Title page

Functional analysis of the UVR8 photoreceptor from the monocot *Zea mays*

Fernández María Belén^a, Lamattina Lorenzo^a, Cassia Raúl^{a*}

^a Instituto de Investigaciones Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata- Consejo Nacional de Investigaciones científicas y Técnicas, CC1245 (7600). Mar del Plata, Argentina.

*Corresponding author: raul.cassia@gmail.com, 54-223-4753030 Int 16

Acknowledgements

We acknowledge Dr. Rius from CEFODI institute for technical support with maize samples.

Funding

This study was funded by the Agencia Nacional de Promoción de Ciencia y Técnica (ANPCYT) grant number 3589, and Universidad Nacional de Mar del Plata grant number EXA 818/17. LL and RC are permanent researcher of CONICET Argentina. MBF has post-doctoral fellowship of Comisión de Investigaciones Científicas (CIC).

Conflict of Interest

The authors declare that they have no conflict of interest.

Abstract

Low UV-B fluence is a signaling stimulus that regulates various physiological processes and induces photomorphogenic responses in plants. The specific UV-B receptor UVR8 is a key component in these processes. Although UVR8 sequence is conserved, few homologs have been cloned and reported to be functional. Here we show the cloning and functional analysis of *Zea mays* UVR8 (*ZmUVR8*). *ZmUVR8* presents 73% of identity with *AtUVR8*, maintaining the key tryptophan responsible of UV-B perception. *ZmUVR8* also contains the VP domain, involved in the interaction with the proteins CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) and REPRESSOR OF UV-B PHOTOMORPHOGENESIS 1 (RUP1). Whereas *UVR8* was expressed in non-irradiated *Arabidopsis* and maize leaves, after 2h of UV-B irradiation, its expression was reduced. The expression of chalcone synthase (*CHS*), involved in flavonoid biosynthesis and regulated by UVR8, was increased in irradiated *Arabidopsis* and maize leaves. *Arabidopsis uvr8-1* null mutant was complemented with *ZmUVR8* driven by the CaMV-35S promoter and fused to eGFP. *ZmUVR8*-eGFP fusion was mainly localized in nuclei of transgenic lines, irrespective of UV-B treatments. UV-B suppressed hypocotyl elongation in WT *Arabidopsis* plants, whereas in *uvr8-1* hypocotyl growth was observed. However, hypocotyl elongation was reduced in UV-B irradiated transgenic lines complemented with *ZmUVR8*. Moreover, *CHS* and transcription factor *HY5* (ELONGATED HYPOCOTYL 5) expression were also restored in these plants. These results confirm that *ZmUVR8* is similar enough to *AtUVR8* to restore UV-B perception and signaling in *Arabidopsis* mutant *uvr8-1*, thus being a functional UV-B photoreceptor.

Keywords

UVR8; UV-B; maize; monocot; *Arabidopsis*.

1. Introduction

Ultraviolet-B (UV-B) radiation is the region between 280 and 315 nm of the total Sun's electromagnetic spectrum. Ultraviolet irradiances reaching the surface of the Archean Earth were higher than the current ones because of the absence of a significant ozone atmosphere (Cnossen et al. 2007). UV-B is higher in terrestrial environments compared to the water column (Rozema et al. 2002). High UV-B doses damages DNA, proteins, lipids, cell membranes, photosynthetic machinery and induces the production of reactive oxygen species (ROS). This affects plant cell integrity and viability, leading to growth retardation and to a decrease in crop yield and quality (Brosché and Strid 2003; Frohnmeyer and Staiger 2003; Jordan 1996). Consequently, plants evolved mechanisms to avoid UV-B damage during the colonization of exposed habitats (Tilbrook et al. 2013). Ancient photosynthetic organisms like cyanobacteria and various eukaryotic algae, use mycosporine-like amino acids (MAAs) as protective compounds against UV-B (Llewellyn and Airs 2010; Rastogi and Incharoensakdi 2013; Rozema et al. 2002). Land plants co-evolved with environmental UV-B levels, and the complexity of UV-B absorbing molecules increased accordingly from algae to higher plants (Rozema et al. 2002). The protein UV RESISTANCE LOCUS (UVR8) was identified as the specific UV-B receptor in plants (Brown et al. 2005). UVR8 signaling significantly contributes to UV-B acclimation responses and the establishment of UV-B tolerance. UVR8 regulates metabolic and developmental processes and induces physiological and photomorphogenic responses. The usually UVR8-mediated UV-B responses are the inhibition of hypocotyl growth and the accumulation of flavonols and anthocyanins. However, nowadays it is suggested that additional physiological responses are modulated by UVR8: phototropism, thermomorphogenesis, circadian clock, auxin signaling, defense, salt stress tolerance, shade avoidance, chloroplast development, stomatal opening, leaf development and downward leaf curling (for a review see (Yin 2017)). Chlorophytes are the earliest organisms with an UV-B photoreceptor, *Chlamydomonas reinhardtii* being the first organism to own a functional UVR8 (Fernandez et al. 2016; Han et al. 2019). Since its discovery in 2005 (Brown et al. 2005), cumulative evidence allows us to consider a landscape of UVR8 interactions. In the absence of UV-B, the UVR8 homodimer is mainly located in the cytoplasm. In the nucleus, the E3 Ubiquitin ligase COP1 (CONSTITUTIVELY PHOTOMORPHOGENIC 1) represses the activity of the

transcription factor HY5 (ELONGATED HYPOCOTYL 5) (Favory et al. 2009). The transcription factor WRKY36 (WRKY DNA-BINDING PROTEIN 36) represses HY5 (Yang et al. 2018), and BES1 (BRI1-EMS-SUPPRESSOR1) and BIM1 (BES1-INTERACTING MYC-LIKE 1) induce BR-responsive gene expressions (Liang et al. 2018).

Following UV-B irradiation, UVR8 rapidly monomerizes and interacts in the nucleus with COP1 and WRKY36. This complex avoids the degradation of HY5, and induces the *HY5* expression. In turn, it triggers the UV-B regulated gene expression, leading to plant acclimation and stress tolerance (Heijde and Ulm 2012). Two of the HY5 upregulated genes are the Repressor of UV-B photomorphogenesis 1 and 2 (RUP1 and RUP2). Interaction of UVR8 with these proteins facilitates UVR8 dimerization, and subsequent inactivation (Heijde and Ulm 2012; Ulm and Jenkins 2015). UVR8 also interacts with BES1 and BIM1, inhibiting the brassinosteroid responsive genes, and reducing the hypocotyl elongation (Sun and Zhu 2018). Recently, it has been shown that the UVR8-COP1 complex participates in the PHYTOCHROME INTERACTING FACTOR (PIF) 4 y 5 degradation, causing the UV-B-induced hypocotyl shortening (Sharma et al. 2019; Tavridou et al. 2019).

