bioRxiv preprint doi: https://doi.org/10.1101/649905; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Molecular cloning and functional analysis of the UV-B photoreceptor gene ZmUVR8 (UVR8 Response locus), from *Zea Mays.*

María Belén Fernández[1], Lorenzo Lamattina[1] and Raúl Cassia[1]*

1: 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:** Raúl Cassia; rocassia@mdp.edu.ar

Summary

Low UV-B fluence is a signalling stimulus that regulates various metabolic and developmental processes, and induces a photomorphogenic response. The specific UV-B receptor UV-B response locus 8 (UVR8) is a key component of this response.

UVR8 and several proteins participating in the UVR8 signalling were first cloned in Arabidopsis. Although UVR8 sequence is conserved, a few UVR8 homologs has been cloned and reported to be functional from green algae, moss and dicots.

Here we show the cloning and functional analysis of *Zea mays* UVR8 (ZmUVR8). ZmUVR8 has 443 amino acid length, a calculated molecular mass of 47.15 kDa, and 73% of identity to AtUVR8. Key trypthophan residues responsible of UV-B perception (W233, 285 and 337) are conserved in ZmUVR8, as well as the VP domain in the C27 region, involved in the interaction with the proteins COP1 (CONSTITUTIVELY PHOTOMORPHOGENIC 1) and RUP1 (Repressor of UV-B photomorphogenesis 1). Blastp analysis shows that all the components of Arabidopsis UVR8 pathway were present in maize.

UVR8 was expressed in non-irradiated Arabidopsis and maize plants. However, after 2h of UV-B irradiation, *AtUVR8* expression was reduced 3-fold, and *ZmUVR8* expression was reduced 6-fold. That indicate a similar regulation of *UVR8* expression under UV-B treatment in Arabidopsis and maize.

Arabidopsis uvr8 *mutant was complemented with ZmUVR8* driven by the CaMV-35S promoter and fused to eGFP (35S::ZmUVR8-eGFP). Whereas UV-B stimulates GFP-UVR8 nuclear accumulation in Arabidopsis, ZmUVR8-GFP fusion was mainly localized in

bioRxiv preprint doi: https://doi.org/10.1101/649905; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

nuclei of transgenic lines, irrespective of UV-B treatments.The hypocotyl length inhibition, is the most commonly phenotype analyzed to investigate the functions of photoreceptors in Arabidopsis. UV-B suppressed hypocotyl growth in wild-type Arabidopsis plants whereas *uvr8-1* was impaired in this response. Hypocotyl elongation was reduced in 35S::ZmUVR8-eGFP lines. These results confirmed that ZmUVR8 is similar enough to AtUVR8 to restore UV-B perception and signalling in Arabidopsis and thus is a functional UV-B photoreceptor

Keywords

UVR8, UV-B, Zea mays

INTRODUCTION

Ultraviolet-B (UV-B) radiation is present in sunlight (280- 315 nm). High doses of UV-B may damage macromolecules, including DNA, and induce the production of reactive oxygen species (ROS), affecting cell integrity and viability [1-3]. Since UV-B penetration in the water column is lower than in terrestrial environments [4], a mechanism to avoid UV-B damage has evolved during the transition of aquatic to land plant. As UV-B was increasing, harboring a potential damage to DNA and photosystem II, a UV-B receptor was necessary to command defense responses for the protection of photosynthetic organisms [5].

The levels of UV radiation on the Archean Earth were several orders of magnitude higher than the current level [6]. Ancient photosynthetic organisms like cyanobacteria and various eukaryotic algae, including some green alga members, had mycosporine-like amino acids (MAAs) which are UV-B protectors [4, 7, 8]. Land plants could co-evolve with ambient UV-B levels through the evolution of UV-B absorbing polyphenolic compounds which increased in complexity from algae to higher plants [4].

Low UV-B fluence is a signalling stimulus that regulates various metabolic and developmental processes and induces a photomorphogenic response regulated by the specific UV-B receptor UV-B response locus 8 (UVR8) [9]. UVR8 is the first photoreceptor described who does not sense light using a prosthetic chromophore. Instead, UV-B perception in UVR8 is mediated by tryptophan residues [10, 11]. UVR8 signalling significantly contributes to UV-B acclimation responses and the establishment of UV-B tolerance. The usually described UVR8-mediated UV-B responses are inhibition of

bioRxiv preprint doi: https://doi.org/10.1101/649905; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

hypocotyl growth, and accumulation of flavonols and anthocyanins. However, additional physiological responses are actually proposed to be modulated by UVR8: phototropism, thermomorphogenesis, circadian clock, auxin signalling, defence, salt stress tolerance, shade avoidance, stomatal opening, leaf development and downward leaf curling (for a review see Yin and Ulm 2017 [12] and references therein).

Several proteins participate in UVR8 signalling cascade. Since its discovery in 2005 [9], cumulative evidences allow us to regard a landscape of UVR8 interactions.

In the absence of UV-B, UVR8 is located in the cytoplasm as a homodimer. In the nucleus, the E3 Ubiquitin ligase COP1 (CONSTITUTIVELY PHOTOMORPHOGENIC 1) repress the activity of the transcription factor HY5 (ELONGATED HYPOCOTYL 5) [13]. The transcription factor WRKY36 (WRKY DNA-BINDING PROTEIN 36) repress HY5 [14], and BES1(BRI1-EMS-SUPPRESSOR1) and BIM1 (BES1-INTERACTING MYC-LIKE 1) induce BR-responsive gene expressions [15]. Following UV-B irradiation, UVR8 changes to monomeric form and interacts in the nucleus with COP1 and WRKY36, avoiding the degradation of HY5 and inducing *HY5* expression. HY5 upregulates the expression of genes associated with UV-B acclimation and stress tolerance [16]. Furthermore, two of these genes are the proteins Repressor of UV-B photomorphogenesis 1 and 2 (RUP1 and RUP2). When UVR8 interacts with RUP1 and RUP, it switches from monomer to dimer, leading to UVR8 inactivation [11, 16]. UVR8 also interact with BES1 and BIM1, inhibiting the brassinosteroid responsive genes, reducing the hypocotyl elongation [17].

