Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Genome-Wide Identification of the SOD Gene Family Reveals Coordinated Antioxidant Regulation and Flavonoid Accumulation in *Astragalus membranaceus* under UV-B Stress

Ming Jiang
Qiqihar Medical University, Qiqihar 16l006, Heilongjiang Province, P.R. China

Jiajia Chen
School of Pharmacy, Qiqihar Medical University, Qiqihar 16l006, Heilongjiang Province, P.R. China

Yue Pan
School of Pharmacy, Qiqihar Medical University, Qiqihar 16l006, Heilongjiang Province, P.R. China

Xiaoxing Ma
School of Pharmacy, Qiqihar Medical University, Qiqihar 16l006, Heilongjiang Province, P.R. China

Yu Jiao
Qiqihar Medical University, Qiqihar 16l006, Heilongjiang Province, P.R. China

Ying Cui
Qiqihar Medical University, Qiqihar 16l006, Heilongjiang Province, P.R. China

Kanchao Yu
Qiqihar Medical University, Qiqihar 16l006, Heilongjiang Province, P.R. China

Yuchen Wang
Qiqihar Medical University, Qiqihar 16l006, Heilongjiang Province, P.R. China

Dong Han
Xinjiang Hongtao Agricultural Development Limited Liability Company, Altay 836500, Xinjiang Uygur Autonomous Region, China

Wenxin Liu
Qiqihar Medical University, Qiqihar 16l006, Heilongjiang Province, P.R. China

Yanshi Ma
Qiqihar Medical University, Qiqihar 16l006, Heilongjiang Province, P.R. China

Feng Su
Qiqihar Medical University, Qiqihar 16l006, Heilongjiang Province, P.R. China

Jicheng Liu
Qiqihar Medical University, Qiqihar 16l006, Heilongjiang Province, P.R. China

Keyong Zhang
zky9977@126.com

Qiqihar Medical University, Qiqihar 16l006, Heilongjiang Province, P.R. China

Hui Li

Qiqihar Medical University, Qiqihar 16l006, Heilongjiang Province, P.R. China

Research Article

Keywords: Astragalus membranaceus, UV-B stress, SOD gene family, antioxidant regulation, flavonoid metabolism, medicinal quality

Posted Date: April 29th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9412338/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: No competing interests reported.

Abstract

Ultraviolet-B (UV-B) radiation induces excessive reactive oxygen species (ROS) in plants, impairing stress tolerance and secondary metabolism. Flavonoids were recognized as key antioxidants in *Astragalus membranaceus*, which help mitigate UV-B damage. However, how antioxidant enzymes interact with flavonoid metabolism under UV-B remains unclear. Using whole-genome data, we identified 14 *AmSOD* genes classified into Cu/Zn-, Fe-, and Mn-SOD subfamilies. These genes share conserved structures but show divergent expression patterns. Under UV-B, *AmCSD10* (Cu/Zn-SOD) was strongly and continuously upregulated, matching increased SOD activity. Transcriptomic, enzyme activity, and metabolomic data were integrated across time points (UV3h-UV18h), revealing 64 flavonoids that shift from stability to accumulation at later stages. Correlation analysis linked 29 flavonoids to six *AmSOD* genes. *AmCSD10* showed the strongest associations with five upregulated flavonoids, contained formononetin, glabranin and aloeresin A. Co-expression networks suggest *AmCSD10* coordinates antioxidant defense and flavonoid biosynthesis via *ERF*- and *WRKY*-type transcription factors. This study identifies an *AmCSD10*-centered module linking ROS detoxification and flavonoid metabolism, highlighting its role in redox control and medicinal compounds accumulation. The findings reveal mechanistic links between stress adaptation and quality formation in *Astragalus*.

Introduction

In recent years, climate change, ozone depletion and atmospheric shifts have increased surface UV radiation across ecosystems, making UV-B stress a key factor affecting plant growth, development and quality [1]. UV-B radiation has shown long-term increases or short-term spikes in mid- to high-latitude and high-altitude regions, threatening agricultural stability and medicinal plant supply [2]. Due to its high energy, UV-B radiation directly damages photosynthetic systems and key molecules [3]. Plants respond rapidly with restricted leaf expansion, lower chlorophyll, reduced photosynthesis and less biomass [4]. Beyond growth inhibition, UV-B radiation affects secondary metabolism in complex ways, particularly by altering levels of functional metabolites. In vegetables, fruit trees and medicinal plants, UV-B treatments boost phenolic compounds and flavonoids, improving nutritional value and pharmacological properties [5]. At the molecular level, UV-B (280–315 nm) disrupts photosystem II, causing rapid reactive oxygen species (ROS) accumulation in chloroplasts and mitochondria. While high ROS levels cause oxidative damage to lipids, proteins, and nucleic acids, moderate levels act as signals to trigger antioxidant defenses and metabolic changes [6]. Plant tolerance to UV-B stress is largely contingent upon the capacity to maintain redox homeostasis between ROS production and scavenging, as well as the efficiency of resource allocation between growth and defense responses [7]. Within specific dose and temporal parameters, UV-B signals may also elicit adaptive defense responses and enhance the accumulation of quality-associated metabolites, demonstrating a characteristic dose-dependent response [8, 9].

ROS homeostasis in plants relies on enzymatic and non-enzymatic antioxidant systems working together. Superoxide dismutase (SOD) is the primary enzyme, converting superoxide anions (O_2^-) into

hydrogen peroxide (H_2O_2) and oxygen (O_2) [10]. Catalase (CAT) and ascorbate peroxidase (APX) then detoxify H_2O_2, reducing the risk of hydroxyl radical (·OH) formation via the Fenton reaction [11]. In the non-enzymatic system, antioxidants such as ascorbic acid, glutathione, and polyphenolic compounds directly neutralize free radicals and sustain cellular redox buffering capacity through regenerative cycles [12]. Antioxidant defense follows a phased pattern under UV-B stress, enzymatic scavenging dominates early while metabolite-based protection increases in mid-to-late stages, setting the cellular tolerance threshold to ROS [13]. SOD is encoded by a multigene family with three main types (Cu/Zn-SOD, Fe-SOD, Mn-SOD) localized to different compartments such as cytoplasm, chloroplasts, and mitochondria, forming a compartmentalized ROS-scavenging network. Advances in plant genomics have shifted SOD research from single-gene studies to genome-wide analyses of evolution and regulation. Comparative studies show that while *SOD* genes are structurally conserved, their member numbers, expression patterns and regulatory mechanisms vary widely across species. It reflected functional diversification and adaptation to hormones and abiotic stresses [14, 15]. *SOD* genes show stress-responsive expression, and their promoters are enriched in light- and hormone-responsive cis-elements in medicinal plants, indicating roles in environmental signaling and antioxidant regulation [16]. Combined with transcriptomic profiling and cis-element analysis under stress, systematic genome-wide identification of *SOD* genes helps pinpoint key regulatory nodes in antioxidant defense.

UV-B radiation boosts non-enzymatic antioxidants by activating secondary metabolism. It is sensed by the UVR8 photoreceptor, triggering signaling that enhances phenylpropanoid and flavonoid pathways with increasing flavonoids, anthocyanins, and phenolic acids [17]. These compounds act as natural sunscreens to reduce UV penetration and directly scavenge ROS by donating hydrogen or electrons. Strong evidence from multiple plant systems shows that UV-B-induced polyphenolic metabolism enhances overall antioxidant capacity with minimal impact on photosynthesis [18]. UV regulation often increases antioxidant metabolites while modulating antioxidant enzyme activities [19]. Higher flavonoid and phenolic levels in *Glycyrrhiza uralensis* coincide with increased SOD and POD activity. While in *Salvia miltiorrhiza*, UV-B enhances rosmarinic acid accumulation by stabilizing key biosynthetic enzymes and regulating their gene expression, demonstrating that light signaling directly controls the production of bioactive compounds [20].

