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PERCEPTION OF SOLAR UV RADIATION AND BLUE LIGHT BY PLANTS: PHOTORECEPTORS, TRANSCRIPTOME AND ENVIRONMENTAL ACCLIMATION

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DOCTORAL DISSERTATION

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Dedicated to my father Prakash Narain Rai

ABSTRACT

Solar UV-B radiation (290–315 nm), UV-A radiation (315–400 nm), and blue light (400–500 nm) regulate multiple aspects of plant growth and development, and these are mediated by different photoreceptors. In plants, UVR8 is described as a UV-B photoreceptor, while cryptochromes (CRYs) are described as UV-A/blue photoreceptors, based on their absorption maxima and action spectra. However, these photoreceptors are also sensitive to other wavelengths outside the wavelengths of maximum absorption. Based on this property, their roles could differ in full-spectrum sunlight than those reported from experiments performed in controlled environments. In sunlight, both UV-B and UV-A/blue photoreceptors are simultaneously activated, and there is a possibility that their signaling pathways interact. However, an interaction between UVR8 and CRYs regulating transcriptome-wide responses remained unexplored. Furthermore, persistent high solar irradiance is often followed by drought in the field, and studies have indicated that UV and drought interact to regulate plant physiological responses. However, an interaction for metabolic and transcript abundance responses has not been well-described. These gaps in knowledge are addressed in my thesis through three main aims: (1) to identify the individual roles of UVR8 and CRYs in the perception of solar UV-B, short-wave UV-A (315–350 nm, UV-A_{sw}), long-wave UV-A (350–400 nm, UV-A_{lw}) radiation, and blue light by plants, (2) to test the interaction between UVR8 and CRYs under solar UV radiation, and (3) to determine if pre-exposure to solar UV radiation could provide acclimation to subsequent drought stress in plants. To achieve the first two aims, I used *Arabidopsis thaliana* wild type and mutants impaired in UVR8 and CRYs photoreceptors and exposed them to different ranges of wavelengths of solar or simulated solar UV radiation and blue light under optical filters. To achieve the third aim, I used two accessions of *Medicago truncatula* (Jemalong A17 and F83005-5). I exposed them to solar UV radiation using optical filters and subjected them to drought stress by restricting watering in a factorial experiment. The results indicated that UVR8 mediates the perception of both UV-B and UV-A_{sw} radiation. In contrast, CRYs mediate the perception of UV-A_{lw} radiation and blue light. A further novel finding is that UVR8 and CRYs interact antagonistically to regulate transcriptome-wide responses under UV-B and UV-A_{sw} radiation. My thesis also provides evidence that UV-B+UV-A_{sw} radiation and mild drought can interact positively to trigger acclimation through an increase in epidermal UV screening in the drought-intolerant accession, F83005-5, and through an increase in transcript abundance of *CHALCONE SYNTHASE* in the moderately drought-tolerant accession, Jemalong A17. Furthermore, all three studies showed a distinct response to solar or simulated solar UV-B+UV-A_{sw} and UV-A_{lw} radiation, suggesting a need to split UV-A into short and long wavelengths for future studies on UV-A radiation.

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Helsinki, 5 October 2020

CONTENTS

ORIGINAL PUBLICATIONS AND MANUSCRIPTS

I. **Neha Rai**, Andrew O'Hara, Daniel Farkas, Omid Safronov, Khuanpiroon Ratanasopa, Fang Wang, Anders V. Lindfors, Gareth I. Jenkins, Tarja Lehto, Jarkko Salojärvi, Mikael Brosché, Åke Strid, Pedro J. Aphalo, Luis O. Morales (2020). The photoreceptor UVR8 mediates the perception of both UV-B and UV-A wavelengths up to 350 nm of sunlight with responsivity moderated by cryptochromes. *Plant, Cell and Environment*. 43(6): 1513–1527.

II. **Neha Rai**, Susanne Neugart, Yan Yan, Fang Wang, Sari M. Siipola, Anders V. Lindfors, Jana Barbro Winkler, Andreas Albert, Mikael Brosché, Tarja Lehto, Luis O. Morales, Pedro J. Aphalo (2019). How do cryptochromes and UVR8 interact in natural and simulated sunlight? *Journal of Experimental Botany*, 70(18): 4975–4990.

III. **Neha Rai**, Susanne Neugart, David Schröter, Anders V. Lindfors, Pedro J. Aphalo. Responses of flavonoids to solar UV radiation and gradual soil drying in two *Medicago truncatula* accessions. (Manuscript).

IV. **Neha Rai**, Luis O. Morales, Pedro J. Aphalo. Perception of UV-A radiation by plants: photoreceptors and mechanisms. (Manuscript).

The publications and manuscripts are referred to in the text by their roman numerals.

AUTHOR'S CONTRIBUTIONS

I. I contributed to the design of experiments, especially in relation to the measurement of transcript abundance by qRT-PCR and RNA-seq. I performed the RNA isolation, cDNA synthesis, qRT-PCR. I analyzed the qRT-PCR data and participated in the analysis of gene expression data together with PJA and LOM. I interpreted the results, and wrote the manuscript together with PJA and LOM.

II. I conceptualized the research and designed the experiments with advice from PJA and LOM. I visited Helmholtz Zentrum (Munich, Germany) for sampling and measurements. I performed the RNA isolation, cDNA synthesis, and qRT-PCR at the University of Helsinki and visited Leibniz Institute of Vegetable and Ornamental Crops (Großbeeren, Germany) to do the secondary metabolite analysis. For this, I prepared the samples for HPLC (HPLC-MS was run by SN). I analyzed all the data and interpreted the results. I wrote the manuscript with advice from PJA, LOM, and TL.

III. I conceptualized the research together with PJA. I designed the experiments with advice from PJA. I performed the field experiment, measurement of petiole length and epidermal flavonoid content, RNA isolation, cDNA synthesis, and qRT-PCR at the University of Helsinki. I visited Leibniz Institute of Vegetable and Ornamental Crops (Großbeeren, Germany) to do the secondary metabolite and abscisic acid analysis. For this, I prepared the samples for HPLC (HPLC-MS was run by SN and DS). I analyzed all the data and interpreted the results. I wrote the manuscript with advice from PJA and LOM.

IV. I conceptualized the layout of the review article. I wrote sections 1, 2, 4, 5, and 6 with advice from PJA and LOM.

ABBREVIATIONS

Abbreviation	Full name
ABA	Abscisic acid
BES1	BRI1-EMS-SUPPRESSOR 1
BICs	BLUE-LIGHT INHIBITOR OF CRYPTOCHROMES
BIM1	BES1-INTERACTING MYC-LIKE 1
bHLH	Basic helix-loop-helix
bZIP	Basic leucine-zipper
CHS	CHALCONE SYNTHASE
CIB1	CRYPTOCHROME-INTERACTING basic helix-loop-helix 1
COP1	CONSTITUTIVE PHOTOMORPHOGENIC 1
CRYs	Cryptochrome 1 and cryptochrome 2
DEGs	Differentially expressed genes
FDR	False discovery rate
GO	Gene ontology
HCA	Hydroxycinnamic acid
HPLC-MS	High performance liquid chromatography mass spectrometry
HY5	ELONGATED HYPOCOTYL 5
HYH	HY5 HOMOLOG
KEGG	Kyoto encyclopedia of genes and genomes
PAR	Photosynthetically active radiation
PHOTs	Phototropin 1 and phototropin 2
PHYs	Phytochromes A-E
PIF	PHYTOCHROME INTERACTING FACTOR
qRT-PCR	Quantitative real time PCR
ROS	Reactive oxygen species
RUP	REPRESSOR OF UV-B PHOTOMORPHOGENESIS
SPA	SUPPRESSOR OF PHYA-105
TF	Transcription factor
UV-B	Ultraviolet-B (290–315 nm)

UV-A	Ultraviolet-A (315–400 nm)
$UV\text{-}A_{sw}$	Short-wave UV-A (315–350 nm)
$UV\text{-}A_{lw}$	Long-wave UV-A (350–400 nm)
UVR8	UV RESISTANCE LOCUS 8
WT	Wild type

1 INTRODUCTION

Plants growing in natural environments are exposed to different signals and cues that regulate responses at the whole plant level. Of those environmental signals and cues, sunlight is of utmost importance as a source of information for plants. The key components of sunlight are ultraviolet (UV, 280–400 nm), photosynthetically active radiation (PAR, 400–700 nm), far-red (FR, 700–780 nm), and infrared radiation (IR, 780–4000 nm).

Based on the ISO standards, wavelengths in the UV region are divided into UV-C (100–280 nm), UV-B (280–315 nm), and UV-A (315–400 nm) radiation (Björn, 2015). However, wavelengths below ~290 nm are absorbed by the ozone layer and atmospheric oxygen and are not detectable at the Earth's surface (Hartley, 1881; Rowland, 2006). UV radiation accounts for 4–4.5% of global ground-level energy irradiance (Escobedo et al., 2011), encompassing a small and very variable UV-B radiation component (0.0–0.2%) and the rest as UV-A radiation (4–4.5%). Wavelengths in the PAR spectral region account for 45 to 50% of global energy irradiance (Jacovides et al., 2003). PAR comprises wavelengths visible to humans as colored light, including violet/blue (400–500 nm), and orange/red (600–700 nm). However, in plant research, these two regions are named as blue light and red light, respectively, although other definitions are sometimes used.

Wavelengths in UV, PAR, and FR spectral regions are perceived by plants through light-sensitive protein photoreceptors. In Arabidopsis, 13 photoreceptors have been identified to this date: phytochromes A-E (PHYs) are the red/far-red light photoreceptors (Fankhauser, 2001); cryptochrome 1 and cryptochrome 2 (CRYs), phototropin 1 and phototropin 2 (PHOTs), and three Zeitlupe proteins are UV-A/blue light photoreceptors (Ahmad and Cashmore, 1993; Lin, 2000; Yu et al., 2010; Christie et al., 2015); and UV RESISTANCE LOCUS 8 (UVR8) is a UV-B photoreceptor (Rizzini et al., 2011).

The assignment of photoreceptors to one or more spectral regions has been based on their stronger absorption of photons in those regions and on monochromatic action spectra. As a result, it is expected that photoreceptors will regulate plant responses in those same spectral regions. It is noteworthy that photoreceptors also absorb in other regions of the spectrum, although more weakly (Figure 1). During the perception of sunlight by plants, different photoreceptors are triggered simultaneously. Upon photon absorption, photoreceptors like UVR8 and CRYs initiate signaling cascades leading to changes in gene expression, which results in plant responses at physiological and biochemical levels (Jenkins, 2017; Yin and Ulm, 2017). This concurrent activation of signaling pathways makes interactions between them possible (Casal, 2000).

Before the studies included in this thesis, the roles of UVR8 and CRYs in plants had been independently assessed under UV-B radiation and blue light, respectively. This approach does not inform on how these photoreceptors would respond to a range of wavelengths covering from UV-B to blue regions of the solar spectrum. It has been assumed that the blue light photoreceptors mediate plant responses to UV-A radiation; however, very few experiments have tested this. Besides, as customary in molecular biology, photoreceptor-mediated plant responses to UV radiation and blue light come from experiments performed in controlled environments, where UV-B:UV-A, UV-A:PAR, and UV-B:PAR ratios are very different from those in natural environments. Therefore, results from these experiments cannot be directly extrapolated to plants growing outdoors.

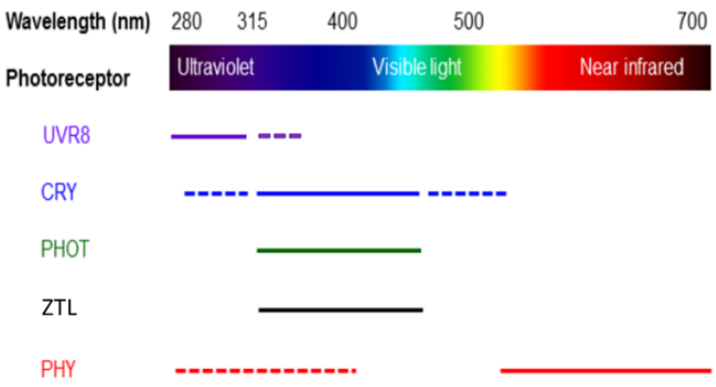

Figure 1 Regions of the solar spectrum where plant photoreceptors absorb strongly (solid lines) or weakly (dashed lines). Modified from Morales (2014).

In an ever-changing natural environment, plants are often exposed to a broad range of abiotic stresses (Pereira, 2016). Of those, drought stress is a major threat to plant growth, especially in mesic and dry habitats (Schwabe and Connor, 2012). Plants being sessile cannot move away from the location of stress to a more favorable location but rather use *in situ* avoidance, escape, and/or tolerance strategies to overcome drought (Farooq et al., 2009; da Silva et al., 2013). Often high UV irradiance under clear sky correlates with high potential evapotranspiration and no precipitation, which are followed by periods of drought stress (Bornman et al., 2019). Besides, it has been reported that UV-B radiation and drought can interact positively or negatively to regulate plants' physiological responses (Comont et al., 2012; Rajabbeigi et al., 2013; Robson et al., 2015).

