

Integrated transcriptomic and metabolomic analyses uncover the key pathways of *Tamarix ramosissima* in adaptation to coppice dune development

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Abstract

Background: *Tamarix ramosissima* is a crucial windbreak and sand-fixing shrub species in the arid deserts of northwest China, where it helps stabilize the ecosystem by trapping dust and enriching soil nutrients. In this study, we systematically investigated the molecular mechanisms underlying *T. ramosissima*'s transition through the four developmental stages (initial, growth, stable, and decline) of coppice dunes through integrated physiological, transcriptomic, and metabolomic analyses.

Results: From the initial to the growth stage of coppice dunes, soil water content (SWC) gradually decreased, whereas the malondialdehyde (MDA) content in *T. ramosissima* increased. *T. ramosissima* could resist drought stress by enhancing soluble protein (SP) and proline (Pro) contents and catalase (CAT) activity. In addition, the decrease in SWC can promote the accumulation of zeatin, dihydrozeatin, and indole-3-acetate in *T. ramosissima*, thereby accelerating starch consumption to meet plant growth demands. Transcriptome and metabolome analyses revealed that *T. ramosissima* primarily coordinates gene expression and metabolite accumulation through the ABC transporters, glycerophospholipid metabolism, and glutathione metabolism pathways to adapt to coppice dune development; meanwhile, four hub genes identified by weighted gene co-expression network analysis (WGCNA) are promising candidates for improving drought tolerance in this species.

Conclusion: This study reveals that *T. ramosissima* can adapt to coppice dune development by coordinating gene expression, metabolite accumulation, and physiological regulations. The results provide an important theoretical basis for ecological restoration in arid regions.

1. Introduction

Global climate change has intensified the spatiotemporal heterogeneity and regional asymmetry of precipitation, profoundly altering the fundamental processes of the global water cycle^[1]. Arid regions face increasingly severe challenges, including shifts in precipitation patterns, elevated evaporative demand, and sustained declines in soil water availability^[2]. These changes drive groundwater depletion and land desertification, threatening vegetation survival and ecosystem stability^[3]. Water scarcity coupled with soil impoverishment constitutes a primary constraint on desert plant growth^[4]. However, coppice dunes can mitigate these stresses by creating a “fertile island effect”, which improves local soil moisture, nutrient availability, and microtopography, thereby providing relatively favorable microhabitats for desert shrubs^[5]. To cope with water stress during the accumulation and development of coppice dune, desert plants typically activate complex antioxidant defense systems. These include enzymatic antioxidants like superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), as well as non-enzymatic substances such as proline (Pro), reduced glutathione, and ascorbic acid, which collectively scavenge excessive reactive oxygen species (ROS) to maintain cellular membrane stability and integrity^[6].

To cope with drought stress and reduce tissue water loss, plants can synthesize osmoprotectants such as Pro, soluble protein (SP), and soluble sugar (SS), thereby lowering cellular osmotic potential, maintaining cell turgor, and facilitating water uptake^[7]. Moreover, under continuous drought stress, plant photosynthesis is inhibited, and the synthesis of non-structural carbohydrates (NSC) is also restricted. When the rate of carbon consumption exceeds the capacity of carbon assimilation, carbon sources become gradually depleted, potentially exposing plants to the risk of carbon starvation and restricting normal physiological processes such as growth and osmotic adjustment^[8]. To balance the dual demands of carbon fixation and water conservation, plants employ adaptive resource allocation strategies. For instance, *Nitraria sibirica* and *Pinus densiflora* reallocate resources from growth to defense mechanisms under drought conditions^[9, 10]. Besides, Phytohormones, serving as central signaling molecules, orchestrate these physiological and metabolic adjustments to enhance drought tolerance^[11]. Therefore, analyzing the integrated physiological and biochemical responses of desert plants is essential for elucidating their adaptation mechanisms to arid environments.