Leguminous plants have specialized metabolic pathways for isoflavone biosynthesis. Isoflavones not only exhibit potent free radical scavenging activity but also play important roles in plant defense and plant-microbe interactions [21]. In soybean tissue culture systems, UV-B irradiation is used to induce isoflavone accumulation, providing a strategy to enhance the production of bioactive metabolites [22]. Formononetin, a representative legume-specific isoflavone, has robust pharmacological evidence supporting its antioxidant properties and is implicated in the activation of endogenous antioxidant systems [23]. *Astragalus membranaceus* (*A. membranaceus*), a prominent medicinal legume, contains a range of bioactive constituents, including flavonoids and isoflavones, triterpenoid saponins, and polysaccharides [24]. UHPLC-MS/MS analyses confirm the presence of multiple flavonoid components in *A. membranaceus*, with antioxidant activity closely correlated to their compositional profile and relative abundance [25]. Furthermore, astragalus polysaccharides exert well-documented biological effects in

antioxidant and immunomodulatory processes [26]. A systematic reviews support the multifaceted pharmacological actions of *A. membranaceus*, mediated through free radical scavenging and modulation of antioxidant defense pathways [27]. Besides roots, aerial tissues of *A. membranaceus* also accumulate bioactive compounds and show strong antioxidant capacity, supporting whole-plant use and comprehensive quality evaluation. UV-B irradiation is an effective elicitor for boosting active constituent levels in medicinal plants. In hairy root cultures of *A. membranaceus*, UV-B treatment significantly enhances the accumulation of multiple isoflavones and increases yields of key compounds like formononetin [28].

Despite extensive research on the chemical composition and pharmacological properties of *A. membranaceus*, a systematic understanding of its molecular mechanisms in antioxidant defense, particularly the coordination between antioxidant enzyme gene families and flavonoid metabolism, remains limited. Given UV-B's role in regulating flavonoid biosynthesis and the central function of SOD in antioxidant defense, this study systematically identified and characterized the *SOD* gene family using whole-genome data. By integrating transcriptomic, metabolomic, and physiological responses to UV irradiation, we assessed the correlation between *SOD* gene expression and flavonoid accumulation, aiming to elucidate how enzymatic and non-enzymatic systems are regulated under UV-B stress. This work provides a molecular basis and identifies potential regulatory targets to enhance stress resilience and medicinal quality in *A. membranaceus*.

Materials and Methods

Plant Materials and UV Stress Treatment

The experimental seeds of *A. membranaceus* were collected from the Greater Khingan Mountains in Heilongjiang Province, China. Species identification was performed by Associate Researcher Li Hui of Qiqihar Medical University. The voucher specimen (No. QMU-AM-2025-123) is deposited in the Public Herbarium of the university's School of Pharmacy (Address: Qiqihar City, Heilongjiang Province, China; Postcode: 161006). Before sowing, seeds were mechanically scarified to improve germination and planted in 9 cm × 9 cm plastic pots. Seedlings were grown in a controlled-environment chamber under standardized conditions, 16 h light/8 h dark photoperiod, 24 ± 2°C during the day, 20 ± 2°C at night, 50–60% relative humidity and a photosynthetic photon flux density (PPFD) of 220 $\mu mol \cdot m^{-2} \cdot s^{-1}$ provided by fluorescent and LED lights. After seven weeks, uniformly developed seedlings with consistent morphology and physiology were selected for UV-B treatment. UV-B exposure was delivered using a calibrated lamp (280–315 nm) at 20 $\mu W \cdot cm^{-2}$, monitored in real time with a digital radiometer. Treatments lasted 3 hours daily, after which plants returned to standard conditions. Five groups were established based on cumulative exposure. The control group (CK, no UV-B) and four treated groups (UV3h, UV6h, UV12h, UV18h) represent total exposures of 3, 6, 12, and 18 hours. After each interval, leaves were harvested from seedlings. For each biological replicate, leaves from 20 seedlings were pooled to reduce individual variation. Each treatment had three independent replicates. Immediately

after collection, leaf tissues were homogenized, mixed, rapidly frozen in liquid nitrogen, and stored at -80°C for biochemical, transcriptomic, and metabolomic analyses.

Identification, Structural Characterization and Subcellular Localization Prediction of *AmSOD* Family

Genomic data for *A. membranaceus* were obtained from NCBI (accession GCA_039519185.1), and *Arabidopsis thaliana* reference genome and annotations from Ensembl Plants. To comprehensively identify SOD genes, a dual approach was used. First, Hidden Markov Models (HMMs) for conserved SOD domains. PF00080 (Cu/Zn-SOD), PF00081 (Fe/Mn-SOD N-terminal), and PF02777 (Fe/Mn-SOD C-terminal) were downloaded from Pfam and scanned against the genome[29]. Second, experimentally validated SOD protein sequences from *A. thaliana* (https://www.arabidopsis.org/) were used as queries in homology searches via TBtools v2.376 to enrich candidate detection[30, 31]. Initial hits were filtered stringently, sequences lacking complete domain structures or showing redundancy were removed. Remaining candidates were validated using NCBI Batch CD-search to confirm essential SOD motifs[32].

Physicochemical properties, molecular weight, theoretical isoelectric point (pI), and amino acid composition were calculated using the “Protein parameter Calc” module in TBtools. Subcellular localization was predicted with Wolf PSORT (https://wolfpsort.hgc.jp), which integrates sequence features to infer targeting[33]. Protein secondary structures (e.g., α-helices, β-sheets) were predicted using GOR4 (https://npsa-prabi.ibcp.fr), and 3D models were generated by homology modeling in SWISS-MODEL (https://swissmodel.expasy.org/) using evolutionarily conserved templates to assess structural stability and active site organization[34, 35]. Transmembrane topology was analyzed using HMMTOP (http://www.enzim.hu/hmmtop/) to exclude membrane-associated candidates for downstream functional studies[36]. Phosphorylation sites were predicted using NetPhos 3.1 (https://services.healthtech.dtu.dk/service.php?NetPhos-3.1) to identify potential regulatory residues affecting enzyme activity under stress[37].

Chromosomal Localization, Phylogenetic Analysis and Synteny of *AmSOD* Genes

Chromosomal localization of the *AmSODs* was extracted from the *A. membranaceus* genome annotation file based on chromosome number, gene start and end positions, and transcriptional orientation. Gene distribution across chromosomes was visualized using TBtools and chromosomes were renamed based on sequential gene order to facilitate genomic navigation and comparison. To identify gene duplication events, the “One Step MCScanX-Super Fast” module in TBtools was used with stringent parameters (E-value ≤ 1×10^{-10}) to detect segmental and tandem duplications. Results were displayed graphically to illustrate genomic expansion patterns. To reconstruct evolutionary relationships, protein sequences of SODs from *A. thaliana* (https://www.arabidopsis.org/) were combined with AmSOD sequences. Full-length amino acid sequences were aligned using MEGA 11.0 and a neighbor-joining (NJ) tree was built with 1,000 bootstrap replicates for node support[38]. All other parameters used default settings to ensure consistency. The tree was refined and visualized using Evolview for improved clarity. For synteny and evolutionary conservation analysis, genome data for rice (*Oryza sativa*), soybean (*Glycine max*), and *A. thaliana* were obtained from Ensembl Plants(https://plants.ensembl.org/index.html) and integrated

with the *A. membranaceus* genome[39]. Cross-species synteny was analyzed using MCScanX in TBtools, identifying conserved syntenic blocks and orthologous gene pairs to infer functional conservation and divergence of SOD genes during legume evolution.

