

RESEARCH ARTICLE

Regulatory mechanisms of amino acid metabolic pathways in *Rhododendron chrysanthum* Pall. Under UV-B stress

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Abstract

UV-B radiation, worsened by ozone layer depletion, threatens plant health. The alpine plant *Rhododendron chrysanthum* has evolved mechanisms to counteract UV-B damage. This study examined the response of *R. chrysanthum* seedlings to UV-B radiation in an artificial climate chamber, focusing on the molecular mechanisms through metabolomics and transcriptomics. We identified 2 distinct amino acids, 35 differentially expressed genes (DEGs), and 2 families of transcription factors (TFs), which involved in amino acids metabolic pathways, including the biosynthesis of phenylalanine, tyrosine, and tryptophan, as well as phenylpropanoid biosynthesis and phenylalanine metabolism. Under UV-B stress, bHLH TFs significantly correlated with the expression of aroD, aroE, TAT, TRP3, trpD, DDC/TDC, PAAS, HCT, CCR, and COMT, thereby accumulating L-phenylalanine and L-tyrosine levels. WRKY TFs significantly correlated with the expression of all these enzymes except COMT, thus accumulating the levels of L-phenylalanine and L-tyrosine. These two families of TFs exhibit both synergistic and antagonistic regulatory roles in amino acid metabolic pathways. The findings presented are significant not only for understanding the UV-B resistance of *R. chrysanthum* but also for serving as a reference for the study of other plant species. This research will contribute to the theoretical foundation necessary for the cultivation of plant varieties with enhanced UV-B stress tolerance.

Introduction

Since the discovery of the Antarctic ozone hole in 1985, stratospheric ozone depletion has become a major global issue, primarily due to increased ultraviolet (UV) radiation reaching the Earth's surface. High levels of UV-B radiation led plants to produce reactive oxygen species (ROS). To mitigate the harmful effects of UV-B, plants synthesize enzymes and metabolites that scavenge ROS or absorb UV-B [1,2].

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UV-B radiation exerts a regulatory influence on plant genomic expression and metabolic processes. Logemann et al. (2000) demonstrated that UV radiation induces the up-regulation of genes encoding key enzymes involved in phenolic synthesis, including 6-phosphogluconate dehydrogenase (6-PDH), 3-deoxy-D-arabinoheptanone glycolate 7-phosphosynthase (DAHPS), aconitase (ACO), phenylalanine ammonia-lyase (PAL), and chalcone synthase (CHS) in celery suspension cells [3]. Sun et al. (2022) reported significant reprogramming of amino acid and carbohydrate metabolic pathways, as well as alterations in gene expression, in the leaves of *Rhododendron chrysanthum* subjected to UV-B stress [4]. Furthermore, Yu et al. (2025) elucidated the critical roles of the flavonoid synthesis pathway and WRKY transcription factors in conferring UV-B resistance in *R. chrysanthum* [5]. Several studies employing multi-omics techniques have highlighted the remodeling of plant metabolic networks in response to UV-B stress. For instance, *Arabidopsis thaliana* exhibited distinct transcriptomic and metabolomic responses to UV-LED irradiation at wavelengths of 280 nm and 310 nm [6]. The Antarctic moss, *Leptobryum pyriforme*, showed significant alterations in gene expression and metabolic profiles following exposure to UV-B radiation [7]. Additionally, the *FtMYB4R1* gene in Tartary buckwheat has been shown to respond to UV-B by regulating flavonoid synthesis [8]. In *Betula platyphylla*, the mitogen-activated protein kinase (MAPK) pathway was utilized to enhance xylem and cell wall integrity, thereby mitigating the effects of UV-B exposure [9].

Plant defense mechanisms against UVB damage encompass the synthesis of UV-absorbing compounds, secondary metabolism, and the regulation of signaling networks. A significant response to UV-B exposure is the increased concentration of UV absorbers in leaves, including flavanols, anthocyanin glycosides, flavonoids, and carotenoids, which serve to protect plant cells from damage [10,11]. Secondary metabolites function as signaling molecules that promote the expression of resistance genes through the regulation of transcription factors [12]. UVB exposure has been shown to induce the synergistic expression of seven structural genes involved in flavonoid synthesis (e.g., CHI, C4H) in the healing tissues of *Mirabilis himalaica*, in conjunction with the bHLH128 transcription factor [13]. Furthermore, UVB irradiation has been observed to alter amino acid metabolism in *Phoenix dactylifera*, impacting the synthesis pathways of secondary metabolites such as flavonoids and phenylpropanes; differentially expressed genes included those related to transcription factors and chloroplast functions [14]. Additionally, UVB radiation induced modifications in the mitochondria of *R. chrysanthum*, which enhanced ATP production and regulated monoterpene biosynthesis pathways, resulting in the accumulation of indole alkaloids [15].

R. chrysanthum, endemic to the Changbai Mountains in China, exhibits remarkable adaptability to extreme environmental conditions, including low winter temperatures, reduced atmospheric pressure, and elevated levels of solar and UV-B radiation, which are significant abiotic stress factors [4]. Consequently, *R. chrysanthum* serves as an important model organism for investigating the mechanisms underlying plant resistance to UV-B radiation.

Current research has identified specific responses to UV-B stress in *R. chrysanthum*, which encompass the regulation of phenylpropanoid and energy metabolism, photosynthesis, hormone networks, and protein modifications, including abscisic acid (ABA) signaling. For instance, UV-B exposure activates key enzyme genes within the phenylpropanoid pathway, such as 4-coumarate-CoA ligase (4CL) and cinnamate-4-hydroxylase (CCR), through the synergistic regulation of the MYB-bHLH-WD40 complex and ABA signaling [16]. Additionally, the remodeling of gene and protein expression in glycolysis and the tricarboxylic acid (TCA) cycle pathways is essential for maintaining energy metabolism homeostasis [17]. G2-like transcription factors contribute to the maintenance of photosynthetic efficiency by regulating the Calvin cycle, while cytokinins and salicylic acid mitigate photo-oxidative damage through both positive and negative regulatory mechanisms [18,19]. Furthermore, acetylation modifications of calcium signaling-related proteins, such as calmodulin-like proteins (CML) and calcium-dependent protein kinase 1 (CPK1), are positively correlated with the accumulation of phenolic compounds [20]. Changes in the expression of 807 proteins and 685 acetylated proteins are implicated in the protection of photosynthesis, with acetylation modifications alleviating UV-B-induced inhibition of photosynthetic activity [21]. ABA plays a role in photosynthetic protection by promoting phenolic synthesis to enhance stress tolerance, and its signaling pathway is modulated by phosphorylation to regulate stomatal responses and overall stress tolerance [22,23].

Despite the insights gained from multi-omics studies regarding the various plant response mechanisms to UV-B radiation, a comprehensive analysis of the systemic defense network in plants against UV-B stress remains insufficient. In particular, the hierarchical regulatory mechanisms involving transcription factors and metabolic pathways warrant further investigation. This study aims to explore the key genes, metabolites, and regulatory networks associated with UV-B stress in *R. chrysanthum*, with a specific emphasis on the reprogramming of amino acid metabolism and the role of regulatory factors. This will be achieved through a joint analysis of transcriptomic and metabolomic data. The findings of this research are expected to provide a theoretical foundation for the cultivation of stress-resistant plant varieties and for ecological restoration efforts in high-altitude arid regions. Furthermore, the results will contribute to sustainable agricultural development and ecological conservation initiatives.

Materials and methods

Plant materials and treatment

The culture conditions and radiation treatments for *R. chrysanthum* seedlings in this study followed the protocol established by Yu et al. (2025) [5]. No field research was conducted because the study exclusively utilized laboratory-cultivated seedlings; consequently, no field permits were required. *R. chrysanthum* seedlings were cultured in artificial climate chambers at 18 °C during the day, with 14 hours of white light, 16 °C at night, 10 hours of darkness, and 60% relative humidity, using 1/4 MS medium. After growing into 8-month-old seedlings, they received radiation treatments. Eight-month-old *R. chrysanthum* seedlings were divided into two groups for radiation treatment. Group M received 50 μmol (photon)/ $\text{m}^2\cdot\text{s}$ PAR, while group N received the same PAR plus 2.3 W/m^2 UV-B. A 400 nm long-pass filter was used for group M and a 295 nm filter for group N. For two days, PAR was irradiated for 48 h in both M and N groups, but UV-B was irradiated for only 8 hours per day in N group. *R. chrysanthum* leaves were collected for transcriptomics and metabolomics analyses. The experiment was repeated three times. The models of long-pass filters and lamps for light sources were detailed in Lyu et al. (2019) [9]. *R. chrysanthum* leaves were stored in liquid nitrogen for transcriptomics and metabolomics analyses.

Widely targeted metabolomics assay

We followed the experimental procedures established by Yu et al. (2025) [5]. *R. chrysanthum* samples were freeze-dried and ground to a powder for 1.5 minutes at 30 Hz using a lyophilizer (Scientz-100F, Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China) and a grinder (MM400, Retsch, Haan, Germany). The samples were ground into a powder at 30 Hz for 1.5 minutes. Fifty mg of powder was weighed, and 1200 μL of 70% methanol was added. After vortexing, the sample

was centrifuged at 12,000 rpm for 3 minutes, filtered through a 0.22 μ m membrane, and stored in an injection vial for liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) analysis. Metabolite profiling data were acquired, and the chromatographic peaks were integrated and adjusted in accordance with Maiwei's WMDB (Metware Database). Metabolites with a variable importance in projection (VIP) > 1 and Fold Change (FC; experimental/control group; N/M) $\geq$ 1.5/FC $\leq$ 0.67 were identified as differential metabolites.

Transcriptomics assay

RNA sequencing was conducted by Shenzhen Huada Gene Science and Technology Research Co. (Shenzhen, China), following methodologies established Yu et al. (2025) [5]. Total RNA was extracted from *R. chrysanthum* leaves using the Tiangen Nano Nano RNA Extraction Kit (DP441). RNA purity and concentration were measured with a NanoDrop 2000 spectrophotometer. The single-strand circular DNA libraries were prepared for analysis on the MGISEQ-2000 platform. Transcriptome sequencing was performed at BGI Genomics Co., Ltd. (Beijing China). The FPKM assessed transcript expression changes post-treatment, while DESeq2 identified differentially expressed genes in *R. chrysanthum* with a fold change (FC) > 1 and a q-value < 0.05. Functional annotation and classification of unigenes used public databases like KEGG, Pfam, and SwissProt, with BLAST for sequence alignment. The Illumina HiSeq platform produced 45.44 million raw reads per sample, yielding 42.21 to 43.51 million clean reads and 6.33 to 6.53 gigabases (Gb) of clean bases, indicating high-quality sequencing.