As exposure to strong UV radiation is frequently followed by drought stress in a natural environment, the most realistic experimental set-up to study UV radiation and

drought interaction are experiments where solar UV radiation is applied both prior to and in parallel to a progressive drought treatment. Furthermore, in addition to physiological responses to UV and drought combinations, measuring biochemical and molecular responses could reveal additional positive interaction between UV radiation and drought, which could provide acclimation in plants. Also, understanding plant responses to UV radiation in conjunction with drought stress would be very informative to understand the role of UV radiation from an ecological perspective.

2 REVIEW OF THE LITERATURE

2.1 Plant responses to UV radiation

Ambient levels of UV-B radiation vary significantly with altitude and solar elevation angle, which depends on latitude and time of the day (Seckmeyer et al., 2007). In addition, they vary with the thickness of the ozone layer and atmosphere, cloud cover, canopy cover, surface reflectance, and scattering and absorbance by aerosol pollutants (Madronich et al., 1998; McKenzie et al., 2007; Seckmeyer et al., 2007).

Highly energetic UV-B photons can damage DNA, RNA, proteins, and membrane lipids (Caldwell et al., 1998; Jansen et al., 1998; Rastogi et al., 2010). High levels of UV-B radiation can cause oxidative damage and consequently lead to the generation of reactive oxygen species (ROS), including singlet oxygen, superoxide radical, and hydrogen peroxide (Dai et al., 1997; Hideg et al., 2002). However, ROS, a damaging agent at high concentrations, can also act as a signaling molecule at low concentrations (Schieber and Chandel, 2014).

Plant UV research in the 1980s used unrealistically high UV-B irradiance and, therefore, frequently reported damaging effects of UV-B radiation on plants. Those experiments informed that high UV-B irradiance could introduce mutagenic lesions in DNA, primarily in the form of cyclobutane-pyrimidine dimers (CPDs) and to a lesser extent in the form of pyrimidine (6, 4) pyrimidone dimers (6-4 PPs) (Britt, 2004; Rastogi et al., 2010). These lesions negatively affect DNA replication and transcription processes since the polymerase enzyme cannot read through CPDs and 6-4 PPs (Britt, 2004; Rastogi et al., 2010). However, plants efficiently repair UV-B-induced DNA damage prior to replication and transcription through a photoreactivation mechanism driven by UV-A and blue light using photolyases (Britt, 2004; Rastogi et al., 2010).

UV-B exposure can decrease photosynthesis (Teramura and Sullivan, 1994). The reported negative effects on photosynthetic activity include decreased transcript abundance of photosynthesis-related genes, reduction of carbon dioxide uptake as a result of an impaired stomatal function, reduction of chlorophyll content, disruption of photosynthetic electron transport due to damage to photosystem II (D1 protein), and damage to Rubisco enzyme (Teramura and Sullivan, 1994). Noteworthy, the extent of damage caused by UV-B radiation depends on the species and its location of growth and origin (Sullivan et al., 1992), developmental stage of the plant (Jordan et al., 1994), UV-B dose and wavelength region (Searles et al., 2001; Ulm et al., 2004; Kalbina et al., 2008; Jenkins, 2009), duration of UV-B exposure and background

irradiation (Searles et al., 2001; Jenkins, 2009), and UV-B:UV-A and UV-B:PAR ratios (Krizek, 2004). As UV-A and PAR mitigate the damaging effects of UV-B radiation, those damaging effects are often observed in experiments performed in controlled growth environments using high UV-B irradiance in a background of low UV-A and PAR irradiance (Caldwell and Flint, 1994; Caldwell et al., 1994). As a result, those experiments tend to show a pronounced effect of UV-B radiation on plants.

The initial response to UV-B radiation occurs at the transcriptional level, where UV-B increases the transcript abundance of genes involved in UV protection and damage repair. Also, many responses regulated by UV-B radiation are similar to those triggered by pathogenesis and herbivory, for example, induction of pathogenesis-related genes and accumulation of hormones (salicylic acid, jasmonic acid, and ethylene) (Brosché and Strid, 2003; Izaguirre et al., 2003, 2007). The regulatory effect of UV-B radiation on plant morphology and development includes the reduction of stem extension growth and leaf expansion plus an increase in leaf thickness and axillary branching (Jenkins, 2009, 2017). UV-B induces accumulation of several phenolics, including flavonoids and hydroxycinnamic acids (HCAs) (Jenkins, 2017). These compounds provide screening against incoming UV radiation by accumulating in the epidermal tissues (Agati and Tattini, 2010). Flavonoids and HCAs also act as antioxidants and accumulate at the sites of production of ROS (vacuole, chloroplast) (Agati and Tattini, 2010). Several enzymatic antioxidants such as glutathione reductase, ascorbate peroxidase, and superoxide dismutase also accumulate in response to UV-B radiation (Landry et al., 1995; Rao et al., 1996). Besides, UV-B increases accumulation of phenolic compounds that protect plants against herbivory (Izaguirre et al., 2003, 2007).

Compared to UV-B radiation, much less is known about the effects of UV-A radiation on plants. This is surprising as there are many times more UV-A photons than UV-B photons in ground-level solar radiation (Escobedo et al., 2011). Moreover, UV-A radiation penetrates more readily into leaf tissues than UV-B radiation (Wilson et al., 2001). Previous studies have shown both stimulatory and inhibitory effects of UV-A radiation on growth, chlorophyll content, and photosynthesis (Tezuka et al., 1993, 1994; Häder, 1996; Krizek et al., 1997, 1998; Mantha et al., 2001; Vass et al., 2002; Tyystjärvi, 2008; Kataria et al., 2013; Verdaguer et al., 2017; Chen et al., 2019; Isner et al., 2019; Qian et al., 2020). The inhibitory effect of UV-A on growth is primarily due to damage to Photosystem II (Mn cluster, D1 and D2 proteins) and the two plastoquinone (Q_A and Q_B) binding sites (Greenberg et al., 1989; Christopher and Mullet, 1994; Turcsányi et al., 2000; Vass et al., 2002; Nayak et al., 2003; Tyystjärvi, 2008). However, it is noteworthy that most of the inhibitory effects of UV-A on photosynthesis come from *in vitro* studies using isolated chloroplasts or thylakoids (Turcsányi et al., 2000; Vass et al., 2002; Verdaguer et al., 2017). In realistic conditions, these adverse effects seem to be ameliorated by the protection provided by

leaf morphology or by the accumulation of UV screening phenolic compounds (Hakala-Yatkin et al., 2010). Furthermore, under both low and high PAR irradiance, UV-A radiation can have a stimulatory effect on photosynthesis through various mechanisms (Mantha et al., 2001; Johnson and Day, 2002; Kataria et al., 2013; Turnbull et al., 2013; Verdaguer et al., 2017). Similar to UV-B, UV-A radiation induces accumulation of anthocyanins and phenolic compounds, including flavonoids and HCAs (Kotilainen et al., 2008, 2009; Guo and Wang, 2010; Morales et al., 2010, 2013; Siipola et al., 2015; Brelsford et al., 2018). Interestingly, it was shown that specific phenolic compounds responded differently to the short-wave UV-A radiation (UV-A_{sw}, 315–350 nm) and long-wave UV-A radiation (UV-A_{lw}, 350–400 nm) (Siipola et al., 2015). At the transcript level, UV-A exposure increases the transcript abundance of several genes associated with flavonoid biosynthesis, including *PHENYLALANINE AMMONIA LYASE* (*PAL*), *CHALCONE SYNTHASE* (*CHS*), *PRODUCTION OF ANTHOCYANIN PIGMENT 1* (*PAP1*), and *DIHYDROFLAVONOL 4 REDUCTASE* (*DFR*) (Fuglevand et al., 1996; Guo and Wang, 2010; Lee et al., 2013; Morales et al., 2013).

Unlike UV-B and UV-A radiation, where the role of photoreceptors has only recently begun to emerge or is not yet well-described, plant responses to blue light are widely discussed pertaining to the roles of photoreceptors (see below).

2.2 Photoreceptor-mediated responses

2.2.1 UVR8

The UV-B photoreceptor UVR8 is a seven-bladed β-propeller protein (Wu et al., 2012). It is different from all other plant photoreceptors as it lacks a bounded cofactor chromophore but instead uses tryptophan amino acids as an intrinsic chromophore for UV-B absorption (Rizzini et al., 2011; Christie et al., 2012; Wu et al., 2012; Jenkins, 2014a, 2014b). In the absence of UV-B, UVR8 exists as a homo-dimer and is mainly present in the cytosol, whereas UV-B exposure leads to UVR8 monomerization and rapid accumulation in the nucleus (Brown et al., 2005; Kaiserli and Jenkins, 2007; Rizzini et al., 2011). The UVR8 protein consists of a C-terminal domain and a β-propeller domain (Christie et al., 2012; Wu et al., 2012). The C27 domain (27 amino acids at C-terminal) of UVR8 monomers bind to CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) to initiate UVR8 signaling (Favory et al., 2009; Cloix et al., 2012). COP1, together with its accessory protein SUPPRESSOR OF PHYA-105 (SPA), functions as a CULLIN4-DAMAGED DNA BINDING PROTEIN 1 (CUL4–DDB1)-based E3 ubiquitin ligase, which is a well-known key repressor of photomorphogenesis in darkness (Lau and Deng, 2012). More recently, it has been shown that the β-propeller domain of UVR8 also interacts with COP1; however, COP1

activity is regulated by interaction with UVR8's C-terminal (Yin et al., 2015). UVR8-COP1 interaction mediates expression of bZIP transcription factors (TFs) ELONGATED HYPOCOTYL 5 (HY5) and HY5 HOMOLOG (HYH) (Favory et al., 2009), which in turn regulate the expression of several UV-B-responsive genes (Ulm et al., 2004; Brown et al., 2005, 2009; Oravecz et al., 2006; Stracke et al., 2010; Binkert et al., 2014). Two of those genes encode for REPRESSOR OF UV-B PHOTOMORPHOGENESIS 1 (RUP1) and RUP2, which are WD40 domain proteins (Gruber et al., 2010). RUP1 and RUP2 interact with UVR8 and facilitate its re-dimerization (Gruber et al., 2010; Heijde and Ulm, 2013; Findlay and Jenkins, 2016). Therefore, UVR8 dimers and monomers exist in constant equilibrium (Findlay and Jenkins, 2016).

UVR8 mediates a variety of plant responses to UV-B radiation. Of those, the most commonly described are inhibition of hypocotyl growth, accumulation of anthocyanin and flavonoids, and changes in gene expression and protein accumulation (Brown et al., 2005; Favory et al., 2009; Morales et al., 2013). Several other UVR8-mediated physiological responses to UV-B radiation include entrainment of the circadian clock (Fehér et al., 2011), phototropism (Vandenbussche et al., 2014), stomatal opening (Tossi et al., 2014), and downward leaf curling (Fierro et al., 2015). UVR8-mediated UV-B signaling represses auxin-responsive genes and could be potentially responsible for the inhibition of hypocotyl elongation response (Vandenbussche et al., 2014). UVR8 is also associated with defense response (Demkura and Ballaré, 2012), osmotic or salt stress tolerance (Fasano et al., 2014), inhibition of shade avoidance (Hayes et al., 2014), and inhibition of thermomorphogenesis (Hayes et al., 2017).

UVR8 physically interacts with BRI1-EMS-SUPPRESSOR 1 (BES1) and BES1-INTERACTING MYC-LIKE 1 (BIM1) TFs in the nucleus (Liang et al., 2018). This interaction is UV-B-independent (Liang et al., 2018); however, the accumulation of UVR8 in the nucleus is UV-B dependent (Kaiserli and Jenkins, 2007). Under UV-B, UVR8-BES1/UVR8-BIM1 interaction inhibits the DNA-binding activity of BES1/BIM1, suppressing the induction of brassinosteroid-induced genes (growth-related genes), which subsequently contributes to the inhibition of hypocotyl elongation (Liang et al., 2018). Similarly, UVR8 also physically interacts with WRKY36 TF in the nucleus, which inhibits WRKY36 DNA-binding activity, thereby promoting *HY5* transcription and inhibition of hypocotyl elongation (Yang et al., 2018).

