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Transcriptomic analysis reveals differential gene expression and metabolic reprogramming in *Coptis teeta* Wall. under drought and heat stress

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Introduction: Abiotic stresses such as drought and heat severely impair plant growth, survival, and secondary-metabolite production, posing a major threat to moisture-dependent alpine medicinal plants. *Coptis teeta* Wall., a critically endangered species of the Eastern Himalayas valued for its isoquinoline alkaloids, is particularly vulnerable to climatic fluctuations.

Methods: In this study, RNA-Seq based transcriptome profiling was performed to decipher the molecular responses of *C. teeta* under drought and heat stress.

Results: Differential expression analysis identified 3,099 DEGs under drought, 816 under heat, and 3,790 under combined stress highlighting a strong and synergistic transcriptional reshaping under environmental extremes. Antioxidant enzymes including ascorbate peroxidase (APX), superoxide dismutase (SOD), and glutathione peroxidase (GPX) were significantly upregulated, whereas thioredoxin and glutaredoxin-associated redox regulators were suppressed, indicating a shift toward peroxidase-driven ROS detoxification. Major transcription factors (TFs) from the NAC, DREB, WRKY, bZIP, and HSF families exhibited strong stress-specific induction, with DREB2A, AREB1, and HSFA2 emerging as central regulatory hubs. Secondary-metabolite biosynthesis was distinctly modulated: early benzylisoquinoline alkaloid (BIA) pathway genes were upregulated, whereas downstream enzymes such as berberine bridge enzyme (BBE) and O-methyltransferases were downregulated, reflecting a stress-induced trade-off between alkaloid diversification and survival. GO and KEGG enrichment further revealed activation of protein folding, oxidoreductase activity, glutathione metabolism, and hormone-signaling pathways.

Discussion: Overall, this study provides the first genome-wide insight into transcriptional networks and metabolic reprogramming in *C. teeta* under abiotic stress, offering essential molecular resources for conservation, stress-tolerance enhancement, and genetic improvement.

KEYWORDS

Coptis teeta, differential gene expression, endangered species, reactive oxygen species, transcriptome analysis

1 Introduction

Drought and heat stress are among the most pervasive environmental constraints threatening global plant productivity, ecological stability, and biodiversity. The accelerating pace of anthropogenic climate change has intensified the frequency, duration, and severity of these stressors, particularly in sensitive mountain ecosystems that are experiencing rapid warming and altered precipitation patterns. In plants, drought and heat disrupt cellular water balance, photosynthetic performance, membrane stability, and metabolic homeostasis, ultimately impairing growth and survival. A major consequence of these stressors is the accumulation of reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2), superoxide radicals ($\text{O}_2^{\bullet-}$), and hydroxyl radicals ($\bullet\text{OH}$), which damage proteins, lipids, and nucleic acids when produced in excess (Dvořák et al., 2021; Regon et al., 2024; Hasnu et al., 2025). Although ROS play essential signaling roles, their overaccumulation under stress necessitates rapid activation of antioxidant defense pathways to maintain redox balance and prevent oxidative injury.

Coptis teeta Wall., a critically endangered medicinal plant belonging to the Ranunculaceae family, is native to the cool, humid, forested slopes of the Eastern Himalayas. It is highly valued for its rich content of benzylisoquinoline alkaloids (BIAs)—particularly berberine, coptisine, and palmatine, which exhibit antimicrobial, anti-inflammatory, antidiabetic, and hepatoprotective properties (Li et al., 2023; Chen et al., 2025). Because *C. teeta* is restricted to narrow ecological niches with high moisture availability, it possesses limited physiological plasticity, rendering it extremely vulnerable to climatic fluctuations, habitat fragmentation, and water-scarcity conditions (Chelleng et al., 2024; Hounsou et al., 2024). Recent ecological reports indicate a steady decline in its natural populations, attributed largely to prolonged dry spells, rising air temperatures, and anthropogenic disturbances across the Himalayan belt (Jangpangi et al., 2025). Given its endangered status and pharmacological significance, understanding the molecular basis of *C. teeta*'s stress responses is crucial for designing conservation strategies and sustaining its phytochemical productivity.

Plants employ multilayered defensive mechanisms to cope with drought and heat, integrating morphological adaptations (e.g., stomatal closure and reduced leaf expansion), physiological responses (osmotic adjustment, transpiration control), and biochemical adjustments involving enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR), along with non-enzymatic antioxidants including ascorbate and glutathione, play central roles in mitigating oxidative stress (Nahar et al., 2018; Rehman et al., 2023). At the molecular level, stress-responsive transcription factors (TFs) such as NAC, DREB/ERF, bZIP, WRKY, MYB, and heat shock factors (HSFs) modulate complex gene regulatory networks to orchestrate protective responses (Zhang et al., 2025). These TFs regulate downstream targets involved

in osmotic adjustment, ROS detoxification, protein refolding, secondary metabolism, and hormone-mediated signaling, thereby integrating cellular responses to external stress cues.

Despite extensive studies in model and crop species, the molecular mechanisms underlying abiotic stress tolerance remain poorly understood in endangered and non-model medicinal plants such as *C. teeta*. This knowledge gap is particularly significant because medicinal plants often exhibit unique metabolic pathways such as BIA biosynthesis that may respond differently to environmental stress (Hasnu et al., 2025). Disruptions in these pathways can influence the quantity and quality of bioactive compounds, affecting both pharmacological value and ecological fitness. Therefore, elucidating stress-induced transcriptional shifts in *C. teeta* is essential for understanding how climate-driven stressors impact its survival, metabolic outputs, and long-term ecological resilience.

Next-generation sequencing (NGS), particularly RNA sequencing (RNA-Seq), provides a powerful platform for exploring genome-wide transcriptional dynamics, identifying differentially expressed genes (DEGs), and uncovering major regulatory hubs involved in abiotic stress responses. This approach is especially valuable in non-model species with limited genomic information, as it enables comprehensive characterization of stress-responsive pathways, TF networks, antioxidant systems, and specialized metabolic processes (Regon et al., 2022, 2024). RNA-Seq further facilitates pathway enrichment analyses, such as Gene Ontology (GO) and KEGG, offering insights into biological processes, molecular functions, and metabolic networks affected by stress.

In this study, we performed transcriptome profiling of *C. teeta* leaves under controlled drought and heat stress conditions to elucidate its molecular response strategies. We identified key DEGs associated with ROS detoxification, osmotic regulation, TF activation, protein homeostasis, and BIA biosynthesis. Integrating differential expression, pathway enrichment, and quantitative real-time PCR (qRT-PCR) validation, we provide a comprehensive molecular framework describing how *C. teeta* adapts to contrasting abiotic stresses. This study fills a critical gap in the molecular understanding of this endangered Himalayan species and lays the foundation for future functional genomics, conservation programs, and climate-resilient cultivation strategies.

2 Materials and methods

2.1 Plant growth and treatment

The *C. teeta* saplings were collected from Myodia Pass, Arunachal Pradesh, India (28.24611° N, 95.91277° E; 2,580 m elevation). Plants were acclimatized for 10 days in a controlled growth chamber at Gauhati University under the following conditions: 22°C, 70% relative humidity (RH), and 8,000 lux

illumination during a 14-h light period, followed by 20°C and 60% RH during a 10-h dark period. After acclimatization, healthy and uniformly growing saplings were subjected to two stress treatments. Drought stress was imposed following [Lama et al. \(2023\)](#) by withholding water until clear morphological symptoms of dehydration appeared, typically after approximately 14 days. Heat stress was applied by exposing plants to 43°C and 70% RH for 6 h, following [Zandalinas et al. \(2017\)](#), until distinct heat-induced morphological symptoms were visible. Visible morphological changes under both stresses were documented photographically. Leaf tissues were harvested immediately after the onset of stress symptoms for RNA extraction and sequencing. Three independent pot-grown plants were used as biological replicates for each treatment and the control group. Representative photographs of stressed and control plants are provided in [Supplementary Figure S1](#).