Statistical analysis

Metabolomics results were plotted using Metware Cloud (<https://cloud.metware.cn>). Boxplots, heatmaps, and histograms were created with OriginPro 2024b. MetaboAnalyst 5.0 was used for VIP value. A VIP plot ranked metabolites based on their discrimination ability between the M and N groups of *R. chrysanthum*. The VIP score is a weighted sum of the squares of the PLS loadings, influenced by the explained Y-variance [5]. The OPLS-DA model was created using the MetaboAnalyst R package, version 1.0.1, after log2 transformation and centering of the raw data. Transcriptomic data were analyzed with the Dr. Tom platform. Pearson correlation analysis assessed correlations between significant metabolites and DEGs from RNA-seq, using a threshold of 0.85 and p < 0.05. Cytoscape (v3.9.1) was used for visualizing correlation networks.

Results

UV-B radiation has a significant impact on the levels of amino acids and derivatives

This study assessed the effects of UV-B radiation on metabolites in *R. chrysanthum* within the M and N groups. Principal component analysis (PCA) revealed strong intra-group repeatability and significant inter-group differences (S1 Fig). Yu et al. (2025) employed orthogonal partial least squares discriminant analysis (OPLS-DA) to identify metabolites associated with leaf UV-B resistance by examining differences between the M and N groups. Their findings indicated that the first principal component of the OPLS-DA analysis explained 28.9% of the variance, while the second principal component accounted for 28.3%, demonstrating reliable results. Differential metabolites were identified with fold change (FC) criteria of $\geq$ 1.5, $\leq$ 0.67, and Variable Importance in Projection (VIP) > 1. The analysis indicated an increase in 355 metabolites and a decrease in 167 under UV-B stress (S1 Fig), with phenolic acids and flavonoids being the most affected, followed by amino acids and derivatives (S1 Fig).

This study identifies 51 differential metabolites (DMs) related to amino acids and derivatives through widely targeted metabolomics (S1 Table in S1 File), with 26 having a cpd_ID and 25 lacking one (S1-S2 Table in S1 File). Fifteen amino acids and derivatives were annotated in KEGG (Fig 1). Two amino acids increased after UV-B stress, linked to three pathways (ko00940, ko00400, ko00360), while thirteen decreased, associated with seven pathways (ko00220, ko01100, ko00310, ko00300, ko00470, ko01110, ko01230). The latter 7 pathways were not analyzed in this study due to the

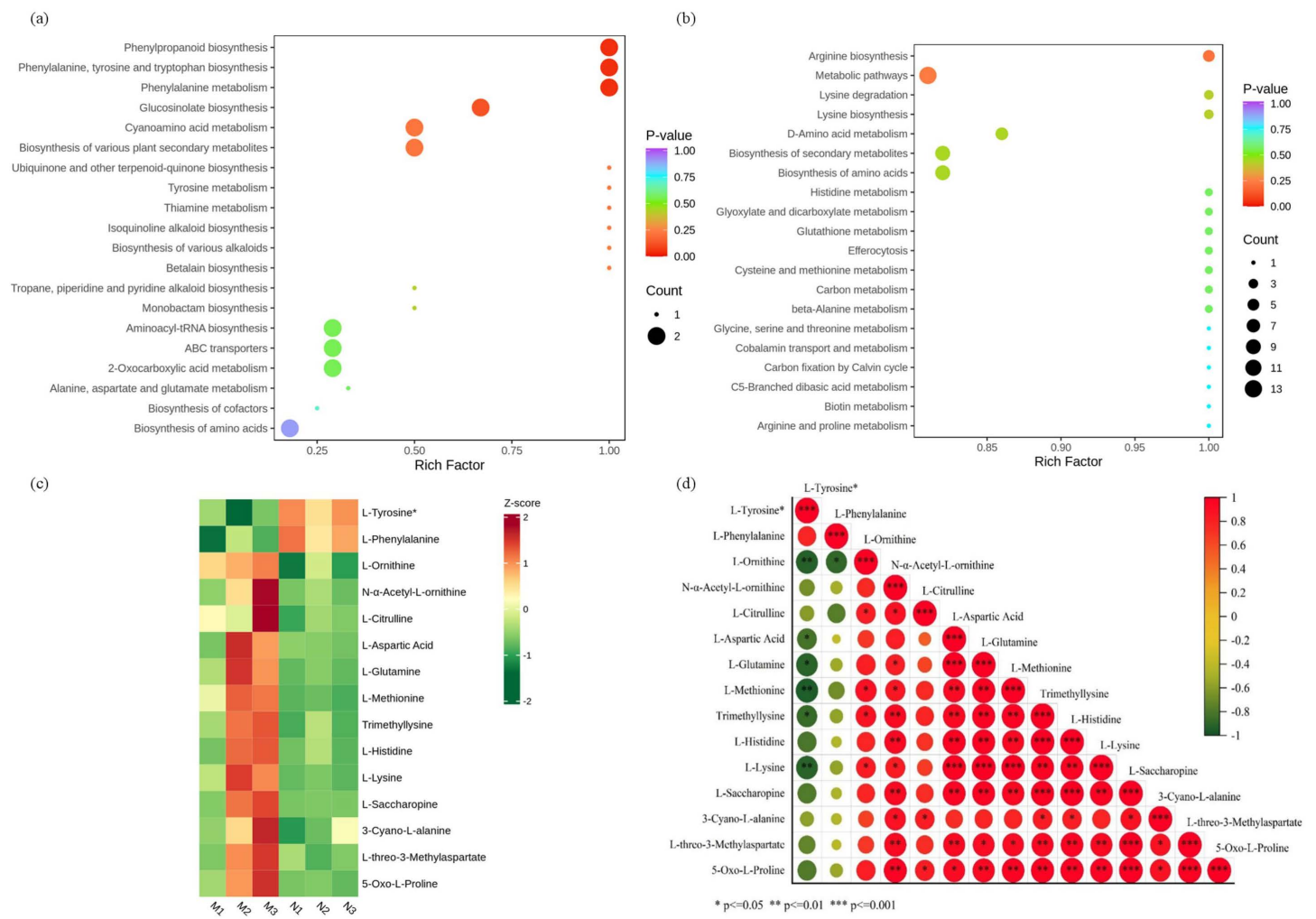


Fig 1. Significant changes in amino acids and derivatives in *R. chrysanthum* leaves under UV-B stress. (a) Bubble map of KEGG enrichment for increased amino acids; (b) Bubble map of KEGG enrichment for decreased amino acids; (c) Heat map clustering of 15 differential amino acids annotated to KEGG; (d) Correlation analysis of 15 differential amino acids annotated to KEGG. Bubble color indicates pathway enrichment significance, while red and green in the heatmap represent high and low metabolite content, respectively. Asterisks indicate significance levels: * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$.

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absence of differentially expressed genes (DEGs) in ko00220, ko00300, and ko00470, the integrative nature of ko01100 and ko01110, the lack of labeled enzymes in ko01230, and only five DEGs in ko00310. In contrast, the former 3 pathways related to L-tyrosine and L-phenylalanine (ko00940, ko00400, ko00360) merit further investigation, as they do not have these issues. Both amino acids increased after UV-B stress and were significantly linked to phenylpropanoid biosynthesis (ko00940), the biosynthesis of phenylalanine, tyrosine, and tryptophan (ko00400), and phenylalanine metabolism (ko00360) (Fig 1a-b and S3 Table in S1 File).

A correlation analysis was conducted to clarify the relationships among 15 amino acids and derivatives (Fig 1D), summarized in S4 Table in S1 File. L-phenylalanine and L-ornithine showed a significant negative correlation ($p \leq 0.05$). L-tyrosine was significantly negatively correlated with six metabolites: L-ornithine, L-methionine, L-lysine, L-aspartic acid, L-glutamine, and trimethyllysine ($p \leq 0.01$; $p \leq 0.05$).

UV-B radiation significantly impacted gene expression related to amino acids

Transcriptome analysis was performed on two groups, M and N, of *R. chrysanthum* to identify genes responsive to UV-B stress. A total of 2,348 differentially expressed genes (DEGs) were identified using a Q-value threshold of less than 0.05 and a fold change greater than 1, including 1,157 upregulated and 1,191 down-regulated DEGs. Among these, 531 DEGs associated with 15 distinct amino acids and derivatives were annotated in the KEGG database (S5 Table in S1 File). Venn diagram analysis revealed that 35 DEGs were related to L-phenylalanine and L-tyrosine (Fig 2a, S6-S7 Table in S1 File). Their expression patterns are shown in a heat map (Fig 2b). Of the 35 DEGs, transcriptional profiling indicated a distinct expression bias: 21 genes (60%) were significantly upregulated, while 14 genes (40%) were down-regulated, as detailed in Fig 2c. This imbalance in activation/suppression ratios suggests a potential prioritization of specific biological pathways under the experimental conditions.

Through protein-protein interaction (PPI) network analysis of 35 candidate genes, a strong correlation was identified between TRINITY_DN2498_c0_g2_i1-B_1 and TRINITY_DN8131_c0_g1_i1-B_2 in the phenylpropanoid biosynthesis pathway. PPI analysis also revealed notable correlations between TRINITY_DN959_c0_g1_i1-B_2 and two metabolic regulators (TRINITY_DN21925_c0_g1_i1-C_1 and TRINI-TY_DN24311_c0_g1_i1-C_1), as shown in Fig 2d and detailed in Table 1. Additionally, in the phenylalanine, tyrosine, and tryptophan biosynthesis pathway, TRINITY_DN5453_c1_g1_i1-C_1 exhibited a strong interaction (967) with TRINI-TY_DN11006_c0_g1_i1-A_2 (Fig 2e, Table 1).

