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

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Abstract

Low UV-B fluence is a signaling stimulus that regulates various physiological processes and induces photomorphogenic responses. The specific UV-B receptor UVR8, is a key component in these processes. Although UVR8 sequence is conserved, few homologs from plants have been cloned and reported to be functional. Here we show the cloning and functional analysis of *Zea mays* UVR8 (*Zm*UVR8) a monocotyledon species. *Zm*UVR8 has 73% of identity to *At*UVR8. Key tryptophan responsible of UV-B perception are conserved, as well as the VP domain, involved in the interaction with the proteins COP1 and RUP1. Whereas UVR8 is expressed in non-irradiated of both *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 *Zm*UVR8 driven by the CaMV-35S promoter and fused to eGFP. *Zm*UVR8-eGFP fusion was mainly localized in nuclei of transgenic lines, irrespective of UV-B treatments. Also, UV-B suppressed hypocotyl growth in WT *Arabidopsis* plants whereas *uvr8-1* was impaired in this response. However, hypocotyl elongation was reduced in UV-B irradiated transgenic lines complemented with *Zm*UVR8. Moreover, CHS and transcription factor HY5 (ELONGATED HYPOCOTYL 5) expression were also restored in these plants. These

results confirmed that *Zm*UVR8 is similar enough to *At*UVR8 to restore UV-B perception and signaling in *Arabidopsis* mutant *uvr8-1* being a functional UV-B photoreceptor.

Keywords

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

1. Introduction

Ultraviolet-B (UV-B) radiation is the part of the solar spectrum included between 280 and 315 nm. At high doses, this radiation damages DNA, proteins, lipids and cell membranes and induces the production of reactive oxygen species (ROS), affecting cell integrity and viability [1-3].

The levels of UV radiation on the Archean Earth were several orders of magnitude higher than the current [4]. Since UV-B penetration in the water column is lower than in terrestrial environments [5], a mechanism to avoid UV-B damage has evolved during the transition of aquatic to land plants. Ancient photosynthetic organisms like cyanobacteria and various eukaryotic algae, contain mycosporine-like amino acids (MAAs) as UV-B protectors [5-7]. As UV-B was increasing, harboring a potential damage to DNA and photosystem II, the appearance of molecules that perceive UV-B radiation was necessary to command defense responses for the protection of photosynthetic organisms [8]. Land plants co-evolved with ambient UV-B levels, and the complexity of UV-B absorbing polyphenolic from algae to higher plants increased [5]. The specific UV-B receptor UV-B response locus 8 (UVR8) was identified mediating plant responses to UV-B [9]. Chlorophytes are the earliest organisms with an UV-B photoreceptor, being *Chlamydomonas reinhardtii* the earliest organism with a functional UVR8 protein [10, 11]. 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 [12, 13].

In contrast to high UV-B fluences, low UV-B fluence is a signaling stimulus that regulates various metabolic and developmental processes and induces a photomorphogenic response. UVR8 signaling 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 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 signaling, defence, salt stress tolerance, shade avoidance, chloroplast development, stomatal opening, leaf development and downward leaf curling (for a review see [14]).

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) [15]. The transcription factor WRKY36 (WRKY DNA-BINDING PROTEIN 36) repress HY5 [16], and BES1 (BRI1-EMS-SUPPRESSOR1) and BIM1 (BES1-INTERACTING MYC-LIKE 1) induce BR-responsive gene expressions [17].

Several proteins participate in UVR8 signaling cascade. Since its discovery in 2005 [9], cumulative evidences allow us to regard a landscape of UVR8 interactions. This interactions occur following UV-B irradiation where 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 [18]. Two HY5 upregulated genes are the 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 [13, 18]. UVR8 also interact with BES1 and BIM1, inhibiting the brassinosteroid responsive genes, reducing the hypocotyl elongation [19].

Arabidopsis UVR8 (*At*UVR8) crystal structure has been determined at high [20-22]. *At*UVR8 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 β-propeller domain and the C-terminal C27 domain of UVR8 are necessary and sufficient for interacting with COP1. Moreover, UVR8 interact also with WRKY36 by its C- terminal (aminoacid 397 to 440) [16]. *At*UVR8 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, being W285 the main UV-B sensor [20-22]. Also, W233 is important both in photoreception and in maintaining exciton coupling, whereas W337 plays an auxiliary role [20, 21]. The “GWRHT” motif from blade 6 may be the most important because it contains W285. This motif is conserved in all UVR8 homologs analyzed [10, 11].

