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Molecular cloning and functional analysis of the UV-B photoreceptor gene *Zm*UVR8 (UVR8 response locus), from the monocot *Zea mays*.

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Key message

We identified the *ZmUVR8* gene from maize. Its expression was downregulated by UV-B. The *Zm*UVR8 protein was highly conserved and is a functional homolog of the *Arabidopsis* UV-B receptor *At*UVR8.

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 proceses. Although UVR8 sequence is conserved, few homologs have 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 (*Zm*UVR8). *Zm*UVR8 has 73% of identity to *At*UVR8. Key trypthophan responsible of UV-B perception are conserved, as well as the VP domain in the C27 region, involved in the interaction with the proteins COP1 and RUP1. Blastp analysis shows that all the components of *Arabidopsis* UVR8 pathway are present in maize. UVR8 was expressed in non-irradiated *Arabidopsis* and maize plants. However, after 2h of UV-B irradiation, UVR8 expression was reduced 3 and 6-fold respectively. This indicates a similar regulation of expression under UV-B treatment in both species. *Arabidopsis* uvr8-1 null mutant was complemented with *Zm*UVR8 driven by the CaMV-35S promoter and fused to eGFP. Whereas UV-B stimulates GFP-UVR8 nuclear accumulation in *Arabidopsis*, *Zm*UVR8-eGFP fusion was mainly localized in 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 wt *Arabidopsis* plants whereas uvr8-1 was impaired in this response. Hypocotyl elongation was reduced in complementary lines. These results confirmed that *Zm*UVR8 is similar enough to *At*UVR8 to restore UV-B perception and signaling in *Arabidopsis* being a functional UV-B photoreceptor.

Keywords

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

Introduction

Ultraviolet-B (UV-B) radiation is present in sunlight (280- 315 nm). High doses of this radiation damage macromolecules, including DNA, and induce the production of reactive oxygen species (ROS), affecting cell integrity and viability (Brosché and Strid 2003; Frohnmeyer and Staiger 2003; Jordan 1996).

Since UV-B penetration in the water column is lower than in terrestrial environments (Rozema et al. 2002), a mechanism to avoid UV-B damage has evolved during the transition of aquatic to land plants. 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 (Tilbrook et al. 2013).

The levels of UV radiation on the Archean Earth were several orders of magnitude higher than the current level (Cnossen et al. 2007). Cyanobacteria and various eukaryotic algae, which are ancient photosynthetic organisms, had mycosporine-like amino acids (MAAs) as UV-B protectors (Llewellyn and Airs 2010; Rastogi and Incharoensakdi 2013; Rozema et al. 2002). Land plants co-evolved with ambient UV-B levels, increasing the complexity of UV-B absorbing polyphenolic from algae to higher plants (Rozema et al. 2002).

Low UV-B fluence is, in contrast, a signaling stimulus that regulates various metabolic and developmental processes and induces a photomorphogenic response. Thus the specific UV-B receptor UV-B response locus 8 (UVR8) was identified mediating plant responses to UV-B (Brown et al. 2005). 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 (O'Hara and Jenkins 2012; Ulm and Jenkins 2015). 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, stomatal opening, leaf development and downward leaf curling (for a review see Yin and Ulm 2017 and references therein).

Several proteins participate in UVR8 signaling cascade. Since its discovery in 2005 (Brown et al. 2005), 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) (Favory et al. 2009). The transcription factor WRKY36 (WRKY DNA-BINDING PROTEIN 36) repress 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 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 (Heijde and Ulm 2012). Two upregulated 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 (Heijde and Ulm 2012; Ulm and Jenkins 2015). UVR8 also interact with BES1 and BIM1, inhibiting the brassinosteroid responsive genes, reducing the hypocotyl elongation (Sun and Zhu 2018).