2.2.2 Cryptochromes

CRYs show structural similarity to the DNA-repair enzyme photolyase (Sancar, 2003). CRYs possess two domains, the photolyase homology region (PHR) domain and a distinctive cryptochrome C-terminus (CCT) domain, which is absent in

photolyase (Christie et al., 2015). The PHR domain is essential in light-sensing, while the CCT domain for signaling (Yang et al., 2000). CRYs bind to two chromophores: the primary sensor flavin adenine dinucleotide in the PHR domain and a pterin derivative 5,10-methenyltetrahydrofolate (Christie et al., 2015). CRY1 is present both in the nucleus and cytoplasm, while CRY2 is exclusively present in the nucleus (Guo et al., 1999; Yu et al., 2007). CRYs exist as inactive monomers in the absence of light and form homodimers and oligomers after light exposure (Wang et al., 2016). They bind to COP1 and SPA proteins through different mechanisms that stabilize HY5 and HYH TFs, in turn regulating the gene expression of a subset of blue-light responsive genes (Yang et al., 2017; Podolec and Ulm, 2018). Two of those genes encode for BLUE-LIGHT INHIBITOR OF CRYPTOCHROME 1 (BIC1) and BIC2 protein, which interact with CRYs and inhibit their dimerization, thereby acting as negative regulators of CRY signaling (Wang et al., 2016, 2017).

Nuclear localized CRYs regulate most of the gene expression response to blue light (Ohgishi et al., 2004; Lin and Todo, 2005). CRY1 mediates the increase in transcript abundance of *CHS* (encoding an enzyme involved in flavonoid biosynthesis) in response to blue light (Jackson and Jenkins, 1995; Fuglevand et al., 1996). Microarray analysis showed that CRY1 and CRY2 function independently to regulate transcription of blue light-induced genes and that many of those genes are involved in light signaling, photosynthetic light reactions, Calvin cycle, phenylpropanoid metabolic pathway, and stress response in plants exposed to blue light (Ohgishi et al., 2004; Kleine et al., 2007). CRY1 mediates inhibition of hypocotyl elongation, inhibition of petiole extension, decrease in leaf width and area, cotyledon development, and accumulation of anthocyanin in response to blue light (Ahmad and Cashmore, 1993; Ahmad et al., 1995; Jackson and Jenkins, 1995; Lin et al., 1995, 1996; Ninu et al., 1999; Weller et al., 2001; Giliberto et al., 2005; Sharma et al., 2014). CRY2 also plays a role in inhibition of hypocotyl elongation and in cotyledon opening under low fluence rates of blue light (Guo et al., 1998; Lin et al., 1998; Giliberto et al., 2005). Besides, both CRY1 and CRY2 act redundantly in controlling flowering time, whereas CRY2 also mediates delay in leaf senescence in response to blue light (Mockler et al., 2003; Meng et al., 2013). From these previous studies, only a few of them have tested roles of CRYs in UV-A mediated responses (Lin et al., 1995, 1996; Fuglevand et al., 1996). Those few studies showed that CRY1 mediates inhibition of hypocotyl length, accumulation of anthocyanin, and induction of *CHS* in response to both UV-A radiation and blue light (Lin et al., 1995, 1996; Fuglevand et al., 1996).

CRYs also physically interact with TFs to regulate plant responses. CRYs bind to bHLH TFs PHYTOCHROME-INTERACTING FACTOR 4 (PIF4) and PIF5 under low blue light to promote growth (Pedmale et al., 2016). CRY2 interacts with CRYPTOCHROME-INTERACTING basic-helix-loop-helix 1 (CIB1) under blue light to promote floral initiation and suppresses the CIB1-mediated leaf senescence (Liu et al., 2008; Meng et al., 2013). CRYs also interact with BES1 and BIM1 TFs in

a blue-light-dependent manner, inhibits their DNA binding activity, leading to suppression of BES1 target genes and inhibition of hypocotyl elongation (Wang et al., 2018).

2.2.3 Other UV-A/blue photoreceptors

PHOTs are another family of UV-A radiation and blue light photoreceptors that consist of an N-terminus photosensory domain and a C-terminus serine-threonine kinase domain (Christie, 2007). At the photosensory terminus, the chromophore flavin mononucleotide binds within the two LOV1 and LOV2 domains (Christie, 2007). PHOTs are activated by blue light through autophosphorylation (Christie, 2007; Christie et al., 2015). PHOTs are primarily localized to the plasma membrane and to the outer membrane of the chloroplast (Christie et al., 2015). Upon blue light exposure, PHOT 1 translocates to the cytosol while PHOT2 to the golgi apparatus (Christie et al., 2015).

PHOT1 and PHOT2 overlap in function to regulate hypocotyl and root phototropism, chloroplast movement/relocation, stomatal opening, and leaf positioning and leaf blade flattening in response to different fluence rates of blue light (Liscum and Briggs, 1995; Briggs et al., 2001; Jarillo et al., 2001; Kagawa et al., 2001; Kinoshita et al., 2001; Sakai et al., 2001; Briggs and Christie, 2002; Sakamoto and Briggs, 2002; Kasahara et al., 2004; Inoue et al., 2008). For hypocotyl phototropism, PHOT2 functions at high light intensities while PHOT1 functions at a broad range of fluence rates (Sakai et al., 2001). For chloroplast movement, both PHOT1 and PHOT2 function redundantly at low light intensity while PHOT2 functions at high light intensity (Jarillo et al., 2001; Kagawa et al., 2001; Higa et al., 2014). PHOT1 is also involved in the rapid inhibition of stem growth in response to blue light (Folta and Spalding, 2001). In addition, both PHOT1 and PHOT2 play roles in blue-light induced growth enhancement under low PAR, suggested to be an outcome of optimization of photosynthetic efficiency (Takemiya et al., 2005). Furthermore, unlike CRYs, PHOTs play a small role in the regulation of gene expression in response to blue light (Briggs and Huala, 1999; Ohgishi et al., 2004). Of these previous studies, Liscum and Briggs, (1995) showed that PHOT1 mediates phototropism in response to UV-A radiation, while other PHOTs-mediated responses have not been described in relation to UV-A radiation.

Other LOV domain-containing proteins include members of the zeitlupe family: Zeitlupe (ztl), Flavin-binding kelch repeat F-box 1 (fkf1), and LOV kelch protein 2 (lkp2), which localize to the cytosol or nucleus (Christie et al., 2015). These proteins are involved in the regulation of the circadian clock and control of the photoperiodic flowering time (Somers et al., 2000; Imaizumi et al., 2003; Baudry et al., 2010).

2.3 Interaction between photosensory pathways

In a polychromatic light environment, interaction between different photosensory pathways allows plants to integrate information to optimize growth and development. Interaction could occur through physical binding between the photoreceptors and/or through signaling components downstream of the photoreceptors. Physical interaction between the photoreceptors would affect all the downstream signaling, while interaction through signaling components would result in different patterns of responses (Robson et al., 2019). Mechanistic evidence for both photoreceptor interaction and signaling component interaction under a specific wavelength of light has been shown by protein interaction assays such as yeast-two-hybrid, biomolecular fluorescence complementation, and co-immunoprecipitation. Furthermore, experiments using photoreceptor-defective mutants grown under different wavelengths of light and measurement of multiple responses can also help to pinpoint interaction.

In the case of CRYs, it has been shown that CRY1 interacts with PHYA, while CRY2 interacts with PHYB (Ahmad et al., 1998; Mas et al., 2000). For UVR8, no physical interaction with other photoreceptors has been reported so far. However, evidence exists for interaction between UVR8 and PHYB signaling (Mazza and Ballare, 2015). Furthermore, there is an indication for interaction between UVR8 signaling and CRY signaling as both UVR8 and CRYs use VP peptide motif, which competes for binding to the WD-40 domain of COP1 (Wang et al., 2001; Favory et al., 2009; Lau et al., 2019). Both UVR8 and CRYs also bind to BES1 and BIM1 TFs (Liang et al., 2018; Wang et al., 2018), and such interaction could be thought of at the same level as the one between PHYB and CRY2 through PIF4 and PIF5 TFs (both PIF4 and PIF5 bind to PHYB and CRY2) (Leivar and Monte, 2014; Pedmale et al., 2016). Thus, the interaction between UVR8 and CRY signaling pathways could exist through a competition between the photoreceptors for binding to the same substrate (COP1, BES1) required to mediate signaling. Both UVR8 and CRY signaling stabilize HY5 and HYH accumulation, which in turn mediate regulation of several genes shared between the two signaling pathways, including those involved in phenolic compounds biosynthesis. Thus, beyond protein-protein physical binding, common signaling components like HY5 and HYH or other TFs could also mediate UVR8-CRY interaction.

2.4 Plant responses to drought stress

Drought occurs when there is a prolonged period of scarce precipitation relative to evapotranspiration, which leads to lower soil water availability than plant demand. Research on plant responses to drought stress has become increasingly important as predicted climate change scenarios suggest a high probability of increase in aridity in

many regions on Earth (Watson et al., 2017). On a global scale, drought occurs in conjunction with high temperature and irradiance and poses a major limitation to plant growth, survival, and productivity (Farooq et al., 2009; Feller and Vaseva, 2014; Daryanto et al., 2016; Lamaoui et al., 2018). In nature, plants are subjected to both short-term (hours to days) and slow-progressive long-term drought (days to months) depending on the local conditions (Chaves et al., 2003).

Drought stress signaling involves the perception of signal likely by the plasma membrane receptors (e.g., transmembrane histidine kinases), which trigger downstream signaling cascade (Tiwari et al., 2017). Once the signal reaches the nucleus, it induces the expression of several TFs and drought-responsive genes, including *ABA-RESPONSIVE ELEMENT* (*ABRE*), *DEHYDRATION-RESPONSIVE ELEMENT-BINDING 2* (*DREB2*), *NAC*, *MYC2*, *MYB2*, *RESPONSIVE TO DEHYDRATION 1* (*ERD1*), *LATE EMBRYOGENESIS ABUNDANT* (*LEA*), and *HEAT SHOCK PROTEINs* (*HSPs*) (Shinozaki and Yamaguchi-Shinozaki, 2007; Tiwari et al., 2017). Moreover, drought induces accumulation of abscisic acid (ABA), which also controls the expression of drought-responsive genes (Shinozaki and Yamaguchi-Shinozaki, 2007; Huang et al., 2008). The changes occurring at the transcript level allow plants to respond to drought at physiological and metabolic scales.

Annual plants that show high developmental plasticity escape drought stress by completing their life cycle before the onset of severe drought (Shavrukov et al., 2017). Annual and perennial plants avoid drought stress by minimizing water loss and maximizing water uptake. Water loss is minimized by closing stomata, reducing light absorbance by rolled leaves, dense trichome cover that increases reflectance, steep leaf angle that decreases radiation interception, reduced shoot growth that decreases leaf area, and shedding of older leaves (Ehleringer and Cooper, 1992; Chaves et al., 2003; Osakabe et al., 2014). Water uptake is maximized by investing resources into the growth of roots (Wasson et al., 2012). Plants tolerate drought through osmotic adjustments, more rigid cell walls, decreased cell size, and partial dormancy (Chaves et al., 2003). Under periods of extreme drought, plants accumulate specialized phenolic compounds like flavones and flavonols to minimize oxidative damage (Hernandez et al., 2004). Higher accumulation of specialized phenolic compounds in response to drought stress is associated with their ability to act as antioxidants in scavenging ROS (Hernandez et al., 2004). These phenolic compounds act as non-enzymatic antioxidants, and their response to drought stress has been less emphasized than the enzymatic antioxidants like ascorbic acid, α-tocopherol, and glutathione. The antioxidant properties of a phenolic compound are determined by the presence of hydroxyl groups and modifications like glycosylation and methylation (Jiang et al., 2016). Based on these structural properties, some compounds that act as good antioxidants are quercetin, luteolin, and their derivatives (Jiang et al., 2016).

2.5 Interaction of UV radiation and drought

During summer months, with a clear sky and low precipitation rate, there is high UV-B irradiance, which is accompanied by subsequent periods of drought. Studies have shown an interaction between UV-B radiation and drought, which affects plant's morphology, physiology, and biochemistry (Comont et al., 2012; Robson et al., 2015; Rodríguez-Calzada et al., 2019). When compared to plant responses regulated by UV-B or drought alone, UV-B and drought can sometimes interact positively or negatively to increase or decrease a response. The effect of interaction depends on the genotype, UV-B dose, strength/duration of drought, the experimental set-up, and timing and order in which the treatments are applied.