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 [23]. Previous work reported that increased UV-B radiation produce a significant reduction in dry matter accumulation and in consequence, affects yield. Also, an increase in flavonoid accumulation and a decrease in chlorophyll content in leaves and a reduction in protein level, sugar and starch of maize seeds was reported [24]. In 2011, Casati et. al. described a transiently upregulation and subsequent downregulation of two UVR8 like genes upon UV-B exposure in maize leaves [25, 26]. However, these genes have little homology to *At*UVR8 as they were identified by homology to rice genome.

Although UVR8 is conserved, and sequences for this gene are found in all the *Viridiplantae* [10, 11], a few UVR8 homologs have 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 molecular cloning, sequence and functional complementation of *Zm*UVR8, the UV-B receptor of *Zea Mays*.

2. Material and Methods

2.1. 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 [27], kindly provided by Dr. Gareth Jenkins (University of Glasgow, Scotland). Prior to germination, seeds were cold treated for 3 days in the dark at 4°C. For expression analysis, plants of three weeks old were used.

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 $\mu mol.m^{-2}\ s^{-1}$. The topmost leaf from V6 developmental stage plants was used for experiments.

2.2. Generation of transgenic *Arabidopsis* plants expressing *Zm*UVR8

*Zm*UVR8 was amplified by PCR using the maize full-length EST ZM_BFb0066P22.r (Arizona Genomics Institute) as template, and specific primers *Zm*UVR8-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::*Zm*UVR8- eGFP) [28]. This vector was introduced in the *Agrobacterium* strain GV3101 by electroporation [29]. Transformation into *uvr8-1* mutant *Arabidopsis* was performed by floral dip [30]. Transformant 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.

2.3. UV-B Treatments

For experiments involving UV-B light treatments, Arabidopsis seedlings were exposed 2h to low fluency white light (PAR: 100 $\mu molm^{-2}s^{-1}$) 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).

2.4. Expression analysis

2.4.1. Gene expression was examined by qRT-PCR

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). 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 [31]. 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 [32].

Table 1 List of primers used in this work.

Primers			Sequence (5´→ 3´)	Amplicon length (pb)	Usage
Cloning	*Zm* UVR8	Fw	CACCGCTCCCGTTCCTCGCGGTGGTCAC	1341	Amplification of full length *Zm* UVR8
		Rv	TGATGACTGCACGCGCATCCTCTTCACGTCGT		
qRT-PCR	*Zm* UVR8	Fw	GTTTGGGTGTCGCCGTC	123	Amplification of *Zm* UVR8
		Rv	CCTCTTCACGTCGTTCTCC		
	Zm Thr	Fw	GGACCAGAAGATTGCAGAAG	104	Thioredoxin- like, maize reference gene
		Rv	CAGCATAGACAGGAGCAATG		
	Zm CHS	Fw	AGCTCGATCGCTAGCTCCTC	196	Maize chalcone synthase
		Rv	TCCCCCTCGATCTTTCTCCC		
	At UVR8	Fw	CCAGATTTCGGGAGGTTGG	102	Amplification of *At* UVR8
		Rv	TTATTGCCGACTCCTACTTGTCC		
	At Actin2	Fw	GTATTGTGCTGGATTCTGGTG	119	*Arabidopsis* reference gene
		Rv	GAGGTAATCAGTAAGGTCACG		
	At CHS	Fw	CAGACAGGACATCGTGGTGGT	102	*Arabidopsis* chalcone synthase
		Rv	ACATGAGTGATCTTTGACTTGG		

2.4.2. *Zm*UVR8-eGFP was detected on immunoblot

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 with or without 3.47 $\mu mol.m^{-2}s^{-1}$ of UV-B were vacuum infiltrated with 5 $\mu g.\ mL^{-1}$ 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 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 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 darkness or white light (PAR: 100 $\mu mol.m^{-2}s^{-1}$) supplemented with or without 3.47 $\mu mol.m^{-2}\ s^{-1}$ UV-B. 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.

2.7. Bioinformatic analysis

Multiple sequence alignments were performed using MAFFT server (http://mafft.cbrc.jp/alignment/server/) and edited with GeneDoc [33].

3. Results

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

Several UVR8 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. In consequence, 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*. Fig. 1a shows the comparison among *Zm*UVR8 and the other UVR8 homologs cloned. *Zm*UVR8 has 443 amino acid 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 (Fig. 1a). Fig. 1b show that *Zm*UVR8 has the same domain profile that *At*UVR8, including the conserved triptophans, the C27 domain and the seven repeated RCC1 domains.

