP2 has a role in photoperiodic flowering. In another more recent publication it was suggested that there is a UVR8-mediated role of RUP2 as a repressor of flowering through regulation of CO activity, a function that helps activating photoperiodic

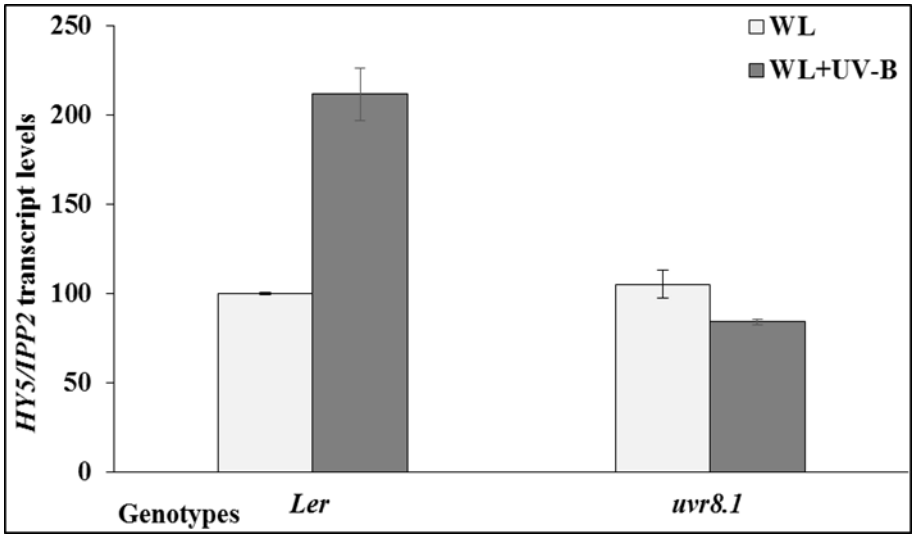
flowering [142]. More specifically it was observed that in SD photoperiods there is an early flowering phenotype of *rup2* and *rup1rup2* mutants, which is specific under WL + UV-B conditions [142]. In this study no involvement of RUP2 was observed under LD WL conditions [142]. Under LD conditions no significant phenotype was observed [142], a result that contradicts the findings of our current study. In the aforementioned study flowering time of OXUVR8 plants was tested in SD under WL and WL + UV-B conditions, but no significant change in flowering time was observed, concluding that under those conditions over-activating the UVR8 pathway does not affect flowering, possibly due to the fact that RUP2 acts to "balance" the repression of flowering [142]. Lastly Arongaus et al., showed a direct interaction between RUP2 and CO, as well as the fact that the early flowering phenotype of *rup2* mutant is CO and FT dependent [142]. This was concluded since the early phenotype or *rup2* mutant plants was supressed in both *rup2 co* and *rup2 ft* mutants [142]. Moreover *FT* transcript levels were once again found to be increased in *rup2* and *rup1rup2* mutant *Arabidopsis* lines compared to wild type plants, this time under SD + UV-B conditions [142]. On the other hand *CO* transcript levels for these mutants were not altered compared to the ones of the wild type [142]. The above data altogether suggested that RUP2 can repress CO binding to the *FT* promoter, thus repressing CO related *FT* expression [142]. In our experiments *rup1rup2* mutants also show increased *FT* transcript levels compared to the wild type under LD WL + UV-B conditions, supporting the early flowering phenotype that was observed under LD conditions. In our case there was also a major downregulation of *CO* transcript in *rup1rup2* mutants under WL + UV-B conditions (Figure 3.16.B). In order to explain these differences it is important to take under consideration the difference between *Arabidopsis* growth under LD and SD conditions. The *CO* expression of WT plants grown under a SD photoperiod peaks at night, while *FT* expression remains low, with the exception of a small peak at dusk, and there is no overlap between *CO* and *FT* expression [72]. Arongaus et al. showed that *rup1rup2* mutants have a peak of *FT* expression in the middle of the night, creating an overlap of the expression of the two genes and supporting their hypothesis [142]. In wild type plants there is a peak and an overlap of *CO* and *FT* expression both at dawn and dusk with the peak at dusk being greater in LD [72]. As mentioned before we present the differences of gene expression at dawn, since it was the time of the day when most differences were observed. In our case the downregulation of *CO* at this timepoint does not support a hypothesis that RUP1 or RUP2 can repress CO binding to the *FT* promoter, explaining the upregulation of *FT* and the early flowering phenotype. It is possible that the upregulation of *FT* is under the regulation of another factor that belongs to the photoperiod or a different flowering-determining pathway (vernalization, autonomous).

3.5.4 UV-B induces late flowering in *elf3-1* and OXPIF4

The early flowering *Arabidospsis* lines *elf3-1* and OXPIF4 demonstrated a late flowering UV-B induced phenotype (Figures 3.13.A and 3.15.A). Gene expression analysis on *elf3-1* indicated an upregulation of *FT*, *CO* and *SOC1* transcript abundance and also a downregulation of *FLC* (Figure 3.13.B). This results altogether contradict its late flowering phenotype, suggesting that possibly there is another factor that contributes to their late flowering phenotype. Moreover ELF3 acts in a zeitnehmer feedback loop and is also known to be involved in its own circadian regulation [83] [181] [182]. For this reason it is possible that the deregulation of the circadian clock caused by the absence of ELF3 inhibits the acclimation process of the plant under UV-B conditions and leads to a later flowering phenotype. To investigate this further, the flowering and acclimation responses of additional key clock components would need to be monitored in WL +/- UV-B.

On the other hand, OXPIF4 showed a reduction in *FT* and *CO* transcript abundance under UV-B which correlates with its late flowering phenotype (Figure 3.15.B), whereas *SOC1* and *FLC* expression was upregulated (Figure 3.15.B). The upregulation of *FLC* under UV-B is a response that has been observed in the majority of mutants tested in this study as well as in the wild type *Col-0*. That observation might suggest that this is a response which is induced by UV-B. A recently published study showed that *FLC* transcript levels are significantly upregulated under WL + UV-B [143] in *Col-0* WT 12-day old planta, an observation that correlates with the findings of our study. Additionally OXPIF4 is known to flower early under WL conditions that demonstrated UV-B induced delayed flowering, indicating that UV-B delays flowering in early flowering *Arabidopsis* lines. It is also possible that ELF3 and its downstream signalling partner PIF4 [183] [168] [81] [180] [181] [182] [184] play a role in UV-B induced early flowering which should be investigated further. Moreover it would be interesting to test other over-expressing early flowering lines (for example the over-expression of CO line –SUC:CO-HA [67]) under UV-B conditions in order to determine if UV-B would cause a reversion of the early flowering UV-B induced phenotype.

A.



B.

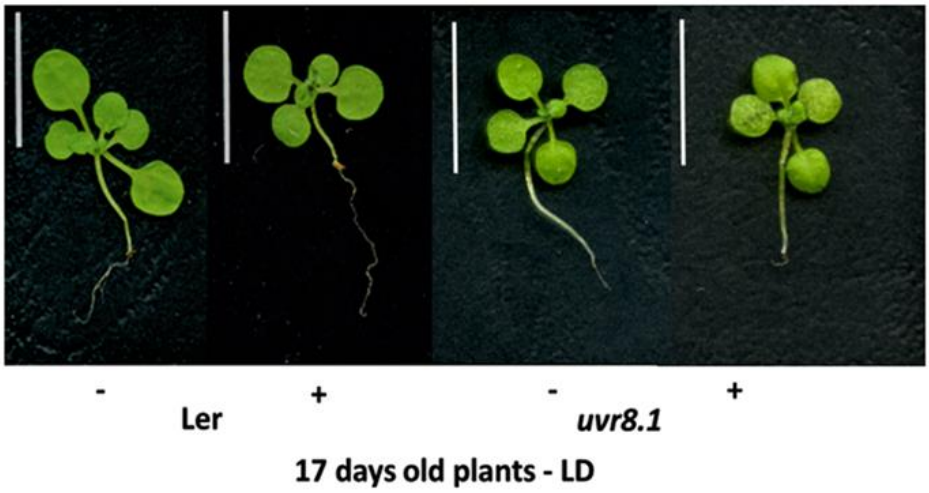


Figure 3. 1 The UV-B regime used for the flowering experiments induces UV-B - dependent photomorphogenesis.

A. qRT-PCR analysis of *HY5* transcript levels in wild-type (*Ler*) and *uvr8-1* mutant plants, normalized with the housekeeping gene *IPP2*. Plants were harvested half an hour after the light onset (ZT 0.5) 12 days after germination. Plants were grown under a LD photoperiod of WL (50 μmol $m^{-2}s^{-1}$) or WL supplemented with UV-B (0.5 μmol $m^{-2}s^{-1}$). Plants grown under WL were used as reference (100%). Data are represented as mean ± SEM. Data are representative of two biological replicates.

B. UV-B-induced hypocotyl inhibition of growth. Wild-type and *uvr8-1* mutant plants were grown for 17 days under LDs of WL (50 μmol $m^{-2}s^{-1}$) or WL supplemented with UV-B (0.5 μmol $m^{-2}s^{-1}$). Data are representative of 2 biological replicates (n = 8 seedlings per experiment).

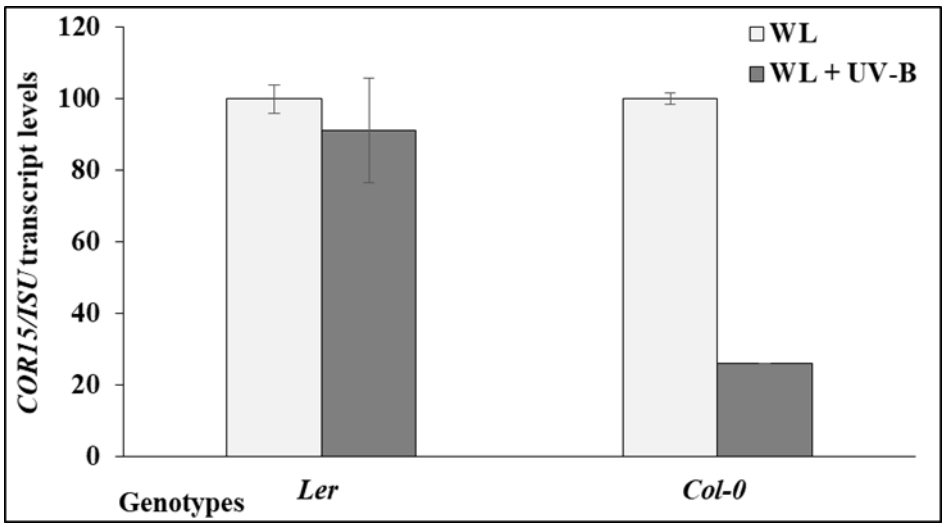
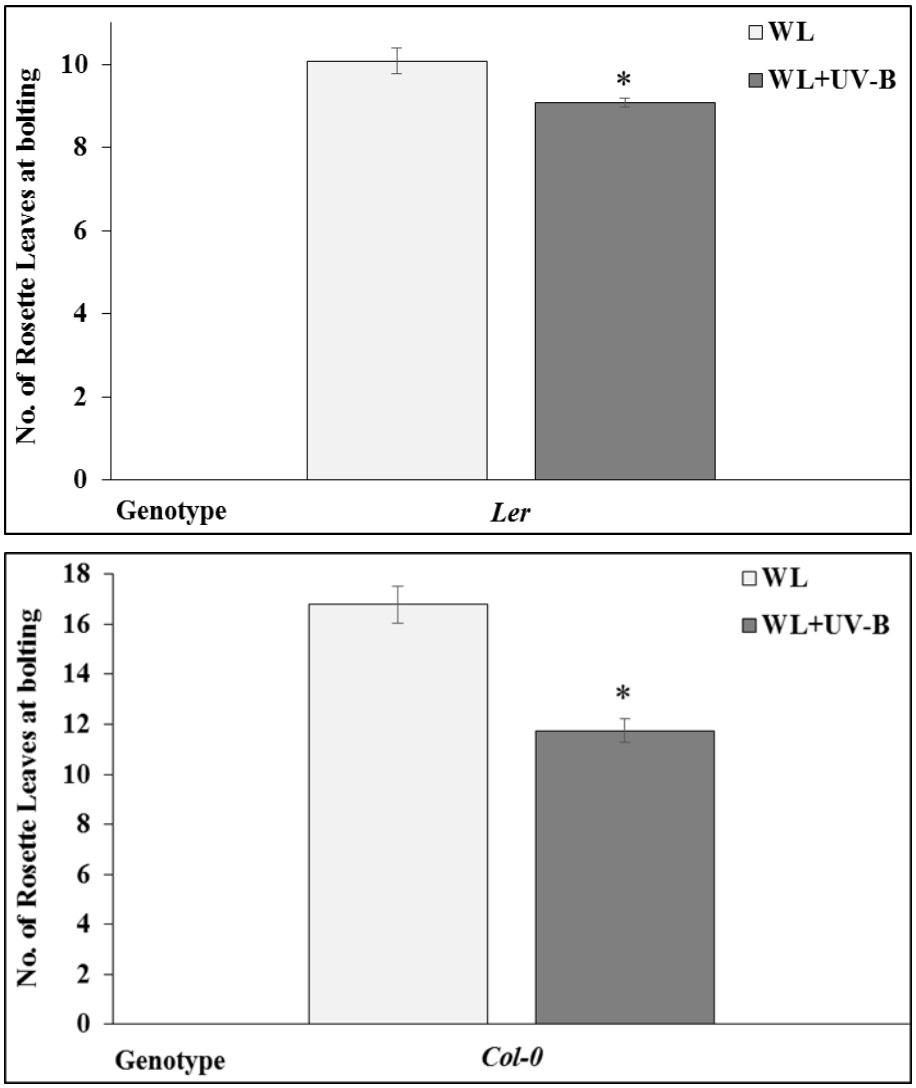
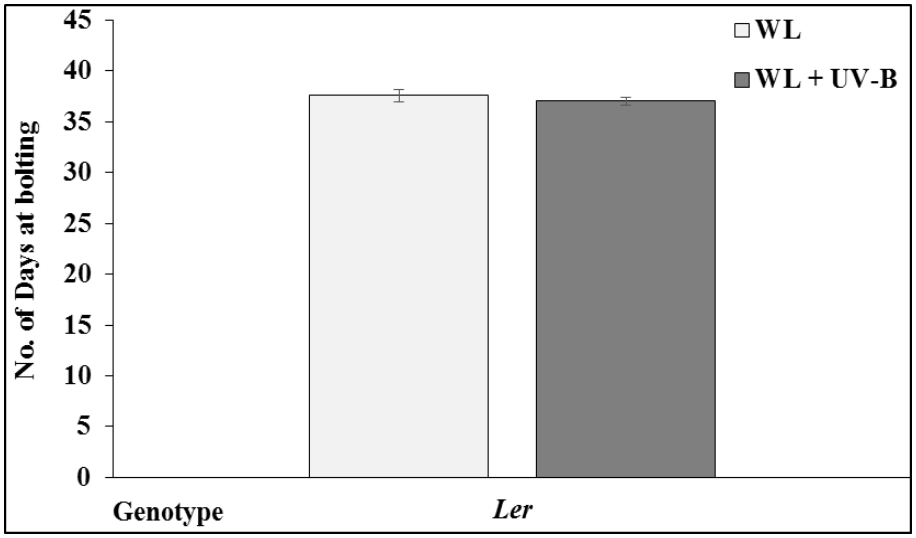


Figure 3. 2 The UV-B regime used for the flowering experiments does not induce stress. qRT-PCR analysis of *COR15* transcript levels normalized with the housekeeping gene *ISU*. Plants were harvested half an hour after the light onset (ZT 0.5) 12 days after germination. Plants were grown under a long day (LD: 16h light/8h dark) photoperiod of WL (50 μmol $m^{-2}s^{-1}$) or WL supplemented with UV-B (0.5 μmol $m^{-2}s^{-1}$). Plants grown under WL were used as reference (100%). Data are represented as mean ± SEM. Data are representative of two biological replicates.

A.


No. of Rosette Leaves at bolting
10
8
6
4
2
0
WL
WL+UV-B
*
Genotype
Ler
No. of Rosette Leaves at bolting
18
16
14
12
10
8
6
4
2
0
WL
WL+UV-B
*
Genotype
Col-0

B.


No. of Days at bolting
45
40
35
30
25
20
15
10
5
0
WL
WL + UV-B
Genotype
Ler

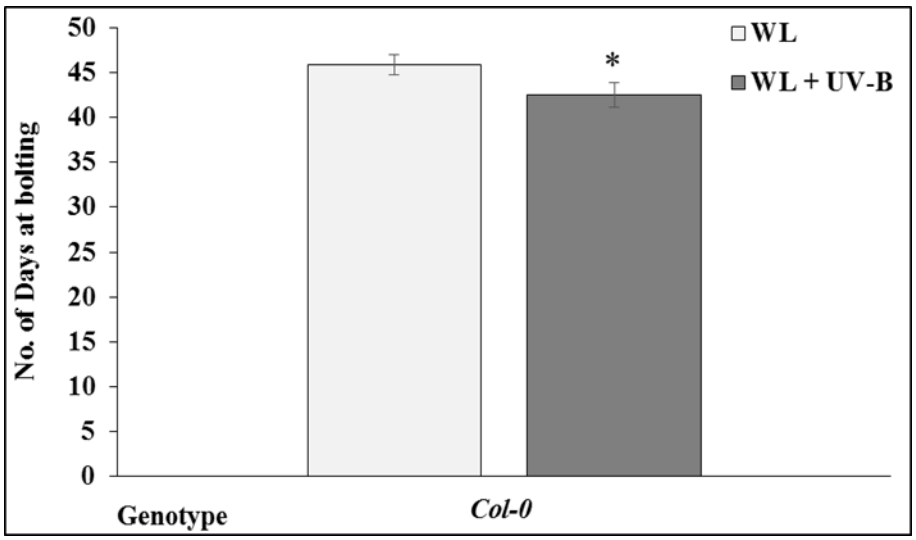


Figure 3. 3 UV-B induces early flowering in *Col-0* and *Ler* ecotypes.

A. Flowering times (as measured by rosette leaf number) of *Col-0* and *Ler* ecotypes grown under a LD photoperiod in WL (50 μmol $m^{-2}s^{-1}$) or WL supplemented with UV-B (0.5 μmol $m^{-2}s^{-1}$). Data are represented as mean ± SEM (n ≥ 15 plants recorded) and an asterisk (*) indicates statistically significant differences ($P < 0.05$) between means.

B. Flowering times (as measured by the number of days at bolting) of *Col-0* and *Ler* ecotypes grown under a LD photoperiod in WL (50 μmol $m^{-2}s^{-1}$) or WL supplemented with UV-B (0.5 μmol $m^{-2}s^{-1}$). Data are represented as mean ± SEM (n ≥ 15 plants recorded). * indicates statistically significant differences ($P < 0.05$) between means. Data are representative of two biological replicates.

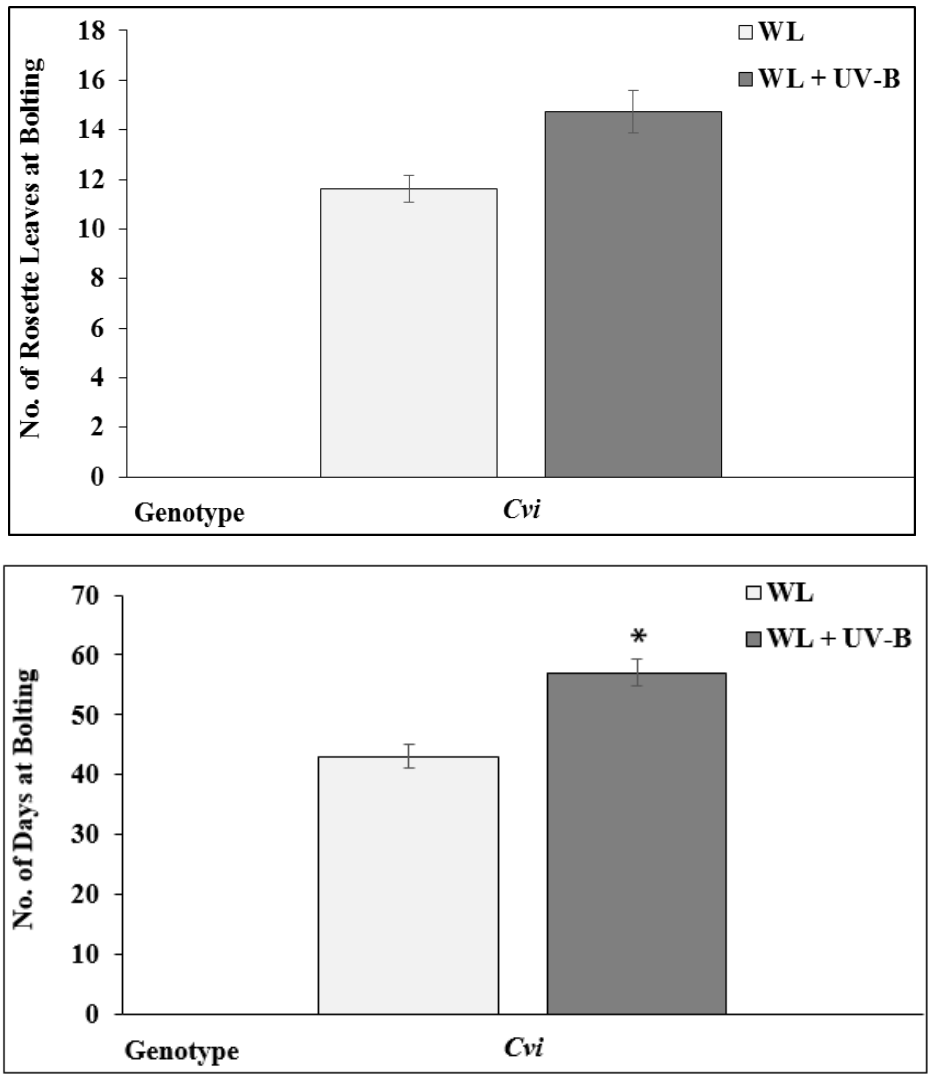
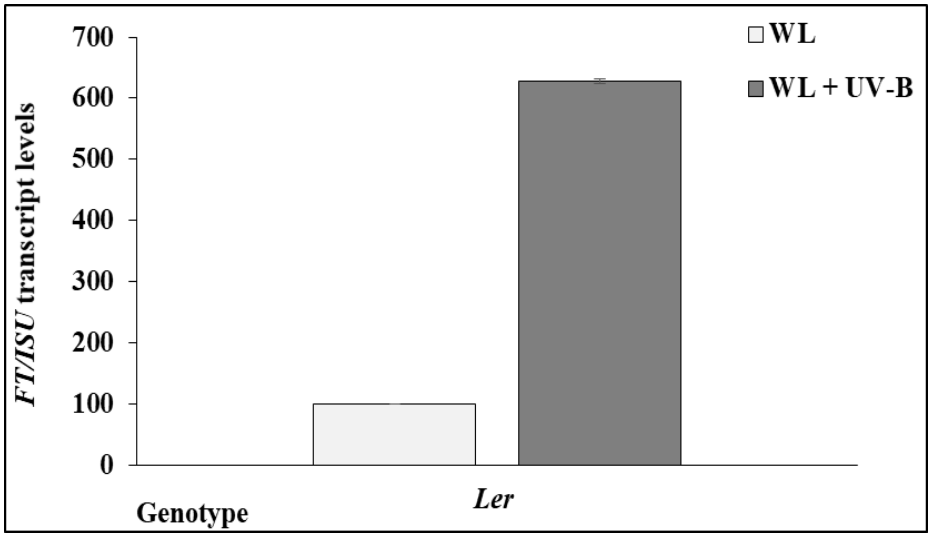
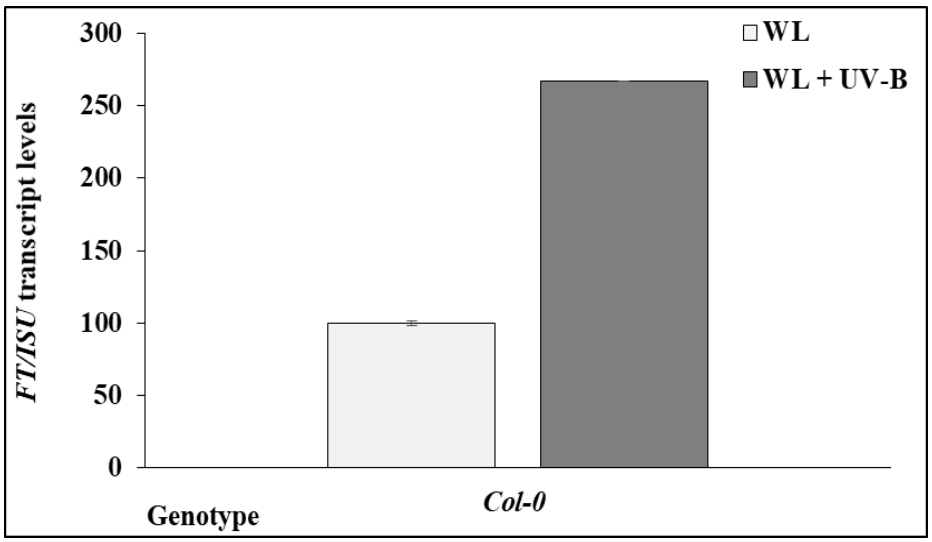


Figure 3. 4 UV-B delays flowering initiation in *Cvi* ecotype.

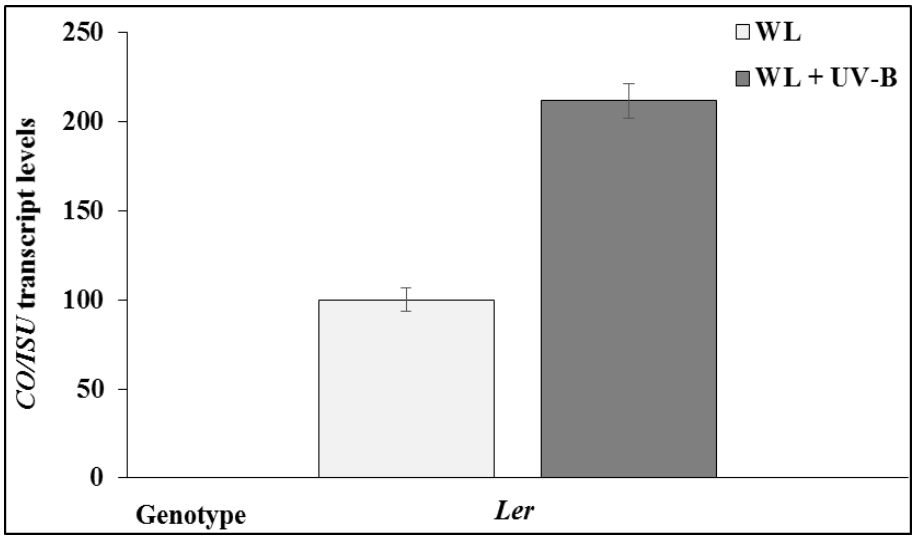
Flowering times (as measured by rosette leaf number and number of days at bolting) of *Cvi* plants grown under a LD photoperiod in WL (50 μmol $m^{-2}s^{-1}$) or WL supplemented with UV-B (0.5 μmol $m^{-2}s^{-1}$). Data are represented as mean ± SEM ($n \geq 15$). * indicates statistically significant differences ($P < 0.05$) between means. Data are representative of two biological replicates.

A.


700
600
500
400
300
200
100
0
FT/ISU transcript levels
WL
WL + UV-B
Genotype
Ler


300
250
200
150
100
50
0
FT/ISU transcript levels
WL
WL + UV-B
Genotype
Col-0

B.


250
200
150
100
50
0
CO/ISU transcript levels
WL
WL + UV-B
Genotype
Ler

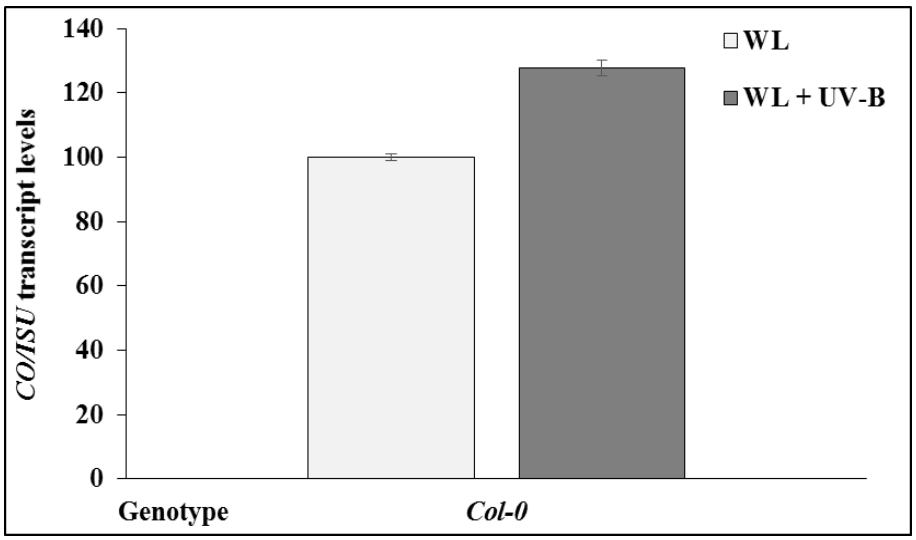
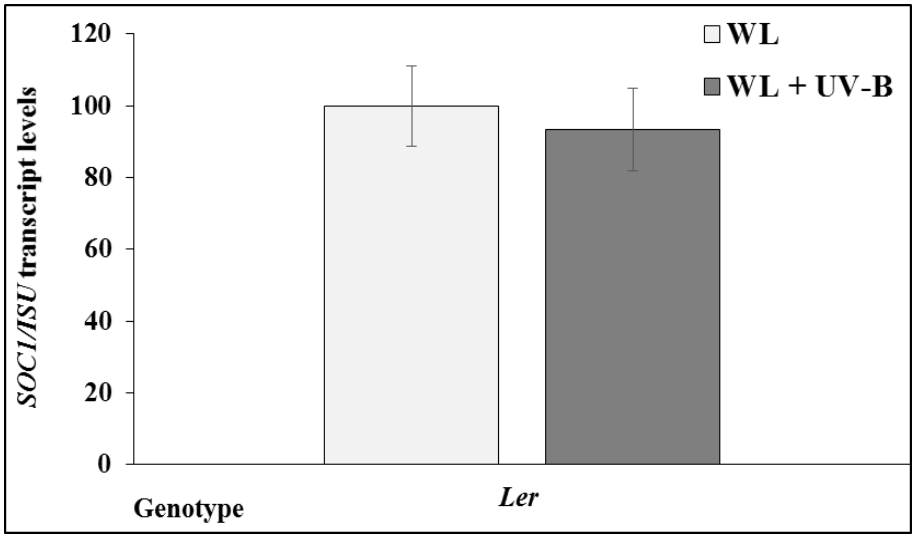
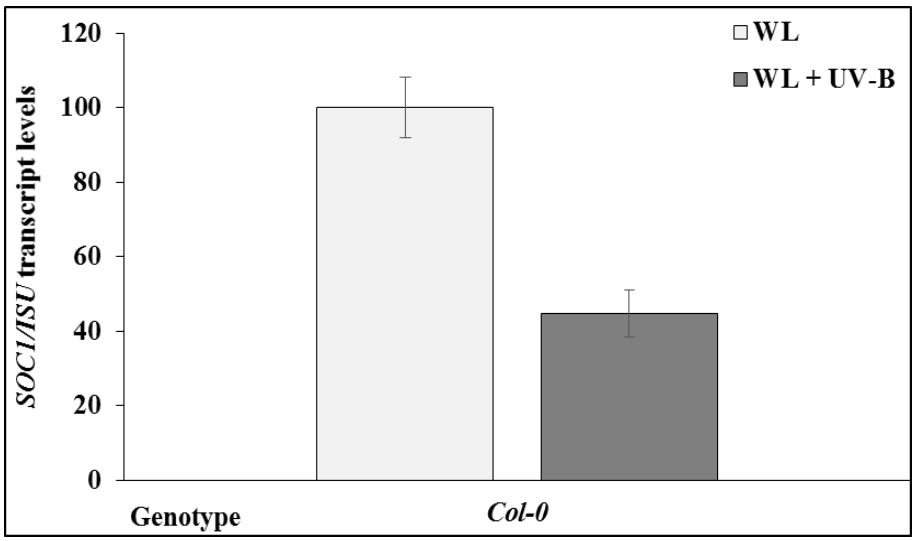


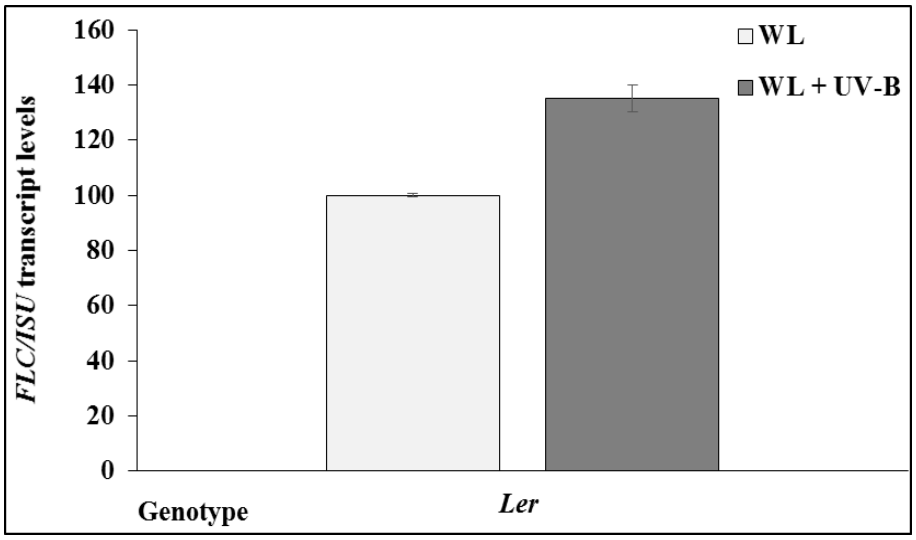
Figure 3. 5 UV-B induces *FT* and *CO* gene expression in *Col-0* and *Ler* ecotypes.
A. qRT-PCR analysis of *FT* transcript levels normalized with the housekeeping gene *ISU*. **B.** qRT-PCR analysis of *CO* transcript levels normalized with the housekeeping gene *ISU*. Plant tissue was collected at ZT 0.5 12 days after germination. Plants were grown under a LD photoperiod of WL (50 μmol $m^{-2}s^{-1}$) or WL supplemented with UV-B (0.5 μmol $m^{-2}s^{-1}$). Plants grown under WL were used as reference (100%). Data are represented as mean ± SEM. Data are representative of two biological replicates.