Arabidopsis UVR8 (AtUVR8) crystal structure has been determined at high resolution [18] [19, 20]. AtUVR8 contains a core domain that forms a seven-bladed -propeller and a flexible C-terminal region of approximately 60 amino acids that contains a C27 region. Both the -propeller domain and the C-terminal C27 domain of UVR8 are necessary and sufficient for interacting with COP1. Moreover, UVR8 interact also with WRKY 36 by its C-terminal (amino acid 397 to 440) [14]. AtUVR8 has 14 tryptophan residues. Each UVR8 monomer contains the conserved pentapeptide repeat Gly-Trp-Arg-His-Thr (GWRHT) in blades 5, 6, and 7. This motif generates a triad of closely packed tryptophans (W233, W285 and W337) which are key for UV-B photoreception, W285 being the main UV-B sensor [18-20]. W233 is also important, both in photoreception and in maintaining exciton coupling, whereas W337 plays an auxiliary role [18, 20]. The “GWRHT” motif from blade 6 may be the most

bioRxiv preprint doi: https://doi.org/10.1101/649905; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

important because it contains W285. This motif is conserved in all UVR8 homologs analyzed [21].

Although UVR8 is conserved, and sequence for this gene are found in all the viridiplantae [21], a few UVR8 homologs has been cloned and reported to be functional from green algae, moss and dicots. Up to now, there are no evidences of UVR8 photoreceptor from monocotyledon plants with confirmed functionality. Here we report the cloning, sequence and functional complementation of ZmUVR8, the UV-B receptor of Zea Mays.

MATERIAL AND METHODS

Plants material and growth conditions

Arabidopsis thaliana used in this study were ecotype Landsberg erecta (Ler), wild-type (Wt) and a uvr8-1 *null* mutant [22], kindly provided by Dr. Gareth Jenkins (University of Glasgow). Prior to germination, seeds were cold treated for 3 days in the dark at 4°C.

Maize (*Zea mays* B73 inbreed line) seeds were kindly provided by Dr. Sofía Eugenia Olmos (INTA Pergamino, Argentina).

Germinated seedlings were grown on soil: vermiculite (3:1, v/v) under a long-day regime (light/dark: 16/8 h) at 25°C. White light PAR: 160 μmol.m^{-2} s^{-1}. The topmost leaf from V6 developmental stage plants was used for experiments.

Generation of ZmUVR8-transgenic *Arabidopsis* plants

ZmUVR8 was amplified by PCR using the maize full-length EST ZM_BFb0066P22.r (Arizona Genomics Institute) as template, and specific primers ZmUVR8-Fw and RvUVR8-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 N-terminal fusion to eGFP (*35S:: ZmUVR8*- eGFP*)* [23]. This vector was introduced in the Agrobacterium strain GV3101 by electroporation [24]. Transformation into uvr8-1 mutant *Arabidopsis* was performed by floral dip [25]. Transformant were selected based on its ability to survive on half- strenght Murashige and Skoog medium supplemented with 1% sucrose containing 15 mg L^{-1} hygromicin. Resistant seedlings were then transferred to soil and grown under conditions described above.

bioRxiv preprint doi: https://doi.org/10.1101/649905; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

The transgenic lines generated were shown through segregation analysis to have the transgene integrated at a single genetic locus. 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.

UV-B Treatments

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

Maize plants were exposed 2h to low fluency white light (PAR: 100 $\mu mol.m^{-2}s^{-1}$) supplemented with 8.81 $\mu mol.m^{-2}s^{-1}$ narrowband UV-B (Philips TL 100W/01) in a controlled environment chamber.

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

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

Expression analysis

The level of transgene expression in each line was examined by qRT-PCR and immunodetection of ZmUVR8- eGFP on imunoblot.

Plant materials were harvested, frozen in liquid nitrogen, and then ground 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). *ZmUVR8* and *AtUVR8* 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 a single product specific melting curve. Negative controls for cDNA synthesis and qRT-PCR reactions were included in all cases. LineReg program was employed

bioRxiv preprint doi: https://doi.org/10.1101/649905; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

for the analysis of gene expression [26]. Results were expressed as N0 which represents the starting concentration of mRNA per sample, expressed in arbitrary fluorescence units. 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 *thiorredoxin h-like*, (Thr) for maize samples as previously reported [27] .

For protein assays, leaves were harvested into liquid nitrogen and proteins extracted in buffer KPO_4 100mM pH 7.4, EDTA 1mM and a cocktail of protease inhibitors. The homogenate was centrifuged for 10 min a 10000 xg at 4°C. Protein concentration was determined by a Bradford assay. Thirty micrograms of total protein were loaded for ZmUVR8 protein expression and separated by 12% denaturating 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 immunoblots were stained with Ponceau S to reveal the Rubisco large subunit (rbcL) as loading control.

Table 1. List of primers used in this work.

	Primers		Sequence	Amplicon lenght (pb)	Usage
Cloning	*Zm*UVR8	Fw	CACCGCTCCCGTTCCTCGCGGTGGTCAC	1341	Amplification of full lenght *Zm*UVR8 cDNA
		Rv	TGATGACTGCACGCGCATCCTCTTCACGTCGT		
qRT-PCR	*Zm*UVR8	Fw	GTTTGGGTGTCGCCGTC	123	Amplification of *Zm*UVR8
		Rv	CCTCTTCACGTCGTTCTCC		
	*Zm*Thr	Fw	GGACCAGAAGATTGCAGAAG	104	Maize reference gene
		Rv	CAGCATAGACAGGAGCAATG		
	*At*UVR8	Fw	CCAGATTTCGGGAGGTTGG	102	Amplification of *At*UVR8
		Rv	TTATTGCCGACTCCTACTTGTCC		
	*At*Actin2	Fw	GTATTGTGCTGGATTCTGGTG	119	*Arabidopsis* reference gene
		Rv	GAGGTAATCAGTAAGGTCACG		

ZmUVR8- eGFP Subcellular Localization

Fifhteen day old plants irradiated 1h with white light with or whitouth 3.47 $\mu mol.m^{-2}s^{-1}$ of UV-B were vacuum infiltrated with 5 µg/mL of Hoescht 33342 (Invitrogen Molecular Probes) in buffer PBS, 0.2% Triton-X100 for 4 min and maintained in shake at 50 rpm and

bioRxiv preprint doi: https://doi.org/10.1101/649905; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

darkness for 1h. Then, samples were washed 3 times with PBS. The subcellular localization of eGFP and Hoescht 33342 were visualized by a confocal laser scanning microscope (Nikon-C1siR Eclipse TiU) under oil (Biopack) with a ×40 objective. Images were taken using the Nikon EZ-C1 3.90 software. eGFP and Hoescht 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. Hoescht 33342 fluorescence was collected at 440/50 nm. The same microscope settings for GFP and Hoescht 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.