Gene Structure, Conserved Motifs, and Cis-Regulatory Elements of *AmSOD*

The genomic and annotation files of *A. membranaceus* were analyzed using TBtools v2.376 to characterize *AmSOD* gene structure. Key features including 5’ and 3’ UTRs, coding sequences (CDS), exons, and introns were mapped and visualized to reveal exon-intron organization. To ensure functional relevance, conserved domains in AmSOD proteins were identified using the NCBI Batch CD-Search tool, confirming signature SOD domain architectures. A comprehensive motif analysis was then performed using the MEME Suite (https://meme-suite.org/meme/tools/meme) to detect evolutionarily conserved motifs[40]. The maximum number of motifs was set to 8 with other parameters at default values, enabling identification of short, functionally important sequence motifs potentially involved in protein stability or catalytic activity. To investigate transcriptional regulation, promoter regions were defined as 2000 bp upstream of the translation start site (ATG) for each gene and analyzed using PlantCARE (http://www.plantcare.co.uk/) to identify putative cis-acting regulatory elements[41]. Focus was placed on stress-responsive, hormone-responsive, and light-responsive elements, indicating potential roles in environmental adaptation and signaling. Finally, the distribution and abundance of these elements across AmSOD promoters were visualized as a heatmap using TBtools, enabling comparative evaluation and revealing potential co-regulatory patterns among subfamilies.

Transcriptional Expression Profiling of AmSOD under UV-B Stress and qRT-PCR Validation

RNA-seq data from *A. membranaceus* under UV-B stress were used to extract FPKM values for *AmSOD* genes. Dynamic expression patterns across treatment groups were analyzed and visualized to identify transcriptional responses to UV-B. To validate the accuracy and reproducibility of RNA-seq results, qRT-PCR was performed using cDNA synthesized from leaf tissues collected at matching time points. Relative expression levels of selected *AmSOD* genes were measured in the control group and under increasing UV-B durations (UV3h, UV6h, UV12h, UV18h) using the $2^{-\Delta\Delta Ct}$ method based on Ct values. Total RNA was extracted from leaf samples using the RNAprep Pure Plant Total RNA Extraction Kit (DP432), followed by DNase I treatment to remove genomic DNA contamination. First-strand cDNA was synthesized with the TIANScript II cDNA First-Strand Synthesis Kit (KR107) following the manufacturer’s protocol. qRT-PCR was carried out using FastFire qPCR PreMix (SYBR Green; FP207) on an ABI QuantStudio 6 Real-Time PCR System. Thermal cycling included initial denaturation at 95°C for 1 min, followed by 40 cycles of 95°C for 5 s and 60°C for 15 s. Each biological replicate was tested in triplicate, with three replicates per treatment to ensure statistical reliability. Seven differentially expressed *AmSOD* genes were selected for validation based on distinct expression trends in RNA-seq. *CYP-2* was used as the reference gene for normalization. Gene-specific primers were designed using Primer Premier 5.0 and synthesized by Shanghai Sangon Biotech Co., Ltd. Primer sequences are provided in Supplementary

Table S1. This integrative approach confirms the reliability of transcriptomic data and supports the regulatory role of *AmSOD* genes in UV-induced oxidative stress response.

Non-targeted Metabolomics Analysis of *A. membranaceus* under UV-B Stress

Non-targeted metabolomics was used to characterize the dynamic metabolic responses of *A. membranaceus* during UV-B stress, including the control group (CK) and four treatment stages (UV3h, UV6h, UV12h, UV18h). Each group had three biological replicates for statistical reliability. Metabolite profiling was performed by Wuhan Metware Biotechnology Co., Ltd. using a UHPLC-MS/MS system consisting of a Shimadzu Nexera X2 UHPLC coupled with a SCIEX TripleTOF 6600 + mass spectrometer. Chromatographic separation was achieved on a Waters ACQUITY UPLC HSS T3 column (1.8 μm, 2.1 mm × 100 mm) at 40°C, with a flow rate of 0.40 mL·min^{-1} and an injection volume of 4 μL. The mobile phase contained 0.1% (v/v) formic acid in water (solvent A) and in acetonitrile (solvent B). Gradient elution was as follows: 0–5.0 min, 5% to 65% B; 5.0–6.0 min, to 99% B; 6.0-7.5 min, hold at 99% B; 7.6–10.0 min, re-equilibration at 5% B. Metabolite detection was conducted using electrospray ionization (ESI) in both positive and negative modes simultaneously to maximize coverage and sensitivity. Raw data were processed for peak identification, alignment, and normalization using MetWare's proprietary pipeline. Differential metabolites were identified using $|\log_2$ fold change (FC)$| \geq 1.5$ and FDR < 0.05 to control false positives from multiple testing. Identified metabolites were annotated via the KEGG database and analyzed for pathway enrichment to reveal key metabolic pathways altered under UV-B stress, linking metabolic changes to physiological and biochemical responses.

Measurement of Antioxidant-Related Physiological Parameters under UV-B Stress

To evaluate the effects of UV-B stress on membrane integrity and antioxidant defense, key physiological parameters were measured in leaves of *A. membranaceus* across treatment groups. Each group included three biological replicates using leaf samples from Section 2.1. Relative electrolyte conductivity (REC), an indicator of membrane damage, was determined by the electrolyte leakage method and calculated as REC (%) = $(C_1/C_2) \times 100\%$, where C_1 is initial conductivity and C_2 is final conductivity after complete cell lysis. For antioxidant enzyme activity and oxidative stress assessment, leaf tissues were rapidly frozen in liquid nitrogen and homogenized to prepare crude enzyme extracts. After centrifugation at 4°C, supernatants were collected for analysis. Activities of SOD, POD, CAT, and MDA content (a marker of lipid peroxidation) were quantified using commercial assay kits (SOD: Nanjing Jiancheng A001-1-2; POD: Nanjing Jiancheng POD-1-Y; CAT: Nanjing Jiancheng A007-1-1; MDA: Nanjing Jiancheng A003-3-1) following manufacturer protocols. SOD activity was measured by its inhibition of nitroblue tetrazolium (NBT) photochemical reduction, POD activity by substrate oxidation rate, CAT activity by H_2O_2 decomposition rate, and MDA content by the thiobarbituric acid (TBA) colorimetric method. All values were normalized to fresh weight (FW) for cross-treatment comparability.

Data Statistical Analysis

All experimental data were organized and subjected to preliminary descriptive statistics using Microsoft Excel. Statistical significance among treatment groups was assessed by one-way analysis of variance (ANOVA), followed by Duncan's multiple range test for post hoc pairwise comparisons, with statistical significance defined as $p < 0.05$. GraphPad Prism (v9.0) was employed for generating publication-quality graphs, while R software (v3.5.1) was used for advanced statistical modeling and multivariate analyses where applicable. The gene-metabolite co-expression network was constructed based on Pearson correlation coefficients ($|r| \geq 0.8$, $p < 0.05$) and visualized using Cytoscape (v3.9.1), enabling identification of highly correlated functional modules.

Results

Identification and Characterization of the *AmSOD* Gene Family in *A. membranaceus*

A genome-wide survey identified 14 *SOD*-encoding genes in *A. membranaceus*, named *AmCSD1-10*, *AmMSD1*, and *AmFSD1-3*, representing the three canonical subfamilies. With ten *CSDs*, one *MSD*, and three *FSDs*, this distribution reflects a functionally diverse yet evolutionarily conserved architecture, consistent with adaptation to oxidative stress across environments. Physicochemical analyses (Supplementary Table S2) revealed structural heterogeneity, protein lengths ranged from 137 to 413 amino acids, molecular weights from 14.35 to 43.85 kDa, and theoretical isoelectric points (pI) from 5.46 to 8.25. Eleven members had instability indices ≤ 40, indicating general stability. AmCSD6, AmCSD8, and AmCSD10 were highly stable (index < 12), while AmCSD2 and AmFSD1 showed higher values (> 45), suggesting regulated turnover. Aliphatic indices (71.68–98.06) indicate broad thermal resilience. GRAVY scores were mostly negative (-0.52 to -0.08) with confirming hydrophilicity. Only AmCSD2, AmCSD3, and AmCSD9 had slightly positive values (0.03–0.11), suggesting mild hydrophobicity and it possibly linked to membrane association or transient targeting. Subcellular localization predictions revealed functional compartmentalization. Chloroplasts housed the largest group (50%, n = 7), including AmCSD1, AmCSD3, AmCSD4, AmCSD9, and all three AmFSDs, consistent with high ROS production in chloroplasts under UV stress. Five members (35.7%, n = 5: AmCSD5-8, AmCSD10) were predicted to be cytoplasmic. AmMSD1 localized to mitochondria and AmCSD2 to the extracellular space, demonstrating broad spatial coverage of ROS scavenging.

