Identification and Expression Analysis of the UV-B Photoreceptor UVR8 Gene in Apocynum venetum and Apocynum cannabinum Postprint

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Abstract

In the process of plant response to Ultraviolet-B (UV-B), the UV-B photoreceptor UVR8 (UV Resistance Locus 8) plays a crucial regulatory role in plant photomorphogenesis and growth metabolism. To investigate the UV-B photoreceptor information in Apocynum plants, this study conducted screening and bioinformatics analysis of the UV-B photoreceptor UVR8 based on whole-genome data of Apocynum venetum and A. cannabinum, and transcriptome data were employed to analyze the expression patterns of UVR8 genes under UV-B stress treatment. The results demonstrated that: (1) A. venetum has 6 UVR8 genes, while A. cannabinum has 5 UVR8 genes, with the former distributed on chromosomes 1, 7, 9, and 11, and the latter on chromosomes 1, 8, and 9. (2) UVR8 proteins are hydrophilic unstable proteins localized in the nucleus, lacking transmembrane structures and signal peptides. The secondary structure is mainly composed of extended strands, random coils, α-helices, and β-turns. The tertiary structures of AvUVR8b and AcUVR8a proteins are most similar to Arabidopsis UVR8 (AtUVR8), and they have the closest phylogenetic relationship with Coffea arabica (CaUVR8) and Coffea eugenioides (CeUVR8). This study found that the gene and protein structures of A. venetum AvUVR8b and A. cannabinum AcUVR8a are highly similar to those of AtUVR8. (3) When both Apocynum plants were treated with a certain dose of UV-B (17.52 kJ·m-2·d-1), the expression levels of AvUVR8b and AcUVR8a were upregulated. Based on this, it is speculated that AvUVR8b plays a major role in A. venetum, and AcUVR8a plays a major role in A. cannabinum in response to UV-B. (4) Analysis of cis-acting elements showed that UVR8 expression is regulated by factors such as light, temperature, water, oxygen, and hormones. This study will lay a foundation for further research on the gene function of UVR8 in Apocynum and provide clues for elucidating the molecular mechanisms of Apocynum plants' adaptation to UV-B.

Full Text

Identification and Expression Analysis of UV-B Photoreceptor UVR8 Genes in *Apocynum venetum* and *A. cannabinum*

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Abstract

The UV-B photoreceptor UVR8 (UV Resistance Locus 8) plays a crucial regulatory role in plant photomorphogenesis, growth, and metabolic processes during plant responses to ultraviolet-B (UV-B) radiation. To investigate UV-B photoreceptor information in *Apocynum* plants, this study screened and performed bioinformatic analysis of UV-B photoreceptor UVR8 using whole-genome data from *Apocynum venetum* and *A. cannabinum*, while analyzing UVR8 gene expression patterns under UV-B stress treatment using transcriptome data. The results showed: (1) *A. venetum* contains six UVR8 genes and *A. cannabinum* contains five, distributed on chromosomes 1, 7, 9, and 11 in the former and chromosomes 1, 8, and 9 in the latter. (2) UVR8 proteins are hydrophilic, unstable proteins localized in the nucleus without transmembrane structures or signal peptides. Secondary structures consist primarily of extended strands, random coils, α-helices, and β-turns. The tertiary structures of AvUVR8b and AcUVR8a proteins are most similar to *Arabidopsis* UVR8 (AtUVR8) and show the closest phylogenetic relationship with *Coffea arabica* (CaUVR8) and *C. eugenioides* (CeUVR8). This study found that the gene and protein structures of *A. venetum* AvUVR8b and *A. cannabinum* AcUVR8a are highly similar to those of AtUVR8. (3) When both *Apocynum* species were treated with a specific UV-B dose (17.52 $kJ \cdot m^{-2} \cdot d^{-1}$), the expression levels of AvUVR8b and AcUVR8a were upregulated. Based on these findings, we speculate that AvUVR8b plays a primary role in UV-B response in *A. venetum*, while AcUVR8a plays a primary role in *A. cannabinum*. (4) Cis-acting element analysis revealed that UVR8 expression is regulated by light, temperature, moisture, oxygen, and hormones. This study provides a foundation for further research on UVR8 gene function in *Apocynum* and offers insights into the molecular mechanisms underlying UV-B adaptation in *Apocynum* plants.