2.2 RNA sequencing

Total RNA was extracted from leaf tissues using the TRIzol reagent (RNAiso, Takara) from three independent biological replicates per treatment (control, drought, and heat). RNA purity and concentration were assessed using a NanoDrop spectrophotometer, and integrity was evaluated on an Agilent 2100 Bioanalyzer. Only samples with an RNA Integrity Number (RIN) ≥ 7.5 were retained for library preparation to ensure high-quality transcriptome sequencing. Strand-specific libraries were prepared using the TruSeq[®] Stranded RNA Library Prep Kit for Plants (Illumina, USA), following the manufacturer's protocol. Libraries meeting quality criteria were sequenced on the Illumina NovaSeq 6000 platform, generating paired-end 150-bp reads (PE150). This sequencing strategy ensured high depth and coverage suitable for robust differential expression analysis and accurate transcript quantification.

2.3 Data processing, reference-based assembly, and differential expression analysis

The quality of raw sequencing reads was evaluated using FastQC v0.11.9, which assessed base quality distribution, GC content, sequence duplication, and overrepresented sequences. Adapter sequences and low-quality bases were removed using Trimmomatic v0.39, ensuring high-quality clean reads suitable for downstream transcriptomic analyses ([Wang et al., 2021](#)). Cleaned reads were aligned to the *C. chinensis* reference genome (BioSample SAMN16094608; Assembly GCA_015680905.1) using the STAR v2.7.1a aligner, which provides high accuracy for spliced read mapping ([Dobin and Gingeras, 2016](#)). The *C. chinensis* genome was selected as the reference because a fully annotated genome for *C. teeta* is not yet available; moreover, both species belong to Ranunculaceae and share conserved BIA biosynthetic pathways,

making *C. chinensis* the most biologically appropriate surrogate for read alignment ([Liu et al., 2021](#)). Alignment files were processed, sorted, and indexed using SAMtools v1.7 ([Kuznetsova et al., 2021](#)). Gene-level read quantification was performed using featureCounts from the Subread v2.0.0 package ([Liao et al., 2014](#)). One control replicate (Control3) exhibited exceptionally low mapping rates and aberrant QC metrics; therefore, to avoid statistical bias and false-positive DEGs, this replicate was excluded from downstream analyses. Differential expression analysis was conducted using DESeq2 v1.32.0 in the R environment ([R Core Team, 2021](#)). Normalization was performed using the median-of-ratios method, and differential expression was assessed using the Wald test. To obtain stable and unbiased effect-size estimates, the apegglm shrinkage estimator was applied to log₂ fold-change values ([Zhu et al., 2019](#)). Genes were classified as differentially expressed when they satisfied both criteria: adjusted p-value (Benjamini–Hochberg FDR) < 0.05 and $|\log_2 \text{fold change}| \geq 1$. Data visualization, including volcano plots and MA plots, was generated using the EnhancedVolcano v1.10.0 package ([Kuznetsova et al., 2021](#)). A complete workflow of sequence quality assessment, trimming, alignment, quantification, and DEG analysis is illustrated in [Supplementary Figure S2](#).

2.4 Annotation

Protein sequences from representative species of the family Ranunculaceae were retrieved from the UniProt database ([UniProt Consortium, 2023](#)). Cleaned transcript sequences were subjected to a local BLASTX search to identify the closest homologs. The top-scoring matches were merged with the corresponding transcript IDs using R ([R Core Team, 2021](#)). GO terms, KEGG pathway identifiers, and additional gene descriptions were assigned based on UniProt annotations and used for downstream functional analysis.

2.5 Gene set enrichment analysis

Functional enrichment analysis of DEGs was performed using the clusterProfiler v4.12.6 package implemented in R v4.4.1 ([Wu et al., 2021](#); [R Core Team, 2021](#)). Overrepresentation analysis (ORA) was conducted using the enrichr() function to identify significantly enriched GO terms among upregulated and downregulated DEGs. Statistical significance was evaluated using the hypergeometric test, and p-values were adjusted using the Benjamini–Hochberg false discovery rate (FDR) correction. GO terms with adjusted p-values ($q < 0.05$) were considered significantly enriched.

2.6 Quantitative real-time PCR analysis

qRT-PCR was performed to validate the differential gene expression patterns identified from the RNA-Seq analysis.

Approximately 100 mg of leaf tissue was collected from control, drought-stressed, and heat-stressed plants, using three independent biological replicates per treatment. Samples were immediately flash-frozen in liquid nitrogen and ground to a fine powder. Total RNA was extracted using the TRIzol reagent following the same protocol used for RNA-Seq. RNA integrity was confirmed, and 1 µg of DNase-treated RNA was used for cDNA synthesis using the Verso cDNA Synthesis Kit. Gene-specific primers were designed using Primer3web (<https://primer3.ut.ee/>), and all primer sequences and amplicon sizes are provided in Table 1. The 18S rRNA gene served as the internal reference due to its stable expression across treatments (Rahman et al., 2021). qRT-PCR reactions were performed using PowerUp SYBR Green Master Mix on a QuantStudio 5 Real-Time PCR System under optimized cycling conditions. Amplification specificity was verified by melt-curve analysis. Relative gene expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method in accordance with MIQE guidelines (Bustin et al., 2009). All measurements were based on three biological replicates, and mean C_t values were used for comparative expression analysis (Kundu et al., 2025b).

2.7 Statistical analysis

Statistical analyses of qRT-PCR gene expression data were performed using three independent biological replicates ($n = 3$) for each treatment. Mean C_t values were normalized to the 18S rRNA reference gene, and relative expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method in accordance with MIQE guidelines. Differences between control and stress-treated (drought or heat) samples were evaluated using a two-tailed Student's t-test implemented in R (R Core Team, 2021). Statistical significance was assigned as $p < 0.05$ (*) and $p < 0.01$ (**). All values are presented as mean $\pm$ standard deviation (SD). Scripts (R script) and command lines (Linux command line) used for data analysis and visualization are provided as Appendix 1-2 for reproducibility of the data.

TABLE 1 List of primers used for qRT-PCR analysis. The table includes gene identifiers, forward and reverse primer sequences, and corresponding amplicon sizes.

Gene	Primer sequences		Amplicon size
	Forward sequences	Reverse sequences	
<i>Ct18S</i>	ACCATAAACGATGCCGACCA	CTTGCAGCCATACTCCC	151
<i>IFM89_005336</i> (AP1)	TTTCCGAACACCATTTGGCAG	GATCGCTTTCATACTGGCCG	181
<i>IFM89_028610</i> (GLP)	CCTGTTTGTGGGCTTTGTGA	TCATAATCACGCCGCTGTTC	176
<i>IFM89_032636</i> (APX)	AGCTCTTCAACGTGCCGAGG	CCTGCAGTTCATCGCTCCA	176
<i>IFM89_038338</i> (NAC)	CATTGGCCTGGAACGCTATC	GCCGTTAACATATGCACGC	151
<i>IFM89_036261</i> (HSP)	TTAGCAAAGATGGAGCGGC	GCCACATGCCAAATCACATC	196
<i>IFM89_016869</i> (DNAJ)	AATTTCCGGGCTGCAACATT	ATCAAACACTTCCGCGCTTT	176
<i>IFM89_013824</i> (APX)	TGGCCGGAGTTGTGCTGTT	GTGTGAGCGCCAGAGAGAGC	196
<i>IFM89_005514</i> (HSF)	AAGAACTGAGCAACGCGTTT	TCTTCCACTTCCACTTCGCT	165

3 Results

3.1 RNA sequencing and reference-based transcriptome assembly

High-quality RNA-Seq data were obtained from leaf tissues of *C. teeta*, generating between 33.30 and 44.33 million clean reads per sample (Supplementary Table S1). After trimming and quality filtering, 62%-80.82% of reads uniquely mapped to the *C. chinensis* reference genome (BioSample SAMN16094608; Assembly GCA_015680905.1), as summarized in Supplementary Tables S1 and S2. The average GC content of the sequencing libraries was approximately 44%. Differential expression analysis identified 3,099 DEGs under drought stress and 816 DEGs under heat stress, whereas 3,790 DEGs were detected when comparing both stress treatments (Figure 1; Supplementary Figure S3; Supplementary Table S2). These datasets provide a comprehensive genome-wide overview of transcriptional variation under the two abiotic stress conditions.