Secondary structure modeling (FIGURE 1A; Supplementary Table S3) showed that AmSOD proteins are primarily composed of random coils (40.87–72.20%) and extended strands (14.65–37.23%). With variable α-helix content (0-39.57%) and no β-turns were detected, supporting rigidity in functional regions. Transmembrane helix prediction indicated limited membrane association: only six proteins (AmCSD1, AmCSD3, AmCSD4, AmCSD9, AmFSD2, AmFSD3) contained 1–3 transmembrane domains. The remaining eight were predicted as soluble, consistent with roles as cytosolic or organellar antioxidants. Tertiary structure modeling (FIGURE 1B) confirmed all 14 AmSOD proteins adopt the conserved SOD fold, featuring a preserved β-barrel core and metal-binding site. It highlighted strong evolutionary conservation of catalytic function despite sequence variation.

Secondary structure features and three-dimensional structure prediction of AmSOD proteins. (A) Schematic representation of the secondary structure distribution of AmSOD proteins. The horizontal axis represents amino acid residue positions. Different colors indicate distinct secondary structure elements, with purple representing α-helices, light blue representing extended strands (β-sheets), and yellow representing random coils. (B) Predicted three-dimensional structures of AmSOD proteins. Regions shown in blue indicate highly confident core structural domains, whereas regions shown in orange represent low-confidence and flexible regions.

Chromosomal Distribution and Gene Duplication Analysis of *AmSOD*

Chromosome localization (FIGURE 2A) showed that 14 *AmSOD* genes are unevenly distributed across seven chromosomes in *A. membranaceus*, indicating non-random genomic organization. While some chromosomes carry multiple genes, others have only one or none, reflecting distribution bias. Chromosome 07 (Chr07) contains the most *AmSOD* genes, four (*AmCSD5*, *AmCSD6*, *AmCSD7*, *AmCSD8*), located adjacent to each other and forming a tandem cluster, suggesting a hotspot for local gene amplification. Chr05 carries three genes (*AmCSD2*, *AmFSD3*, *AmCSD3*), while Chr03 and Chr09 each have two. Single genes are located on Chr01, Chr02, and Chr06 and no SOD homologs were found on Chr04 or Chr08. Duplication analysis using MCScanX (FIGURE 2B) identified two tandemly duplicated pairs (*AmCSD5/AmCSD6 and AmCSD7/AmCSD8*) both within the Chr07 cluster. These pairs show close physical proximity and high sequence similarity, meeting criteria for tandem duplication. No segmental duplications or whole-genome duplication (WGD)-derived paralogs were detected, indicating that tandem duplication has likely driven the expansion and evolution of the *AmSOD* family in *A. membranaceus*.

Chromosomal distribution and duplication analysis of *AmSOD* genes in *A.membranaceus*. (A) Chromosomal localization of the *AmSOD* gene family. The positions of the 14 AmSOD genes are mapped onto seven chromosomes (Chr01, Chr02, Chr03, Chr05, Chr06, Chr07 and Chr09). Gene names are indicated next to their corresponding chromosomal locations. (B) Duplication analysis of *AmSOD* genes based on MCScanX. Gray lines represent collinear relationships among chromosomal regions, and red lines indicate tandemly duplicated *AmSOD* gene pairs. The color scale on the right represents gene density across chromosomes.

Phylogenetic and Comparative Synteny Analysis of *SOD* Genes across Species

The SOD protein sequences from *Arabidopsis thaliana* and *Astragalus mongholicus* were employed to construct a neighbor-joining (NJ) phylogenetic tree, with visualization enhanced using Evolview (https://www.evolgenius.info/evolview/)[42] (FIGURE 3A; Supplementary Table S4). All SOD proteins clustered into three distinct clades, Group I (Cu/Zn-SOD), Group II (Fe-SOD), and Group III (Mn-SOD), corresponding exactly to their metal cofactor specificities, indicating strong structural and functional conservation during evolution. Within each clade, *AmSOD* genes generally grouped with their *A. thaliana* orthologs, forming well-supported monophyletic lineages. High bootstrap values (> 80%) at key nodes, such as AmCSD1-AtCSD1 and AmCSD2-AtCSD2, highlight strong sequence conservation and likely conserved functions. Notably, some Fe-SOD members, including AmFSD2 and AtFSD3, showed close

phylogenetic relationships, suggesting potential functional redundancy or recent divergence. In contrast, several branches in Group II had moderate support (40–80), indicating a more complex evolutionary history for Fe-SOD genes, possibly due to accelerated divergence, lineage-specific duplications, or incomplete lineage sorting.

To further assess evolutionary conservation, comparative synteny analysis was conducted among *A. membranaceus*, *A. thaliana*, *G. max*, and *O. sativa* (FIGURE 3B). The results showed more conserved syntenic gene pairs between *A. membranaceus* and *G. max*, than between *A. membranaceus* and *O. sativa*, reflecting greater genomic collinearity and stronger conservation within dicots, especially among closely related species. Intragenomic synteny analysis also revealed tandemly duplicated SOD gene pairs in *A. membranaceus*, primarily located on Chr07. These findings align with earlier chromosomal distribution and duplication analyses, supporting the role of tandem duplication in the local expansion and diversification of the SOD gene family in *A. membranaceus*.

Phylogenetic relationships and interspecific synteny analysis of *AmSOD* genes. (A) Phylogenetic tree of SOD proteins from *A. membranaceus* and *A. thaliana* constructed using the Neighbor-Joining (NJ) method. Bootstrap values are indicated by colored circles at the nodes, representing different confidence levels. The SOD proteins are clustered into three major groups corresponding to Cu/Zn-SOD (Group I), Fe-SOD (Group II), and Mn-SOD (Group III). (B) Synteny analysis of SOD genes among *A. membranaceus*, *A. thaliana*, *G. max*, and *O. sativa*. Conserved collinear blocks are shown by gray lines, while red lines highlight syntenic relationships involving SOD genes. The distribution of syntenic gene pairs illustrates the evolutionary conservation and divergence of SOD genes among dicot and monocot species.

Structural Organization, Conserved Motifs, and Cis-Regulatory Elements of *AmSOD*

Integrated analysis of conserved domains, gene structures, and motif architecture classified the 14 *AmSOD* genes in *A. membranaceus* into three subfamilies. This classification aligns closely with the phylogenetic clades ,supporting robust functional grouping (FIGURE 4). All AmSOD proteins contain a canonical SOD domain, confirming their identity as superoxide dismutases and highlighting structural conservation essential for enzymatic activity. Gene structure analysis revealed variation in intron number (0–8) and exon length (FIGURE 4D), indicating architectural diversity within the family. This heterogeneity may contribute to differential transcriptional regulation or alternative splicing across subfamilies. Conserved motif profiling identified both shared and subfamily-specific features (FIGURE 4B, E). Genes within the same clade generally share conserved motif compositions and arrangements, indicating functional coherence. All CSD proteins contain Motifs 1–4, likely corresponding to core structural and catalytic elements. In contrast, FSD and MSD members have distinct motif combinations, potentially underlying their specific metal cofactor binding and subcellular targeting. Notably, AmCSD9 contains an additional, lineage-specific motif 8 not found in other CSDs, suggesting neofunctionalization or regulatory specialization.