Keywords: *Apocynum venetum*, *A. cannabinum*, UVR8 gene, expression analysis, bioinformatic analysis

Introduction

Sunlight serves not only as an energy source for photosynthesis but also as a crucial environmental factor regulating plant growth, circadian rhythms, and metabolite synthesis (Frohnmeyer et al., 2003; Jenkins, 2014a,b). As a component of solar radiation, ultraviolet (UV) light is classified by wavelength into long-wave UV-A (320–400 nm), medium-wave UV-B (280–320 nm), and short-wave UV-C (100–280 nm) (Liu et al., 2012; Chen et al., 2021). While UV-A reaches the Earth's surface directly without causing significant biological effects, and UV-C is largely absorbed by the atmosphere before reaching the surface due to its short wavelength and poor penetration, UV-B is mostly absorbed by the ozone layer and exhibits dual effects as biologically effective radiation. High-intensity UV-B acts as a stress factor that damages biomolecules such as DNA, proteins, and lipids, potentially causing plant death, whereas low-intensity UV-B serves as a signaling regulator that plays important roles in plant photomorphogenesis and metabolic processes (Frohnmeyer, 2003; Shamala et al., 2020).

The UV-B photoreceptor UVR8 was first identified in 2002 through screening of UV-B hypersensitive *Arabidopsis* mutants (uvr8-1) (Kliebenstein et al., 2002) and was confirmed in 2011 as a specific UV-B photoreceptor (Rizzini et al., 2011). Current research on UVR8 structure and function has been conducted primarily in *Arabidopsis*, revealing that UVR8 protein forms a homodimer structure linked by salt bridges, with each monomer consisting of seven β-propeller blades arranged longitudinally in a ring structure formed by seven blade-like structures (Christie et al., 2012; Bao, 2016). Highly conserved UVR8 tryptophan residues (W) maintain protein structural stability and function in UV-B signal reception and transduction (Jenkins, 2014a; Zhang et al., 2019; Li et al., 2020), with AtUVR8 directly receiving UV-B through W233 and W258 without requiring other cofactors as chromophores (Rizzini, 2011; O' Hara et al., 2012; Jenkins, 2014b; Yang et al., 2018). In the absence of UV-B irradiation, UVR8 exists as a dimer in the cytoplasm; upon UV-B irradiation, salt bridges break to form monomers that translocate to the nucleus (Wu et al., 2012) and form a UVR8-COP1-SPA complex dissociated from the CUL4-DDB1 (cullin4-damaged DNA binding protein 1) E3 ubiquitin ligase (Rizzini, 2011; Huang et al., 2013; Vanesa et al., 2019), thereby reducing COP1-mediated degradation of HY5 (Long Hypocotyl 5) (Huang, 2013) while promoting expression of transcription factors such as HY5, HYH (HY5 Homolog), and MYB, which stimulate transcription of enzyme genes involved in flavonoid synthesis (Hartmann et al., 2005; Qian, 2019; Shamala, 2020; Ling et al., 2021). When UVR8-mediated downstream genes are overexpressed, a negative feedback mechanism is activated, such as inducing transcription of RUP1 (repressor of UV-B photomorphogenesis 1)/RUP2 and STO/BBX24 (Salt Tolerance/BBX24) (Jenkins, 2014b; Parihar et al., 2015; Li et al., 2015). UVR8 interacts with RUP1/RUP2 to promote its dimerization (Cloix et al., 2012; Hideg et al., 2013), enabling

timely response to UV-B light signals.