UVR8 is the first photoreceptor described that perceives UV-B through tryptophan residues instead of a prosthetic chromophore (O'Hara and Jenkins 2012; Ulm and Jenkins 2015). Two *Arabidopsis* UVR8 (*AtUVR8*) high-resolution crystal structures have been determined using different crystallization conditions. However, they proved to be nearly identical in tertiary and quaternary structure (Christie et al. 2012; Wu et al. 2012; Yang et al. 2016; Zeng et al. 2015). *AtUVR8* contains a core domain that forms a seven-bladed β -propeller and a flexible C-terminal region of approximately 60 amino acids that contains a C27 region. Both the β -propeller domain and the C-terminal C27 domain of UVR8 are necessary and sufficient for interacting with COP1. Moreover, UVR8 interacts also with WRKY36 by its C-terminal (aminoacids 397 to 440) (Yang et al. 2018). *AtUVR8* has fourteen tryptophan residues, seven of which are exposed to the dimer interface. It also contains three conserved pentapeptide repeats with the motif "GWRHT" in blades 5, 6, and 7. This motif generates a closely clustered triad of tryptophans (W233, W285 and W337) which are the most important for UV-B photoreception (Christie et al. 2012; Wu et al. 2012; Zeng et al. 2015). Structural and mutagenesis studies show a primary role for W285 and W233 in UV-B perception, whereas

W337 is not essential in this process (Christie et al. 2012; Sun and Zhu 2018; Wu et al. 2012). The motif “GWRHT” from blade 6 contains W285 and is conserved in all UVR8 homologs analyzed (Fernandez et al. 2016; Han et al. 2019).

Zea mays also known as corn, is a cereal grain of agronomic importance, and has been used as a model organism in basic and applied research for nearly a century (Strable and Scanlon 2009). Previous work shows that increased UV-B radiation produces a significant reduction in dry matter accumulation and, consequently, affects yield. Moreover, an increase in flavonoid accumulation, a decrease in chlorophyll content in leaves and a reduction in protein level, sugar and starch of maize seeds have also been reported (Gao et al. 2004). In 2011, Casati et. al. described a transiently upregulation and subsequent downregulation of two UVR8- like genes upon UV-B exposure in maize leaves (Casati et al. 2011a; Casati et al. 2011b). However, these genes have little homology to *AtUVR8* as they were identified by homology to rice genome.

Although UVR8 is conserved, and sequences for this gene are found in all the *Viridiplantae* (Fernandez et al. 2016; Han et al. 2019), a few UVR8 homologs have been cloned and reported to be functional from green algae, moss and dicots. Up to now, there is no evidence of UVR8 photoreceptor from monocotyledon plants with confirmed functionality. Here, we report the molecular cloning, sequence and functional complementation of *ZmUVR8*, the UV-B receptor of *Zea Mays*.

1. Material and Methods

1.1. Plants material and growth conditions

Seeds of wild- type (WT) *Arabidopsis thaliana* ecotype *Landsberg erecta* (Ler) and the *uvr8-1* null mutant (Cloix et al. 2012) were kindly provided by Dr. Gareth Jenkins (University of Glasgow, Scotland). Seeds were surface sterilized in 30% (v/v) commercial bleach for one minute, rinsed with distilled sterile water and stratified for 72h at 4°C in darkness. Germinated seedlings were grown on agar plates containing half- strength Murashige and Skoog (MS) salts or in soil/ perlite/ vermiculite (3:1:1, v/v) under white light (160 $\mu\text{mol.m}^{-2}\text{s}^{-1}$, fluorescent tubes) and long- day regime (light/dark: 16/8h) at 25°C in an environment controlled chamber.

Maize (*Zea mays* B73 inbred line) seeds were kindly provided by Dr. Sofia Eugenia Olmos (INTA Pergamino, Argentina). Seeds were surface sterilized with 30% (v/v) commercial bleach for 20 minutes and rinsed in distilled water. Subsequently, seeds were germinated on water saturated filter paper in Petri dishes for 4-5 days and maintained at 25°C. Germinated seedlings were grown on soil/ vermiculite (3:1, v/v) under a long- day regime (light/dark: 16/8 h) at 25°C in an environment controlled chamber with white light at 160 $\mu\text{mol.m}^{-2} \text{s}^{-1}$. The topmost leaf from V6 developmental stage (when sixth leaf are visible in the leaf whorl) plants was used for experiments.

1.2. Generation of transgenic *Arabidopsis* plants expressing *ZmUVR8*

The full length coding sequence from *ZmUVR8* was amplified by PCR using the maize full-length EST ZM_BFb0066P22.r (Arizona Genomics Institute) as template, and the specific primers *ZmUVR8*-Fw and *ZmUVR8*Rv (Table 1). The amplified cDNA was cloned into the entry pENTR/D-TOPO vector and confirmed sequence, orientation and reading frame by DNA sequencing (Macrogen). The obtained entry clone was recombined with the Gateway pH7FWG2 binary destination vector for 35S-driven expression in plants, with C-terminal fusion to eGFP (35S::*ZmUVR8*- eGFP) (Karimi et al. 2002). This vector was introduced in the *Agrobacterium* strain GV3101 by electroporation (Koncz and Schell 1986). Transformation into *uvr8-1* mutant *Arabidopsis* was performed by floral dip (Clough and Bent 1998). Transformants were selected based on its ability to survive on half- strength Murashige and Skoog medium supplemented with 1% sucrose containing 15 mg L⁻¹ hygromycin. Resistant seedlings were then transferred to soil and grown under conditions described above.

The transgenic lines generated were shown to have the transgene integrated at a single genetic locus through segregation analysis. Homozygous T3 (#6.5) and T4 (#5.1.7) independent lines were obtained by self-crossing and were used for the experiments. The level of transgene expression in each line was examined by qRT-PCR and immunoblot.

1.3. UV-B Treatments

For experiments involving UV-B light treatments, *Arabidopsis* seedlings were exposed 2h to white light (100 $\mu\text{mol.m}^{-2}\text{s}^{-1}$, fluorescent tubes) supplemented with 3.47 $\mu\text{mol m}^{-2}\text{s}^{-1}$ narrowband UV-B (Philips TL 100W/01) in a controlled environment chamber. The UV-B

dose used is similar to the radiation measured from sunlight at midday in Mar del Plata summer (38.0055° S, 57.5426° W).

Maize plants were exposed for 0, 2 and 4h to white light (100 $\mu\text{mol.m}^{-2}\text{s}^{-1}$, fluorescent tubes) supplemented with 8.81 $\mu\text{mol m}^{-2}\text{s}^{-1}$ narrowband UV-B (Philips TL 100W/01) in a controlled environment chamber.

The spectral irradiance was determined with an Ultraviolet- B photo-radiometer (Delta ohm HD2102.1).

2.4. Expression analysis

For gene expression quantification, plant materials were harvested, frozen in liquid nitrogen and grounded under RNase-free conditions. Total RNA was extracted using TRIzol method, and treated with DNase I (Invitrogen) at 37°C for 30 min, following the manufacturer's instructions. Then, the RNA was reverse-transcribed using the M-MLV reverse transcriptase (Thermo) following the manufacturer's instructions. cDNA obtained was used for quantitative RT-PCR using Power SYBR Green PCR mix and a StepOne machine (Applied Biosystems). Primers used are listed in Table 1. PCR conditions were: 10 min at 95 °C, 40 cycles of 15 s at 95 °C and 1 min at 60°C. After amplification, a melting curve analysis was performed, which resulted in a single product specific melting curve. Negative controls for cDNA synthesis and qRT-PCR reactions were included in all cases. LineReg program was employed for the analysis of gene expression (Ruijter et al. 2009). The transcript relative quantifications were determined from the ratio between the starting concentration value of analyzed mRNA and the reference genes *actin2* for *Arabidopsis* samples or thioredoxin- like (Thr) for maize as previously reported (Casati and Walbot 2004). The data shown are representative of at least three independent experiments.