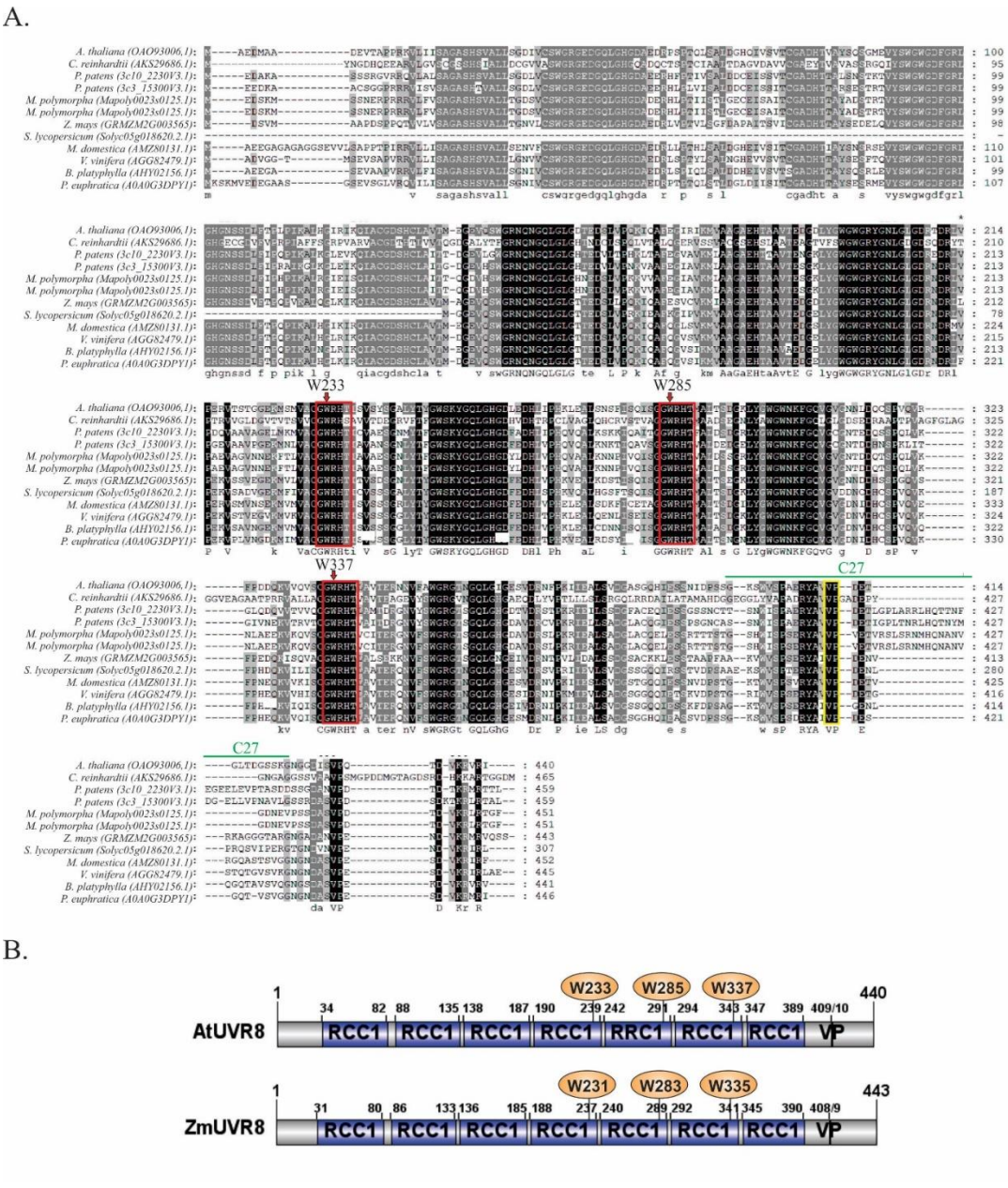

Fig. 1 *Zm*UVR8 sequence and structural domains. A. Multiple sequence alignment of *At*UVR8 homologs. Protein sequences that have been cloned and complemented *uvr8-1* mutant were aligned with MAFFT (http://mafft.cbrc.jp/alignment/server/) and edited with GeneDoc [33]. Red boxes indicate "GWRHT" motifs 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 minor identity. Yellow box show the "VP" domain in the C27 domain, important for interaction with COP1 and RUP proteins. **B)** Schematic representation of structural domains of the *At*UVR8 and *Zm*UVR8 proteins. Analysis of amino-acid sequences was performed in the National Center for Biotechnology Information (NCBI) database. RCC1 (pfam00415), Regulator of

chromosome condensation (RCC1) repeat. Arrows indicate tryptophans important in UV-B perception (W233, 285 and 337). VP: Valine-Proline domain.

Blastp analysis in Table 2 shows that all the components of *Arabidopsis* UVR8 signaling pathway are 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 *Zm*UVR8 should be a functional UV-B receptor, triggering a UVR8 signaling cascade in maize.