A.


120
100
80
60
40
20
0
SOC1/ISU transcript levels
WL
WL + UV-B
Genotype
Ler


120
100
80
60
40
20
0
SOC1/ISU transcript levels
WL
WL + UV-B
Genotype
Col-0

B.


160
140
120
100
80
60
40
20
0
FLC/ISU transcript levels
WL
WL + UV-B
Genotype
Ler

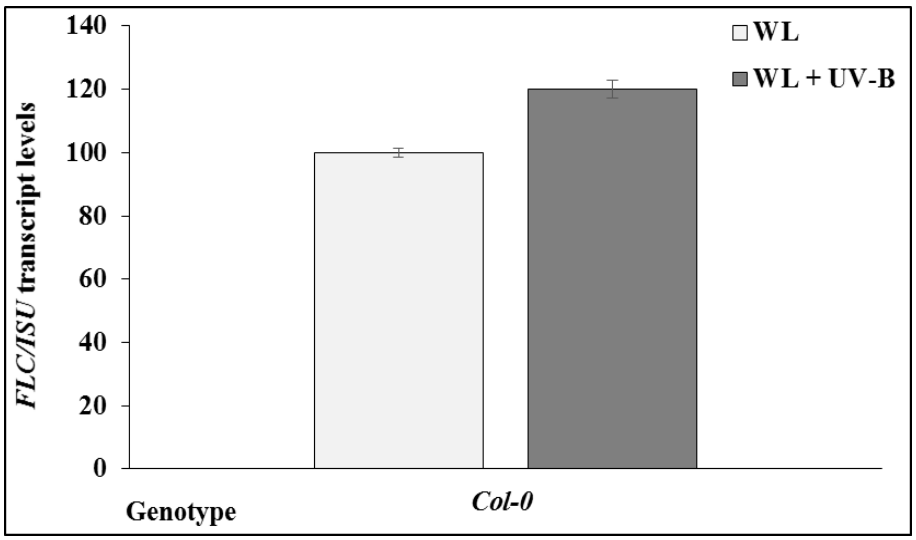
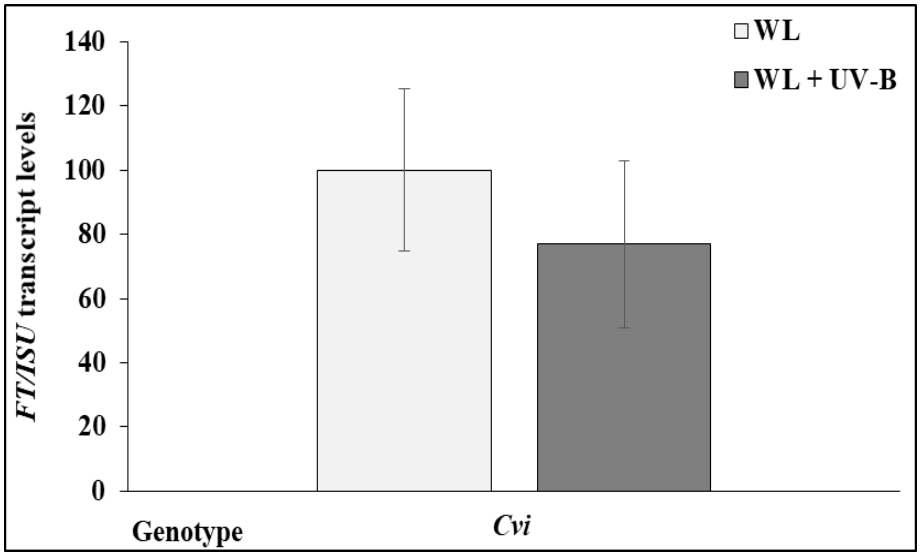


Figure 3. 6 The effect of UV-B on *SOC1* and *FLC* transcript abundance in *Col-0* and *Ler* ecotypes.

A. qRT-PCR analysis of *SOC1* transcript levels normalized with the housekeeping gene *ISU*. **B.** qRT-PCR analysis of *FLC* transcript levels normalized with housekeeping gene *ISU*. Plants were harvested at ZT 0.5 12 days after germination. Plant tissue was collected at ZT 0.5 12 days after germination. Plants were grown under a LD photoperiod of WL (50 μmol $m^{-2}s^{-1}$) or WL supplemented with UV-B (0.5 μmol $m^{-2}s^{-1}$). Plants grown under WL were used as reference (100%). Data are represented as mean ± SEM. Data are representative of two biological replicates.

A.



B.

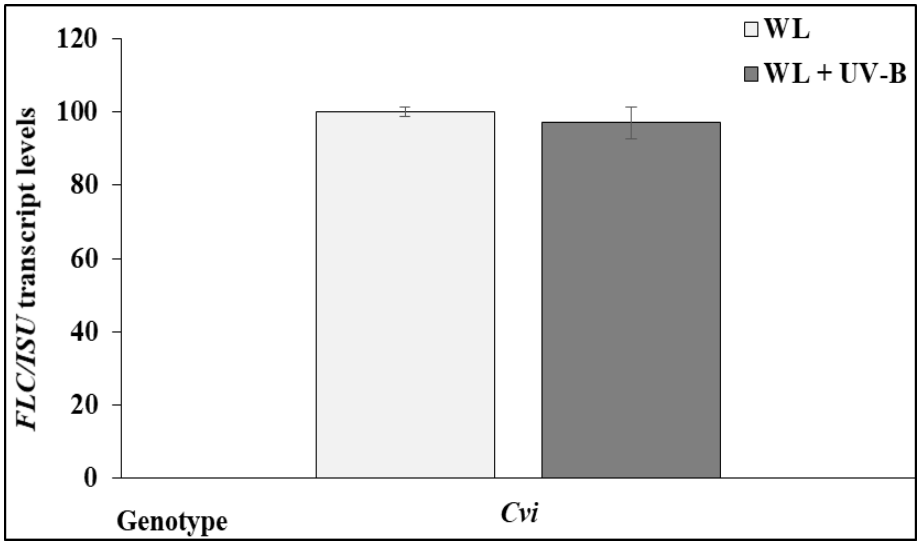
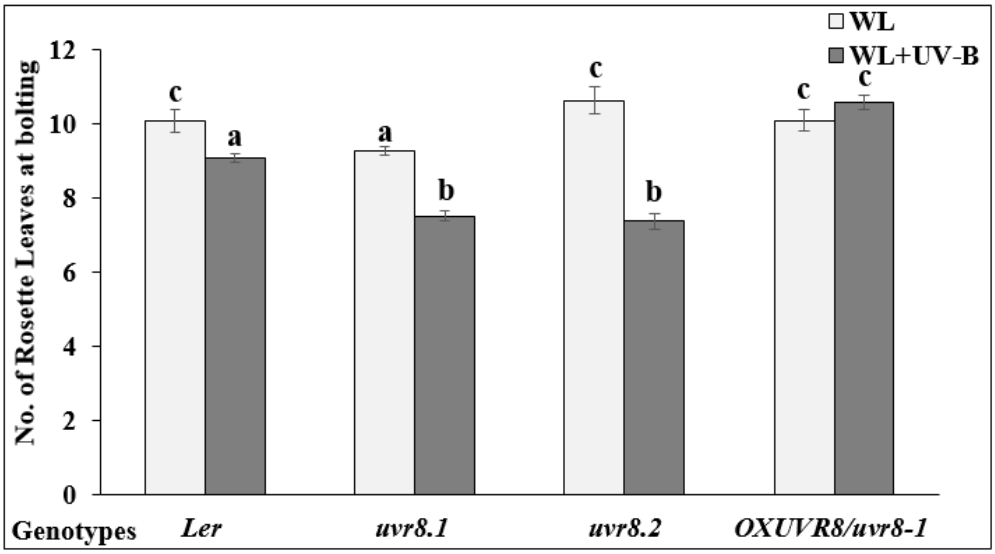


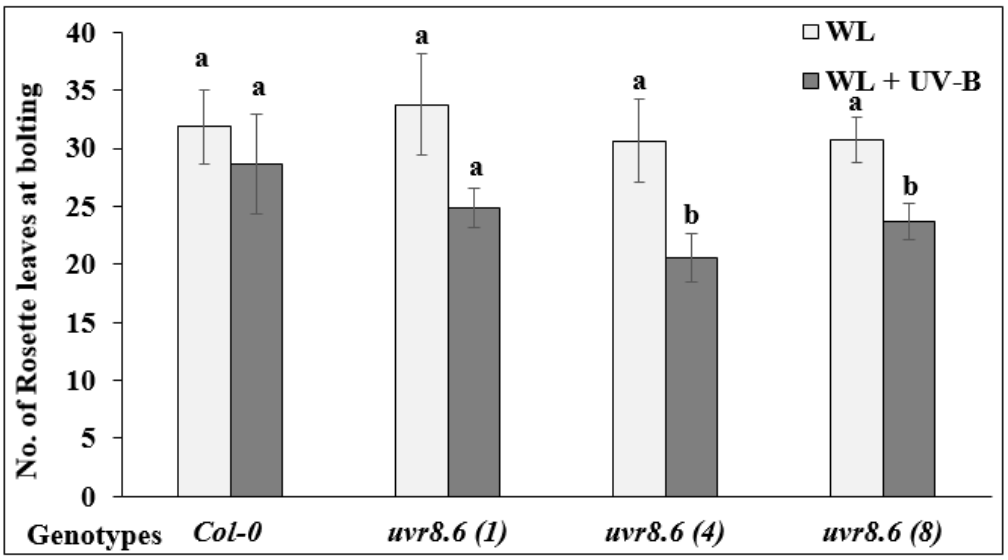
Figure 3. 7 UV-B effect on *FT* and *FLC* transcript abundance in *Cvi* ecotype.

A. qRT-PCR analysis of *FT* transcript levels normalized with the housekeeping gene *ISU*.

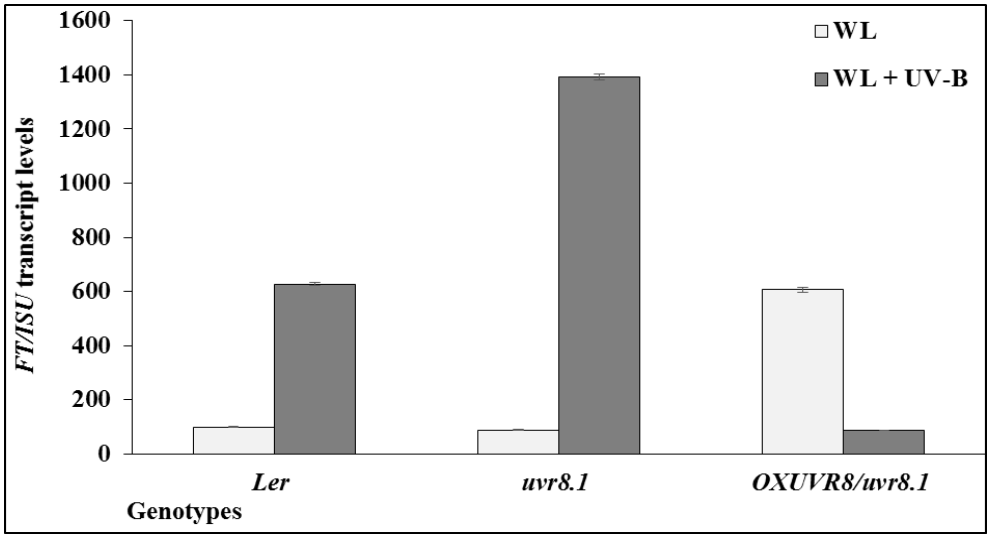
B. qRT-PCR analysis of *FLC* transcript levels normalized with housekeeping gene *ISU*. Plants were harvested at ZT 0.5 12 days after germination. Plant tissue was collected at ZT 0.5 12 days after germination. Plants were grown under a LD photoperiod of WL (50 µmol $m^{-2}s^{-1}$) or WL supplemented with UV-B (0.5 µmol $m^{-2}s^{-1}$). Plants grown under WL were used as reference (100%). Data are represented as mean ± SEM. Data are representative of two biological replicates.

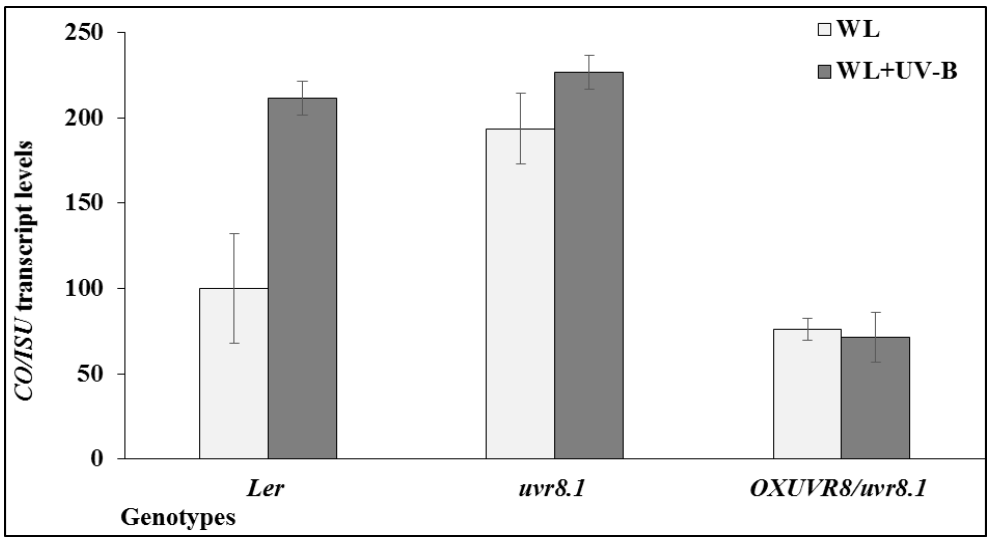
A.

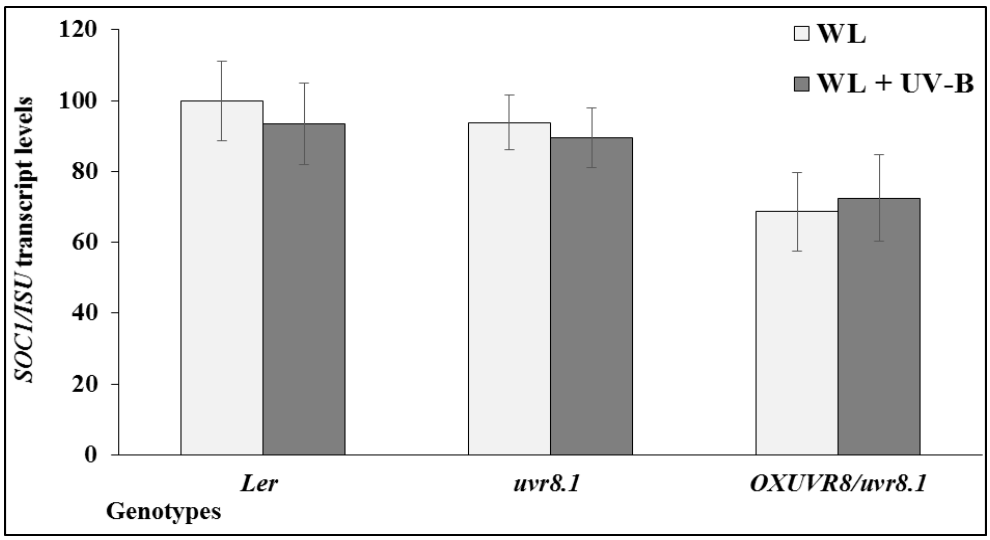

WL
WL+UV-B
No. of Rosette Leaves at bolting
0
2
4
6
8
10
12
c
a
a
b
c
b
c
c
Genotypes
Ler
uvr8.1
uvr8.2
OXUVR8/uvr8-1


WL
WL + UV-B
No. of Rosette leaves at bolting
0
5
10
15
20
25
30
35
40
a
a
a
a
a
b
a
b
Genotypes
Col-0
uvr8.6 (1)
uvr8.6 (4)
uvr8.6 (8)

B.


1600
1400
1200
1000
800
600
400
200
0
FT/ISU transcript levels
WL
WL + UV-B
Ler
uvr8.1
OXUVR8/uvr8.1
Genotypes


250
200
150
100
50
0
CO/ISU transcript levels
WL
WL+UV-B
Ler
uvr8.1
OXUVR8/uvr8.1
Genotypes


120
100
80
60
40
20
0
SOC1/ISU transcript levels
WL
WL + UV-B
Ler
uvr8.1
OXUVR8/uvr8.1
Genotypes

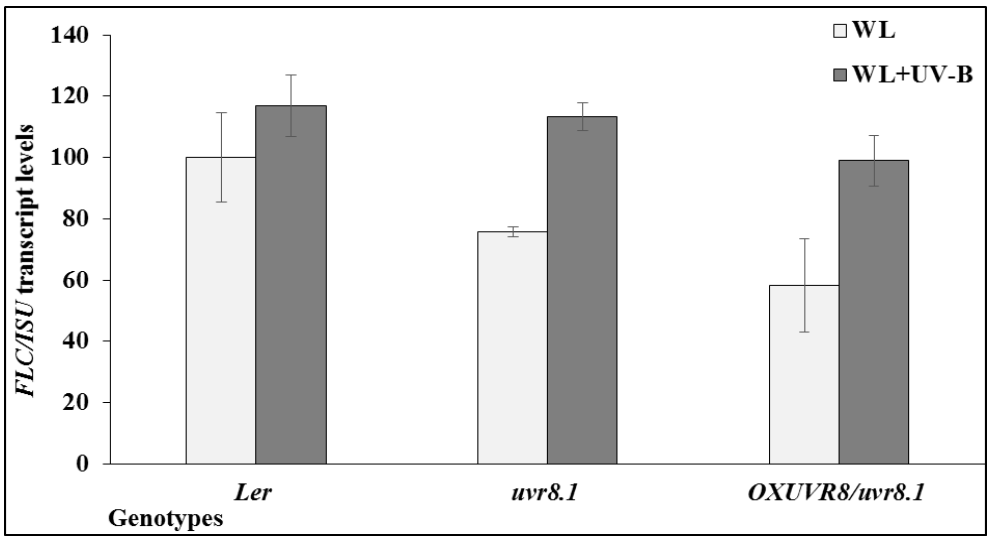


Figure 3. 8 UVR8 acts as a negative regulator of flowering initiation.

A. Flowering time of wild-type (*Ler* and *Col-0*), *uvr8-1*, *uvr8-2*, *uvr8-6* and OX-UVR8 plants grown under a LD photoperiod in WL (50 μmol $m^{-2}s^{-1}$) or WL supplemented with UV-B (0.5 μmol $m^{-2}s^{-1}$). Data are represented as mean ± SEM ($n \geq 15$). Different letters indicate statistically significant differences ($P < 0.05$) between means. The different *uvr8-6* lines are all homozygous lines containing a mutation in the UVR8 gene, in a *Col-0* background.

B. qRT-PCR analysis of *FT*, *CO*, *SOC1* and *FLC* transcript levels normalized with the housekeeping gene *ISU* in wild-type (*Ler*), *uvr8-1*, *uvr8-2* and OX-UVR8 plants. Plant tissue was collected at ZT 0.5 12 days after germination. Plants were grown under a LD photoperiod of WL (50 μmol $m^{-2}s^{-1}$) or WL supplemented with UV-B (0.5 μmol $m^{-2}s^{-1}$). Plants grown under WL were used as reference (100%). Data are represented as mean ± SEM. Data are representative of two biological replicates.

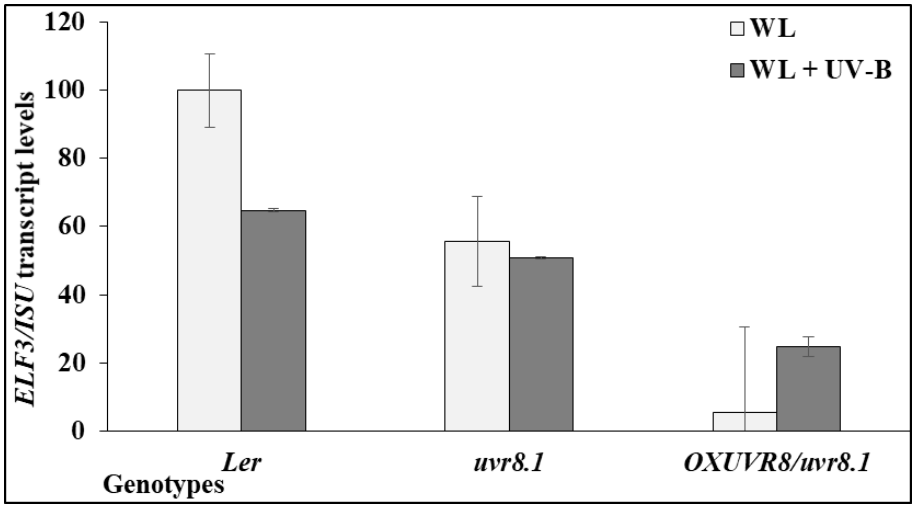


Figure 3. 9 The effect of UV-B on *ELF3* transcript abundance.

qRT-PCR analysis of *ELF3* transcript levels normalized with housekeeping gene *ISU* in wild-type (*Ler*), *uvr8-1*, *uvr8-2* and OX-UVR8 plants. Plants were grown under a LD photoperiod of WL (50 µmol $m^{-2}s^{-1}$) or WL supplemented with UV-B (0.5 µmol $m^{-2}s^{-1}$). Plants grown under WL were used as reference (100%). Data are represented as mean ± SEM. Data are representative of two biological replicates.

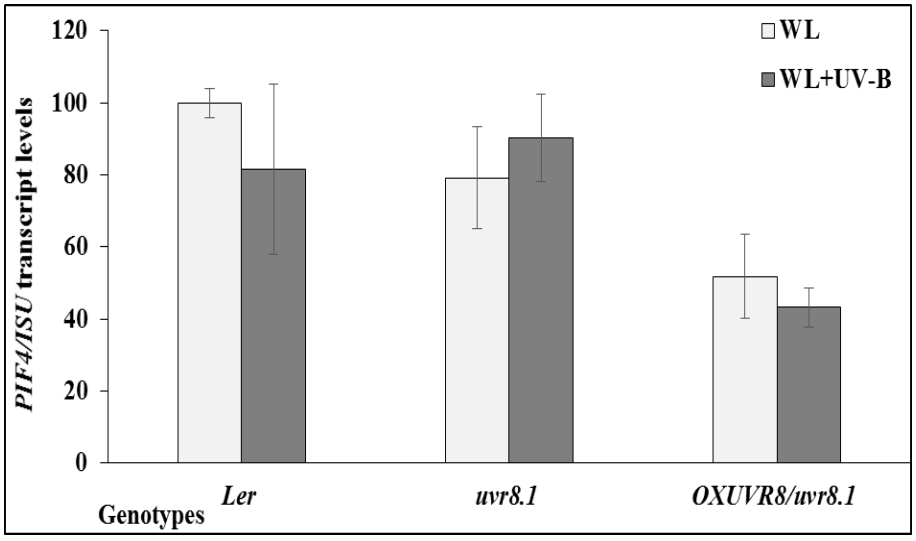


Figure 3. 10 The effect of UV-B on PIF4 transcript abundance.

qRT-PCR analysis of *PIF4*, a gene that encodes a transcription factor that promotes hypocotyl elongation but gets degraded under UV-B, transcript levels normalized with housekeeping gene *ISU* in wild-type (*Ler*), *uvr8-1*, *uvr8-2* and OX-UVR8 plants. Plant tissue was collected at ZT 0.5 12 days after germination. Plants were grown under a LD photoperiod of WL (50 µmol $m^{-2}s^{-1}$) or WL supplemented with UV-B (0.5 µmol $m^{-2}s^{-1}$). Plants grown under WL were used as

reference (100%). Data are represented as mean ± SEM. Data are representative of two biological replicates.

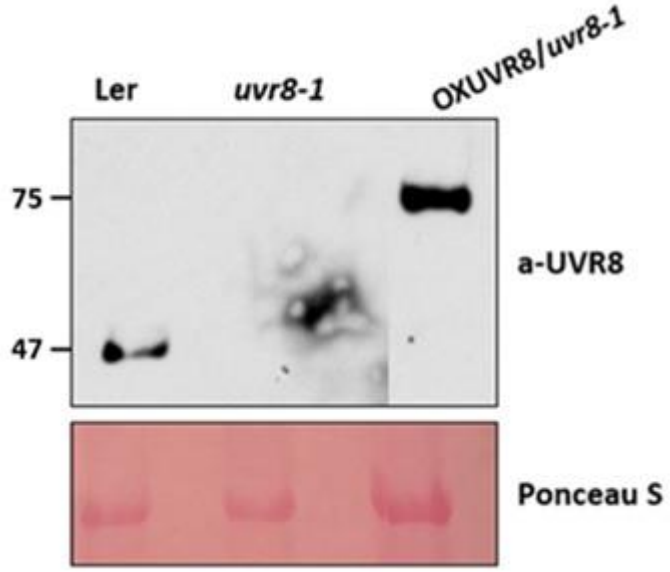
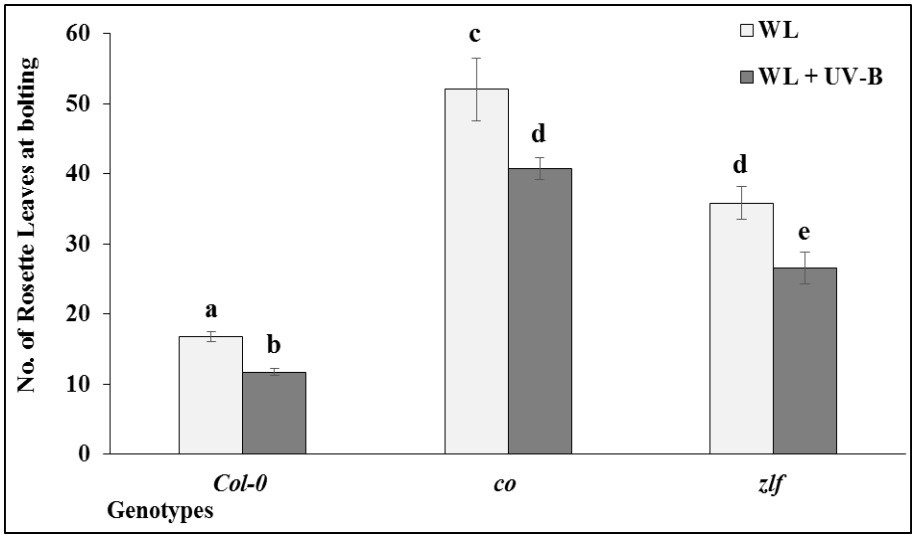
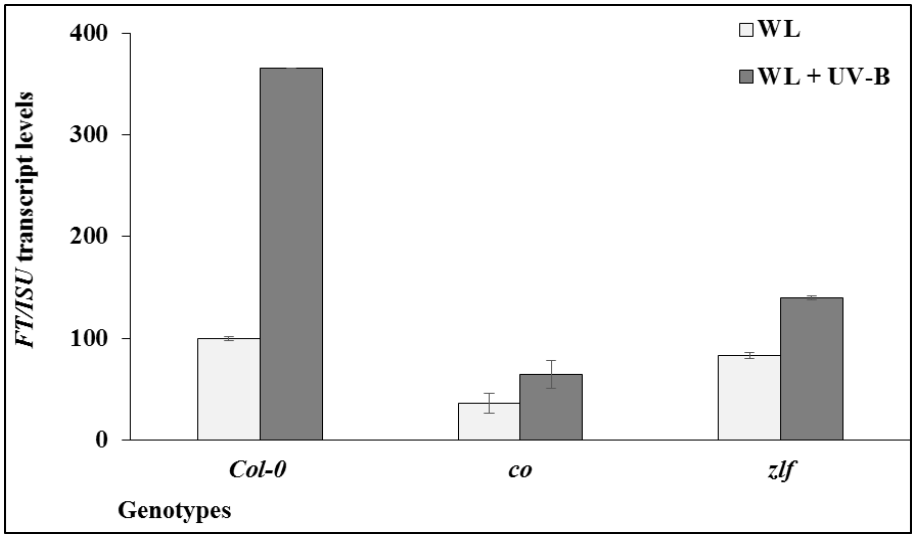


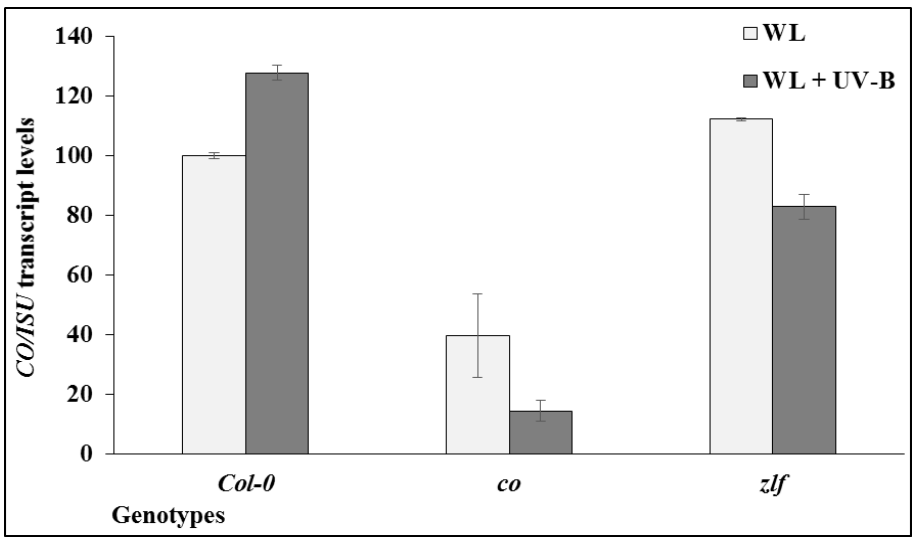
Figure 3. 11 Western blot analysis of UVR8 protein levels in the genotypes examined. Total protein was extracted from wild type (*Ler*), *uvr8-1*, and OX-UVR8 lines grown in WL. A Ponceau Stain (RbcL) is shown below as a loading control and an anti-UVR8 antibody [127] was used for detecting UVR8 protein.

A.


No. of Rosette Leaves at bolting
60
50
40
30
20
10
0
a
b
c
d
d
e
WL
WL + UV-B
Col-0
co
zlf
Genotypes

B.