Recent advances in high-throughput sequencing have accelerated research into plant stress resistance. Integrated transcriptomic and metabolomic analyses have emerged as powerful tools for deciphering the underlying molecular mechanisms^[12, 13]. Specifically, Transcriptomics reveals the genetic regulation of metabolite biosynthesis, transport, and signaling^[14], whereas metabolomics identifies and quantifies small-molecule metabolites, linking them to physiological changes^[15]. Consequently, their combined analysis can reconstruct the complex regulatory network of "gene expression—metabolite accumulation—phenotypic response", enabling comprehensive elucidation of plant adaptation to adverse environments^[16]. This integrated strategy has been widely applied to study drought adaptation in desert plants such as *Haloxylon ammodendron*, *Haloxylon persicum*, *Salix psammophila*, and *Tamarix taklamakanensis*^[17, 18, 19]. However, Species-specific genetic backgrounds drive distinct adaptive mechanisms. For instance, *H. ammodendron* and *H. persicum* enhance drought tolerance by upregulating pathways related to glycolysis/gluconeogenesis and amino acid synthesis^[19]. In contrast, drought represses photosynthesis in *Nitraria sibirica* through the downregulation of genes encoding key components, including photosystem II (e.g., *PsbP*, *PsbQ*, *PsbR*, *PsbY*, *Psb27*), photosystem I (e.g., *PsaD*, *PsaF*, *PsaG*, *PsaH*, *PsaK*, *PsaO*), photosynthetic electron transport (*PetF*), and light-harvesting antenna proteins^[10]. Meanwhile, *Ammopiptanthus nanus* enhances drought resistance via ABA signaling transduction, achieved by downregulating *PYR/PYL* and *PP2C* genes and promoting ABA accumulation^[20]. Collectively, these studies lay a crucial foundation for understanding the drought response mechanisms of desert plants.

T. ramosissima, a keystone windbreak and sand-fixing shrub in arid regions, forms distinctive coppice dunes through long-term wind-sand interactions and plays a pivotal role in soil and water conservation, desertification control, and biodiversity maintenance^[21, 22]. The formation of these dunes is an extremely protracted process, often spanning centuries to over a millennium, during which dune heights increase from tens of centimeters to tens of meters. Based on morphological characteristics, the

development of *Tamarix* coppice dunes typically proceeds through four stages: initial, growth, stable, and decline^[23, 24]. Notably, at the decline stage of coppice dunes, *T. ramosissima* exhibits dwarfism, self-thinning, and degradation, ultimately losing its sand-fixing capacity^[23]. This transformation turns the dunes into sources of sandstorms, threatening both ecosystem integrity and human well-being. Consequently, in the context of global climate change, deciphering the physiological and molecular adaptation of *T. ramosissima* to dune development is therefore critical for desert ecosystem conservation. Current research on *Tamarix* coppice dunes has primarily focused on soil nutrients, water-use strategies, and photosynthetic responses^[23, 25, 26]. However, the associated physiological metabolism, gene regulatory networks, and metabolite accumulation patterns in *T. ramosissima* throughout dune development remain largely uncharacterized.

Therefore, this study focused on *T. ramosissima* inhabiting coppice dunes at different developmental stages in the southwestern Gurbantunggut Desert. By integrating physiological trait analysis with transcriptomics and metabolomics, we elucidated the adaptive mechanisms underlying its response to dune development. Specifically, we addressed the following two questions: (1) What is the relationship between the physiological traits of *T. ramosissima* and soil water content during this process? (2) What are the molecular bases of *T. ramosissima*'s adaptation to coppice dune development? Our findings advance the understanding of the physiological and molecular regulatory mechanisms behind *T. ramosissima*'s adaptation to dune development and provide valuable genetic resources for breeding drought-tolerant plants.