Table 1 List of primers used in this work.

Primers			Sequence (5'→3')	Amplicon length (pb)	Usage
Cloning	<i>Zm</i> UVR8	Fw	CACCGCTCCCGTTCCTCGCGGTGGTCAC	1341	Amplification of full length <i>Zm</i> UVR8
		Rv	TGATGACTGCACGCGCATCCTCTTCACGTCGT		
qRT-PCR	<i>Zm</i> UVR8	Fw	GTTTGGGTGTCGCCGTC	123	Amplification of <i>Zm</i> UVR8
		Rv	CCTCTTCACGTCGTTCTCC		
	<i>Zm</i> Thr	Fw	GGACCAGAAAGATTGCAGAAAG	104	Thioredoxin- like, maize reference gene
		Rv	CAGCATAGACAGGAGCAATG		
	<i>Zm</i> CHS	Fw	AGCTCGATCGCTAGCTCCTC	196	Maize chalcone synthase
		Rv	TCCCCCTCGATCTTTCTCCC		
	<i>At</i> UVR8	Fw	CCAGATTTTCGGGAGGTTGG	102	Amplification of <i>At</i> UVR8
		Rv	TTATTGCCGACTCCTACTTGTC		
	<i>At</i> Actin2	Fw	GTATTGTGCTGGATTCTGGTG	119	<i>Arabidopsis</i> reference gene
		Rv	GAGGTAATCAGTAAGGTCAAG		
	<i>At</i> CHS	Fw	CAGACAGGACATCGTGGTGGT	102	<i>Arabidopsis</i> chalcone synthase
		Rv	ACATGAGTGATCTTTGACTTGG		

For protein assays, leaves were harvested into liquid nitrogen and proteins extracted in 100mM buffer KPO₄ (pH 7.4), 1mM EDTA and a cocktail of protease inhibitors. The homogenate was centrifuged for 10 min a 10000 x g at 4°C. Protein concentration was determined by a Bradford assay. Thirty micrograms of total protein were loaded for *Zm*UVR8 protein expression and separated by 12% denaturing SDS-PAGE. Immunoblots were incubated with anti-GFP antiserum as primary antibody. After several washes, a secondary anti- mouse antibody conjugated to alkaline phosphatase and developed by NBT/BCIP staining. The membranes were stained with Ponceau S to reveal the Rubisco large subunit (rbcl) as loading control.

2.5. *Zm*UVR8- eGFP Subcellular Localization

Fifteen day old plants irradiated 1h with white light or white light supplemented with UV-B (3.47 $\mu\text{mol.m}^{-2}\text{s}^{-1}$), were vacuum infiltrated with 5 $\mu\text{g. mL}^{-1}$ of Hoechst 33342 (Invitrogen Molecular Probes) in buffer PBS, 0.2% Triton-X100 for 4 min and maintained in shake at 50 rpm and darkness for 1h. Then, samples were washed 3 times with PBS. The subcellular localization of eGFP and Hoechst 33342 was visualized by a confocal laser scanning microscope (Nikon-C1siR Eclipse TiU) under oil (Biopack) with a $\times 40$ objective. Images were taken using the Nikon EZ-C1 3.90 software. eGFP and Hoechst were excited using an argon laser at 488 nm and a laser at 408 nm, respectively. eGFP emission was collected

between 515 and 530 nm to avoid crosstalk with chloroplast autofluorescence. Hoechst 33342 fluorescence was collected at 440/50 nm. The same microscope settings for GFP and Hoechst 33342 detection were used before and after UV-B illumination. Colocalization analysis was performed on two independent transgenic lines. The data shown are representative of at least three independent experiments.

2.6. Hypocotyl length measurement

Seedlings were grown for 5 days on agar plates of half strength Murashige and Skoog (MS) salts containing 1% sucrose in white light or white light supplemented with $3.47 \mu\text{mol.m}^{-2} \text{ s}^{-1}$ UV-B. Photographs were taken after treatments and hypocotyl lengths were measured using the ImageJ software (<http://rsb.info.nih.gov/ij>). Three independent biological replicates were performed for all experiments using at least 10 seedlings for each replicate ($n \geq 30$).

2.7. Bioinformatic analysis

Multiple sequence alignments were performed using MAFFT server (<http://mafft.cbrc.jp/alignment/server/>) and edited with GeneDoc (Nicholas and Nicholas 1997).

1. Results

3.1. An *At*UVR8 homolog with a conserved structure is found in maize.

UVR8 sequences from green algae (*C. reinhardtii* (Tilbrook et al. 2016)), moss (*P. patens* (Soriano et al. 2018)), liverwort (*M. polymorpha* (Soriano et al. 2018)) and dicots (*S. lycopersicum* (Li et al. 2018a), *M. domestica* (Zhao et al. 2016), *V. vinifera* (Liu et al. 2014), *B. platyphylla* (Li et al. 2018b) and *P. euphratica* (Mao et al. 2015)) have been cloned, characterized, and shown to restore the loss-of-function of the UVR8 null mutant *uvr8-1* (Kliebenstein et al. 2002).

However, no monocot functional UVR8 protein has been reported so far. To this end, the *Arabidopsis* protein sequence (AAD43920.1) was used as bait in a PSI-BLASTp for searching the UVR8 homolog in maize, using the NCBI (www.ncbi.nlm.nih.gov/) and Phytozome databases (www.phytozome.jgi.doe.gov/pz/portal.html). One sequence was found (GRMZM2G003565), herein named “*Zm*UVR8”. Figure 1A shows the comparison between *Zm*UVR8 and the other functional UVR8 homologs. *Zm*UVR8 has 443 amino acids

length, a calculated molecular mass of 47.15 kDa, and 73% of identity to *At*UVR8. Key tryptophan residues responsible of UV-B perception (W233, 285 and 337) are conserved in *Zm*UVR8, as well as the VP domain in the C27 region, involved in the interaction with COP1 and RUP (Figure 1A). Figure 1B shows that *Zm*UVR8 has the same domain profile than *At*UVR8, including the conserved tryptophans, the C27 domain and the seven repeated RCC1 domains.

A.

B.

248

Fig. 1 *Zm*UVR8 sequence and structural domains. **A.** Multiple sequence alignment of *At*UVR8 homologs. Sequences of proteins that restore the loss-of-function of the *uvr8-1* null mutant were aligned with MAFFT (<http://mafft.cbrc.jp/alignment/server/>) and edited with GeneDoc (Nicholas and Nicholas 1997). Red boxes indicate “GWRHT” motifs and the VP domain. The accession number of each sequence is given next to the species name. Conserved residues common to all sequences are shadowed in black and less identity is shown in gray scale. Capital letter indicates 100% of homology whereas lowercase indicates minor identity. The yellow box shows the “VP” domain in the C27 domain, important for interaction with COP1 and RUP proteins. **B)** Schematic representation of structural domains of *At*UVR8 and *Zm*UVR8 proteins. Analysis of amino-acid sequences was performed at the National Center for Biotechnology Information (NCBI) database. RCC1 (pfam00415), Regulator of chromosome condensation (RCC1) repeat. Arrows indicate significant tryptophans in UV-B perception (W233, 285 and 337). VP: Valine-Proline domain.