FT/ISU transcript levels
400
300
200
100
0
WL
WL + UV-B
Col-0
co
zlf
Genotypes


CO/ISU transcript levels
140
120
100
80
60
40
20
0
WL
WL + UV-B
Col-0
co
zlf
Genotypes

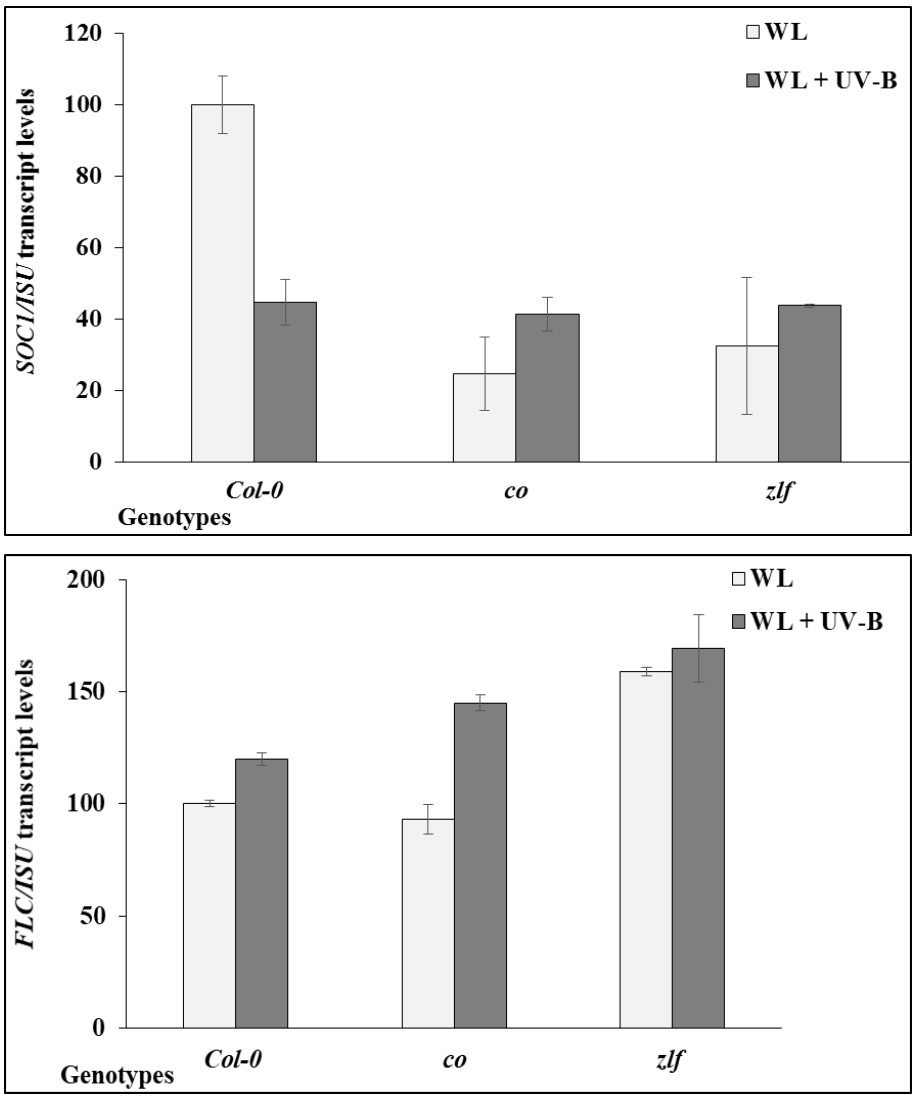
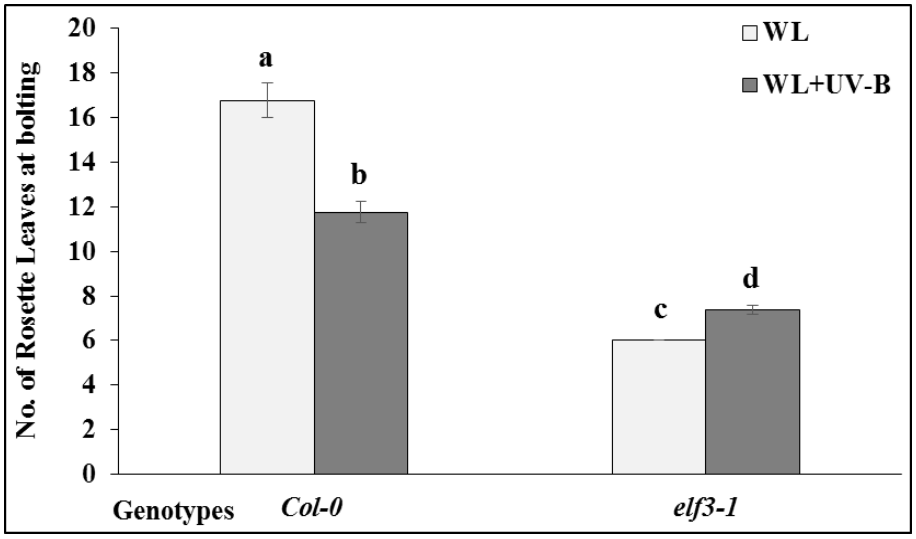


Figure 3. 12 UV-B accelerates flowering initiation of late flowering mutants.

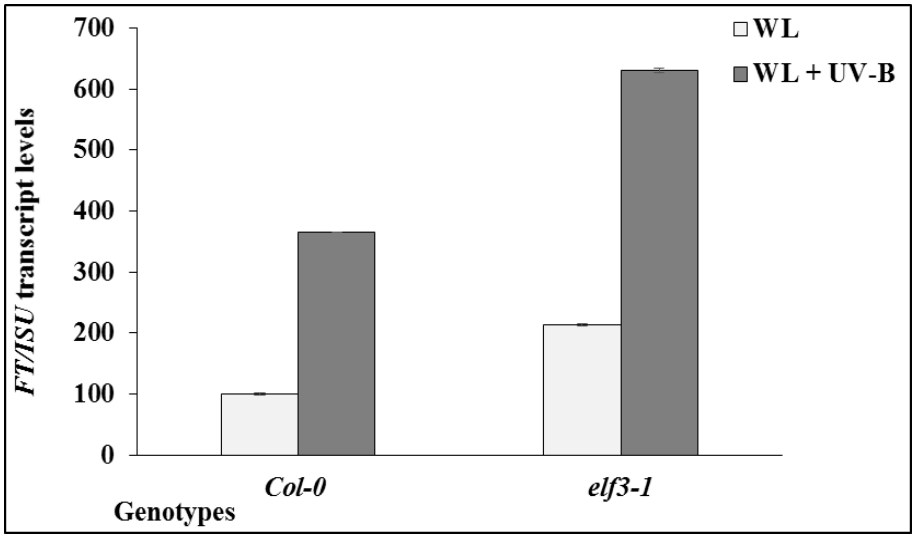
A. Flowering time of wild-type (*Col-0*), *co* and *zlf* plants grown under a LD photoperiod in WL (50 µmol $m^{-2}s^{-1}$) or WL supplemented with UV-B (0.5 µmol $m^{-2}s^{-1}$). Data are represented as mean ± SEM (n ≥ 15). Different letters indicate statistically significant differences ($P < 0.05$) between means.

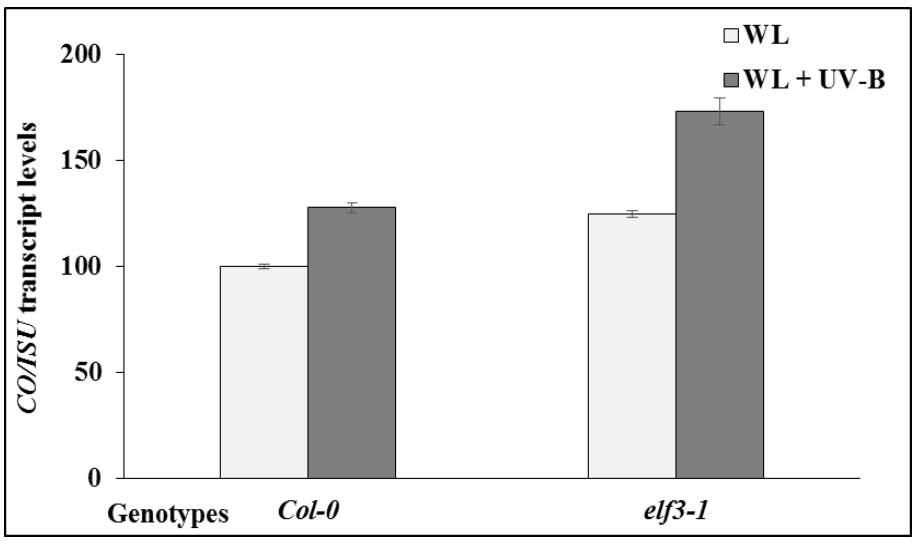
B. qRT-PCR analysis of *FT*, *CO*, *SOC1* and *FLC* transcript levels normalized with the housekeeping gene *ISU* in wild-type (*Col-0*), *co* and *zlf* plants. Plant tissue was collected at ZT 0.5 12 days after germination. Plants were grown under a LD photoperiod of WL (50 µmol $m^{-2}s^{-1}$) or WL supplemented with UV-B (0.5 µmol $m^{-2}s^{-1}$). Plants grown under WL were used as reference (100%). Data are represented as mean ± SEM. Data are representative of two biological replicates.

A.


No. of Rosette Leaves at bolting
20
18
16
14
12
10
8
6
4
2
0
a
b
c
d
WL
WL+UV-B
Genotypes
Col-0
elf3-1

B.


FT/ISU transcript levels
700
600
500
400
300
200
100
0
WL
WL + UV-B
Col-0
elf3-1
Genotypes


CO/ISU transcript levels
200
150
100
50
0
WL
WL + UV-B
Genotypes
Col-0
elf3-1

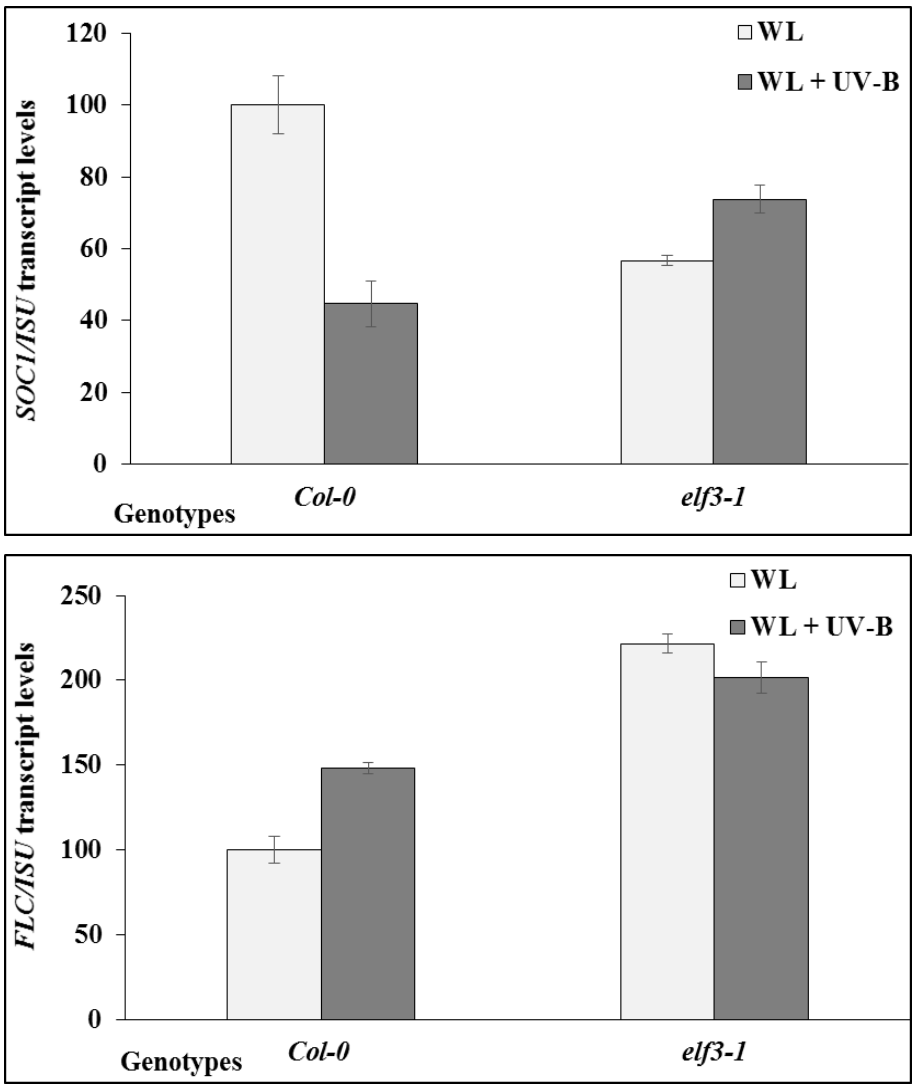
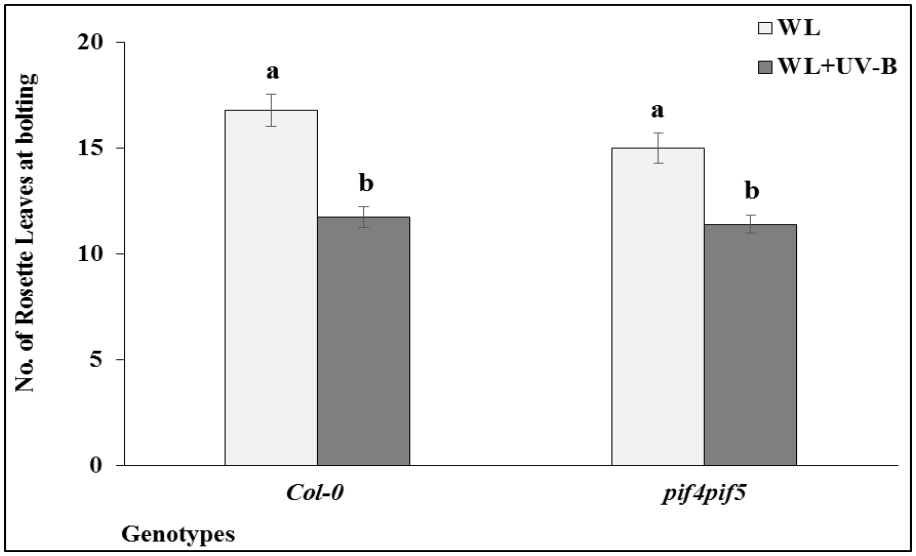


Figure 3. 13 UV-B delays flowering initiation of early flowering mutants.

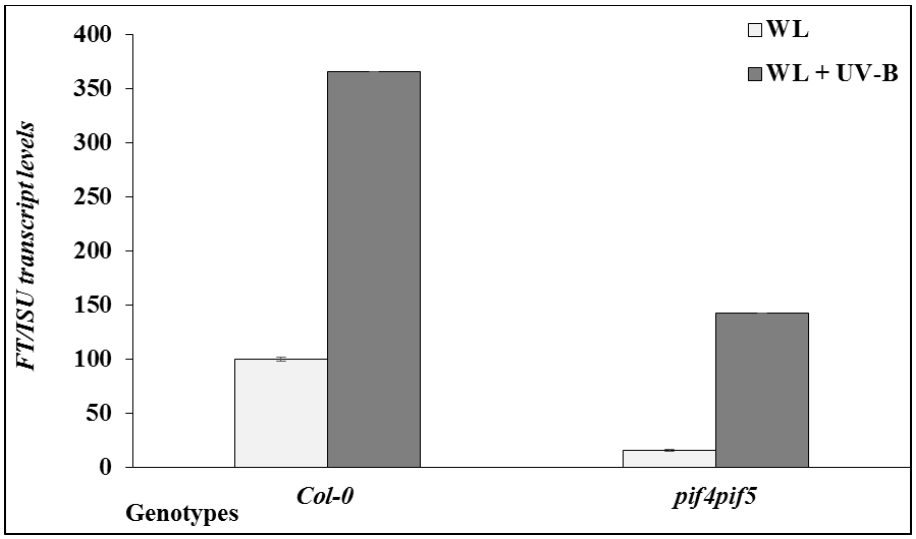
A. Flowering time of wild-type (*Col-0*) and *elf3-1* plants grown under a LD photoperiod in WL (50 µmol $m^{-2}s^{-1}$) or WL supplemented with UV-B (0.5 µmol $m^{-2}s^{-1}$). Data are represented as mean ± SEM (n ≥ 15). Different letters indicate statistically significant differences ($P < 0.05$) between means.

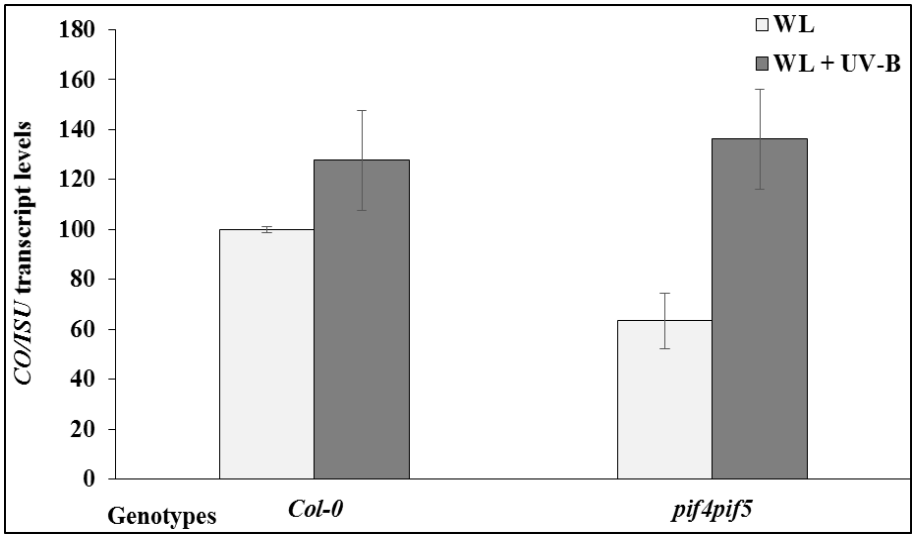
B. qRT-PCR analysis of *FT*, *CO*, *SOC1* and *FLC* transcript levels normalized with housekeeping gene *ISU* in wild-type (*Col-0*) and *elf3-1* plants. Plant tissue was collected at ZT 0.5 12 days after germination. Plants were grown under a LD photoperiod of WL (50 µmol $m^{-2}s^{-1}$) or WL supplemented with UV-B (0.5 µmol $m^{-2}s^{-1}$). Plants grown under WL were used as reference (100%). Data are represented as mean ± SEM. Data are representative of two biological replicates.

A.


WL
WL+UV-B
No. of Rosette Leaves at bolting
20
15
10
5
0
a
b
a
b
Col-0
pif4pif5
Genotypes

B.


WL
WL + UV-B
FT/ISU transcript levels
400
350
300
250
200
150
100
50
0
Col-0
pif4pif5
Genotypes


WL
WL + UV-B
CO/ISU transcript levels
180
160
140
120
100
80
60
40
20
0
Col-0
pif4pif5
Genotypes

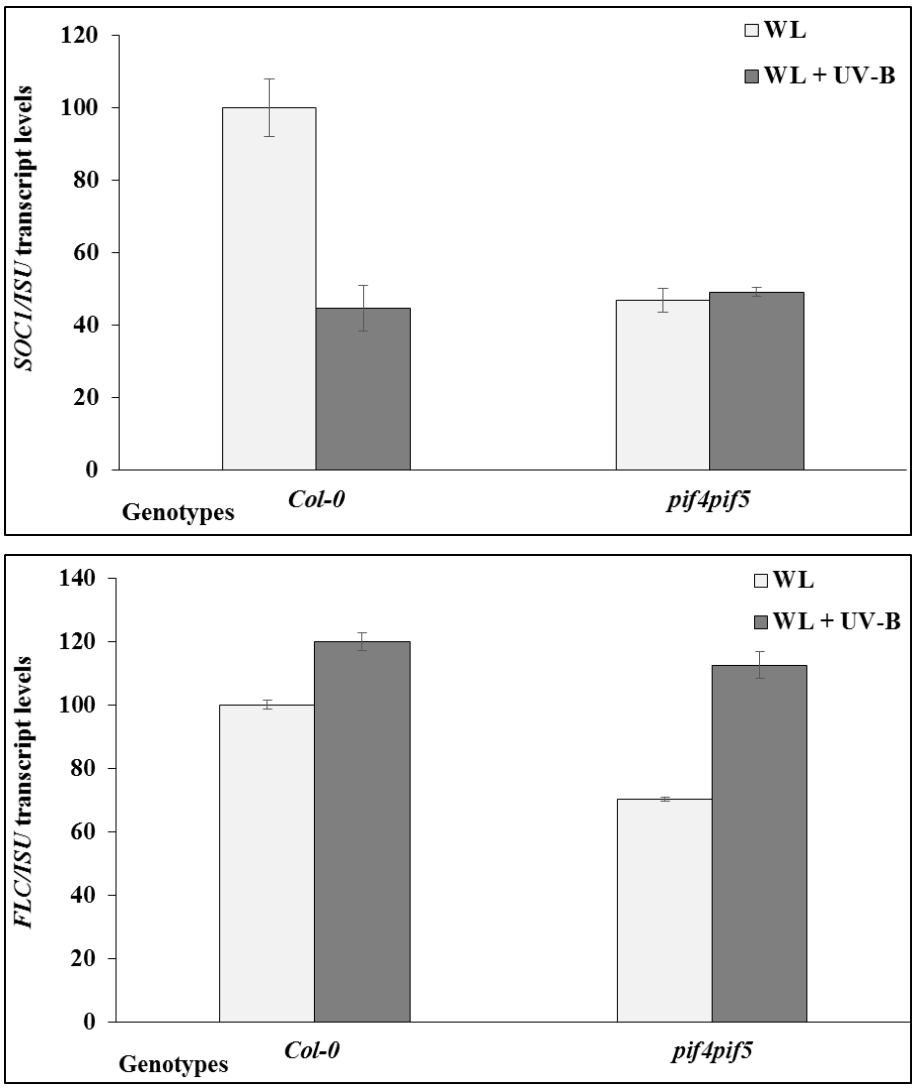
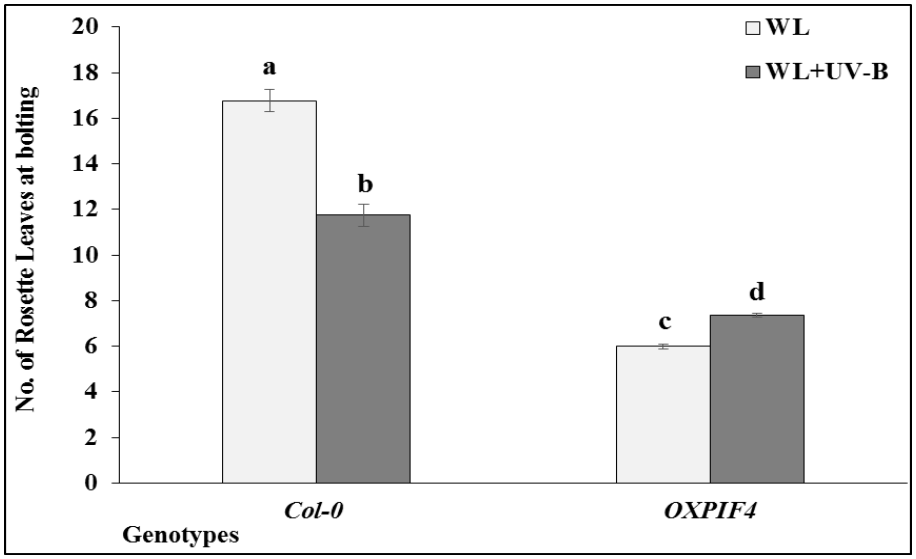


Figure 3. 14 UV-B induces early flowering in *pif4pif5*.

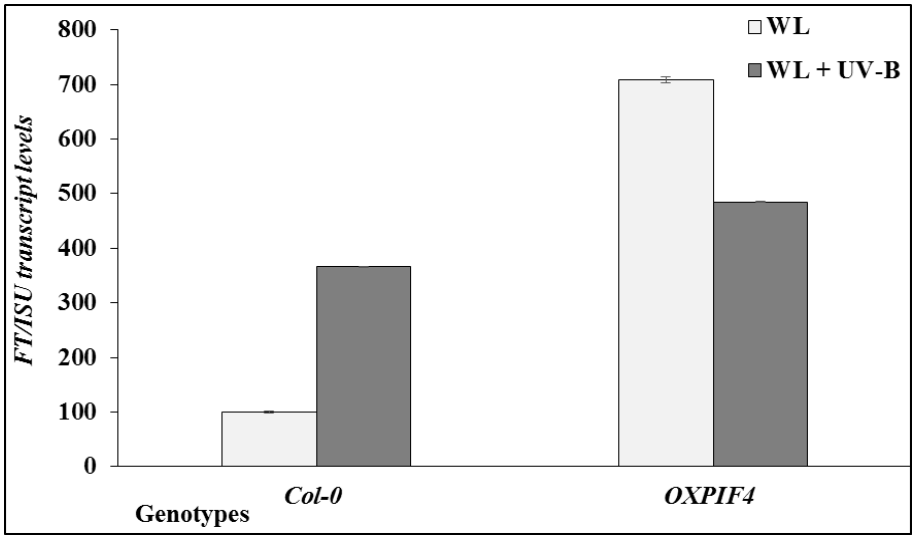
A. Flowering time of wild-type (*Col-0*) and *pif4pif5* plants grown under a LD photoperiod in WL (50 µmol $m^{-2}s^{-1}$) or WL supplemented with UV-B (0.5 µmol $m^{-2}s^{-1}$). Data are represented as mean ± SEM (n ≥ 15). Different letters indicate statistically significant differences ($P < 0.05$) between means.

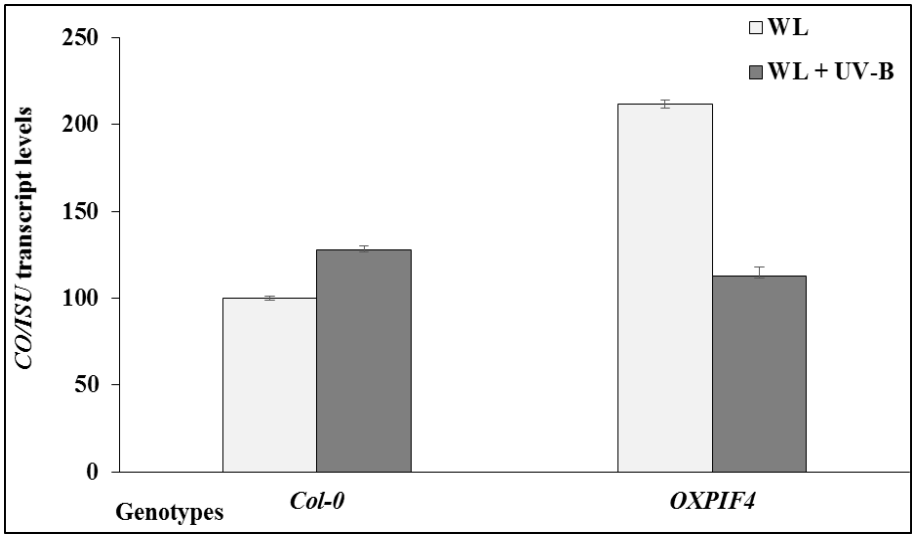
B. qRT-PCR analysis of *FT*, *CO*, *SOC1* and *FLC* transcript levels normalized with housekeeping gene *ISU* in wild-type (*Col-0*) and *elf3-1* plants. Plant tissue was collected at ZT 0.5 12 days after germination. Plants were grown under a LD photoperiod of WL (50 µmol $m^{-2}s^{-1}$) or WL supplemented with UV-B (0.5 µmol $m^{-2}s^{-1}$). Plants grown under WL were used as reference (100%). Data are represented as mean ± SEM. Data are representative of two biological replicates.

A.


No. of Rosette Leaves at bolting
20
18
16
14
12
10
8
6
4
2
0
a
b
c
d
WL
WL+UV-B
Col-0
OXPIF4
Genotypes

B.


FT/ISU transcript levels
800
700
600
500
400
300
200
100
0
WL
WL + UV-B
Col-0
OXPIF4
Genotypes


CO/ISU transcript levels
250
200
150
100
50
0
WL
WL + UV-B
Col-0
OXPIF4
Genotypes

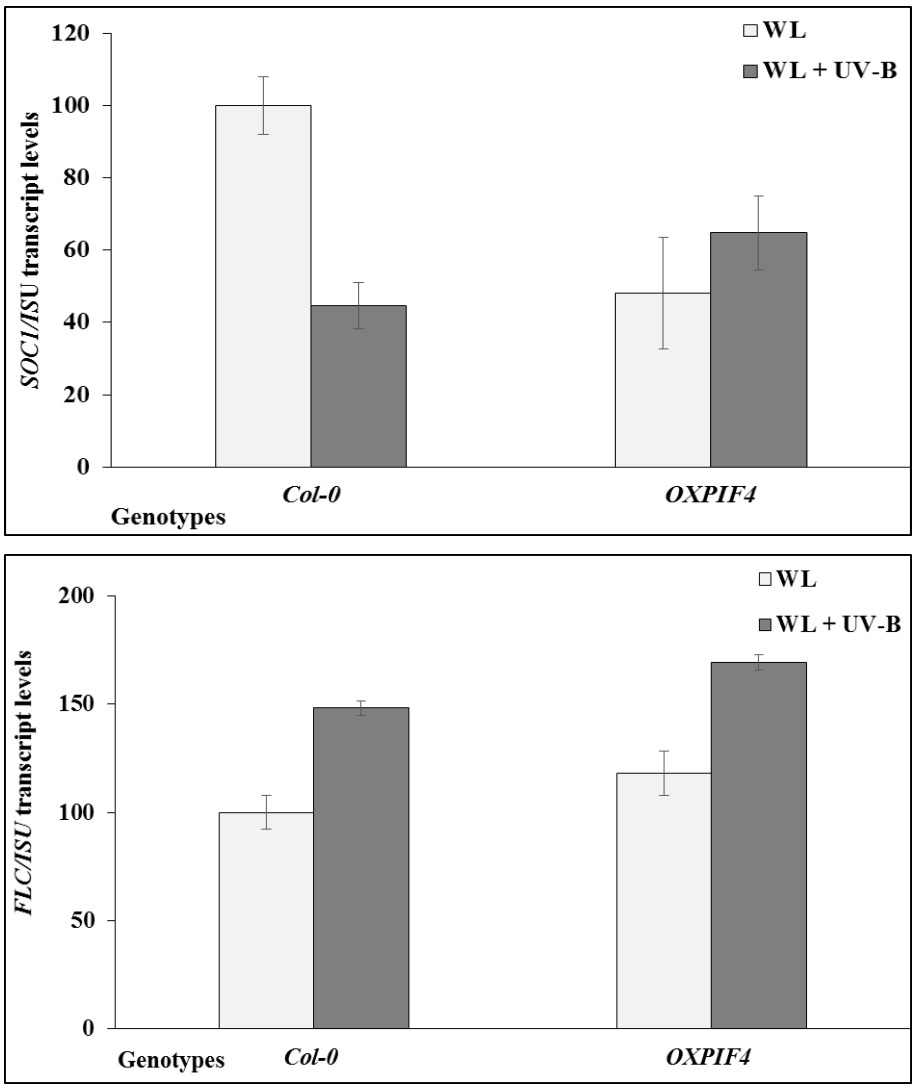
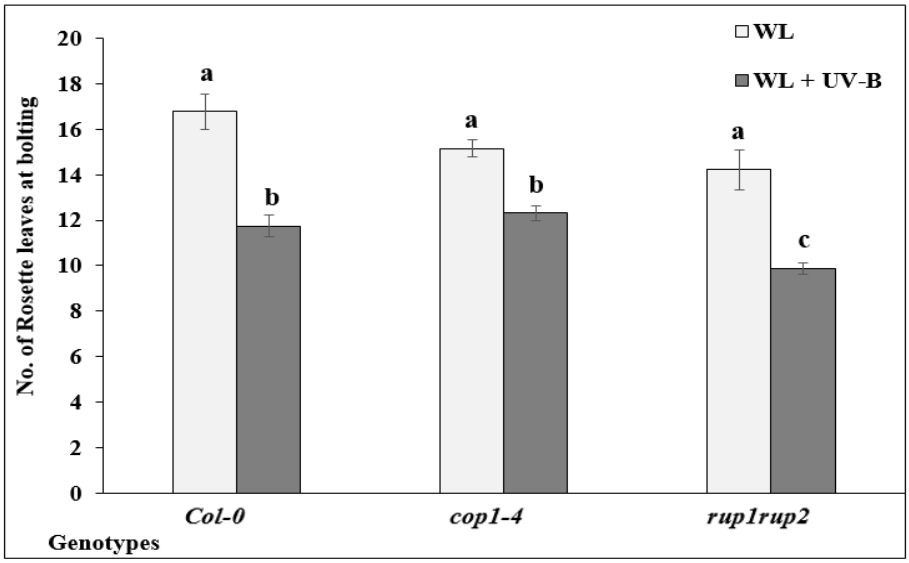


Figure 3. 15 Overexpression of PIF4 delays UV-B induced flowering.

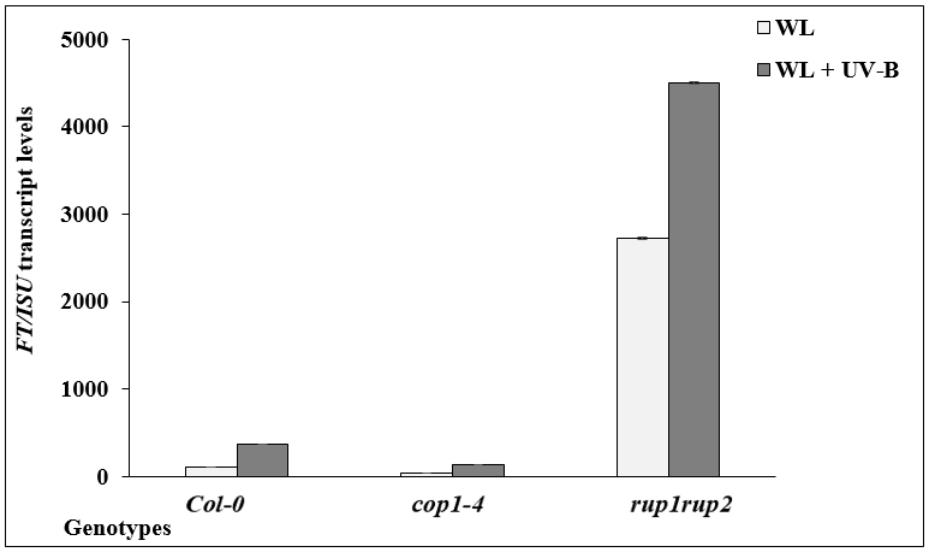
A. Flowering time of wild-type (*Col-0*) and OX-PIF4 plants grown under a LD photoperiod in WL (50 µmol $m^{-2}s^{-1}$) or WL supplemented with UV-B (0.5 µmol $m^{-2}s^{-1}$). Data are represented as mean ± SEM ($n \geq 15$). Different letters indicate statistically significant differences ($P < 0.05$) between means.