Arabidopsis UVR8 (*At*UVR8) crystal structure has been determined at high resolution (Wu et al. 2012) (Christie et al. 2012; Zeng et al. 2015). *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) (Yang et al. 2018). *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 (Christie et al. 2012; Wu, et al. 2012; Zeng, et al. 2015). Also, W233 is important both in photoreception and in maintaining exciton coupling, whereas W337 plays an auxiliary role (Christie et al. 2012; Wu, et al. 2012). The "GWRHT" motif from blade 6 may be the most important because it contains W285. This motif is conserved in all UVR8 homologs analyzed (Fernandez et al. 2016).

Although UVR8 is conserved, and sequences for this gene are found in all the viridiplantae (Fernandez et al. 2016), 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 molecular cloning, sequence and functional complementation of *Zm*UVR8, 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 (Cloix et al. 2012), 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.

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 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 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) (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). 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.

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 µmol.m-2s-1) supplemented with 3.47 µmol.m-2s-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 µmol.m-2s-1) supplemented with 8.81 µmol.m-2s-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 *Zm*UVR8- 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 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 samples as previously reported (Casati and Walbot 2004) .

For protein assays, leaves were harvested into liquid nitrogen and proteins extracted in buffer KPO4 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
		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		

*Zm*UVR8- eGFP Subcellular Localization

Fifhteen day old plants irradiated 1h with white light with or whitouth 3.47 µmol.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 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 µmol.m^{-2}s^{-1}) supplemented with or without 3.47 µmol.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.

Bioinformatic analysis

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

Results

An *At*UVR8 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. 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 trypthophan 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.

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

***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 (Favory, et al. 2009; Rizzini, et al. 2011). 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. 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. That indicate a similar regulation of *UVR8* expression under UV-B treatment in *Arabidopsis* and maize plants. Table 3 shows that UV-B has no effect on *UVR8* expression in *M. polymorpha*. Moreover, UV-B upregulates transcript levels in *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	Dose	Reference
Arabidopsis thaliana	Leaf	Downregulated after 2h of treatment	3.47 µmol 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 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			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			Mao et. al., 2015

Nd: not determined.

Expression of *Zm*UVR8 in the uvr8 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. We obtained two independent T3 and T4 homocygous lines (#6.5 and #5.1.7 respectively).

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. *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. Fig. 4 shows that *Zm*UVR8-eGFP localization differs from that of *At*GFP-UVR8 in *Arabidopsis*. Whereas UV-B stimulates *At*GFP-UVR8 nuclear accumulation in *Arabidopsis* (Kaiserli and Jenkins 2007), *Zm*UVR8-eGFP fusion is 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 (Soriano et al. 2018).

_Zm_UVR8 complements the _Arabidopsis_ UVR8 null mutant

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 the uvr8-1 mutant is impaired in the response. **E**xpression of wt UVR8 fused to GFP or yellow fluorescent protein (YFP) in uvr8-1 plants functionally complements the mutant phenotype (Favory et al. 2009; Huang et al. 2014; O'Hara and Jenkins 2012). 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* is impaired in this 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 in *Arabidopsis*. 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.

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.

Two maize UV-B-responsive genes were reported as *UVR8* homologs by comparation with rice genome by Casati et. al. in 2011 (Casati et al. 2011; Casati et al. 2011). 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 (Fernandez et al. 2016).

ZmUVR8 expression is regulated by UV-B. 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. That is similar to *Betula platyphylla* UVR8 (*BpUVR8*) expression, which is induced by UV-B, and decreased after 9h of continuous irradiation (Li et al. 2018). 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 be due to the different UV-B intensities used in the experiments and tissues analyzed.

*At*UVR8 is a stable protein (Heilmann and Jenkins 2013). 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 (Ren et al. 2019).

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 (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 *Zm*UVR8- 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 (Kaiserli and Jenkins 2007), but *Zm*UVR8- 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. (Soriano et al. 2018), that generated two lines of *Arabidopsis* uvr8-1 complemented with *Marchantia polymorpha* (GFP-MpUVR8). In both lines, UVR8 is constitutively expressed in nuclei. In the same article, authors show in one line of *Arabidopsis* uvr8-1 complemented with *Physcomitrella patens,* GFP-PpUVR8 is also localised in nuclei in the absence of UV-B. Interestingly, both proteins are functional after UV-B irradiation and able to complement the *At*UVR8-1 mutant. There is no information about localization of UVR8 homologs in *Arabidopsis* uvr8 complemented lines.