Hypocotyl length measurement

Seedlings were grown for 5 days on agar plates of half strength Murashige and Skoog (MS) salts containing 1% sucrose in darkness or white light (PAR: 100 $\mu mol.m^{-2}s^{-1}$) supplemented with or whitouth 3.47 μmol.m-2 s-1 UV-B. Photographs were taken after treatments and hypocotyl lengths were measured using the ImageJ sotware (http://rsb.info.nih.gov/ij). At least three independent biological replicates were performed for all experiments.

Bioinformatic analysis

Multiple sequence alignments were performed using MAFFT server (http://mafft.cbrc.jp/alignment/server/) and edited with GeneDoc [28].

RESULTS

An AtUVR8 homolog with a conserved structure is found in maize.

Several UVR8 protein sequences from green algae, moss, liverworth, and dicots species have been cloned and characterized (C. reinhardtii, *P.patens, M. polymorpha, S. lycopersicum, M. domestica, V. vinifera, B. platyphylla* and *P. euphratica*). However, none monocot UVR8 protein has been reported up to now. The *Arabidopsis* protein sequence (AAD43920.1) was used as template in a PSI-BLASTp to search the UVR8 homolog in maize. One sequence was found (GRMZM2G003565), herein named as *ZmUVR8*. Figure 1A shows the

bioRxiv preprint doi: https://doi.org/10.1101/649905; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

comparison among ZmUVR8 and the other UVR8 homologs cloned. ZmUVR8 has 443 amino acid length, a calculated molecular mass of 47.15 kDa, and 73% of identity to AtUVR8. Key trypthophan residues responsible of UV-B perception (W233, 285 and 337) were conserved in ZmUVR8, as well as the VP domain in the C27 region, involved in the interaction with COP1 and RUP.

Figure 1 B show that ZmUVR8 has the same domain profile that AtUVR8, including the conserved triptophans, the C27 domain and the seven repeated RCC1 domains.

bioRxiv preprint doi: https://doi.org/10.1101/649905; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

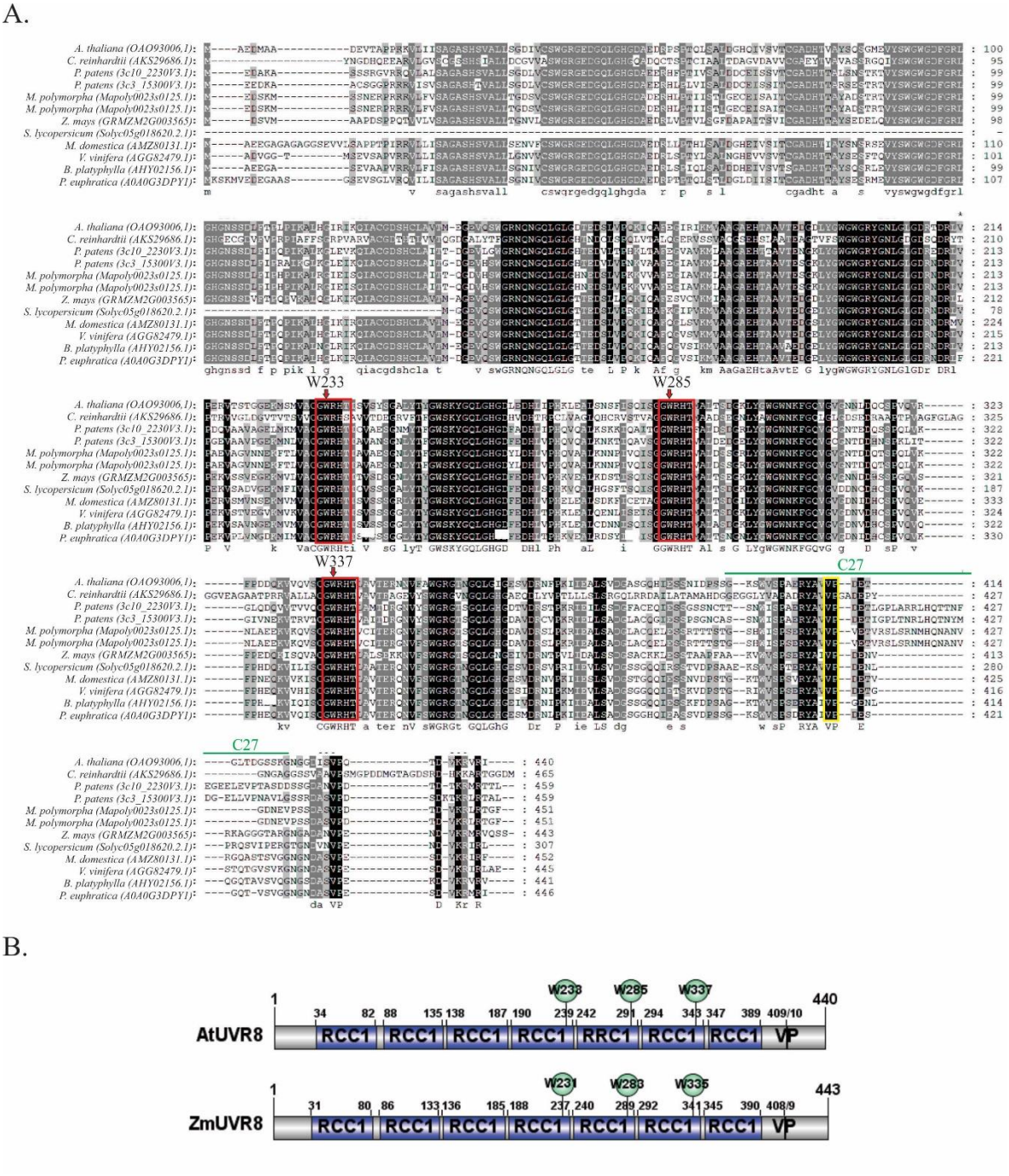

Figure 1. ZmUVR8 sequence and structural domains. A. Multiple sequence alignment of *At*UVR8 homologs. Protein sequences that have been cloned and complemented *uvr8-1* mutant were aligned with MAFFT (http://mafft.cbrc.jp/alignment/server/) and edited with GeneDoc [28]. Boxes indicate "GWRHT" motifs 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. Yellow box show the "VP" domain in the C27 domain, important for interaction with COP1 and RUP proteins. **B)** Sechematic representation of structural domains of the AtUVR8 and ZmUVR8 proteins. Analysis of amino-

bioRxiv preprint doi: https://doi.org/10.1101/649905; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

acid sequences was performed in the National Center for Biotechnology Information (NCBI) database. RCC1 (pfam00415), Regulator of chromosome condensation (RCC1) repeat.