3.2 DEGs in leaves

Analysis of leaf transcriptomes revealed distinct sets of differentially expressed genes (DEGs) under drought and heat stress (Supplementary Table S2). Under drought stress, several well-known stress-responsive genes were markedly upregulated, including ZAT12, SnRK2.6, DREB2A, AREB1, and LEA14, indicating activation of dehydration-associated transcriptional pathways. Heat stress induced a separate group of approximately 22 heat-responsive genes, predominantly belonging to the HSP20, HSP40 (DNAJ), HSP70/Clp, and HSF families. Genes such as HSP isoform 1, DNAJ, and ClpA/ClpB were highly expressed, whereas specific members of the HSP20 family and selected HSP-domain proteins were downregulated (Supplementary Table S2). Functional grouping of DEGs showed a broad representation of molecular chaperones and stress-associated proteins, including sHSPs, DNAJ

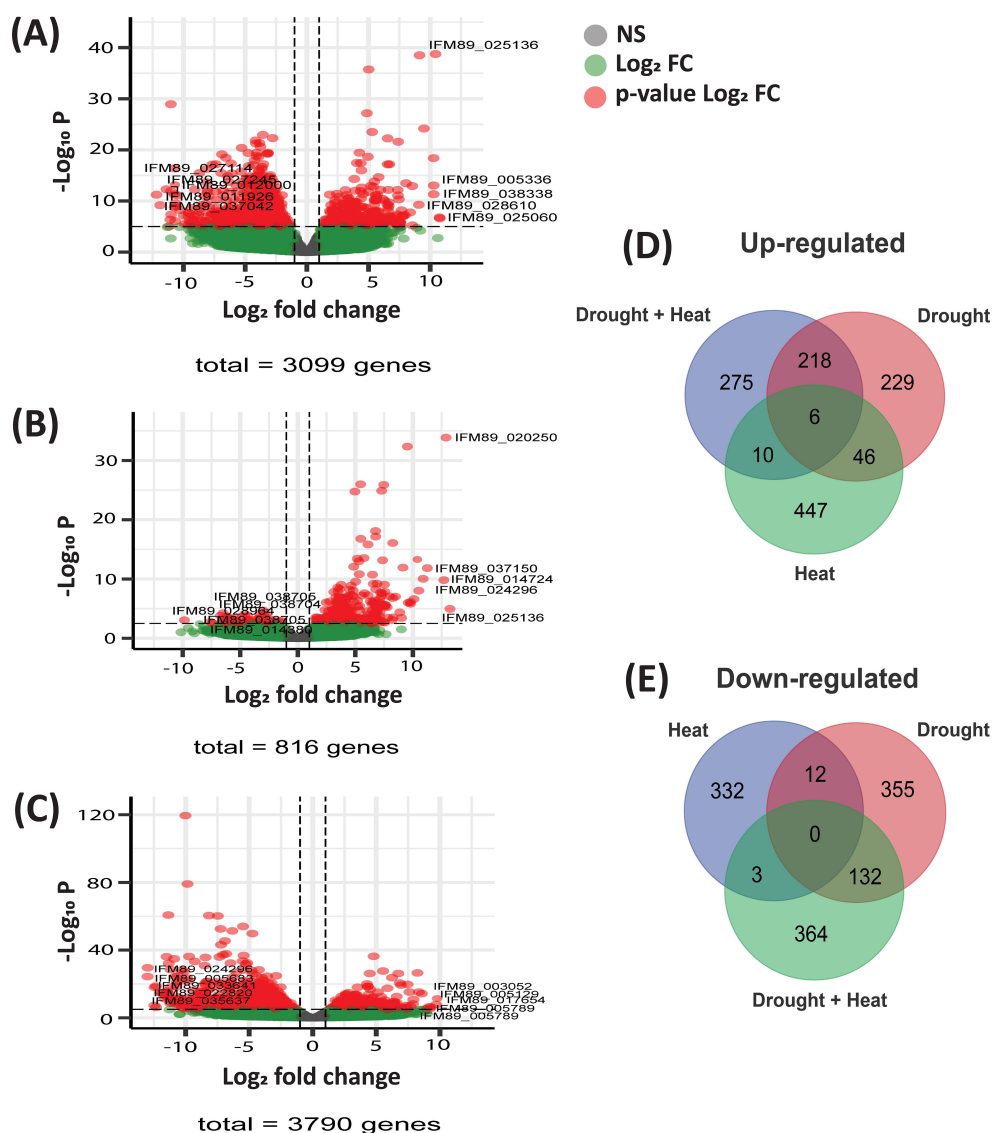


FIGURE 1

DEGs in *C. teeta* leaves under drought and heat stress. (A–C) Volcano plots showing DEGs under (A) drought vs. control (total = 3,099 DEGs) (B) heat vs. control (total = 816 DEGs), and (C) combined drought + heat vs. control (total = 3,790 DEGs). Each point represents an individual gene; the x-axis denotes $\log_2$ fold change ($\log_2 FC$), and the y-axis indicates $-\log_{10}$ (adjusted p-value). Red points represent significantly DEGs (Benjamini–Hochberg FDR < 0.05 and $|\log_2 FC| \geq 1$); green points show genes with $|\log_2 FC| \geq 1$ but not reaching the adjusted p-value threshold; gray points represent non-significant genes. Representative highly responsive genes are annotated on the plots. Volcano plots were generated using EnhancedVolcano and ggplot2 in R. (D, E) Venn diagrams illustrating the overlap among the top 500 most significantly upregulated (D) and top 500 most significantly downregulated (E) DEGs across drought, heat, and combined drought + heat treatments. Overlaps reflect shared and stress-specific gene responses. Venn diagrams were created using the VIB/UGent Bioinformatics & Evolutionary Genomics tool. All analyses are based on $n = 3$ biological replicates per treatment. Complete DEG lists, exact gene identifiers, and QC metrics are provided in [Supplementary Tables S1–S4](#).

proteins, and Clp ATP-dependent chaperones. Several TF families, including HSF, DREB/ERF, and zinc-finger proteins, were also differentially expressed, along with genes associated with osmoprotectant metabolism such as galactinol synthase ([Supplementary Table S3](#)). Comparative analysis of the top 500 upregulated DEGs demonstrated strong treatment specificity: 447 genes were uniquely upregulated in Control vs. Heat, 275 genes in Drought vs. Heat, and 229 genes in Control vs. Drought. Only six genes were commonly upregulated across all three comparisons.

Downregulated DEGs also exhibited distinct patterns, with 364, 355, and 332 genes uniquely suppressed in Drought vs. Heat, Control vs. Drought, and Control vs. Heat, respectively, and only 12 genes overlapping between two comparisons. No gene was commonly downregulated across all three comparisons. Direct comparison of drought and heat treatments identified only 122 shared DEGs ([Supplementary Figure S4](#)), confirming that each stress condition elicited largely distinct transcriptional responses in leaves ([Figure 1C, D](#)).

3.3 DEGs involved in ROS and scavenging

Oxidative stress-associated transcriptional adjustments were evident under both drought and heat treatments, reflecting a stress-induced imbalance between ROS production and detoxification. Under drought stress, several components of the thioredoxin (TRX) system including TRXh, TRXf, and TRX-like isoforms were consistently downregulated, indicating attenuation of thiol-disulfide exchange-mediated redox buffering. In contrast, multiple enzymatic antioxidants, including APX, SOD, and glutathione peroxidase (GPX), were significantly upregulated (Supplementary Table S4). This opposing regulation suggests that drought triggers a metabolic shift from TRX-dependent redox signaling toward peroxidase-mediated detoxification to maintain ROS homeostasis.