Most of the UV-B-drought combination experiments have been performed in a way where both treatments were applied simultaneously. In experiments where supplemental UV-B was provided through lamps, UV-B radiation and drought interacted to increase yield and relative water content of spring wheat (Feng et al., 2007), whereas interacted to decrease rosette biomass, leaf area, growth rate, and net assimilation rate in Arabidopsis (Comont et al., 2012). In an experimental set-up where UV-B radiation was applied first using fluorescent lamps, and drought afterward, UV-B and drought interacted to decrease stem length, and increase leaf thickness and accumulation of luteolin in chili pepper (Rodríguez-Calzada et al., 2019). At the transcript level, no interaction was detected for the accumulation of *PAL* and *CHS* (Rodríguez-Calzada et al., 2019). On the other hand, UV radiation and drought appeared to interact positively to increase the abundance of a gene related to oxidative response *Mn-SUPEROXIDE DISMUTASE* (*Mn-SOD)* (Rodríguez-Calzada et al., 2019). In a field experiment using UV absorbing filters, UV-B and drought interacted positively to improve water-use efficiency and to increase the height of seedlings plus the number and length of leaves in silver birch seedlings (Robson et al., 2015).

3 AIMS AND HYPOTHESES

Our knowledge on the perception of UV-B radiation, UV-A radiation, and blue light, and the interaction between UV radiation and drought stress are mostly based on experiments performed in controlled environments using artificial light sources. The main aim of my thesis is to expand this knowledge for plants growing in field conditions where they receive a full spectrum of sunlight.

The specific aims were:

1. To identify the individual roles of UVR8 and CRYs in the perception of solar UV-B, short-wave UV-A (315–350 nm, UV-A_{sw}), long-wave UV-A (350–400 nm, UV-A_{lw}) radiation, and blue light by plants (I).
2. To test the interaction between UVR8 and CRYs under UV radiation (I, II).
3. To determine if pre-exposure to solar UV radiation could provide acclimation to subsequent drought stress in plants (III).

The following hypotheses were tested:

1. UVR8, but not CRYs, is required for the perception of solar UV-B and UV-A_{sw} radiation (I).
2. CRYs, but not UVR8, are required for the perception of solar UV-A_{lw} radiation and blue light (I).
3. UVR8 and CRY signaling pathways interact to modulate plant responses to UV-B and UV-A_{sw} radiation (I, II).
4. Pre-exposure of plants to solar UV-B+UV-A_{sw} radiation provides acclimation to subsequent drought stress (III).

4 MATERIALS AND METHODS

The materials and methods used in this dissertation are described in detail in papers I-IV.

4.1 Plant material

Two different model plant species were used in the experiments: *Arabidopsis thaliana* (thale cress) and *Medicago truncatula* (barrel medic or barrel clover) (Figure 2A, B). *Arabidopsis thaliana* is a facultative long-day plant that belongs to the *Brassicaceae* family and has a determinate growth. *Medicago truncatula* is also a long-day plant that belongs to the *Fabaceae* family and has an indeterminate growth.

Table 1 Plant species and their genotypes or accessions used in the study.

Species	Genotype/Accession	Description	Publication or Manuscript
Arabidopsis thaliana	Landsberg *erecta* (L*er*)	wild type (WT)	I, II
	uvr8-2	UVR8 photoreceptor mutant	I, II
	cry1cry2	cryptochrome 1 and 2 photoreceptor mutant	I, II
	cry1cry2uvr8-2	cryptochrome 1, 2 and UVR8 triple mutant	II
Medicago truncatula	Jemalong A17	Australian cultivar, mildly drought-tolerant	III
	F83005-5	French natural accession, drought-sensitive	III

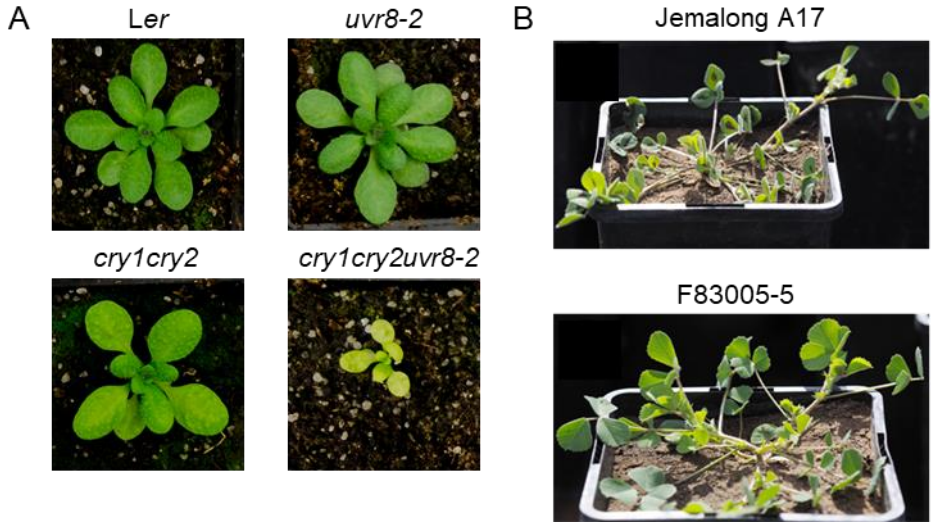

Figure 2 Representative photographs of *Arabidopsis thaliana* and *Medicago truncatula* plants. (A) 17 days old *Arabidopsis thaliana* wild type (L*er*) and photoreceptor mutants (*uvr8-2*, *cry1cry2*, *cry1cry2uvr8-2*) grown under a filter attenuating UV-B radiation, and (B) 45 days old *Medicago truncatula* (Jemalong A17 and F83005-5) grown under a filter allowing all wavelengths.

4.2 Plant cultivation and treatments

An experiment for the paper I was performed in the growth room and the field area of the University of Helsinki (Figure 3A). Arabidopsis plants were grown in growth rooms for two weeks under 12 hours photoperiod at 23°C/19°C and 70%/90% relative humidity (day/night) with 280 μmol m^{-2} s^{-1} white light irradiance, without UV. For exposure to 6 hours and 12 hours of solar UV and blue light, plants were moved to the field and kept under long-pass filters (Figure 3A). Five different kinds of filters were used in the experiment to assess the effects of UV-B, UV-A, UV-A_{sw}, UV-A_{lw}, blue, UV-B+UV-A_{sw}, and UV-A_{lw}+blue (Figure 4). Plant rosette material was harvested after 6 hours (solar noon) and 12 hours (late afternoon) to assess transcript abundance. For *in vitro* monomerization of UVR8, Arabidopsis protein was expressed and purified from *Nicotiana benthamiana*. Purified UVR8 protein was exposed to different wavelengths in UV-B and UV-A regions using a UV tunable laser and then quantified to assess monomer-dimer status.

An experiment for the paper II was performed in a sun simulator chamber at Helmholtz Zentrum (Munich, Germany) (Figure 3B). Arabidopsis plants were grown in the sun simulator, where different kinds of lamps were connected in separately controlled groups to allow the simulation of the diurnal variation in solar irradiance. The sun simulator was under a 10 hours photoperiod at 21 °C/19 °C air temperature and 65%/80% relative humidity (day/night). Two different cuvettes in the chamber were used to create short-term (6 hours) and long-term (17 days) UV and blue treatments

using long-pass filters (Figure 4). Photographs of plants were taken after 17 days to assess growth. Plants were harvested after 6 hours and 17 days for transcript abundance and secondary metabolite analysis. Transcript abundance analysis was carried out at the University of Helsinki. Secondary metabolite analysis was carried out at the Leibniz Institute of Vegetable and Ornamental Crops (Großbeeren, Germany). In addition, an outdoor experiment was performed to assess growth and survival of WT and photoreceptor mutants after 17, 20, 24, and 27 days of exposure to UV radiation and blue light (II).

An experiment for the paper III was performed in the field area of the University of Helsinki using *Medicago truncatula*. Plants were germinated under three different long-pass filters. These filters were used to assess the effects of UV-B+UV-A_{sw} and UV-A_{lw} radiation. Plants were kept well-watered for nearly one month and later one set of plants were subjected to drought by withholding water for the next seven days. During these seven days, both drought and UV treatments were simultaneously applied. Plants were measured for epidermal screening on Day 1 and Day 7 of the water-withholding period. Leaves (blade and petiole) were harvested at Day 0 and Day 7 of the water-withholding period to assess transcript abundance, secondary metabolites, and ABA accumulation. Transcript abundance analysis was carried out at the University of Helsinki. Secondary metabolite and ABA analysis were carried out at the Leibniz Institute of Vegetable and Ornamental Crops (Großbeeren, Germany).

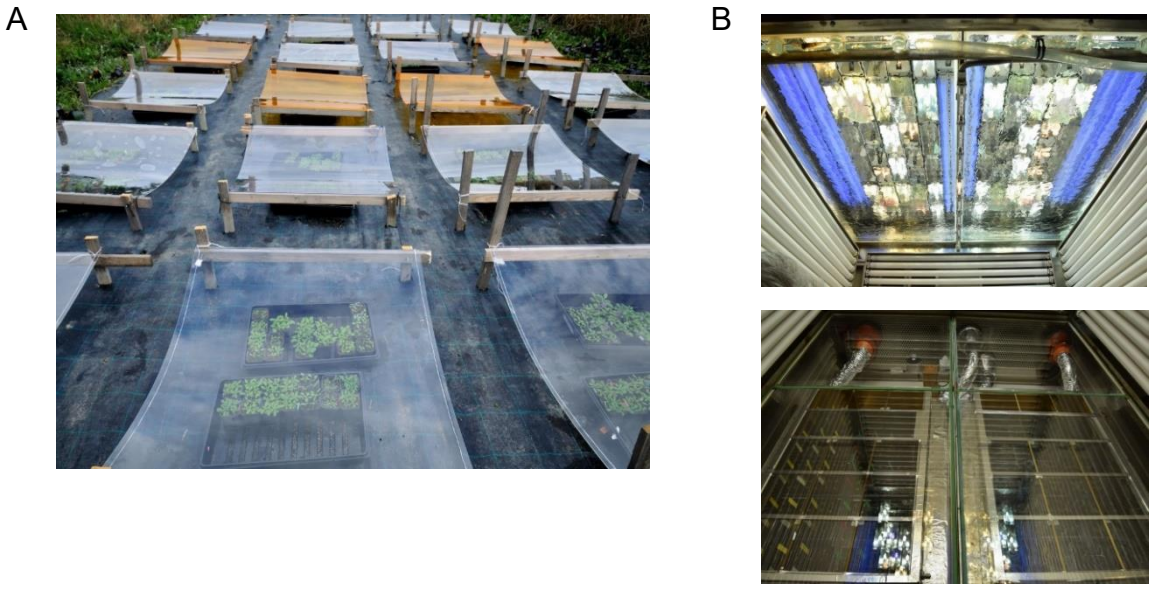

Figure 3 Photographs of the experimental set-up (A) field experiment, (B) sun-simulator experiment.

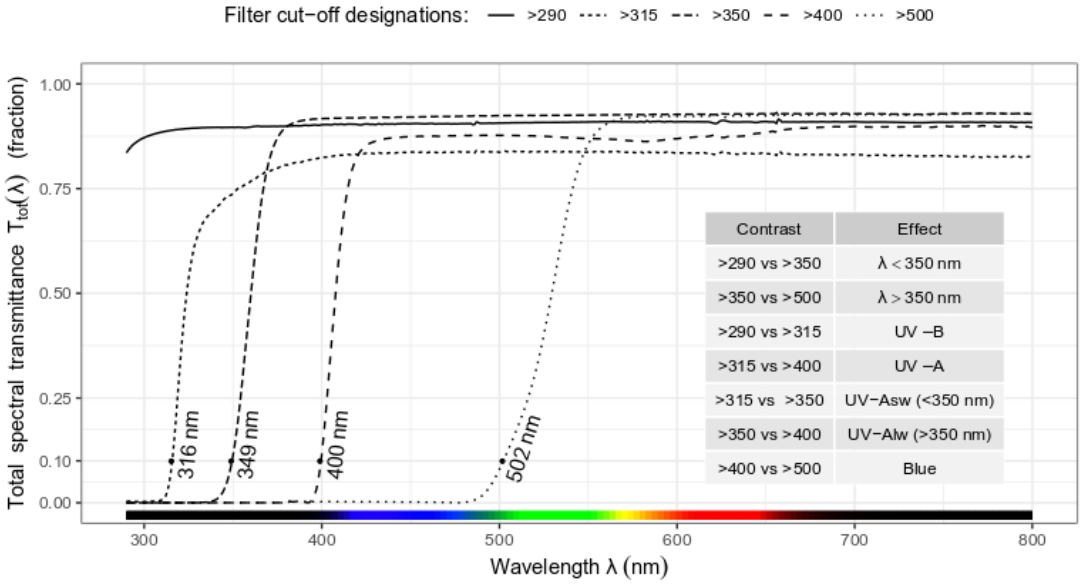

Figure 4 Transmittance of filters used in this study and the statistical contrasts between pairs of filter treatments used to assess the effects of different ranges of wavelengths in solar radiation. Effect of all the wavebands were tested in paper I, effect of UV-B, UV-A, UV-A_{sw}, UV-A_{lw} radiation, and blue light were tested in paper II, and effect of UV-B+UV-A_{sw} (λ < 350) and UV-A_{lw} were tested in paper III. Figure from Rai et al. (2020).