Blastp analysis in Table 2 shows that all the components of *Arabidopsis* UVR8 pathway were present in maize: COP1 (70% identity), HY5 (68%), HYH (48%), RUP1 (45%), RUP2 (46%), WRKY36 (35%), BES1 (50%) and BIM1 (41%). These results indicate that ZmUVR8 should be a functional UV-B recepetor, triggering a UVR8 signalling cascade in maize.

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

Table 2. Table 2: Identification of Arabidopsis UVR8 signaling homologs in maize.
Proteins involved in UVR8 signaling cascade from Arabidopsis were used as query in BLASTp analysis restricting the search to Z. mays.

ZmUVR8 and *AtUVR8* expression are regulated by UV-B irradiation.

Previous studies show that AtUVR8 is a ubiquitously expressed gene, allowing plants to immediately respond to UV-B exposure [13, 29]. We analyzed UVR8 expression in *Arabidopsis* and maize plants irradiated with 3.47 and 8.81 $\mu mol.m^{-2}$ s^{-1} of UV-B respectively. Quantitative RT-PCR in Figure 2 shows that UVR8 occur in non-irradiated plants. However, after 2h of UV-B irradiation, *AtUVR8* expression was reduced 3-fold, and *ZmUVR8* expression was reduced 6-fold. That indicate a similar regulation of *UVR8* expression under UV-B treatment in *Arabidopsis* and maize plants. However, Table 3 shows that UV-B has no effect on *UVR8* expression in M. polymorpha and V. vinifera. Moreover, UV-B upregulates transcript levels in M. domestica and B. platyphylla. These findings suggest the existence of different regulatory mechanisms for *UVR8* expression.

bioRxiv preprint doi: https://doi.org/10.1101/649905; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

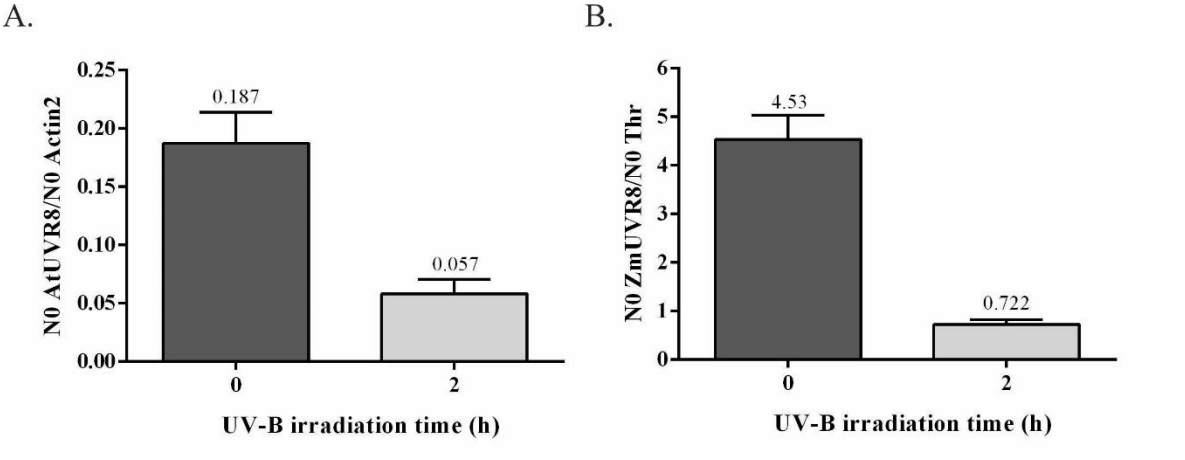

Figure 2 . AtUVR8 and ZmUVR8 expression in response to UV-B. UVR8 transcript level of *A. thaliana* **(A)** and maize **(B)** control leaves or treated for 2h with 3.47 and 8.81 µmol.m-2 s-1 of UV-B irradiation respectively were analyzed by qRT-PCR. LineReg program was employed for the analysis of gene expression. N0 represents the starting concentration per sample, expressed in arbitrary fluorescence units. Expression of Actin2 and Thr were used for Arabidopsis and maize normalization respectively. Primers are listed in Table 1. Error bars indicate the standard desviation over three biological replicates, with each replicate containing two technical replicates.

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

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

ZmUVR8 complements the uvr8 null mutant

To elucidate 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 *Arabidopsis* uvr8-1 null mutant. We obtained two independent T3 and T4 homocygous lines (#6.5 and #5.1.7 respectively).

bioRxiv preprint doi: https://doi.org/10.1101/649905; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Figure 3A shows that both lines expresed *ZmUVR8* mRNA, with line #6.5 having the highest level. However, immunoblot with anti-GFP antibody in Figure 3B shows that ZmUVR8-eGFP was present in both transgenic lines in similar amount. ZmUVR8-eGFP migrated aberrantly at an apparent molecular mass of approximately 65 kDa, which is smaller than predicted for the combination of fully denatured UVR8 and eGFP (47.1 kDa+ 29 kDa = 76. 16 kDa) (Fig. 3B). Figure 3c shows that phenotype of transgenic lines did not displays differences with Wt and uvr8.1 after 30 days.