Apocynum venetum and *A. cannabinum* are perennial herbs or subshrubs in the Apocynaceae family, exhibiting strong stress resistance to drought, salinity, and poor soil conditions (Wang et al., 2012). Known as the "king of wild fibers," their textiles offer breathability, warmth, antistatic properties, and UV protection. As medicinal plants, they can be used entirely for lowering blood pressure, lipids, and glucose while providing anti-aging effects (Li et al., 2018), and their leaves can be processed into health tea. Flavonoids are the main medicinal components in *Apocynum* plants (Zhang, 2021), with complex synthesis pathways influenced by both endogenous genes and environmental factors. Against the backdrop of global climate change, plants face increasingly severe UV stress, making investigation of plant UV-B response mechanisms and stress reactions extremely important. While partial studies on UV-B photoreceptor UVR8 structure and function have been conducted in green algae, alfalfa, soybean, and ginkgo, no reports exist on UVR8 research in *Apocynum* plants. Based on whole-genome data from both *Apocynum* species, this study screens UVR8 genes through bioinformatic analysis and investigates UVR8 gene expression patterns using transcriptome data to address: (1) UVR8 gene structure, cis-acting elements, and chromosomal location; (2) UVR8 conserved domains, protein structure, and physicochemical properties; (3) UVR8 phosphorylation sites and phylogenetic relationships; and (4) UVR8 gene expression patterns under UV-B stress. This research aims to provide deeper insights into UVR8 function and its role in UV-B response mechanisms and medicinal component synthesis in *Apocynum*.

1.1 Materials and Data Sources

UVR8 gene and protein sequences for both *Apocynum* species were obtained from whole-genome sequencing data generated previously in our laboratory (Song et al., 2019; Song, 2020). The AtUVR8 protein sequence (Protein: AT5G63860.1) was downloaded from the *Arabidopsis thaliana* database TAIR (https://www.arabidopsis.org/).

1.2.1 Screening of UVR8 Genes in Both *Apocynum* Species

Using whole-genome annotation results from IPRSCAN, KEGG, NR, and Swissport for both *Apocynum venetum* and *A. cannabinum*, we screened for gene and protein sequences annotated as UV-B photoreceptor UVR8. BioEdit software was used for analysis, with the AtUVR8 protein sequence as a seed sequence for local BLAST alignment (E-value $<$ 1e-10, Identity $\geq$ 30%) to select optimal UVR8 protein sequences. Pfam (https://pfam.xfam.org/search) and SMART (http://smart.embl-heidelberg.de/) software were then employed for domain verification, with redundant sequences removed to finally obtain UVR8 gene and

protein sequences for both species.

1.2.2 Chromosomal Localization of UVR8 Genes in Both *Apocynum* Species

Based on whole-genome annotation files for both species, positional information of UVR8 genes on chromosomes was obtained, and chromosomal localization maps were drawn online using MG2C software (Chao et al., 2021) (http://mg2c.iask.in/mg2c_{v2}.1/).

1.2.3 Analysis of Gene Structure and Conserved Domains of UVR8 Genes

Exon and intron positions for UVR8 genes were referenced from genome annotation gff3 files, and gene structures were analyzed using GSDS 2.0 (Hu et al., 2015) (http://gsds.gao-lab.org/index.php). Conserved protein domains were predicted using MEME software (Bailey et al., 2009) (http://meme-suite.org/tools/meme).

1.2.4 Analysis of Basic Physicochemical Properties of UVR8 Proteins

Online ExPASy software (Gasteiger et al., 2003) (https://web.expasy.org/cgi-bin/protparam/protpara) was used to analyze amino acid number, molecular weight, theoretical isoelectric point, instability index, and aliphatic amino acid index of UVR8 proteins.

1.2.5 Protein Structure Analysis and Subcellular Localization of UVR8 Proteins

SOPMA (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_{automat}.pl?page=npsa_{sopma}.html) was used to predict secondary structure, with Phyre2 (Kelley et al., 2015) (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index) analyzing tertiary structure using AtUVR8 protein as a template. Nuclear localization was predicted using Cell PLoc 2.0 (http://www.csbio.sjtu.edu.cn/bioinf/CellPLoc-2/) with Euk-PLoc 2.0.

1.2.6 Prediction of Transmembrane Structure, Signal Peptide, and Phosphorylation Sites in UVR8 Proteins

Transmembrane structure was analyzed using TMHMM 2.0 (http://www.cbs.dtu.dk/services/TMHMM/), signal peptide prediction was performed using SignalP 5.0 (https://services.healthtech.dtu.dk/service.php?Signa 5.0), and phosphorylation site types and numbers were analyzed using NetPhos 3.1 (http://www.cbs.dtu.dk/services/NetPhos/).

1.2.7 Analysis of Cis-Acting Elements in UVR8 Genes

TBtools (Chengjie et al., 2020) was used to obtain 2,000 bp upstream sequences of UVR8 genes as promoter sequences. PlantCARE (http://bioinformatics.psd.ugent.be/webtools/plantcare/ht was used for prediction, with TBtools drawing maps to analyze positions and quantities of major cis-elements.