Heat stress induced a distinct oxidative transcriptional signature characterized by strong upregulation of several class III peroxidases, highlighting enhanced H_2O_2 detoxification and activation of canonical heat-responsive antioxidant defenses. Members of the Fe(II)/2-oxoglutarate (2OG)-dependent dioxygenase superfamily exhibited both induction and suppression, reflecting their context-specific roles in ROS signaling and secondary metabolism. Downregulation of flavanone 3-hydroxylase (F3H) and gibberellin 2-oxidase (GA2ox1) suggests heat-induced adjustments in flavonoid biosynthesis and gibberellin turnover that may contribute to altered growth-defense balance (Supplementary Table S5).

Comparative analysis of drought- and heat-responsive transcripts further supported divergent redox strategies (Supplementary Table S6). The most strongly induced genes across treatments included peroxidases and L-APX, emphasizing the central importance of peroxidase-driven ROS detoxification in

C. teeta. In parallel, increased expression of several 2OG-dependent dioxygenases including GA2ox isoforms linked redox signaling with hormone modulation. Conversely, broad repression of the TRX system including TRX-domain proteins, TRX-dependent peroxiredoxins (PRX), and DSBA-like proteins indicates suppression of thiol-based antioxidant mechanisms under both stresses. Downregulation of GPX and glutaredoxin-dependent PRX genes further indicates a stress-induced shift away from glutathione-TRX cycling toward alternative antioxidant pathways. Together, these patterns demonstrate that *C. teeta* employs stress-specific redox strategies: Drought preferentially activates peroxidase-centered ROS removal, whereas heat induces a combined peroxidase-dioxygenase response while concurrently suppressing the TRX/PRX network. This reconfiguration of antioxidant pathways highlights a flexible redox regulatory system essential for maintaining cellular homeostasis under distinct abiotic stress conditions.

Transcriptomic profiling revealed extensive modulation of TF families under both drought and heat stress, demonstrating large-scale reprogramming of regulatory networks in *C. teeta*. Under drought conditions, members of the NAC family particularly NAM, ATAF1/2, and CUC2 were strongly induced, highlighting their central roles in mediating dehydration tolerance and coordinating downstream stress-responsive pathways. The AP2/ERF superfamily, especially DREB-like TFs, also showed pronounced upregulation, indicating rapid activation of ABA-independent pathways central to drought perception and transcriptional control. In addition, WRKY and bZIP TFs, both known regulators of oxidative stress signaling and defense activation, were significantly upregulated (Figure 2A; Supplementary Table S7). The MYB family exhibited a mixed regulatory pattern, with subsets involved in secondary metabolism showing repression

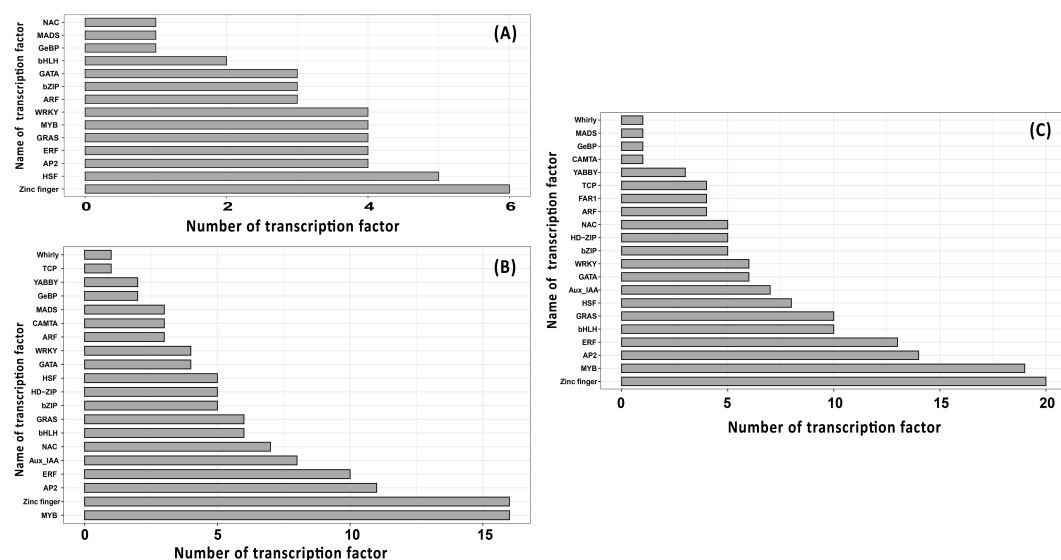


FIGURE 2

Inverted bar graphs showing the distribution of TF families differentially expressed in *C. teeta* under abiotic stress. (A) TF families expressed under heat stress, (B) TF families expressed under drought stress, and (C) TF families identified in the comparative heat-drought dataset. Bars represent the total number of TFs belonging to each family, illustrating stress-specific and shared transcriptional regulatory responses.

whereas others associated with stress signaling were induced. In contrast, growth-associated TF families including GRAS (GAI, RGA, and SCR) and ARF were largely downregulated, consistent with the suppression of growth and developmental processes during drought stress.

Heat stress triggered a distinct transcriptional response dominated by canonical thermotolerance regulators. Heat shock factors (HSFs), including HSF-A2 and other HSF-type DNA-binding proteins, showed marked induction (Figure 2B), consistent with their well-established roles in controlling the expression of heat shock proteins (HSPs) and maintaining proteostasis. Zinc finger (ZF) TFs, including ZPR1-type and SWIM-type proteins, were also strongly upregulated, suggesting enhanced protein stability, signaling, and transcriptional regulation under elevated temperatures. TFs from the MYB, bZIP, WRKY, GRAS, and AP2/ERF families displayed significant modulation as well, indicating that heat stress activates a broad network balancing protective molecular responses with metabolic adjustments (Supplementary Table S8).

Comparative analysis of drought and heat transcriptomes revealed overlapping but distinct TF signatures (Figure 2C; Supplementary Table S9). While both stresses induced major stress-associated families (NAC, WRKY, bZIP, and AP2/ERF),

heat imposed a more focused activation of HSF-HSP pathways, whereas drought elicited broader developmental, hormonal, and redox-associated TF responses. These patterns underscore the presence of both shared and stress-specific regulatory frameworks that enable *C. teeta* to tailor its physiological and molecular responses to different abiotic challenges.

3.4 DEGs related to berberine and coptisine (secondary metabolite) pathway

C. teeta is well known for its high accumulation of BIAs, particularly coptisine and berberine (Figure 3), which contribute markedly to its medicinal value. Transcriptomic profiling under drought stress revealed strong reprogramming of BIA pathway genes, indicating substantial restructuring of precursor flux and downstream enzymatic conversions. Early pathway enzymes, especially S-norcochlorine synthase (NCS; EC 4.2.1.78), which catalyzes the rate-limiting condensation step leading to (S)-norcochlorine were significantly upregulated. Multiple NCS isoforms showed highly significant adjusted p-values, confirming strong transcriptional activation and enhanced entry-point flux into the BIA biosynthetic pathway during drought.