4.3 Methods

In this dissertation, state-of-the-art molecular biology methods were used to study the roles of UVR8 and CRYs photoreceptors in mediating plant responses to solar UV radiation and blue light, and interactive effects of solar UV radiation and drought (see details in Table 2). RNA-seq data analysis was performed in Chipster and R, using different tools and packages (see the details in the paper I). Analysis for the rest of data was performed in R using linear mixed-effects models (nlme package) (I-III). Factorial ANOVA was used to assess the significance of the main effect of factors (treatment, genotype/accession, and time) and the different double and triple interactions between the factors (I-III). When ANOVA showed significant main effect and/or interaction between two or more factors ($P \leq 0.05$), responses were assessed by taking a subset of the data and fitting simpler models for each subset, as required (I-III). When the effect of filter treatment was significant, the individual effect of different wavebands (e.g., UV-B: 290 nm vs 350 nm, UV-A_{sw}: 315 nm vs 350 nm, UV-A_{lw}: 350 nm vs 400 nm, and blue: 400 nm vs 500 nm, see Figure 4 inset table for all the comparisons) was assessed by using function fit.contrast from package gmodels 2.18.1 and *P*-values were adjusted with function p.adjust in R (I-III).

Table 2 Methods used in this dissertation.

Purpose/ Assessment	Method	Species	Publication/ Manuscript
RNA isolation	Extraction kit or Trizol method	Arabidopsis, *Medicago truncatula*	I, II, III
Gene transcript abundance	qRT-PCR	Arabidopsis, *Medicago truncatula*	I, II, III
Gene transcript abundance	RNA-seq	Arabidopsis	I
Phenolic compounds composition and quantification	HPLC-MS	Arabidopsis, *Medicago truncatula*	II, III
ABA quantification	HPLC-MS	*Medicago truncatula*	III
Epidermal absorbance	*In vivo* dual excitation fluorescence	*Medicago truncatula*	III
Rosette area	Image analysis	Arabidopsis	II
Survival	Visual scoring	Arabidopsis	II
Protein quantification	Immunoblotting	*Nicotiana benthamiana*	I
Absorption spectrum of UVR8 protein	Spectrophotometry	*Escherichia coli*	I
Estimates of photons absorbed	Radiation transfer modeling		I, IV
Soil moisture	Time-domain reflectometry (TDR)		III
Irradiance	Spectroradiometery		I, II, III, IV
Transmittance of filters	Spectrophotometery		I, II, III

5 RESULTS AND DISCUSSIONS

5.1 Perception of solar UV radiation and blue light

The perception of light of a given wavelength through a photoreceptor requires the absorption of enough photons of that wavelength. The fraction of photons that are absorbed is conventionally presented as the absorption spectrum of a photoreceptor protein. To assess the absorption spectrum of UVR8, recombinant Arabidopsis UVR8 was expressed and purified from *Escherichia coli* (I). This absorption spectrum extended beyond that measured by Christie et al. (2012) and showed that UVR8 absorbs wavelengths in the UV-A region (I). The newly measured absorption spectrum for UVR8 (I) and the previously published for CRY2 (Banerjee et al., 2007), together with the solar spectra, were used to estimate the relative number of photons absorbed by UVR8 (I, IV) and CRY2 (I) in the whole UV region. As there are more photons in the UV-A and blue regions of sunlight than in the UV-B region (I), the estimates showed that UVR8 could absorb photons in the UV-B, UV-A_{sw}, UV-A_{lw}, and even in the blue region (I). In contrast, CRY2 absorbed very few photons in UV-A_{sw}, some in UV-A_{lw}, and most photons in the blue region (I). Moreover, UVR8 can be expected to absorb photons in the UV-A_{sw} region throughout the day, also when the sun is low in the sky (IV), suggesting that UV-A_{sw} photons perceived by UVR8 may contribute to responses to solar radiation at low sun elevations when UV-B photon irradiance is very low (IV).

Once excited by the absorption of UV-B photons, UVR8 monomerizes from a homodimer to monomers (Rizzini et al., 2011). Díaz-Ramos et al. (2018) reported UVR8 monomerization up to 310 nm. However they did not investigate monomerization at wavelengths >310 nm. As UVR8 monomers are required for signaling and response (Rizzini et al., 2011), the monomer-dimer status of UVR8 protein expressed in and purified from *Nicotiana benthamiana* was assessed for wavelengths in both UV-B and UV-A regions (I). The results showed that UVR8 monomerizes in wavelengths between 300–335 nm, which extends beyond that shown by Díaz-Ramos et al. (2018). The lack of monomerization between 335–350 nm suggests that UV-A photons of wavelengths >335 nm may not have enough energy to trigger the monomerization of UVR8 (I).

Upon exposure to UV-B radiation, UVR8 monomers interact with COP1 to initiate UVR8 signaling, which consequently leads to changes in transcript abundance (Brown et al., 2005; Favory et al., 2009). In my study, transcriptome-wide analysis in Arabidopsis showed that UVR8 mediated changes in transcript abundance of more than 90% of differentially expressed genes (DEGs) responding to solar UV-B

radiation, and of more than 60% of DEGs responding to solar UV-A radiation (I). On the other hand, CRYs mediated changes in transcript abundance of 95% DEGs in response to solar blue light, while lack of CRYs enhanced the number of DEGs by 25% in response to solar UV-A radiation (I). It indicated that photoreceptor-mediated gene expression differed for blue light and UV-A radiation. Assessment of two ranges of wavelengths within UV-A (UV-A_{sw} and UV-A_{lw}) showed that UVR8 mediated changes in transcript abundance of 95% DEGs responding to solar UV-A_{sw} in the WT, whereas the number of DEGs responding to solar UV-A_{lw} increased nearly three times when functional UVR8 was absent (I). The DEGs responding to solar UV-A_{lw} in WT were too few to draw any conclusions (I). Using 350 nm to split the UV spectral region further confirmed that UVR8 mediated perception of UV-B and UV-A_{sw} radiation while CRYs mediated perception of UV-A_{lw} radiation and blue light (I, Figure 5). These results are consistent with an earlier study showing that UVR8 mediates regulation of gene expression not only in response to solar UV-B radiation but also in response to solar UV-A radiation (Morales et al., 2013) where the role of UVR8 was assumed to be in connection with CRYs. Therefore, results of my study make it necessary to consider UVR8 as a photoreceptor for both UV-B and UV-A_{sw} radiation in sunlight.

Transcript accumulation of selected UV-B marker genes (*CHI*, *CHS*, *HY5*, *RUP2*, and *SPS1*) measured with qRT-PCR showed that UVR8 mediated an increase in their transcript abundance in response to solar UV-B and UV-A_{sw} radiation but not in response to solar UV-A_{lw} radiation (I). Under simulated sunlight, UVR8 mediated induction of *RUP2* in response to 6 hours of UV-B radiation. However, for UV-A_{sw}, no increase in transcript abundance was detected for any genes in both WT and *uvr8-2* (II). For UV-B radiation, our results agree with previous research, which showed that UVR8 mediated the induction of these genes in response to UV-B (Brown et al., 2005, 2009; Favory et al., 2009; Gruber et al., 2010). For UV-A radiation, Brown et al. (2005) showed that the higher transcript abundance of *CHS* in response to UV-A was not UVR8-dependent. The discrepancy between my results and Brown et al. (2005) could be due to difference in the spectral quality of UV-A radiation source. In Brown et al. (2005), UV-A radiation was provided by fluorescent tubes which emit photons between 350 nm and 400 nm wavelength (i.e., UV-A_{lw}), and transcript abundance of *CHS* in response to UV-A_{lw} radiation could be mediated by CRYs.

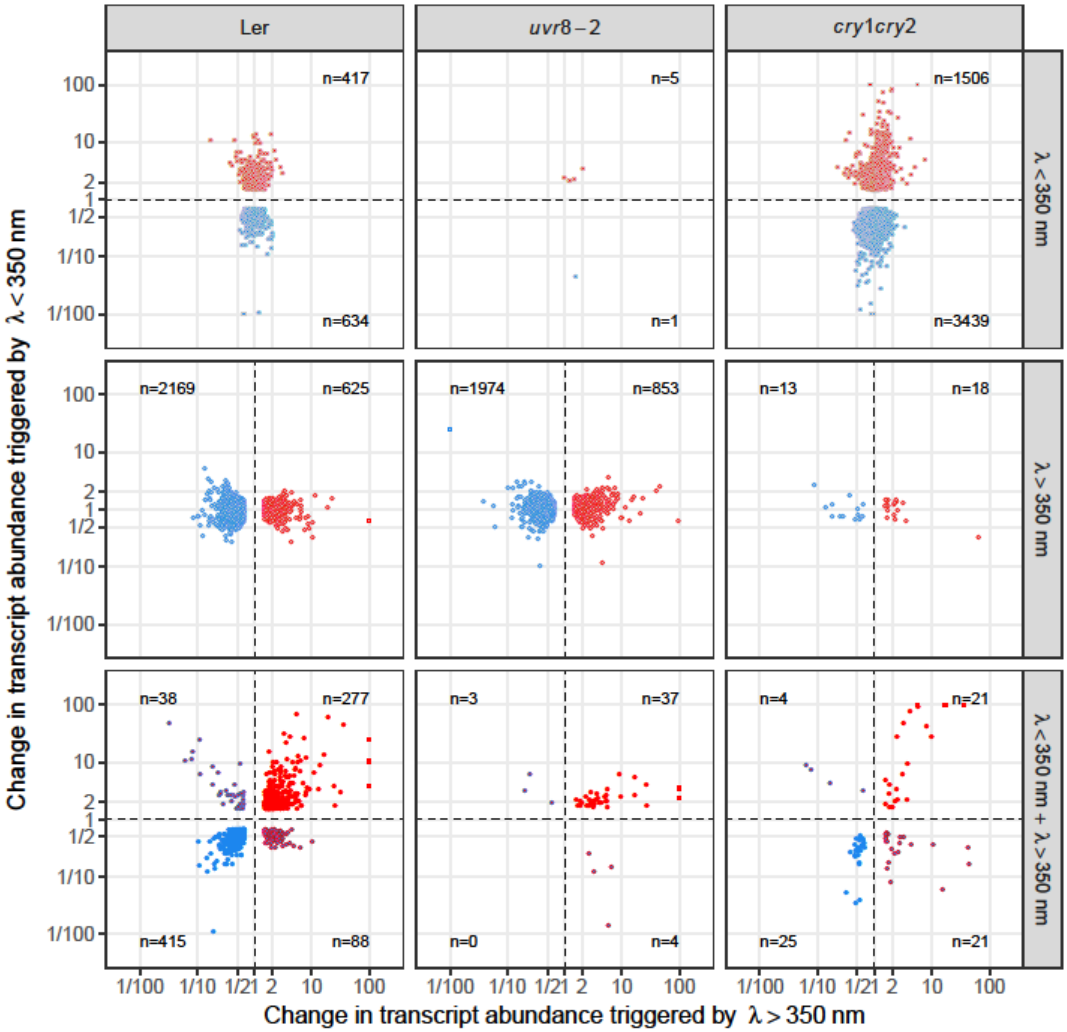

Figure 5 A plot showing differential gene expression in response to λ < 350 nm (UV-B+UV-A_{sw}), λ > 350 nm (UV-A_{lw}+blue), and to both λ < 350 nm + λ > 350 nm (DEGs shared by both). |logFC| > $\log_2(1.5)$ and FDR < 0.05.

Another finding that came out from my study is that the role of UVR8 in mediating transcript accumulation in response to solar UV-B radiation varied during the day (I). For example, to increase transcript levels of *CHI*, *HY5*, *RUP2*, and *SPS1*, UVR8 was required at solar noon, whereas, for *CHS*, it was required both at solar noon and late afternoon (I). Changes in transcript abundance for *CHS*, *CHI*, *ELIP2*, *HY5*, *RUP2*, and *SPS1* in response to solar UV-A_{lw} radiation were minimal and unclear at all time points and therefore, could not be assigned to UVR8 or CRYs (I). CRYs mediated an increase in transcript abundance of *CHS*, *CHI*, *ELIP2*, *RUP2*, and *PAP1* in response to blue light at both solar noon and late afternoon and of *HY5* and *SPS1* only at solar noon (I). Under simulated sunlight, CRYs mediated an increase in abundance of *CHS*, *HY5*, *RUP2*, and *SPS1* in response to 6 hours of blue light (II), similar to that in response to solar blue light (I). CRYs also mediated an increase in transcript abundance of *ELIP2* both after 6 hours and 17 days of blue light (II). The CRYs-mediated increase in

transcript abundance under solar blue light is consistent with that in controlled conditions (Fuglevand et al., 1996; Ohgishi et al., 2004; Kleine et al., 2007; Gruber et al., 2010). In the context of the time of day, UVR8 mediated transcript abundance in response to UV-B and UV-A_{sw} radiation primarily at solar noon (except *CHS*), while CRYs mediated transcript abundance at both solar noon and in the late afternoon. In the context of the duration of exposure, transcript abundance was more responsive at short-term exposure of 6 hours than at long-term exposure of 17 days, which could be due to the acclimation in plants growing under UV radiation and blue light treatments for long term starting from germination of seedlings.