1.2.8 Phylogenetic Analysis of UVR8 Proteins

NCBI BLASTn (https://www.ncbi.nlm.nih.gov/) was used for homology searches to obtain UVR8 gene sequences from both species. A phylogenetic tree was constructed using MEGA 11.0 software, with Clustal W for multiple sequence alignment and the Neighbor-Joining (NJ) method (bootstrap value set to 1,000, other parameters default). ITQL (https://itol.embl.de) was used for visualization.

1.2.9 Analysis of UVR8 Gene Expression

Both *Apocynum* species were potted in a greenhouse in spring 2021 (forest soil and nutrient soil mixed 1:1) with conventional field management. Based on Gao et al. (2019) classification of UV-B radiation doses and considering summer UV-B intensity in Yinchuan, Ningxia and the strong stress resistance of both species, natural light (containing UV-B intensity of approximately 8–11 $W \cdot m^{-2}$) was set as the control, with additional UV-B radiation treatment applied. For 30–40 cm tall plants, UV-B tubes (Philips TL 100W/01) were installed 0.5 m above the canopy, with treatments applied four times each at 10:00 AM and 2:00 PM daily, each lasting 10 minutes with 10-minute intervals. The increased UV-B radiation dose detected at the canopy was 17.52 $kJ \cdot m^{-2} \cdot d^{-1}$ at 3.65 $W \cdot m^{-2}$ (equivalent to a 33.2–45.6% increase in summer UV-B intensity in Yinchuan). A 0.1 mm cellulose acetate film covered the tubes to block UV-C below 280 nm, with UV-B intensity measured using a Lutron UV-340A radiometer. Samples of upper mature leaves were collected at 0, 0.5, 1, 4, and 7 days (d0, d0.5, d1, d4, d7) of UV-B treatment, with three biological replicates per sample stored at -80°C. Transcriptome data from our previous experiments (not yet published)

were used to analyze UVR8 gene expression, with TBtools used to construct expression heatmaps.

2 Results and Analysis

2.1 Screening of UVR8 Genes in Both *Apocynum* Species

From whole-genome sequencing data, we initially screened 10 UVR8 protein sequences from *A. venetum* and 14 from *A. cannabinum*. Using *Arabidopsis* AtUVR8 protein as a seed sequence for sequence alignment and online domain analysis, we finally obtained six UVR8 genes from *A. venetum* (AvUVR8a–f) and five from *A. cannabinum* (AcUVR8a–e) .

2.2 Chromosomal Localization of UVR8 Genes

Both *Apocynum* species have 11 chromosomes. MG2C software was used to draw chromosomal localization maps, showing no tandem duplication among UVR8 genes. AcUVR8 genes were unevenly distributed across three chromosomes, with AcUVR8e on chromosome 8 and two genes each on chromosomes 1 (AcUVR8b and AcUVR8c) and 9 (AcUVR8a and AcUVR8d). AvUVR8 genes were distributed across four chromosomes, with AvUVR8d and AvUVR8c on chromosomes 7 and 11, respectively, and two genes each on chromosomes 1 (AvUVR8f and AvUVR8a) and 9 (AvUVR8b and AvUVR8e) [Figure 1: see original paper].

2.3 Gene Structure and Conserved Motif Analysis

Analysis of gff3 annotation files using GSDS 2.0 revealed that all UVR8 genes contain upstream/downstream non-coding regions, introns, and exons, but differ in length and exon number [Figure 2: see original paper]. AvUVR8 genes ranged from 5,115–12,672 bp with 6–16 exons (AvUVR8c had the most at 16, AvUVR8a the fewest at 6). AcUVR8 genes ranged from 5,876–11,585 bp with 6–15 exons (AcUVR8d had the most at 15, AcUVR8b the fewest at 6). MEME analysis with seven motifs (default parameters) showed AvUVR8 proteins contained 4–7 motifs, with AvUVR8b, AvUVR8e, and AvUVR8f having the most (7) and AvUVR8c the fewest (4). AcUVR8 proteins contained 6–7 motifs, with AcUVR8b having the fewest (6) and the other four having 7. While UVR8 motifs differed within species, similarity existed between species, such as between AvUVR8a and AcUVR8b, AvUVR8b and AcUVR8a, AvUVR8e and AcUVR8d, and AvUVR8f and AcUVR8c in motif number, position, and amino acid composition [Figure 3: see original paper].