No obvious nuclear localization signal (NLS) is found in UVR8, and there is no consensus about the mechanism of UVR8 translocation (Yin 2017). Moreover, it was proved that the presence of UVR8 in the nucleus is required but not sufficient for its function. Kaiserli & Jenkins (Kaiserli and Jenkins 2007) 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 (Kim et al. 1998). The hypocotyl growth of the *uvr8* 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 (Li et al. 2018; Liu et al. 2014; Mao et al. 2015; Soriano et al. 2018; Tilbrook et al. 2016; Zhao et al. 2016).

*Zm*UVR8 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, 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 maize.

Conclusion

We identified the *ZmUVR8* gene from *Zea mays* (*Zm*UVR8) using multiple sequence comparison analysis. *ZmUVR8* gene expression was modulated by UV-B. The *Zm*UVR8 protein was highly conserved and has shown to be a functional homolog of the *Arabidopsis* UV-B receptor *At*UVR8.

Author Contributions

MF performed 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.

Conflict of interest

The authors declare that they have no conflict of interest.

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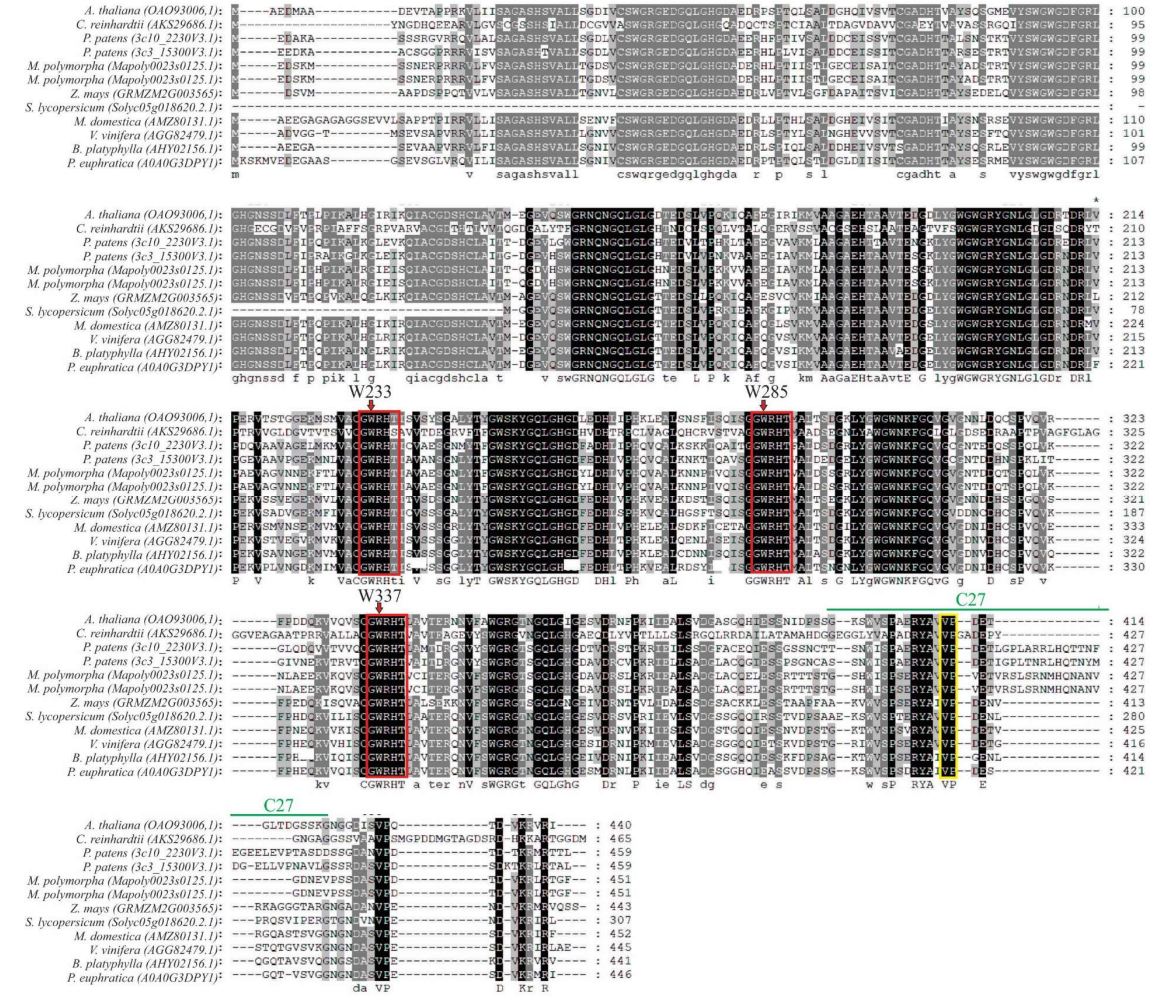
Figures