Consistent results from both RNA-seq and qRT-PCR after 6 hours of exposure to solar UV radiation and blue light confirmed that functional UVR8 mediates the regulation of gene expression in response to both UV-B and UV-A_{sw} radiation but not to UV-A_{lw} radiation (I). Results also showed that UV-A_{sw} was more effective in inducing a response than UV-A_{lw}, both at the level of the number of genes and in the transcript abundance of individual genes (I). CRYs are the primary regulators of gene expression in response to solar blue light (I), which is consistent with previous research using artificial blue light (Ohgishi et al., 2004; Yu et al., 2010). The absence of CRYs-mediated response to UV-A_{sw} radiation and a relatively weak/unclear response to UV-A_{lw} radiation suggested a minor role of CRYs in the regulation of gene expression to solar UV-A radiation (I, II). Consequently, these results showed that the photoreceptor-mediated transcriptome-wide responses to UV-A radiation are not the same as those to blue light.

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis and Gene Ontology (GO) analysis performed on the DEGs showed that UVR8 mediated the regulation of genes associated with different biosynthetic and metabolic pathways (eg., phenylalanine, phenylpropanoid, flavonoid, diterpenoid, and coumarin biosynthesis; arachidonic acid, glyoxylate, bicarboxylate, carbon, and nitrogen metabolism, carbon fixation) in response to both solar UV-B and UV-A_{sw} radiation (I, Figure 6). In this list, some pathways, including phenylpropanoid and flavonoid biosynthesis, are only partially UVR8-dependent under UV-B radiation, however completely UVR8-dependent under UV-A_{sw} (I, Figure 6). CRYs mediated the regulation of genes associated with photosynthesis; carbon fixation in photosynthetic organisms; carbon, glyoxylate, dicarboxylate, glycine, serine, and threonine metabolism in response to blue light (I, Figure 6). Some processes that were only partially CRYs-dependent in response to blue light were flavonoid biosynthesis, vitamin B6 metabolism, and plant hormone signal transduction (I). These results indicated that UVR8- and CRYs-mediated changes in transcript abundance correspond more to acclimation responses than to stress responses in sunlight. Therefore, UVR8 and CRYs could provide acclimation to plants exposed to solar UV-B+UV-A_{sw} radiation and blue light, respectively.

Previous experiments using Affymetrix Arabidopsis ATH1 GeneChips and microarray analysis have shown that UVR8 mediates transcript accumulation of genes associated with UV-B tolerance, such as genes involved in phenylpropanoid biosynthesis and photo-repair of UV-B-induced damage in response to UV-B radiation (Brown et al., 2005; Favory et al., 2009). CRYs mediate transcript accumulation of genes involved in light signaling, photosynthetic light reaction, Calvin cycle, and phenylpropanoid metabolic pathway in response to blue light (Ohgishi et al., 2004; Kleine et al., 2007). Although extremely informative, these studies do not cover the full range of transcriptome-wide responses to UV radiation or blue light, as those previously used techniques only detect a small proportion of DEGs from the genome.

The *in silico* enrichment analysis of DNA-binding motifs can inform about the putative involvement of TFs in the regulation of gene expression (McLeay and Bailey, 2010). Motif enrichment analysis was performed on DEGs from different genotype and UV/blue treatment combinations. This analysis revealed that BES1, HY5, PIF1, PIF3, PIF4, PIF7, and several other bZIP and bHLH TFs could regulate the UVR8-mediated gene expression in response to UV-B radiation and CRYs-mediated gene expression in response to blue light (I). Earlier research has shown that TF BES1 binds to both UVR8 and CRYs in response to UV-B radiation and blue light, respectively, to suppress the brassinosteroid-responsive genes and inhibit the hypocotyl elongation (Liang et al., 2018; Wang et al., 2018). Another TF, HY5 is a common master regulator to both UV-B/UVR8 and blue/CRY signaling pathways (Brown et al., 2009; Gangappa and Botto, 2016). Therefore, it could be expected that BES1 and HY5 TFs were enriched for UVR8-mediated or CRYs-mediated transcript accumulation in response to UV-B radiation and blue light, respectively (I). MYB TFs are known regulators of flavonoid biosynthesis (Stracke et al., 2007), and were enriched for DEGs responding to UV-B and UV-A_{sw} radiation (I). DREB1 and STZ TFs, which are known to regulate gene expression in response to drought and salt tolerance (Shinozaki and Yamaguchi-Shinozaki, 2007), were induced by solar UV-B radiation and blue light (I). This means that different wavelengths of the solar spectrum could trigger acclimation to different abiotic stresses through regulation of gene expression.

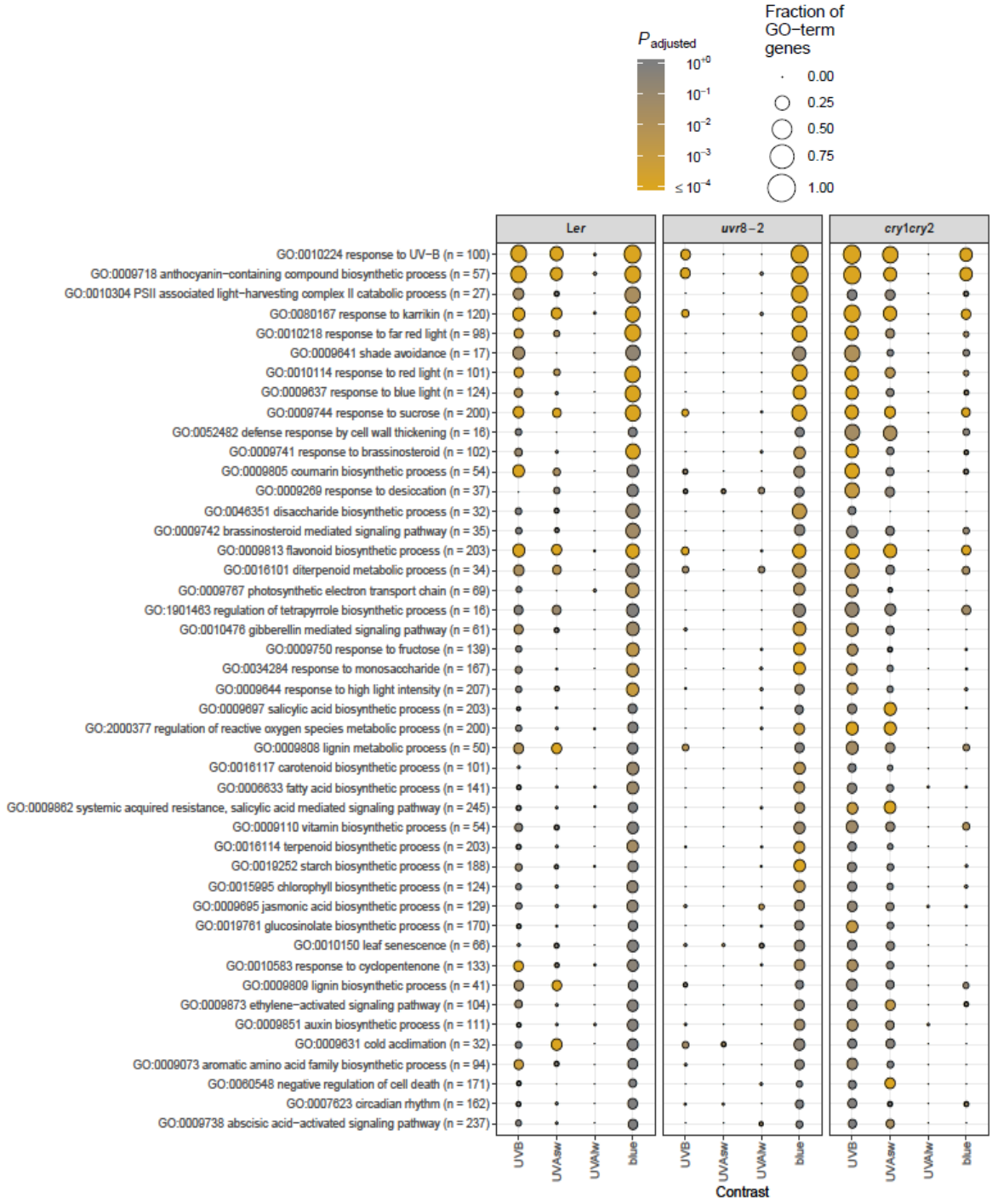

Figure 6 Gene Ontology analysis showing the top 45 GO terms. GO term with *P*-value < 0.01 for at least one contrast and genotype combination are included in the figure. The area of the circles shows a fraction of DEGs in a particular GO term against the total number of genes annotated for that GO term. The saturation of the color of circles shows *P*-value on a log scale after setting all the *P*-values $<10^{-4}$ to 10^{-4}. In the figure, n refers to the total number of genes annotated for that GO term in the database. The terms were ordered by the average of fractions of DEGs. Therefore, the most responsive pathways to waveband contrasts come at the top of the figure.

Flavonoids and HCAs composition and concentration from whole leaf extracts were assessed using HPLC. The sum of all HCAs showed a high concentration under all filter treatments and genotypes and, therefore, could not be attributed to the action of UVR8 or CRYs (II). The flavonoid compounds were either glycoside derivatives of quercetin or kaempferol; kaempferol derivatives were present in higher concentrations than quercetin derivatives (II). UVR8 mediated an increase in the concentration of both quercetin and kaempferol derivatives in response to 17 days of UV-B radiation (II). This UVR8-mediated induction was only weakly observed after 6 hours (II). Furthermore, CRYs mediated a slight increase in the concentration of quercetin derivatives in response to 6 hours of UV-A_{lw} radiation (II). Earlier research performed outdoors or using LEDs showed that both solar UV-B and UV-A radiation could mediate the accumulation of flavonoids and HCAs (Kotilainen et al., 2008, 2009; Morales et al., 2013; Siipola et al., 2015), and induction in response to UV-A radiation required both UVR8 and CRYs (Brelsford et al., 2018). The increase in the concentration of quercetin derivatives or kaempferol derivatives in response to blue light was minimal or not significant at both time points and, therefore, could not be assigned to UVR8 or CRYs (II). This result is in contrast with results from pea plants grown outdoors where blue light played a dominant role in the induction of flavonoids compared to UV-B and UV-A radiation (Siipola et al., 2015). The discrepancy (lack of response to UV-A radiation and blue light) between my study and others could be due to a difference in genotype, growth conditions, illumination protocol, and time of measurement of response. Furthermore, unlike in the simulated-sun condition, in outdoor conditions, better antioxidants like the quercetin derivatives were more abundant than the kaempferol derivatives (Morales et al., 2013; Siipola et al., 2015). My results taken together with previous studies suggest that in sunlight, the accumulation of the different quercetin or kaempferol derivatives contribute both to antioxidant capacity and to UV screening.

Growth and survival are important determinants of plant fitness in a natural environment. UVR8 and CRYs regulate growth in response to UV-B radiation and blue light, respectively (Christie et al., 2015; Jenkins, 2017). Furthermore, it has been well-established that the absence of UVR8 or CRYs is not lethal for plants (Mao et al., 2005; Morales et al., 2013). It was proposed that other signaling pathways than UVR8 signaling could be promoting growth in the *uvr8-2* mutant under UV-B radiation (Morales et al., 2013). In my study, under the full spectrum sunlight, when plants had either functional UVR8 or CRYs they had healthy growth similar to the WT (II). However, when both UVR8 and CRYs activity was impaired (*cry1cry2uvr8-2* mutant), plants could not survive in response to UV-B radiation (II). Besides, when both UVR8 and CRYs activity was impaired, growth was drastically retarded in response to UV-A_{sw} and UV-A_{lw} radiation but not in response to blue light (II). A similar lethal response was observed in the *cry1uvr8-2* mutant exposed to artificial UV-B radiation (Tissot and Ulm, 2020). My results indicated that both UVR8 and CRYs could substitute for each other to promote growth and survival in response to

UV-B, UV-A_{sw}, and UV-A_{lw} radiation under a realistic light environment; therefore reveal novel roles of CRYs under UV-B, UV-A_{sw}, and UV-A_{lw} and of UVR8 under UV-A_{sw} and UV-A_{lw} in regulating growth (II). Furthermore, these results also demonstrate that photoreceptor-mediated regulation of growth in response to UV-A radiation and blue light are different.