2.4 Physicochemical Properties of UVR8 Proteins

ExPASy prediction revealed AvUVR8 proteins contain 420–534 amino acids, with molecular weights of 45,846.10–56,827.20 Da and theoretical pI of 5.55–8.41 . AvUVR8c had the highest values (534 aa, 56,827.20 Da, pI 8.41), while AvUVR8d had the lowest (420 aa, 45,846.10 Da). All AvUVR8 proteins were unstable (instability index < 40), with AvUVR8b showing the highest instability (38.89) and AvUVR8d the lowest (31.12). Aliphatic indices ranged from 63.50–85.17, and grand average of hydropathicity values were -0.629 to -0.134 (<0), indicating hydrophilic proteins. Only AvUVR8a, AvUVR8b, and AvUVR8f had pI < 7 (acidic proteins), while the other three were basic.

AcUVR8 proteins contained 388–488 amino acids, with molecular weights of 41,778.96–53,233.19 Da and pI of 5.42–8.08. AcUVR8b had the highest values (488 aa, 53,233.19 Da, pI 8.08), while AcUVR8e had the lowest (388 aa, 41,778.96 Da, pI 5.42). All AcUVR8 proteins were unstable (instability index < 40), with AcUVR8a showing the highest instability (37.70) and AcUVR8b the lowest (31.56). Aliphatic indices ranged from 77.74–82.42, and hydropathicity values were -0.047 to -0.275 (<0), indicating hydrophilic proteins. Only AcUVR8b and AcUVR8d had pI > 7 (basic proteins), while the other three were acidic.

2.5 Protein Structure Analysis and Subcellular Localization

SOPMA analysis revealed UVR8 secondary structure comprises α-helices, β-turns, extended strands, and random coils [TABLE:3, FIGURE:4]. Random coils were most abundant (45.49–58.05%), followed by extended strands (22.28–28.04%), with β-turns being least abundant (7.40–9.90%). Cell PLoc 2.0 prediction showed all UVR8 proteins localize to the nucleus.

Tertiary structure prediction using Phyre2 with AtUVR8 as a template revealed that only AvUVR8b and AcUVR8a share similarity with AtUVR8, forming a seven-bladed β-propeller structure from seven complete RCC1 conserved motifs. Other UVR8 proteins could not form complete seven-bladed β-propeller structures due to incomplete or missing RCC1 motifs, suggesting AvUVR8b and AcUVR8a play important roles in UV-B response [Figure 5: see original paper].

2.6 Transmembrane Structure, Signal Peptide, and Phosphorylation Site Analysis

TMHMM 2.0, SignalP 5.0, and NetPhos 3.1 analyses revealed no transmembrane structures or signal peptides in UVR8 proteins. Phosphorylation site prediction showed AvUVR8 proteins contain 35–50 sites (13–31 serine, 10–19 threonine, and 4–8 tyrosine residues), with AvUVR8a having the most (50) and AvUVR8d the fewest (35). AcUVR8 proteins contain 31–51 sites (12–31 serine, 10–17

threonine, and 2–8 tyrosine residues), with AcUVR8 having the most (51) and AcUVR8d the fewest (31) [Figure 6: see original paper].

2.7 Cis-Acting Element Analysis

PlantCARE analysis of 2,000 bp upstream sequences revealed cis-elements involved in light response, hormone reaction, stress response, and growth regulation [Figure 7: see original paper]. Light-responsive elements were most abundant (117), including ATC-motif, Box 4, I-box, TCT-motif, GA-motif, GT1-motif, G-box, AT1-motif, and ACE. Hormone-responsive elements (44) included TATC-box, GARE-motif, and P-box for gibberellin; CGTCA-motif and TGACG-motif for methyl jasmonate; TCA-element for salicylic acid; ABRE for abscisic acid; and TGA-element for auxin. Stress-responsive elements (38) included ARE for anaerobic response, WUN-motif for wounding, MBS for drought, LTR for low temperature, and TC-rich repeats for defense. Growth-regulatory elements (18) were least abundant, including circadian, CAT-box, GCN4-motif, and O2-site. This indicates UVR8 expression is regulated by light, temperature, moisture, oxygen, and hormones.