2 Materials and methods

2.1. Study site

The Gurbantunggut Desert (84°50'–91°20' E, 44°15'–46°50' N), located in the central Junggar Basin of Xinjiang, covers an area of approximately 4.88×10^4 km² (Fig. 1). It is dominated by fixed and semi-fixed dunes, which account for about 97% of its total area, making it the largest desert of this type in China^[27]. The region experiences a typical temperate continental climate characterized by hot summers and cold, snowy winters, with a stable snow cover period lasting 100–150 days. The annual average temperature ranges from 6 to 10 °C, while annual precipitation averages between 70 and 150 mm, far below the annual potential evaporation, which exceeds 2,000 mm. Vegetation coverage is relatively high, ranging from 40% to 50%, and includes over 200 species of drought-tolerant and ephemeral plants^[27]. Dominant species consist of xerophytic and super-xerophytic shrubs, such as *Haloxylon ammodendron*, *Haloxylon persicum*, *Eremosparton songoricum*, and *Agriophyllum squarrosum*.

2.2. Plot set up and sample collection

With local department permission, we established a 5 km × 5 km experimental plot in the Mosuowan Desert Area (44°55' N, 86°12' E), at the southwestern edge of the Gurbantunggut Desert (Fig. 1). Using

established dune classification criteria, we adopted the space-for-time substitution method and selected four developmental stages of *T. ramosissima* coppice dunes: initial, growth, stable, and decline [23, 28]. For each stage, three replicate dunes were chosen, totaling 12 coppice dunes. Mature, intact, and pest-free leaf samples of *T. ramosissima* were collected from the east-oriented middle canopy of the selected dunes. Sampling took place between 07:00 and 10:00 local time on July 12, 2024. All samples were immediately flash-frozen in liquid nitrogen and then transported to the laboratory for storage at -80 °C for subsequent analysis. We also measured the soil water content (SWC) at 0–500 cm depth in coppice dunes of each developmental stage. Results showed that SWC decreased continuously as dune development progressed (**Table S1**). In addition, a meteorological station within 5 km of the study area confirmed that no precipitation occurred in the 37 days before sampling. This ensured that sampling took place under extreme aridity and high temperatures, minimizing the confounding effects of short-term climatic variability.

2.3. Physiological indicator determination

Following the method of Chang and Lv [10], after 0.1 g of plant leaf tissue was weighed, the activities of SOD, CAT, and POD were determined using the nitroblue tetrazolium (NBT) reduction, spectrophotometric, and guaiacol methods, respectively; the contents of Pro, SS, SP, and MDA were quantified using the sulfosalicylic acid, anthrone colorimetric, Coomassie brilliant blue (G-250), and thiobarbituric acid (TBA) methods, respectively. Starch (ST) content was measured by acid hydrolysis followed by anthrone colorimetric quantification and conversion to starch equivalents. Non-structural carbohydrates (NSC) content was calculated as the sum of SS and ST [29]. All assays were performed with commercial kits (Grace Biotechnology Co., Ltd., Suzhou, China).

2.4. Metabolomic analysis

Approximately 50 mg of frozen leaf tissue was ground at 45 Hz for 10 min, followed by sonication in an ice-water bath for 10 min and centrifugation at 12,000 rpm (4°C) for 15 min. The supernatant was collected, vacuum-dried, and then reconstituted in the extraction solution. The mixture was vortexed for 30 s, sonicated for 10 min in an ice-water bath, and centrifuged again. The final supernatant was used for LC-MS/MS analysis. Raw data files were processed using Progenesis Q1 software for peak extraction and alignment. Metabolites were identified by searching against the integrated METLIN database and public repositories, with confirmation based on theoretical fragment matching. They were subsequently annotated using the Kyoto Encyclopedia of Genes and Genomes (KEGG Database), the Human Metabolome Database (HMDB Database), and the Lipid Metabolites and Pathways Strategy (LIPID MAPS). Differentially accumulated metabolites (DAMs) were screened based on the following criteria: a variable importance in projection (VIP) value > 1.0 from orthogonal partial least squares-discriminant analysis (OPLS-DA), fold change ≥ 1 , and $p < 0.05$.