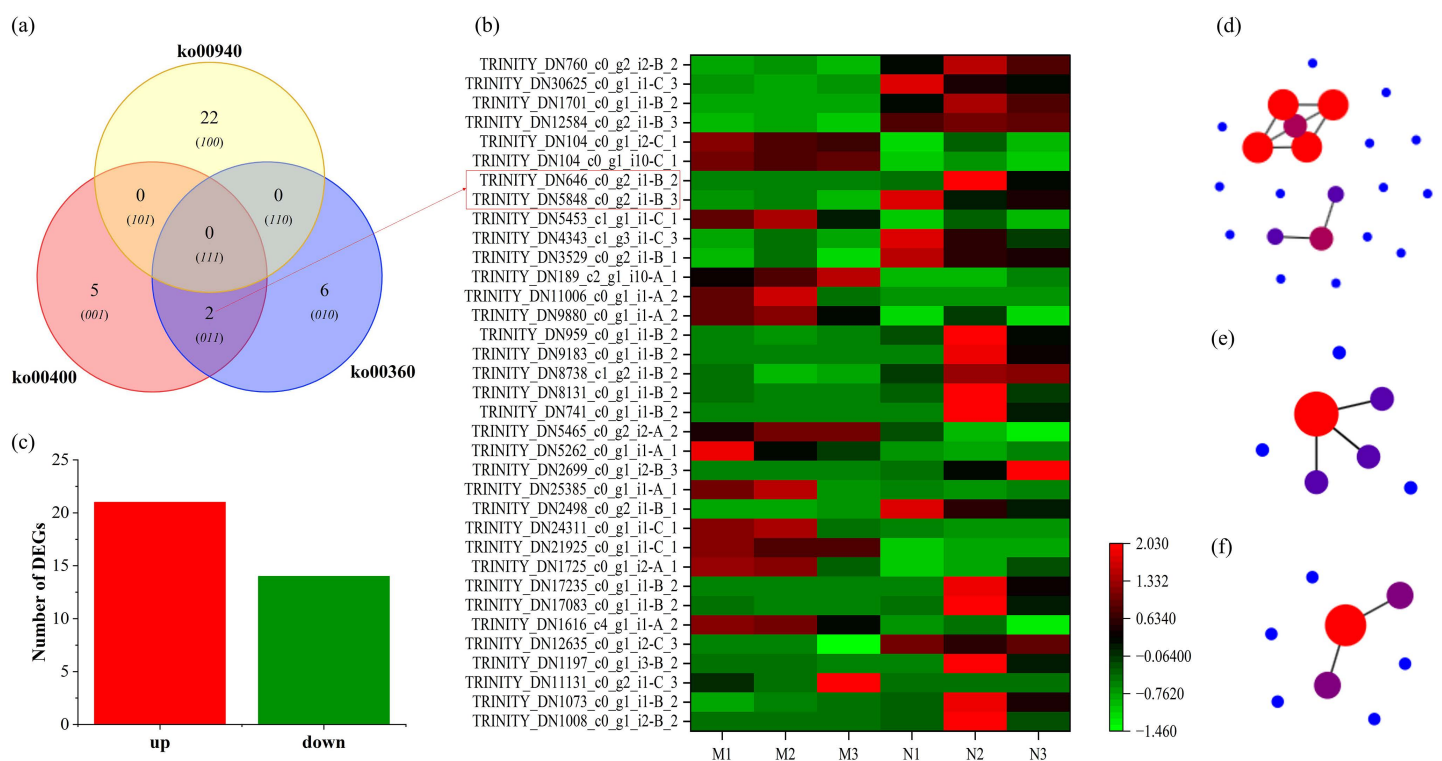


Fig 2. Changes in differential expressed genes in *R. chrysanthum* following UV-B stress. (a) Venn diagram of 35 DEGs linked to 15 differential amino acids and derivatives annotated in the KEGG database. (b) Heat map of 35 DEGs linked to L-phenylalanine and L-tyrosine synthesis. (c) Statistics on 35 DEGs related to L-phenylalanine and L-tyrosine. (d) Protein-protein interaction network of 22 DEGs in phenylpropanoid biosynthesis. (e) Protein-protein interaction network map of 7 DEGs involved in phenylalanine, tyrosine, and tryptophan biosynthesis. (f) Protein-protein interaction network map of 8 DEGs in phenylalanine metabolism. In the heatmap, redder colors indicate higher DEG levels, and greener colors indicate lower levels.

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Table 1. Protein–protein interaction scores for 35 DEGs linked to L-phenylalanine and L-tyrosine.

KEGG pathway	Gene1	Gene2	Interaction Score
phenylpropanoid biosynthesis (ko00940)	TRINITY_DN2498_c0_g2_i1-B_1	TRINITY_DN8131_c0_g1_i1-B_2	983
	TRINITY_DN959_c0_g1_i1-B_2	TRINITY_DN21925_c0_g1_i1-C_1	961
	TRINITY_DN959_c0_g1_i1-B_2	TRINITY_DN24311_c0_g1_i1-C_1	961
	TRINITY_DN1616_c4_g1_i1-A_2	TRINITY_DN21925_c0_g1_i1-C_1	945
	TRINITY_DN1616_c4_g1_i1-A_2	TRINITY_DN24311_c0_g1_i1-C_1	945
	TRINITY_DN959_c0_g1_i1-B_2	TRINITY_DN1616_c4_g1_i1-A_2	937
	TRINITY_DN17235_c0_g1_i1-B_2	TRINITY_DN21925_c0_g1_i1-C_1	897
	TRINITY_DN17235_c0_g1_i1-B_2	TRINITY_DN24311_c0_g1_i1-C_1	897
	TRINITY_DN1008_c0_g1_i2-B_2	TRINITY_DN2498_c0_g2_i1-B_1	884
phenylalanine, tyrosine and tryptophan biosynthesis (ko00400)	TRINITY_DN5453_c1_g1_i1-C_1	TRINITY_DN11006_c0_g1_i1-A_2	967
	TRINITY_DN5848_c0_g2_i1-B_3	TRINITY_DN5453_c1_g1_i1-C_1	863
	TRINITY_DN646_c0_g2_i1-B_2	TRINITY_DN5453_c1_g1_i1-C_1	863
phenylalanine metabolism (ko00360)	TRINITY_DN12584_c0_g2_i1-B_3	TRINITY_DN5848_c0_g2_i1-B_3	792
	TRINITY_DN12584_c0_g2_i1-B_3	TRINITY_DN646_c0_g2_i1-B_2	792

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As shown in [Tables 1](#) and [2](#), in the phenylpropanoid biosynthesis pathway, TRINITY_DN2498_c0_g2_i1-B_1 and TRINITY_DN8131_c0_g1_i1-B_2 interact directly, encoding coniferyl-alcohol glucosyltransferase (UGT72E) and peroxidase (E1.11.1.7), respectively. In the phenylalanine, tyrosine, and tryptophan biosynthesis pathway, TRINITY_DN5453_c1_g1_i1-C_1 interacts with TRINITY_DN11006_c0_g1_i1-A_2 and TRINITY_DN5848_c0_g2_i1-B_3. The former encodes anthranilate synthase component I (TRP3), and the latter encode anthranilate phosphoribosyltransferase (trpD) and tyrosine aminotransferase (TAT). In the phenylalanine metabolism pathway, TRINITY_DN12584_c0_g2_i1-B_3 and TRINITY_DN5848_c0_g2_i1-B_3 interact directly, encoding primary-amine oxidase (AOC3/AOC2/tynA) and TAT, respectively. [Table 2](#) presents information on DEGs encoding 15 enzymes related to L-phenylalanine and L-tyrosine.

Differential amino acids and derivatives correlate with differentially expressed genes

After identifying two KEGG-annotated metabolites with significant accumulation, we conducted systematic multi-omics integration to resolve metabolite-gene regulatory relationships across three stress-responsive pathways ([Fig 3](#)). Network topology analysis revealed distinct interaction patterns: (1) Phenylpropanoid biosynthesis exhibited the highest connectivity with 13 significant metabolite-gene interactions (5 positive and 8 negative correlations), suggesting complex feedback regulation ([Fig 3a](#)). (2) Phenylalanine, tyrosine, and tryptophan biosynthesis contained 6 directed edges (balanced 3:3 positive and negative correlations), indicating potential compensatory regulation of aromatic amino acid precursors ([Fig 3b](#)). (3) Phenylalanine metabolism demonstrated 10 coordinated relationships (6 positive and 4 negative), with particularly dense connectivity around tyrosine nodes. Notably, gene families associated with L-tyrosine bio-synthesis were more represented than those linked to L-phenylalanine, implying a metabolic prioritization of tyrosine-derived compounds (e.g., ROS-scavenging secondary metabolites) during UV-B adaptation ([Fig 3c](#)).

bHLH and WRKY transcription factors are key regulators in *R. chrysanthum*'s UV-B adaptation

To examine changes in the amino acids-related pathways of *R. chrysanthum* under UV-B stress, we identified DEGs classified as TFs ([Fig 4a-b](#)). Multi-omics correlation analysis ([Fig 5](#)) identified nine key TFs based on edge density: (1) Core signaling modules: bHLH (128) and WRKY (121); (2) Secondary metabolic regulators: GRAS (96), AP2-EREBP (91), and NAC (87); (3) Novel candidates: C2C2-GATA (75), G2-like (66), TCP (58), and MYB (51) ([Fig 4c](#), [Fig 5a-i](#)). Notably, bHLH

Table 2. Information regarding the genes that encode essential enzymes involved in the amino acid metabolic pathways.

Name	Enzyme	Gene ID	log2 (N/M)	Q-Value (M vs. N)
peroxidase	[EC:1.11.1.7]	TRINITY_DN21925_c0_g1_i1-C_1	-1.401	0.015
		TRINITY_DN741_c0_g1_i1-B_2	8.033	0.008
		TRINITY_DN8131_c0_g1_i1-B_2	4.883	0.006
cinnamoyl-CoA reductase	[EC:1.2.1.44]	TRINITY_DN8738_c1_g2_i1-B_2	2.144	0.005
caffeic acid 3-O-methyltransferase/ acetylserotonin O-methyltransferase	[EC:2.1.1.68/ 2.1.1.4]	TRINITY_DN9880_c0_g1_i1-A_2	-0.965	0.020
shikimate O-hydroxycinnamoyltransferase	[EC:2.3.1.133]	TRINITY_DN11131_c0_g2_i1-C_3	-6.092	0.020
coniferyl-alcohol glucosyltransferase	[EC:2.4.1.111]	TRINITY_DN2498_c0_g2_i1-B_1	1.268	0.012
cinnamyl-alcohol dehydrogenase	[EC:1.1.1.195]	TRINITY_DN959_c0_g1_i1-B_2	1.469	0.048
caffeoyl-CoA O-methyltransferase	[EC:2.1.1.104]	TRINITY_DN5262_c0_g1_i1-A_1	-1.753	0.009
anthranilate phosphoribosyltransferase	[EC:2.4.2.18]	TRINITY_DN11006_c0_g1_i1-A_2	-2.236	0.001
anthranilate synthase component I	[EC:4.1.3.27]	TRINITY_DN5453_c1_g1_i1-C_1	-1.503	0.009
3-dehydroquinate dehydratase/ shikimate dehydrogenase	[EC:4.2.1.10/ 1.1.1.25]	TRINITY_DN3529_c0_g2_i1-B_1	1.506	0.000
3-dehydroquinate dehydratase/ shikimate dehydrogenase	[EC:4.2.1.10/ 1.1.1.25]	TRINITY_DN4343_c1_g3_i1-C_3	1.078	0.042
tyrosine aminotransferase	[EC:2.6.1.5]	TRINITY_DN5848_c0_g2_i1-B_3	1.248	0.004
aromatic-L-amino-acid/L-tryptophan decarboxylase	[EC:4.1.1.28/ 4.1.1.105]	TRINITY_DN104_c0_g1_i10-C_1	-3.490	0.000
aromatic-L-amino-acid/L-tryptophan decarboxylase	[EC:4.1.1.28/ 4.1.1.105]	TRINITY_DN104_c0_g1_i2-C_1	-2.059	0.009
primary-amine oxidase	[EC:1.4.3.21]	TRINITY_DN12584_c0_g2_i1-B_3	0.703	0.000
phenylacetaldehyde synthase	[EC:4.1.1.109]	TRINITY_DN30625_c0_g1_i1-C_3	1.488	0.001