A.



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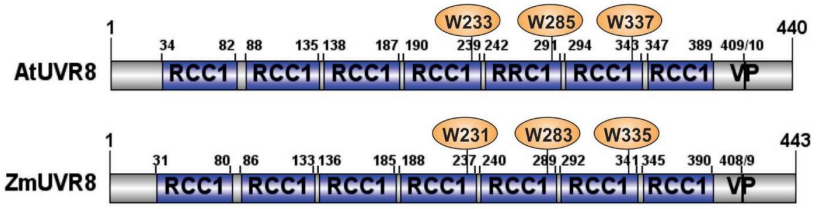


Fig. 1 *Zm*UVR8 sequence and structural domains. A. Multiple sequence alignment of *At*UVR8 homologs. Protein sequences that have been cloned and complemented *uvr8-1* mutant were aligned with MAFFT (http://mafft.cbrc.jp/alignment/server/) and edited with GeneDoc (Nicholas and Nicholas 1997). Red boxes 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 minor indentity. 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 *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 tryphtophans important in UV-B perception (W233, 285 and 337). VP: Valine-Proline domain.

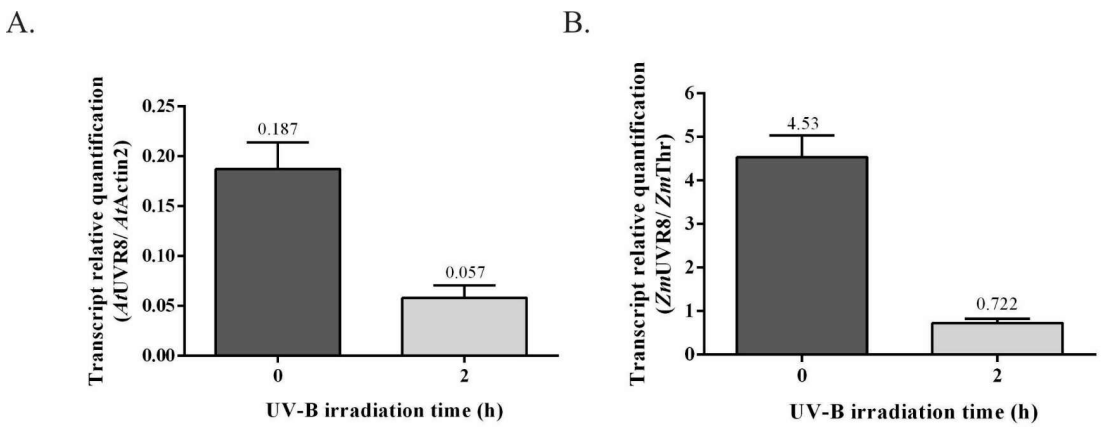


Fig. 2 Expression of *At*UVR8 and *Zm*UVR8 in response to UV-B. UVR8 transcript level in *A. thaliana* **(A)** and maize **(B)** control leaves or treated for 2h with 3.47 and 8.81 µmol.m^{-2} $^{s-1}$ of UV-B irradiation respectively were analyzed by qRT-PCR. Expression of Actin2 and thioredoxin- like (Thr) were used for *Arabidopsis* and maize normalization respectively. Primers are listed in Table 1. Error bars indicate the standard desviation over three biological replicates, with each replicate containing two technical replicates.