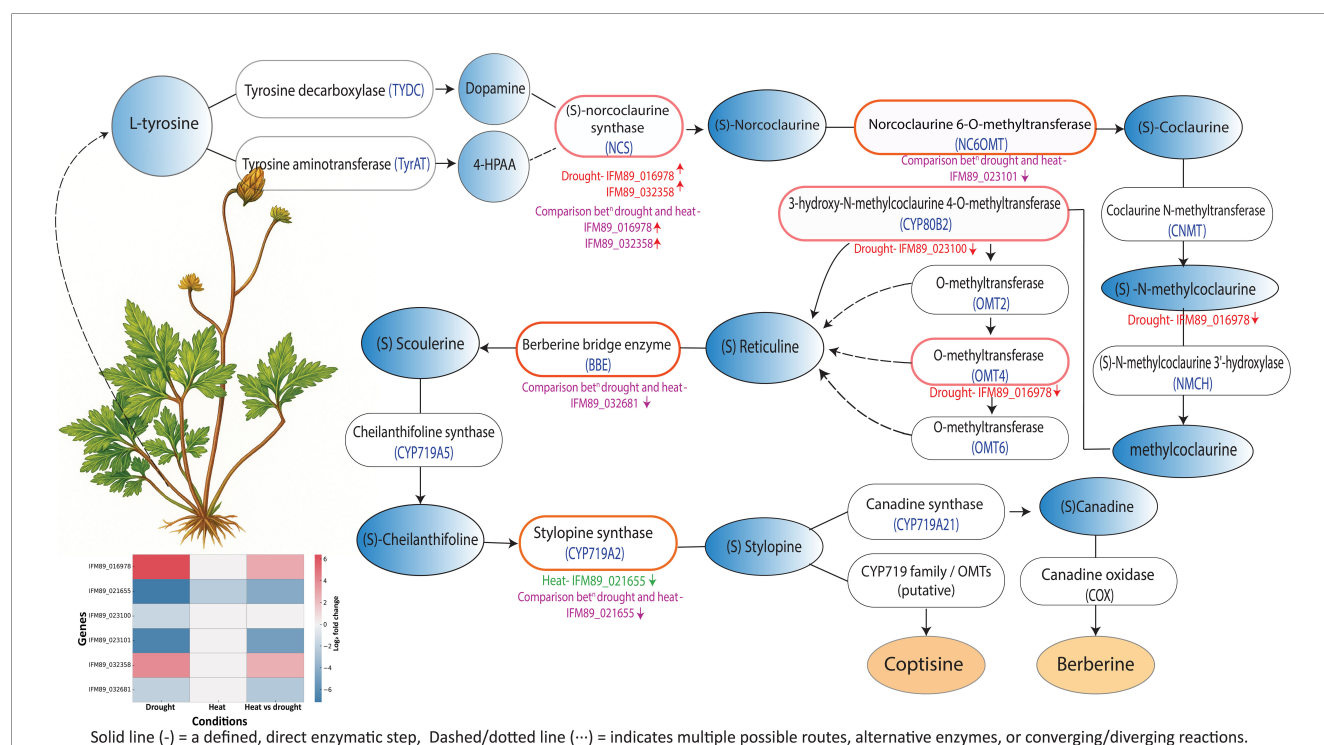


FIGURE 3

Biosynthetic pathway of coptisine and berberine in *C. teeta* under drought and heat stress. The schematic illustrates the BIA pathway beginning from L-tyrosine and progressing through key enzymatic steps to form the major alkaloids coptisine and berberine. DEGs encoding major pathway enzymes including NCS, norcochlorine 6-O-methyltransferase (NC6OMT), berberine BBE, CYP80B2, multiple OMTs, CYP719A family enzymes, and canadine oxidase (COX) are shown adjacent to their corresponding reactions with arrows indicating the direction of flux. Upregulated or downregulated transcripts under drought, heat, or comparative stress conditions are annotated beside each enzyme. Solid lines denote experimentally established enzymatic conversions, whereas dashed or dotted lines represent alternative pathways, putative enzyme steps, or diverging/converging metabolic routes within the broader BIA network.

In contrast, several key O-methyltransferases (OMTs; EC 2.1.1.x) responsible for mid- and late-stage alkaloid methylation were consistently and significantly downregulated. These included norcoclaurine 6-O-methyltransferase, S-adenosyl-L-methionine-dependent OMTs, and caffeic acid 3-O-methyltransferase, all of which modulate essential structural diversification steps in BIA biosynthesis. Furthermore, oxidative enzymes such as berberine bridge enzyme (BBE) and multiple cytochrome P450 monooxygenases displayed marked transcriptional suppression, indicating diminished oxidative conversions required for progression toward berberine and coptisine. Downregulation of stylopine synthase (CYP719A family) further supports a broad attenuation of specialized alkaloid biosynthesis, consistent with stress-induced metabolic reallocation toward cellular protection processes (e.g., ROS detoxification and osmotic adjustment).

Comparative drought-heat analysis revealed that early induction of NCS was maintained across both stress treatments, suggesting shared enhancement of precursor biosynthesis. However, downstream enzymes including BBE, stylopine synthase, NCS 1-like isoforms, and norcoclaurine 6-O-methyltransferase were strongly repressed in both drought and heat datasets. This indicates a pronounced metabolic bottleneck in intermediate conversion steps, restricting flux toward end products such as berberine and coptisine under stress conditions. Collectively, these coordinated transcriptional shifts suggest that although initial steps of BIA biosynthesis remain active, the completion of specialized alkaloid formation is limited, likely due to strategic redirection of metabolic resources toward antioxidant mechanisms and maintenance of cellular homeostasis (Supplementary Table S10).

3.5 Gene-enrichment analysis of the DEGs under heat and drought stress

GO enrichment analysis revealed distinct stress-specific functional reprogramming under drought and heat treatments. Under drought stress, upregulated DEGs were predominantly enriched in biological processes related to lipid transport, transmembrane transport, endoplasmic reticulum-to-Golgi vesicle-mediated transport, protein glycosylation, response to stimulus, and lignin catabolic processes. Corresponding molecular functions included transmembrane transporter activity, serine-type carboxypeptidase activity, dipeptide and tripeptide transporter activity, channel activity, lipid binding, NAD⁺ binding, translation initiation factor activity, and multiple carbohydrate transport and phosphatase activities.

In contrast, drought significantly downregulated core processes associated with translation, photosynthesis, chlorophyll biosynthesis, photosystem I/II activity, chloroplast organization, and response to light stimulus. Suppression of the molecular function structural constituent of ribosome further indicated strong inhibition of protein synthesis and photosynthetic activity,

reflecting major energy-conservation and metabolic restructuring during water limitation (Supplementary Figures S5-S6; Supplementary Tables S11-S12).

Heat stress produced a markedly different enrichment profile. Upregulated DEGs were associated with protein folding, chaperone-mediated protein refolding, cellular response to heat, protein import into mitochondria, response to H₂O₂, and defense response, highlighting activation of a robust proteostasis network. Enriched molecular functions included unfolded protein binding, ATP-dependent chaperone activity, peptidyl-prolyl cis-trans isomerase activity, and nucleic acid binding consistent with strong induction of heat-responsive molecular chaperones (Supplementary Figures S7-S8; Supplementary Tables S11-S12). Conversely, heat stress downregulated biological processes related to protein phosphorylation and secondary metabolite biosynthesis, along with molecular functions such as purine transport and ubiquitin-protein transferase activity, suggesting suppression of metabolically expensive pathways under elevated temperatures.

Comparative GO analysis further highlighted the distinctiveness of these stress responses (Supplementary Figures S9-S10). Drought uniquely enhanced protein glycosylation, fucose metabolism, and protein phosphorylation, whereas heat imposed stronger activation of protein folding and chaperone-assisted proteostasis. Drought caused a more pronounced decline in photosynthesis, translation, and chloroplast organization, whereas heat maintained higher activity of protein quality-control pathways. Collectively, these enrichment patterns demonstrate that drought primarily suppresses energy-intensive photosynthetic and translational machinery while stimulating extensive intracellular transport and cell-wall remodeling. In contrast, heat stress activates a focused proteostasis-centered defense system. This divergence underscores the stress-specific physiological strategies deployed by *C. teeta* to maintain cellular stability under contrasting abiotic challenges.