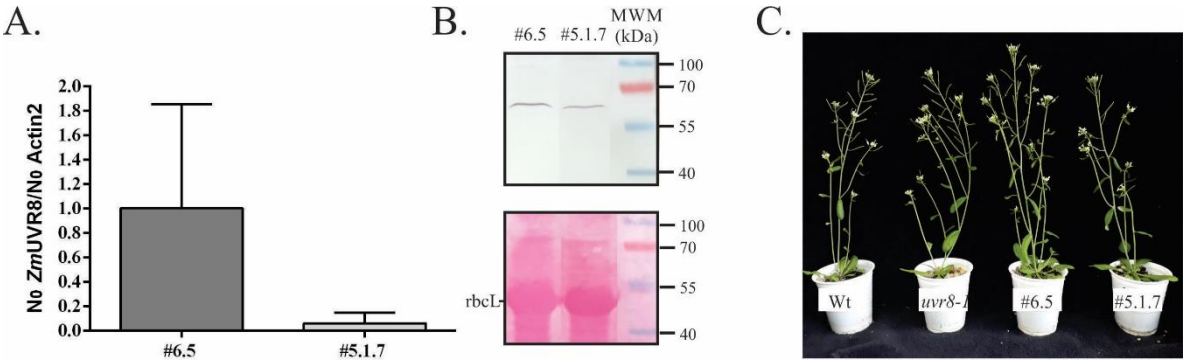

Figure 3. 35S::ZmUVR8-eGFP expression analysis in two homozygous lines. A. ZmUVR8 transgene transcript level of *A. thaliana* leaves was analyzed by qRT-PCR. LineReg program was employed for the analysis of gene expression. Expression of Actin2 was used for normalization. Primers are listed in Table 1. Error bars indicate the standard desviation over three biological replicates, with each replicate containing two technical replicates. **B.** Denaturating immunoblot from Arabidopsis transgenic lines #6.5 and #5.1.7 protein extracts. Inmunodetection was performed using anti-GFP antiserum. Stained Rubisco large subunit (rbcL) is shown as a loading control (lower panel). **C.** 30 day old wt, uvr8-1 mutant and #6.5 and #5.1.7 transgenic lines.

The sub-cellular localization of ZmUVR8-eGFP, was analyzed using a confocal laser scanning microscope. Figure 4 shows that ZmUVR8-eGFP localization differs from *Arabidopsis*. Whereas UV-B stimulates GFP-UVR8 nuclear accumulation in *Arabidopsis* (Kaiserli & Jenkins, 2007), ZmUVR8-GFP fusion was mainly localized in nuclei of #6.5 and #5.1.7 lines, irrespective of UV-B treatments. This result is in accordance to the liverwort Marchantia GFP-UVR8 fusion localization previously reported [30].

bioRxiv preprint doi: https://doi.org/10.1101/649905; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

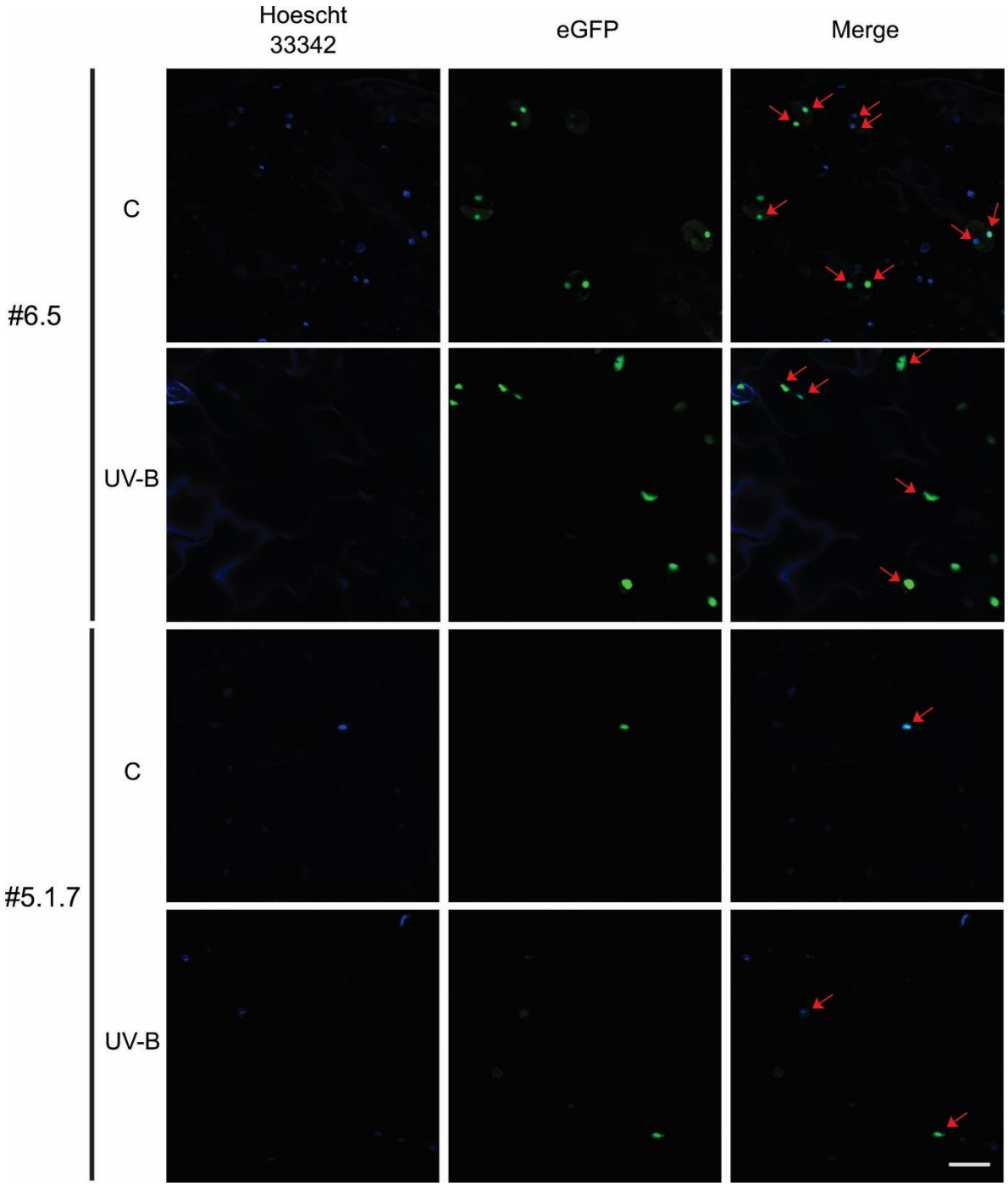

Figure 4. Subcellular localization of eGFP-ZmUVR8. Cellular localization of ZmUVR8-eGFP fusions revealed by confocal laser microscopy. Non-irradiated control (C) plants or irradiated with (whithe light + 3.47 $\mu mol.m^{-2}s^{-1}$ UV-B for 1 h were vacuum infiltrated with Hoescht 33342 which specifically stain nuclei. eGFP and Hoescht 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. Hoescht 33342 fluorescence was

bioRxiv preprint doi: https://doi.org/10.1101/649905; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

collected at 440/50 nm. The same microscope settings for GFP and Hoescht 33342 detection were used before and after UV-B illumination. The arrows indicate colocalization. Bar, 25 µm.