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

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

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 plants to immediately respond to UV-B exposure [15, 34]. UVR8 expression was analyzed in *Arabidopsis* and maize plants irradiated with 3.47 and 8.81 $\mu mol.m^{-2}\ s^{-1}$ of UV-B respectively. Fig. 2 shows quantitative RT-PCR analysis of UVR8 expression in irradiated and non- irradiated leaves. After 2h of UV-B irradiation, *At*UVR8 expression was reduced 3 fold and *ZmUVR8* expression was reduced 6 fold. However, CHS expression was increased in both species after UV-B. These indicate a similar regulation of UVR8 and CHS expression under UV-B treatment in *Arabidopsis* and maize plants.

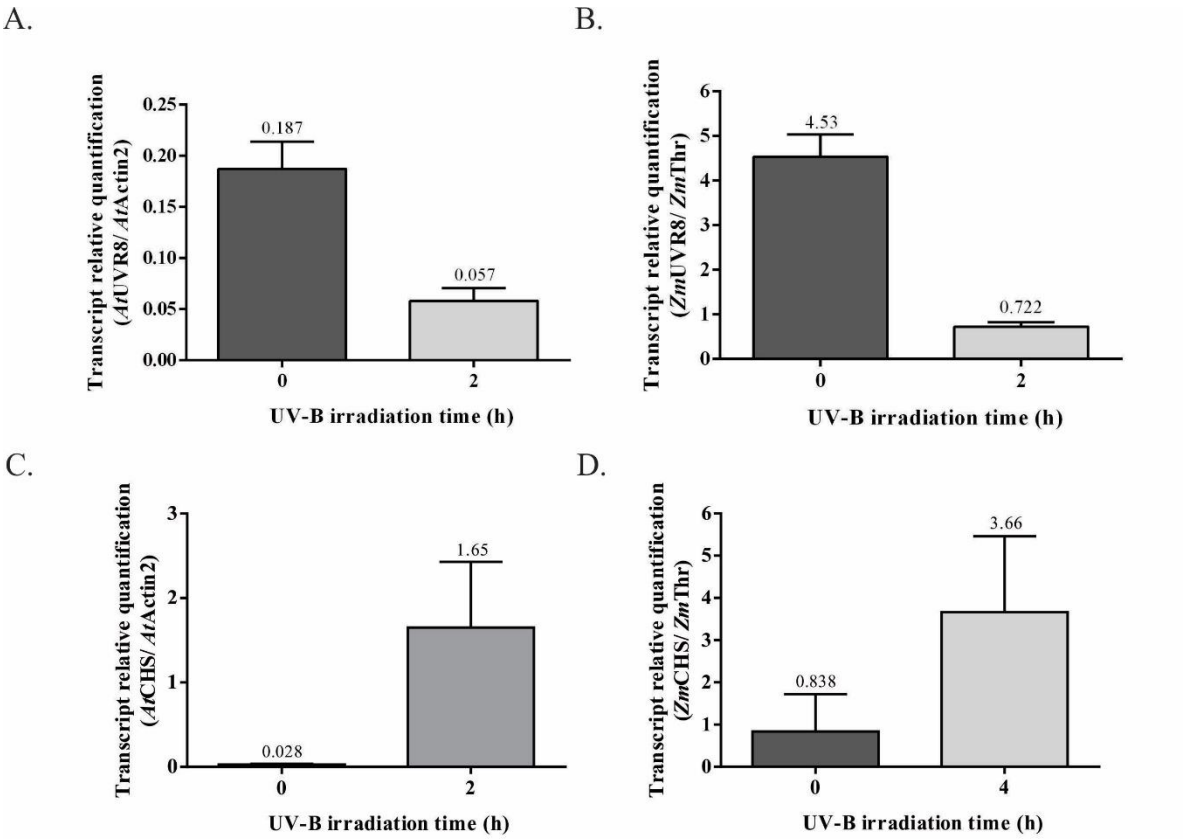

Fig. 2 Expression of UVR8 and CHS in response to UV-B in *Arabidopsis* and maize plants. UVR8 transcript level in *A. thaliana* **(A)** and maize **(B)** control leaves or treated for 2h with 3.47 and 8.81 µmol.m^{-2} s^{-1} of UV-B irradiation respectively were analyzed by qRT-PCR. Expression of Actin2 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.