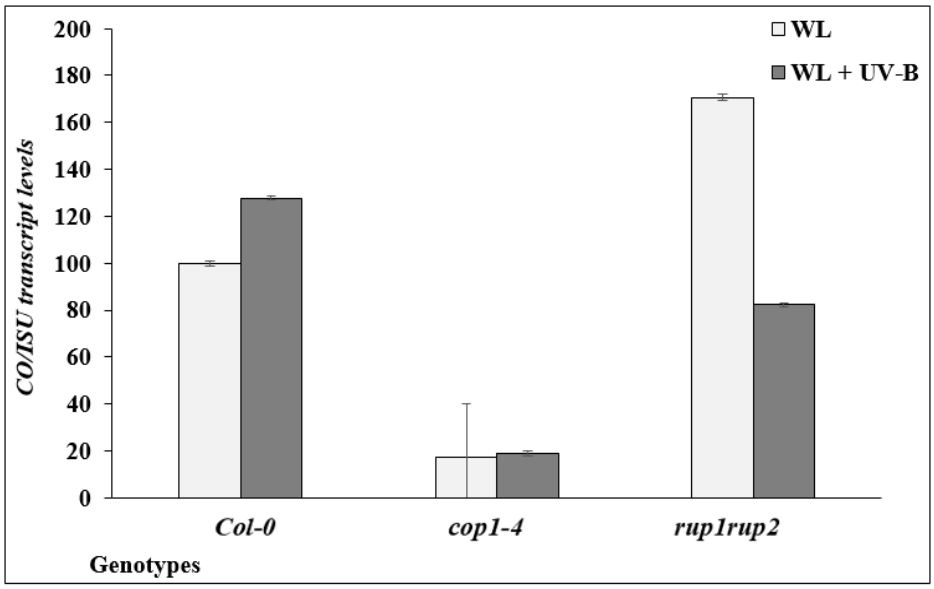
B. qRT-PCR analysis of *FT*, *CO*, *SOC1* and *FLC* transcript levels normalized with housekeeping gene *ISU* in wild-type (*Col-0*) and OX-PIF4 plants. Plant tissue was collected at ZT 0.5 12 days after germination. Plants were grown under a LD photoperiod of WL (50 µmol $m^{-2}s^{-1}$) or WL supplemented with UV-B (0.5 µmol $m^{-2}s^{-1}$). Plants grown under WL were used as reference (100%). Data are represented as mean ± SEM. Data are representative of two biological replicates.

A.


WL
WL + UV-B
No. of Rosette leaves at bolting
20
18
16
14
12
10
8
6
4
2
0
a
b
a
b
a
c
Col-0
cop1-4
rup1rup2
Genotypes

B.


WL
WL + UV-B
FT/ISU transcript levels
5000
4000
3000
2000
1000
0
Col-0
cop1-4
rup1rup2
Genotypes


WL
WL + UV-B
CO/ISU transcript levels
200
180
160
140
120
100
80
60
40
20
0
Col-0
cop1-4
rup1rup2
Genotypes

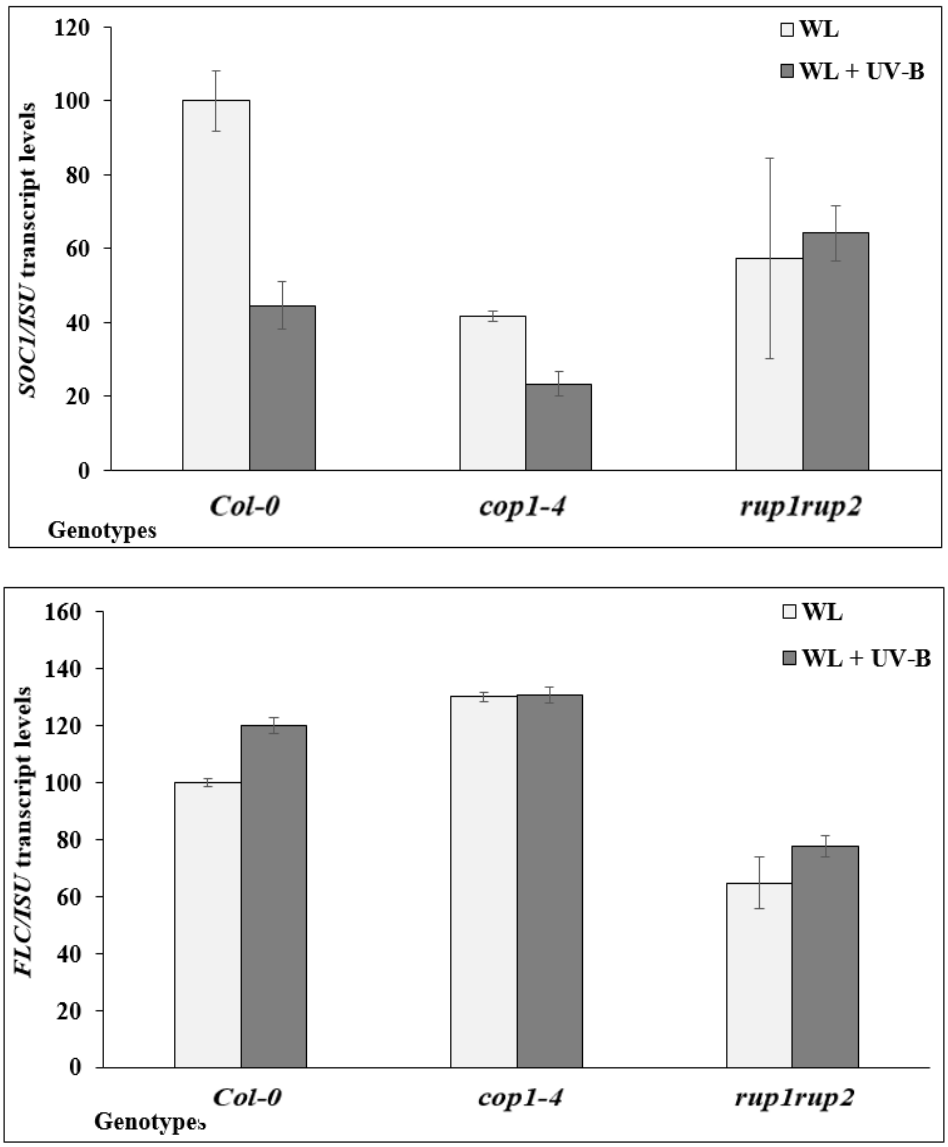


Figure 3. 16 COP1 and RUP proteins are not necessary for the UV-B-induced flowering initiation.

A. Flowering time of wild-type (*Col-0*), *cop1-4* and *rup1rup2* plants grown under a LD photoperiod in WL (50 µmol $m^{-2}s^{-1}$) or WL supplemented with UV-B (0.5 µmol $m^{-2}s^{-1}$). Data are represented as mean ± SEM ($n \geq 15$). Different letters indicate statistically significant differences ($P < 0.05$) between means.

B. qRT-PCR analysis of *FT*, *CO*, *SOC1* and *FLC* transcript levels normalized with housekeeping gene *ISU* in wild-type (*Col-0*), *cop1-4* and *rup1rup2* plants. Plants were harvested at ZT 0.5 12 days after germination. Plants were grown under a LD photoperiod of WL (50 µmol $m^{-2}s^{-1}$) or WL supplemented with UV-B (0.5 µmol $m^{-2}s^{-1}$). Plants grown under WL were used as reference (100%). Data are represented as mean ± SEM. Data are representative of two biological replicates.

Chapter 4: The role of UV-B in regulating flowering under vegetative shade

4.1 Introduction

Plants often have to adjust their architecture, morphology, growth patterns and development in response to environmental stresses since they are immobile organisms, thus they are not able to move away from pressure factors. One such factor is shade, since it is very common for plants to grow in close proximity to each other or under a canopy. Plants are able to perceive differences in the origin of shade from an inanimate object and shade that comes from another plant competing for sunlight in the future [185]. Under the shade of a neighbour plant light quality shifts [186]. Far red light is present in a higher irradiance than red light since the red wavelengths are absorbed by the chlorophyll of the neighbouring plants, while far red wavelengths are reflected or transmitted, or in some cases both [187] [188]. The described responses lead to a decrease in the ratio of red to far red irradiation, a phenomenon characterised as far red enrichment [189]. Specifically shade has been correlated with a R:FR ratio less than 1, while non shade WL conditions have been described to occur when the R:FR ratio is above 1 [190]. *Arabidopsis* copes with shade via a group of responses known as the shade avoidance syndrome (SAS) [189]. These include the stimulation of elongation growth which is associated with reduced leaf development and leaf elevation, increased apical dominance and a reduction in branching [187] [188].

Shade also has an effect in flowering time in *Arabidopsis* species [149]. More specifically under vegetative shade conditions there is an acceleration of flowering time, specifically under LD photoperiods [149]. Far red light enrichment acts as a promoter of flowering initiation via reduction of Pfr levels of PHYTOCHROMES B, C, D and E [191] [192] [193] [194] [195] [196]. PHYTOCHROME photoreceptors are responsible for perceiving red and far red irradiation. In *Arabidopsis* PHYs are encoded from a family of five genes (PHYA-PHYB) [197] [198] [28] [199] [22]. PHYs can exist in two forms: the Pr form which absorbs red light at a peak of 666 nm, and the Pfr form which is active and initiates biological responses and absorbs at a peak of 730 nm [186]. Pr can be converted to Pfr due to a conformational change triggered by red light [200]. Pfr can be converted back to Pr when absorbing far red light [200]. In a given R:FR ration usually a dynamic equilibrium between the two forms exists [186]. Under shade conditions there is an increase of Pr which subsequently activates shade avoidance signal transduction pathways and leads to accelerated flowering under LD inductive photoperiods [201] [202] [203] [204] [205] [206] [149] . Early flowering

under low R:FR is not orchestrated only by one signal [149]. On the contrary, shade-induced flowering is a response initiated by an accumulation of different opposing as well as quantitative influences such as: the R:FR ratio and shade treatment which leads to a corresponding Pfr reduction, the genetic background of the plant which causes variations in the amount of the repression of flowering and very importantly the day length [149]. For example it has been demonstrated that under vegetative shade conditions repression of *FLC* is bypassed and the presence of high FLC levels is overridden in terms of flowering [149]. Additionally, an increase in *FT* abundance was observed under shade [149]. This response was found to be CO-dependent, as CO protein is stabilized very rapidly upon a low R:FR treatment and an increase in CO expression is observed after a longer exposure under vegetative shade conditions [149]. Moreover it was observed that shade has the ability to enhance the expression of genes that are involved in flowering only at certain times within a 24-hour photoperiod [149 207 208].

Currently there is not a lot of information on the effect of UV-B irradiation in regulating vegetative shade induced flowering. A delay of flowering has been observed in wild type plants under low R:FR + UV-B conditions compared to the plants growing only under low R:FR [144]. Also *uvr8* mutants have been observed to flower around the same time under low R:FR and low R:FR that has been supplemented with UV-B [144].

4.2 The effect of UV-B in regulating flowering initiation under vegetative shade

In order to assess the effects of non-stress inducing UV-B irradiation under vegetative shade conditions we conducted flowering experiments under LD photoperiods of shade (WL = 35 μmol $m^{-2}s^{-1}$, R:FR = 0.14) and Shade (WL = 35 μmol m^{-2} s^{-1}, R:FR = 0.14) that was supplemented with UV-B (= 0.7 μmol $m^{-2}s^{-1}$) conditions. The shade created by the conditions we used is considered as moderate towards strong but not severe (e.g R:FR = 0.05) [209 210]. Actually the reported ratios of different canopies of vegetation have been reported to range between 0.05 - 0.7 [186 188]. The R:FR ratio of usual daylight is around 1.15 and can vary throughout the year, because of different weather conditions [186 188]. *Arabidopsis* natural ecotypes *Ler, Col-0* and *Cvi* were selected for detailed examination of flowering initiation in response to UV-B. Additionally we investigated the flowering time of two *uvr8* mutant lines and a UVR8 over-expressing line. We also investigated if UV-B radiation initiates any changes in flowering time of *Arabidopsis thaliana* lines that were either mutant or over-expressed genes that are involved in flowering and / or UVR8 function.

4.3 The role of UV-B in flowering initiation under vegetative shade in different Arabidopsis ecotypes

As mentioned previously three *Arabidopsis* ecotypes where chosen for detailed monitoring of their flowering initiation under shade in the presence or absence of supplementary UV-B. Our results indicate that UV-B irradiation does not affect flowering time of *Ler* and *Cvi* ecotypes, but delays flowering of *Col-0* ecotype (Figures 4.1-4.3. In all experiments described in Chapter 4, flowering time was again measured by counting the number of rosette leaves the plant had the day the first bolt (over 2 cm) appeared as well as the number of days that passed from germination to bolting. As depicted in Figure 4.1 there is no significant difference between flowering initiation in *Ler* under low R:FR and low R:FR that was supplemented with UV-B. Figure 4.3 presents flowering time of *Cvi* ecotype under these conditions. Even though there is a reduction of the number of rosette leaves under low R:FR + UV-B conditions compared to low R:FR, the difference was not calculated as statistically significant. As presented in Figure 4.2 UV-B radiation leads to a late flowering phenotype of *Col-0*.

4.4 The role of UV-B in regulating vegetative shade flowering in *UVR8* mutants and overexpressors

UVR8 is the photoreceptor responsible for sensing and mediating physiological responses to UV-B irradiation[114], thus we wanted to investigate the role of this photoreceptor in inducing vegetative shade flowering initiation in *Arabidopsis thaliana* by examining *uvr8* mutant and over-expressing lines.

We conducted flowering experiments using the same UV-B intensity as described previously on *Ler* wild-type plants and selected *uvr8* mutant and over-expressing lines. More specifically we used one UVR8 over-expressing line, OXUVR8, and two different *uvr8* mutant alleles: *uvr8-1* and *uvr8-2*, which have all been described previously (Chapters 2 and 3). *uvr8-1* showed no significant difference in flowering time between low R:FR and low R:FR that was supplemented with UV-B (Figure 4.4). On the other hand *uvr8-2* mutant line showed a statistically significant delay in flowering initiation in low R:FR + UV-B (Figure 4.4). It is important, however, to note that the difference in the number of leaves constitutes to less than one leaf, but this difference was still calculated as statistically significant. Moreover, exposure of OXUVR8 to low R:FR+UV-B resulted in delayed flower compared to low R:FR only (Figure 4.4). This delay was also calculated as statistically significant.

4.5 The effect of UV-B on flowering initiation under vegetative shade in photoperiodic flowering mutants

To assess the effect of UV-B on photoperiodic flowering initiation under vegetative shade conditions, we examined the phenotype of two mutants lacking essential flowering-inducing components, *co* and *zlf*, as well as the early flowering *elf3-1* mutant line, which lacks the flowering repressor ELF3. Our results shown in Figure 4.5 indicate that there is no significant difference in flowering initiation in *co* and *zlf* lines that were grown under a low R:FR +/- UV-B. On the contrary, *elf3-1* line showed a delay in flowering time, similar to the delay observed in the wild type *Col-0*.

4.6 The effect of UV-B on vegetative shade flowering initiation in *pif* mutants and overexpressors

As a next step we investigated the effect of UV-B on flowering initiation under shade conditions in *pif4* single mutant, *pif4pif5* double mutant as well as in OXPIF4. PIFs are important transcription factors acting downstream of phytochromes and mediating signalling cross-talk among light, hormone, clock and temperature pathways [173 174 211 212 12 213]. As depicted in Figure 4.6 a delay in flowering initiation was observed in all three lines, *pif4*, *pif4pif5*, OXPIF4, under low R:FR + UV-B, compared to the ones grown under low R:FR only. The difference in the average leaf number at the time of bolting in *pif4* and *pif4pif5* was similar to *Col-0*. On the other hand the difference in leaf number of OXPIF4 line grown under low R:FR + UV-B, compared to the ones grown only under low R:FR, constitutes of less than a leaf, but was still calculated as statistically significant.

4.7 How does UV-B irradiation affect flowering initiation of UV-B signalling mutants under shade conditions?

Lastly in order to complete the vegetative shade initiation set of flowering experiments, we tried to have an insight of the way UV-B affects vegetative shade flowering initiation of our two *Arabidopsis* lines that lack important genes which are involved in the UV-B signalling pathway. Therefore we investigated flowering initiation of *cop1* and *rup1rup2* mutant lines. Our results which are depicted in Figure 4.7 demonstrate that there is a late flowering phenotype similar to the wild type, observed for both lines under low R:FR + UV-B conditions compared low R:FR growth conditions, where UV-B wasn't used.

4.8 Discussion

This chapter focused on the investigation of the potential changes in flowering time mediated by supplementary UV-B in *Arabidopsis* lines grown under vegetative shade. During Shade avoidance there is an acceleration in flowering time that has been observed before [149] and this is a general response we also observed in our experiments (Figure 4.8). Under shade conditions PIF stability is increased (PIF4 protein for example is degraded under light [100]). This increase and particularly the one of PIF4 leads to an increased expression of genes that are associated with the elongation growth (like genes involved with the synthesis of gibberellin) [150]. In *pif4pif5* mutant line both the auxin concentration and the overall shade avoidance response is reduced [214], since this mutant is hypersensitive to far-red light and it is proven that PIF4 and PIF5 redundantly control the far-red light responses [215]. PIF4 also can control the thermosensory activation of flowering initiation under SD growth conditions [100]. At high temperatures (27 ^{o}C - 28 ^{o}C) *PIF4* transcript expression is upregulated and it regulates *FT* through PIF4 binding to its promoter, in a temperature dependent way and under SD growth conditions [100]. At lower temperatures (12 ^{o}C) there is a decrease of PIF4 binding to the promoter *FT* which leads to a late flowering phenotype of *35S::PIF4* line at SD growth conditions [100]. It is believed that the temperature dependent regulation of *FT* transcript by PIF4 under SDs is controlled at two levels [100]. The first one is the chromatin accessibility of the *FT* promoter and the second is the regulation of PIF4 protein activity [100].

Also PIF4 and PIF5 get degraded under shade that is supplemented with UV-B [144]. This results in reduced auxin activity and stem elongation. Moreover UVR8-COP1 interaction up-regulates transcription of *HY5* and *HYH* genes which results in the inhibition of petiole elongation and leaf elevation [144]. Also at high temperatures UV-B irradiation inhibits thermomorphogenesis in a UVR8 dependent manner at LDs [89]. The specific interaction of UVR8 and COP1 that occurs under UV-B irradiation reduces the levels of *PIF4* transcript abundance and subsequently PIF4 proteins levels [89]. UV-B irradiation also leads to the stabilization of basic helix-loop-helix protein LONG HYPOCOTYL IN FAR RED (HFR1), a factor that binds to PIF4 and inhibits its action [89].

The results acquired from the aforementioned experiments, suggest that UV-B delays vegetative shade flowering time in the *Col-0* (WT) (Figure 4.2.A). Also there is a delay in flowering time in characterized early flowering mutants *elf3-1* (Figure 4.5) as well as in light signalling mutants *pif4*, *pif4pif5* (Figure 4.6) and UV-B signalling mutants *cop1-4* and

rup1rup2 (Figure 4.7), suggesting that these genes are not involved in UV-B mediated late flowering.

Ler and *Cvi* ecotypes showed no significant changes in flowering initiation under low R:FR and low R:FR supplemented with UV-B (Figures 4.1 and 4.3), suggesting that UV-B does not affect their flowering time. The results of our experiments for the *Ler* accession contradict a previously shown delay in flowering time under low R: FR + UV-B [144]. Results acquired from investigation of the potential role of UVR8 in vegetative shade flowering initiation enhanced our already formed hypothesis that UVR8 acts as a negative regulator of flowering time. Our hypothesis was supported by the delayed flowering time of the OXUVR8 line under shade that was supplemented with UV-B. Also the nature of the UVR8 protein mutation was found to be connected with their flowering phenotype under UV-B. The null mutant *uvr8-1* depicted no significant change in flowering initiation under UV-B or not while the *uvr8-2* which still produces a truncated protein displayed a gradual delay of flowering compared to OXUVR8 under low R:FR + UV-B.

4.8.1 The effect of UV-B in regulating vegetative shade flowering initiation varies amongst different *Arabidopsis* ecotypes

The role of UV-B in regulating vegetative shade initiation of flowering has not been widely investigated. It has been shown that there is a delay in flowering time under UV-B and low R:FR conditions compared to only low R:FR conditions in the *Ler* ecotype [144]. In the current study there was no significant difference observed in the particular ecotype (Figure 4.1). A possible explanation for this difference between these two observations could possibly derive from the differences in the light conditions used while conducting the flowering experiments. More specifically, the current study used a R:FR ratio of 0.14. The selection of this ratio was based on published literature as proven to be capable of initiating shade avoidance responses and early flowering in *Arabidopsis thaliana* [149]. Moreover the UV-B intensity that was used was 0.7 μmol $m^{-2}s^{-1}$ (an intensity that has been proven to mediate UVR8 photomorphogenesis but not induce stress and close to the UV-B intensity of a sunny day in Glasgow). For this reason it is possible that a much lower R:FR ratio and a higher UV-B radiation, similar to the ones used in the study by Hayes et al. (0.05 and ≈ 1 μmol m^{-2} s^{-1} respectively), could possibly lead to a delay in flowering time, thus it is important to repeat the flowering experiments in the future, testing different low R:FR and UV-B conditions. UV-B was not observed to have any effect on flowering time in another *Arabidopsis* ecotype examined, *Cvi* (Figure 4.3). Whereas a delay in flowering was observed for the *Col-0* under low R:FR + UV-B (Figure 4.2). All three

ecotypes that were chosen derive from very diverse environments and possibly this is an explanation of their differences in flowering time. *Ler* as mentioned before was originally found in Germany and Europe [162] and is subjected to LD and SD photoperiods depending on the seasons throughout the year (timeanddate website). *Col-0* which was found in Columbia in Missouri [162] is also under LD and SD photoperiods as the seasons change throughout the year (timeanddate website). On the other hand *Cvi* ecotype originates from Cape Verde islands [163] and is under a 12h light and 12h darkness photoperiod all year long (timeanddate website). The above factors could possibly be responsible for the differences observed in flowering time, since light has been established to be able to regulate flowering time [18].

4.8.2 The role of UVR8 in mediating UV-B induced delayed vegetative shade flowering

UVR8 is the only photoreceptor known to absorb UV-B irradiation and mediate photomorphogenic and photoprotective responses [117]. In the current study the potential role of UVR8 in flowering initiation under vegetative shade was investigated thought flowering experiments under low R:FR ± UV-B, of two *uvr8* mutant and an overexpressing, OXUVR8, line. Our results suggest that there is no significant difference in flowering time under a low R:FR + UV-B compared to low R:FR alone in *uvr8-1*. On the other hand *uvr8-2* showed a very mild delayed flowering phenotype under low R:FR + UV-B (Figure 4.4). This delay which was consisted of less than one leaf, was nevertheless calculated to be statistically significant, based on the number of plants that was counted.

The difference in the phenotype of these two mutants maybe can be explained considering the difference in their mutation. As it has been mentioned before *uvr8.2* allele produces a truncated but non-functional UVR8 protein [126], whereas *uvr8-1* is a null allele [122]. The null allele of course does not produce any UVR8 protein at all, as a result we do not expect to see any phenotype related to the UVR8 protein. On the other hand in the *uvr8-2* mutant line there is *UVR8* transcript that is being produced. Also the truncation of the UVR8 protein possibly results in a defective protein structure or affects the ability of the protein's interaction with some factors. This might explain the delay in flowering time, we observe for the *uvr8-2* mutant *Arabidopsis* line. The fact that we see a gradual increase of delay in flowering initiation between the *uvr8-2* mutant and the OXUVR8 correlates with the assumption that UVR8 could possibly regulate flowering in a negative way.

In a recent study by Hayes et al. it was observed that there is no significant difference in flowering time of *uvr8-1* grown under low R:FR and low R:FR + UV-B [144], a result that correlates with our own findings of the current thesis. A delay in flowering time under shade + UV-B was also observed in the OXUVR8 mutant (Figure 4.4), and the difference in the leaf rosette number under the two conditions was greater than the *uvr8-2* mutant, suggesting that this is a truly significant difference. This result could further support the hypothesis that UVR8 is a negative regulator of flowering, since once it is overexpressed there is a significant delay in flowering time even under vegetative shade conditions.

4.8.3 UV-B delays flowering in most photoperiodic and light signalling mutant and over-expressing lines examined

To investigate how shade-induced flowering is affected by the presence of UV-B we monitored flowering initiation of various lines that either lack or overexpress genes that are involved in flowering and/or UV-B and light signalling. Our flowering experiments in shade +/- UV-B showed that that supplementary UV-B causes a delay in flowering time *elf3-1* and OXPIF4 (Figures 4.5 and 4.6), as well as in light signalling mutants *pif4* and *pif4pif5* (Figure 4.6) and UV-B signalling mutants *cop1-4* and *rup1rup2* (Figure 4.7).The above results are similar to the wild type phenotype (*Col-0)* suggesting that the aforementioned genes are not involved in the UV-B induced delay of flowering time. Additionally under sole vegetative shade conditions all of the lines described above flower about the same time as the wild type *Col-0* with the exception of *cop1-4* that flowers later. COP1 has been shown to be critical for the shade avoidance response in *Arabidopsis* seedlings. Under vegetative shade conditions it has been observed that *cop1-4* mutants flower slightly later than the wild type *Col-0*, even though the difference was not found to be statistically significant [216]. The conditions of vegetative shade used in the study were similar to our experiments (R:FR ratio = 0.15 and white light provided by fluorescent tubes [216]). For this reason it would be interesting to repeat the flowering experiments with the *cop1-4* mutant line in the future in order to characterize better this response. Perhaps this aggravated late flowering phenotype was due to the age of seeds that were used, which resulted in a delayed germination. For future repeats it would be better to use freshly harvested seeds and potentially grow the plants in plates first, before transferring to soil in order to ensure that all of the plants that will be transferred to soil will have germinated exactly the same time.

4.8.4 UV-B does not affect shade-induced flowering initiation in *co* and *zlf* mutants

Finally we assessed how vegetative shade-induced flowering is regulated by UV-B in the well characterized late photoperiodic flowering mutants, *co* and *zlf*. Flowering time experiments under LD in low R:FR ± UV-B growth conditions showed no difference in flowering time (Figure 4.5). Contrary to the late flowering phenotype observed in *Col-0* in response to shade + UV-B, *co* and *zlf* mutants showed insensitivity to UV-B. An explanation for this response could be due to the acceleration of flowering that occurs under vegetative shade conditions [149]. Under low R:FR conditions plants flower earlier than when subjected to plain WL [149], and so do *co* and *zlf*. But these lines are still late flowering even under shade conditions compared to the wild type *Arabidopsis*. For this reason it is possible that the late flowering effect caused by UV-B is diminished in these two lines, since their late flowering phenotype prohibits a significant difference in flowering time to occur. Actually it has been shown that *co* mutants have a lessened ability for flowering acceleration under low R:FR conditions and that this response is not only a secondary product of their late flowering phenotype but also an indication of how important the photoperiodic pathway really is when it comes to shade induction of early flowering [149]. In the same study the abundance of *FT* transcript levels was also monitored [149]. Under low R:FR both morning and evening *FT* peaks were found to be higher [149]. This response was not observed thought for the *co* mutants suggesting that *FT* induction requires *CO* [149]. When *CO* mRNA levels were monitored under shade conditions it was found that although there is a R:FR mediated increase of transcript especially at dawn, dusk and during the dark period, but overall levels do not rise as much as *FT* [149]. As it was mentioned before ZEITLUPE is crucial in order to maintain a normal circadian clock periodicity [48]. GIGANTEA (GI) is the one which stabilizes ZTL in vivo. Under a low R:FR radio *GI* transcript expression is altered [149]. Specifically there is a delay in the peak of *GI* mRNA levels after dawn [149]. Since *ZTL* is a downstream actor of *GI* [54] possibly its expression could be affected as well and possibly the entrainment of the clock under R:FR conditions could occur slightly differently. From our experiments we can conclude that probably *CO* and the *ZEITLUPE/LKP2/FKF1* genes are not involved in the UV-B induced delay of flowering time under vegetative shade conditions.

4.8.5 The effect of UV-B irradiation on flowering initiation under high and low R:FR.

Concluding the set of experiments conducted under low R:FR ± UV-B, it is important to compare the results that we acquired with the flowering results for the experiments that were conducted under WL ± UV-B in order to be able to understand the bigger picture in terms of how are the mutants used in our experiments are behaving under different R:FR conditions. Even though the WL conditions that were used between these two sets of experiments are different (50 μmol m^{-2} s^{-1} for WL ± UV-B experiments and 35 μmol m^{-2} s^{-1} for shade ± UV-B experiments) we can easily observe an acceleration of flowering time under shade for both the wild type, mutant and over expressing *Arabidopsis* lines that were used (Figure 4.8). The two wild type lines that were tested presented an early phenotype under WL + UV-B. Under low R:FR + UV-B conditions one of them (*Col-0*) presented a reverse phenotype of delay in flowering but the response that was the strongest was the one observed in high R:FR. The two *uvr8* mutant lines also behave differently under shade compared to non-shade conditions. *uvr8-1* which flowers earlier under WL + UV-B, while there is no difference in its flowering initiation under shade + UV-B, perhaps because the accelerated flowering that occurs anyway under shade does not give enough time for a specific phenotype to show up. Additionally the nature of this line's mutation might affect the phenotype. For the *uvr8-2* mutant line there is also a reversion of the UV-B induced phenotype under non shade and shade conditions, suggesting that in the absence of UVR8 different light conditions affect flowering in a different way under UV-B irradiation. This observation could lead to the hypothesis that UVR8 is necessary for the control and coordination of flowering time under UV-B irradiation conditions. The OXUVR8 on the other hand is the only line that displays the same phenotype of delayed flowering under both shade and non-shade conditions. This response supports the hypothesis that UVR8 is a negative regulator of flowering and when over-expressed delays flowering in high and low R:FR conditions.

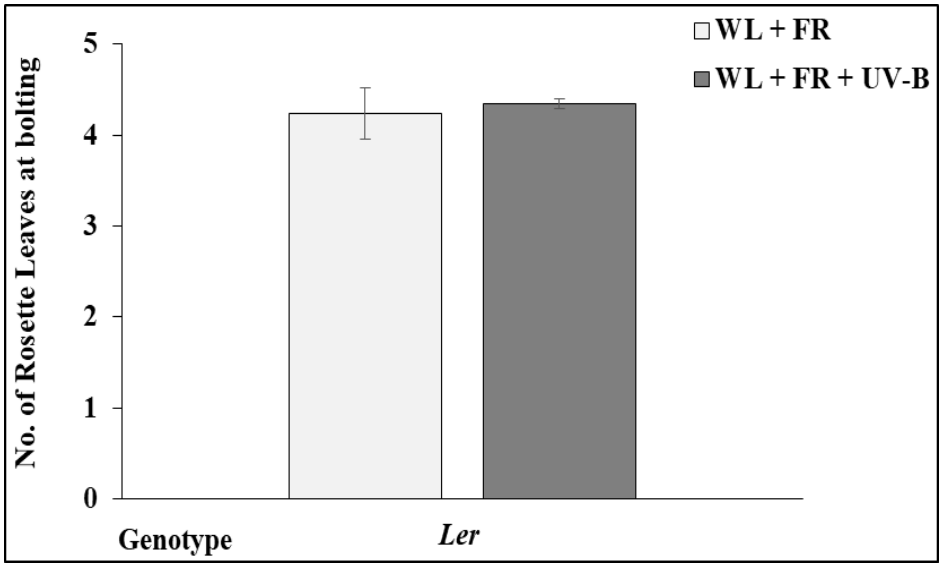
Under shade conditions most of the other mutants and the over-expressing line (*elf3-1*, *pif4*, *pif4pif5*, OXPIF4, *cop1-4*, *rup1rup2*) we used including the wild type *Col-0* displayed the same delay in flowering time under shade + UV-B, with only the exception of late flowering mutants *co* and *zlf* as mentioned before. The UV-B induced delay in flowering time is therefore a uniform response under shade and thus we can conclude that UV-B irradiation delays flowering under low R:FR supplemented with UV-B irradiation. Under high R:FR the response shifts and we can observe that UV-B causes early flowering in all of the mutant lines and the wild type

Col-0, this time with the exception of early flowering lines *elf3-1* and OXPIF4. It is interesting that *Arabidopsis* flowering mutants (late and early flowering), seem to mask the effect of UV-B in flowering. This fact could lead to the hypothesis that the flowering type of the *Arabidopsis thaliana* genotype that is being assessed for flowering initiation +/- UV-B, consists an important parameter in order for the UV-B flowering effect to be detectable. To further support this hypothesis testing the flowering initiation on other early and late flowering *Arabidopsis* lines under both high and low R:FR is recommended.

4.9 Future perspectives

For future experiments, an important next step would be to repeat the flowering experiments under different low R:FR conditions. Since vegetative shade has proven to have an acceleration effect in flowering [149], it would be very interesting to examine if lower R:FR ratios cause an equivalent acceleration of flowering time. In a study that has been published by Hayes et al, flowering experiments were conducted under shade conditions where the R:FR ratio was 0.05 [144]. Their results indicated the wild type line that was tested (*Ler*) flowered later compared to our experiments (a difference of about 2 leaves [144]), even with a lower R:FR ratio than the one used in ours studies. Still growth parameters and chambers can differ. For that reason the investigation of flowering under several shade conditions, is recommended. Additionally transcript expression analysis experiments should be conducted in order to monitor the RNA abundance of the main flowering regulators genes, such as *FT* and *CO*, which both of them are essential parts of photoperiodic flowering [18]. In addition to these factors, *SOC1* abundance could be investigated since this factor is another positive regulator of flowering [30]. *FLC* transcript levels would be really interesting to check, especially since we have already observed an upregulation of *FLC* transcript under UV-B in most of the *Arabidopsis* lines that we examined. We also observed that there is a UV-B specific repression of *ELF3* which is partially regulated by UVR8, and this is why *ELF3* transcript levels should also be examined in a future experiment. Finally, since it has been demonstrated that PIF4 and PIF5 are degraded under UV-B irradiation [144] it would be useful to evaluate their transcript levels in order to investigate if this response happens on a transcriptional level.