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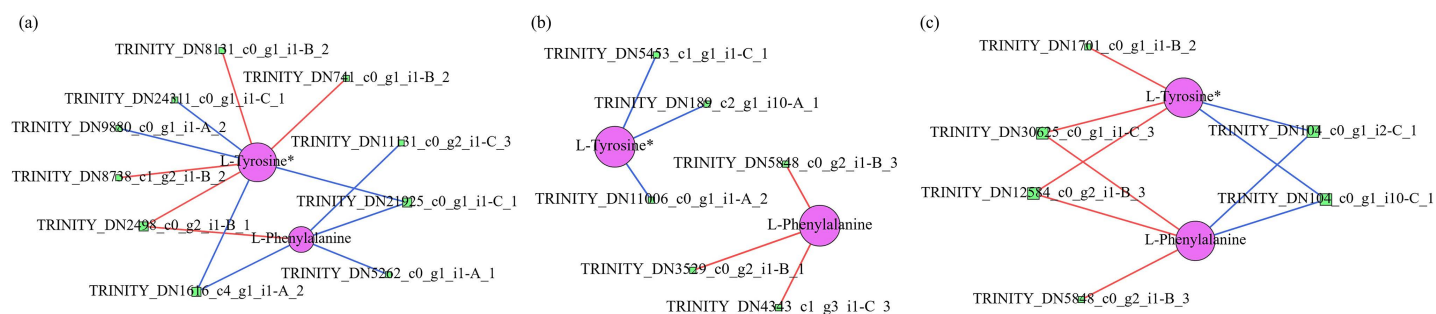


Fig 3. Transcriptomic and metabolomic analysis. (a–c) Correlation analysis of metabolites with ko00940, ko00400, and ko00360 pathway-related genes. Pearson correlation analysis was conducted with a threshold of 0.8, $p < 0.05$. Pink nodes represent metabolites, green nodes represent genes, red edges indicate positive correlations, and blue edges indicate negative correlations.

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and WRKY TFs exhibited the highest edge densities, highlighting their pivotal roles in orchestrating stress-responsive transcriptional reprogramming under UV-B stress.

Within the bHLH TF family, eleven members form a robust interaction network involving 2 amino acids and 30 DEGs, totaling 109 interaction edges. Four core TFs, including TRINITY_DN5624_c0_g2_i2-C_2, each contribute 13–14 interactions, serving as key nodes in this regulatory network. Similarly, 10 WRKY family TFs create 102 interaction edges with the same amino acids and DEGs. Notably, TRINITY_DN321_c1_g2_i1-B_3 (19 edges) and TRINITY_DN21369_c0_g1_i1-C_3 (15 edges) act as central molecules, highlighting their dominant roles in the network (Fig. 5a–b).

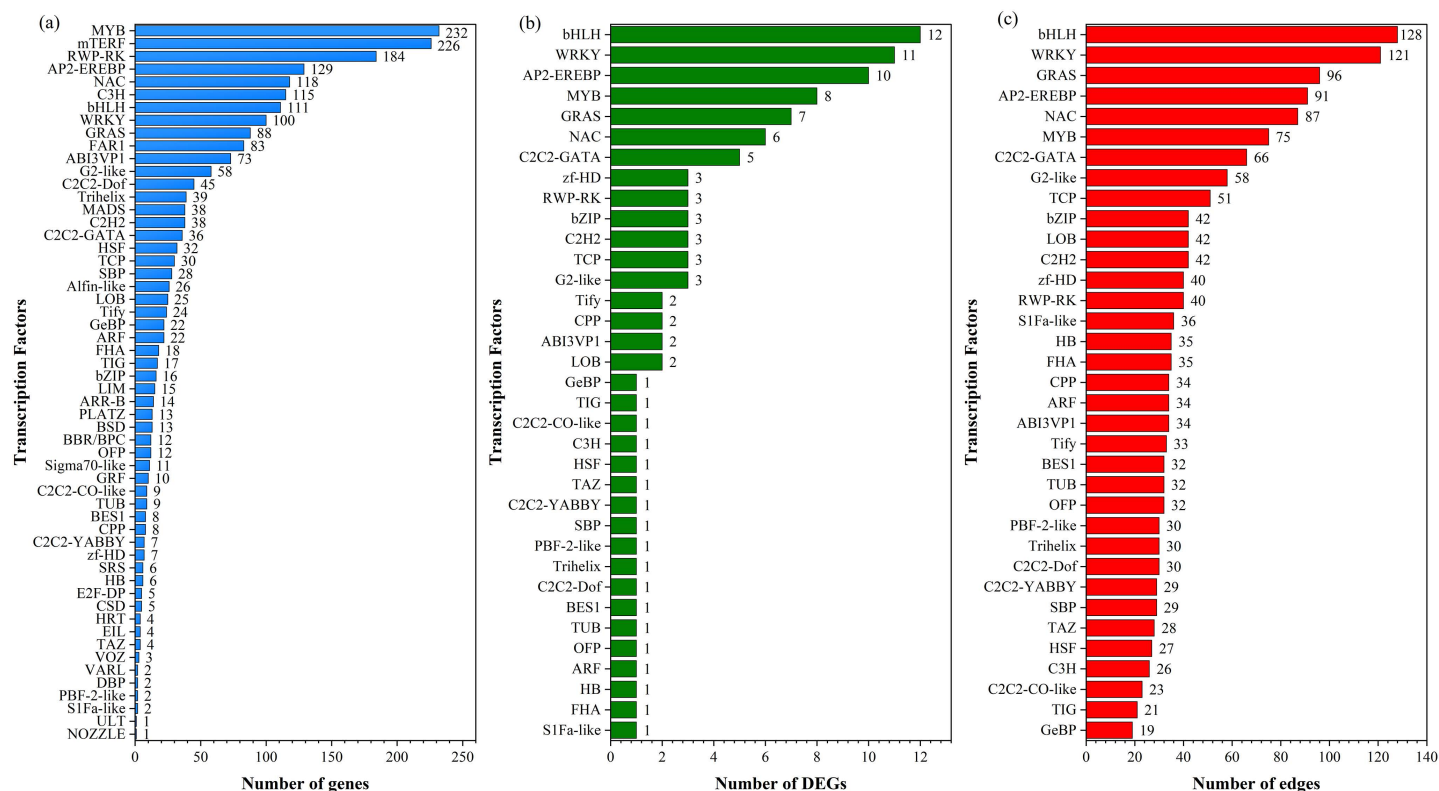


Fig 4. Transcription factor analysis. (a) Number of genes identified as transcription factors (TFs); (b) Number of differentially expressed genes (DEGs) identified as TFs; (c) Edges from the multi-omics correlation analysis of 2 differential metabolites (2 DMs), 35 DEGs, and 35 TFs.

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Secondary metabolism regulatory module includes transcription factors from the GRAS (6 TFs) and AP2-EREB (7 TFs) families, forming 77 and 72 interaction edges, respectively, with 2 amino acids and 29 DEGs, highlighting their role in secondary metabolism regulation. Additionally, six NAC family transcription factors interacted with L-phenylalanine and 23 DEGs, creating 68 interaction edges, suggesting their involvement in secondary metabolite synthesis via phenylalanine metabolism regulation (Fig. 5c-e).

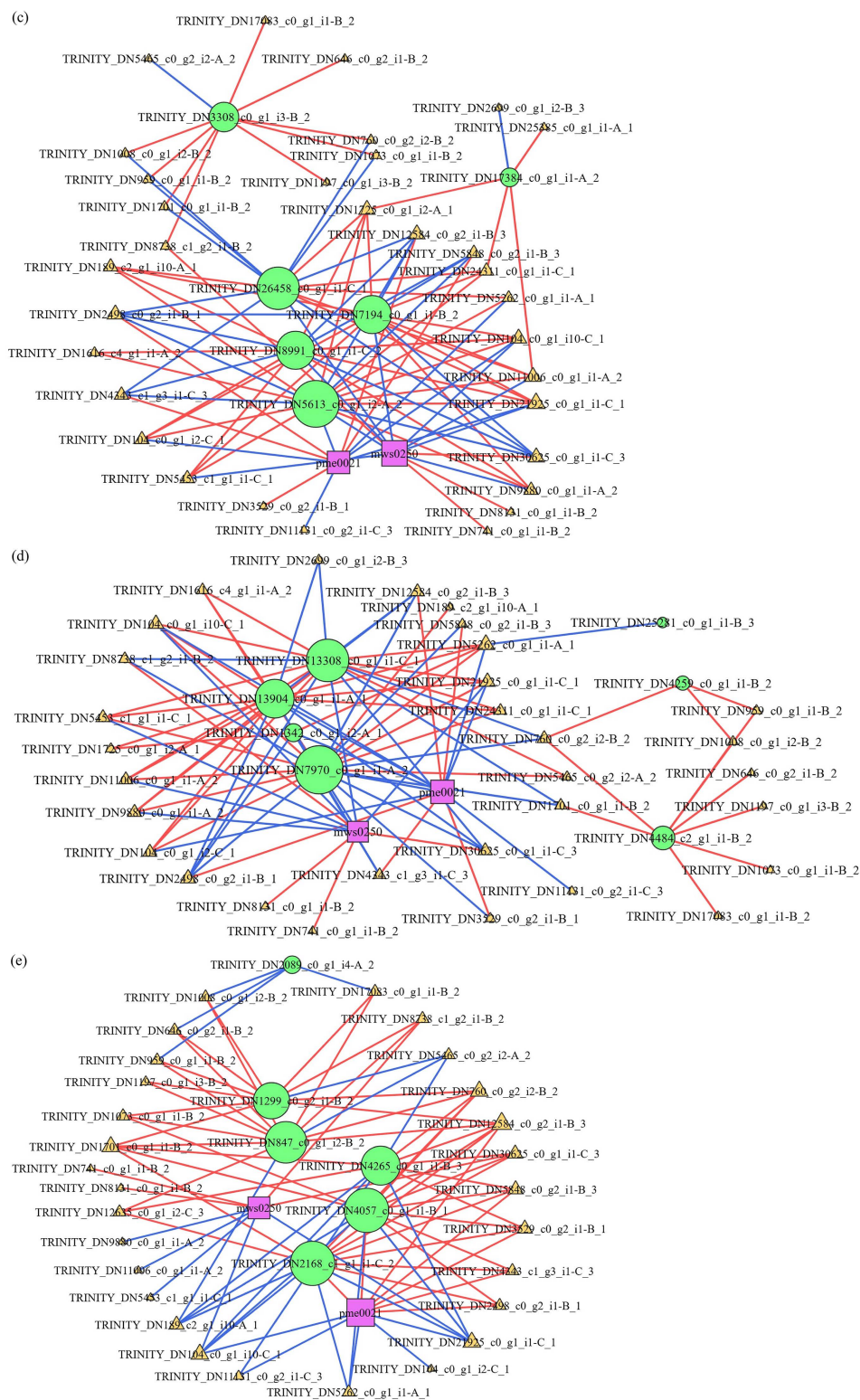
Seven MYB TF family formed 56 interaction edges involving two amino acids and 24 DEGs, regulating distinct metabolic pathways. The C2C2-GATA and G2-like TF families were linked to L-tyrosine and 26 DEGs, while the TCP family targeted L-phenylalanine and 25 DEGs. Core TFs from these families coordinate the regulation of specific amino acids, creating specialized regulatory branches that control amino acid metabolism and downstream DEG expression (Fig. 5f-i).