Table 3 shows that UV-B has no effect on UVR8 expression in *M. polymorpha* and *V. vinifera* grapes. Moreover, UV-B upregulates transcript levels in different tissues from *M. domestica* and *B. platyphylla*. These findings 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
Arabidopsis thaliana	Leaf	Downregulated after 2h of treatment	3.47 $\mu mol\ m^{-2}\ s^{-1}$	Figure 2
Chlamydomonas reinhardtii	nd			Tilbrook et. al., 2016
Physcomitrella patens	nd			Soriano et. al., 2018
Marchantia polymorpha	Plant	No significant effect of treatment after 3	3 $\mu mol\ m^{-2}\ s^{-1}$	Soriano et. al., 2018 Kondou et. al., 2019
Zea mays	Leaf	Downregulated after 2h of treatment	8.81 $\mu mol\ m^{-2}\ s^{-1}$	Figure 2
Solanum lycopersicum	nd			Li et. al., 2018
Malus domestica	Fruit skin	Upregulation after 6h of treatment	1.5 $\mu mol\ m^{-2}\ s^{-1}$	Zhao et. al, 2016
	Apple callus	Upregulation after 1h of treatment	1.5 $\mu mol\ m^{-2}\ s^{-1}$	
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 $\mu mol\ m^{-2}\ s^{-1}$	Li et. al., 2018
Populus euphratica	nd			Mao et. al., 2015

Nd: not determined.

3.3. Expression of *Zm*UVR8 in the *uvr8-1* null mutant

To get 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::*Zm*UVR8-eGFP). This construct was used to transform *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. Transgene expression was determined by quantitative RT-PCR. Fig. 3a shows that both lines expressed *ZmUVR8* mRNA, with line #6.5 having the highest level. However, immunoblot with anti-GFP antibody in Fig 3b shows that *Zm*UVR8-eGFP was present in both transgenic lines in similar amount.

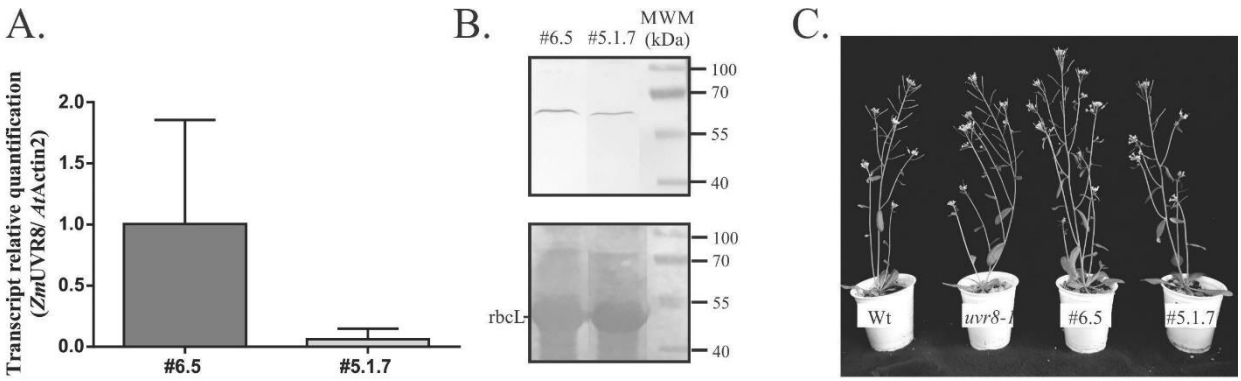

Fig. 3 35S::*Zm*UVR8-eGFP expression analysis in two *Arabidopsis* homozygous lines. A. *Zm*UVR8 transgene transcript level was analyzed by qRT-PCR in transgenic *A. thaliana* leaves. Expression of Actin2 was used for normalization. Primers used are listed in Table 1. Error bars indicate the standard deviation 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 (upper panel). Inmunodetection 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.

*Zm*UVR8-eGFP migrates 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). Fig. 3c shows no evident differences between phenotypes of transgenic lines with WT and *uvr8-1* after 30 days.

The sub-cellular localization of *Zm*UVR8-eGFP, was analyzed using a confocal laser scanning microscope. Hoechst 33342 staining was used to nuclear staining. Fig. 4 shows that *Zm*UVR8-eGFP fusion is mainly localized in nuclei of #6.5 and #5.1.7 lines, irrespective of UV-B treatments.