A.



B.

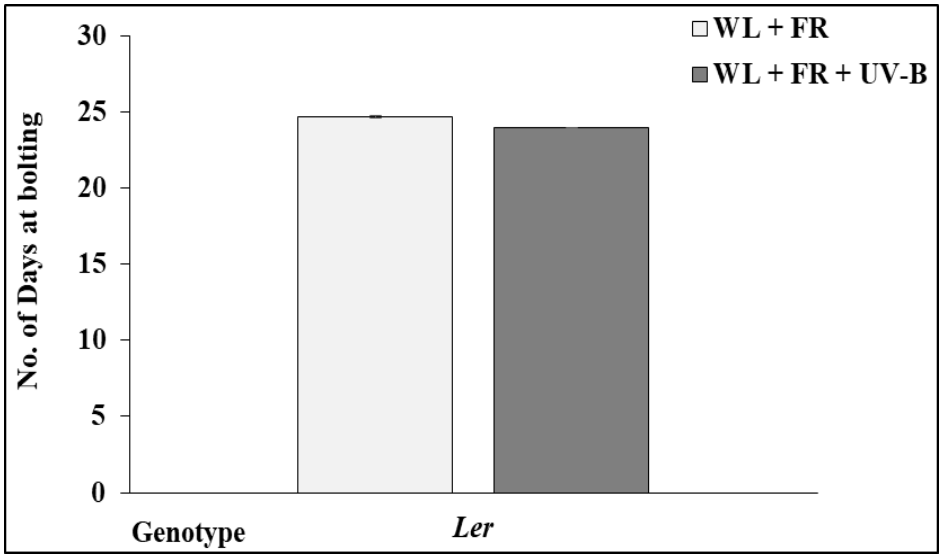
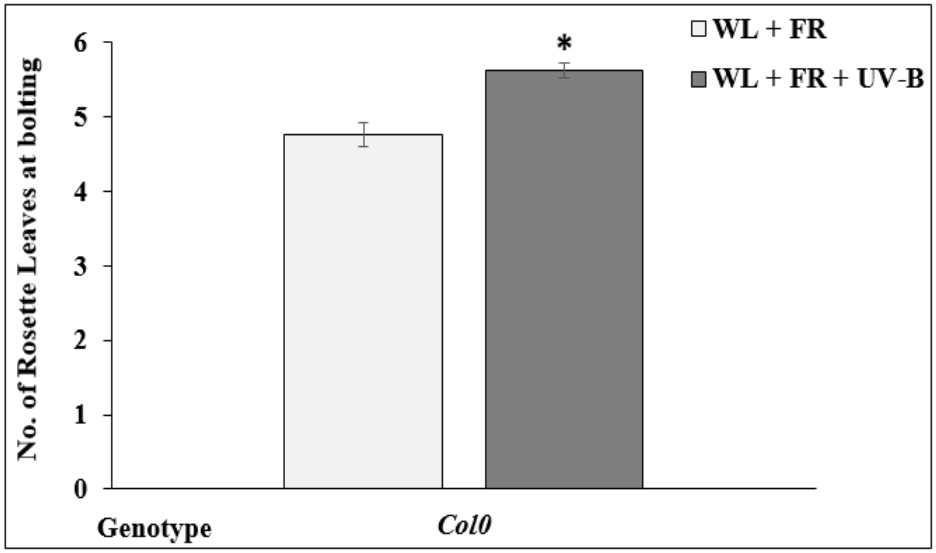


Figure 4. 1 UV-B effect on vegetative shade flowering initiation in *Ler* ecotype.

A. Flowering times (as measured by rosette leaf number) of *Ler* ecotype grown under a LD photoperiod in R:FR = 0.14 and WL (35 µmol m^{-2} s^{-1}) or under R:FR = 0.14 and WL (35 µmol $m^{-2}s^{-1}$) for 9 days and then supplemented with UV-B (0.7 µmol m^{-2} s^{-1}). Data are represented as mean ± SEM (n ≥ 15 plants recorded).

B. Flowering times (as measured by the number of days at bolting) of *Ler* ecotype grown under R:FR = 0.14 and WL (35 µmol m^{-2} s^{-1}) or under R:FR = 0.14 and WL (35 µmol m^{-2} s^{-1}) for 9 days and then supplemented with UV-B (0.7 µmol m^{-2} s^{-1}). Data are represented as mean ± SEM (n ≥ 15 plants recorded). Data are representative of two biological replicates.

A.



B.

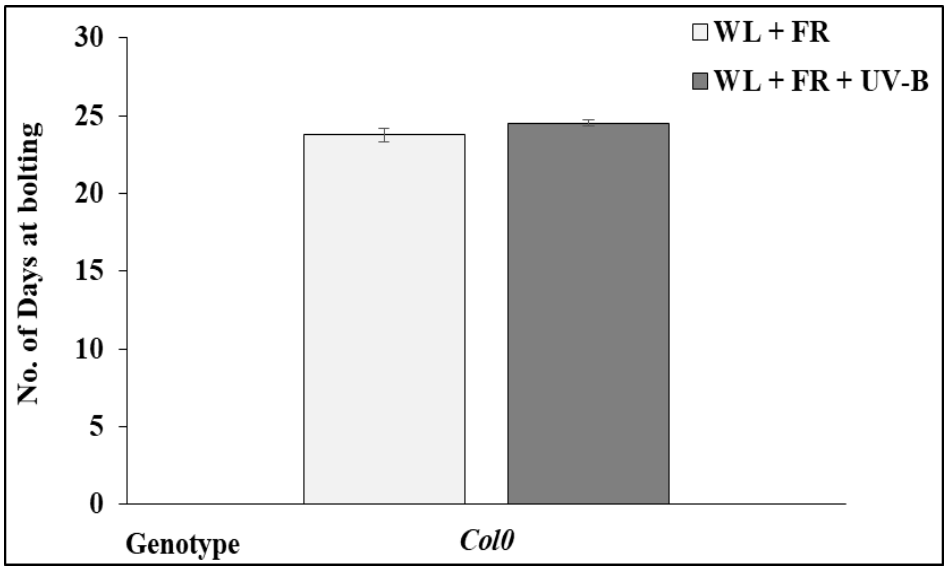
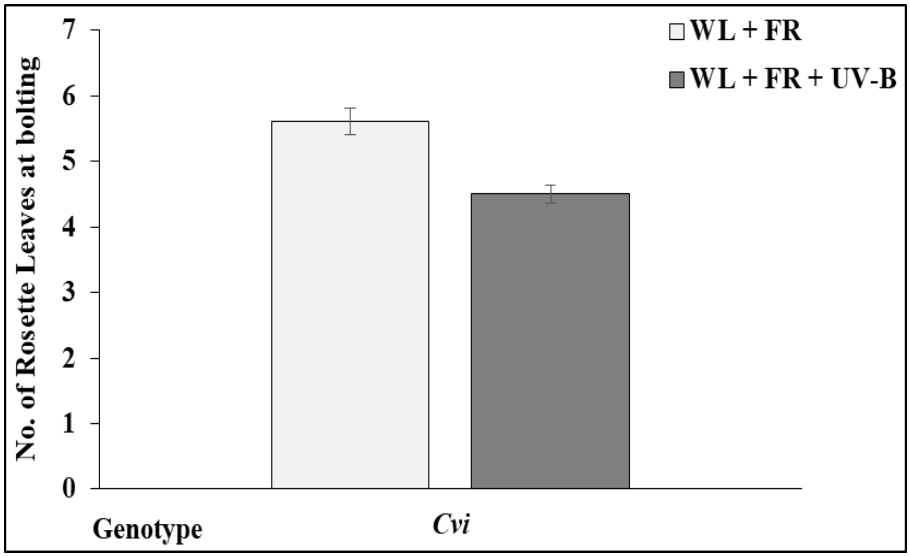


Figure 4. 2 UV-B effect on vegetative shade flowering initiation in *Col-0* ecotype.

A. Flowering times (as measured by rosette leaf number) of *Col-0* ecotype grown under a LD photoperiod in R:FR = 0.14 and WL (35 µmol $m^{-2}s^{-1}$) or under R:FR = 0.14 and WL (35 µmol $m^{-2}s^{-1}$) for 9 days and then supplemented with UV-B (0.7 µmol $m^{-2}s^{-1}$). Data are represented as mean ± SEM (n ≥ 15 plants recorded) and an asterisk (*) indicates statistically significant differences ($P < 0.05$) between means.

B. Flowering times (as measured by the number of days at bolting) of *Col0* ecotype grown under R:FR = 0.14 and WL (35 µmol $m^{-2}s^{-1}$) or under R:FR = 0.14 and WL (35 µmol $m^{-2}s^{-1}$) for 9 days and then supplemented with UV-B (0.7 µmol $m^{-2}s^{-1}$). Data are represented as mean ± SEM (n ≥ 15 plants recorded). Data are representative of two biological replicates.

A.



B.

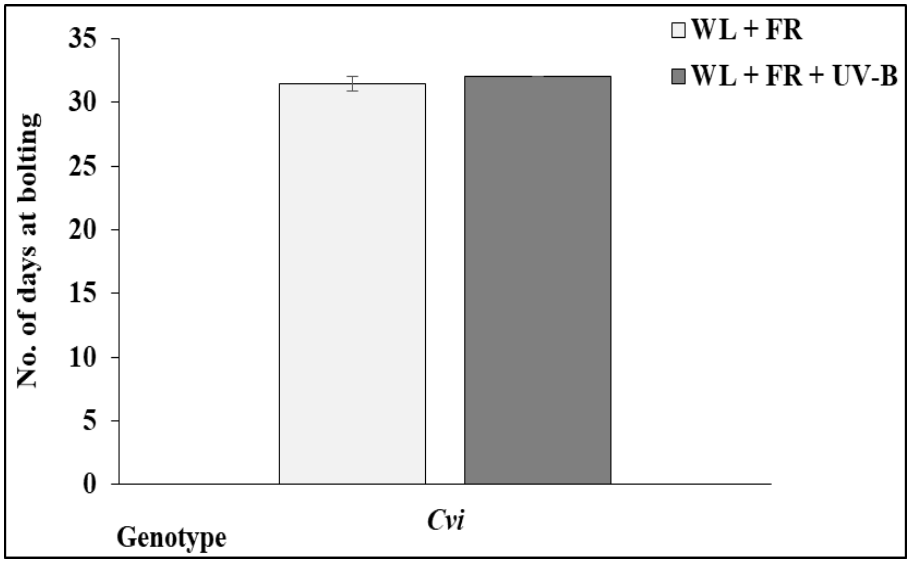


Figure 4. 3 UV-B effect on vegetative shade flowering initiation in *Cvi* ecotype.

A. Flowering times (as measured by rosette leaf number) of *Cvi* ecotype grown under a LD photoperiod in R:FR = 0.14 and WL (35 µmol m^{-2} s^{-1}) or under R:FR = 0.14 and WL (35 µmol $m^{-2}s^{-1}$) for 9 days and then supplemented with UV-B (0.7 µmol m^{-2} s^{-1}). Data are represented as mean ± SEM (n ≥ 15 plants recorded).

B. Flowering times (as measured by the number of days at bolting) of *Cvi* ecotype grown under R:FR = 0.14 and WL (35 µmol m^{-2} s^{-1}) or under R:FR = 0.14 and WL (35 µmol m^{-2} s^{-1}) for 9 days and then supplemented with UV-B (0. µmol m^{-2} s^{-1}). Data are represented as mean ± SEM (n ≥ 15 plants recorded). Data are representative of two biological replicates.

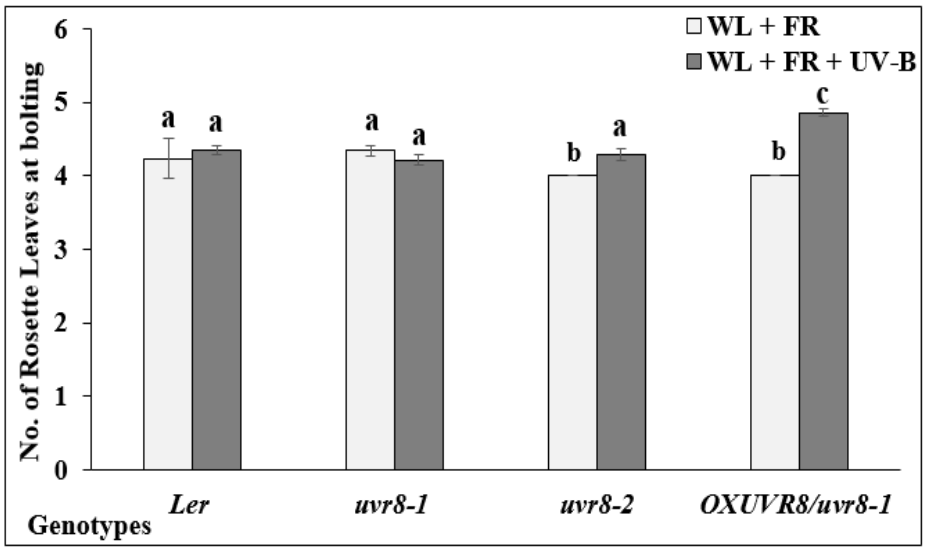


Figure 4. 4 The role of UVR8 in regulating flowering initiation under shade in *uvr8* mutants and over-expressers.

Flowering times (as measured by rosette leaf number) of wild-type (*Ler*), *uvr8-1*, *uvr8-2* and OX-UVR8 plants grown under a LD photoperiod in R:FR = 0.14 and WL (35 μmol m^{-2} s^{-1}) or under R:FR = 0.14 and WL (35 μmol m^{-2} s^{-1}) for 9 days and then supplemented with UV-B (0.7 μmol m^{-2} s^{-1}). Data are represented as mean ± SEM (n ≥ 15 plants recorded). Different letters indicate statistically significant differences ($P < 0.05$) between means. Data are representative of two biological replicates.

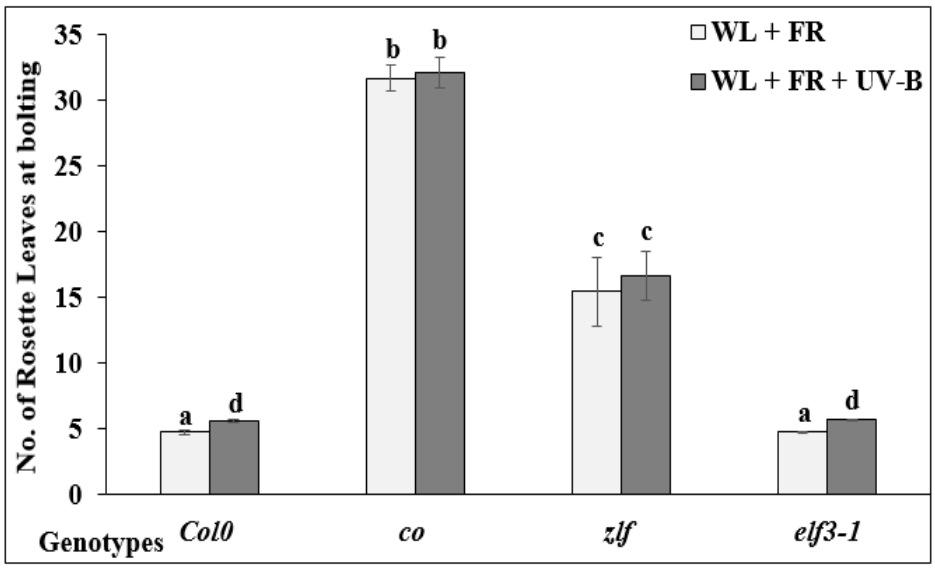


Figure 4. 5 The effect of UV-B in regulating vegetative shade flowering initiation in photoperiodic flowering mutants.

Flowering times (as measured by rosette leaf number) of wild-type (*Ler*), *co*, *zlf* and *elf3-1* plants grown under a LD photoperiod in R:FR = 0.14 and WL (35 μmol $m^{-2}s^{-1}$) or under R:FR = 0.14 and WL (35 μmol $m^{-2}s^{-1}$) for 9 days and then supplemented with UV-B (0.7 μmol $m^{-2}s^{-1}$). Data are represented as mean ± SEM (n ≥ 15 plants recorded). Different letters indicate statistically significant differences ($P < 0.05$) between means. Data are representative of two biological replicates

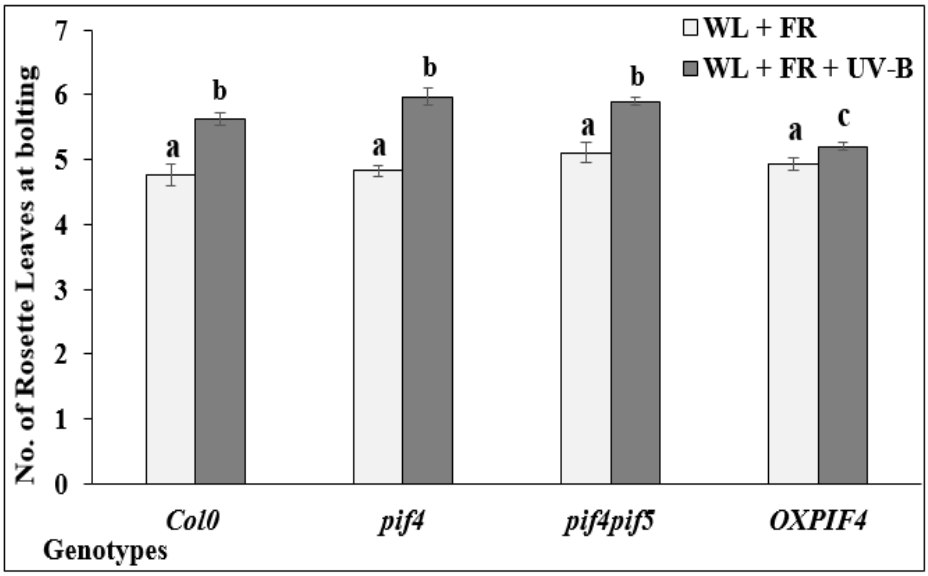


Figure 4. 6 UV-B delays vegetative shade flowering initiation in light signalling mutants. Flowering times (as measured by rosette leaf number) of wild-type (Ler), *pif4*, *pif4pif5* and OX-PIFA plants grown under a LD photoperiod in R:FR = 0.14 and WL (35 μmol $m^{-2}s^{-1}$) or under R:FR = 0.14 and WL (35 μmol $m^{-2}s^{-1}$) for 9 days and then supplemented with UV-B (0.7 μmol $m^{-2}s^{-1}$). Data are represented as mean ± SEM (n ≥ 15 plants recorded). Different letters indicate statistically significant differences ($P < 0.05$) between means. Data are representative of two biological replicates.

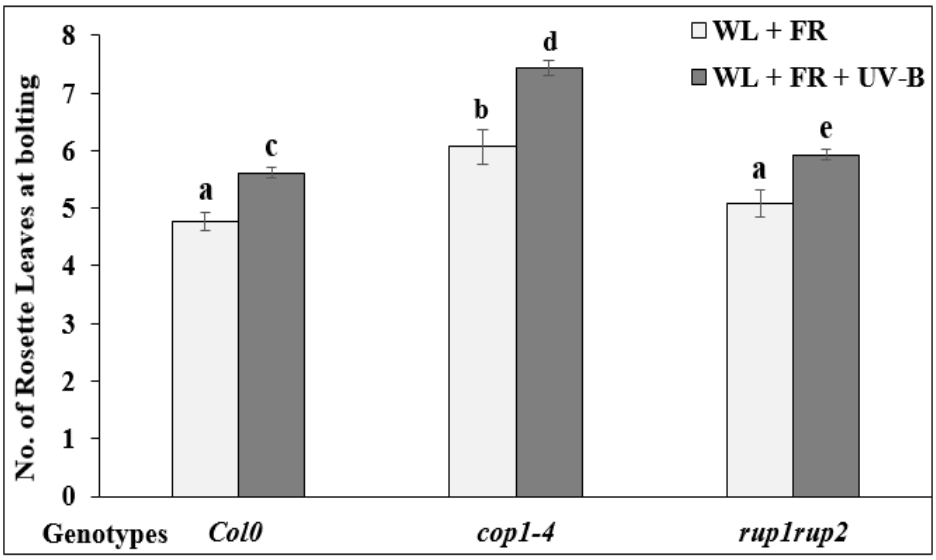
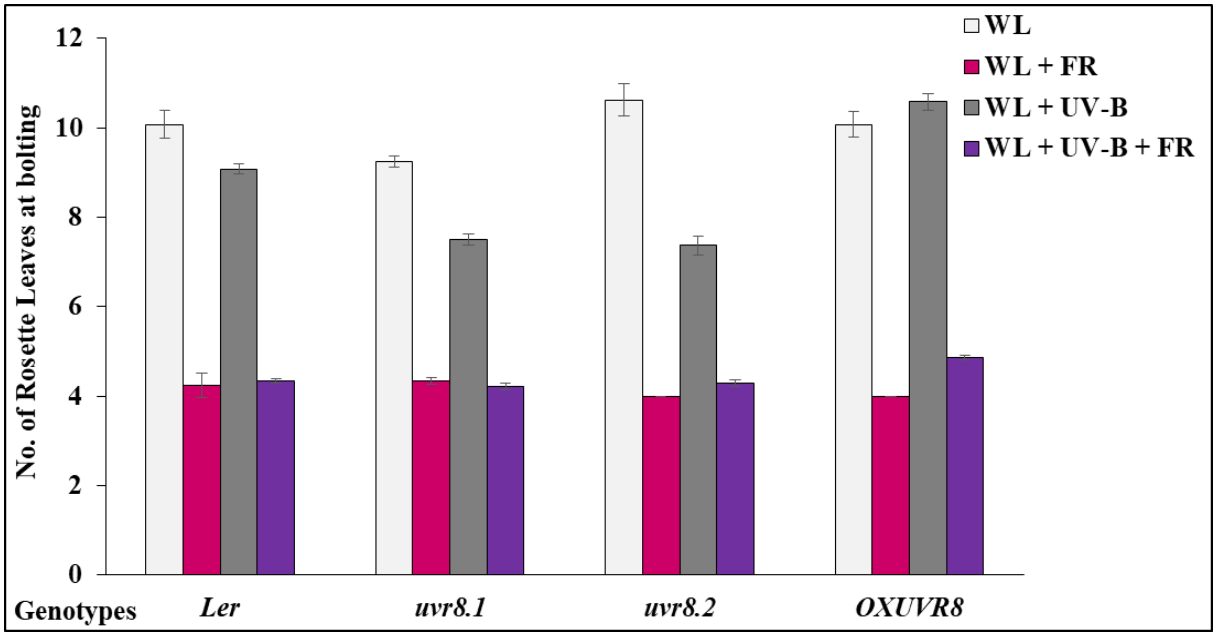


Figure 4. 7 UV-B delays vegetative shade flowering initiation in UV-B signalling mutants. Flowering times (as measured by rosette leaf number) of wild-type (*Ler*), *cop1-4* and *rup1rup2* plants grown under a LD photoperiod in R:FR = 0.14 and WL (35 µmol m^{-2} s^{-1}) or under R:FR = 0.14 and WL (35 µmol m^{-2} s^{-1}) for 9 days and then supplemented with UV-B (0.7 µmol m^{-2} s^{-1}). Data are represented as mean ± SEM (n ≥ 15 plants recorded). Different letters indicate statistically significant differences ($P < 0.05$) between means. Data are representative of two biological replicates.

A.



B.

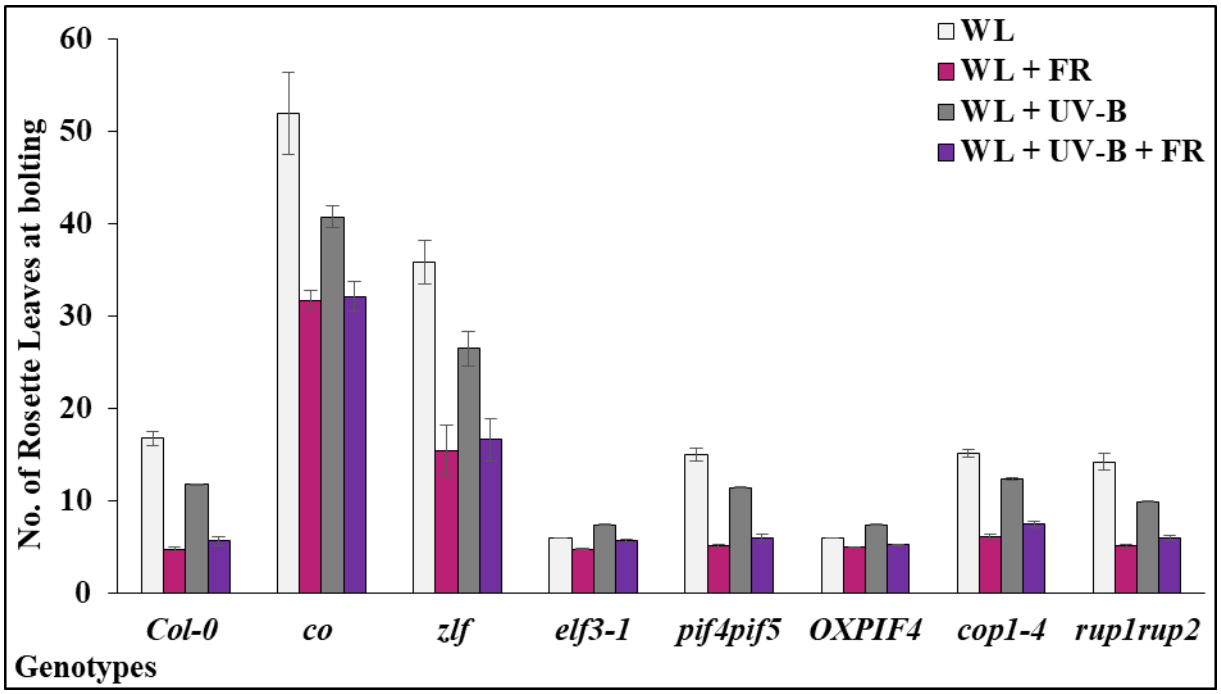


Figure 4. 8 The effect of supplementary UV-B on flowering initiation under high and low R:FR.

A. Flowering time (as measured by rosette leaf number) of wild-type (*Ler*), *uvr8.1*, *uvr8.2* and OXUVR8 plants grown under grown under a LD photoperiod in WL (50 µmol $m^{-2}s^{-1}$), WL supplemented with UV-B (0.5 µmol $m^{-2}s^{-1}$), R:FR = 0.14 and WL (35 µmol $m^{-2}s^{-1}$) or under R:FR = 0.14 and WL (35 µmol $m^{-2}s^{-1}$) for 9 days and then supplemented with UV-B (0.5 µmol $m^{-2}s^{-1}$). Data are represented as mean ± SEM (n ≥ 15 plants recorded). Data are representative of two biological replicates.

B. Flowering time (as measured by rosette leaf number) of wild-type (*Col-0*), *co, zlf*, *elf3-1*, *pif4pif5*, OXPIF4, *cop1-4* and *rup1rup2* plants grown under grown under a LD photoperiod in WL (50 μmol $m^{-2}s^{-1}$), WL supplemented with UV-B (0.5 μmol $m^{-2}s^{-1}$), R:FR = 0.14 and WL (35 μmol $m^{-2}s^{-1}$) or under R:FR = 0.14 and WL (35 μmol $m^{-2}s^{-1}$) for 9 days and then supplemented with UV-B (0.5 μmol $m^{-2}s^{-1}$). Data are represented as mean ± SEM ($n \geq 15$ plants recorded). Data are representative of two biological replicates.

Chapter 5: Investigation of potential UVR8 interactions

5.1 Introduction

UVR8 until most recently had been found to have one major interactor COP1. COP1 does accumulate in the nucleus under WL supplemented with UV-B irradiation similar to monomeric UVR8 [125]. Their interaction is essential for the activation of a gene named *HY5* and subsequently its partially redundant gene *HYH* [125]. The activation of these genes through the interaction of UVR8 and COP1 leads to the expression of genes that are UV-B responsive and encode proteins that provide both UV-B acclimation and protection as well as repair from damage cause by UV-B [125].

UVR8 has also been associated with PIF4 and PIF5, since it has been proven that these PIFs get degraded under WL that is supplemented with UV-B [144]. Nevertheless it was shown that this does not happen through a physical interaction of UVR8 with PIFs, but probably this is happening via a yet not identified pathway [144].

CO is a very important positive regulator of flowering [18]. Very recently it was shown that it can interact with RUP2 [142]. RUP2 is a protein that along with RUP1 can regulate UVR8 in a negative way by reverting it to its inactive dimeric form [125]. RUP2 was found to be able to inhibit flowering under SD conditions and WL that was supplemented with UV-B [142]. Furthermore, this recent study confirmed that RUP2 interacts with the full length CO protein in order to mediate the repression of CO activity [142]. UVR8 was shown to be important for this regulation since the lines that lacked both the RUP2 and UVR8 protein did not depict any different flowering phenotype than the wild type [142].

5.2 Does UVR8 interact with CO?

To determine a potential interaction between UVR8 and CO, we performed a yeast-two-hybrid assay. Our proteins of interest were cloned previously in the according prey (PDEST22) or bait (PDEST32) vectors. The interactions were tested under two conditions: darkness and UV-B irradiation (0.1 $\mu molm^{-2}s^{-1}$ [144]). In order to ensure the assay's efficiency known interactions were included in our assay; UVR8 homodimerisation under darkness and UVR8 - COP1 interaction under UV-B irradiation. Additionally the interaction of the protein TZP (TANDEM ZINC-FINGER PLUS 3) and ZFHD10 (ZINC-FINGER HOMEOBOX PROTEIN 10) was used as a positive control since these two proteins have a strong interaction [217] as well as the interaction between CO and GI proteins [218]. Four different concentrations of the selective factor

3-Amino-1,2,4-triazole and two dilutions of the transformed yeast cells were used. Apart from the interactions were both of the plasmids contained the desirable proteins, interactions between a protein and an empty plasmid vector were tested, in order to determine the level of auto-activation of each protein and make genuine conclusions. The results of this assay were unfortunately pretty inconclusive (Figure 5.1). The known interactions with UVR8 were not visible under neither of the conditions used, in none of the concentrations of the selective factor or the dilutions that were used (Figure 5.1 A and B). Even though we can observe some interaction between UVR8 and CO especially under darkness (Figure 5.1 A), this would be a false positive, since there is auto-activation of both proteins observed, especially when CO is fused to the binding domain (Figure 5.1 A and B). The interaction between TZP and ZFHD10 was ultimately the only one that could be observed and confirmed from this experiment (Figure 5.1 A and B).

5.3 Generation of transgenic lines to assess the role of UVR8 on CO protein abundance

As described above we wanted to have a better insight on the impact of UVR8 on the abundance of CO protein. Since we did not possess a functional CO antibody at the time, the process of generating transgenic lines was considered necessary. The first step was to transform the vector that was fused to our protein of interest CO – labelled also with a mCitrine (YFP) fluorescent tag – in competent *A. tumefaciens* cells and then to *uvr8* mutant *Arabidopsis* through a floral dip transformation. Afterwards for the next two generations seeds collected from each individual plant were grown and selected with the appropriate antibiotic. T2 generation was subdued to a 3:1 selection according to Mendelian genetics and the lines with the best p value (< 0.05) were selected to be grown further. Additionally western blot analysis was conducted using an anti – GFP antibody in order to verify the presence of CO protein (Figure 5.3 A – a band is present around 70 kDa which is the expected size of CO (41, 986 kDa) tagged with YFP (26, 4 kDa)). T3 seeds that were harvested from the independent lines selected previously were selected for 100% resistance to the appropriate antibiotic to ensure they are now homozygous for the *CO* insertion. Western blot analysis was once more conducted to confirm the existence of CO protein (Figure 5.3 B). The selected seeds were grown further to provide the T4 generation which would be 100% homozygous CO-mCitrine in *uvr8.1* background.

5.4 Generation of transgenic lines to assess a genetic interaction between UVR8 and PIFs

In order to access further if PIFs have a more active role in UVR8 induced flowering we initiated the generation of UVR8-GFP/ *pif4pif5* transgenic Arabidopsis lines. In order to achieve that, we transformed the appropriate vector containing UVR8 fused with a GFP tag first in competent *A. tumefaciens* cells and subsequently to *pif4pif5* mutant *Arabidopsis* through a floral dip transformation. The T2 and T3 generation of transgenic seeds were selected exactly as described previously. Western blot analysis was performed for each generation of *Arabidopsis* in order to verify the existence of UVR8 protein (Figure 5.2) A and B – a band is present around 75 kDa which is the expected size of UVR8 (47, 118 kDa) tagged with GFP (26, 9 kDa)). The seeds originating from each selected line were grown further to provide the T4 generation which would consist of 100% homozygous UVR8-GFP in *pif4pif5* background.

5.5 Isolating and genotyping *uvr8* mutant alleles in *Col-0*

As mentioned before flowering experiments using an *uvr8* mutant allele in the Arabidopsis *Col-0* background was essential for our study. For this reason, we acquired *uvr8-6* (SALK_033468) seeds. Genotyping of *uvr8-6* was performed and results are depicted in Figure 5.3. Specifically, we were able to identify six independent lines that were homozygous for the *uvr8-6* mutant allele depicted with numbers: 1, 4, 5, 9, 12 and 14, in Figure 5.3. Three of them were selected to be tested in a flowering experiment, the results of which have been described in Chapter 3.3.