2.5. Transcriptomic analysis

Total RNA was extracted using the Polysaccharide-Polyphenol Plant Total RNA Extraction Kit (Tiangen, China). mRNA libraries were constructed and sequenced on the Illumina NovaSeq Xplus platform. Raw

reads were filtered to remove adapters and low-quality bases, yielding clean reads. De novo transcriptome assembly was performed using Trinity software^[30]. The assembled transcripts were clustered to remove redundancy using TGICL, generating a set of unigenes. Functional annotation of the unigenes was performed using BLAST searches against multiple databases, including NR, Swiss-Prot, COG, KOG, eggNOG, KEGG, GO, and Pfam. Transcription factors (TFs) were predicted using iTAK software^[31]. Gene expression levels were quantified using the FPKM metric. Differentially expressed genes (DEGs) were identified with the DESeq2 package, applying thresholds of $|\log_2FC| \geq 1$ and False Discovery Rate (FDR) < 0.01.

2.6. Quantitative Real-Time PCR (qRT-PCR) validation

A total of 12 DEGs associated with drought stress were randomly selected for quantitative real-time PCR (qRT-PCR) validation. Total RNA was extracted using a total plant RNA extraction kit (FOREGENE, China), and first-strand cDNA was synthesized with a corresponding reverse transcription kit (FOREGENE, China). qRT-PCR assays were performed using SYBR Green I Master Mix (FOREGENE, China) with *Actin* serving as the reference gene. The primer sequences used are listed in **Table S2**. Relative expression levels were calculated using the $2^{-\Delta\Delta CT}$ method based on three independent biological replicates.

2.7. Statistical analysis

Statistical analyses of physiological data were performed using IBM SPSS Statistics 26. One-way analysis of variance (ANOVA) was conducted, followed by Duncan's multiple-range test for post hoc comparisons, with a significance level of $p < 0.05$. Graphs were constructed using Origin 2024. Hub genes within key co-expression modules were identified with the degree algorithm in the cytoHubba plugin for Cytoscape 3.7.1. Venn diagrams were generated using TBtools. All other bioinformatics analyses were performed on an online platform (<https://www.biocloud.net/>).

3. Results

3.1. Leaf physiological traits

Leaf physiological and biochemical traits of *T. ramosissima* varied significantly across the developmental stages of coppice dunes (Fig. 2). SP and Pro contents were significantly higher at the decline stage of coppice dunes compared to the initial and growth stages of coppice dunes ($p < 0.05$; Fig. 2A, C). In contrast, SS content was highest at the growth stage of coppice dunes and lowest at the decline stage of coppice dunes ($p < 0.05$; Fig. 2B). ST content of *T. ramosissima* exhibited a gradual decrease with dune development, reaching its lowest level at the decline stage of coppice dunes ($p < 0.05$), which represented a 52.6% reduction from the initial stage of coppice dunes (Fig. 2D). NSC content was significantly higher at initial (24.68 mg/g) and growth (24.37 mg/g) stages of coppice dunes than at stable and decline stages of coppice dunes ($p < 0.05$; Fig. 2E). Regarding antioxidant capacity, SOD activity peaked at growth and stable stages of coppice dunes ($p < 0.05$) and was lowest at the decline stage of coppice dunes (Fig. 2I). In contrast, CAT activity showed the opposite trend, being

highest at the decline stage of coppice dunes (Fig. 2H). MDA content was highest in plants at the decline stage of coppice dunes (27.66 nmol/g; $p < 0.05$), indicating the most severe membrane lipid peroxidation under environmental stress (Fig. 2F). These results indicate that the development of coppice dunes significantly affects the physiological characteristics of *T. ramosissima*.

3.2. Metabolomic analysis

Principal Component Analysis (PCA) revealed clear separation among the four dune developmental stages, with biological replicates closely clustered within the 95% confidence ellipse (Fig. 3A). Furthermore, hierarchical clustering analysis demonstrated distinct, stage-specific patterns of metabolite accumulation across the developmental stages of coppice dunes (Fig. 3B).