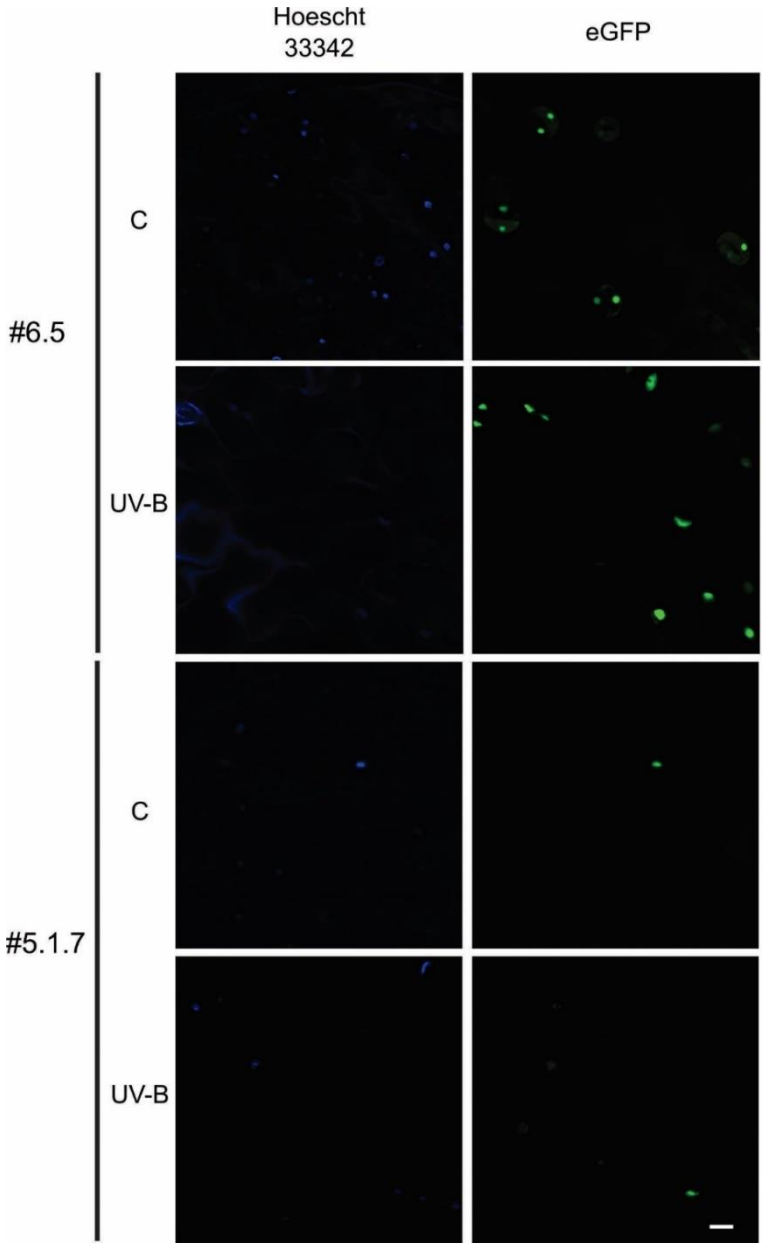

Fig. 4 Subcellular localization of eGFP-*Zm*UVR8. Cellular localization of *Zm*UVR8-eGFP fusions revealed by confocal laser microscopy. Non-irradiated control (C) *Arabidopsis* plants or irradiated with white light + 3.47 µmol.m^{-2}s^{-1} UV-B for 1 h, were vacuum infiltrated with Hoechst 33342 which 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 *Zm*UVR8-eGFP.

3.4. *Zm*UVR8 complements the *Arabidopsis* UVR8 null mutant

The hypocotyl length inhibition is frequently used to analyze to investigate the photoreceptors functionality in *Arabidopsis*. UV-B suppresses hypocotyl growth in wild-type *Arabidopsis* plants whereas the *uvr8-1* mutant is impaired in the response. Expression of WT UVR8 fused to GFP or yellow fluorescent protein (YFP) in *uvr8-1* plants functionally complements the mutant phenotype [12, 15, 35]. Fig. 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 WT *Arabidopsis* plants whereas *uvr8-1* has no response. Fig. 5 also shows that hypocotyl elongation was reduced 20% in line #6.5 and 15% (similar to WT) in line #5.1.7 indicating that *Zm*UVR8 is a positive regulator in UV-B induced photomorphogenesis.