3.6 Validation of gene expression

qRT-PCR analysis confirmed the RNA-Seq-derived expression patterns for the eight selected stress-responsive genes. Under drought stress, ZAT12 (*IFM89_038338*), DREB2A (*IFM89_022243*), APX (*IFM89_032636*), and the NAC TF as NAC (*IFM89_038338*) showed substantial increases in transcript abundance relative to the control, consistent with enhanced oxidative-stress mitigation and activation of ABA-dependent and ABA-independent dehydration-responsive pathways. Under heat stress, the classical protein-quality control machinery was strongly induced: HSP (*IFM89_036261*) and ClpA/ClpB (*IFM89_03867*) exhibited pronounced upregulation, accompanied by increased expression of their upstream regulatory factor HSF (*IFM89_005514*). These results validate the reliability of the RNA-Seq dataset and demonstrate that drought and heat trigger distinct yet well-defined molecular programs involving ROS detoxification, transcriptional regulation, and proteostasis (Figure 4).

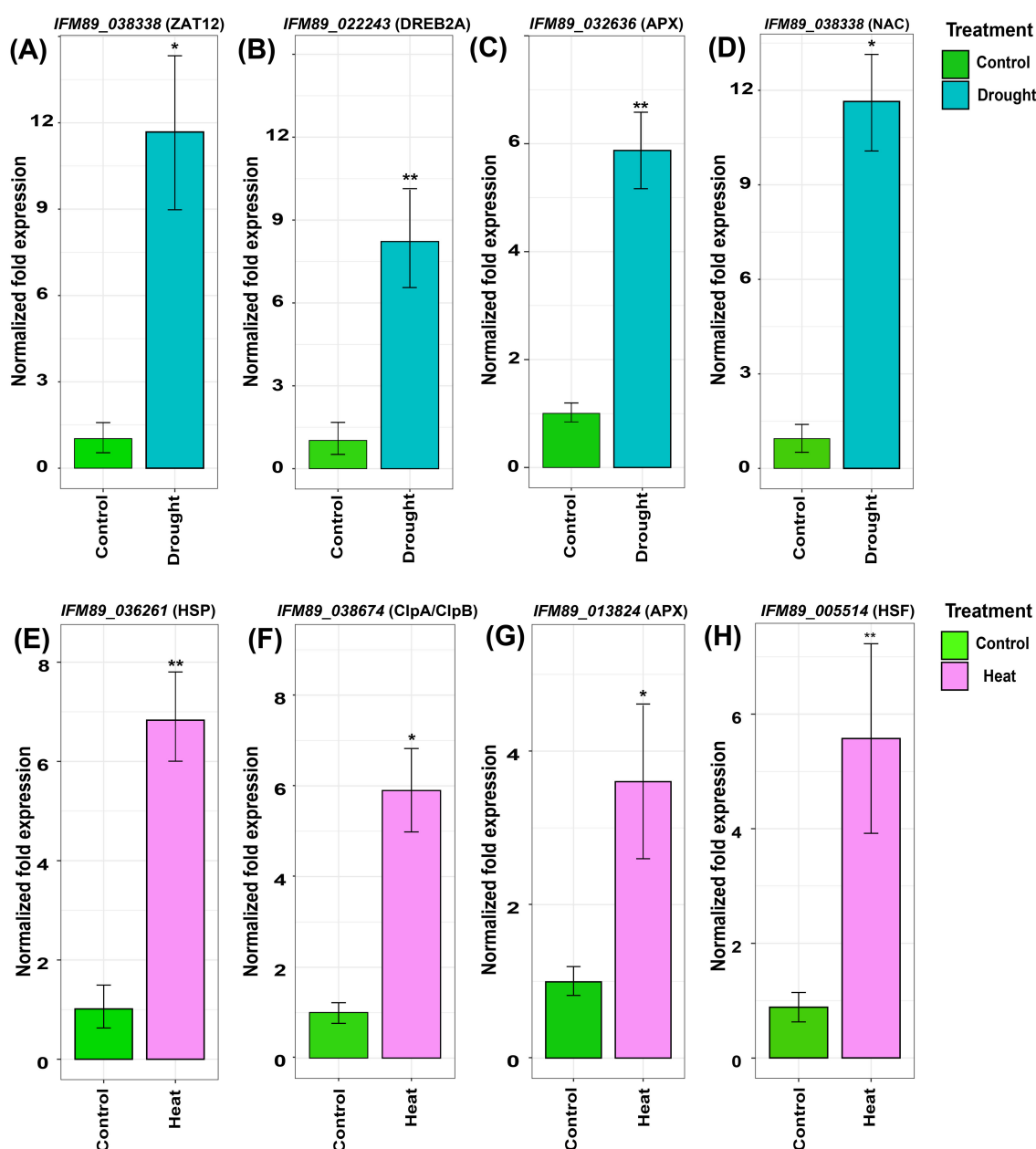


FIGURE 4

Validation of RNA-Seq results using quantitative real-time PCR (qRT-PCR). Expression profiles of four drought (A–D) and heat (E–H) stress-responsive genes were validated by qRT-PCR to confirm transcriptome-derived differential expression patterns. Gene expression levels were normalized against the 18S rRNA internal reference gene, and relative fold changes were calculated using the $2^{-\Delta\Delta C_t}$ method following MIQE guidelines. Bars represent the mean $\pm$ standard deviation (SD) from three independent biological replicates ($n = 3$). Statistical significance between control and stress-treated samples (drought or heat) was assessed using a two-tailed Student's *t*-test implemented in R. Significant differences are indicated as $p < 0.05$ (*) and $p < 0.01$ (**).

4 Discussion

The increasing frequency and intensity of abiotic stresses such as drought and heat, exacerbated by anthropogenic climate change, pose substantial threats to global plant biodiversity (Mittler et al., 2025). Species with narrow ecological niches, including *C. teeta*, are particularly vulnerable because of their restricted distribution, limited physiological plasticity, and fragmented populations (Manish, 2022). This study provides the first comprehensive transcriptomic analysis of

C. teeta under drought and heat stress, revealing coordinated molecular, metabolic, and physiological reprogramming in response to these environmental challenges (Figure 5).

Drought elicited a stronger transcriptomic response (3,099 DEGs) than heat (816 DEGs), suggesting that water deficit imposes a greater physiological burden on *C. teeta*. Similar sensitivity has been reported in moisture-dependent Himalayan taxa adapted to shaded, humid understories (Sato et al., 2024). Under drought, upregulation of GO categories associated with lipid