Blastp analysis in Table 2 shows that sequences with high percentage of identity with the components of the *Arabidopsis* UVR8 signaling pathway were found in maize: COP1 (70%), HY5 (68%), HYH (48%), RUP1 (45%), RUP2 (46%), WRKY36 (35%), BES1 (50%) and BIM1 (41%). These results indicate that *Zm*UVR8 could be a functional UV-B receptor, triggering a UVR8 signaling cascade in maize.

Table 2. Identification of maize homologs to the *Arabidopsis* UVR8 signaling pathway. Proteins involved in UVR8 signaling cascade from *Arabidopsis* were used as bait in BLASTp analysis restricting the search to *Z. mays* and the non-redundant protein sequences database from NCBI.

<i>Arabidopsis</i> protein	Bait ID	<i>Zea mays</i> identification	Identity (%)	E- value	Accession
COP1	NP_180854.1	Ubiquitin ligase protein COP1	70	0.0	AQK75277.1
Hy5	NP_001330553.1	Uncharacterized protein LOC100286123	68	9,00E-48	NP_001152483.1
HyH	NP_850605.1	Uncharacterized protein LOC100286123 isoform X1	48	3,00E-27	XP_008643871.1
RUP1	NP_200038.1	WD repeat-containing protein RUP2	45	5,00E-89	PWZ22039.1
RUP2	NP_568435.2	WD repeat-containing protein RUP2	46	2,00E-91	AQK67844.1
WRKY36	NP_564976.1	Probable WRKY transcription factor 31	35	4,00E-47	XP_008655458.1
BES1	NP_973863.1	BES1/BZR1 protein	50	7,00E-69	NP_001151195.2
BIM1	NP_001190259.1	Unknown	41	3,00E-49	ACN34591.1

3.2. *Zm*UVR8 and *At*UVR8 expression are downregulated by UV-B irradiation.

Previous studies show that *At*UVR8 is a ubiquitously expressed gene, allowing an immediate reaction of plants to UV-B exposure (Favory et al. 2009; Rizzini et al. 2011). UVR8

expression was analyzed in *Arabidopsis* and maize plants irradiated with 3.47 and 8.81 $\mu\text{mol.m}^{-2} \text{s}^{-1}$ of UV-B respectively. Figure 2 shows quantitative RT-PCR analysis of UVR8 expression in irradiated and non-irradiated leaves. After 2h of UV-B irradiation, UVR8 expression was reduced 3-fold in *Arabidopsis* and 6-fold in maize, respectively. However, CHS expression was increased in both species after UV-B. These results indicate a similar regulation of UVR8 and CHS expression under UV-B treatment both in *Arabidopsis* and maize plants.

Arabidopsis

Maize

Fig. 2 Expression of UVR8 and CHS in response to UV-B in *Arabidopsis* and maize plants. UVR8 and CHS transcript levels were analyzed by qRT-PCR. Three week-old *Arabidopsis* and maize plants were irradiated during 2 and 4h with 3.47 and 8.81 $\mu\text{mol.m}^{-2} \text{s}^{-1}$ of UV-B respectively. Non-irradiated plants were used as control. Expression of Actin2 and thioredoxin- like (Thr) were used for *Arabidopsis* and maize

normalization respectively. Primers are listed in Table 1. Error bars indicate the standard deviation over three biological replicates (n=3).

Table 3 compares the expression of *ZmUVR8* with those of the other functional homologues. Whereas UV-B has no effect on *UVR8* expression in *M. polymorpha* and *V. vinifera* grapes, it upregulates transcript levels in different tissues from *M. domestica* and *B. platyphylla*. These findings, together with results shown in Figure 2, where *AtUVR8* and *ZmUVR8* transcript levels were downregulated by this radiation, suggest the existence of different regulatory mechanisms for *UVR8* expression in different plant species and tissues.

Table 3. *UVR8* expression after UV-B stress in plant with confirmed *UVR8* functional homologs.

Specie	Tissue	UVR8 expression in respopnse to UV-B	UV-B dose	Reference
<i>Arabidopsis thaliana</i>	Leaf	Downregulated after 2h of treatment	3.47 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Figure 2
<i>Chlamydomonas reinhardtii</i>	nd			Tilbrook et. al., 2016
<i>Physcomitrella patens</i>	nd			Soriano et. al., 2018
<i>Marchantia polymorpha</i>	Plant	No significant effect of treatment after 3	3 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Soriano et. al., 2018 Kondou et. al., 2019
<i>Zea mays</i>	Leaf	Downregulated after 2h of treatment	8.81 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Figure 2
<i>Solanum lycopersicum</i>	nd			Li et. al., 2018
<i>Malus domestica</i>	Fruit skin	Upregulation after 6h of treatment	1.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Zhao et. al, 2016
	Apple callus	Upregulation after 1h of treatment	1.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$	
<i>Vitis vinifera</i>	Fruit	Not influenced by UV-B	Field radiation	Liu et. al., 2014
<i>Betula platyphylla</i>	Tissue culture seedling	Upregulation after 6h of treatment	1.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Li et. al., 2018
<i>Populus euphratica</i>	nd			Mao et. al., 2015

Nd: not determined.

3.3 Transgenic *ZmUVR8* expression in the *uvr8-1* null mutant

For insights into the *in vivo* role of *ZmUVR8*, we cloned the *ZmUVR8* cDNA into the pH7FWG2 plant expression vector driven by the CaMV-35S promoter and fused to eGFP (35S::*ZmUVR8*-eGFP). This construct was used to transform the *Arabidopsis uvr8-1* null mutant. After repeated selection on hygromycin and microscopy eGFP-screening, two independent T3 and T4 homozygous lines (#6.5 and #5.1.7 respectively) were obtained. *ZmUVR8* expression was determined by quantitative RT-PCR using specific primers that

314 did not amplify *AtUVR8* (Online resource S1). Figure 3A illustrates that both lines
315 expressed *ZmUVR8* mRNA, line #6.5 reaching the highest level. Immunoblot with anti-
316 GFP antibody in Figure 3B shows that *ZmUVR8*-eGFP was present in both transgenic
317 lines.

Fig. 3 35S::*ZmUVR8*-eGFP expression analysis in two *Arabidopsis* homozygous lines. **A.** Leaves from three-weeks-old transgenic *Arabidopsis* plants were used to analyze *ZmUVR8* transgene transcript level by qRT-PCR. Expression of *Actin2* was used for normalization. Primers are listed in Table 1. Error bars indicate the standard deviation over three biological replicates. **B.** Denaturing immunoblot from *Arabidopsis* transgenic lines #6.5 and #5.1.7 protein extracts (upper panel). Immunodetection was performed using anti-GFP antiserum. Stained Rubisco large subunit (*rbcL*) is shown as a loading control (lower panel). **C.** Thirty-days-old WT, *uvr8-1* mutant and #6.5 and #5.1.7 transgenic *Arabidopsis* lines.