The hypocotyl length inhibition, is the most commonly phenotype analyzed to investigate the functions of photoreceptors in Arabidopsis. UV-B suppresses hypocotyl growth in wild-type Arabidopsis plants whereas uvr8-1 is impaired in the response. Expression of wild-type UVR8 fused to GFP or yellow fluorescent protein (YFP) in uvr8-1 plants functionally complements the mutant phenotype (Favory et al., 2009; O'Hara & Jenkins, 2012; Huang et al., 2014). Figure 5 shows no differences among hypocotyl length of wt, uvr8-1 and transgenic lines grown under white light. As previously reported, UV-B suppressed hypocotyl growth in wild-type *Arabidopsis* plants whereas *uvr8-1* was impaired in this response. Figure 5 also shows that hypocotyl elongation was reduced 20% in line #6.5 and 15% (similar to wt) in line #5.1.7. These results demonstrate that ZmUVR8 is a positive regulator in UV-B induced photomorphogenesis in plants. That confirmed that Zm-UVR8 is similar enough to At-UVR8 to restore UV-B perception and signaling in Arabidopsis and thus is a functional UV-B photoreceptor.

bioRxiv preprint doi: https://doi.org/10.1101/649905; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

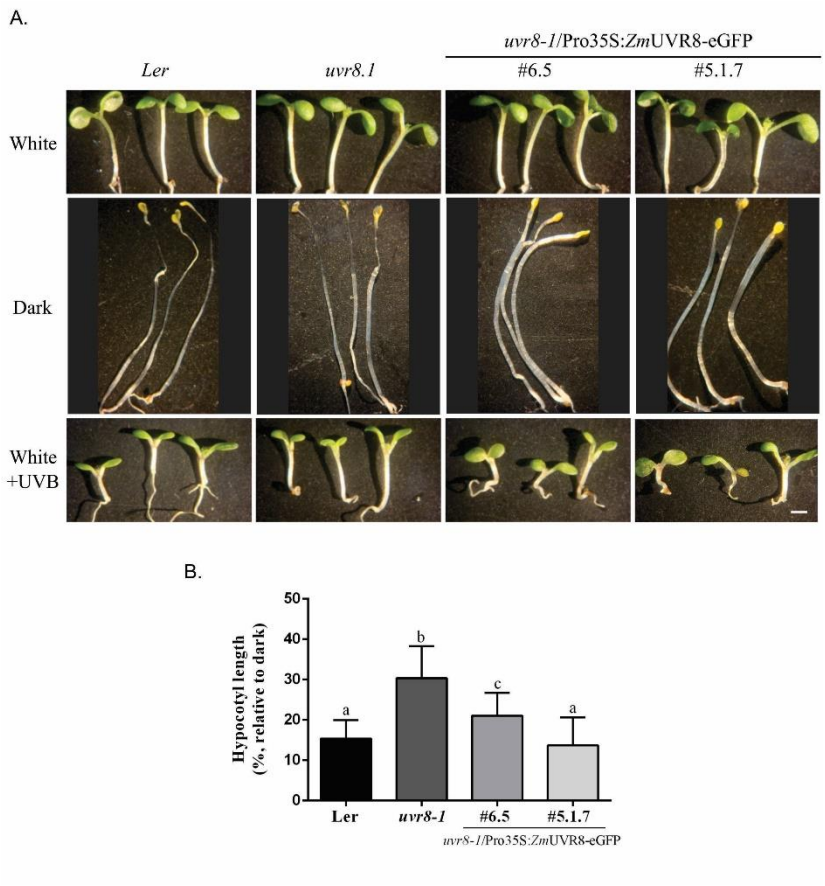

Figure 5. Functional complementation assay of eGFP-ZmUVR8 in an *Arabidopsis* uvr8-1 mutant. A. Phenotypes of the WT, uvr8-1 null mutant and transgenic lines grown under white, UV-B-light and darkness conditions **B.** Comparison of the hypocotyl length of WT, uvr8-1 and transgenic lines (#6.5 and #5.1.7) of *Arabidopsis* seedlings grown under UV-B light and darkness conditions. The scale bar represents 1mm. Photographs were taken after treatments and hypocotyl lengths were measured using the ImageJ software (http://rsb.info.nih.gov/ij). At least three independent biological replicates were performed for all experiments.

Discussion

In this work, we have cloned a maize functional homologue of the Arabidopsis UV-B receptor, named as ZmUVR8, the first UVR8 protein from monocots.

Two maize UV-B-responsive genes were reported as *UVR8* homologs by Casati et al in 2011. Previously, these genes were identified by comparation with rice genome. However, these genes have low homology with AtUVR8. We have found ZmUVR8 after maize sequence was recently completed. ZmUVR8 has a conserved sequence and modular structure, in concordance with several UVR8 homologs [21].

bioRxiv preprint doi: https://doi.org/10.1101/649905; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

ZmUVR8 expression was regulated by UV-B. Although *UVR8* was reported as constitutive in Arabidospsis, we show here that *UVR8* transcript decreased after 2 hours both in Arabidopsis and maize leaves. That is similar to *Betula platyphylla* UVR8 (*BpUVR8*) expression, which is induced by UV-B, and decreased after 9h of continuous irradiation [31]. On the other hand, *UVR8* expression is increased by UV-B in the hypocotyls of radish sprouts [32]. Variations in UVR8 expression were also reported in UV-B treated fruit skin and apple callus ([33]). Differences observed in UVR8 expression in the literature, could be due to the different UV-B intensities used in the experiments.

AtUVR8 is a stable protein [34]. It could be possible that once translated, gene expression will be decreased to avoid UVR8 over accumulation in Arabidopsis and maize. A huge amount of UVR8 may influence the activity of COP1 and RUP as E3 ligases [35].