3.3. DAMs statistics

Across all pairwise comparisons encompassing the four dune developmental stages, a total of 4,504 DAMs were identified (**Table S3**). Specifically, 1,981 DAMs (1,076 upregulated, 905 downregulated), 547 DAMs (248 upregulated, 299 downregulated), 987 DAMs (423 upregulated, 564 downregulated), 867 DAMs (260 upregulated, 607 downregulated), 795 DAMs (284 upregulated, 511 downregulated), and 755 DAMs (459 upregulated, 296 downregulated) were identified in the pairwise comparisons of ISL vs GSL, ISL vs SSL, ISL vs DSL, GSL vs SSL, GSL vs DSL, and SSL vs DSL, respectively. Notably, the number of DAMs in *T. ramosissima* gradually decreased across the comparisons of ISL vs GSL, GSL vs SSL, and SSL vs DSL, indicating that coppice dune development suppresses the metabolic regulatory capacity of *T. ramosissima*.

3.4. Phytohormone content changes

Based on previous reports linking specific metabolites to drought response, we focused on analyzing changes in plant hormone levels among drought-related DAMs. Venn analysis was performed on DAMs involved in the plant hormone signal transduction pathway. The results demonstrated that zeatin and indole-3-acetate were commonly identified across three comparison groups: ISL vs GSL, GSL vs SSL, and SSL vs DSL (Fig. 4A). In contrast, Dihydrozeatin was exclusively shared between the ISL vs GSL and SSL vs DSL groups (Fig. 4A). Subsequently, the abundance dynamics of these three hormones were visualized to illustrate their accumulation patterns. We found that zeatin and indole-3-acetate levels exhibited a “rise–fall–rise” pattern across the dune developmental stages (Fig. 4B, D). Moreover, dihydrozeatin levels increased from the initial stage to the growth stage and from the stable stage to the decline stage (Fig. 4C). Notably, all three hormones reached their peak concentrations at the decline stage of coppice dunes (Fig. 4B-D). These results suggest that *T. ramosissima* actively modulates its endogenous hormone homeostasis to activate stress-responsive signaling pathways, thereby adapting to the changing environment during coppice dune development.

3.5. Transcriptome sequencing

Transcriptome sequencing generated 72.52 Gb of high-quality clean data, with Q30 scores exceeding 95.26% and GC content above 43.19%, confirming high sequencing accuracy (**Table S4**). De novo assembly yielded 99,886 unigenes with an N50 length of 2,038 bp, indicating good transcriptome continuity. High consistency among biological replicates was confirmed by Pearson correlation coefficients ($R^2 > 0.95$; Fig. 5A).

3.6. Functional annotation of unigenes

Among the assembled unigenes, 45,428 (45.48% of total) were successfully annotated against at least one of the eight public databases, while 7,036 unigenes obtained annotations from all databases (Fig. 5B). The eggNOG database provided the highest number of unique annotations, whereas the Gene Ontology (GO) database contributed the fewest exclusive annotations.

3.7. DEGs statistics

Across all pairwise comparisons encompassing the four dune developmental stages, a total of 24,881 DEGs were identified. Among of 15,302 DEGs (12,915 upregulated, 2,387 downregulated), 9,243 DEGs (6,978 upregulated, 2,265 downregulated), 14,325 DEGs (11,653 upregulated, 2,672 downregulated), 15,594 DEGs (3,370 upregulated, 12,224 downregulated), 15,974 DEGs (7,128 upregulated, 8,846 downregulated), and 15,504 DEGs (10,897 upregulated, 4,607 downregulated) were identified in the pairwise comparisons of ISL vs GSL, ISL vs SSL, ISL vs DSL, GSL vs SSL, GSL vs DSL, and SSL vs DSL, respectively (Fig. 6A). Notably, upregulated DEGs predominated in comparisons involving ISL vs GSL and SSL vs DSL, whereas downregulated genes were markedly more abundant in GSL vs SSL. This pattern suggests that the progression from the growth to the stable stage of coppice dune is associated with a broad suppression of core metabolic processes. Transcription factor (TF) prediction identified hundreds of differentially expressed TFs in each comparison, with total counts ranging from 373 to 634 (Fig. 6B). The C2H2 and bHLH families were consistently the most abundant among these TFs across all comparisons, indicating their pivotal roles in regulating *T. ramosissima*'s response to coppice dune development.