5.6 Discussion

The study in this chapter is mainly focused in the investigation of possible UVR8 protein interactors either when UVR8 is inactive under darkness or active under UV-B irradiation. The approach used was based on the identification of protein interactions using the yeast-two-hybrid system. Our results from this assay were inconclusive and we were not able to identify any true interactions. The conditions we used to test the reactions under UV-B light have been proven successful to mediate UVR8 – COP1 before [144]. However, in this study an alternative yeast-two-hybrid system was used (Mav203 yeast strain instead of AH109; pDEST22 and pDEST32 instead of pGBKT7 and pGADT7 encoding Gal4 – AD) [144]. The selection on the plates was also different from our study, since we used 3-Amino-1,2,4-triazole as a selective factor in plates that lacked Tryptophan (W) and Histidine (H), while in the study by Hayes et

al. selective plates lacking Tryptophan (W) and Histidine (H) were used as low-stringency in order to detect weaker interactions and selective plates lacking Tryptophan (W), Histidine (H) and Adenine (A) were used as high-stringency for the detection of stronger interactions [144]. The difference in the yeast and selection systems used may be the reason that our results that derived from this assay were inconclusive. It is possible that the plasmids we used as vectors affected the ability of some proteins to interact with each other. In the future if this assay is repeated the yeast-two-hybrid system from the original study would be the best option for carrying out the experiment. The protein expression levels of all constructs expressed in yeast was confirmed by western blot analysis (data not shown).

5.7 Future Work

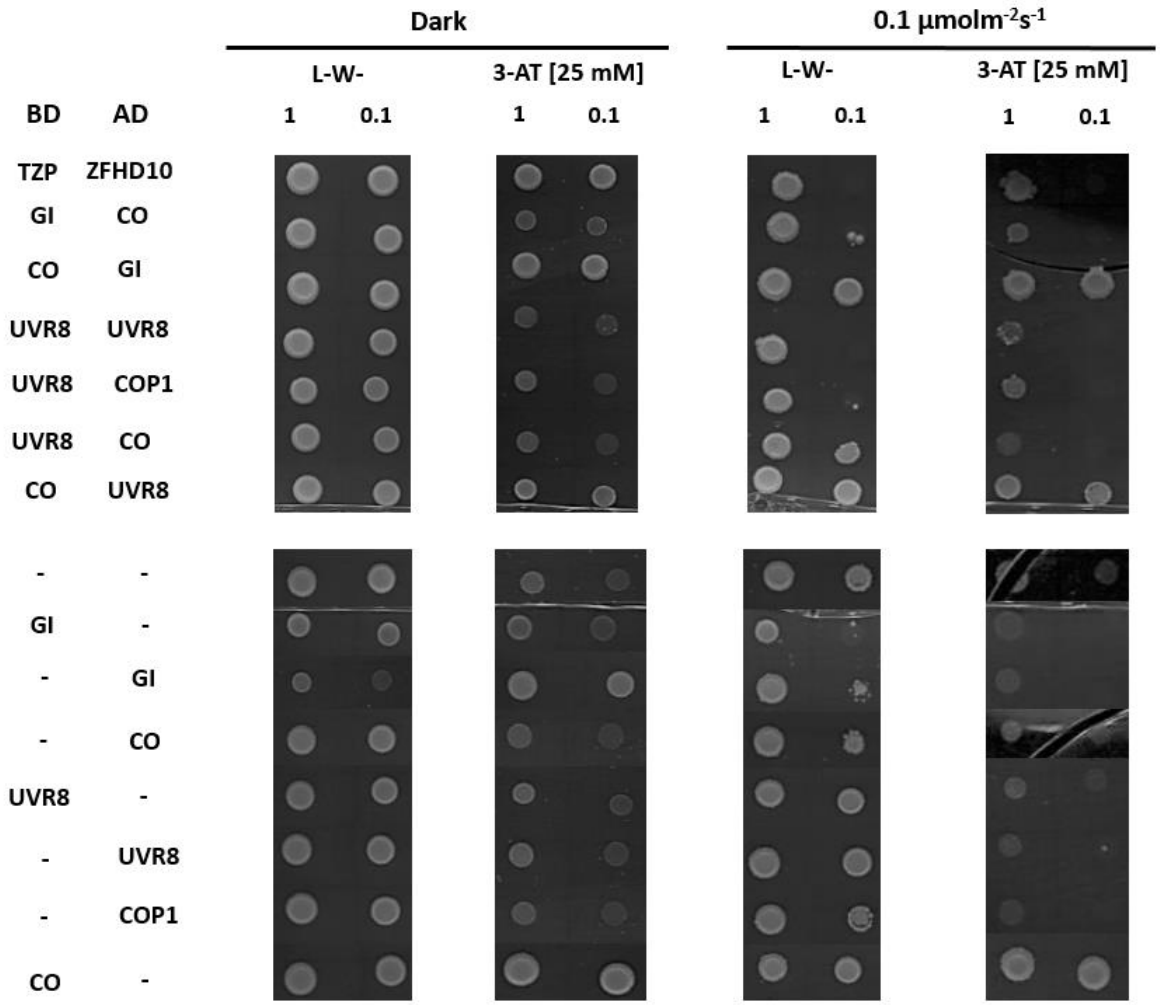
Further work would be required in order to investigate possible interactions between UVR8 and other proteins. Another way to investigate this would be to perform protein complex immunoprecipitation to evaluate if there is an immediate interaction of CO with UVR8. For this cause, protein extracts could be isolated from transgenic lines expressing GFP-UVR8 and/or CO-RFP with the appropriate controls in the presence or absence of UV-B. An anti-GFP antibody could be used to pull-down GFP-UVR8 and an anti-RFP antibody to assess if CO-RFP is immunoprecipitated with UVR8-GFP. This assay could also be performed using the *Nicotiana benthamiana* transient expression system. A third alternative method that could be considered is the Bimolecular Fluorescence Complementation (BiFC), where protein interactions can be investigated through the reconstitution of the emission of a fluorescent signal [219]. This system has been used before to investigate UVR8-COP1 interactions [141]. Since UVR8 and CO are both nuclear proteins [122] [220], the nuclei of cells cotransfected with a C-terminal fluorescent protein (such as cCFP) fused with UVR8 and an N-terminal fluorescent protein (such as nYFP) fused with CO plasmids, could be investigated for the detection of fluorescence signal, suggesting reconstitution of the fluorophore upon interaction of the two proteins.

For the case of the CO-mCitrine/*uvr8.1* transgenic *Arabidopsis* line we generated, the idea behind constructing this line was to investigate if UV-B mediated early flowering and induction of *FT* occurs through an effect of UVR8 on CO protein abundance. During the realization of the current project though, a study by Arongaus et al. was able to confirm that RUP2 can interact with the full length of CO in order to mediate the repression of CO activity and thus lead to the inhibition of flowering under SD in WL that has been supplemented with UV-B [142],

suggesting that probably since RUP2 interacts with CO in a UVR8 dependent manner there is no immediate interaction between the photoreceptor protein and CO, but nevertheless it would be interesting to test the hypothesis of this potential interaction. Once the final generation of fully homozygous seeds will be collected further experiments can be performed including this transgenic line. An experiment that could be designed using the CO-mCitrine/*uvr8.1* transgenic *Arabidopsis* line is the quantification of CO protein levels of this line compared with a CO-mCitrine/*Col-0 Arabidopsis* line (provided by Professor's Lucio Conti group) as a control (after the exposure of plants under LD of UV-B irradiation and collection of tissue at ZT 0.5, since it is the timepoint for which we have observed the most significant changes in the expression of flowering regulator genes under UV-B). The above could be achieved through the detection of the protein with an anti-GFP antibody followed by quantification of each line's western blot analysis, in order to compare the relative protein abundance and make suggestions about the possible interaction of UVR8 and CO proteins.

As mentioned earlier UVR8-GFP/*pif4pif5* transgenic lines were also constructed. This line was generated in order to investigate further the relation between UVR8 and PIF4, since PIF4 is able to control flowering initiation under high temperatures (27 ºC – 28 ºC) by activating directly *FT* gene that promotes flowering [212]. Specifically PIF4 is able to bind to the promoter of *FT* in a temperature-dependent manner [212]. Under high temperatures supplemented with UV-B irradiation, the interaction of UVR8 and COP1 supresses the accumulation of *PIF4* transcript and subsequently of PIF4 protein [89]. At 28 ºC PIF4 protein is not completely degraded but its function is severely affected [89]. Additionally under cooler temperatures UV-B irradiation initiates degradation of PIF4 and PIF5 [144]. Utilizing the UVR8-GFP/*pif4pif5* line we could perform flowering experiments monitoring the effect of UVR8 overexpression in flowering initiation and *FT* transcript abundance the absence of PIF4 and PIF5 under WL and WL that is supplemented with UV-B and in response to different temperatures (22 ºC versus 28 ºC). This experiment would asses if PIF4 and PIF5 are important for mediating UVR8-induced UV-B-dependent control of flowering. A triple *uvr8 pif4 pif5* mutant would also address a possible genetic interaction among these proteins. Furthermore, direct binding of GFP-UVR8 on the *FT* promoter could also be examined by Chromatin Immunoprecipitation assays on GFP-UVR8/*uvr8* and UVR8-GFP/*pif4pif5* lines. Again, the necessity of the PIFs would be assessed for this binding in UVR8-GFP/*pif4pif5*.

A.


Dark
0.1 µmolm-2s-1
L-W-
3-AT [25 mM]
L-W-
3-AT [25 mM]
BD
AD
1
0.1
1
0.1
1
0.1
1
0.1
TZP ZFHD10
GI CO
CO GI
UVR8 UVR8
UVR8 COP1
UVR8 CO
CO UVR8
- -
GI -
- GI
- CO
UVR8 -
- UVR8
- COP1
CO -

B.

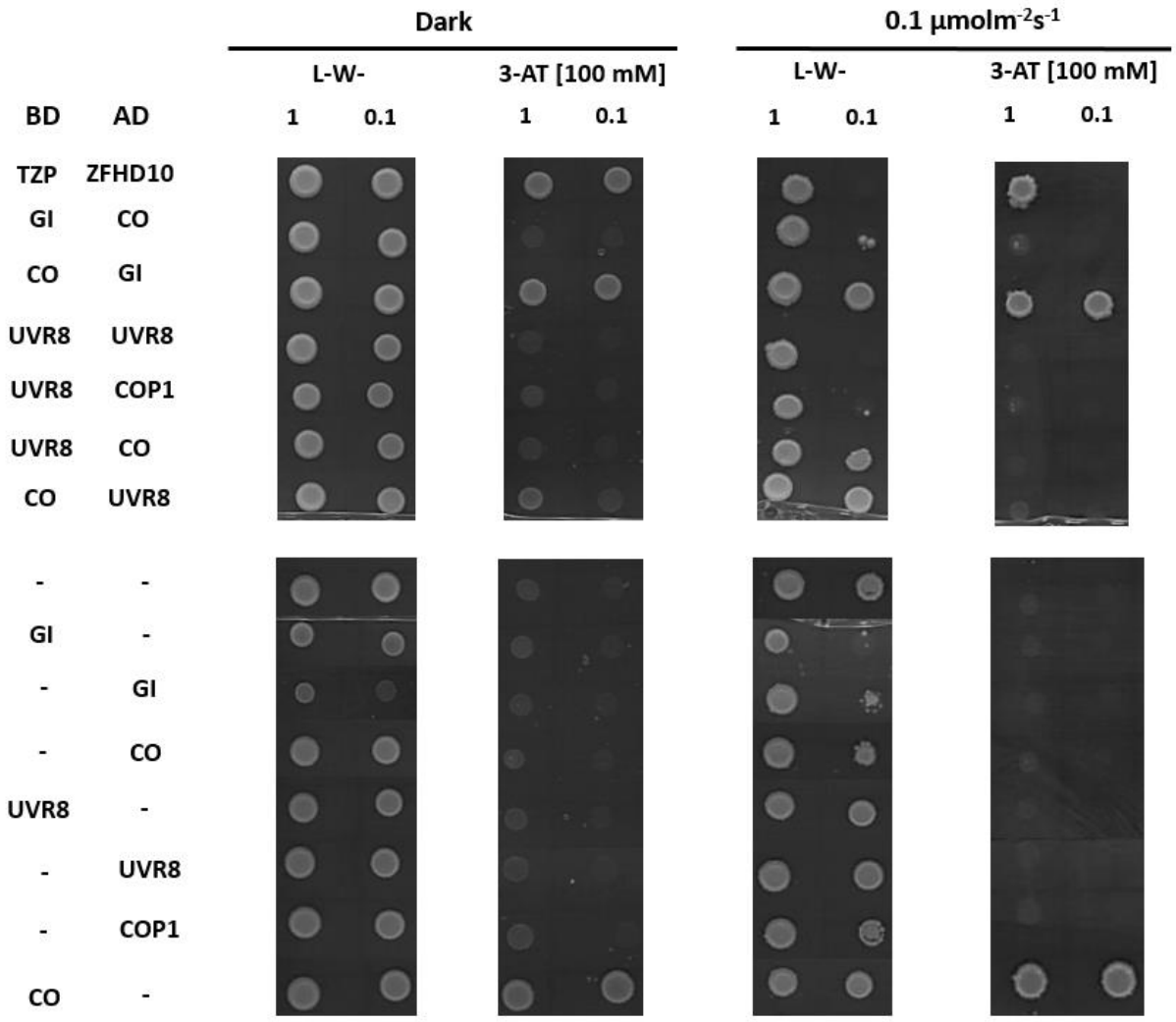
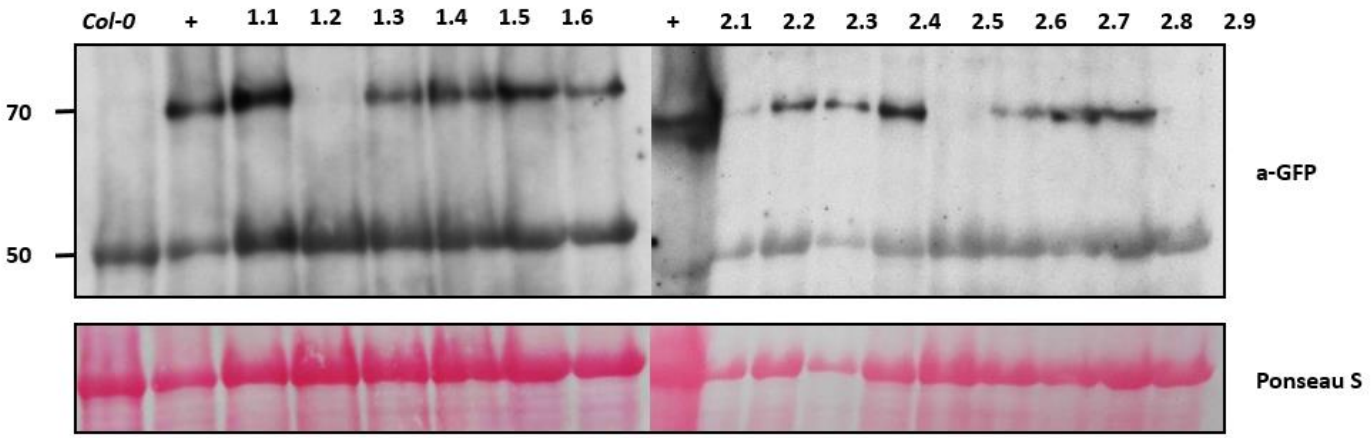


Figure 5. 1 Yeast – two – hybrid analysis of putative UVR8 interactions.

A. and **B.** Yeast-two-hybrid analysis of interactions between the indicated test proteins fused to pDEST32-UVR8 (GAL4 DNA binding domain -bait) and pDEST22; GAL4 activation domain s- prey) on media selecting for positive transformation (L^-W^-) and media selective for a positive interaction ($L^-W^-H^-$ 25 mM 3-AT) (A) or ($L^-W^-H^-$ 100 mM 3-AT) (B). 3-AT = 3-Amino-1,2,4-triazole. Two dilutions (decreasing from left to right) are shown for each light treatment.

A.



B.

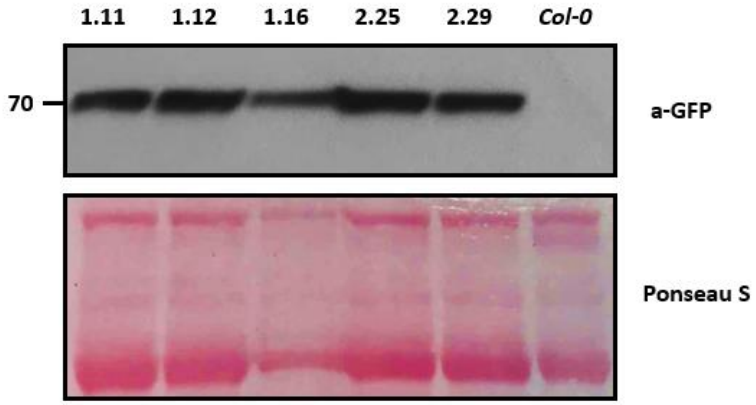


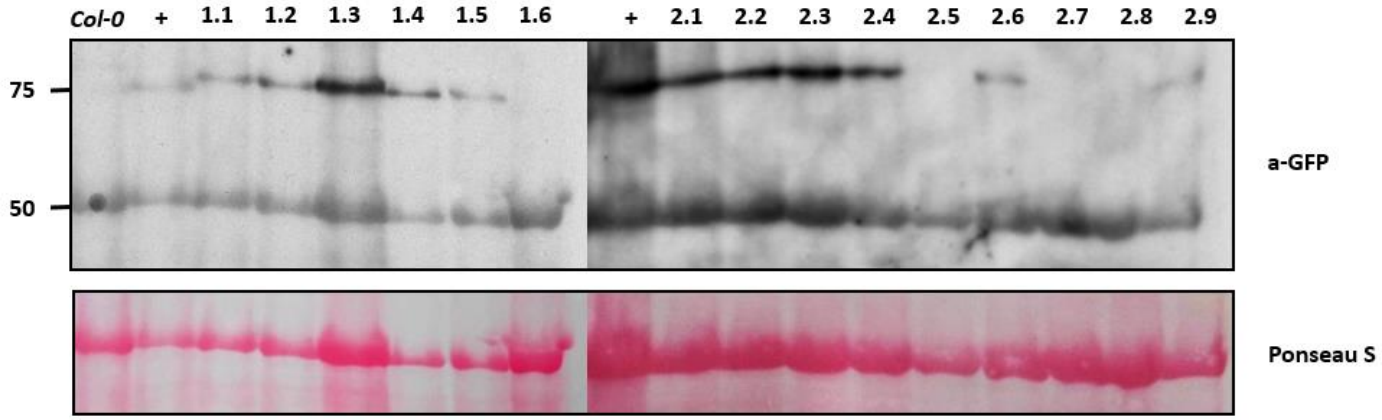
Figure 5. 2 Monitoring CO-mCitrine abundance in CO-mCitrine/*uvr8.1* transgenic lines.

A. Western blot analysis of CO-mCitrine protein levels of independent T2 transgenic *Arabidopsis* lines showing 3:1 antibiotic resistance segregation in order to determine the lines that carry single GFP-CO insertions. Different initial numbers indicate origin from different parental *Arabidopsis* plants and + indicates the positive control that was used.

B. Western blot analysis of CO-mCitrine protein levels of independent T3 transgenic *Arabidopsis* lines showing 100% antibiotic resistance segregation in order to determine the lines that are homozygous for the GFP-CO insertion. Different initial numbers indicate origin from different parental *Arabidopsis* plants.

Total protein was extracted from wild type (*Col-0*), GFP-UVR8 (positive control) and independent T2 and T3 CO-mCitrine / *uvr8.1* transgenic lines grown under WL. A Ponceau Stain (RbcL) is shown below as a loading control and an anti-GFP antibody [159], was used for detecting CO-mCitrine protein.

A.



B.

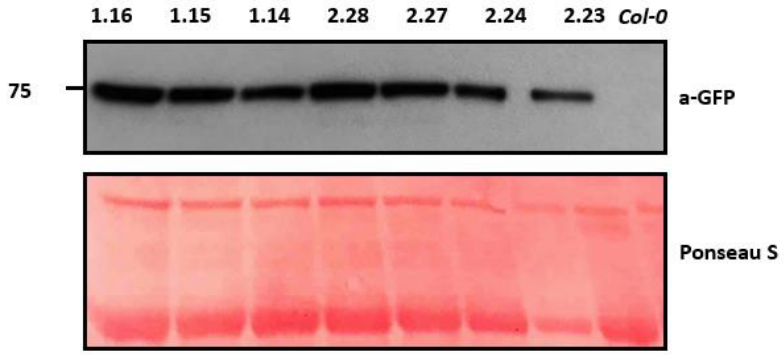
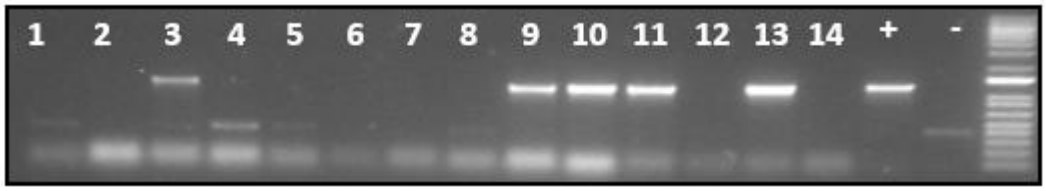


Figure 5. 3 Monitoring UVR8-GFP protein levels in UVR8-GFP /*pif4pif5* transgenic lines.
A. Western blot analysis of UVR8 levels from T2 transgenic *Arabidopsis* levels of independent T2 transgenic *Arabidopsis* lines showing 3:1 antibiotic resistance segregation in order to determine the lines that carry single GFP-UVR8 insertions. Different initial numbers indicate origin from different parental *Arabidopsis* plants and + indicates the positive control that was used.
B. Western blot analysis of UVR8 levels from T2 transgenic *Arabidopsis* levels of independent T3 transgenic *Arabidopsis* lines showing 100% antibiotic resistance segregation in order to determine the lines that are homozygous for the GFP-UVR8 insertion. Different initial numbers indicate origin from different parental *Arabidopsis* plants.

Total protein was extracted from wild type (*Col-0*), GFP-UVR8 (positive control) and independent T2 and T3 GFP-UVR8/*pif4pif5* transgenic *Arabidopsis* lines grown under WL. A Ponceau Stain (RbcL) is shown below as a loading control and an anti-GFP antibody [159], was used for detecting GFP-UVR8 protein.

A.



B.

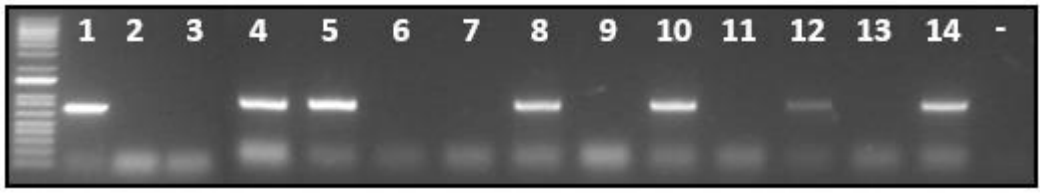


Figure 5. 4 Genotyping of homozygous *uvr8-6* (*Col-0)* plants.

A. PCR analysis of possible homozygous *uvr8-6* T-DNA insertion lines in a *Col-0* background, using *UVR8* gene specific primers in combination with the left border primer LBb1.3.

B. PCR analysis of possible homozygous *uvr8-6* mutant lines, using *UVR8* gene specific primers. Plants were grown under a LD photoperiod of WL. + and – represent the positive and negative sample reactions (negative reactions did not contain any DNA, while the positive one contained DNA of a sample already known to bind within the primers' region). Number from 1 to 14 represent the number of individual plants that was examined. As homozygous *Arabidopsis* plants are considered the ones that presented one single band when amplified with the *UVR8* specific primers and no band when amplified with the *UVR8* gene specific primers in combination with the left border primer LBb1.3. As heterozygous lines are considered the ones that presented a band when amplified with both primer combinations. As wild type plants are considered the ones that depicted a single band when amplified with the *UVR8* gene specific primers in combination with the left border primer LBb1.3. The ladder that was used is the Quick-Load Purple 1 kb DNA Ladder from BioLabs.

Chapter 6: Final Discussion

6.1 Introduction

UV-B is an integral part of the electromagnetic spectrum that reaches the earth and is thus present in everyday natural sunlight. Plants receive UV-B irradiation on a daily basis, and for this reason they have developed mechanisms of photo-protection when the UV-B intensity is high enough to provoke the initiation of deleterious effects to the plant, as well as acclimation and photomorphogenic responses under non-damaging levels of UV-B [115] [3] [114]. UVR8 is responsible for perceiving UV-B irradiation and responding to it, since so far, is the only genetically encoded known UV-B receptor that exists in nature [136]. UVR8 can act as a regulator of gene transcription by initiating transcriptional events that lead to the biosynthesis of flavonoids -which act as the plant's sunscreen- , and, damage repair proteins [124].

Even though the importance of UV-B radiation and its receptor UVR8 in regulating plant photoprotection and photomorphogenesis are already established [114] [136] [115], there is not much known about the role of either in controlling flowering initiation. Flowering is the transition of the plant from vegetative to reproductive development and of course is one of the most crucial events in a plants life-cycle [18]. For this reason, the possible effect of UV-B radiation in flowering initiation was examined. Furthermore, since UV-B has been shown to modulate vegetative shade responses with regards to hypocotyl elongation and petiole positioning, the effect of UV-B on shade-induced flowering initiation was also tested. Additionally, the role of UVR8 in this process was also investigated, as a means of providing information for a potential mechanism of UVR8 action in determination of flowering time. Our approach included flowering experiments for monitoring of flowering time initiation and transcription expression analysis that were conducted in specific timepoints of the day (ZT05 and ZT15), in order to also monitor the diurnal periodicity of this responses. Our data suggested that early in the day is when we can observe most of the UVR8 mediated UV-B responses regarding the transcript abundance of the flowering-related factors of our interest, compared to later in the day when these effects of UV-B are not so evident. This observation was in agreement with results from previous flowering experiments which have been conducted in our group by past lab members (Kaiserli lab unpublished data). Even though for some of the genes we tested their peak expression time is not in the early morning this is the timepoint we observed that the effect of UV-B expression on *FT* was more profound (data not shown).

6.2 UV-B affects photoperiodic flowering differently in various *Arabidopsis* ecotypes

Flowering experiments were conducted under the presence or absence of UV-B irradiation that was non-damaging to the plants. The intensity of the radiation that was chosen reflects realistic UV-B irradiation conditions, since it was measured as the intensity a plant in Glasgow would receive on a moderately sunny day. In order to ensure that the chosen amount of UV-B irradiation did not induce any stress responses which could potentially cause early flowering, we monitored the expression levels of the stress-induced gene *COR15*, and showed no UV-B induced expression under the conditions used for our flowering experiments (Figure 3.2)

In this study the determination of flowering time was achieved through the calculation of the number of rosette leaves each plant had at the day the first flower bud appeared. Our results suggested that out of the three *Arabidopsis* accessions that were tested two of them (*Ler* and *Col-0*) depicted an early flowering phenotype when irradiated with UV-B (Figure 3.3) while the third one (*Cvi*), depicted a late flowering phenotype instead (Figure 3.4). On top of this observation, transcript expression analysis on some of the main flowering regulating genes (*FT*, *CO*, *SOC1*, *FLC*) suggested that a concomitant UV-B dependent upregulation of *FT* and *CO* is observed in *Ler* and *Col-0* ecotypes (Figure 3.5), while *FT* gets downregulated in the late flowering *Cvi* accession (Figure 3.7.A).

This data indicate that different environments of origin are crucial for adaptation of different *Arabidopsis* responses. All three ecotypes originate from very different growth conditions. *Cvi* is an islandic population and Cape Verde islands are known to exhibit a particular geography that includes volcanic and plutonic rocks [164] as well as a photoperiod of 12h of light and 12h of darkness all year long (https://www.timeanddate.com/sun/cape-verde). Additionally the climate in Cape Verde islands demonstrates hardly any changes in temperature during the course of a year – the temperature during the hottest months does not exceed 28 ºC and the colder months does not drop below 23 ºC – (https://www.holiday-weather.com/cape_verde/averages). On top of that *Cvi* commonly found altitudes and latitudes differs from the ones of *Ler* and *Col-0* (data from The *Arabidopsis* Information Resource). Cvi can be found in higher altitudes compared to *Col-0* and shorter latitudes compared to *Col-0* and *Ler* –which exhibit vert similar altitudes– (data from The *Arabidopsis* Information Resource). All the above factors have potentially resulted in this particular phenotype to have developed different characteristics when it comes to flowering time regulation. A possible explanation could be that some ecotypes interpret UV-B irradiation as a probable harmful factor even when

the intensity of the irradiation is not harmful yet but is still perceived as an alarming factor especially if these ecotypes experience a variation of photoperiodic length during the course of the year like *Ler* and *Col-0*. In this case early flowering might be triggered as a way of the plant to secure its reproduction. On the other hand, ecotypes like *Cvi* which face no change in photoperiodic length and probably experience less variation in the sunlight they perceive, might have adapted differently in interpreting UV-B irradiation signals. In any case it has been found that it is very common for *Arabidopsis thaliana* species to develop variations both phenotypically and genetically, due to their wide distribution [163]. It would be interesting to examine the genetic variations exhibited in the *UVR8* locus in the Cvi ecotype.

Nevertheless it has been also observed that under UV-B irradiation both *Ler* and *Col-0* exhibit a late flowering phenotype compared to the corresponding plants grown solely under WL [143] [144]. It is important to note though, that even if these results do not agree with our own observations, the experimental conditions that have been used in every case were significantly different. We conducted our flowering experiments under a continuous irradiation of UV-B under LD photoperiods, while in the case that *Col-0* observed a delayed phenotype under UV-B [143], experiments were conducted both under LD and SD conditions under an irradiation intensity almost 20 times greater than ours. Furthermore the duration of the UV-B irradiation was not continuous, but only covered a single hour interval during the day [143]. On the other hand the conditions where *Ler* was found to manifest an early flowering phenotype under UV-B irradiation, were also slightly different than the growth conditions in our own experiments, since the intensity of the irradiation and the commencement of the UV-B treatment differed [144]. The variability of all the described conditions, has probably contributed to the variation of phenotypes observed in all three studies.

6.3 UVR8 acts as a negative regulator of flowering

In case of UVR8 we already know that it is the only genetically encoded UV-B photoreceptor known to date [4], but its role in flowering initiation has yet to be determined. In order to better understand if UVR8 actually affects flowering initiation, we investigated flowering time of plants that either lack or over-express UVR8. Our results suggest that UVR8 can act as a negative regulator of flowering. This conclusion was based on the early flowering phenotype that *uvr8* mutant alleles demonstrate when grown under WL that was supplemented with UV-B compared to growth solely under WL conditions (Figure 3.8.A). On the contrary, plants that over-expressed UVR8 demonstrated a delay in flowering initiation and even though it was not

calculated as statistically significant, we can still see that these plants flower later than the wild type (Figure 3.8.A).

These observations are in agreement with our transcript analysis findings since transcript abundance of both flowering inducers *FT* and *CO* was upregulated in *uvr8* mutants, whilst downregulated in UVR8 overexpressors (Figure 3.8.B), suggesting that these two factors are involved in UV-B dependent regulation of flowering. The flowering promoter *SOC1* was excluded for participating in this response since there was no significant fluctuation in its transcript levels between the different phenotypes tested in the presence and absence of UV-B (Figure 3.8.B). The flowering repressor *FLC* was also excluded from participating in this response for the same reason. Interestingly though, a UV-B specific upregulation of this factor was observed in all plant phenotypes tested (Figure 3.8.B) suggesting that UV-B might trigger a morning specific induction of *FLC,* which ultimately does not cause a flowering time delay, in LD but could have a role in SD or vernalisation.

The effect of UV-B radiation in flowering was investigated by Dotto et al. Even if some of our observations are not in agreement with that study, there were some common responses such as an upregulation of *FT* and *FLC* transcript levels and unchanged *SOC1* transcript levels of *uvr8* mutants grown under WL + UV-B compared to WL [143]. The study by Dotto et al. focused mainly on a possible mechanism that leads to *FLC* upregulation through the control of the age flowering pathway that ultimately delays flowering in their experimental conditions under UV-B irradiation [143].

The significance of our findings is that we provide evidence that another flowering pathway in *Arabidopsis*, the photoperiodic one could potentially be involved in the regulation of flowering time under UV-B irradiation, through UVR8-specific mediated responses.

Further investigation of flowering regulator genes revealed that there is a UV-B specific repression of *ELF3* and is at least partially regulated by UVR8. Wild type plants that were grown under UV-B depicted a decrease in *ELF3* transcript levels. This UV-B specific decrease was not present in *uvr8* mutants but the *ELF3* transcript levels of this line were found to be downregulated under bot WL and WL+UV-B. On the other hand, in UVR8 over-expressing lines *ELF3* transcript levels were once again significantly reduced in response to UV-B (Figure 3.9). Since ELF3 is a core clock component, this data provide information that the circadian clock could also be involved in the regulation of flowering under UV-B irradiation. An interaction between UV-B and circadian clock light signalling pathways has already been demonstrated [140].