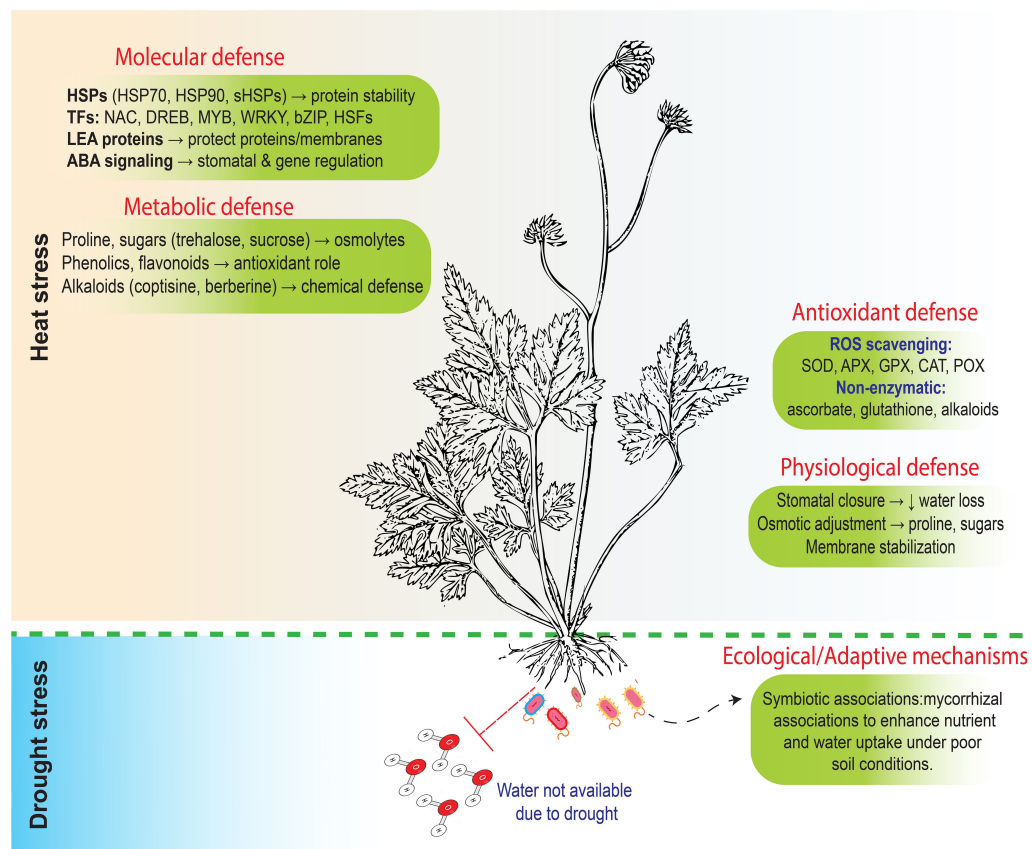


FIGURE 5

Hypothetical defense model of *C. teeta* under drought and heat stress. Environmental stress triggers a coordinated defense strategy involving molecular, metabolic, physiological, and ecological responses. Molecular defenses include activation of HSPs, key TFs (NAC, DREB/ERF, WRKY, bZIP, HSFs), LEA proteins, and ABA-mediated signaling. Metabolic adjustments involve accumulation of osmoprotectants (proline, trehalose, sucrose), phenolics, flavonoids, and alkaloid-based chemical defenses (coptisine, berberine). Antioxidant protection is provided by enzymatic ROS-scavenging systems (SOD, APX, GPX, CAT, POX) and non-enzymatic components (ascorbate, glutathione, alkaloids). Physiological responses include stomatal closure, osmotic adjustment, and membrane stabilization to limit water loss and cellular damage. Ecological/adaptive mechanisms such as mycorrhizal associations further enhance nutrient acquisition and water uptake. Together, these integrated responses enable *C. teeta* to tolerate drought and heat stress.

transport, transmembrane transport, and ER-to-Golgi vesicle-mediated transport indicates substantial membrane remodeling and intracellular trafficking adjustments. Upregulated families included lipid transfer proteins (LTPs), aquaporins (PIPs and TIPs), nitrate/peptide transporters (NRT/PTR), ABC transporters, and laccases pointing to enhanced solute movement, lignin reinforcement, and cellular resource mobilization (Supplementary Figures S5–S6). Conversely, processes related to photosynthesis, chlorophyll biosynthesis, chloroplast organization, and translation were strongly downregulated, reflecting suppression of energy-intensive pathways during drought. Reduced expression of chlorophyll a/b binding proteins, PSII components (e.g., PsbS), thylakoid lumen proteins, and multiple ribosomal proteins highlights impaired photosynthetic capacity and protein synthesis under water limitation.

Heat stress triggered a more targeted but robust proteostasis-centered response. GO enrichment revealed strong activation of protein folding, refolding, oligomerization, and other chaperone-mediated processes consistent with the induction of sHSPs, HSFs,

and HSP70/Clp chaperones (Supplementary Figures S7–S8). Enrichment of molecular functions such as unfolded protein binding and ATP-dependent chaperone activity supports their role in preventing denaturation and aggregation under thermal stress (Kan et al., 2023; Tang et al., 2022; Kundu et al., 2025b). Unlike drought, heat stress suppressed pathways associated with protein phosphorylation and secondary metabolism, indicating diversion of cellular energy toward thermal protection rather than metabolic specialization.

The differential induction of drought-responsive genes including ZAT12, SnRK2.6/OST1, DREB2A, AREB1/ABF2, and LEA14 demonstrates integration of ABA-dependent and ABA-independent pathways (Kim et al., 2024; Yin et al., 2021). SnRK2.6/OST1 promotes ABA signaling and stomatal closure, whereas ABF/AREB factors drive osmoprotective responses. DREB2A activates dehydration-responsive pathways independently of ABA, and ZAT12 regulates redox signaling by buffering drought-induced oxidative bursts. Late-stage induction of *LEA14* contributes to stabilization of proteins and membranes during dehydration (Nakashima et al., 2025). Together,

these genes represent a temporal cascade from early signaling (OST1, ZAT12) to transcriptional regulation (AREB1, DREB2A) and late structural protection (LEA14) that enhances drought tolerance.

Oxidative stress mitigation emerged as a central shared response to both stresses. Upregulation of key enzymatic antioxidants including APX, SOD, and GPX highlights the activation of core ROS-detoxification pathways (Dumanović et al., 2021). APX activity is particularly important for reducing H_2O_2 via the ascorbate-glutathione cycle (Zandi and Schnug, 2022; Kundu et al., 2025a). Conversely, downregulation of TRX and peroxiredoxin (PRX) genes suggests suppression of thiol-mediated redox signaling under stress-induced energy constraints (Sevilla et al., 2023) (Figure 6). This shift may reflect preferential reliance on peroxidase-based H_2O_2 detoxification during stress.

TFs represent major regulatory nodes orchestrating the observed transcriptomic shifts. Drought strongly induced NAC, DREB/ERF, WRKY, and bZIP TFs, each linked to dehydration tolerance, osmoprotection, and ROS regulation (Kim et al., 2024; Nakashima et al., 2025; Zeng et al., 2024). Heat predominantly

activated HSFs and downstream sHSPs, along with HSP70 and ClpB chaperones involved in thermotolerance (Kan et al., 2023; Liu et al., 2023). Upregulation of BAG and p23 co-chaperones further supports coordinated proteostasis under heat stress (Farid et al., 2025). Shared induction of NAC, WRKY, and AP2/ERF TFs under both stresses suggests cross-talk between drought and heat signaling networks (Sato et al., 2024). These patterns are consistent with regulatory frameworks described in *Arabidopsis thaliana*, rice, *C. sinensis*, and other plants (Raja et al., 2020; Li et al., 2020; Sun et al., 2021; Zhang et al., 2022).

Secondary metabolism, particularly BIA biosynthesis, was strongly affected under both stresses. Upregulation of NCS, the entry-point enzyme suggests enhanced precursor formation during drought (Shah et al., 2025). However, downstream enzymes such as berberine BBE, OMTs, and CYP719A family oxidases were consistently downregulated, indicating metabolic bottlenecks limiting the conversion of precursors to mature alkaloids (Tjallinks et al., 2024; Menéndez-Perdomo and Facchini, 2020). These shifts reflect common growth-defense trade-offs where