2.8 Phylogenetic Analysis of UVR8 Proteins

Since UVR8 was first discovered in *Arabidopsis* (Kliebenstein, 2002) and subsequently found in other plants, we performed homology searches using NCBI BLASTn, downloading 94 UVR8 sequences from 42 plant species. MEGA 11.0 with ClustalW alignment and Neighbor-Joining method (bootstrap = 1,000) constructed a phylogenetic tree dividing into three subgroups. AvUVR8a and AcUVR8b occupied a separate subgroup from AtUVR8, indicating a distant relationship. AvUVR8b and AcUVR8a clustered together, showing closest relationship with *Coffea arabica* (CaUVR8) and *C. eugenioides* (CeUVR8), followed by *Chrysanthemum lavandulifolium* (ClUVR8), *Tripterygium wilfordii* (TwUVR8), *Macadamia integrifolia* (MiUVR8), *Ipomoea triloba* (ItUVR8), and *I. nil* (InUVR8). In the main cluster of *Apocynum* UVR8 proteins, the closest relationships were with *Arabidopsis* (AtUVR8) and *Ziziphus jujuba* (ZjUVR8), followed by *Theobroma cacao* (TcUVR8), *Durio zibethinus* (DzUVR8), *Hibiscus syriacus* (HsUVR8), *Gossypium hirsutum* (GhUVR8), *G. arboreum* (GaUVR8), and *G. raimondii* (GrUVR8) [Figure 8: see original paper], indicating clear homologous relationships among UVR8 proteins in this subgroup.

2.9 UVR8 Gene Expression Analysis

TBtools-generated heatmaps of UVR8 expression under UV-B stress (d0 as control) showed that in *A. venetum*, AvUVR8b, AvUVR8c, and AvUVR8e were

upregulated, with AvUVR8b peaking at d7, AvUVR8e peaking at d1 then declining, and AvUVR8c showing an upward trend. AvUVR8a, AvUVR8d, and AvUVR8f were downregulated, with AvUVR8a peaking at d1 and lowest at d4, AvUVR8d highest at d0 and lowest at d7, and AvUVR8f highest at d1 and lowest at d7 [Figure 9: see original paper]. In *A. cannabinum*, AcUVR8a and AcUVR8b were upregulated, with AcUVR8a showing an increasing trend and AcUVR8b peaking at d0.5. AcUVR8c and AcUVR8d showed large fluctuations, with AcUVR8c declining then increasing from d0.5 (lowest) to d7 (highest), and AcUVR8d peaking at d1 and lowest at d0.5. AcUVR8e was downregulated, highest at d0 and lowest at d4 [Figure 10: see original paper]. This suggests AvUVR8b, AvUVR8c, and AvUVR8e play important roles in *A. venetum* UV-B response, while AcUVR8a and AcUVR8b are important in *A. cannabinum*.

3 Discussion and Conclusion

As human society develops, ozone layer depletion has enhanced surface UV-B radiation (Caldwell et al., 1989), affecting plant photosynthesis rates, metabolism, and ecological functions (Bao, 2016), thereby threatening growth, development, and crop yield. As a specific UV-B photoreceptor, studying UVR8 structure, function, and response mechanisms is essential for crops (Wargent et al., 2013). Under UV-B, uvr8-1 mutants show reduced induction of key genes for flavonoid and anthocyanin synthesis, with no upregulation of chalcone synthase mRNA and protein expression (Kliebenstein, 2002). UVR8 overexpression enhances UV-B-mediated photomorphogenesis and increases UV-B adaptation and tolerance (Favory et al., 2009). Studies show UVR8 improves plant adaptability and stress resistance by regulating multiple life activities during UV-B response (Jenkins, 2014b; Vandenbussche et al., 2014), with low-dose UV-B inhibiting hypocotyl and root growth (Frohnmeyer, 2003; Wellmann, 1976) while promoting synthesis of UV-B "sunscreen" flavonoids to enhance adaptability (Winkel-Shirley, 2002; Hartmann et al., 2005; Gruber et al., 2010). UV-B damage repair mainly involves antioxidant systems and enzymatic DNA damage repair (Jenkins, 2014b), providing clues for studying *Apocynum* UVR8 function and UV-B regulatory networks.