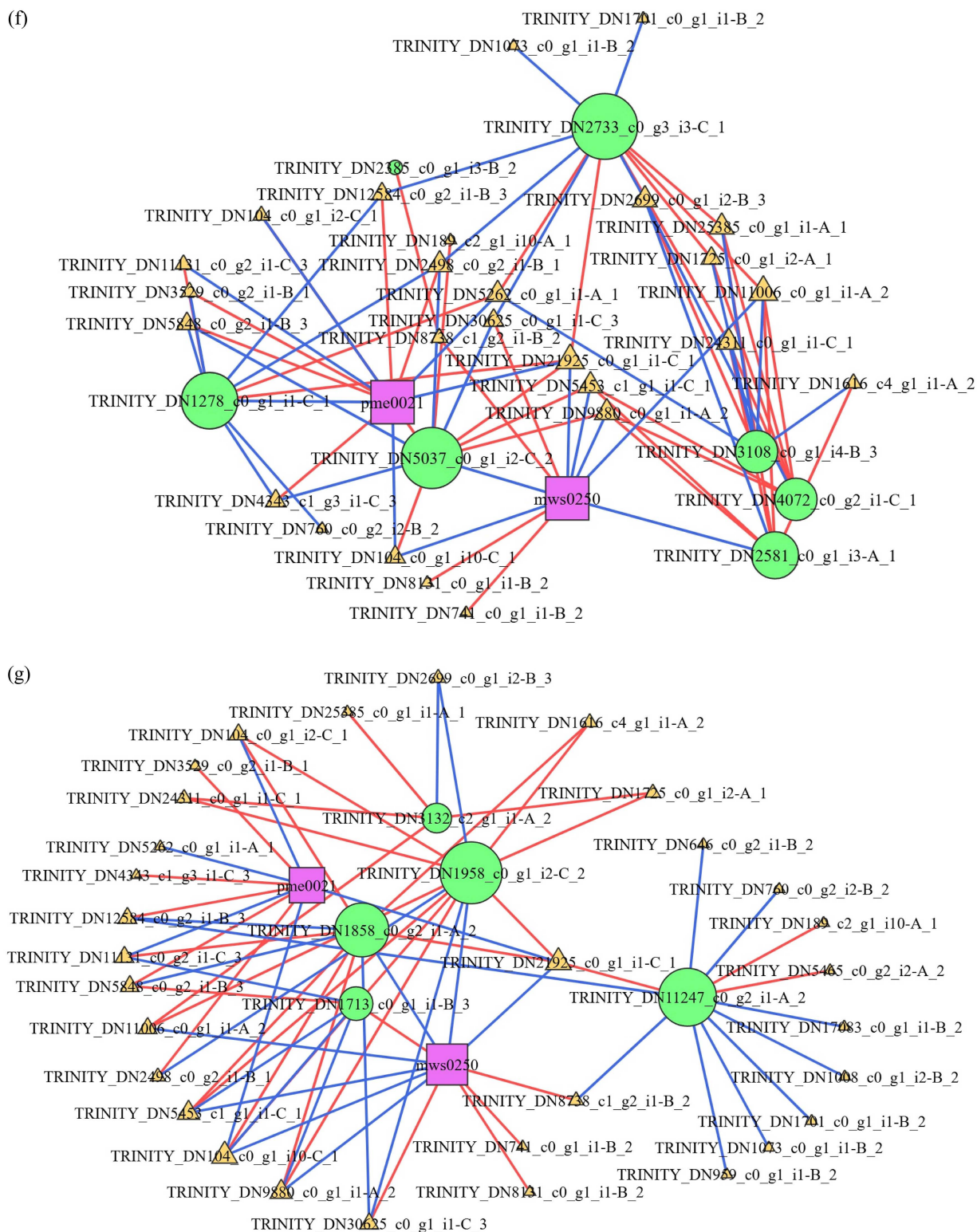
This analysis identifies key regulatory nodes, marked by TFs with strong interactions, along with distinctive molecules such as amino acids and DEGs across nine TF families. It reveals a hierarchical regulatory network involving TFs, amino acids, and DEGs, providing crucial targets to understand plant metabolic regulation and pinpoint key pathways.

bHLH and WRKY TFs potentially regulate key enzyme genes in amino acid synthesis pathways

Thirty-five DEGs were linked to three KEGG-enriched amino acid pathways, with 18 DEGs encoding enzymes (Table 2). We correlated the significantly altered bHLH and WRKY TFs after UV-B irradiation with these 18 DEGs (Fig 6–8).







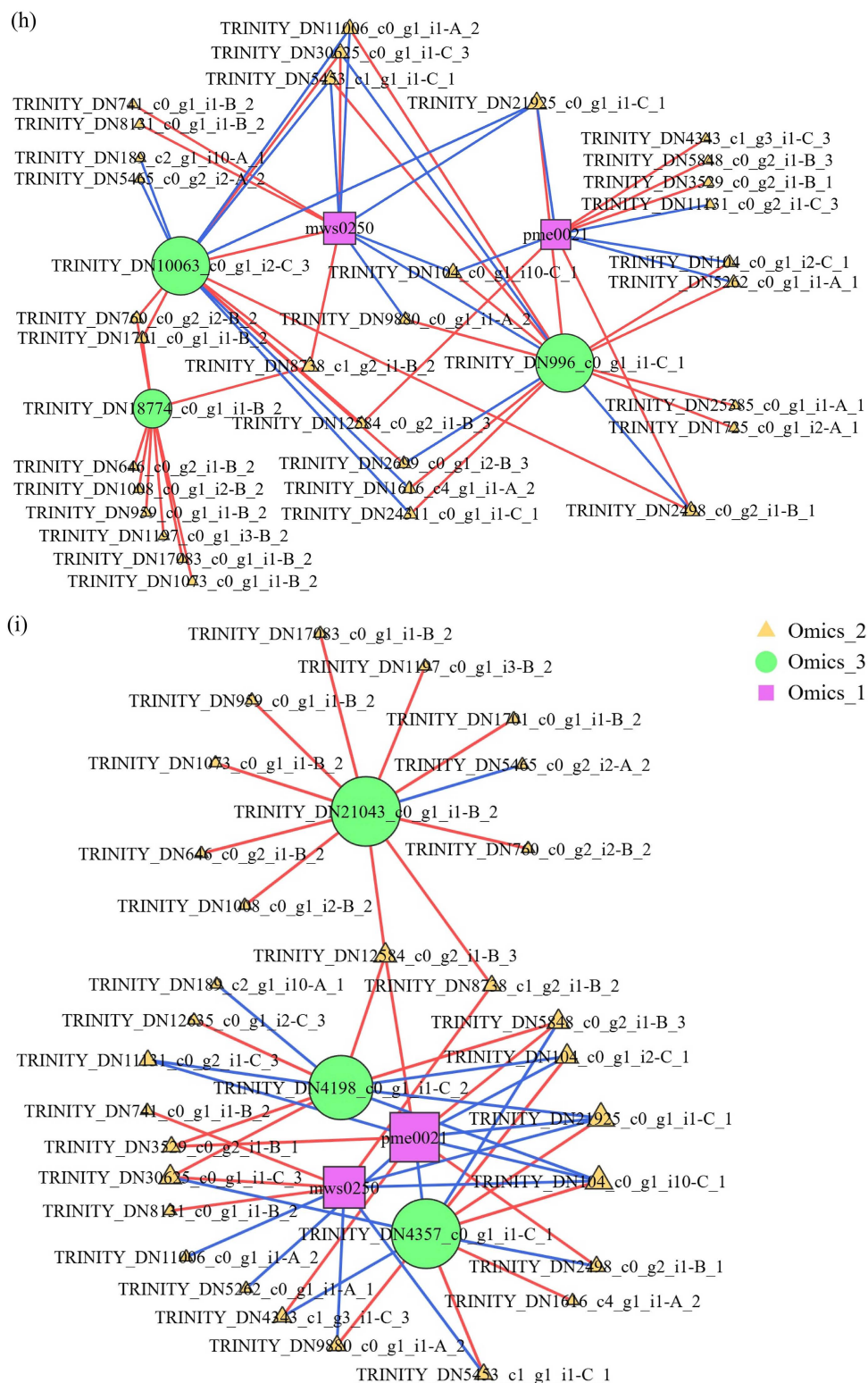


Fig 5. Multi-omics correlation analysis of 2DMs, 35 DEGs, and 9 TFs: (a) bHLH; (b) WRKY; (c) GRAS; (d) AP2-EREBP; (e) NAC; (f) MYB; (g) C2C2-GATA; (h) G2-like; (i) TCP. TFs are transcription factors; Differentially metabolites (DMs) are indicated by pink boxes, differentially expressed genes (DEGs) are represented by orange triangles, and transcription factors (TFs) are denoted by green circles.

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In the phenylpropanoid biosynthesis pathway (Fig 6a), six bHLH TFs correlated with peroxidase (E1.11.1.7). Three bHLH TFs were negatively correlated with cinnamoyl-CoA reductase (CCR), while one was positively and two negatively correlated with caffeic acid 3-O-methyltransferase/acetylserotonin O-methyltransferase (COMT). Four bHLH TFs correlated with shikimate O-hydroxycinnamoyltransferase (HCT) and coniferyl-alcohol glucosyltransferase (UGT72E). Two bHLH TFs were positively correlated with caffeoyl-CoA O-methyltransferase (E2.1.1.104). Additionally, four WRKY TFs correlated with peroxidase, six WRKY TFs were positively correlated with CCR, and one WRKY TF was negatively correlated with HCT. Two WRKY TFs correlated with UGT72E and E2.1.1.104 (Fig 6b).