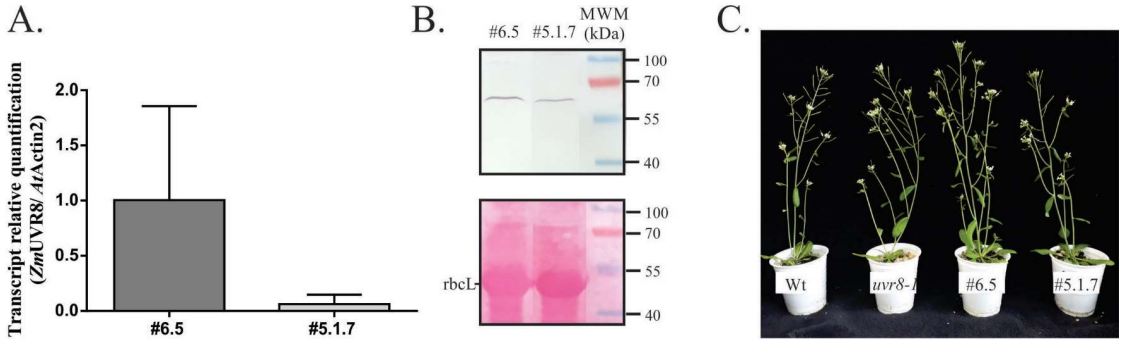


Fig. 3 35S::*Zm*UVR8-eGFP expression analysis in two *Arabidopsis* homozygous lines. A. *Zm*UVR8 transgene transcript level was analyzed by qRT-PCR in transgenic *A. thaliana* leaves. Expression of Actin2 was used for normalization. Primers used are listed in Table 1. Error bars indicate the standard 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 (upper panel). Inmunodetection was performed using anti-GFP antiserum. Stained Rubisco large subunit (rbcL) is shown as a loading control (lower panel). **C.** Thirty day old wt, uvr8-1 mutant and #6.5 and #5.1.7 transgenic *Arabidopsis* lines.