5.2 Interaction between UVR8 and CRYs

In a polychromatic light environment, different photoreceptors are activated simultaneously, and there is a possibility of interaction in the signaling pathways activated by them (Wade et al., 2002). The effect of interaction on UVR8 and CRY signaling in response to solar/simulated-solar UV-B and UV-A_{sw} radiation was assessed transcriptome-wide (I) and for selected genes (I, II). Transcriptomics analysis showed that the absence of CRYs resulted in an increase in the number of DEGs by three and seven times in response to UV-B and UV-A_{sw} radiation, respectively. Transcript accumulation by qRT-PCR of selected genes showed that absence of CRYs resulted in an increase in transcript abundance of *CHS*, *RUP2*, *SPS1*, *CHI*, and *F3'H* in response to UV-B radiation while that of *CHS*, *ELIP2*, *RUP2*, and *SPS1* in response to UV-A_{sw} radiation (I, II). This enhanced transcript abundance in *cry1cry2* was absent in *uvr8-2* and *cry1cry2uvr8-2* (II). The enhanced gene expression response was also evident for several KEGG pathways and GO terms (photosynthesis; plant hormone signal transduction; ribosome biogenesis; flavonoid biosynthesis; response to karrikin, brassinosteroid, and desiccation; and regulation of ROS metabolic process) (I) (Figure 6). These results indicated that CRY signaling activated by blue light negatively regulates UVR8 signaling induced by UV-B and UV-A_{sw} radiation, and this negative regulation has an effect on a variety of plant processes.

TFs that could be putatively involved in the enhanced transcript abundance response to UV-B and/or UV-A_{sw} radiation were BES1, HY5, HAT5, PIF1, PIF3, PIF4, PIF7, several other bZIPs, bHLHs, ATHBs, BPCs, TCPs, and WRKYs. Among these, BES1, HY5, and PIFs regulate gene expression downstream of both UVR8 and CRYs (Hayes et al., 2014; Gangappa and Botto, 2016; Pedmale et al., 2016; Liang et al., 2018; Wang et al., 2018) (I). However, the plethora of other TFs identified in my study could also present multiple points of interaction between UVR8 and CRY signaling.

Interaction between signaling pathways can also occur through other components downstream of photoreceptors. In the case of UVR8 and CRYs, both of them interact with COP1 (Podolec and Ulm, 2018). Moreover, both UVR8 and CRYs use the VP-peptide motif to compete for binding the WD40 domain of COP1 (Lau et al., 2019). These suggest that COP1 could be involved in the interaction between UVR8 and CRY signaling. RUPs act as negative regulators of UVR8 signaling by facilitating

redimerization of UVR8 monomers, while BICs are negative regulators of CRY signaling as they inhibit CRYs dimerization (Gruber et al., 2010; Heijde and Ulm, 2012; Findlay and Jenkins, 2016; Wang et al., 2017). Lack of CRYs resulted in an increase in transcript abundance of *RUP1* and *RUP2* in response to UV-A_{sw}, indicating that the interaction between UVR8 and CRY signaling pathways may involve RUPs (I). More recently, it was shown that CRY signaling activated by blue light, mediated an increase in *RUP1* and *RUP2* transcript abundance and RUP2 protein accumulation, consequently enhancing UVR8 redimerization (Tissot & Ulm, 2020). Reciprocally, UVR8 signaling activated by UV-B radiation mediated an increase in *BIC1* and *BIC2*, and overexpression of BIC1 and BIC2 suppresses the CRYs-mediated UVR8 redimerization (Tissot & Ulm, 2020). My results, together with this recently published study, confirm the interaction between UVR8 and CRY signaling pathways, with the involvement of RUPs and BICs. Noteworthy, one cannot rule out the possibility that physical interactions between the photoreceptors or other mechanisms of interaction are also involved.

5.3 Interaction between solar UV radiation and drought

Paper I informed about UVR8 and CRYs-mediated transcriptome-wide responses to UV-B+UV-A_{sw} and UV-A_{lw}+blue, respectively. KEGG and GO analysis showed that some of these responses are associated with changes at metabolic level (e.g., synthesis of flavonoids), which could provide acclimation for various stressors, including drought (I). Motif enrichment analysis showed that several drought-related TFs (DREBs) could putatively regulate gene expression in response to UV radiation (I), suggesting an interaction between solar UV radiation and drought at the transcriptional level. To further assess this interaction, I checked if differential gene expression mediated by UVR8 and UV-B+UV-A_{sw} radiation in my study (I) overlaps to those mediated by drought stress, as reported in two published studies using Arabidopsis (Huang et al., 2008; Harb et al., 2010). Comparing the RNA-seq data from paper I with Huang et al. (2008) showed that of the total 1869 DEGs responding to UV-B+UV-A_{sw} radiation (I), 285 DEGs were common to those responding to drought (Huang et al., 2008), from which 279 DEGs were those regulated by UVR8 (Figure 7A, subset "X"). A similar comparison with Harb et al. (2010) showed that 978 DEGs responding to UV-B+UV-A_{sw} radiation were common to drought from which 911 DEGs were those regulated by UVR8 (Figure 7B, subset "Y"). These results suggest that the interaction between UV-B+UV-A_{sw} radiation and drought at transcription level could be UVR8-dependent (Figure 7A, B). The DEGs corresponding to "X" and "Y" subsets were also compared to find the common DEGs between the three studies (UVR8-, UV-B+UV-A_{sw}-, and drought-mediated DEGs) (Figure 7C, subset "Z"). GO analysis was performed on 218 DEGs corresponding to subset "Z" to identify the biological processes which are regulated by UVR8, UV-B+UV-A_{sw} radiation, and

drought stress (Figure 8). This analysis showed that the shared UVR8-, UV-B+UV-A_{sw}-, and drought-mediated DEGs were enriched for GO terms related to response to abiotic stress, secondary metabolic process, and carbohydrate metabolic process (Figure 8).

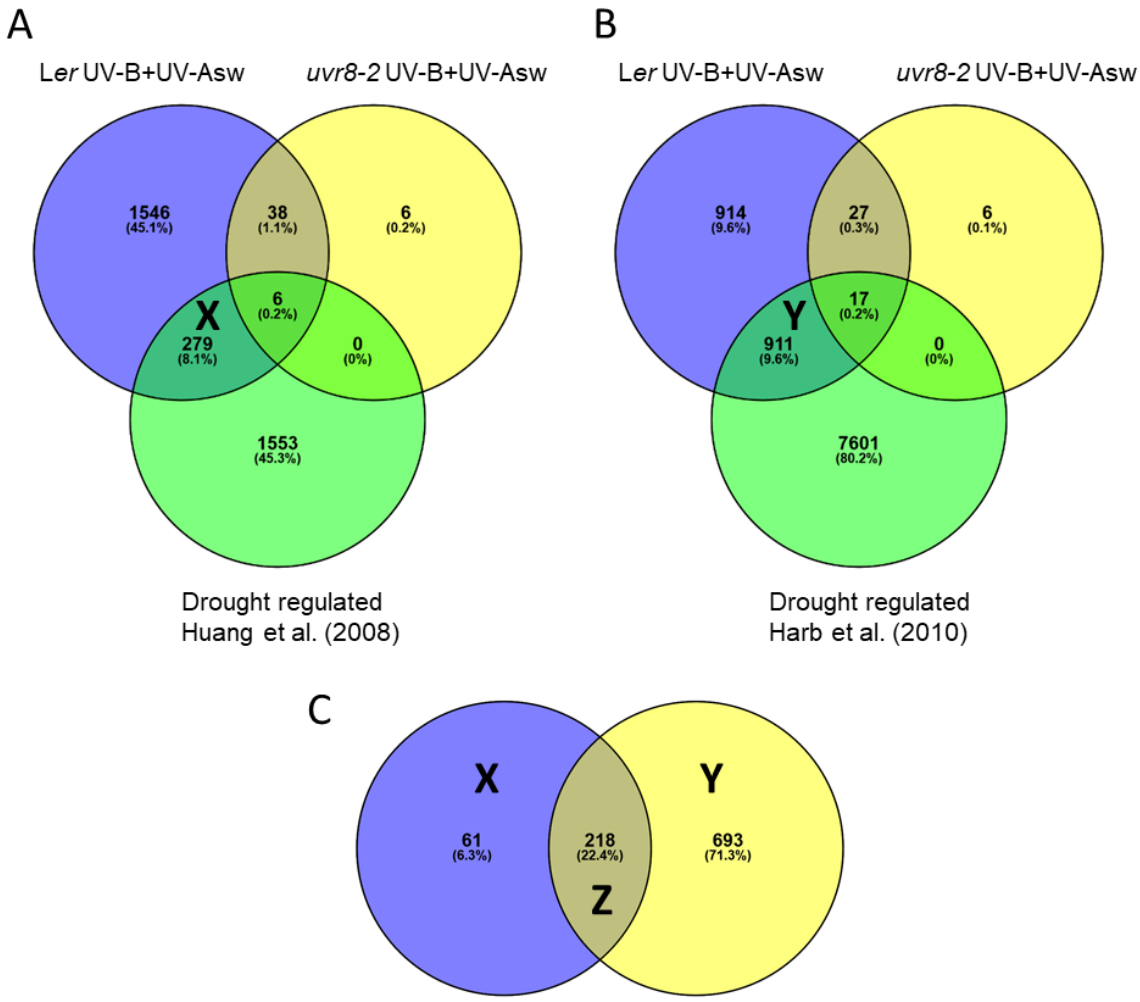

Figure 7 DEGs that are regulated by UVR8, UV-B+UV-A_{sw} radiation, and drought (FDR < 0.05) (A) DEGs responding to UV-B+UV-A_{sw} radiation in WT and *uvr8-2* from paper (I) and to drought in WT from Huang et al. (2008), (B) DEGs responding to UV-B+UV-A_{sw} radiation in WT and *uvr8-2* from paper (I) and to drought in WT from Harb et al. (2010), (C) UVR8-mediated DEGs responding to UV-B+UV-A_{sw} radiation from paper (I) and to drought from the two studies.

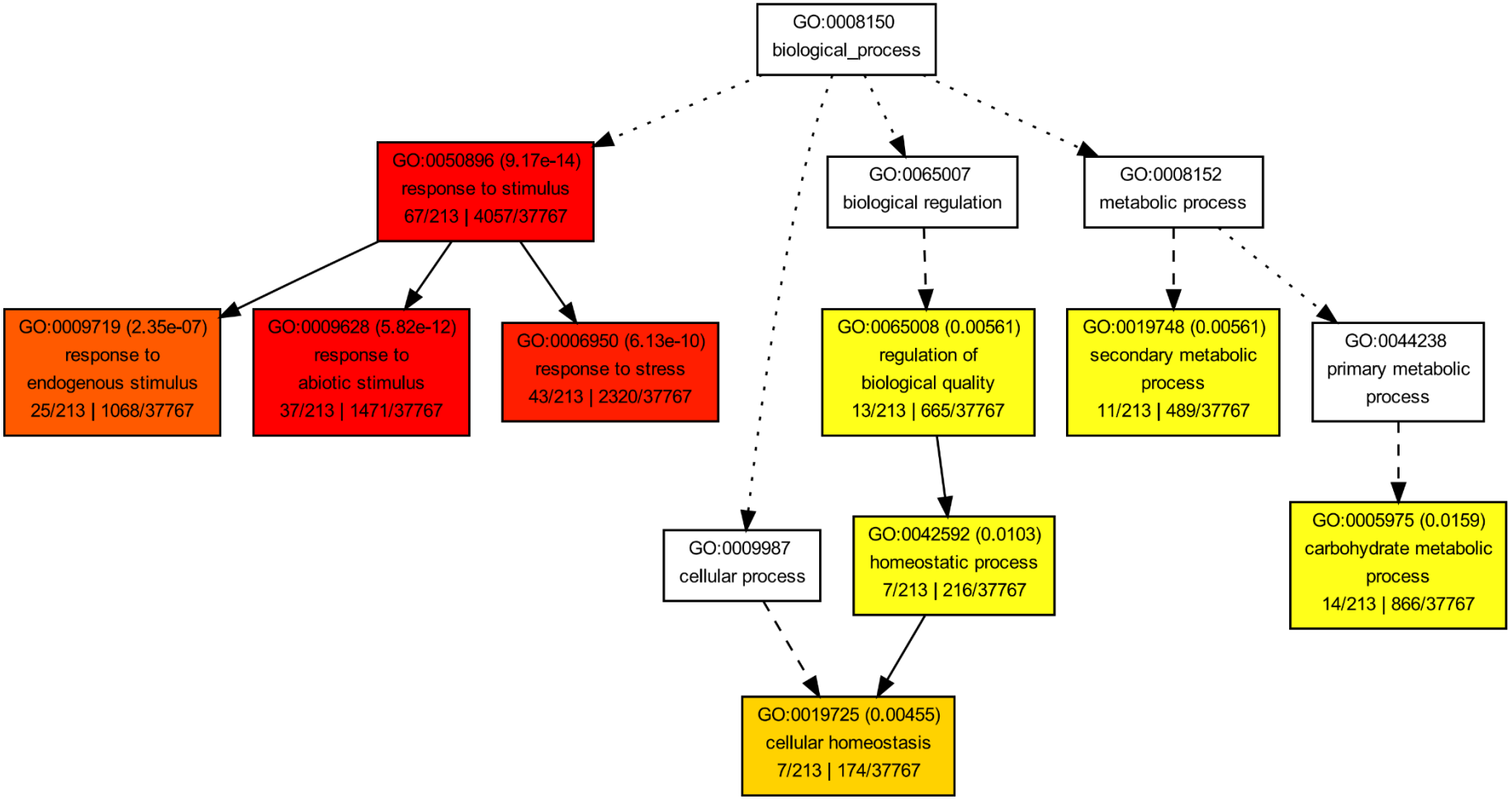
GO:0008150
biological_process
GO:0050896 (9.17e-14)
response to stimulus
67/213 | 4057/37767
GO:0065007
biological regulation
GO:0008152
metabolic process
GO:0009719 (2.35e-07)
response to
endogenous stimulus
25/213 | 1068/37767
GO:0009628 (5.82e-12)
response to
abiotic stimulus
37/213 | 1471/37767
GO:0006950 (6.13e-10)
response to stress
43/213 | 2320/37767
GO:0065008 (0.00561)
regulation of
biological quality
13/213 | 665/37767
GO:0019748 (0.00561)
secondary metabolic
process
11/213 | 489/37767
GO:0044238
primary metabolic
process
GO:0009987
cellular process
GO:0042592 (0.0103)
homeostatic process
7/213 | 216/37767
GO:0005975 (0.0159)
carbohydrate metabolic
process
14/213 | 866/37767
GO:0019725 (0.00455)
cellular homeostasis
7/213 | 174/37767