In the phenylalanine, tyrosine, and tryptophan biosynthesis pathway, four bHLH TFs were positively correlated with anthranilate phosphoribosyltransferase (trpD). Six bHLH TFs correlated with anthranilate synthase component I (TRP3), and five bHLH TFs correlated with 3-dehydroquinate dehydratase/shikimate dehydrogenase (aroD/aroE). Four bHLH TFs were also correlated with tyrosine aminotransferase (TAT) (Fig 7a). Additionally, three WRKY TFs correlated with trpD, while one WRKY TF was positively correlated with TRP3, one with aroD/aroE, and three with TAT (Fig 7b).

In the phenylalanine metabolism pathway, five bHLH TFs correlated with aromatic L-amino acid/L-tryptophan decarboxylase (DDC/TDC). Six bHLH TFs correlated with primary amine oxidase (AOC3/AOC2/tyrA), and three bHLH TFs were linked to phenylacetaldehyde synthase (PAAS) and DDC/TDC (Fig 8a). Additionally, three WRKY TFs negatively correlated with DDC/TDC, while six WRKY TFs positively correlated with AOC3/AOC2/tyrA. Three WRKY TFs were associated with PAAS and DDC/TDC (Fig 8b).

To understand the relationship between DMs and DEGs, we combined KEGG-annotated amino acids with DEGs to construct a network, clarifying the links between gene expression and amino acids accumulation in *R. chrysanthum* leaves under UV-B stress. Fig 6–8 reveals significant increases in L-phenylalanine and L-tyrosine levels after UV-B stress, associated with three metabolic pathways: phenylalanine, tyrosine, and tryptophan biosynthesis; phenylalanine metabolism; and phenylpropanoid biosynthesis.

In the phenylalanine, tyrosine, and tryptophan biosynthesis pathway, increased levels of L-phenylalanine and L-tyrosine is linked to elevated levels of aroD/aroE, and TAT, and reduced levels of TRP3 and trpD. The bHLH and WRKY TFs may influence the expression of these enzymes, contributing to the increased levels of L-phenylalanine and L-tyrosine (Fig 6).

In the phenylpropanoid biosynthesis pathway (Fig 7), HCT and CCR, responsible for L-phenylalanine catabolism, were down-regulated (−1.81). Similarly, enzymes for L-tyrosine catabolism, including COMT (−0.96), HCT (−12.18), and CCR (2.14), were also down-regulated (−11). Elevated levels of L-phenylalanine and L-tyrosine in the phenylalanine metabolism pathway are due to the inhibition of enzymes (Fig 8). The decrease in DDC/TDC (−2.06) surpasses the increase in PAAS (1.49). bHLH and WRKY TFs may inhibit the catabolism of these amino acids by affecting the relevant genes.

Discussion

UV-B radiation is a significant abiotic stressor for plant growth, exacerbated by ozone layer depletion. The alpine plant *R. chrysanthum* has developed adaptive mechanisms to intense UV-B exposure [5,24]. Our previous research investigated the responses of phenolic acids and flavonoids in *R. chrysanthum* to UV-B stress [5]. Zhou et al. (2021) found that flavonoids, organic acids, amino acids, and fatty acids may protect *R. chrysanthum* from UV-B stress [2]. Yu et al. (2025) showed that WRKY transcription factors regulate flavonoid accumulation, reducing photosynthesis impairment in *R. chrysanthum* leaves under UV-B stress [8].

Amino acids are vital for plant resistance to abiotic stresses and are precursors of secondary metabolites related to UV-B radiation resistance. This study aims to investigate changes in amino acids and derivatives in *R. chrysanthum* leaves under UV-B stress and the related transcriptional regulatory mechanisms. In this work, we found 188 amino acids and derivatives, with 51 showing significant differences between groups M and N, indicating that UV-B radiation affected their accumulation. L-phenylalanine and L-tyrosine were the most elevated, suggesting they may help *R. chrysanthum* adapt to UV-B stress. L-phenylalanine and L-tyrosine initiate the phenylpropanoid metabolic pathway, aiding plant

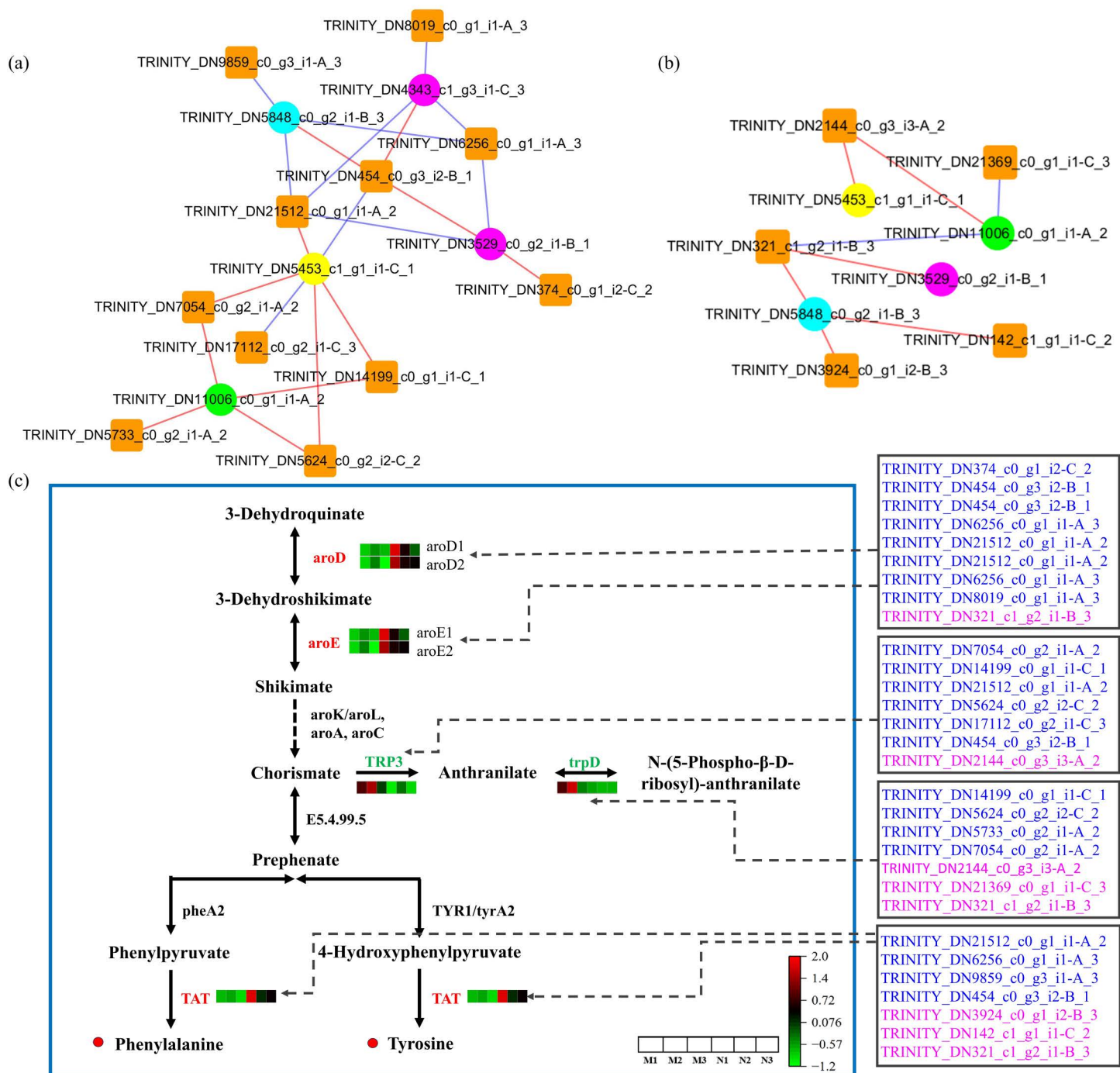


Fig 6. bHLH and WRKY transcription factors enhance UV-B stress tolerance in *R. chrysanthumby* regulating key enzyme genes in the phenylalanine, tyrosine, and tryptophan biosynthesis. (a/b) bHLH/ WRKY TFs are linked to key enzymes genes in the phenylalanine, tyrosine, and tryptophan biosynthesis; The Pearson correlation analysis used a threshold of 0.85 and $p < 0.05$. Boxes represent TFs, and circles denote genes for 16 enzymes. Red edges show positive correlations, while blue edges indicate negative correlations. (c) The heat map shows the expression profile of DEGs. Green denotes down-regulation; red indicates up-regulation. The point shows the change in compound content in *R. chrysanthum* under UV-B radiation, with red dots for up-regulation and green dots for down-regulation. The blue font indicates the bHLH transcription factor, and the pink font denotes WRKY, both of which potentially regulate genes encoding enzymes in the amino acid metabolic pathway.