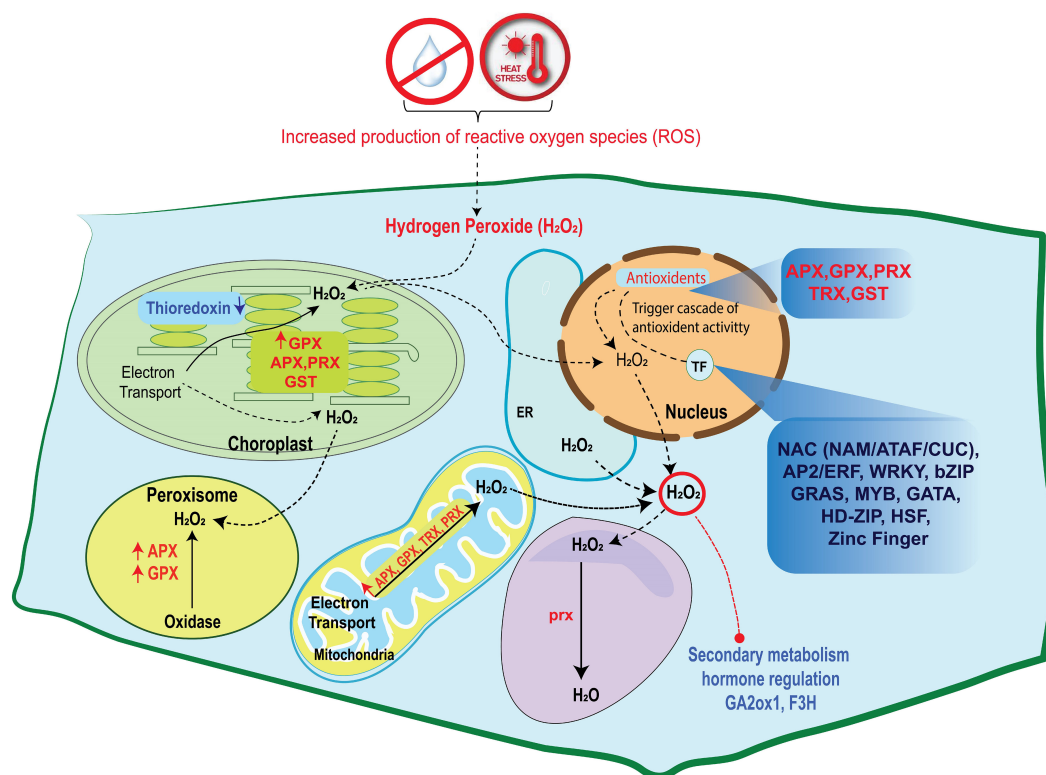


FIGURE 6

Integrated model of ROS generation, detoxification, and redox-regulated signaling in *C. teeta* under drought and heat stress. Drought and heat stress increase the production of ROS, particularly H_2O_2 , in chloroplasts, mitochondria, and peroxisomes. Excess H_2O_2 is detoxified primarily through enhanced activity of key antioxidant enzymes APX, GPX, PRX, and glutathione S-transferases (GST), whereas TRX-dependent redox buffering is concurrently suppressed. H_2O_2 also functions as a mobile signaling molecule, diffusing into the cytosol and nucleus where it activates major stress-responsive TF families including NAC, AP2/ERF, WRKY, bZIP, MYB, GATA, HD-ZIP, HSF, and zinc-finger proteins. These transcriptional regulators coordinate downstream antioxidant defenses, hormone-associated pathways (e.g., GA2ox1), and secondary-metabolite adjustments (e.g., F3H), thereby integrating redox signaling with broader cellular stress responses. Together, this model illustrates how *C. teeta* balances ROS detoxification with H_2O_2 -mediated signal transduction to maintain redox homeostasis and enhance tolerance under drought and heat stress.

energetic resources are redirected toward stress mitigation rather than secondary metabolite diversification (Yadav et al., 2021).

Plant hormone signaling pathways including ABA, JA, ethylene, SA, and brassinosteroids were enriched, revealing complex hormonal cross talk. ABA signaling via PYR/PYL-PP2C-SnRK2 modules plays a central role in drought responses, whereas JA/ET pathways contribute to stress defense and ROS modulation (Kanehisa et al., 2023). Activation of MAPK cascades further integrates ABA, Ca^{2+} , and ROS signals, converging on major TF families such as DREB/AREB, NAC, WRKY, and ZAT12 (Zhao et al., 2025b). qRT-PCR validation supported the transcriptome results: Drought preferentially induced ZAT12, DREB2A, and APX, reflecting redox stress and dehydration signaling, whereas heat strongly upregulated HSPs and HSFs, confirming activation of canonical thermotolerance pathways. NAC induction across both stresses reinforces its role as a central regulator of stress-responsive transcriptional networks.

Collectively, our findings reveal that drought and heat elicit partially overlapping yet mechanistically distinct responses in *C. teeta*. Drought suppresses photosynthesis and protein synthesis while inducing transport, membrane remodeling, and oxidative defense pathways; heat triggers a highly specialized proteostasis-centered response. Integration of transcriptional, hormonal, and metabolic adjustments underscores the adaptive strategies that enable *C. teeta* to survive contrasting abiotic stresses, providing foundational insights for conservation, ecological resilience studies, and potential molecular breeding of related medicinal species.

5 Conclusion

This study provides the first comprehensive transcriptomic framework describing how *C. teeta* responds to drought and heat stress, revealing both shared and stress-specific molecular strategies. Drought triggered the strongest transcriptional reprogramming, characterized by suppression of photosynthesis, chloroplast organization, and translation, alongside enhanced lipid transport, vesicle trafficking, aquaporin activity, and cell-wall remodeling, indicating major energy conservation and cellular stabilization mechanisms. Heat stress, by contrast, induced a focused proteostasis-centered response dominated by strong activation of HSF-HSP chaperone pathways and ATP-dependent refolding machinery, reflecting the central importance of molecular chaperones for thermotolerance. Both stresses activated robust ROS-detoxification systems through APX, SOD, and GPX, coupled with repression of TRX/PRX-mediated thiol-redox pathways, suggesting a strategic shift toward peroxidase-driven antioxidant protection. Major transcription factors including NAC, DREB/ERF, WRKY, bZIP, and HSF acted as regulatory hubs coordinating hormonal, metabolic, and redox signaling, with qRT-PCR validating their stress-specific expression. The BIA pathway exhibited a clear trade-off: Early precursor synthesis (e.g., NCS) remained active, whereas downstream enzymes such as BBE and OMTs were suppressed,

indicating constrained specialized metabolite production under resource-limited conditions. Overall, *C. teeta* deploys a combination of conserved defenses (ROS detoxification, transcriptional activation, hormone signaling) and distinct stress-specific strategies—energy conservation and membrane remodeling under drought, and proteostasis stabilization under heat. The candidate genes and pathways identified here provide valuable targets for conservation genomics, metabolic engineering, and climate-resilient propagation. This transcriptome resource establishes a foundational platform for future research and long-term preservation of this ecologically and medicinally important species.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: NCBI BioProject PRJNA1313294.

Author contributions

MN: Data curation, Formal Analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. DB: Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing. PR: Conceptualization, Formal Analysis, Resources, Validation, Visualization, Writing – original draft. DG: Formal Analysis, Investigation, Methodology, Writing – original draft. KK: Formal Analysis, Investigation, Methodology, Writing – original draft. KB: Investigation, Methodology, Software, Writing – review & editing. BK: Data curation, Formal Analysis, Investigation, Methodology, Software, Writing – review & editing. BT: Conceptualization, Funding acquisition, Investigation, Resources, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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SUPPLEMENTARY TABLE 1

Summary of RNA-seq datasets from *C. teeta*, including total read counts (in millions) and the percentage of uniquely mapped reads to the *C. chinensis* reference genome for each sample under control, drought, and heat stress conditions.

SUPPLEMENTARY TABLE 2

Total differentially expressed gene and their annotation in all sample (Control, Heat, Drought condition).

SUPPLEMENTARY TABLE 3

Differentially expressed specific genes under heat and drought stress and their annotation.

SUPPLEMENTARY TABLE 4

Differentially expressed antioxidant responsive genes under drought stress and their annotation.

SUPPLEMENTARY TABLE 5

Differentially expressed antioxidant responsive genes under heat stress and their annotation.

SUPPLEMENTARY TABLE 6

Comparative analysis of differentially expressed antioxidant responsive genes between heat and drought stress and their annotation.

SUPPLEMENTARY TABLE 7

Differentially expressed transcription factors under drought stress and their annotation.

SUPPLEMENTARY TABLE 8

Differentially expressed transcription factor under heat stress and their annotation.

SUPPLEMENTARY TABLE 9

Comparative analysis of differentially expressed transcription factor between heat and drought and annotation.

SUPPLEMENTARY TABLE 10

Differentially expressed coptisine and berberine biosynthesis responsive genes under heat and drought condition and their annotation.

SUPPLEMENTARY TABLE 11

Gene Ontology (GO) enrichment analysis of upregulated genes under drought and heat stress, showing associated biological processes, molecular functions, cellular component and statistical significance.

SUPPLEMENTARY TABLE 12

Gene Ontology (GO) enrichment analysis of downregulated genes under drought and heat stress, showing associated biological processes, molecular functions, cellular component and statistical significance.

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