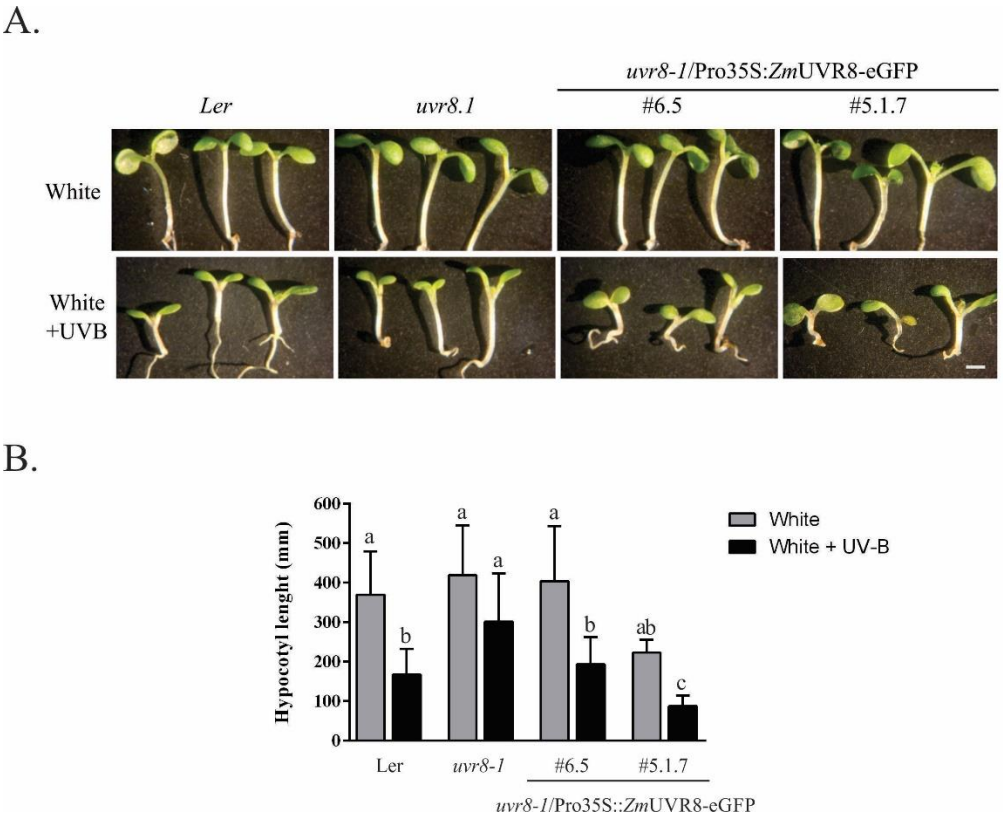

Fig. 5 Functional complementation assay of eGFP-*Zm*UVR8 expressed in *Arabidopsis* uvr8-1 mutant. A. Phenotypes of the WT, *uvr8-1* null mutant and transgenic lines grown under white or UV-B-light conditions. The scale bar represents 1mm. Photographs were taken after treatments and the hypocotyl lengths were measured using the ImageJ software (http://rsb.info.nih.gov/ij). **B.** Comparison of the hypocotyl length of WT, uvr8-1 mutant and transgenic lines (#6.5 and #5.1.7 in uvr8-1 background) of *Arabidopsis* seedlings grown under withe and UV-B supplemented conditions. Grey bars: -UV-

B, black bars: +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). Three independent biological replicates were performed, using al least 10 seedlings for each replicate.

*3.5. Zm*UVR8 promotes CHS and HY5 gene expression in *uvr8-1 Arabidopsis* mutant

In response to UV-B, plants increase the concentration of phenolic compounds involved in UV-B protection such as flavonoids. The enzyme CHS catalyzes the first step of flavonoid biosynthesis and is regulated by the UVR8 signaling pathway [36, 37]. UVR8-COP1-SPA complexes induce the expression of the transcription factor HY5 which is a positive regulator of CHS gene expression [36]. In order to determine if *Zm*UVR8 is able to induce CHS and HY5 gene expression under UV-B irradiation, three week old Ler, *uvr8-1* and #6.5 transgenic line plants were irradiated with white light supplemented with UV-B for 2 hours. CHS and HY5 gene expression was determined by quantitative RT-PCR. Transgenic line #6.5 was chosen for analysis of CHS and HY5 gene expression. CHS and HY5 gene expression was then determined by quantitative RT-PCR. Figure 6 shows that under white light irradiation, CHS and HY5 have low basal expression in all plants. After UV-B irradiation, the expression of both genes increased in WT plants. However, the uvr8-1 mutant was impaired in this response. UV-B-irradiated line #6.5 increased CHS and HY5 expression. These results, demonstrate that *Zm*UVR8 is able to induce UVR8 signaling in response to UV-B.