Cis-regulatory element analysis of the 2000 bp upstream promoter region of each *AmSOD* gene revealed that all members contain diverse numbers and combinations of cis-acting elements, indicating complex

transcriptional regulation (FIGURE 5). These elements were grouped into four functional categories, hormone-responsive, stress-responsive, light-responsive, and those linked to basal transcription or development. Most *AmSOD* promoters contain multiple hormone- and stress-responsive elements, suggesting integrated regulation by environmental and physiological signals. The abscisic acid-responsive element (ABRE) and MYB binding site (MBS) were common across promoters, highlighting conserved regulatory features likely involved in coordinated expression under abiotic stresses such as drought and oxidative challenge. Light-responsive elements were present in all promoters and relatively abundant, indicating a strong link between *AmSOD* expression and light-mediated signaling, potentially connecting their function to photosynthesis-associated redox homeostasis and circadian regulation.

Gene structure, conserved motifs and domain composition of *AmSOD* genes. (A) Conserved domain composition of AmSOD proteins. Different colored boxes represent distinct conserved domains identified in AmSOD proteins. (B) Conserved motif distribution of AmSOD proteins. Colored boxes indicate different conserved motifs (Motif 1–8) identified by MEME analysis. (C) Length comparison of AmSOD protein sequences. Green boxes represent protein length, while gray boxes indicate untranslated regions. (D) Exon–intron structures of AmSOD genes. Green boxes represent exons, black lines indicate introns, and yellow boxes represent untranslated regions (UTRs). (E) Sequence logos of conserved motifs identified in AmSOD proteins, showing amino acid conservation patterns within each motif.

Cis-acting regulatory element analysis of *AmSOD* gene promoters. Distribution and frequency of predicted cis-acting regulatory elements in the 2000 bp upstream promoter regions of *AmSOD* genes. Different colors indicate distinct categories of cis-elements, including hormone-responsive elements, stress-responsive elements, light-responsive elements, and elements related to basic transcriptional regulation and development. The color scale represents the number of cis-elements identified in each promoter region.

Expression Profiling of *AmSOD* Genes under UV-B Stress and qRT-PCR Validation

RNA-seq-based transcriptomic analysis was used to examine the expression dynamics of the 14 *AmSOD* genes in *A. membranaceus* leaves under UV-B stress (FIGURE 6A; Supplementary Table S5). Seven genes showed significant transcriptional changes during UV-B exposure, indicating differential responsiveness within the family. Heatmap visualization revealed that *AmCSD7*, *AmFSD1*, *AmFSD2*, *AmFSD3*, and *AmCSD3* were moderately up-regulated at the early stage (UV3h), followed by progressive down-regulation as treatment extended (UV6h-UV12h). Although transcript levels partially recovered after prolonged exposure (UV18h), they remained below control levels, suggesting transient induction followed by suppression under sustained stress. In contrast, *AmCSD9* and *AmCSD10* exhibited continuous up-regulation throughout the time course. Notably, *AmCSD10* peaked at UV18h, showing a strong and sustained response to chronic UV irradiation, indicative of a role in long-term oxidative defense. To validate RNA-seq results, qRT-PCR was performed on the seven differentially expressed genes. The qRT-PCR data closely matched the transcriptome profiles (FIGURE 6B). *AmCSD3*, *AmCSD7*, *AmFSD1*, *AmFSD2*, and *AmFSD3* were up-regulated at UV3h, declined to lowest levels at UV12h, and

showed modest recovery at UV18h. *AmCSD9* and *AmCSD10* increased steadily over time. Statistical analysis confirmed significant differences between each treatment and the control for all tested genes. *AmCSD10* showed highly significant upregulation ($p < 0.01$) at both UV12h and UV18h, supporting its role as a key responsive gene in prolonged UV-B stress.

Expression profiles of *AmSOD* genes under UV stress and qRT-PCR validation. (A) Heatmap showing the expression patterns of *AmSOD* genes based on RNA-seq data under different UV-B treatment durations. Expression levels are presented as row-normalized FPKM values. Color scale indicates relative expression levels, with red and green representing high and low expression, respectively. (B) qRT-PCR validation of selected *AmSOD* genes under UV stress. Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method and normalized to the internal reference gene. Data represent the mean ± SD of three biological replicates. Asterisks indicate statistically significant differences compared with the control (*$p < 0.05$*, **$p < 0.01$*, ***$p < 0.001$*).

Construction of the *AmSOD* Co-expression Network and Functional Enrichment Analysis

To explore the transcriptional regulatory landscape of the *AmSOD* gene family, a co-expression network was constructed using transcriptome data from *A. membranaceus*, integrating expression correlations between *AmSOD* genes and transcription factors (TFs) (FIGURE 7A; Supplementary Table S6). Several *AmSOD* genes (*AmCSD10*, *AmFSD1*, *AmFSD2*, and *AmCSD9*) showed strong positive correlations with multiple TFs (Pearson correlation coefficient > 0.96), indicating highly coordinated transcriptional regulation. Notably, *AmCSD10* exhibited the strongest association with *WOX8*, a *WOX* family TF ($R = 0.991$), suggesting a potential link to developmental or stress-responsive pathways regulated by WOX proteins. *AmFSD1* and *AmFSD2* were also co-expressed with several TFs, including *RNJ, YAB5, WHY1, C3H43*, and *TCP7*. Intriguingly, *WHY1*, TCP7, and *YAB5* showed consistent co-expression across multiple *AmSOD* genes, implying their role as shared regulators in oxidative stress responses.

To identify associated biological functions, Gene Ontology (GO) and KEGG pathway enrichment analyses were conducted on the highly correlated *AmSOD* and interacting TFs. GO results (FIGURE 7B) revealed significant enrichment in processes related to redox homeostasis, transcription regulation, stress responses, and molecular functions such as metal ion binding, it is consistent with canonical SOD enzyme roles. KEGG analysis further showed enrichment in key pathways, including plant hormone signal transduction, phenylalanine/tyrosine/tryptophan biosynthesis, circadian rhythm, MAPK signaling, and biosynthesis of plant secondary metabolites (FIGURE 7C). These findings indicate that the *AmSOD* gene family is embedded in a tightly regulated transcriptional network and functionally linked to diverse physiological processes. Co-expression with stress- and hormone-related TFs, along with pathway enrichment, supports a role for *AmSOD* genes in coordinating multi-layered responses to environmental challenges in *A. membranaceus*.

Co-expression network construction and functional enrichment analysis of *AmSOD* genes. (A) Co-expression network between *AmSOD* genes and transcription factors based on transcriptome data. Nodes represent *AmSOD* genes or transcription factors, and edges indicate significant positive

correlations (R > 0.96). (B) Gene Ontology (GO) enrichment analysis of highly correlated. (C) KEGG pathway enrichment analysis of the co-expressed gene set.

AmSOD Regulates Flavonoid Accumulation in *A. membranaceus* under UV-B Stress

Flavonoid dynamics in *A. membranaceus* were analyzed using UHPLC-MS/MS under UV-B stress. A total of 64 flavonoids and derivatives were identified, classified into 26 flavones, 12 flavonols, 8 isoflavones, and 18 substituted flavonoid derivatives (FIGURE 8A; Supplementary Table S7). Relative to CK, flavonoid accumulation showed a phased response. At 3 h (UV3h), metabolism remained largely stable 38 compounds unchanged, 18 decreased, and only 8 upregulated, indicating weak induction by short-term UV stress. By 6 h (UV6h), responses intensified slightly 43 unchanged, 13 upregulated, 8 downregulated, suggesting early activation of flavonoid regulation. At 12 h (UV12h), 19 compounds were significantly upregulated, 36 unchanged, and 9 downregulated, indicating stronger activation under sustained exposure. Under prolonged stress (UV18h), the most pronounced changes occurred 19 compounds continuously upregulated, 14 downregulated, and 31 stable. Notably, several flavonoids and their glycosylated derivatives accumulated to high levels, reflecting a shift from low- to high-amplitude regulation during chronic UV stress.