Online resource S2 shows no positive GFP signal in WT lines, ensuring the specificity of *ZmUVR8*-eGFP detection. Figure 3C shows that *ZmUVR8* has no detrimental effects on plant development, because no evident phenotypic differences were found among WT, *uvr8-1*, #6.5 and #5.1.7, after 30 days.

The sub-cellular localization of *ZmUVR8*-eGFP, was analyzed using a confocal laser scanning microscope. Hoechst 33342 staining was employed for nuclear staining. Figure 4

338 shows that *ZmUVR8*-eGFP fusion was mainly localized in nuclei of #6.5 and #5.1.7 lines,
339 irrespective of UV-B treatments.

Fig. 4 Subcellular localization of eGFP-*ZmUVR8*. Cellular localization of *ZmUVR8*-eGFP fusions revealed by confocal laser microscopy. Two-weeks-old *Arabidopsis* plants were irradiated with 100 $\mu\text{mol m}^{-2}\text{s}^{-1}$ of white light (C), or white light plus 3.47 $\mu\text{mol m}^{-2}\text{s}^{-1}$ UV-B (UV-B) for 1 h. Hoechst 33342 was vacuum infiltrated to specifically stain nuclei. eGFP and Hoechst were excited using a laser at 488 nm and 408 nm, respectively. eGFP emission was collected between 515 and 530 nm to avoid crosstalk with chloroplast autofluorescence. Hoechst 33342 fluorescence was collected at 440/50 nm. The same microscope settings for GFP and Hoechst 33342 detection were used before and after UV-B illumination. Bar: 25 μm . #6.5 and #5.1.7: transgenic *Arabidopsis* lines expressing *ZmUVR8*-eGFP. Arrows indicate co-localization.

3.4. *ZmUVR8* complements the *Arabidopsis uvr8-1* null mutant

The hypocotyl length inhibition is frequently used to analyze photoreceptors functionality in *Arabidopsis*. UV-B suppresses hypocotyl growth in wild-type *Arabidopsis* plants, whereas the *uvr8-1* mutant is impaired in this response (Favory et al. 2009). Expression of WT *AtUVR8* fused to GFP or yellow fluorescent protein (YFP) in *uvr8-1* plants, restores the WT phenotype (Favory et al. 2009; Huang et al. 2014; O'Hara and Jenkins 2012). The recovering of this morphogenic response was established as a parameter of functionality for several UVR8 homologs (Kondou et al. 2019; Li et al. 2018a; Li et al. 2018b; Liu et al. 2014; Mao et al. 2015; Soriano et al. 2018; Tilbrook et al. 2016; Zhao et al. 2016). Figure 5 shows no differences among hypocotyl length of WT, *uvr8-1* and transgenic lines grown under white light. Although #5.1.7 line grown under white light appears slightly shorter than *uvr8-1* and #6.5 at the seedling stage, no differences were observed in 30-days-old plants (Fig. 3C). Figure 5 B shows that, under UV-B irradiation, the reduction in hypocotyl length was of 54% in WT plants. In contrast, the reduction in *uvr8-1* mutant was only of 31%. However, hypocotyl elongation was reduced 50% in line #6.5 and 60% in line #5.1.7, similar to WT plants (Fig. 5B). These results indicate that *ZmUVR8* restore the *uvr8-1* null mutant phenotype under UV-B radiation demonstrating that is a positive regulator in UV-B induced photomorphogenesis.

Fig. 5 Functional complementation assay of eGFP-ZmUVR8 expressed in *Arabidopsis uvr8-1* mutant. **A.** Phenotypes of the *Arabidopsis* WT, *uvr8-1*, and #6.5 and #5.1.7 lines grown under $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ of white light, or white light plus $3.47 \mu\text{mol m}^{-2} \text{s}^{-1}$ of UV-B. The scale bar represents 1mm. Photographs were taken after treatments. **B.** The hypocotyl lengths were measured using the ImageJ software (<http://rsb.info.nih.gov/ij>). Grey bars: white light, black bars: white light plus UV-B. Error bars indicate standard deviation. Different letters show significant differences between treatments (Kruskal- Wallis One-way ANOVA on ranks. Multiple comparisons: Dunn's method, $p < 0.05$). Three independent biological replicates were performed, $n \geq 30$.

3.5.ZmUVR8 promotes CHS and HY5 gene expression in *uvr8-1 Arabidopsis* mutant

In response to UV-B, plants increase the concentration of UV-B-absorbing phenolic compounds such as flavonoids. The enzyme chalcone synthase (CHS) catalyzes the first step of flavonoid biosynthesis and is regulated by the UVR8 signaling pathway (Jenkins 2014;

Yonekura-Sakakibara et al. 2019). UVR8-COP1-SPA complexes induce the expression of the transcription factor HY5, which is a positive regulator of *CHS* expression (Jenkins 2014). In order to determine if *ZmUVR8* is able to induce *CHS* and *HY5* expression in response to UV-B, three-weeks-old Ler, *uvr8-1* and #6.5 transgenic line plants were irradiated with white light or white light supplemented with UV-B for 2 hours. *CHS* and *HY5* expression was determined by quantitative RT-PCR. Figure 6 illustrates that, under white light irradiation, *CHS* and *HY5* have low basal expression in all plants. After UV-B irradiation, the expression of both genes was increased in WT plants, but the *uvr8-1* mutant was impaired in this response. However, UV-B-irradiated #6.5 line increased *CHS* and *HY5* expression (Fig. 6). These results, demonstrate that *ZmUVR8* was able to trigger the UVR8 signaling in response to UV-B.

Fig. 6 Expression levels of *CHS* and *HY5* in response to UV-B in WT, *uvr8-1* mutant and transgenic line #6.5 under UV-B irradiation. Three-weeks-old WT, *uvr8-1* and #6.5 transgenic line plants were exposed to white light, or white light supplemented with 3.47 $\mu\text{mol.m}^{-2} \text{s}^{-1}$ of UV-B for 2h. The expression of *CHS* and *HY5* in leaves was determined by qRT-PCR. Expression of Actin2 was used for normalization. Error bars indicate the standard deviation of at least two biological replicates.

Discussion

In this work we have cloned the maize *ZmUVR8*, the first functional UVR8 protein from monocots homologue to the *Arabidopsis* UV-B receptor.

Two maize UV-B-responsive genes were previously reported as UVR8-like homologs by comparison with rice genome (Casati et al. 2011a; Casati et al. 2011b). However, these genes present less than 34% of identity with *AtUVR8*, and tryptophans 233 and 285, involved in UV-B perception, are not conserved (not shown). After the maize genome sequencing was completed, we found *ZmUVR8* and, by phylogenetic studies, we concluded that a unique UVR8 sequence was present in maize (Fernandez et al. 2016). *ZmUVR8* has a conserved sequence and a modular structure, in concordance with several UVR8 homologs (Fernandez et al. 2016). In addition, we found that maize proteins involved in UVR8 signaling have more than 35% of identity with those from *Arabidopsis* (Table 2). Considering that 30% of identity is enough to infer that two proteins are homologous (Pearson 2014), we suggest that the UVR8 signaling cascade might be present in maize. Furthermore, phylogenetic studies demonstrate that UVR8, SPAs and HY5 proteins are conserved from chlorophytes to angiosperms (Fernandez et al. 2016; Han et al. 2019). Functional studies will be necessary to confirm these hypotheses.