Transcript analysis was also conducted in order to determine if there are differences in transcript abundance of *PIF4*, which encodes yet another transcription factor that has been associated with flowering and is knowingly degraded under UV-B irradiation. Our results suggest that there is no significant effect of UV-B on *PIF4* transcripts (Figure 3.10). This is not surprising as PIF4 may be regulated primarily at the protein level, but there is also a possibility that the reduction in PIF4 protein levels. UVR8 is probably involved in this process since it has already been published that PIF4 and UVR8 directly interact [144].

6.4 UV-B accelerates flowering in a variety of flowering and signalling mutants

A great part of this work also focused on determining the effect of UV-B on the flowering time of different Arabidopsis mutants of key protein components of photoperiodic flowering and/or light signalling. UV-B irradiation was found to induce early flowering in many of these lines, leading to the conclusion that these factors could not be involved in UV-B specific acceleration of flowering.

A UV-B induced acceleration of flowering was observed in the late flowering mutants *co* and *zlf* (Figure 3.12.A) which also demonstrated a UV-B dependent induction of *FT* and *SOC1* as well (Figure 3.12.B). The observation especially for *co* mutant line could possibly suggest that even if the photoperiod pathway is most likely involved in UV-B specific acceleration of flowering, it may involve additional factors other than CO that induce *FT*. This could suggest a putative mechanism where UVR8 bypasses CO in the photoperiodic flowering pathway by interacting directly with the *FT* promoter or by interacting with another transcription factor that induces *FT* expression.

Another factor that has been shown to be involved in flowering induction especially in a temperature depending manner is PIF4 [89]. Our results suggested that *pif4pif5* mutants present the same early flowering phenotype under UV-B conditions as WT (Figure 3.14.A), thus suggesting that PIF4 is not essential for UV-B induced early flowering. This line demonstrated elevated transcript levels of *FT* and *CO* under WL+UV-B conditions similar to WT, an observation which supports their early flowering phenotype and the hypothesis that FT actually mediates this UV-B dependent early flowering response. *FLC* transcript levels were found to be elevated in *pif4pif5*, a response common for most of the *Arabidopsis* lines tested in our study, suggesting that this might be a more general reaction under UV-B conditions.

Another set of mutants for UV-B signalling components *cop1-4* and *rup1rup2* we investigated demonstrated an upregulation of *FT* transcript levels under WL + UV-B compared to WL,

which supports their early flowering phenotype (Figure 3.16.B). *FLC* transcript levels did not appear to change in the *cop1-4* line (Figure 3.16.B), suggesting that the UV-B specific *FLC* induction is somehow regulated by COP1. Mutants *rup1rup2* on the other hand did present an upregulation of *FLC* transcript levels under WL+UV-B.

RUP2 has been very recently been associated with flowering regulation under UV-B conditions [142]. Earlier studies showed that RUP1 and RUP2 regulate floral transition under WL [137]. While RUP1 was shown to not have an important role in flowering, RUP2 was found to repress flowering [137]. Strangely the over-expression of RUP1 or RUP2 accelerated flowering in LD [137]. *Rup2* mutants demonstrated to have an early flowering phenotype as well as RUP2 over-expressing plants, indicating a more complex control mechanism [137]. Both factors were found to be controlled by the circadian clock [137]. The more recent study by Arongaus et al. suggests that *rup2* mutants flower at the same time as WT under WL LD conditions but under SD + UV-B conditions, *rup2* and *rup1rup2* lines flower earlier than wild type plants in a UVR8 dependent manner[142]. They prove that RUP2 and CO can interact directly and that the early phenotype of *rup2* is dependent on both FT and CO [142]. An upregulation of *FT* transcript abundance in *rup2* and *rup1rup2* mutants was observed under SD +UV-B [142]. Interestingly this observation correlates with our own findings for flowering experiments conducted under LD photoperiods. *Rup1rup2*, however, in our set of experiments demonstrated a major downregulation of *CO* transcript levels and even though in the study by Arongaus et al. *CO* transcript levels were not found to be affected in any of the mutant lines they tested, including *rup1rup2*, *rup2* and *uvr8rup1rup2* [142], this downregulation of *CO* as well as our previous observations of an early flowering phenotype of *co* mutant line grown under WL+UV-B compare to growth under sole WL, does not support the SD hypothesis of CO binding to the *FT* promoter and thus leading to the upregulation of *FT* and the early flowering phenotype. For this reason, it is always important to consider that the photoperiod length is crucial for controlling flowering time since the expression patterns of flowering inducing genes differ greatly between SD and LD. Under a short day photoperiod *CO* expression of WT plants peaks at ZT15 and there is no overlap with the expression of *FT* that generally remains in low levels except from a slight peak at dusk [72]. Arongaus et al. showed that *rup1rup2* mutants have indeed a peak of *FT* expression in the middle of the night, creating an overlap of the expression of the two genes and supporting their hypothesis that RUP2 has actually the ability to repress CO binding to the *FT* promoter [142]. The expression patterns of *FT* and *CO* are altered under LD, since there is both a peak and an overlap of *CO* and *FT* expression at dawn and dusk, with the peak at dusk being greater [72]. Still our observations were mainly made at dawn (ZT0.5), since

it was the timepoint we observed the most significant difference in the expression of the genes of interest. The above results lead to the observation that probably under LD it is not CO but another factor that promotes FT and leads to the UV-B induced early flowering phenotype that we observed.

6.5 UV-B delays the early flowering phenotype of *elf3-1* and OXPIF4

In contrast to all the previous observations mutants investigated, two of the mutants we tested, the early flowering *elf3-1* and OXPIF4 demonstrated a delay in flowering initiation under WL + UV-B compared to WL (Figures 3.13.A and 3.15.A). The gene expression profile of *elf3-1* indicated that under UV-B all three main flowering promoting genes *FT*, *CO* and *SOC1* were surprisingly upregulated and *FCL* transcript levels were downregulated (an exception to the general downregulation of *FLC* that was observed under UV-B) (Figure 3.13.B). All these responses contradict the late flowering phenotype of this line. Two explanations for this observation could be possible. Firstly, an unknown factor could be involved in this UV-B induced delay of flowering. Alternatively, it is important to consider the fact that ELF3 is an important component of the circadian clock. On top of that ELF3 is involved in its own circadian regulation [83] [181]. In an *elf3* mutant though the circadian clock is majorly deregulated possibly affecting the plant's flowering time [83] [181] .

The genetic profile of OXPIF4 indicated that there is a reduction of transcript level of both *FT* and *CO* transcript abundance along with an upregulation of *FLC* (Figure 3.15.B). PIF4 is a downstream signalling partner of ELF3 [183], indicating that these two factors might be putative mediators of UV-B induced early flowering, but this hypothesis would need to be investigated further. Our results altogether indicate that UV-B has clearly a different effect in flowering initiation of early flowering mutant lines and for this reason it would be really interesting to perform additional experiments on additional early flowering lines in order to monitor their flowering behaviour as well under UV-B irradiation (possibly *SUCpro*::CO-HA also known as the over-expressing CO line [184]).

6.6 UVR8 affects vegetative shade flowering in an ecotype-specific manner

Another part of this work focused on the study of the way flowering initiation is affected under vegetative shade growth conditions that have been subjected to UV-B irradiation. Our results suggest that when low R:FR conditions are supplemented with UV-B, the latter does not further affect the flowering behaviour of *Arabidopsis thaliana* accessions *Cvi* and *Ler*. However, UV-B delays the shade-induced flowering initiation in *Col-0* (Figures 4.1, 2 and 3). As mentioned

and discussed before these three accessions originate from very different environments [162] and light conditions and most likely these plants have adapted differently in their unique environments. Besides, it is already known that variation in both physiological and genetic traits amongst *Arabidopsis thaliana* natural accessions is very common and was one of the main reasons that aspired work with *Arabidopsis* [162].

In a recently published study by Hayes et al. it was observed that under low R:FR growth conditions supplemented with UV-B irradiation (vegetative shade) + UV-B) *Ler* demonstrates a significant delay in flowering [144], an observation that did not occur in our experiments. Thus, it is really important to consider the significance of the different parameters used in a flowering experiment, such as the intensity of the total fluence rate, wavelength content or the duration of the treatment and the developmental stage when plants were exposed to. If these conditions vary in between different flowering experiments, it is possible that different responses could potentially be observed. In our case we tested a "milder" treatment (lower UV-B intensity and moderate shade), which probably could be the cause of the difference between the two observations.

6.7 The role of UVR8 in mediating UV-B induced delayed vegetative shade flowering

As discussed previously we also wanted to investigate if there is a link between the photoreceptor UVR8 in the UV-B moderation of shade-induced flowering. Our experiments examined flowering initiation under WL + FR (shade) and WL + FR + UV-B (shade + UV-B) in two *uvr8* mutant alleles and a UVR8 transgenic over-expressing line. While *uvr8-1* showed no significant change in shade-induced flowering, *uvr8-2* and OXUVR8 showed a delay in shade-induced flowering in the presence of UV-B compared to shade alone (Figure 4.4). The absence of a change in flowering initiation of the *uvr8-1* line under WL+ FR + UV-B growth conditions, compared to WL+ FR conditions has been observed before [144].

The difference in the response of the two mutant alleles can possibly derive from their different mutations. *uvr8-1* is a null allele and consequently does not express any protein at all, whilst *UVR8* transcript can still be produced in *uvr8-2*. The truncation protein version of UVR8 in this mutant may result in a defective protein structure or incapacitation of its ability to interact with or bind other factors leading to a dominant negative phenotype.

Additionally the more severe delay in flowering initiation of the OXUVR8 line supports the hypothesis that UVR8 regulates flowering in a negative way, since once UVR8 is

overexpressed there is a significant delay in flowering time observed when plants are grown under UV-B irradiation, even under vegetative shade conditions (in addition to the delay in flowering time OXUVR8 demonstrated under WL+ UV-B growth conditions).

6.8 UV-B delays vegetative shade induced flowering in photoperiodic and light signalling mutant and over-expressing lines

As highlighted before we also wanted to shed some light on the role of UV-B on flowering phenotypes of plants that harbour mutations in important genes involved in light signalling and photoperiodic flowering. Interestingly, under low R:FR growth conditions supplemented with UV-B, most of the mutants examined demonstrated a delay in flowering time similar to the one of their wild type *Col-0*. Lines including early flowering *elf3-1* and OXPIF4 (Figures 4.5 and 4.6), as well as light signalling mutants *pif4* and *pif4pif5* (Figure 4.6) and UV-B signalling mutants *cop1-4* and *rup1rup2* (Figure 4.7), all demonstrated delayed flowering under WL+ FR+ UV-B compared to WL +FR comparable to WT. These observations could possibly suggest that the corresponding genes do not have a significant role in this response, but further transcriptomic analysis needs to be performed in order to identify that key factors responsible. Nevertheless the late flowering phenotype of all these mutants under shade that was supplemented with UV-B is not as severe as the early flowering phenotype of the same lines under WL+ UV-B, suggesting that under vegetative shade conditions UV-B does not majorly affect flowering time, probably because of the fact that when subjected to low R:FR growth conditions, plants flower earlier anyway, especially under LD photoperiods [149]. This acceleration in flowering time probably prevails and overrides any further changes occurring with respect to flowering initiation.

6.9 UV-B does not affect vegetative shade flowering initiation in late flowering mutants

Another novel observation is that the late flowering photoperiodic mutants *co* [221] and *zlf* [49] are not affected with respect to flowering initiation under vegetative shade supplemented with UV-B (Figure 4.5).. This observation supports the hypothesis that the early flowering response which occurs in response to vegetative shade does not allow further major differences in flowering time initiation to take place. We show that *co* and *zlf*, not only demonstrate late flowering under WL, but also show a reduced rate of flowering acceleration under low R:FR supplemented with UV-B [149]. However, the afore mentioned late flowering mutants partially

retain a shade-induced flowering acceleration under low R:FR conditions compared to high R:FR (Figures 4.5 and 4.8) suggesting that the shade-induced flowering pathways overrides the effect of UV-B and is potentially distinct from the CO-regulated photoperiodic flowering pathway.

Overall, we can conclude that both the wild type and mutant lines we investigated throughout this study behave differently under high R:FR and low R:FR growth conditions with regards to flowering time control under UV-B irradiation. Under high R:FR supplemented with UV-B there is an acceleration of flowering time observed in *Arabidopsis thaliana Ler* and *Col-0* ecotypes, while flowering initiation of *Cvi* is not affected (Figures 3.3 and 3.4). Under low R:FR conditions supplemented with UV-B *Ler* and *Cvi* flowering initiation is not significantly affected, but *Col-0* demonstrates delayed flowering compared to shade alone (Figures 4.1, 4.2 and 4.3). Under high R:FR+ UV-B conditions both *uvr8* mutant lines with the two different alleles demonstrate an early flowering phenotype and UVR8 overexpression causes a delay in flowering time (Figure 3.8). Under low R:FR+ UV-B conditions the flowering time of the null *uvr8-1* mutant is not affected, while *uvr8-2* coding for a truncated non-functional but stable protein [128] demonstrates a slight delay in flowering time (Figure 4.4). OXUVR8 shows a more severe delay in flowering time, suggesting that UVR8 acts indeed as a negative regulator of flowering time.

Under high R:FR+ UV-B conditions most of the lines examined (photoperiodic, light and/or UV-B signalling mutants and over-expressors) were early flowering (Figures 3.12, 3.14 and 3.16) with the only exception of the early flowering mutants *elf3-1* and OXPIF4 (Figures 3.13 and 3.15). On the contrary, the majority of the lines examined under low R:FR+ UV-B conditions showed delayed flowering phenotypes (Figures 4.5, 4.6 and 4.7) apart from late flowering lines *co* and *zlf* (Figure 4.5). The above results are interesting since it is hinted that *Arabidopsis thaliana* lines with abnormal flowering phenotypes (early or late flowering) behave differently under UV-B mediated flowering responses.

6.10 Final Conclusions

The major conclusions based on the data collected from this study are the following:

1. UV-B promotes flowering initiation under LD supplemented with low and physiologically relevant levels of UV-B irradiation (0.5 μmol m^{-2} s^{-1}).
2. UV-B induced flowering acceleration in *Arabidopsis thaliana* is ecotype dependent.

3. UV-B induces the expression of the flowering promoter *FT* under LDs.
4. UV-B mediates an increase in the expression of the flowering repressor *FLC* under LDs, but its effect on photoperiodic flowering initiation is negligible.
5. UVR8 acts as a negative regulator of UV-B induced early flowering, since *uvr8* mutants show early flowering, elevated *FT* and *CO* levels under WL supplemented with UV-B, while OXUVR8 demonstrates delayed flowering and reduced *FT* and *CO* levels, under LDs photoperiods.
6. Under a LD photoperiod UV-B mediates the repression of *ELF3* that is partially regulated by UVR8.
7. Supplementation of shade with UV-B has a different effect on different *uvr8* mutant alleles, under LD growth conditions.
8. UVR8 acts a negative regulator of shade-induced flowering in response to supplementary UV-B, under LDs.
9. The effect of UV-B dependent flowering regulation is either supressed or reverted in mutant *Arabidopsis thaliana* lines with altered flowering phenotypes (early and late flowering mutants). Under high R:FR + UV-B LD growth conditions early flowering mutant *elf3-1* and over-expressing OXPIF4 lines, presented a late flowering phenotype compered to high R:FR LD growth conditions. On the other hand, *co, zlf, pif4pif5, cop1-4* and *rup1rup2* demonstrated an early flowering phenotype when UV-B irradiation was present in the high R:FR light treatment compared to when it was absent. Under low R:FR + UV-B LD growth conditions late flowering mutants *co* and *zlf* presented no difference in flowering time compared to sole low R:FR LD growth conditions. By contrast *Arabidopsis* lines *elf3-1, pif4, pif4pif5,* OXPIF4, *cop1-4* and *rup1rup2* depicted a late flowering phenotype under low R:FR + UV-B LD growth conditions compared to low R:FR LD growth conditions not supplemented with UV-B irradiation.

Based on all of the above conclusions a preliminary and simplified model of UVR8 action can be formulated (Figure 6.1). However further experiments need to be performed in order to identify the mechanism and factors that are involved in the UV-B specific control of flowering time in *Arabidopsis thaliana*, through the action of UVR8.

6.11 Future work

Although the work of the current study has provided valuable information on the way UV-B irradiations affects flowering initiation in *Arabidopsis thaliana*, future experiments need to be

performed in order to fully elucidate the molecular mechanism, as well as the physiological significance of these flowering time alternations.

One of the major priorities is to investigate possible interactions of UVR8 with other flowering-controlling protein components and promoters of flowering inducing genes. Does UVR8 associate directly with the *FT* promoter or through the action of known transcriptional regulators (e.g. CO, PIF4). Does UVR8 block positive regulators from inducing FT expression in the presence of UV-B? Chromatin immunoprecipitation assays could address these questions. Also, although, we were not able to test potential interactions between UVR8 and known flowering signalling components through the yeast-two-hybrid system, alternative ways could be utilized such as co-immunoprecipitation in *Nicotiana benthamiana* and Arabidopsis, and BiFC, a technique that employs the emission of a fluorescent signal in order to investigate direct protein interactions. [219]

We also generated two *Arabidopsis thaliana* transgenic lines CO-mCitrine/*uvr8.1* and UVR8-GFP/ *pif4pif5*/, to monitor CO protein abundance as well as UVR8 efficiency in promoting *FT* expression in the absence of PIFs. Since the generation of these lines is a process that requires a period of time exceeded the duration of this project, we were not able to perform any experiments. The experiments we had designed utilizing these two lines involve quantification of CO protein levels compared to a CO-mCitrine/*Col-0 Arabidopsis* line in order to investigate if UV-B mediated early flowering and induction of *FT* can occur through an effect of UVR8 on CO protein abundance. Furthermore, flowering experiments on GFP-UVR8/*pif4pif5* in WL+/- UV-B to further investigate the role of PIF4 and PIF5 in UV-B mediated flowering initiation.

PIF4 and PIF5 are factors of great interest of their involvement in UV-B flowering initiation process , since PIF4 has already been demonstrated to control flowering initiation under high temperatures (27 ^{o}C – 28 ^{o}C) by activating directly *FT* [212]. On top of that under UV-B irradiation both PIF4 and PIF5 factors get degraded [144]. It would be interesting to test the effect of UV-B and the role of UVR8 in high temperature-induced flowering.

Lastly in order to better characterize the UV-B induced flowering phenotype of *Arabidopsis thaliana* lines under vegetative shade conditions, transcript expression analysis for the main flowering promoter and repressor genes (*FT*, *CO*, *SOC1*, *FLC*), could be performed in completion to the flowering experiments we conducted.

All of the above proposed experiments aim to ultimately uncover a novel mechanism for UV-B specific flowering initiation, including all the factors and pathways that integrate resulting in modulating flowering in response to diverse environmental stimuli. It is of great importance to achieve a clearer view on all the different flowering pathways that are associated with the UV-B mediated flowering behaviour, and, most importantly to determine the role of UVR8 in this process, as it seems to be a key factor of UV-B regulation of flowering time.

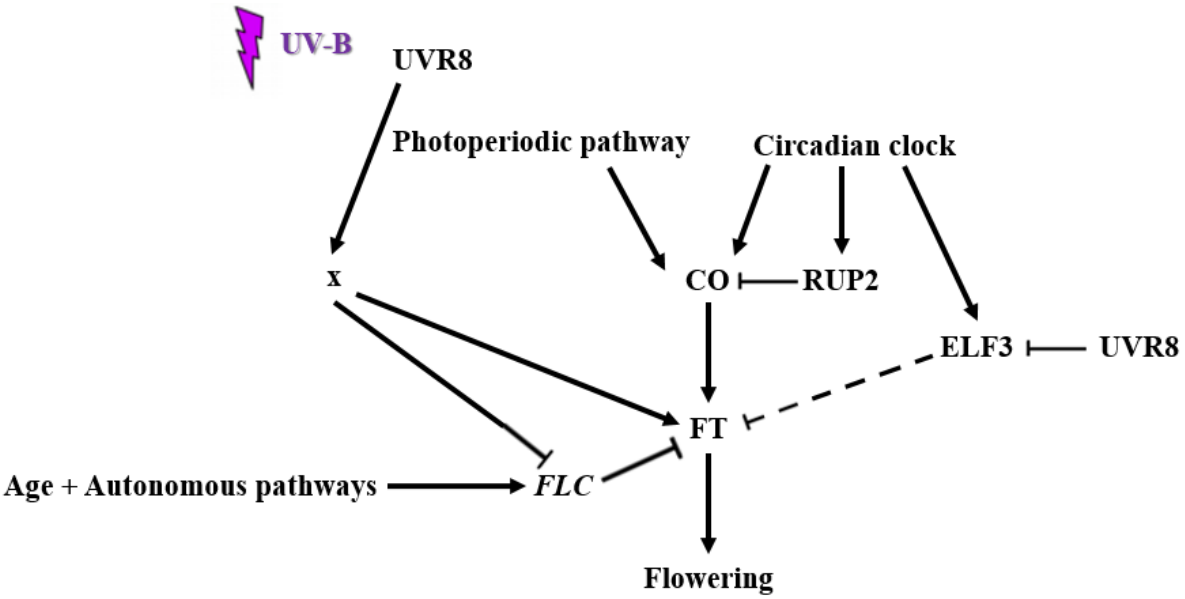


Figure 6. 1 Schematic representation of UV-B mediated control of flowering time (factors and flowering pathways involved)

Upon UV-B irradiation multiple flowering pathways control flowering time. The age and autonomous pathways lead to an upregulation of *FLC* (as demonstrated by Dotto et al [91]), which acts as an *FT* repressor. CO and FT are flowering promoting factors of the photoperiodic pathway. CO is repressed by RUP2- a negative regulator of UVR8 signalling (as demonstrated by Arongaus et al. [142]), a circadian clock component. ELF3, another circadian clock component also represses *FT*, indirectly by targeting CO for degradation. UVR8 represses *ELF3* and possibly interacts with a factor that has not yet been identified in order to promote *FT* and repress *FLC* gene expression.

Appendix

7.1 Supplemental Figures

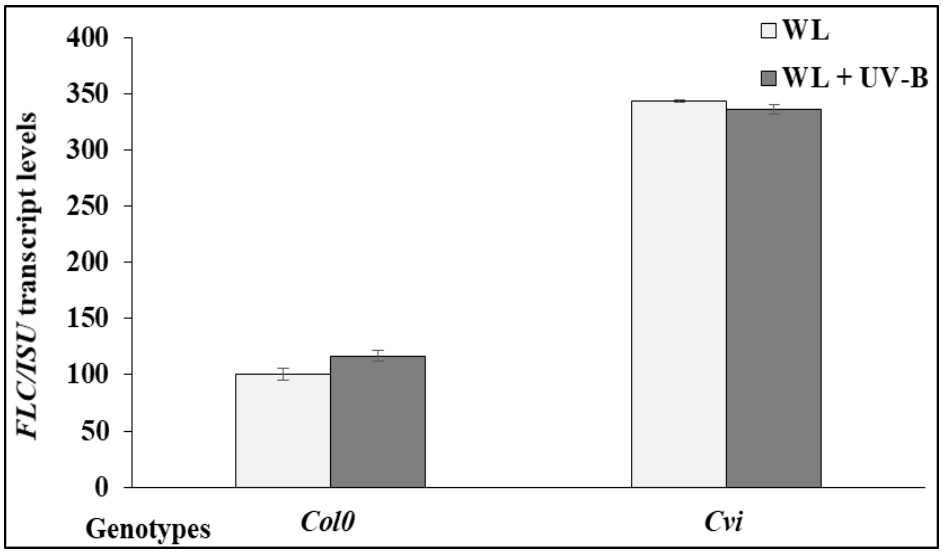


Figure S3. 1 *Cvi* shows a constitutive increase in *FLC* transcript levels.
qRT-PCR analysis *FLC* transcript levels normalized with housekeeping gene *ISU* in wild-type *Col-0* and *Cvi* plants. Plants were harvested at ZT 0.5 12 days after germination. Plants were grown under a LD photoperiod of WL (50 µmol $m^{-2}s^{-1}$) or WL supplemented with UV-B (0.5 µmol $m^{-2}s^{-1}$). Plants grown under WL were used as reference (100%). Data are represented as mean ± SEM. Data are representative of two biological replicates.

BIBLIOGRAPHY CITED

1. Fu, Q. RADIATION (SOLAR). 1859–1863 (1996).
2. Gruijl, F. R. De. HEALTH EFFECTS FROM SOLAR UV RADIATION. **72,** 177–196 (1997).
3. Dotto, M. & Casati, P. Developmental reprogramming by UV-B radiation in plants. *Plant Sci.* **264,** 96–101 (2017).
4. Rizzini, L. *et al.* Perception of UV-B by the arabidopsis UVR8 protein. *Science (80-.).* **332,** 103–106 (2011).
5. Sullivan, J. A. & Deng, X. W. From seed to seed: The role of photoreceptors in Arabidopsis development. *Dev. Biol.* **260,** 289–297 (2003).
6. Chory, J. Light signal transduction : an infinite spectrum of possibilities. 982–991 (2010). doi:10.1111/j.1365-313X.2009.04105.x
7. Montgomery, B. L. & Lagarias, J. C. Phytochrome ancestry : sensors of bilins and light Phytochromes : a definition. **1385,** 1–10 (2002).
8. Hoffmann, M. H. Biogeography of Arabidopsis thaliana. 125–134 (2002).
9. Chory, J. *et al.* From seed germination to flowering, light controls plant development via the pigment phytochrome. **93,** 12066–12071 (1996).
10. Parks, B. M. The Red Side of Photomorphogenesis 1. **133,** 1437–1444 (2003).
11. Sakai, T. & Haga, K. Molecular Genetic Analysis of Phototropism in Arabidopsis Review. **53,** 1517–1534 (2012).
12. Casal, J. J. Shade Avoidance. 1–19 (2012). doi:10.1199/tab.0157
13. Briggs, W. R. & Christie, J. M. Phototropins 1 and 2: versatile plant blue-light receptors.
14. Higaki, T., Rasmussen, H. . & Carpenter, W. . A Study of Some Morphological and Anatomical Aspects of Anthurium andreaum Lind. (1984).
15. Kasahara, M., Kagawa, T. & Oikawa, K. Chloroplast avoidance movement reduces photodamage in plants. **420,** 829–832 (2002).
16. Kagawa, T. & Wada, M. Blue Light-Induced Chloroplast Relocation. **43,** 367–371 (2002).
17. Kim, J. A., Kim, H., Choi, S., Jang, J. & Jeong, M. The Importance of the Circadian Clock in Regulating Plant Metabolism. (2017). doi:10.3390/ijms18122680
18. Fornara, F., de Montaigu, A. & Coupland, G. SnapShot: Control of flowering in arabidopsis. *Cell* **141,** 3–5 (2010).

19. Foster, R. G. *et al.* Circadian photoreception in the retinally degenerate mouse (rd / rd). (1991).
20. Andreas, M., Yang, X., Ayers, R. A. & Moffat, K. Structure and Function of Plant Photoreceptors. (2010). doi:10.1146/annurev-arplant-042809-112259
21. Sullivan, S., Hart, J. E., Rasch, P., Walker, C. H. & Christie, J. M. Phytochrome A Mediates Blue-Light Enhancement of Second-Positive Phototropism in Arabidopsis. **7,** 1–12 (2016).
22. Rockwell, N. C., Su, Y.-S. & Lagarias, J. C. Phytochrome Structure and Signaling Mechanisms. *Annu. Rev. Plant Biol.* **57,** 837–858 (2006).
23. Yamaguchi, R., Nakamura, M., Mochizuki, N., Kay, S. A. & Nagatani, A. Light-dependent Translocation of a Phytochrome B-GFP Fusion Protein to the Nucleus in Transgenic Arabidopsis. **145,** 437–445 (1999).
24. Sakamoto, K. & Nagatani, A. Nuclear localization activity of phytochrome B.
25. Kircher, S. *et al.* Light Quality – Dependent Nuclear Import of the Plant Photoreceptors Phytochrome A and B. **11,** 1445–1456 (1999).
26. Ecker, J. R. & Quail, P. H. The Arabidopsis Phytochrome-Interacting Factor PIF7 , Together with PIF3 and PIF4 , Regulates Responses to Prolonged Red Light by Modulating phyB Levels. **20,** 337–352 (2008).
27. Ni, M., Tepperman, J. M., Quail, P. H., Ars, U. & Gene, P. PIF3 , a Phytochrome-Interacting Factor Necessary for Normal Photoinduced Signal Transduction , Is a Novel Basic Helix-Loop-Helix Protein. **95,** 657–667 (1998).
28. Quail, P. H. Phytochrome photosensory signalling networks. *Nat. Rev. Mol. Cell Biol.* **3,** 85–93 (2002).
29. Jung, J.-H. *et al.* Phytochromes function as thermosensors in Arabidopsis. *Science (80-.).* **354,** 886 LP-889 (2016).
30. Lee, J. & Lee, I. Regulation and function of SOC1, a flowering pathway integrator. *J. Exp. Bot.* **61,** 2247–2254 (2010).
31. Lin, C. Photoreceptors and Regulation of Flowering Time. (2000).
32. Yu, X., Liu, H., Klejnot, J. & Lin, C. The Cryptochrome Blue Light Receptors. 1–27 (2010).
33. Kleine, T., Lockhart, P. & Batschauer, A. An Arabidopsis protein closely related to Synechocystis cryptochrome is targeted to organelles. (2003). doi:10.1046/j.1365-313X.2003.01787.x
34. Hitomi, K. *et al.* Bacterial cryptochrome and photolyase : characterization of two

photolyase-like genes of Synechocystis sp . **28,** 2353–2362 (2000).

35. Brudler, R. *et al.* Identification of a New Cryptochrome Class : Structure , Function , and Evolution. **11,** 59–67 (2003).
36. Veluchamy, S. & Rollins, J. A. A CRY-DASH-type photolyase / cryptochrome from Sclerotinia sclerotiorum mediates minor UV-A-specific effects on development. **45,** 1265–1276 (2008).
37. Worthington, E. N. *et al.* Purification and Characterization of Three Members of the Photolyase / Cryptochrome Family Blue-light Photoreceptors from Vibrio cholerae *. **278,** 39143–39154 (2003).
38. Huang, Y. *et al.* Crystal structure of cryptochrome 3 from Arabidopsis thaliana and its implications for photolyase activity. (2006).
39. Klar, T. *et al.* Cryptochrome 3 from Arabidopsis thaliana : Structural and Functional Analysis of its Complex with a Folate Light Antenna. 954–964 (2007). doi:10.1016/j.jmb.2006.11.066
40. Pokorny, R. *et al.* Recognition and repair of UV lesions in loop structures of duplex DNA by DASH-type cryptochrome. **105,** 21023–21027 (2008).
41. Wu, G. & Spalding, E. P. Separate functions for nuclear and cytoplasmic cryptochrome 1 during photomorphogenesis of Arabidopsis seedlings. **104,** 18813–18818 (2007).
42. Guo, H., Duong, H., Ma, N. & Lin, C. The Arabidopsis blue light receptor cryptochrome 2 is a nuclear protein regulated by a blue light-dependent post-transcriptional mechanism. **19,** (1999).
43. Kleiner, O., Kircher, S., Harter, K. & Batschauer, A. Nuclear localization of the Arabidopsis blue light receptor cryptochrome 2. **19,** 289–296 (1999).
44. Yu, X. *et al.* Arabidopsis Cryptochrome 2 Completes Its Posttranslational Life Cycle in the Nucleus. **19,** 3146–3156 (2007).
45. Cashmore, A. R. Cryptochromes : Blue Light Receptors for Plants and Animals. **760,** (2013).
46. Sakai, T. *et al.* Arabidopsis nph1 and npl1 : Blue light receptors that mediate both phototropism and chloroplast relocation. (2001).
47. Takemiya, A., Inoue, S., Doi, M., Kinoshita, T. & Shimazaki, K. Phototropins Promote Plant Growth in Response to Blue Light in Low Light Environments. **17,** 1120–1127 (2005).
48. Kim, W. Y. *et al.* ZEITLUPE is a circadian photoreceptor stabilized by GIGANTEA

in blue light. *Nature* **449,** 356–360 (2007).

49. Baudry, A. *et al.* F-Box Proteins FKF1 and LKP2 Act in Concert with ZEITLUPE to Control Arabidopsis Clock Progression. *Plant Cell* **22,** 606–622 (2010).
50. Schultz, T. F., Kiyosue, T., Yanovsky, M., Wada, M. & Kay, S. A. A Role for LKP2 in the Circadian Clock of Arabidopsis. **13,** 2659–2670 (2001).
51. Imaizumi, T. FKF1 F-Box Protein Mediates Cyclic Degradation of a Repressor of CONSTANS in Arabidopsis. **293,** (2014).
52. Song, Y. H., Smith, R. W., To, B. J., Millar, A. J. & Imaizumib, T. FKF1 conveys crucial timing information for CONSTANS stabilization in the photoperiodic flowering. **336,** 1045–1049 (2013).
53. Sawa, M., Nusinow, D. A., Kay, S. A. & Imaizumib, T. FKF1 and GIGANTEA complex formation is required for daylength measurement in Arabidopsis. **318,** 261–265 (2013).
54. Fornara, F. *et al.* Arabidopsis DOF Transcription Factors Act Redundantly to Reduce CONSTANS Expression and Are Essential for a Photoperiodic Flowering Response. *Dev. Cell* **17,** 75–86 (2009).
55. Zoltowski, B. D. & Imaizumi, T. Structure and function of the ZTL/FKF1/LKP2 group proteins in Arabidopsis. **1,** 1–24 (2015).
56. Nelson, D. C., Lasswell, J., Rogg, L. E., Cohen, M. A. & Bartel, B. FKF1 , a Clock-Controlled Gene that Regulates the Transition to Flowering in Arabidopsis. **101,** 331–340 (2000).
57. Takase, T. *et al.* LOV KELCH PROTEIN2 and ZEITLUPE repress Arabidopsis photoperiodic flowering under non-inductive conditions , dependent on FLAVIN-BINDING KELCH REPEAT F-BOX1. 608–621 (2011). doi:10.1111/j.1365-313X.2011.04618.x
58. Somers, D. E., Kim, W.-Y. & Geng, R. The F-Box Protein ZEITLUPE Confers Dosage-Dependent Control on the Circadian Clock, Photomorphogenesis, and Flowering Time. *Plant Cell* **16,** 769 LP-782 (2004).
59. Schultz, T. F., Kiyosue, T., Yanovsky, M., Wada, M. & Kay, S. A. A Role for LKP2 in the Circadian Clock of Arabidopsis. *Plant Cell* **13,** 2659 LP-2670 (2001).
60. Imaizumi, T., Schultz, T. F., Harmon, F. G., Ho, L. A. & Kay, S. A. FKF1 F-Box Protein Mediates Cyclic Degradation of a Repressor of CONSTANS in Arabidopsis *Science (80-.).* **309,** 293 LP-297 (2005).