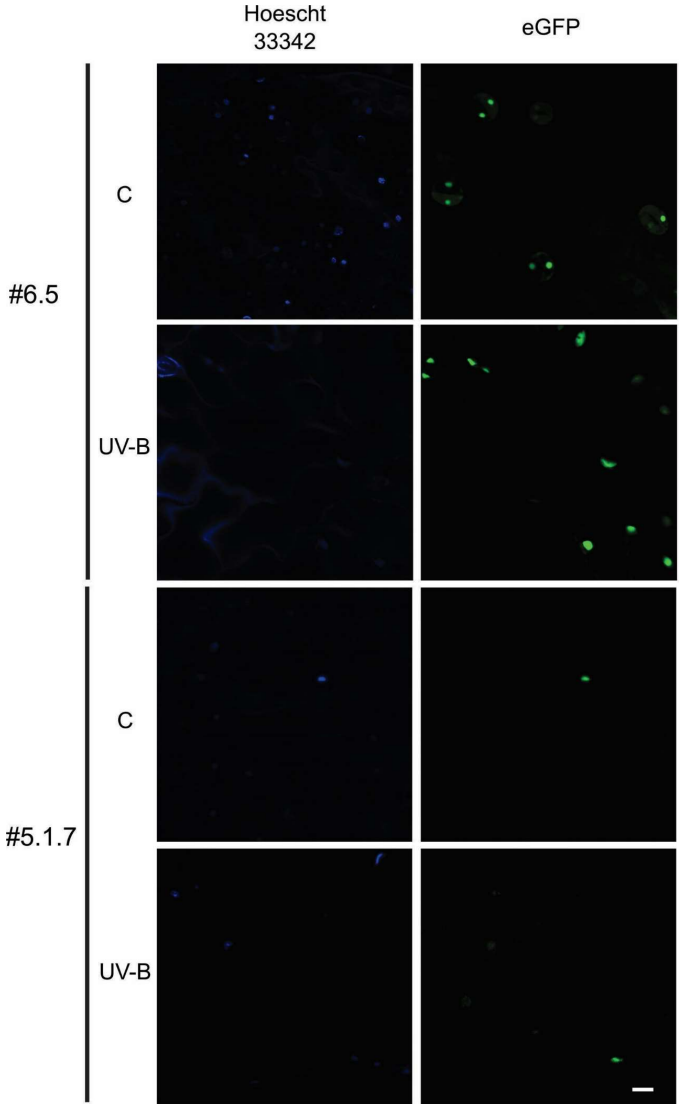
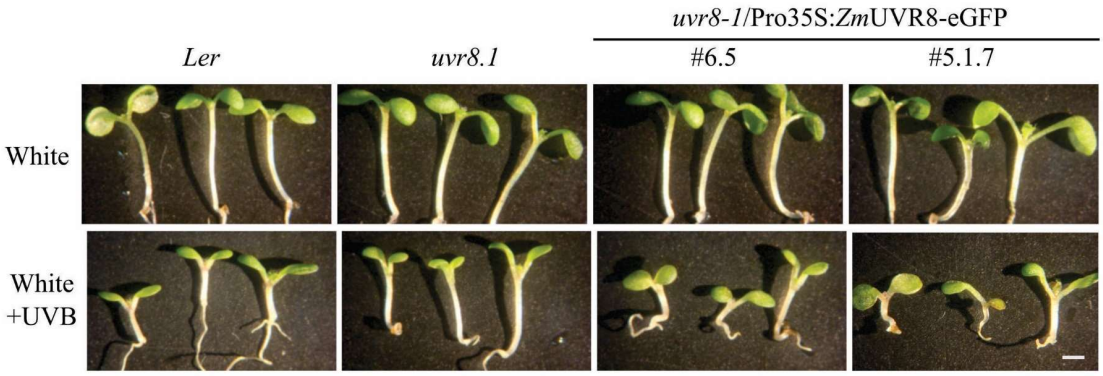


Fig. 4 Subcellular localization of eGFP-*Zm*UVR8. Cellular localization of *Zm*UVR8-eGFP fusions revealed by confocal laser microscopy. Non-irradiated control (C) plants or irradiated with white light + 3.47 µmol.m^{-2}s^{-1} UV-B for 1 h, were vacuum infiltrated with Hoescht 33342 which specifically 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 collected at 440/50 nm. The same microscope settings for GFP and Hoescht 33342 detection were used before and after UV-B illumination. Bar, 25 µm.

A.



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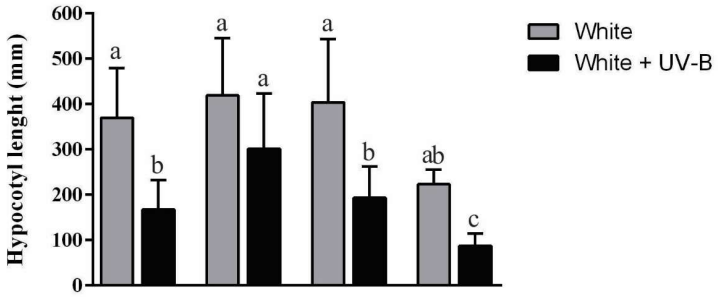

Fig. 5 Functional complementation assay of eGFP-*Zm*UVR8 expressed in *Arabidopsis* uvr8-1 mutant. A. Phenotypes of the WT, uvr8-1 null mutant and transgenic lines grown under white or UV-B-light conditions. The scale bar represents 1mm. Photographs were taken after treatments and the hypocotyl lengths 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). At least three independent biological replicates were performed for all experiments.