Although *UVR8* was reported as constitutive in *Arabidopsis*, we show herein that *UVR8* transcript decreased both in *Arabidopsis* and maize leaves after 2 hours of UV-B radiation. Likewise, *Betula platyphylla* UVR8 (*BpUVR8*) expression is induced by UV-B, and decreases after 9h of continuous irradiation (Li et al. 2018b). On the other hand, *UVR8* expression is increased by UV-B in the hypocotyls of radish sprouts (Wu et al. 2016). Variations in *UVR8* expression were also reported in UV-B-treated fruit skin and apple callus (Mao et al. 2015). Differences observed in *UVR8* expression in the literature could result from the different UV-B intensities used in the experiments and tissues analyzed. *AtUVR8* is a stable protein (Heilmann and Jenkins 2013) and a huge amount of UVR8 may influence the activity of COP1 and RUP as E3 ligases (Ren et al. 2019). It may be possible that after UVR8 was translated, gene expression could be decreased to avoid UVR8 overaccumulation in *Arabidopsis* and maize.

Little is known about the existence of different regulatory mechanisms for *UVR8* expression. Wu et. al. (2016) proposed that UV-B enhances the production of H₂O₂, thus increasing the level of NO to further magnify the *UVR8* expression. It was recently reported that *AtUVR8* expression may be modulated by blue light in UV-B-irradiated *Arabidopsis*. Cryptochrome 1 (Cry1) mutant *hy4* shows reduced *UVR8* expression in response to UV-B, suggesting a linkage between *cry1*, *UVR8* and UV-B (Khudyakova et al. 2019). Besides, it has been observed that *ZmUVR8* expression is increased by waterlogged in non-UV-B-irradiated maize root (Rajhi et al. 2011).

We obtained two independent homozygous *Arabidopsis* lines (#6.5 and #5.1.7), where *ZmUVR8*-eGFP complemented the *uvr8-1* null mutant both in hypocotyl elongation assay and induction of UVR8-regulated genes. *Arabidopsis* GFP-UVR8 shows increased nuclear localization following UV-B treatment of plants (Kaiserli and Jenkins 2007), but *ZmUVR8*-eGFP was constitutively located in nuclei in #6.5 and #5.1.7 lines.

No obvious nuclear localization signal (NLS) is found in UVR8, and there is no consensus about the mechanism of UVR8 translocation (Yin 2017). Yin et. al. (2016) reported that UVR8 interaction with COP1 is necessary for UVR8 translocation to nucleus, as well as for UVR8 signaling in response to UV-B (Yin et al. 2016). Moreover, it was demonstrated that the presence of UVR8 in the nucleus is necessary but not sufficient for its function. Kaiserli & Jenkins (Kaiserli and Jenkins 2007) fused a NLS to GFP-UVR8 and observed that the constitutive nuclear localization of NLS-GFP-UVR8 is insufficient to promote *HY5* expression in the absence of UV-B. Therefore, even though *ZmUVR8* has nuclear localization irrespective of UV-B treatment, this radiation is necessary for downstream signaling as previously reported for *AtUVR8*.

UV-B irradiation is not a mere stress signal but can also serve as an environmental stimulus to direct growth and development. A well-established UV-B morphogenic effect is the reduction of hypocotyl elongation (Kim et al. 1998). The hypocotyl growth of the *uvr8-1* mutant seedlings, in stark contrast to wild-type seedlings, was not inhibited by UV-B (Favory et al. 2009). The restoring of this morphogenic response was established as a parameter of complementation by functional UVR8 homologs (Kondou et al. 2019; Li et al.

2018a; Li et al. 2018b; Liu et al. 2014; Mao et al. 2015; Soriano et al. 2018; Tilbrook et al. 2016; Zhao et al. 2016).

ZmUVR8 restored the impaired UV-B hypocotyl growth suppression, and the *CHS* and *HY5* expression in *uvr8-1*, working as an effective component of the UVR8 pathway. These results demonstrate that similar signaling responses to UV-B are present in monocots and dicots plants. Consequently, a canonical UVR8 pathway could be present in monocotyledons.

Conclusion

We identified the *ZmUVR8* gene from *Zea mays* (*ZmUVR8*) using multiple sequence comparison analysis. *ZmUVR8* gene expression is modulated by UV-B. The *ZmUVR8* protein is highly conserved and has proven to be a functional homolog of the *Arabidopsis* UV-B receptor *AtUVR8*.

494 Supplementary figures

Fig. S1 Sequence alignment of AtUVR8 and ZmUVR8 transcripts. UVR8 transcript sequences from maize (GRMZM2G003565_T03) and *Arabidopsis* (NM_125781.4) were aligned using MAFFT software (<http://mafft.cbrc.jp/alignment/server/>) and edited with GeneDoc (Nicholas and Nicholas 1997). Forward (Fw) and reverse (Rv) ZmUVR8 primers used for quantitative RT-PCR are shown in red and yellow respectively. Conserved residues common to both sequences are shadowed in black and less identity is shown in gray scale. Capital letter indicates 100% of homology whereas lowercase indicates minor identity.

Fig. S2 *ZmUVR8* detection by immunoblot. Non-boiled total protein extracts from Ler, *uvr8-1* and #6.5 transgenic line were separated on 12%SDS-PAGE and immunoblot was probed with anti-GFP antibody (left panel). Ponceau staining is shown as loading control (right panel). MWM: molecular weight markers.

Author Contributions

MF conducted experiments, interpreted data, drew figures, and collaborated in writing the manuscript. RC conceived the project and wrote the paper. LL supervised and improved the manuscript.