61. Jenkins, G. I. Structure and function of the UV-B photoreceptor UVR8. *Curr. Opin. Struct. Biol.* **29,** 52–57 (2014).
62. Simpson, G. G. The autonomous pathway : epigenetic and post-transcriptional gene regulation in the control of Arabidopsis flowering time. (2004). doi:10.1016/j.pbi.2004.07.002
63. Hedden, P. & Mutasa-go, E. Gibberellin as a factor in floral regulatory networks. **60,** 1979–1989 (2009).
64. Garner, W. W. & Allard, H. A. EFFECT OF THE RELATIVE LENGTH OF DAY AND NIGHT AND OTHER FACTORS OF THE ENVIRONMENT ON GROWTH AND REPRODUCTION IN PLANTS. **18,** 1920 (1920).
65. Andrés, F. & Coupland, G. The genetic basis of flowering responses to seasonal cues. *Nat. Publ. Gr.* **13,** 627–639 (2012).
66. Redei, G. P. Supervital mutants of Arabidopsis. (1962).
67. Song, Y. H., Lee, I., Lee, S. Y., Imaizumi, T. & Hong, J. C. CONSTANS and ASYMMETRIC LEAVES 1 complex is involved in the induction of FLOWERING LOCUS T in photoperiodic flowering in Arabidopsis. *Plant J.* **69,** 332–342 (2012).
68. Zhu, Y., Liu, L., Shen, L. & Yu, H. NaKR1 regulates long-distance movement of FLOWERING LOCUS T in Arabidopsis. **2,** (2016).
69. Samach, A. *et al.* Distinct Roles of CONSTANS Target Genes in Reproductive Development of Arabidopsis. *Science (80-.).* **288,** 1613 LP-1616 (2000).
70. Simon, R., Coupland, G., Centre, J. I., Lane, C. & Nr, N. Activation of floral meristem identity genes in Arabidopsis. **384,** 59–62 (1996).
71. Yanovsky, M. J. & Kay, S. A. Living by the calendar: How plants know when to flower. *Nat. Rev. Mol. Cell Biol.* **4,** 265–275 (2003).
72. Sablowski, R. *et al.* Regulation of the floral transition at the shoot apical meristem of Arabidopsis as studied by genetics and next generation sequencing. *Nature* **10,** 1089–1092 (2010).
73. Song, Y. H., Smith, R. W., Millar, A. J., Imaizumib, T. & To, B. J. FKF1 Conveys Timing Information for CONSTANS stabilization in Photoperiodic Flowering. 1045–1050
74. Seaton, D. D. *et al.* Linked circadian outputs control elongation growth and development in response to photoperiod and temperature. *Mol. Syst. Biol.* 1–20 (2014). doi:10.15252/msb.20145766
75. Jang, S. *et al.* Arabidopsis COP1 shapes the temporal pattern of CO accumulation

conferring a photoperiodic flowering response. *EMBO J.* **27,** 1277–1288 (2008).

76. Laubinger, S. Arabidopsis SPA proteins regulate photoperiodic flowering and interact with the floral inducer CONSTANS to regulate its stability. *Development* **133,** 3213–3222 (2006).
77. Valverde, F. *et al.* Photoreceptor Regulation of CONSTANS Protein in Photoperiodic Flowering. *Science (80-.).* **303,** 1003 LP-1006 (2004).
78. Zuo, Z., Liu, H., Liu, B., Liu, X. & Lin, C. Blue Light-Dependent Interaction of CRY2 with SPA1 Regulates COP1 activity and Floral Initiation in Arabidopsis. **21,** 841–847 (2011).
79. Wang, H., Ma, L.-G., Li, J.-M., Zhao, H.-Y. & Deng, X. W. Direct Interaction of Arabidopsis Cryptochromes with COP1 in Light Control Development. *Science (80-.).* **294,** 154 LP-158 (2001).
80. Hicks, K. A., Albertson, T. M. & Wagner, D. R. EARLY FLOWERING3 Encodes a Novel Protein That Regulates Circadian Clock Function and Flowering in Arabidopsis. *Plant Cell* **13,** 1281 (2001).
81. Nusinow, D. A. *et al.* The ELF4–ELF3–LUX complex links the circadian clock to diurnal control of hypocotyl growth. *Nature* **475,** 398 (2011).
82. Hicks, K. A., Albertson, T. M. & Wagner, D. R. EARLY FLOWERING3 Encodes a Novel Protein That Regulates Circadian Clock Function and Flowering in Arabidopsis. **13,** 1281–1292 (2001).
83. McWatters, H. G., Bastow, R. M., Hall, A. & Millar, A. J. The ELF3 zeitnehmer regulates light signalling to the circadian clock. *Nature* **408,** 716 (2000).
84. Covington, M. F. *et al.* ELF3 Modulates Resetting of the Circadian Clock in Arabidopsis. **13,** 1305–1315 (2001).
85. Song, Y. H. *et al.* Molecular basis of flowering under natural long-day conditions in Arabidopsis. doi:10.1038/s41477-018-0253-3
86. Sawa, M. & Kay, S. A. GIGANTEA directly activates Flowering Locus T in Arabidopsis thaliana. *Proc. Natl. Acad. Sci.* **108,** 11698 LP-11703 (2011).
87. Liu, H. *et al.* Photoexcited CRY2 Interacts with CIB1 to Regulate Transcription and Floral Initiation in Arabidopsis. *Science (80-.).* **322,** 1535 LP-1539 (2008).
88. Thines, B. C., Youn, Y., Duarte, M. I. & Harmon, F. G. The time of day effects of warm temperature on flowering time involve PIF4 and PIF5. **65,** 1141–1151 (2014).
89. Hayes, S. *et al.* UV-B Perceived by the UVR8 Photoreceptor Inhibits Plant Thermomorphogenesis. *Curr. Biol.* **27,** 120–127 (2017).

90. Turck, F., Martienssen, R. A., Coupland, G., Colot, V. & Buisine, N. Arabidopsis TFL2 / LHP1 Specifically Associates with Genes Marked by Trimethylation of Histone H3 Lysine 27. **3,** (2007).
91. Dotto, M., Gómez, M. S., Soto, M. S. & Casati, P. UV-B radiation delays flowering time through changes in the PRC2 complex activity and miR156 levels in *Arabidopsis thaliana*. *Plant. Cell Environ.* (2018). doi:10.1111/pce.13166
92. Liu, L. *et al.* FTIP1 Is an Essential Regulator Required for Florigen Transport. **10,** (2012).
93. Abe, M. *et al.* FD, a bZIP Protein Mediating Signals from the Floral Pathway Integrator FT at the Shoot Apex. *Science (80-.).* **309,** 1052 LP-1056 (2005).
94. Lee, J., Oh, M., Park, H., Lee, I. & Metabolism, P. SOC1 translocated to the nucleus by interaction with AGL24 directly regulates LEAFY. 832–843 (2008). doi:10.1111/j.1365-313X.2008.03552.x
95. Yamaguchi, A. *et al.* The MicroRNA-Regulated SBP-Box Transcription Factor SPL3 Is a Direct Upstream Activator. *Dev. Cell* **17,** 268–278 (2009).
96. Jung, J. *et al.* The SOC1-SPL module integrates photoperiod and gibberellic acid signals to control flowering time in Arabidopsis. 577–588 (2012). doi:10.1111/j.1365-313X.2011.04813.x
97. Wigge, P. A. *et al.* Integration of spatial and temporal information during floral induction in Arabidopsis. *Science (80-.).* **309,** 1056–1059 (2005).
98. Conti, L. & Bradley, D. TERMINAL FLOWER1 Is a Mobile Signal Controlling Arabidopsis Architecture. **19,** 767–778 (2007).
99. Wing, W., Ho, H. & Weigel, D. Structural Features Determining Flower-Promoting Activity of Arabidopsis FLOWERING LOCUS T. **26,** 552–564 (2014).
100. Kumar, S. V. *et al.* Transcription factor PIF4 controls the thermosensory activation of flowering. 2–6 (2012). doi:10.1038/nature10928
101. Levy, Y. Y., Mesnage, S., Mylne, J. S., Gendall, A. R. & Dean, C. Multiple Roles of Arabidopsis VRN1 in Vernalization and Flowering Time Control. *Science (80-.).* **297,** 243 LP-246 (2002).
102. Song, J., Irwin, J. & Dean, C. Remembering the Prolonged Cold of Winter Minireview. *CURBIO* **23,** R807–R811 (2013).
103. Shindo, C., Lister, C., Crevillen, P., Nordborg, M. & Dean, C. Variation in the epigenetic silencing of FLC contributes to natural variation in Arabidopsis vernalization response. 3079–3083 (2006). doi:10.1101/gad.405306.therefore

104. Yang, H., Howard, M. & Dean, C. Antagonistic Roles for H3K36me3 and H3K27me3 in the Cold-Induced Epigenetic Switch at Arabidopsis FLC. *Curr. Biol.* **24,** 1793–1797 (2014).
105. Gendall, A. R., Levy, Y. Y., Wilson, A., Dean, C. & Agamous-like, O. O. F. C. The VERNALIZATION 2 Gene Mediates the Epigenetic Regulation of Vernalization in Arabidopsis. **107,** 525–535 (2001).
106. Kim, D. & Sung, S. Polycomb-Mediated Gene Silencing in Arabidopsis thaliana. **37,** 841–850 (2014).
107. De Lucia, F., Crevillen, P., Jones, A. M. E., Greb, T. & Dean, C. A PHD-Polycomb Repressive Complex 2 triggers the epigenetic silencing of FLC during vernalization. *Proc. Natl. Acad. Sci.* **105,** 16831 LP-16836 (2008).
108. Hennig, L. Keeping the gate closed : functions of the polycomb repressive complex PRC2 in development. 121–132 (2015). doi:10.1111/tpj.12828
109. Wu, G. *et al.* The sequential action of miR156 and miR172 regulates developmental timing in Arabidopsis. **138,** 750–759 (2010).
110. Pil, J. J., Seo, J., Ki, S. & Park, C. miR172 signals are incorporated into the miR156 signaling pathway at the SPL3 / 4 / 5 genes in Arabidopsis developmental transitions. 35–45 (2011). doi:10.1007/s11103-011-9759-z
111. Wang, J., Czech, B. & Weigel, D. miR156-Regulated SPL Transcription Factors Define an Endogenous Flowering Pathway in Arabidopsis thaliana. *Cell* **138,** 738–749 (2009).
112. Hyun, Y., Richter, R. & Coupland, G. Competence to Flower: Age-Controlled Sensitivity to Environmental Cues. (2016). doi:10.1104/pp.16.01523
113. Aukerman, M. J. & Sakai, H. Regulation of Flowering Time and Floral Organ Identity by a MicroRNA and Its APETALA2 Like Target Genes. *Plant Cell* **15,** 2730 LP-2741 (2003).
114. Kliebenstein, D. J., Lim, J. E., Landry, L. G. & Last, R. L. Arabidopsis Regulates Ultraviolet-B Signal Transduction and Tolerance and Contains Sequence Similarity to Human Regulator of Chromatin Condensation 1. *Plant Physiol.* **130,** 234 LP-243 (2002).
115. Brown, B. A. *et al.* A UV-B-specific signaling component orchestrates plant UV protection. *Proc. Natl. Acad. Sci. U. S. A.* **102,** 18225 LP-18230 (2005).
116. Favory, J. *et al.* Interaction of COP1 and UVR8 regulates stress acclimation in Arabidopsis. 591–601 (2009). doi:10.1038/emboj.2009.4

117. Christie, J. M. *et al.* Plant UVR8 Photoreceptor Senses UV-B by Tryptophan-Mediated Disruption of Cross-Dimer Salt Bridges. **335,** 1492–1496 (2012).
118. Di Wu *et al.* Structural basis of ultraviolet-B perception by UVR8. *Nature* **484,** 214 (2012).
119. Zeng, X. *et al.* Dynamic crystallography reveals early signalling events in ultraviolet photoreceptor UVR8. *Nat. Plants* **1,** 14006 (2015).
120. Takaaki, M. *et al.* Reaction dynamics of the UV-B photosensor UVR8. (2015). doi:10.1039/C5PP00012B
121. Zhong, D. et al. Quenching Dynamics of Ultraviolet-Light Perception by UVR8 Photoreceptor. 8–11 (2014).
122. Kaiserli, E. & Jenkins, G. I. UV-B Promotes Rapid Nuclear Translocation of the UV-B–Specific Signaling Component UVR8 and Activates Its Function in the Nucleus. *Plant Cell* **19,** 2662 LP-2673 (2007).
123. Heilmann, M. *et al.* Dimer/monomer status and in vivo function of salt-bridge mutants of the plant UV-B photoreceptor UVR8. *Plant J.* **88,** 71–81 (2016).
124. Cloix, C. & Jenkins, G. I. Interaction of the Arabidopsis UV-B-specific signaling component UVR8 with chromatin. *Mol. Plant* **1,** 118–128 (2008).
125. Heijde, M. & Ulm, R. UV-B photoreceptor-mediated signalling in plants. *Trends Plant Sci.* **17,** 230–237 (2012).
126. Cloix, C. *et al.* C-terminal region of the UV-B photoreceptor UVR8 initiates signaling through interaction with the COP1 protein. *Proc. Natl. Acad. Sci.* **109,** 16366 LP-16370 (2012).
127. Yin, R., Arongaus, A. B., Binkert, M. & Ulm, R. Two Distinct Domains of the UVR8 Photoreceptor Interact with COP1 to Initiate UV-B Signaling in Arabidopsis. *Plant Cell Online* **27,** 202–213 (2015).
128. Brown, B. A. *et al.* A UV-B-specific signaling component orchestrates plant UV protection. *Proc. Natl. Acad. Sci.* **102,** 18225–18230 (2005).
129. Osterlund, M. T., Hardtke, C. S., Wei, N. & Deng, X. W. Targeted destabilization of HY5 during light-regulated development of Arabidopsis. *Nature* **405,** 462 (2000).
130. Saijo, Y. *et al.* The COP1 – SPA1 interaction defines a critical step in phytochrome A-mediated regulation of HY5 activity. 2642–2647 (2003). doi:10.1101/gad.1122903.2642
131. Yi, C. & Deng, X. W. COP1 – from plant photomorphogen- esis to mammalian tumorigenesis. **15,** 9–16 (2005).

132. Jenkins, G. I. Photomorphogenic Responses to Ultraviolet-B light. doi:10.1111/pce.12934
133. Huang, X. *et al.* Arabidopsis FHY3 and HY5 Positively Mediate Induction of COP1 Transcription in Response to Photomorphogenic UV-B Light. *Plant Cell* **24,** 4590 LP-4606 (2012).
134. Stracke, R. *et al.* The Arabidopsis bZIP transcription factor HY5 regulates expression of the PFG1 / MYB12 gene in response to light and ultraviolet-B radiation. 88–103 (2010). doi:10.1111/j.1365-3040.2009.02061.x
135. Brown, B. A. & Jenkins, G. I. 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 Physiol.* **146,** 576 LP-588 (2008).
136. Yin, R. & Ulm, R. How plants cope with UV-B: from perception to response. *Curr. Opin. Plant Biol.* **37,** 42–48 (2017).
137. Wang, W., Yang, D. & Feldmann, K. A. EFO1 and EFO2, encoding putative WD-domain proteins, have overlapping and distinct roles in the regulation of vegetative development and flowering of Arabidopsis. *J. Exp. Bot.* **62,** 1077–1088 (2011).
138. Heijde, M. & Ulm, R. Reversion of the Arabidopsis UV-B photoreceptor UVR8 to the homodimeric ground state. **2012,** (2012).
139. Fornara, F. *et al.* SnapShot : Control of Flowering in Arabidopsis SnapShot : Control of Flowering in Arabidopsis. 3–5 (2010). doi:10.1016/j.cell.2010.04.024
140. Hajdu, A., Binkert, M., Davis, S. J., Scha, E. & Ulm, R. Functional interaction of the circadian clock and UV RESISTANCE LOCUS 8-controlled UV-B signaling pathways in Arabidopsis thaliana. **1,** 37–48 (2011).
141. Favory, J. *et al.* Interaction of COP1 and UVR8 regulates UV-B induced photomorphogenesis and stress acclimation in Arabidopsis. 591–601 (2009). doi:10.1038/emboj.2009.4
142. Arongaus, A. B. *et al.* Arabidopsis RUP2 represses UVR8-mediated flowering in noninductive photoperiods. *Genes Dev.* **32,** 1332–1343 (2018).
143. Dotto, M., Gómez, M. S. & Casati, P. UV - B radiation delays flowering time through changes in the PRC2 complex activity and miR156 levels in Arabidopsis thaliana. 1–13 (2018). doi:10.1111/pce.13166
144. Hayes, S., Velanis, C. N., Jenkins, G. I. & Franklin, K. A. UV-B detected by the UVR8 photoreceptor antagonizes auxin signaling and plant shade avoidance. *Proc. Natl. Acad. Sci.* **111,** 11894–11899 (2014).

145. Yan, A., Pan, J., An, L., Gan, Y. & Feng, H. The responses of trichome mutants to enhanced ultraviolet-B radiation in Arabidopsis thaliana. *J. Photochem. Photobiol. B Biol.* **113,** 29–35 (2012).
146. Mark, U., Saile-Mark, M. & Tevini, M. Effects of solar UVB radiation on growth, flowering and yield of central and southern European maize cultivars (Zea mays L.). *Photochem. Photobiol.* **64,** 457–463 (1996).
147. Del-Castillo-Alonso, M. Á. *et al.* Effects of ambient solar UV radiation on grapevine leaf physiology and berry phenolic composition along one entire season under Mediterranean field conditions. *Plant Physiol. Biochem.* **109,** 374–386 (2016).
148. Rajendiran, K. & Ramanujam, M. P. Improvement of biomass partitioning, flowering and yield by triadimefon in UV-B stressed Vigna radiata (L.) Wilczek. *Biol. Plant.* **48,** 145–148 (2004).
149. Cerda, P. D., Amasino, R. M. & Wollenberg, A. C. Acceleration of Flowering during Shade Avoidance in Arabidopsis Alters the Balance between FLOWERING LOCUS C -Mediated Repression and Photoperiodic Induction of Flowering 1 [W][OA]. **148,** 1681–1694 (2008).
150. Keara A. Franklin. Shade avoidance. *New Phytol.* **179,** 930–944 (2008).
151. Turck, F., Fornara, F. & Coupland, G. Regulation and Identity of Florigen : FLOWERING LOCUS T moves center stage. (2008). doi:10.1146/annurev.arplant.59.032607.092755
152. Kutschera, U. & Briggs, W. R. Seedling development in buckwheat and the discovery of the photomorphogenic shade-avoidance response. *Plant Biol.* **15,** 931–940 (2013).
153. Vanhaelewyn, L. *et al.* REPRESSOR OF ULTRAVIOLET-B PHOTOMORPHOGENESIS function allows efficient phototropin mediated ultraviolet-B phototropism in etiolated seedlings. *Plant Sci.* **252,** 215–221 (2016).
154. Gruber, H. *et al.* Negative feedback regulation of UV-B-induced photomorphogenesis and stress acclimation in Arabidopsis. *Proc. Natl. Acad. Sci.* **107,** 20132–20137 (2010).
155. McNellis, T. W. *et al.* Genetic and molecular analysis of an allelic series of cop1 mutants suggests functional roles for the multiple protein domains. *Plant Cell* **6,** 487–500 (1994).
156. Zagotta, M. T., Shannon, S., Jacobs, C. & Meeks-Wagner, D. R. Early-flowering mutants of *Arabidopsis thaliana*. *Aust. J. Plant Physiol.* **19,** 411–418 (1992).
157. Koini, M. A. *et al.* High Temperature-Mediated Adaptations in Plant Architecture

Require the bHLH Transcription Factor PIF4. *Curr. Biol.* **19,** 408–413 (2009).

158. Rodrı, M. *et al.* LETTERS A molecular framework for light and gibberellin control of cell elongation. **451,** (2008).
159. Kaiserli, E. *et al.* Integration of light and photoperiodic signaling in transcriptional Nuclear Foci. **35,** 311–321 (2015).
160. Brosché, M. & Strid, Å. Molecular events following perception of ultraviolet-B radiation by plants. *Physiol. Plant.* **117,** 1–10 (2003).
161. Lin, C. & Thomashow, M. F. DNA Sequence Analysis of a Complementary DNA for Cold-Regulated Arabidopsis Gene cor15 and Characterization of the COR15 Polypeptidel. 519–525 (1992).
162. Koornneef, M., Meinke, D., Linne, C. & West, R. The development of Arabidopsis as a model plant. 909–921 (2010). doi:10.1111/j.1365-313X.2009.04086.x
163. Alonso-blanco, C. & Koornneef, M. Naturally occurring variation in Arabidopsis : an underexploited resource for plant genetics. **1385,** 13–15 (2000).
164. *The Peace Corps Welcomes you to Cape Verde*. *October* (2009).
165. Yoo, S. J., Yoo, S. Y., Lee, J. S. & Ahn, J. H. CONSTANS Activates SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 through FLOWERING LOCUS T to Promote Flowering. **139,** 770–778 (2005).
166. Liu, X. L. ELF3 Encodes a Circadian Clock-Regulated Nuclear Protein That Functions in an Arabidopsis PHYB Signal Transduction Pathway. *Plant Cell Online* **13,** 1293–1304 (2001).
167. Tilbrook, K. *et al.* The UVR8 UV-B Photoreceptor: Perception, Signaling and Response. *Arab. B.* **11,** e0164 (2013).
168. Nozue, K. *et al.* Rhythmic growth explained by coincidence between internal and external cues. *Nature* **448,** 358 (2007).
169. Kumar, S. V., Lucyshyn, D., Jaeger, K. E., Alós, E. & Alvey, E. PHYTOCHROME INTERACTING FACTOR4 controls the thermosensory activation of flowering. **484,** 242–245 (2016).
170. Más, P., Kim, W.-Y., Somers, D. E. & Kay, S. A. Targeted degradation of TOC1 by ZTL modulates circadian function in Arabidopsis thaliana. *Nature* **426,** 567 (2003).
171. Somers, D. E., Schultz, T. F., Milnamow, M. & Kay, S. A. ZEITLUPE encodes a novel clock-associated PAS protein from Arabidopsis. *Cell* **101,** 319–329 (2000).
172. Wang, Y. H. How effective is T-DNA insertional mutagenesis in Arabidopsis ? **1,** 11–20 (2008).

173. Pedmale, U. V. *et al.* Cryptochromes Interact Directly with PIFs to Control Plant Growth in Limiting Blue Light. *Cell* **164,** 233–245 (2016).
174. Khanna, R. *et al.* The Basic Helix-Loop-Helix Transcription Factor PIF5 Acts on Ethylene Biosynthesis and Phytochrome Signaling by Distinct Mechanisms. *Plant Cell Online* **19,** 3915–3929 (2007).
175. Lorrain, S., Allen, T., Duek, P. D., Whitelam, G. C. & Fankhauser, C. Phytochrome-mediated inhibition of shade avoidance involves degradation of growth-promoting bHLH transcription factors. *Plant J.* **53,** 312–323 (2008).
176. Oravecz, A. CONSTITUTIVELY PHOTOMORPHOGENIC1 Is Required for the UV-B Response in Arabidopsis. *Plant Cell Online* **18,** 1975–1990 (2006).
177. Hardtke, C. S. HY5 stability and activity in Arabidopsis is regulated by phosphorylation in its COP1 binding domain. *EMBO J.* **19,** 4997–5006 (2000).
178. Gazzani, S. Analysis of the Molecular Basis of Flowering Time Variation in Arabidopsis Accessions. *Plant Physiol.* **132,** 1107–1114 (2003).
179. Mockler, T. *et al.* Regulation of photoperiodic flowering by Arabidopsis photoreceptors. *PNAS* **100,** 2140–2145 (2003).
180. Thines, B. C., Youn, Y., Duarte, M. I. & Harmon, F. G. The time of day effects of warm temperature on flowering time involve PIF4 and PIF5. *J. Exp. Bot.* **65,** 1141–1151 (2014).
181. Thines, B. & Harmon, F. G. Ambient temperature response establishes ELF3 as a required component of the core Arabidopsis circadian clock. **107,** 3257–3262 (2010).
182. Dixon, L. E., Knox, K., Kozma-bognar, L., Southern, M. M. & Pokhilko, A. Report Temporal Repression of Core Circadian Genes Is Mediated through EARLY FLOWERING 3 in Arabidopsis. *Curr. Biol.* **21,** 120–125 (2011).
183. Raschke, A. *et al.* Natural variants of ELF3 affect thermomorphogenesis by transcriptionally modulating PIF4 -dependent auxin response genes. *BMC Plant Biol.* **5,** 1–10 (2015).
184. Mizuno, T. *et al.* Ambient Temperature Signal Feeds into the Circadian Clock Transcriptional Circuitry Through the EC Night-Time Repressor in Arabidopsis thaliana. **55,** 958–976 (2014).
185. Roig-Villanova, I. & Martínez-García, J. F. Plant Responses to Vegetation Proximity: A Whole Life Avoiding Shade. *Front. Plant Sci.* **7,** 1–10 (2016).
186. Smith, H. Light quality, Photopereception, and Plant Strategy. 481–518 (1982).
187. Ballaré, C. L. Keeping up with the neighbours: Phytochrome sensing and other

signalling mechanisms. *Trends Plant Sci.* **4,** 97–102 (1999).

188. Franklin, K. A. & Whitelam, G. C. Phytochromes and shade-avoidance responses in plants. *Ann. Bot.* **96,** 169–175 (2005).
189. Smith, H. & Whitelam, G. C. The shade avoidance syndrome: Multiple responses mediated by multiple phytochromes. *Plant, Cell Environ.* **20,** 840–844 (1997).
190. Jaillais, Y. & Chory, J. Unraveling the paradoxes of plant hormone signaling integration. *Nat. Struct. Mol. Biol.* **17,** 642–645 (2010).
191. Whitelam, G. C. & Smith, H. Retention of Phytochrome-Mediated Shade Avoidance Responses in Phytochrome-Deficient Mutants of Arabidopsis, Cucumber and Tomato. *J. Plant Physiol.* **139,** 119–125 (1991).
192. Devlin, P. F. *et al.* Phytochrome D acts in the shade-avoidance syndrome in Arabidopsis by controlling elongation growth and flowering time. *Plant Physiol.* **119,** 909–15 (1999).
193. Robson, P., Whitelam, G. C. & Smith, H. Selected Components of the Shade-Avoidance Syndrome Are Displayed in a Normal Manner in Mutants of Arabidopsis thaliana and Brassica rapa Deficient in Phytochrome B. *Plant Physiol.* **102,** 1179–1184 (1993).
194. Franklin, K. A. Mutant Analyses Define Multiple Roles for Phytochrome C in Arabidopsis Photomorphogenesis. *Plant Cell Online* **15,** 1981–1989 (2003).
195. Halliday, K. J., Salter, M. G., Thingnaes, E. & Whitelam, G. C. Phytochrome control of flowering is temperature sensitive and correlates with expression of the floral integrator FT. *Plant J.* **33,** 875–885 (2003).
196. Franklin, K. A. Phytochromes B, D, and E Act Redundantly to Control Multiple Physiological Responses in Arabidopsis. *Plant Physiol.* **131,** 1340–1346 (2003).
197. Sharrock, R. A. & Quail, P. H. Novel phytochrome sequences in Arabidopsis thaliana: structure, evolution, and differential expression of a plant regulatory photoreceptor family. *Genes Dev.* **3,** 1745–1757 (1989).
198. Clack, T., Mathews, S. & Sharrock, R. A. The phytochrome apoprotein family in Arabidopsis is encoded by five genes: the sequences and expression of PHYD and PHYE. *Plant Mol. Biol.* **25,** 413–427 (1994).
199. Mathews, S. Phytochrome-mediated development in land plants: Red light sensing evolves to meet the challenges of changing light environments. *Mol. Ecol.* **15,** 3483–3503 (2006).
200. Rockwell, N. C. The Structure of Phytochrome: A Picture Is Worth a Thousand

Spectra. *Plant Cell Online* **18,** 4–14 (2006).

201. Goto, N., Kumagai, T. & Koornneef, M. Flowering responses to light- breaks in photomorphogenic mutants of Arabidopsis thaliana, a long- day plant. *Physiol. Plant.* **83,** 209–215 (1991).
202. Nagatani, A., Reed, J. W. & Chory, J. Isolation and Initial Characterization of Arabidopsis Mutants That Are Deficient in Phytochrome A. *Plant Physiol.* **102,** 269–277 (1993).
203. Aukerman, M. J. A Deletion in the PHYD Gene of the Arabidopsis Wassilewskija Ecotype Defines a Role for Phytochrome D in Red/Far-Red Light Sensing. *Plant Cell Online* **9,** 1317–1326 (1997).
204. Devlin, P. F. Phytochrome E Influences Internode Elongation and Flowering Time in Arabidopsis. *Plant Cell Online* **10,** 1479–1488 (1998).
205. Monte, E. *et al.* Isolation and Characterization of phyC mutants in Arabidopsis reveals complex crosstalk between phytochrome signaling pathways. *Plant Cell* **15,** 1962–1980 (2003).
206. Balasubramanian, S. *et al.* The PHYTOCHROME C photoreceptor gene mediates natural variation in flowering and growth responses of Arabidopsis thaliana. *Nat. Genet.* **38,** 711 (2006).
207. Salter, M. G., Franklin, K. A. & Whitelam, G. C. Gating of the rapid shade-avoidance response by the circadian clock in plants. *Nature* **426,** 680 (2003).
208. Franklin, K. A. & Whitelam, G. C. Light-quality regulation of freezing tolerance in Arabidopsis thaliana. *Nat. Genet.* **39,** 1410–1413 (2007).
209. Schwartz, C. J., Lee, J. & Amasino, R. Variation in shade-induced flowering in Arabidopsis thaliana results from FLOWERING LOCUS T allelic variation. 1–18 (2017).
210. Craig, D. S. & Runkle, E. S. A Moderate to High Red to Far-red Light Ratio from Light-emitting Diodes Controls Flowering of Short-day Plants. **138,** 167–172 (2013).
211. Lucas, M. De. PIFs get BRright : PHYTOCHROME INTERACTING FACTORs as integrators of light and hormonal signals. (2014).
212. Proveniers, M. C. G. & Zanten, M. Van. High temperature acclimation through PIF4 signaling. *Trends Plant Sci.* **18,** 59–64 (2013).
213. Ezer, D. *et al.* The Evening Complex coordinates environmental and endogenous signals in Arabidopsis. (2017). doi:10.1038/nplants.2017.87.The
214. Hornitschek, P. *et al.* Phytochrome interacting factors 4 and 5 control seedling growth

in changing light conditions by directly controlling auxin signaling. *Plant J.* **71,** 699–711 (2012).

215. Choi, H. & Oh, E. PIF4 Integrates Multiple Environmental and Hormonal Signals for Plant Growth Regulation in Arabidopsis. *Mol. Cells* **39,** 587–593 (2016).
216. Hoecker, U. Arabidopsis COP1 and SPA genes are essential for plant elongation but not for acceleration of flowering time in response to a low R:FR ratio. (2012). doi:10.1104/pp.112.207233
217. Perrella, G. *et al.* ZINC-FINGER interactions mediate transcriptional regulation of hypocotyl growth in *Arabidopsis*. *Proc. Natl. Acad. Sci.* **115,** E4503–E4511 (2018).
218. Song, Y. H. *et al.* Distinct roles of FKF1, GIGANTEA, and ZEITLUPE proteins in the regulation of CONSTANS stability in Arabidopsis photoperiodic flowering. *Proc. Natl. Acad. Sci.* **111,** 17672–17677 (2014).
219. Kerppola, T. K. Design and Implementation of Bimolecular Fluorescence Complementation (BiFC) Assays for the Visualization of Protein Interactions in Living Cells. **1,** 1278–1286 (2006).
220. Robson, F. *et al.* Functional importance of conserved domains in the flowering-time gene CONSTANS demonstrated by analysis of mutant alleles and transgenic plants. *Plant J.* **28,** 619–631 (2001).
221. Koornneef, M., Hanhart, C. J. & Veen, J. H. Van Der. A genetic and physiological analysis of late flowering mutants in Arabidopsis thaliana. 57–66 (1991).