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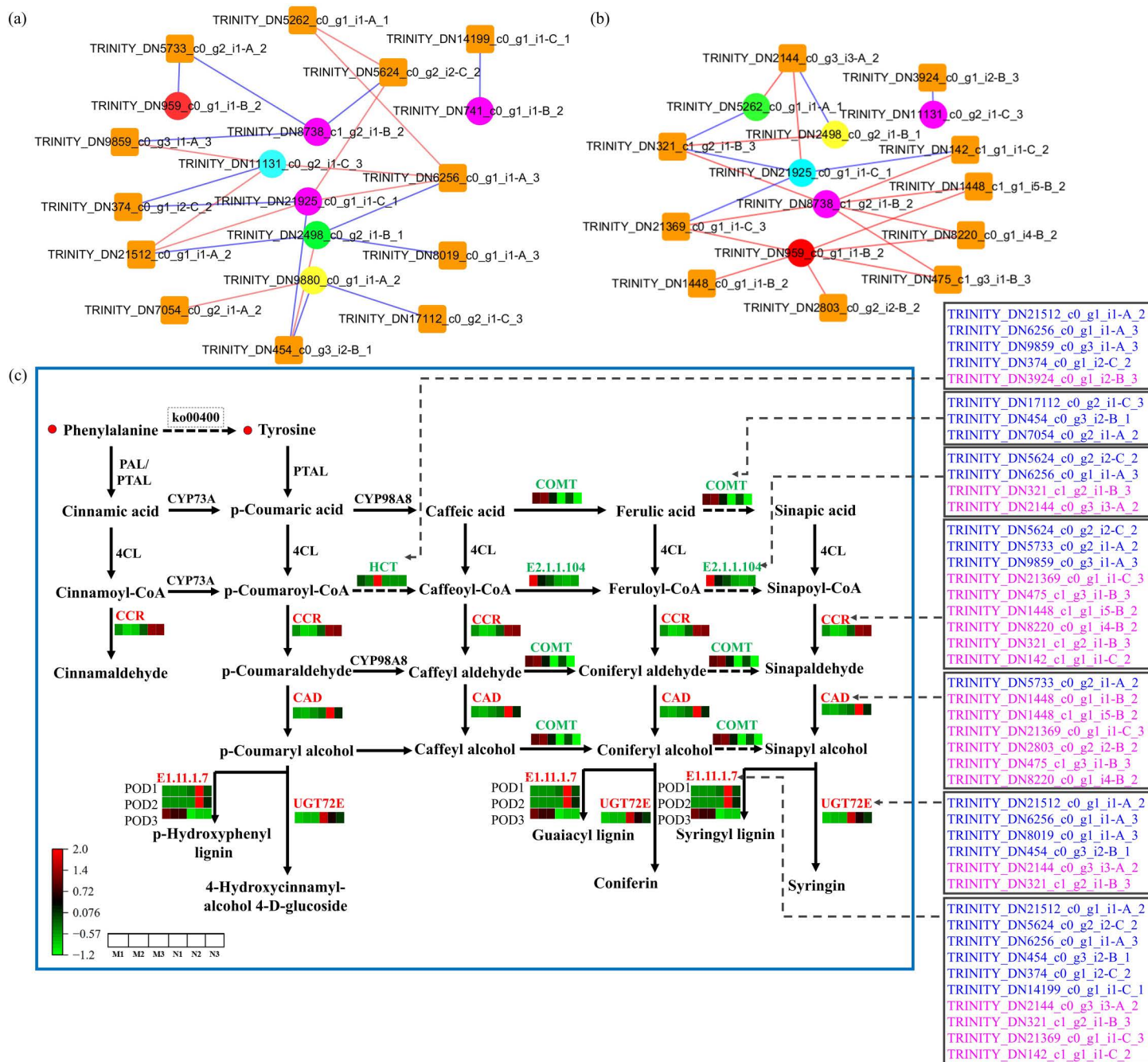


Fig 7. bHLH and WRKY transcription factors enhance UV-B stress tolerance in *R. chrysanthemum* by regulating key enzyme genes in the phenylpropanoid biosynthesis. (a/b) bHLH/ WRKY TFs are linked to key enzymes genes in the phenylpropanoid biosynthesis; The Pearson correlation analysis used a threshold of 0.85 and $p < 0.05$. Boxes represent TFs, and circles denote genes for 16 enzymes. Red edges show positive correlations, while blue edges indicate negative correlations. (c) The heatmap shows the expression profile of DEGs. Green denotes down-regulation; red indicates up-regulation. The point shows the change in compound content in *R. chrysanthemum* under UV-B radiation, with red dots for up-regulation and green dots for down-regulation. The blue font indicates the bHLH transcription factor, and the pink font denotes WRKY, both of which potentially regulate genes encoding enzymes in the amino acid metabolic pathway.

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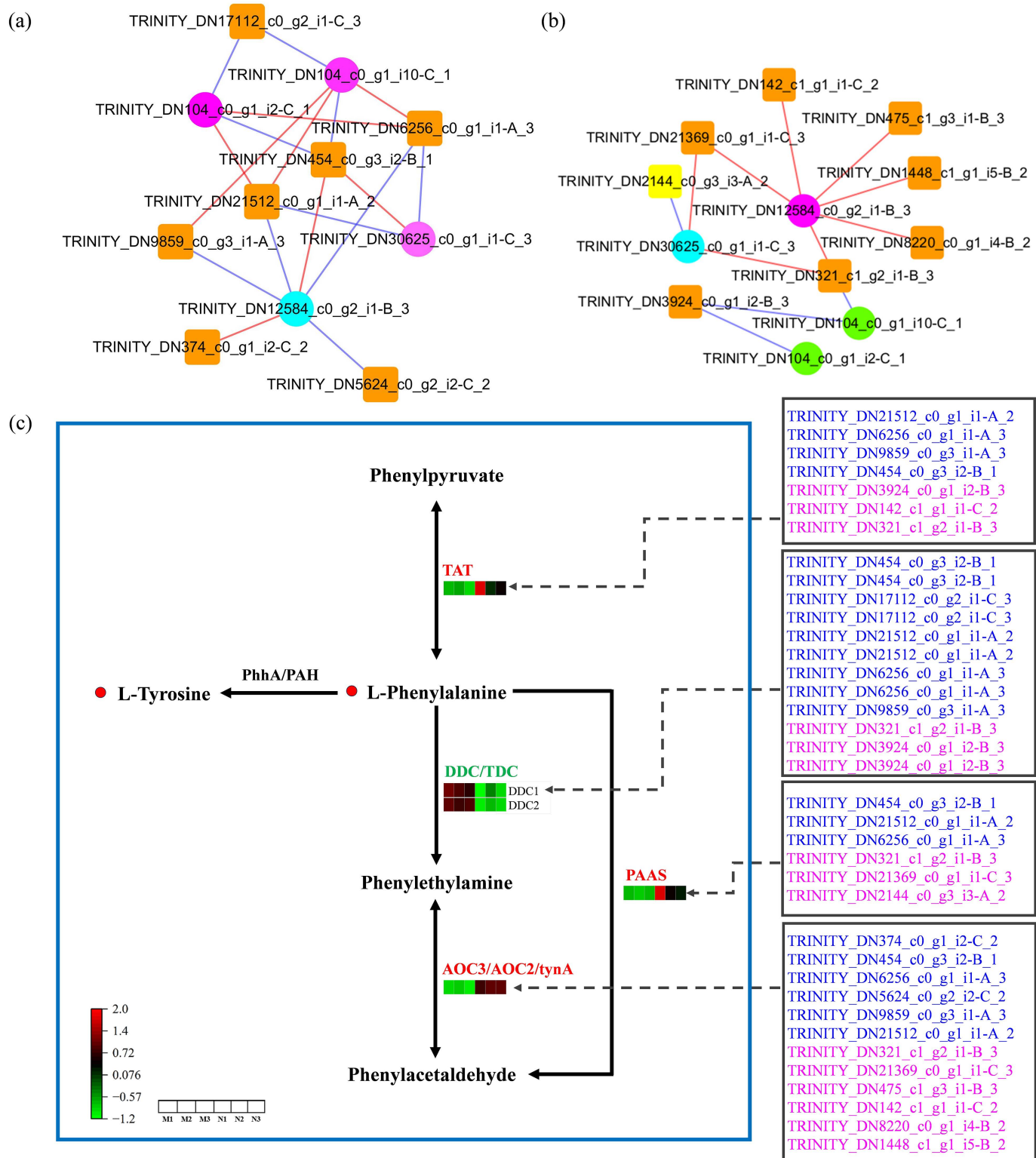


Fig 8. bHLH and WRKY transcription factors enhance UV-B stress tolerance in *R. chrysanthemum* by regulating key enzyme genes in the phenylalanine metabolism. (a/b) bHLH/ WRKY TFs are linked to key enzymes genes in the phenylalanine metabolism; The Pearson correlation analysis used a threshold of 0.85 and $p < 0.05$. Boxes represent TFs, and circles denote genes for 16 enzymes. Red edges show positive correlations, while

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responses to UVB stress by synthesizing secondary metabolites. These include UV-B-absorbing phenolic compounds, antioxidants, and lignin, which modifies and thickens cell walls [25]. Tyrosine and phenylalanine levels rise after 24 hours of UV-B exposure but decline with extended treatment [26]. *Arabidopsis thaliana* enhances flavonoid and anthocyanin synthesis by regulating L-phenylalanine and L-tyrosine pathways under UV-B stress, mitigating damage [27]. L-phenylalanine and L-tyrosine influence wheat's secondary metabolism, activating phenylalanine ammonia lyase (PAL)-related pathways and promoting flavonoids and phenolic acids synthesis. This indicates their potential as inducers to improve wheat's stress tolerance [28]. Metabolomic studies show that UV-B stress triggers amino acid reprogramming and ABA-mediated hormonal crosstalk in *R. chrysanthum* leaves [29].

UV-B stress altered 51 amino acids and derivatives in *R. chrysanthum*, with 15 annotated in the KEGG database and 36 not, suggesting they may be unique adaptive variations. Correlation analyses showed that trimethyllysine, 5-Oxo-L-proline, N- α -acetyl-L-ornithine, L-methionine, L-lysine, and L-saccharopine had correlations of 10 or more with L-threo-3-methylaspartate, marking them as central metabolites among the 15 DMs. Of the 15 differential metabolites, L-phenylalanine and L-tyrosine were significantly enriched in three pathways, while the other 13 metabolites, enriched in seven pathways, could not be analyzed further (Fig 1). Thus, we focused on L-phenylalanine and L-tyrosine and their associated pathways: phenylpropanoid biosynthesis, phenylalanine, tyrosine, and tryptophan biosynthesis, and phenylalanine metabolism. The phenylpropanoid pathway is vital in plants, comprising over 20% of total cellular metabolism [30], with L-phenylalanine as a precursor in the phenylpropane pathway. The phenylpropane pathway produces phenolic compounds such as phenolic acids, flavonoids, and lignans, either directly or through branches like the flavonoid and monolignol pathways. To cope with environmental stresses like drought and UVB radiation, plants modify their biosynthetic pathways by altering gene expression and enzyme activity, impacting secondary metabolite production [31]. UVB radiation activates signaling pathways that influence gene expression in the phenylpropane pathway, involving receptor proteins and the mitogen-activated protein kinase (MAPK) cascade [31]. In rice, UVB stress significantly remodels metabolic pathways, particularly those related to phenylpropanes and amino acids [32]. This study found that UVB radiation increased L-phenylalanine (L-Phe) synthesis in *R. chrysanthum*, enhancing the phenylpropane pathway. L-Tyrosine (L-Tyr), related to L-Phe, may also be indirectly affected, as increased L-Phe levels raise precursors for L-Tyr synthesis.

bHLH transcription factors protect plants from UV-B stress by influencing the biosynthesis of flavonoids, anthocyanins, terpenoids, and alkaloids [3,28,33,34]. In peach fruit pericarp, they respond to UV-B signaling and collaborate with other factors like HFR1 to stabilize light responses via the UVR8 pathway [34]. In *Arabidopsis*, MYC2 interacts with bHLH and other families, including ETHYLENE INSENSITIVE 3 [35]. MYB factors regulating thioglucoside biosynthesis are hypothesized to interact with ALC, ICE1, PIF4, and JAM2, while other bHLH-type factors regulate secondary metabolism [36–38]. bHLH proteins can also activate anthocyanin biosynthesis genes by forming a complex with MYB proteins and TTG1 [39–40]. In *R. chrysanthum* leaves, UV-B irradiation and ABA treatment significantly impacted the phenylpropanoid biosynthesis pathway, activating key enzyme genes like 4CL, CCR, and HCT, highlighting the role of the MYB-bHLH-WD40 (MBW) complex in regulating this pathway and its interactions with ABA signaling [16].