Figure 8 GO analysis on DEGs regulated by UVR8, UV-B+UV-A_{sw} radiation, and drought (corresponding to DEGs in subset "Z" from Figure 7C). The colors of boxes represent significance level, red for extremely significant (FDR < 0.0001) and yellow for moderately significant (FDR < 0.05), while white is for non-significant GO terms. Numbers for each significant GO term in the boxes represent: number of DEGs detected for a GO term / total number of DEGs | number of DEGs for a GO term in the database / total number of DEGs in the database. GO analysis was performed using AgriGO (Tian et al., 2017).

This data mining together with published research on interactive effects of UV and drought at the physiological level (Robson et al., 2015), developed the motivation to study the effects of UV radiation, drought, and their interaction on the accumulation of flavonoids and transcript abundance of *CHS* and abiotic-stress regulated genes in two different accessions of *Medicago truncatula* Jemalong A17 (mild-drought tolerant) and F83005-5 (drought-sensitive) (Limami et al., 2007) (III). A shift from using *Arabidopsis thaliana* to *Medicago truncatula* was driven by the reason that Arabidopsis plants have a shorter life cycle than *Medicago truncatula*, and is a long day plant; as a result, it flowers very early when exposed to solar UV radiation and drought. Using *Medicago truncatula* allowed us to expose the plants to long-term solar UV radiation, followed by the application of progressive soil-drying without flowering. As it was established from my previous studies that the same photoreceptor UVR8 mediates responses to both UV-B and UV-A_{sw} radiation (I), here, I applied UV-B+UV-A_{sw} as one radiation treatment while UV-A_{lw} as the second radiation treatment (III). Plants were germinated under different UV radiation treatments for 30 days, and later one set of plants was subjected to progressive soil-drying for seven days, and another set was watered regularly. The soil-drying treatment was applied together with ongoing UV treatment (III).

Epidermal UV-A absorbance of leaves is a measure of UV-A screening and is correlated with the accumulation of epidermal flavonoids (Goulas et al., 2004). The concentration of epidermal flavonoids per unit area, inferred from *in vivo* absorbance, was higher in F83005-5 than in Jemalong A17, independently of the treatments (III). There was an increase in the epidermal flavonoid content per unit area in response to UV-B+UV-A_{sw} radiation in Jemalong A17, an effect that was similar in both well-watered and drought-treated plants (III). This indicated that the gradual drought imposed in our experiment could not trigger a response different or in addition to the response to UV-B+UV-A_{sw} radiation in Jemalong A17 (III). On the other hand, there was an increase in epidermal flavonoids in response to UV-B+UV-A_{sw} radiation and a decrease in response to UV-A_{lw} radiation in well-watered plants of F83005-5 (III). In contrast, no such effect was observed in drought-treated F83005-5 plants as they had similarly high epidermal flavonoid contents (III). It indicated that drought could supersede any pre-existing effect of UV radiation and trigger the accumulation of large amounts of flavonoids in the leaf epidermis of F83005-5 plants (III). Thus, the effect

of UV radiation was predominant in drought-tolerant accession Jemalong A17, while the effect of drought was predominant in drought-sensitive accession F83005-5 for the accumulation of epidermal flavonoid. The inducing effect of UV-B (+UV-A_{sw}) radiation on the epidermal flavonoid content is consistent with previous research in silver birch (Morales et al., 2010), pea (Siipola et al., 2015), and fava bean (Yan et al., 2019), however, the effect of drought was not investigated in these studies. Although no photoreceptor mutants have been used in paper III, based on the conclusions from the paper I that UVR8 and CRYs are required in the perception of UV-B+UV-A_{sw} and UV-A_{lw}+blue radiation, respectively, it can be speculated that UVR8 mediated changes in epidermal flavonoid content in response to UV-B+UV-A_{sw} radiation, while CRYs mediated it in response to UV-A_{lw} radiation in *Medicago truncatula*.

Unlike in Arabidopsis, pea, fava bean, and birch seedlings, where the individual phenolic compounds are HCAs and derivatives of quercetin and kaempferol (II) (Morales et al., 2010, 2013; Siipola et al., 2015; Yan et al., 2019), in *Medicago truncatula*, they are derivatives of apigenin, luteolin, chrysoeriol, and tricin and often acylated with coumaroyl or feruloyl hydroxycinnamoyl groups (III). These phenolic aglycones belong to the flavone group and act both as UV screens and antioxidants (Jiang et al., 2016). Among the four aglycones, luteolin is known to be a better antioxidant than others due to the presence of two hydroxyl groups in its backbone structure (Jiang et al., 2016). The luteolin concentration was higher in Jemalong A17 than in F83005 across all treatments, suggesting a mechanism for better drought-tolerance in Jemalong A17 than in F83005. The total of all luteolin derivatives showed an increment in response to drought stress, particularly under filter >290 nm for the two accessions, However, this increase was detected significant in F83005-5. These results suggest a positive effect of UV-B+UV-A_{sw} radiation and drought on the accumulation of luteolin derivatives (III). A similar response was also reported in a previous study where drought increased the concentration of luteolin in UV-B-treated chili pepper plants (Rodríguez-Calzada et al., 2019).

My study showed drastic differences in the composition and concentration of phenolic compounds between the two accessions (III). Jemalong A17 had a larger number of distinct apigenin derivatives than F83005-5, while F83005-5 had a larger number of tricin derivatives than Jemalong A17 (III). In Jemalong A17, three apigenin derivatives were present at a concentration >2 µmol/g dw, while other compounds (including the ones from different aglycone groups) were present in a concentration <2 µmol/g dw. Similarly, in F83005-5, three tricin derivatives were at concentrations >2 µmol/g dw, while other compounds were present at lower concentrations (III). Two out of 12 compounds in Jemalong and three out of 19 compounds in F83005-5 showed an effect of UV radiation or drought (III). Consistently with an earlier study in fava beans where only the UV radiation effect was tested (Yan et al., 2019), the specific phenolic compounds in my study showed induction in response to UV-B+UV-A_{sw} radiation in well-watered plants or drought-treated plants (IV). While UV-B+UV-A_{sw}

radiation always showed either inducing effect or no effect on the concentration of specific compounds, UV-A_{lw} radiation showed an inducing effect, no effect, or an additional inhibitory effect in a few cases (III). This demonstrated that the split of wavelength in the UV region at 350 nm showed a distinct response at the level of secondary metabolite accumulation for the two spectral regions (315–350 nm, 350–400 nm), making it necessary to split UV-A radiation between short and long wavelengths for future plant studies. Furthermore, it can be speculated that these distinct responses could be regulated by different photoreceptors, i.e., UVR8 and CRYs. Moreover, only for one compound in Jemalong A17, there was a significant effect of drought, and drought superseded UV effects (III). Therefore, at the level of individual phenolic compounds from the whole leaf, UV radiation had a predominating effect compared to drought.

CHS enzyme converts acetyl CoA to naringenin chalcone, which is a precursor of flavone compounds, including apigenin, luteolin, chrysoeriol, and tricin (Jiang et al., 2016). My study showed that there was an increase in transcript abundance of *CHS* in response to UV-B+UV-A_{sw} radiation in drought-treated Jemalong A17 plants, but not in well-watered ones (III). Furthermore, there was a higher transcript abundance of *CHS* in drought-treated plants than in well-watered plants under filters >290 nm and >350 nm. The fact that drought mediated the induction of *CHS* in solar UV-B+UV-A_{sw}-dependent manner indicated a positive interaction between UV-B+UV-A_{sw} radiation and drought. In contrast, no effect of UV or drought treatment on *CHS* abundance was observed for F83005-5, once again showing the contrast between the two accessions. Earlier studies have shown that UV-B radiation mediated induction of transcript abundance of *CHS* in Arabidopsis (Brown et al., 2005; Favory et al., 2009; Morales et al., 2013) and chili pepper (Rodríguez-Calzada et al., 2019), however, the studies in Arabidopsis did not investigate the effect of drought while the study in chili pepper could not detect an interaction between UV-B radiation and drought. An increase in flavonoid biosynthesis in response to drought stress was not accompanied by an increase in transcript abundance of stress-responsive genes (*COR47*, *CRK10*, *ELIP1*, *HSP70*) and ABA concentration (III), which is consistent with results in Arabidopsis (Nakabayashi et al., 2014). Concerning my experimental set-up, this lack of increase in the transcript abundance of stress-responsive genes could be a result of mild drought stress, or because the time of expression for these genes did not match the time of sampling.

6 CONCLUSIONS AND FUTURE PERSPECTIVES

It has been well accepted that UVR8 is a UV-B photoreceptor, while CRYs are UV-A/blue light photoreceptors. However, these roles were assigned mainly based on experiments performed in unrealistic light conditions which differ from sunlight. My thesis challenges these roles by providing evidence that in sunlight, UVR8 mediates the perception of both UV-B and UV-A_{sw} radiation while CRYs mediate perception of UV-A_{lw} radiation and blue light (I). Thus it also demonstrates that the results on the roles of UVR8 and CRYs photoreceptors are different in sunlight than under unrealistic conditions in controlled environments, and this conclusion is likely to apply to other plant photoreceptors. Therefore, for future studies on photoreceptor-mediated light perception, it will be useful to systematically complement controlled-environment experiments with field experiments, using wavelengths in different spectral regions of UV-A radiation. Furthermore, my thesis also reveals the need to split the UV-A waveband into UV-A_{sw} and UV-A_{lw} for future studies on plants' responses to UV-A radiation.

In sunlight, different photoreceptors are activated at the same time by different wavelengths of light, and there is an indication that the downstream signaling pathways interact. However, my study presents the first evidence for a very strong antagonistic interaction between UVR8 and CRYs under both solar and simulated solar UV-B and UV-A_{sw} radiation (I, II). This interaction could occur through signaling components downstream of photoreceptors such as RUPs and TFs; however, to confirm this mechanism, experimental evidence for these molecular interactions in solar UV radiation is needed.

In the field, high UV irradiance often occurs in conjunction with drought, and there is evidence that UV-B radiation and drought interact to regulate plant physiology. However, very limited information existed at the secondary metabolite and transcript level. My study provides evidence that UV-B+UV-A_{sw} radiation and mild drought can interact positively to trigger acclimation through an increase in epidermal UV screening in the drought-intolerant accession, F83005-5, and through an increase in luteolin and transcript abundance of *CHS* in the moderately drought-tolerant accession, Jemalong A17 (III). As the expression of the *CHS* gene and consequent flavonoid biosynthesis is a photomorphogenic response to UV-B+UV-A_{sw} mediated by UVR8, future transcriptome-wide analysis using photoreceptor mutants in a suitable model plant species such as *Medicago truncatula* would be required to study the roles of UVR8 and CRYs in plants' acclimation to different combinations of UV radiation and drought.

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