Little is known about the existence of different regulatory mechanisms for *UVR8* expression. Wu et al (2015) [32] proposed that UV-B enhances the production of H_2O_2, which increases the level of NO to further magnify the *UVR8* expression. It was recently reported that At*UVR8* expression may be modulated by blue light in UV-B-irradiated Arabidopsis. Moreover, cryptochrome 1 (Cry1) mutant *hy4* shows reduced *UVR8* expression, suggesting that *UVR8* may be regulated by Cry1 in the UV-B response [36]. Besides, *ZmUVR8* expression is increased by waterlogged in non-UV-B-irradiated maize root [37].

We obtained two independent homozygous lines (#6.5 and #5.1.7), where ZmUVR8- eGFP complement the uvr8- null mutant. Although *ZmUVR8* had different expression in both lines, the amount of this protein was similar. Arabidopsis GFP-UVR8 shows increased nuclear localisation following UV-B treatment of plants [38], but ZmUVR8- eGFP was constitutively located in nuclei in #6.5 and #5.1.7 lines. Our results are in agreement with that of Soriano et al (2018) [30], that generated two lines of uvr8- complemented with *Marchantia polymorpha* (GFP-MpUVR8). In both lines, UVR8 is constitutively expressed in nuclei. In the same article, they show that in one of two lines of uvr8- complemented with *Physcomitrella patens*, GFP-PpUVR8 is also localised in nuclei in the absence of UV-B. However, both proteins are functional after UV-B irradiation. There is little information about other UVR8 homologs localisation in uvr8- complemented lines.

No obvious nuclear localization signal (NLS) is found in UVR8, and there is no consensus about the mechanism of UVR8 translocation [12]. Moreover, it was proved that the

bioRxiv preprint doi: https://doi.org/10.1101/649905; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

presence of UVR8 in the nucleus is required but not sufficient for its function. Kaiserli & Jenkins, (2007) [38] fused an 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.

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 [39]. The hypocotyl growth of the *uvr8* mutant seedlings, in stark contrast to wild-type seedlings, was not inhibited by UV-B [13]. The restoring of this morphogenic response was established as a parameter of complementation by functional UVR8 homologs [30, 31, 33, 40-42].

ZmUVR8 complement the impaired UV-B response of the uvr8-1 mutant with respect to hypocotyl growth suppression, working as an effective component of the UVR8 pathway.

Thus, together with the identification of homologs of components of the UVR8 pathway in maize, indicate that a functional UVR8 pathway is present in maize.

Conclusion

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

Acknowledgements

This work was supported 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 is fellowship of Comisión de Investigaciones Científicas (CIC)

References

[1] Jordan B.R., The effects of ultraviolet-B radiation on plants: a molecular perspective, Advances in Botany 22 (1996) 98-138.

bioRxiv preprint doi: https://doi.org/10.1101/649905; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

[2] Brosché M., Strid A., Molecular events following perception of ultraviolet-B radiation by plants, Physiologia Plantarum 117 (2003) 1-10.
[3] Frohnmeyer H., Staiger D., Ultraviolet-B radiation-mediated responses in plants. Balancing damage and protection, Plant Physiol 133 (2003) 1420-1428.
[4] Rozema J., Bjorn L.O., Bornman J.F., Gaberscik A., Hader D.P., Trost T., Germ M., Klisch M., Groniger A., Sinha R.P., Lebert M., He Y.Y., Buffoni-Hall R., de Bakker N.V., van de Staaij J., Meijkamp B.B., The role of UV-B radiation in aquatic and terrestrial ecosystems--an experimental and functional analysis of the evolution of UV-absorbing compounds, J Photochem Photobiol B 66 (2002) 2-12.
[5] Tilbrook K., Arongaus A.B., Binkert M., Heijde M., Yin R., Ulm R., The UVR8 UV-B Photoreceptor: Perception, Signaling and Response, Arabidopsis Book 11 (2013) e0164.
[6] Cnossen I., Sanz-Forcada J., Favata F., Witasse O., Zegers T., Arnold N.F., Habitat of early life: Solar X-ray and UV radiation at Earth's surface 4–3.5 billion years ago, (2007).
[7] Llewellyn C.A., Airs R.L., Distribution and abundance of MAAs in 33 species of microalgae across 13 classes, Mar Drugs 8 (2010) 1273-1291.
[8] Rastogi R.P., Incharoensakdi A., UV radiation-induced accumulation of photoprotective compounds in the green alga Tetraspora sp. CU2551, Plant Physiol Biochem 70 (2013) 7-13.
[9] Brown B.A., Cloix C., Jiang G.H., Kaiserli E., Herzyk P., Kliebenstein D.J., Jenkins G.I., A UV-B-specific signaling component orchestrates plant UV protection, Proc Natl Acad Sci U S A 102 (2005) 18225-18230.
[10] O'Hara A., Jenkins G.I., In vivo function of tryptophans in the Arabidopsis UV-B photoreceptor UVR8, Plant Cell 24 (2012) 3755-3766.
[11] Ulm R., Jenkins G.I., Q&A: How do plants sense and respond to UV-B radiation?, BMC Biol 13 (2015) 45.
[12] Yin R., Cooling Down Thermomorphogenesis by UV-B Signaling, Trends Plant Sci 22 (2017) 447-449.
[13] Favory J.J., Stec A., Gruber H., Rizzini L., Oravecz A., Funk M., Albert A., Cloix C., Jenkins G.I., Oakeley E.J., Seidlitz H.K., Nagy F., Ulm R., Interaction of COP1 and UVR8 regulates UV-B-induced photomorphogenesis and stress acclimation in Arabidopsis, EMBO J 28 (2009) 591-601.
[14] Yang Y., Liang T., Zhang L., Shao K., Gu X., Shang R., Shi N., Li X., Zhang P., Liu H., UVR8 interacts with WRKY36 to regulate HY5 transcription and hypocotyl elongation in Arabidopsis, Nat Plants 4 (2018) 98-107.
[15] Liang T., Mei S., Shi C., Yang Y., Peng Y., Ma L., Wang F., Li X., Huang X., Yin Y., Liu H., UVR8 Interacts with BES1 and BIM1 to Regulate Transcription and Photomorphogenesis in Arabidopsis, Dev Cell 44 (2018) 512-523 e515.
[16] Heijde M., Ulm R., UV-B photoreceptor-mediated signalling in plants, Trends Plant Sci 17 (2012) 230-237.
[17] Sun K., Zhu Z., Illuminating the Nucleus: UVR8 Interacts with More, Trends Plant Sci 23 (2018) 279-281.
[18] Wu D., Hu Q., Yan Z., Chen W., Yan C., Huang X., Zhang J., Yang P., Deng H., Wang J., Deng X., Shi Y., Structural basis of ultraviolet-B perception by UVR8, Nature 484 (2012) 214-219.
[19] Zeng X., Ren Z., Wu Q., Fan J., Peng P.P., Tang K., Zhang R., Zhao K.H., Yang X., Dynamic Crystallography Reveals Early Signalling Events in Ultraviolet Photoreceptor UVR8, Nat Plants 1 (2015).
[20] Christie J.M., Arvai A.S., Baxter K.J., Heilmann M., Pratt A.J., O'Hara A., Kelly S.M., Hothorn M., Smith B.O., Hitomi K., Jenkins G.I., Getzoff E.D., Plant UVR8 photoreceptor senses UV-B by tryptophan-mediated disruption of cross-dimer salt bridges, Science 335 (2012) 1492-1496.