WRKY is a large family of transcriptional regulators in plants, crucial for growth, development, secondary metabolite synthesis, and stress tolerance [41]. These factors regulate genes involved in the biosynthesis of phenylalanine, alkaloids, and terpenoids, influencing secondary metabolite production. They also modify biosynthesis pathways in response to UV-B-induced plant defense [12,38,42,43]. For example, WRKY36 interacts with UVR8 in *Arabidopsis* to repress HY5 transcription and promote hypocotyl elongation [44]. Following UV-B radiation, UVR8 monomers interact with transcription

factors like BES1, BIM1, and WRKY36 in the nucleus. WRKY overexpression regulates the biosynthesis of secondary metabolites, such as volatile terpenes by WRKY3 and WRKY6 [45], and kamacin by WRKY33 from *Arabidopsis thaliana* [41]. WRKY also influences the biosynthesis of flavanols, proanthocyanidins, and terpenoid indole alkaloids [43,46,47]. A recent study confirmed WRKY's role in flavonoid biosynthesis in *R. chrysanthum* under UV-B radiation.

The current study illustrates that the transcription factors bHLH and WRKY are crucial in regulating phenylpropanoid biosynthesis, as well as the biosynthesis of phenylalanine, tyrosine, and tryptophan, and the metabolism of phenylalanine. UV-B radiation affects the expression of these factors, regulating genes that encode enzymes in these pathways and influencing the accumulation of L-phenylalanine and L-tyrosine (Fig 5). bHLH and WRKY factors exhibit both synergistic and antagonistic effects on amino acid synthesis. For example, TRINITY_DN374_c0_g1_i2-C_2 (bHLH) and six WRKY factors synergistically regulate L-phenylalanine and L-tyrosine biosynthesis, while TRINITY_DN321_c1_g2_i1-B_3 (WRKY) and five bHLH factors antagonistically regulate these pathways (Fig 6–8). Fig 6–8 illustrates the relationships among genes, transcription factors, and metabolites, revealing regulatory mechanisms for amino acid accumulation in *R. chrysanthum* under UV-B stress. The pathway involves 2 amino acids, 15 DEGs, and 2 TFs (bHLH and WRKY). These TFs influence the expression of several enzymes, including 3-dehydroquinate dehydratase/shikimate dehydrogenase (aroD/aroE), tyrosine aminotransferase (TAT), anthranilate synthase component I (TRP3), anthranilate phosphoribosyltransferase (trpD), aromatic-L-amino-acid/L-tryptophan decarboxylase (DDC/TDC), phenylacetaldehyde synthase (PAAS), shikimate O--hydroxycinnamoyltransferase (HCT), cinnamoyl-CoA reductase (CCR), and caffeic acid 3-O-methyltransferase (COMT). Consequently, this modulation impacts the accumulation of L-phenylalanine and L-tyrosine. It is important to note that CCR is a crucial enzyme in the biosynthesis of lignin. Additionally, HCT play a role in the synthesis of plant lignin and cell walls, while UGT72E and E2.1.1.104 are also involved in lignin synthesis. UV-B radiation in blueberry healing tissues induces the expression of key genes in the phenylpropanoid pathway, affecting the accumulation of flavonoids derived from L-Phe and L-Tyr [48]. Transcription factors bHLH74 and bHLH25 may negatively regulate flavonoid biosynthesis by repressing related genes or interacting with MYB proteins [48]. Jaiswal et al. (2021) indicated that UVB-induced transcriptome alterations depend on the UVB signaling cascade and secondary metabolic pathways, such as flavonoids and phenylpropanoids [49]. In rice, UV-B stress affects flavonoid content linked to phenylpropanoid biosynthesis, with OsbZIP18 serving as a key transcription factor for this process [37].

The PPI network analysis of 35 DEGs identified TRINITY_DN2498_c0_g2_i1-B_1 (UGT72E) and TRINITY_DN8131_c0_g1_i1-B_2 (peroxidase) as key components in the phenylpropanoid biosynthesis pathway, with an interaction score of 983. It also revealed significant correlations between TRINITY_DN959_c0_g1_i1-B_2 (cinnamyl-alcohol dehydrogenase, CAD) and two peroxidase-encoding genes (interaction score of 961). In the phenylalanine, tyrosine, and tryptophan biosynthesis pathway, TRINITY_DN5453_c1_g1_i1-C_1 (TRP3) strongly interacted with TRINITY_DN11006_c0_g1_i1-A_2 (trpD), yielding an interaction score of 967 (Fig 2, Table 1). In the phenylalanine metabolic pathway, TRINITY_DN12584_c0_g2_i1-B_3 (AOC3/AOC2/tyrA) and TRINITY_DN5848_c0_g2_i1-B_3 (TAT) had an interaction score of 792 (Fig 2, Table 1). It is important to note that CAD is vital in the phenylpropanoid biosynthesis pathway, converting cinnamaldehyde to cinnamyl alcohol, a lignin monomer precursor. High-scoring interactions suggest that gene pairs mentioned above play a central regulatory role in amino acid synthesis pathways, impacting plant metabolism and UV-B stress tolerance. We recommend prioritizing experimental verification of direct interactions and combining metabolomic and structural biology techniques to analyze molecular mechanisms, supporting crop improvement and natural product synthesis.

This study primarily aims to identify “candidate nodes” within core regulatory networks through broad-targeted metabolomics. The subsequent research plan involves: (1) designing specific primers for quantitative PCR (qPCR) experiments targeting core regulatory transcription factors (e.g., the bHLH family member TRINITY_DN5624_c0_g2_i2-C_2 and the WRKY family member TRINITY_DN321_c1_g2_i1-B_3), as well as key DEGs, to validate the correlation between their transcriptional expression levels and metabolite variations; and (2) performing time-series targeted metabolomics focusing on characteristic amino acids such as L-phenylalanine and L-tyrosine, along with downstream secondary metabolites, to

dynamically monitor their concentration changes under different treatment conditions, thereby further elucidating the temporal dynamics of the regulatory networks.

Conclusion

The effect of UV-B radiation on *R. chrysanthum* seedlings was investigated in an artificial climate chamber using widely targeted metabolomics and transcriptomics to elucidate molecular mechanisms. The findings indicated that, in response to UV-B stress, the transcription factors bHLH and WRKY may regulate enzyme genes in amino acid metabolic pathways, including the biosynthesis of phenylalanine, tyrosine, and tryptophan, as well as phenylpropanoid biosynthesis and phenylalanine metabolism. Consequently, this regulation impacts the levels of L-phenylalanine and L-tyrosine. High-scoring PPI interactions suggest these genes are central to amino acid synthesis, affecting plant metabolism and UV-B stress tolerance. This research will support the experimental validation of direct protein interactions and the breeding of UV-B resistant plant species.

Abbreviations

The following abbreviations are used in this manuscript:

Name	Abbreviations	No.
differential metabolites	DMs	
deferentially expressed genes	DEGs	
transcription factors	TFs	
phenylalanine, tyrosine and tryptophan biosynthesis		ko00400
phenylpropanoid biosynthesis		ko00940
phenylalanine metabolism		ko00360
shikimate kinase	aroK/aroL	[EC:2.7.1.71]
3-phosphoshikimate 1-carboxyvinyltransferase	aroA	[EC:2.5.1.19]
chorismate synthase	aroC	[EC:4.2.3.5]
chorismate mutase	E5.4.99.5	[EC:5.4.99.5]
prephenate dehydratase	pheA2	[EC:4.2.1.51]
prephenate dehydrogenase (NADP+)	TYR1	[EC:1.3.1.13]
prephenate dehydrogenase	tyrA2	[EC:1.3.1.12]
tyrosine aminotransferase	TAT	[EC:2.6.1.5]
3-dehydroquinate dehydratase I	aroD	[EC:4.2.1.10]
shikimate dehydrogenase	aroE	[EC:1.1.1.25]
anthranilate synthase	TRP3	[EC:4.1.3.27]
anthranilate phosphoribosyltransferase	trpD	[EC:2.4.2.18]
phenylalanine ammonia-lyase	PAL	[EC:4.3.1.24]
phenylalanine/tyrosine ammonia-lyase	PTAL	[EC:4.3.1.25]
trans-cinnamate 4-monooxygenase	CYP73A	[EC:1.14.14.91]
cytochrome P450 family 98 sub-family A8	CYP98A8	[EC:1.14.13.-]
4-coumarate--CoA ligase	4CL	[EC:6.2.1.12]
cinnamoyl-CoA reductase	CCR	[EC:1.2.1.44]
cinnamyl-alcohol dehydrogenase	CAD	[EC:1.1.1.195]

Name	Abbreviations	No.
shikimate O-hydroxycinnamoyltransferase	HCT	[EC:2.3.1.133]
caffeic acid 3-O-methyltransferase/ acetylserotonin O-methyltransferase	COMT	[EC:2.1.1.68 2.1.1.4]
caffeoyl-CoA O-methyltransferase	E2.1.1.104	[EC:2.1.1.104]
coniferyl-alcohol glucosyltransferase	UGT72E	[EC:2.4.1.111]
peroxidase	E1.11.1.7	[EC:1.11.1.7]
aromatic-L-amino-acid/L-tryptophan decarboxylase	DDC/TDC	[EC:4.1.1.28 4.1.1.105]
primary-amine oxidase	AOC3/ AOC2/tynA	[EC:1.4.3.21]
phenylacetaldehyde synthase	PAAS	[EC:4.1.1.109]
phenylalanine-4-hydroxylase	PhhA/PAH	[EC:1.14.16.1]

Supporting information

S1 Fig. Metabolomic changes in *R. chrysanthum* after UV-B stress. (a) PCA analysis; (b) Volcano map; (c) Differential metabolite classification statistical chart. In the volcano and multiplicity-of-difference bar graphs, metabolites with increased contents are shown in red and metabolites with decreased contents are shown in green. Data from [5] (JPG)

S1 File. S1-S8 Tables.
(XLSX)

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Writing – original draft: Jie Lyu, Xiangqun Li.

Writing – review & editing: Jie Lyu, Hongwei Xu.

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