References

- Brosché M, Strid A (2003) Molecular events following perception of ultraviolet-B radiation by plants. *Physiol Plant* 117:1-10
- Brown BA, Cloix C, Jiang GH, Kaiserli E, Herzyk P, Kliebenstein DJ, Jenkins GI (2005) A UV-B-specific signaling component orchestrates plant UV protection. *Proc Natl Acad Sci U S A* 102:18225-18230 doi:0507187102 [pii] 10.1073/pnas.0507187102
- Casati P, Campi M, Morrow DJ, Fernandes JF, Walbot V (2011a) Transcriptomic, proteomic and metabolomic analysis of UV-B signaling in maize. *BMC Genomics* 12:321 doi:1471-2164-12-321 [pii] 10.1186/1471-2164-12-321
- Casati P, Morrow DJ, Fernandes JF, Walbot V (2011b) Rapid Maize Leaf and Immature Ear Responses to UV-B Radiation. *Front Plant Sci* 2:33 doi:10.3389/fpls.2011.00033
- Casati P, Walbot V (2004) Rapid transcriptome responses of maize (*Zea mays*) to UV-B in irradiated and shielded tissues. *Genome Biol* 5:R16 doi:10.1186/gb-2004-5-3-r16 gb-2004-5-3-r16 [pii]

535 Cloix C et al. (2012) C-terminal region of the UV-B photoreceptor UVR8 initiates signaling through
536 interaction with the COP1 protein. *Proc Natl Acad Sci U S A* 109:16366-16370
537 doi:1210898109 [pii] 10.1073/pnas.1210898109

538 Clough SJ, Bent AF (1998) Floral dip: a simplified method for *Agrobacterium*-mediated
539 transformation of *Arabidopsis thaliana*. *Plant J* 16:735-743

540 Cnossen I, Sanz-Forcada J, Favata F, Witasse O, Zegers T, Arnold NF (2007) Habitat of early life: Solar
541 X-ray and UV radiation at Earth's surface 4–3.5 billion years ago. *Geophys Res Lett* 112:E2

542 Christie JM et al. (2012) Plant UVR8 photoreceptor senses UV-B by tryptophan-mediated disruption
543 of cross-dimer salt bridges. *Science* 335:1492-1496 doi:science.1218091 [pii]
544 10.1126/science.1218091

545 Favory JJ et al. (2009) Interaction of COP1 and UVR8 regulates UV-B-induced photomorphogenesis
546 and stress acclimation in *Arabidopsis*. *EMBO J* 28:591-601 doi:emboj20094 [pii]
547 10.1038/emboj.2009.4

548 Fernandez MB, Tossi V, Lamattina L, Cassia R (2016) A Comprehensive Phylogeny Reveals Functional
549 Conservation of the UV-B Photoreceptor UVR8 from Green Algae to Higher. *Plants Front*
550 *Plant Sci* 7:1698 doi:10.3389/fpls.2016.01698

551 Frohnmeier H, Staiger D (2003) Ultraviolet-B radiation-mediated responses in plants. Balancing
552 damage and protection. *Plant Physiol* 133:1420-1428 doi:10.1104/pp.103.030049
553 133/4/1420 [pii]

554 Gao W, Zheng Y, Slusser JR, Heisler GM, Grant RH, Xu J, He D (2004) Effects of supplementary
555 ultraviolet-B irradiance on maize yield and qualities: a field experiment. *Photochem*
556 *Photobiol* 80:127-131 doi:10.1562/2004-05-03-RA-156.1
557 2004-05-03-RA-156 [pii]

558 Han X, Chang X, Zhang Z, Chen H, He H, Zhong B, Deng XW (2019) Origin and Evolution of Core
559 Components Responsible for Monitoring Light Environment Changes during Plant
560 Terrestrialization. *Mol Plant* 12:847-862 doi:S1674-2052(19)30135-2 [pii]
561 10.1016/j.molp.2019.04.006

562 Heijde M, Ulm R (2012) UV-B photoreceptor-mediated signalling in plants *Trends Plant Sci* 17:230-
563 237 doi:S1360-1385(12)00008-8 [pii] 10.1016/j.tplants.2012.01.007

564 Heilmann M, Jenkins GI (2013) Rapid reversion from monomer to dimer regenerates the ultraviolet-
565 B photoreceptor UV RESISTANCE LOCUS8 in intact *Arabidopsis* plants. *Plant Physiol* 161:547-
566 555 doi:pp.112.206805 [pii]
567 10.1104/pp.112.206805

568 Huang X, Yang P, Ouyang X, Chen L, Deng XW (2014) Photoactivated UVR8-COP1 module determines
569 photomorphogenic UV-B signaling output in *Arabidopsis*. *PLoS Genet* 10:e1004218
570 doi:10.1371/journal.pgen.1004218 PGENETICS-D-13-02962 [pii]

571 Jenkins GI (2014) The UV-B photoreceptor UVR8: from structure to physiology *Plant Cell* 26:21-37
572 doi:tpc.113.119446 [pii] 10.1105/tpc.113.119446

573 Jordan BR (1996) The effects of ultraviolet-B radiation on plants: a molecular perspective. *Advances*
574 *in Botany* 22:98-138

575 Kaiserli E, Jenkins GI (2007) UV-B promotes rapid nuclear translocation of the *Arabidopsis* UV-B
576 specific signaling component UVR8 and activates its function in the nucleus. *Plant Cell*
577 19:2662-2673 doi:tpc.107.053330 [pii] 10.1105/tpc.107.053330

578 Karimi M, Inze D, Depicker A (2002) GATEWAY vectors for *Agrobacterium*-mediated plant
579 transformation. *Trends Plant Sci* 7:193-195 doi:S1360-1385(02)02251-3 [pii]

580 Khudyakova AY et al. (2019) Impact of UV-B radiation on the photosystem II activity, pro-
581 /antioxidant balance and expression of light-activated genes in *Arabidopsis thaliana* hy4

mutants grown under light of different spectral composition. J Photochem Photobiol B 194:14-20 doi:S1011-1344(18)31461-1 [pii] 10.1016/j.jphotobiol.2019.02.003

Kim BC, Tennessen DJ, Last RL (1998) UV-B-induced photomorphogenesis in *Arabidopsis thaliana*. Plant J 15:667-674

Kliebenstein DJ, Lim JE, Landry LG, Last RL (2002) *Arabidopsis* UVR8 regulates ultraviolet-B signal transduction and tolerance and contains sequence similarity to human regulator of chromatin condensation 1. Plant Physiol 130:234-243 doi:10.1104/pp.005041

Koncz C, Schell J (1986) The promoter of TL-DNA gene 5 controls the tissue- specific expression of chimaeric gene carried by a novel type of *Agrobacterium* binary vector. Mol gen genet 204:383-396

Kondou Y et al. (2019) Physiological function of photoreceptor UVR8 in UV-B tolerance in the liverwort *Marchantia polymorpha*. Planta 249:1349-1364 doi:10.1007/s00425-019-03090-w10.1007/s00425-019-03090-w [pii]

Li H et al. (2018a) Tomato UV-B receptor SlUVR8 mediates plant acclimation to UV-B radiation and enhances fruit chloroplast development via regulating SlGLK2. Sci Rep 8:6097 doi:10.1038/s41598-018-24309-y 10.1038/s41598-018-24309-y [pii]

Li X et al. (2018b) Molecular cloning and functional analysis of a UV-B photoreceptor gene, BpUVR8 (UV Resistance Locus 8), from birch and its role in ABA response. Plant Sci 274:294-308 doi:S0168-9452(17)31104-4 [pii] 10.1016/j.plantsci.2018.06.006

Liang T et al. (2018) UVR8 Interacts with BES1 and BIM1 to Regulate Transcription and Photomorphogenesis in *Arabidopsis*. Dev Cell 44:512-523 e515 doi:S1534-5807(17)31079-1 [pii] 10.1016/j.devcel.2017.12.028

Liu L, Gregan S, Winefield C, Jordan B (2014) From UVR8 to flavonol synthase: UV-B-induced gene expression in *Sauvignon* blanc grape berry. Plant Cell Environ 38:905-919 doi:10.1111/pce.12349

Llewellyn CA, Ains RL (2010) Distribution and abundance of MAAs in 33 species of microalgae across 13 classes. Mar Drugs 8:1273-1291 doi:10.3390/md8041273

Mao K, Wang L, Li YY, Wu R (2015) Molecular Cloning and Functional Analysis of UV RESISTANCE LOCUS 8 (PeUVR8) from *Populus euphratica*. PLoS One 10:e0132390 doi:10.1371/journal.pone.0132390 PONE-D-15-04341 [pii]

Nicholas KB, Nicholas HBJ (1997) GeneDoc: a tool for editing and annotating multiple sequence alignments.