To assess the potential regulatory role of *AmSOD* genes, correlation analysis was performed between *AmSOD* expression and flavonoid accumulation. Of the 64 flavonoids, 29 showed significant positive correlations (|r| > 0.7) with six *AmSOD* genes (FIGURE 8B; Supplementary Table S8). Garcinone E exhibited the strongest correlation with *AmCSD9* and *AmCSD3* (r > 0.95), followed by 6-Geranylchrysin, which also correlated highly with both genes (r > 0.90). In the *Fe-SOD* subfamily, Petunidin 3-(6"-acetylglucoside) showed the highest correlation with *AmFSD3* (r > 0.95), while Albanin A also positively associated with *AmFSD3*. These key flavonoids occupied central positions in the co-accumulation network and displayed coordinated temporal trends with specific *AmSOD* genes across the time course, suggesting a functional link between SOD-mediated redox homeostasis and flavonoid biosynthesis.

Changes in flavonoid accumulation patterns and *AmSOD*-flavonoid co-expression network under UV-B stress. (A) Bar chart showing the numbers of up-regulated, down-regulated, and non-significantly changed flavonoid metabolites in *A. membranaceus* leaves at different UV-B treatment durations compared with the control group. (B) *AmSOD*-flavonoid co-expression network constructed based on Pearson correlation analysis (|r| > 0.7), illustrating the associations between six *AmSOD* genes (orange nodes) and correlated flavonoid metabolites (green nodes) under UV-B stress. Edges indicate significant positive correlations between gene expression levels and metabolite accumulation.

Integrated Co-expression Network of *AmCSD10*, TFs, and Flavonoid Metabolites and Its Role in UV Stress Response

To elucidate the molecular mechanism of SOD-mediated flavonoid regulation in *A. membranaceus* under UV-B stress, an integrative analysis was conducted using data from Sections 3.6 and 3.7. Flavonoid metabolites with strong upregulation (r > 0.8) and transcription factors showing high co-expression (r >

0.95) were selected for further analysis. Results showed that *AmCSD10* was significantly co-expressed with 18 transcription factors including *ERF11, WRKY57*, and *C3H44* and five key flavonoids such as Formononetin, Glabranin, and Aloeresin A (FIGURE 9A; Supplementary Table S9). These metabolites accumulated markedly under UV stress, especially at later stages (UV12h and UV18h), suggesting roles in long-term adaptation. A comprehensive co-expression network linking *AmCSD10*, TFs, and flavonoids was constructed, illustrating coordinated regulation among redox homeostasis, transcriptional control, and secondary metabolism. This network reveals how UV-induced oxidative stress responses may enhance both stress resilience and medicinal compound accumulation in *A. membranaceus*.

To validate the biological relevance of this module, key physiological parameters and flavonoid levels were measured. Data revealed a close relationship between *AmCSD10* expression and flavonoid accumulation. Notably, changes in Formononetin, Glabranin, and Aloeresin A levels were significantly correlated with SOD enzyme activity, supporting the functional link between antioxidant gene expression and metabolic reprogramming (FIGURE 9B). Prolonged UV-B exposure increased activities of major antioxidant enzymes SOD, POD, and CAT while MDA content decreased, indicating effective suppression of oxidative damage and activation of the cellular antioxidant system. These findings suggest that *AmCSD10* acts as a central regulatory node, potentially modulating the biosynthesis of pharmacologically important flavonoids through interactions with specific TFs. By enhancing antioxidant capacity and promoting protective metabolite accumulation, AmCSD10 likely contributes to improved UV-B tolerance and increased production of medicinal compounds in *A. membranaceus*.

AmCSD10-centered co-expression network and physiological validation under UV-B stress. (A) Pearson correlation-based co-expression network of *AmCSD10* with transcription factors (r > 0.95) and significantly upregulated flavonoid metabolites (*r > 0.80*) under UV-B treatment. (B) Changes in antioxidant-related physiological parameters and representative flavonoid metabolite accumulation in response to different UV-B treatment durations. Values represent mean ± SD (n = 3). Significant differences relative to the control are indicated (**p < 0.05*, ***p < 0.01*, ****p < 0.001*).

Discussion

UV-B radiation imposes sustained physiological and metabolic perturbations on plants by disrupting the homeostasis between ROS generation and detoxification. In medicinal plants, where secondary metabolite profiles directly determine pharmacological efficacy, this redox imbalance not only compromises stress resilience but also critically modulates the biosynthesis and accumulation of bioactive compounds [43]. The UV-B response in *A. membranaceus* is integrative, involving coordinated modulation of the enzymatic antioxidant system, transcriptional networks, and flavonoid biosynthetic pathways. It collectively forming a robust, multi-tiered defense architecture. As the primary enzymatic scavenger of superoxide anions, the *SOD* gene family exhibits pronounced structural conservation across angiosperms [44]. The *AmSOD* gene repertoire, including Cu/Zn-SOD, Fe-SOD, and Mn-SOD subfamilies, mirrors that of model species like *Arabidopsis thaliana* and *Glycine max* in gene copy number and domain organization [45]. AmSOD members show substantial divergence in chromosomal

localization, intron-exon architecture, and promoter cis-element composition, indicating functional specialization and context-dependent transcriptional regulation [46]. The enrichment of light- and phytohormone-responsive elements in *AmSOD* promoters supports differential induction under UV-B stress and links these genes to photomorphogenic and hormonal signaling integration [47].

During UV-B exposure, AmSOD members showed divergent expression, with *AmCSD10* as the most responsive isoform. Its sustained upregulation closely matched total SOD activity, positioning *AmCSD10* as a central regulator of ROS homeostasis. This key isoform-auxiliary isoforms model aligns with findings in other species, where specific Cu/Zn-SODs dominate under photooxidative stress and others serve constitutive or tissue-specific roles [48]. This hierarchical deployment optimizes antioxidant capacity while minimizing energy costs, a critical adaptation under resource limitation. Enzymatic antioxidant responses are linked to overall cellular physiology. The enhanced SOD and CAT activities during early-to-mid UV-B exposure suppressed MDA accumulation, reducing lipid peroxidation and preserving cellular integrity[49]. This rapid, enzyme-driven defense is the frontline response to acute oxidative stress. Under prolonged UV-B exposure, enzymatic capacity is overwhelmed, requiring a shift to non-enzymatic antioxidant mechanisms. Non-targeted metabolomic profiling revealed this biphasic pattern: minimal metabolic changes occurred during initial UV-B exposure, while flavonoid accumulation, including isoflavones and flavanones, increased robustly over time. This delayed metabolic reprogramming follows the established phased model of plant antioxidant defense, with enzymatic clearance dominating early responses and secondary metabolite synthesis becoming more prominent under prolonged stress [50]. Flavonoids provide dual protection, they absorb harmful UV-B wavelengths as endogenous screens and stabilize photosynthetic membranes against photooxidative damage [51].

Correlation network analysis revealed a synergistic relationship between AmSOD activity and flavonoid metabolism beyond co-occurrence, indicating functional interdependence. *AmCSD10* showed strong positive correlations with multiple UV-induced flavonoids, suggesting its activity supports a redox environment favorable for flavonoid biosynthesis. Conversely, accumulating flavonoids likely buffer residual ROS, reducing demand on high-turnover enzymatic systems. This reciprocal reinforcement is an emergent feature of integrated stress adaptation and is increasingly recognized as a hallmark of robust, metabolically flexible stress tolerance [52]. Among correlated metabolites, formononetin, glabranin, and aloeresin A showed strong and temporally consistent association with AmCSD10 expression. Formononetin, a legume-derived isoflavonoid and canonical phytoalexin, is strongly induced by abiotic stresses like UV-B and contributes to plant antioxidant defense by detoxifying ROS and screening UV radiation. In *A. membranaceus* hairy roots, UV-B treatment increased total isoflavonoids, enhanced extract antioxidant activity, and upregulated key biosynthetic genes [53]. These results confirm isoflavonoids' stress-protective, redox-modulating role. Mechanistically, stress-induced oxidative bursts activate MPK3/MPK6, driving expression of isoflavonoid genes and boosting formononetin production. Similarly, glabranin and aloeresin A show membrane stabilization and direct ROS scavenging in biochemical assays[54]. Their coordinated late-phase accumulation reinforces the functional centrality of *AmCSD10* in orchestrating a temporally resolved defense program. At the transcriptional level, *AmCSD10* expression was strongly correlated with stress-responsive transcription factor families,

especially ERF and WRKY proteins, known as master regulators that link environmental signals to antioxidant gene expression and phenylpropanoid/flavonoid pathway activation[55, 56]. By co-regulating upstream antioxidant effectors and downstream metabolic enzymes, these transcription factors enable synchronized, system-level coordination of redox homeostasis and specialized metabolism [57]. The "*AmCSD10*-TFs-flavonoid" co-expression network reconstructed here represents a concrete molecular implementation of this hierarchical control logic in *A. membranaceus*. Based on genome-wide annotation, transcriptomics, metabolomics, and physiological data, we propose a unifying model of UV-B stress activates an *AmCSD10*-centered regulatory hub that simultaneously enhances enzymatic ROS detoxification and reprograms flavonoid metabolism, achieving both oxidative damage mitigation and pharmacologically relevant isoflavone accumulation (FIGURE 10). This mechanism reconciles adaptive fitness with medicinal quality, offering a mechanistically grounded strategy for optimizing the cultivation and post-harvest processing of high-value medicinal plants under controlled abiotic stress regimes.