This study screened UVR8 protein sequences from whole-genome data of both species, using AtUVR8 as a seed sequence to obtain six *A. venetum* and five *A. cannabinum* UVR8 genes for bioinformatic analysis and expression pattern analysis under UV-B stress. UVR8 genes were unevenly distributed across multiple chromosomes without tandem duplication. While UVR8 protein sequences differed within species, similarity existed between species, such as between AvUVR8a and AcUVR8b, and AvUVR8b and AcUVR8a, showing high similarity in motif number, position, and type. Reports indicate that key amino acid residues in UVR8 proteins from different species are highly similar, suggesting relatively conserved UVR8 proteins during evolution (Yang et al., 2018) and similar molecular functions for UV protection in photosynthetic organisms

(Rizzini, 2011). In this study, UVR8 protein secondary structures were similar, but ring-shaped tertiary structures differed. Only AvUVR8b and AcUVR8a showed tertiary structures most similar to AtUVR8, forming a seven-bladed β-propeller from seven complete RCC1 motifs, consistent with reported UVR8 structure studies (Jenkins, 2014a; Bao, 2016; Zhang et al., 2019). Other UVR8 proteins may have gradually degenerated during evolution, resulting in incomplete or missing RCC1 motifs that prevent formation of complete seven-bladed β-propeller structures, suggesting AvUVR8b and AcUVR8a may play major roles in UV-B response. Phosphorylation of *Apocynum* UVR8 proteins was dominated by serine modification, involving threonine and tyrosine modifications. UVR8 proteins were unstable hydrophilic proteins lacking signal peptides and transmembrane structures, consistent with studies in *Haematococcus pluvialis*, rice, and other species (Bao, 2016; Zhang et al., 2019). Studies show hydrophilic proteins contain numerous hydrophilic amino acids that enhance plant stress resistance to drought, low temperature, and high salinity when overexpressed (Liu, 2019), suggesting UVR8 may participate in plant stress resistance processes.

The UVR8 protein phylogenetic tree divided into three subgroups, with AvUVR8a and AcUVR8b occupying a separate subgroup from AtUVR8, indicating a distant relationship. In the main cluster of AvUVR8/AcUVR8, the closest relationships were with *Arabidopsis* (AtUVR8) and *Ziziphus jujuba* (ZjUVR8), while centrally clustered AvUVR8b and AcUVR8a showed closest relationship with *Coffea arabica* (CaUVR8) and *C. eugenioides* (CeUVR8). This indicates clear homologous relationships among UVR8 proteins in the main *Apocynum* cluster. Cis-acting element sequences upstream of genes regulate expression by binding transcription factors, involving promoters, enhancers, regulatory elements, and inducible elements (Liu et al., 2022). Our analysis revealed UVR8 expression is regulated not only by light but also by temperature, moisture, oxygen, and endogenous hormones, indicating UVR8 participates in regulating plant growth, development, and stress resistance. Studies show appropriate UV-B doses positively regulate plant growth, quality improvement, preservation, and stress resistance (Jenkins, 2009; Liu et al., 2020). In tomato, UV-B pre-treatment significantly increased superoxide dismutase (SOD) and catalase (CAT) gene expression and enzyme activity. Silencing UVR8 function downregulated UV-B-activated SOD and CAT expression, inhibiting UV-B-alleviated oxidative stress and cold damage, demonstrating UVR8 involvement in UV-B-induced cold tolerance and antioxidant enzyme activity dependence on UVR8 (Jiang et al., 2022). Under UV-B stress, expression analysis revealed upregulation of AvUVR8b, AvUVR8c, AvUVR8e, AcUVR8a, and AcUVR8b, with AvUVR8c and AcUVR8a showing increasing trends with treatment duration, indicating these genes participate in plant UV-B response.

In summary, AvUVR8b and AcUVR8a protein structures and conserved motifs are most similar to AtUVR8, and their expression is upregulated under UV-B stress. We speculate that AvUVR8b plays a primary role in UV-B response in *A. venetum*, while AcUVR8a plays a primary role in *A. cannabinum*. Focus-

ing on AvUVR8b and AcUVR8a for subsequent functional analysis and UV-B stress response studies will provide insights into the molecular mechanisms and regulatory networks of UV-B response in *Apocynum*.

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