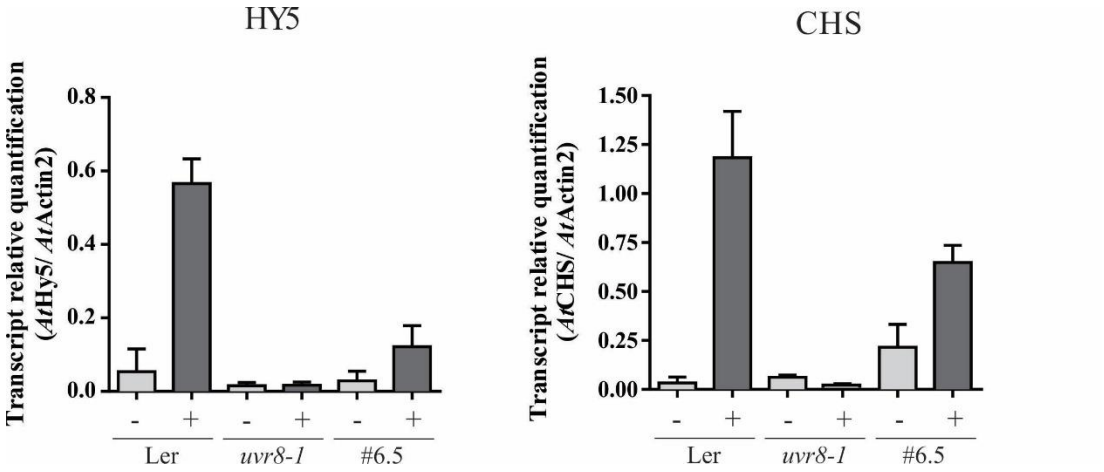

Fig. 6 Expression levels of CHS and HY5 in response to UV-B in WT, *uvr8-1* mutant and transgenic line #6.5 under UV-B irradiation. Three week old WT, *uvr8-1* and #6.5 transgenic line plants were exposed to

with light supplemented with 3.47 μmol.m^{-2} s^{-1} of UV-B for 2 h and the expression of CHS and HY5 was determined by qRT-PCR. Expression of Actin2 was used for normalization. Error bars indicate the standard deviation of at least two biological replicates.

4. Discussion

In this work, we have cloned a maize functional homologue of the *Arabidopsis* UV-B receptor, named as *Zm*UVR8, the first UVR8 protein from monocots. Previously, two maize UV-B-responsive genes were reported as *UVR8* homologs by comparison with rice genome by Casati et. al. in 2011 [25, 26]. However, these genes have low homology with *At*UVR8. We have found *Zm*UVR8 after maize sequence was recently completed. *Zm*UVR8 has a conserved sequence and modular structure, in concordance with several UVR8 homologs [10].

In this work, we have shown that *ZmUVR8* expression is regulated by UV-B (Fig. 2). Although *UVR8* was reported as constitutive in *Arabidospsis*, we show here that *UVR8* transcript decreased both in *Arabidopsis* and maize leaves after 2 hours of UV-B radiation (Fig. 2). That is similar to *Betula platyphylla* UVR8 (*BpUVR8*) expression, which is induced by UV-B, and decreased after 9h of continuous irradiation [38]. On the other hand, *UVR8* expression is increased by UV-B in the hypocotyls of radish sprouts [39]. Variations in UVR8 expression were also reported in UV-B treated fruit skin and apple callus [40]. Differences observed in UVR8 expression in the literature, could be due to the different UV-B intensities used in the experiments and tissues analyzed.

*At*UVR8 is a stable protein [41]. 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 [42].

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 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*. Cryptochrome 1 (Cry1) mutant *hy4* shows reduced *UVR8* expression in response to UV-B, suggesting a linkage between cry1, *UVR8* and UV-B [43]. Besides, it has been observed that *ZmUVR8* expression is increased by waterlogged in non-UV-B-irradiated maize root [44].

We obtained two independent homozygous *Arabidopsis* lines (#6.5 and #5.1.7), where *Zm*UVR8- eGFP complement the *uvr8-1* null mutant. Although *ZmUVR8* had different expression in both lines, the amount of this protein was similar (Fig. 3). *Arabidopsis* GFP-UVR8 shows increased nuclear localization following UV-B treatment of plants [45], but *Zm*UVR8- eGFP was constitutively located in nuclei in #6.5 and #5.1.7 lines (Fig. 4).

No obvious nuclear localization signal (NLS) is found in UVR8, and there is no consensus about the mechanism of UVR8 translocation [14]. Moreover, it was proved that the presence of UVR8 in the nucleus is required but not sufficient for its function. Kaiserli & Jenkins [45] 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 [46]. The hypocotyl growth of the *uvr8* mutant seedlings, in stark contrast to wild-type seedlings, was not inhibited by UV-B [15]. The restoring of this morphogenic response was established as a parameter of complementation by functional UVR8 homologs [38, 40, 47-52].

*Zm*UVR8 complement the impaired UV-B response of the *uvr8-1* mutant with respect to hypocotyl growth suppression (Fig. 5) and CHS and HY5 gene expression (Fig. 6), 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.

Thus, the identification of homologs of components of the UVR8 pathway in maize and the ability of *Zm*UVR8 to complement *Arabidopsis* uvr8 mutant indicate that a functional and canonical UVR8 pathway could be present in monocotyledons.

5. Conclusion

We identified the *ZmUVR8* gene from *Zea mays* (*Zm*UVR8) using multiple sequence comparison analysis. *ZmUVR8* gene expression is modulated by UV-B. The *Zm*UVR8

protein is highly conserved and has shown to be a functional homolog of the *Arabidopsis* UV-B receptor *At*UVR8.

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.

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Conflict of interest

The authors declare that they have no conflict of interest.

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