Proposed regulatory model of *AmCSD10*-mediated antioxidant defense and flavonoid accumulation in *A. membranaceus* under UV-B stress.

Conclusion

This study characterized the composition, evolution, and expression divergence of the SOD gene family in *A. membranaceus* under UV-B stress. The SOD family is structurally conserved but functionally specialized at the regulatory level. AmCSD10 showed sustained upregulation during UV-B stress, closely correlated with antioxidant enzyme activity, highlighting its key role in maintaining ROS homeostasis. Combined metabolomic, co-expression, and physiological analyses reveal that *AmCSD10* promotes the accumulation of bioactive flavonoids, particularly isoflavones, through synergistic interactions with transcription factor families, while concurrently reducing oxidative damage and enhancing medicinal quality. This work clarifies the molecular link between antioxidant defense and flavonoid metabolism. These findings provide candidate genes and metabolic biomarkers for enhancing stress resilience and phytochemical quality in medicinal plants under UV-B, and support strategies for optimizing *A. membranaceus* quality through environmental or molecular breeding approaches.

Declarations

Ethical approval

This publication does not report any research involving human participants or animals conducted by the authors.

Authorship contribution

Conceptualization, H.L. and M.J.; writing—original draft, H.L. and M.J.; software, investigation, J.C., Y.P., X.M., Y.C. and D.H.; formal analysis, M.J., Y.M., F.S. and J.L.; funding acquisition, M.J.; writing—review and

editing, Y.J., Y.W. and W.L.; data collection, K.Z. and K.Y.; supervision, project administration, methodology, M.J. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This work was supported by Heilongjiang Provincial Department of Education (Grant No. YQJH2024283); Natural Science Foundation of Heilongjiang Province of China (Grant No. ZL2024H018); Project of Qiqihar Science and Technology Bureau (Grant No. LSFGG-2025127/LSFGG-2025126); Construction Project of Dominant Characteristic Disciplines of Qiqihar Medical University (QYZDXK-007).

Author Contribution

Conceptualization, H.L. and M.J.; writing—original draft, H.L. and M.J.; software, investigation, J.C., Y.P., X.M., Y.C. and D.H.; formal analysis, M.J., Y.M., F.S. and J.L.; funding acquisition, M.J.; writing—review and editing, Y.J., Y.W. and W.L.; data collection, K.Z. and K.Y.; supervision, project administration, methodology, M.J. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

During the preparation of this work the authors used Google AI Studio in order to improve language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Data Availability

The original contributions presented in this study are included in the article/Supplementary Materials; further inquiries can be directed to the corresponding author.

References

1. Chen Z, Dong Y, Huang X. Plant responses to UV-B radiation: signaling, acclimation and stress tolerance. Stress Biology. 2022;2(1):51.

2. Tan Y, Duan Y, Chi Q, Wang R, Yin Y, Cui D, et al. The role of reactive oxygen species in plant response to radiation. Int J Mol Sci. 2023;24(4):3346.
3. Milić Komić S, Živanović B, Dumanović J, Kolarž P, Sedlarević Zorić A, Morina F, et al. Differential antioxidant response to supplemental UV-B irradiation and sunlight in three basil varieties. Int J Mol Sci. 2023;24(20):15350.
4. Li M, Kim C. Chloroplast ROS and stress signaling. Plant Commun. 2022;3(1).
5. Rao MJ, Duan M, Zhou C, Jiao J, Cheng P, Yang L, et al. Antioxidant defense system in plants: reactive oxygen species production, signaling, and scavenging during abiotic stress-induced oxidative damage. Horticulturae. 2025;11(5):477.
6. Skowron E, Trojak M, Pacak I. Effects of UV-B and UV-C spectrum supplementation on the antioxidant properties and photosynthetic activity of lettuce cultivars. Int J Mol Sci. 2024;25(17):9298.
7. Kimura S, Kaya H, Hashimoto K, Wrzaczek M, Kuchitsu K. Quantitative analysis for ROS-producing activity and regulation of plant NADPH oxidases in HEK293T cells. Reactive Oxygen Species in Plants. Methods and Protocols: Springer; 2022. pp. 107–22.
8. Liu W, Jenkins GI. Recent advances in UV-B signalling: interaction of proteins with the UVR8 photoreceptor. J Exp Bot. 2025;76(3):873–81.
9. Vanhaelewyn L, Van Der Straeten D, De Coninck B, Vandenbussche F. Ultraviolet radiation from a plant perspective: The plant-microorganism context. Front Plant Sci. 2020;11:597642.
10. Cannea FB, Padiglia A. Antioxidant defense systems in plants: Mechanisms, regulation, and biotechnological strategies for enhanced oxidative stress tolerance. Life. 2025;15(8):1293.
11. Mittler R. ROS are good. Trends Plant Sci. 2017;22(1):11–9.
12. Wang P, Liu WC, Han C, Wang S, Bai MY, Song CP. Reactive oxygen species: multidimensional regulators of plant adaptation to abiotic stress and development. J Integr Plant Biol. 2024;66(3):330–67.
13. Bulut M, Tohge T, Chen W, Luo J, Wang Z, Huang X, et al. Learning from nature: phytochemical strategies to protect against UV-B damage. Nat Commun. 2025;16(1):9927.
14. Su W, Raza A, Gao A, Jia Z, Zhang Y, Hussain MA, et al. Genome-wide analysis and expression profile of superoxide dismutase (SOD) gene family in rapeseed (Brassica napus L.) under different hormones and abiotic stress conditions. Antioxidants. 2021;10(8):1182.
15. Rehman S, Rashid A, Manzoor MA, Li L, Sun W, Riaz MW, et al. Genome-wide evolution and comparative analysis of superoxide dismutase gene family in Cucurbitaceae and expression analysis of Lagenaria siceraria under multiple abiotic stresses. Front Genet. 2022;12:784878.
16. Yu P, Ma C, Gao Q, Yan D, Liu Y, Xu J et al. Genome-wide identification and expression profiles of the superoxide dismutase (SOD) genes in Lentinula edodes. Int J Biol Macromol. 2025:147032.
17. Hideg É, Jansen MA, Strid Å. UV-B exposure, ROS, and stress: inseparable companions or loosely linked associates? Trends Plant Sci. 2013;18(2):107–15.