bioRxiv preprint doi: https://doi.org/10.1101/649905; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

[21] Fernandez M.B., Tossi V., Lamattina L., Cassia R., A Comprehensive Phylogeny Reveals Functional Conservation of the UV-B Photoreceptor UVR8 from Green Algae to Higher Plants, Front Plant Sci 7 (2016) 1698.
[22] Cloix C., Kaiserli E., Heilmann M., Baxter K.J., Brown B.A., O'Hara A., Smith B.O., Christie J.M., Jenkins G.I., C-terminal region of the UV-B photoreceptor UVR8 initiates signaling through interaction with the COP1 protein, Proc Natl Acad Sci U S A 109 (2012) 16366-16370.
[23] Karimi M., Inze D., Depicker A., GATEWAY vectors for Agrobacterium-mediated plant transformation, Trends Plant Sci 7 (2002) 193-195.
[24] Koncz C., Schell J., The promoter of TL-DNA gene 5 controls the tissue- specific expression of chimaeric gene carried by a novel type of Agrobacterium binary vector, Molecular and general genetics 204 (1986) 383-396.
[25] Clough S.J., Bent A.F., Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana, Plant J 16 (1998) 735-743.
[26] Ruijter J.M., Ramakers C., Hoogaars W.M., Karlen Y., Bakker O., van den Hoff M.J., Moorman A.F., Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data, Nucleic Acids Res 37 (2009) e45.
[27] Casati P., Walbot V., Rapid transcriptome responses of maize (Zea mays) to UV-B in irradiated and shielded tissues, Genome Biol 5 (2004) R16.
[28] Nicholas K.B., Nicholas H.B.J., GeneDoc: a tool for editing and annotating multiple sequence alignments., (1997).
[29] Rizzini L., Favory J.J., Cloix C., Faggionato D., O'Hara A., Kaiserli E., Baumeister R., Schafer E., Nagy F., Jenkins G.I., Ulm R., Perception of UV-B by the Arabidopsis UVR8 protein, Science 332 (2011) 103-106.
[30] Soriano G., Cloix C., Heilmann M., Nunez-Olivera E., Martinez-Abaigar J., Jenkins G.I., Evolutionary conservation of structure and function of the UVR8 photoreceptor from the liverwort Marchantia polymorpha and the moss Physcomitrella patens, New Phytol 217 (2018) 151-162.
[31] Li X., Ma M., Shao W., Wang H., Fan R., Chen X., Wang X., Zhan Y., Zeng F., 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 (2018) 294-308.
[32] Wu Q., Su N., Zhang X., Liu Y., Cui J., Liang Y., Hydrogen peroxide, nitric oxide and UV RESISTANCE LOCUS8 interact to mediate UV-B-induced anthocyanin biosynthesis in radish sprouts, Sci Rep 6 (2016) 29164.
[33] Mao K., Wang L., Li Y.Y., Wu R., Molecular Cloning and Functional Analysis of UV RESISTANCE LOCUS 8 (PeUVR8) from Populus euphratica, PLoS One 10 (2015) e0132390.
[34] Heilmann M., Jenkins G.I., Rapid reversion from monomer to dimer regenerates the ultraviolet-B photoreceptor UV RESISTANCE LOCUS8 in intact Arabidopsis plants, Plant Physiol 161 (2013) 547-555.
[35] Ren H., Han J., Yang P., Mao W., Liu X., Qiu L., Qian C., Liu Y., Chen Z., Ouyang X., Chen X., Deng X.W., Huang X., Two E3 ligases antagonistically regulate the UV-B response in Arabidopsis, Proc Natl Acad Sci U S A (2019).
[36] Khudyakova A.Y., Kreslavski V.D., Shmarev A.N., Lyubimov V.Y., Shirshikova G.N., Pashkovskiy P.P., Kuznetsov V.V., Allakhverdiev S.I., Impact of UV-B radiation on the photosystem II activity, pro-/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 (2019) 14-20.
[37] Rajhi I., Yamauchi T., Takahashi H., Nishiuchi S., Shiono K., Watanabe R., Mliki A., Nagamura Y., Tsutsumi N., Nishizawa N.K., Nakazono M., Identification of genes expressed in maize root

bioRxiv preprint doi: https://doi.org/10.1101/649905; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

cortical cells during lysigenous aerenchyma formation using laser microdissection and microarray analyses, New Phytol 190 (2011) 351-368.

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

[39] Kim B.C., Tennessen D.J., Last R.L., UV-B-induced photomorphogenesis in Arabidopsis thaliana, Plant J 15 (1998) 667-674.

[40] Liu L., Gregan S., Winefield C., Jordan B., From UVR8 to flavonol synthase: UV-B-induced gene expression in Sauvignon blanc grape berry, Plant Cell Environ 38 (2014) 905-919.

[41] Zhao C., Mao K., You C.X., Zhao X.Y., Wang S.H., Li Y.Y., Hao Y.J., Molecular cloning and functional analysis of a UV-B photoreceptor gene, MdUVR8 (UV Resistance Locus 8), from apple, Plant Sci 247 (2016) 115-126.

[42] Tilbrook K., Dubois M., Crocco C.D., Yin R., Chappuis R., Allorent G., Schmid-Siegert E., Goldschmidt-Clermont M., Ulm R., UV-B perception and acclimation in Chlamydomonas reinhardtii, Plant Cell (2016).