O'Hara A, Jenkins GI (2012) In vivo function of tryptophans in the *Arabidopsis* UV-B photoreceptor UVR8 Plant Cell 24:3755-3766 doi:tpc.112.101451 [pii] 10.1105/tpc.112.101451

Pearson WR (2014) An introduction to sequence similarity ("homology") searching. Curr Protoc Bioinformatics Chapter 3:Unit3 1 doi:10.1002/0471250953.bi0301s42

Rajhi I et al. (2011) Identification of genes expressed in maize root cortical cells during lysigenous aerenchyma formation using laser microdissection and microarray analyses. New Phytol 190:351-368 doi:10.1111/j.1469-8137.2010.03535.x

Rastogi RP, Incharoensakdi A (2013) UV radiation-induced accumulation of photoprotective compounds in the green alga *Tetraspora* sp. CU2551. Plant Physiol Biochem 70:7-13 doi:S0981-9428(13)00159-9 [pii] 10.1016/j.plaphy.2013.04.021

Ren H et al. (2019) Two E3 ligases antagonistically regulate the UV-B response in *Arabidopsis*. Proc Natl Acad Sci U S A doi:1816268116 [pii] 10.1073/pnas.1816268116

Rizzini L et al. (2011) Perception of UV-B by the *Arabidopsis* UVR8 protein Science 332:103-106 doi:332/6025/103 [pii] 10.1126/science.1200660

629 Rozema J et al. (2002) The role of UV-B radiation in aquatic and terrestrial ecosystems--an
630 experimental and functional analysis of the evolution of UV-absorbing compounds. J
631 Photochem Photobiol B 66:2-12 doi:S101113440100269X [pii]
632 Ruijter JM, Ramakers C, Hoogaars WM, Karlen Y, Bakker O, van den Hoff MJ, Moorman AF (2009)
633 Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data.
634 Nucleic Acids Res 37:e45 doi:gkp045 [pii] 10.1093/nar/gkp045
635 Sharma A, Sharma B, Hayes S, Kerner K, Hoecker U, Jenkins GI, Franklin KA (2019) UVR8 disrupts
636 stabilisation of PIF5 by COP1 to inhibit plant stem elongation in sunlight. Nat Commun
637 10:4417 doi:10.1038/s41467-019-12369-1 10.1038/s41467-019-12369-1 [pii]
638 Soriano G, Cloix C, Heilmann M, Nunez-Olivera E, Martinez-Abaigar J, Jenkins GI (2018) Evolutionary
639 conservation of structure and function of the UVR8 photoreceptor from the liverwort
640 *Marchantia polymorpha* and the moss *Physcomitrella patens*. New Phytol 217:151-162
641 doi:10.1111/nph.14767
642 Strable J, Scanlon MJ (2009) Maize (*Zea mays*): a model organism for basic and applied research in
643 plant biology. Cold Spring Harb Protoc 2009:pdb emo132 doi:2009/10/pdb.emo132 [pii]
644 10.1101/pdb.emo132
645 Sun K, Zhu Z (2018) Illuminating the Nucleus: UVR8 Interacts with More. Trends Plant Sci 23:279-
646 281 doi:S1360-1385(18)30044-X [pii] 10.1016/j.tplants.2018.03.002
647 Tavridou E, Pireyre M, Ulm R (2019) Degradation of the transcription factors PIF4 and PIF5 under
648 UV-B promotes UVR8-mediated inhibition of hypocotyl growth in *Arabidopsis*. Plant J
649 doi:10.1111/tpj.14556
650 Tilbrook K, Arongaus AB, Binkert M, Heijde M, Yin R, Ulm R (2013) The UVR8 UV-B Photoreceptor:
651 Perception, Signaling and Response Arabidopsis Book 11:e0164 doi:10.1199/tab.0164
652 Tilbrook K et al. (2016) UV-B perception and acclimation in *Chlamydomonas reinhardtii*. Plant Cell
653 doi:tpc.15.00287 [pii] 10.1105/tpc.15.00287
654 Ulm R, Jenkins GI (2015) Q&A: How do plants sense and respond to UV-B radiation? BMC Biol 13:45
655 doi:10.1186/s12915-015-0156-y [pii]
656 Wu D et al. (2012) Structural basis of ultraviolet-B perception by UVR8. Nature 484:214-219
657 doi:nature10931 [pii] 10.1038/nature10931
658 Wu Q, Su N, Zhang X, Liu Y, Cui J, Liang Y (2016) Hydrogen peroxide, nitric oxide and UV RESISTANCE
659 LOCUS8 interact to mediate UV-B-induced anthocyanin biosynthesis in radish sprouts. Sci
660 Rep 6:29164 doi:srep29164 [pii] 10.1038/srep29164
661 Yang X, Montano S, Ren Z (2016) How does photoreceptor UVR8 perceive a UV- B signal? Photochem
662 Photobiol 91:993- 1003
663 Yang Y et al. (2018) UVR8 interacts with WRKY36 to regulate HY5 transcription and hypocotyl
664 elongation in *Arabidopsis*. Nat Plants 4:98-107 doi:10.1038/s41477-017-0099-0
665 10.1038/s41477-017-0099-0 [pii]
666 Yin R (2017) Cooling Down Thermomorphogenesis by UV-B Signaling. Trends Plant Sci 22:447-449
667 doi:S1360-1385(17)30068-7 [pii] 10.1016/j.tplants.2017.04.003
668 Yin R, Skvortsova MY, Loubery S, Ulm R (2016) COP1 is required for UV-B-induced nuclear
669 accumulation of the UVR8 photoreceptor Proc Natl Acad Sci U S A 113:E4415-4422
670 doi:1607074113 [pii] 10.1073/pnas.1607074113
671 Yonekura-Sakakibara K, Higashi Y, Nakabayashi R (2019) The Origin and Evolution of Plant Flavonoid
672 Metabolism. Front Plant Sci 10:943 doi:10.3389/fpls.2019.00943
673 Zeng X et al. (2015) Dynamic Crystallography Reveals Early Signalling Events in Ultraviolet
674 Photoreceptor UVR8. Nat Plants 1 doi:10.1038/nplants.2014.6

675 Zhao C, Mao K, You CX, Zhao XY, Wang SH, Li YY, Hao YJ (2016) Molecular cloning and functional
 676 analysis of a UV-B photoreceptor gene, MdUVR8 (UV Resistance Locus 8), from apple. Plant
 677 Sci 247:115-126 doi:S0168-9452(16)30037-1 [pii] 10.1016/j.plantsci.2016.03.006