18. Tian X, Hu M, Yang J, Yin Y, Fang W. Ultraviolet-B radiation stimulates flavonoid biosynthesis and antioxidant systems in buckwheat sprouts. Foods. 2024;13(22):3650.
19. Zhou X, Li C, Su Y, Zhang D, Li M. Multi-omics reveal UV-B irradiation plays positive roles in flavonoids and phenolic accumulation in Glycyrrhiza inflata. Ind Crops Prod. 2025;238:122322.
20. Yu H, Zhang S, Chen Y, Li Y, Tian H, Wu Y, et al. UV-B enhances phenolic acid biosynthesis in Salvia miltiorrhiza through dual-level regulation of rosmarinic acid synthase. Ind Crops Prod. 2025;237:122311.
21. Gong F, Yu W, Zeng Q, Dong J, Cao K, Xu H, et al. Rhododendron chrysanthum's primary metabolites are converted to phenolics more quickly when exposed to UV-B radiation. Biomolecules. 2023;13(12):1700.
22. Wang M, Liu G, Guo T, Xie C, Wang P, Yang R. UV-B radiation enhances isoflavone accumulation and antioxidant capacity of soybean calluses. Front Nutr. 2023;10:1139698.
23. Ding M, Bao Y, Liang H, Zhang X, Li B, Yang R, et al. Potential mechanisms of formononetin against inflammation and oxidative stress: A review. Front Pharmacol. 2024;15:1368765.
24. Son S-R, Cho S-Y, Bae J, Yeon G-B, Lee H-S, Hwang H et al. Astragalus membranaceus sprouts and their unique constituents regulate reactive oxygen species production, inflammation-related senescence-associated secretory phenotype components, and extracellular matrix in fibroblasts. Food Res Int. 2026:118390.
25. Sheng Z, Jiang Y, Liu J, Yang B. UHPLC–MS/MS analysis on flavonoids composition in Astragalus membranaceus and their antioxidant activity. Antioxidants. 2021;10(11):1852.
26. Chen T, Xie L, Shen M, Yu Q, Chen Y. Recent advances in Astragalus polysaccharides: Structural characterization, bioactivities and gut microbiota modulation effects. Trends Food Sci Technol. 2024;153:104707.
27. Yao J, Peng T, Shao C, Liu Y, Lin H, Liu Y. The antioxidant action of astragali radix: its active components and molecular basis. Molecules. 2024;29(8):1691.
28. Jiao J, Gai Q-Y, Wang W, Luo M, Gu C-B, Fu Y-J, et al. Ultraviolet radiation-elicited enhancement of isoflavonoid accumulation, biosynthetic gene expression, and antioxidant activity in Astragalus membranaceus hairy root cultures. J Agric Food Chem. 2015;63(37):8216–24.
29. Huo C, He L, Yu T, Ji X, Li R, Zhu S, et al. The superoxide dismutase gene family in Nicotiana tabacum: genome-wide identification, characterization, expression profiling and functional analysis in response to heavy metal stress. Front Plant Sci. 2022;13:904105.
30. Zhang Q, Ding K, Lv T, Wang X, Yin Q, Zhang Y, et al. Scientific large language models: A survey on biological & chemical domains. ACM-CSUR. 2025;57(6):1–38.
31. Chen C, Wu Y, Li J, Wang X, Zeng Z, Xu J, et al. TBtools-II: A one for all, all for one bioinformatics platform for biological big-data mining. Mol Plant. 2023;16(11):1733–42.
32. Yang M, Derbyshire MK, Yamashita RA, Marchler-Bauer A. NCBI's conserved domain database and tools for protein domain analysis. Curr protocols Bioinf. 2020;69(1):e90.

33. Horton P, Park K-J, Obayashi T, Fujita N, Harada H, Adams-Collier C, et al. WoLF PSORT: protein localization predictor. Nucleic Acids Res. 2007;35(suppl2):W585–7.
34. Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, et al. SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic Acids Res. 2018;46(W1):W296–303.
35. Amir A, Kapoor N, Kumar H, Tariq M, Siddiqui MA. In silico, Physico-chemical characterization and analysis of Sandalwood proteins. Int J Agricultural Invention. 2018;3(2):150–7.
36. Tusnady GE, Simon I. The HMMTOP transmembrane topology prediction server. Bioinformatics. 2001;17(9):849–50.
37. Blom N, Gammeltoft S, Brunak S. Sequence and structure-based prediction of eukaryotic protein phosphorylation sites. J Mol Biol. 1999;294(5):1351–62.
38. Tamura K, Stecher G, Kumar S. MEGA11: molecular evolutionary genetics analysis version 11. Mol Biol Evol. 2021;38(7):3022–7.
39. Yates AD, Allen J, Amode RM, Azov AG, Barba M, Becerra A, et al. Ensembl Genomes 2022: an expanding genome resource for non-vertebrates. Nucleic Acids Res. 2022;50(D1):D996–1003.
40. Bailey TL, Williams N, Misleh C, Li WW. MEME: discovering and analyzing DNA and protein sequence motifs. Nucleic Acids Res. 2006;34(suppl2):W369–73.
41. Rombauts S, Déhais P, Van Montagu M, Rouzé P. PlantCARE, a plant cis-acting regulatory element database. Nucleic Acids Res. 1999;27(1):295–6.
42. Subramanian B, Gao S, Lercher MJ, Hu S, Chen W-H. Evolview v3: a webserver for visualization, annotation, and management of phylogenetic trees. Nucleic Acids Res. 2019;47(W1):W270–5.
43. Foyer CH. Reactive oxygen species, oxidative signaling and the regulation of photosynthesis. Environ Exp Bot. 2018;154:134–42.
44. Gill SS, Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem. 2010;48(12):909–30.
45. Yu W, Kong G, Chao J, Yin T, Tian H, Ya H, et al. Genome-wide identification of the rubber tree superoxide dismutase (SOD) gene family and analysis of its expression under abiotic stress. PeerJ. 2022;10:e14251.
46. Panchy N, Lehti-Shiu M, Shiu S-H. Evolution of gene duplication in plants. Plant Physiol. 2016;171(4):2294–316.
47. Hayes S, Velanis CN, Jenkins GI, Franklin KA. UV-B detected by the UVR8 photoreceptor antagonizes auxin signaling and plant shade avoidance. Proceedings of the National Academy of Sciences. 2014;111(32):11894-9.
48. Jin H, Zhu X, Zhang M, Zheng H, Chen Y, Yao D, et al. Cu/Zn/histidine supramolecular assemblies with optimized Cu catalytic sites as an alternative of superoxide dismutase. Nat Commun. 2025;16(1):10075.

49. Pathak J, Rajneesh, Ahmed H, Singh DK, Singh PR, Kumar D et al. Oxidative stress and antioxidant defense in plants exposed to ultraviolet radiation. Reactive oxygen, nitrogen and sulfur species in plants: production, metabolism, signaling and defense mechanisms. 2019:371–420.
50. Noctor G, Mhamdi A, Foyer CH. The roles of reactive oxygen metabolism in drought: not so cut and dried. Plant Physiol. 2014;164(4):1636–48.
51. Agati G, Tattini M. Multiple functional roles of flavonoids in photoprotection. New Phytol. 2010;186(4):786–93.
52. Sharma SK, Singh D, Pandey H, Jatav RB, Singh V, Pandey D. An overview of roles of enzymatic and nonenzymatic antioxidants in plant. Antioxid De?F plants: Mol basis Regul. 2022:1–13.
53. Jiao J, Gai QY, Wang W, Luo M, Gu CB, Fu YJ, et al. Ultraviolet Radiation-Elicited Enhancement of Isoflavonoid Accumulation, Biosynthetic Gene Expression, and Antioxidant Activity in Astragalus membranaceus Hairy Root Cultures. J Agric Food Chem. 2015;63(37):8216–24.
54. Patil JR, Mhatre KJ, Yadav K, Yadav LS, Srivastava S, Nikalje GC. Flavonoids in plant-environment interactions and stress responses. Discover Plants. 2024;1(1):68.
55. Javed T, Gao SJ. WRKY transcription factors in plant defense. Trends Genet. 2023;39(10):787–801.
56. Wu Y, Li X, Zhang J, Zhao H, Tan S, Xu W, et al. ERF subfamily transcription factors and their function in plant responses to abiotic stresses. Front Plant Sci. 2022;13:1042084.
57. Cao Y, Mei Y, Zhang R, Zhong Z, Yang X, Xu C, et al. Transcriptional regulation of flavonol biosynthesis in plants. Hortic Res. 2024;11(4):uhae043.