3.8. qRT-PCR validation

The reliability of the RNA-seq expression data was validated by qRT-PCR analysis of 12 randomly selected DEGs related to drought stress. The results exhibited a high correlation ($R^2 > 0.94$) between the two methods (**Fig. S1**), confirming the high consistency and robustness of the transcriptomic dataset.

3.9. Integrated transcriptomic and metabolomic analysis identifies key pathways

Integrated transcriptomic and metabolomic analysis consistently revealed three co-enriched pathways across ISL vs GSL, ISL vs SSL, and ISL vs DSL, including glutathione metabolism (ko00480), ABC transporters (ko02010), and glycerophospholipid metabolism (ko00564) (Fig. 7A–C). This highlights

their crucial roles in *T. ramosissima*'s adaptation to the development of coppice dunes. DEGs expression within these pathways displayed distinct stage-specific patterns. In the glutathione metabolism pathway, most genes encoding glutathione S-transferase (16 upregulated, 3 downregulated), dehydroascorbate reductase (2 upregulated), and glutathione peroxidase (7 upregulated) were upregulated in ISL vs GSL; most were downregulated in GSL vs SSL (glutathione S-transferase: 4 upregulated, 14 downregulated; dehydroascorbate reductase: 2 downregulated; glutathione peroxidase: 3 upregulated, 7 downregulated); and re-upregulated in SSL vs DSL (glutathione S-transferase: 16 upregulated, 12 downregulated; dehydroascorbate reductase: 2 upregulated, 1 downregulated; glutathione peroxidase: 4 upregulated, 2 downregulated) (for detailed gene counts, see **Table S5**). Correspondingly, the GSH/GSSG ratio (reduced-to-oxidized glutathione ratio) decreased from GSL to SSL but increased from SSL to DSL, reflecting dynamic shifts in cellular redox state (**Table S5**). Similarly, in both the ABC transporters and glycerophospholipid metabolism pathways, the predominant expression pattern for most genes was upregulated in ISL vs GSL, downregulated in GSL vs SSL, and subsequently re-upregulated in SSL vs DSL (**Table S6 and S7**). Venn analysis of metabolites associated with these pathways identified 17 key differential metabolites (Fig. 7D, **Table S8**).

3.10. Hub gene identification via weighted gene co-expression network analysis (WGCNA)

To identify key gene modules linked to coppice dune development, we performed WGCNA by correlating DEGs expression matrices with the abundance of 17 key DAMs. Based on expression patterns, DEGs were clustered into eight distinct modules (Fig. 8A), and a heatmap visualized the correlation strengths between these gene modules and the DAMs (Fig. 8B). Among them, the MEturquoise and MEyellow modules showed the most significant correlations with the DAMs. Four drought response hub genes were identified based on gene connectivity within modules and the published literature. Of these, two were derived from the MEturquoise module (Fig. 9A, B), while the remaining two originated from the MEyellow module (Fig. 9C, D). These were *TRINITY_DN1805_c0_g1* (*SAP4*-like), *TRINITY_DN740_c1_g1* (*EXO70B1*), *TRINITY_DN2662_c2_g1* (*MAPKK5*-like), and *TRINITY_DN4872_c1_g2* (*bHLH47*-like).

3.11. Co-expression network analysis of molecular levels, physiological traits, and SWC

A co-expression network was constructed based on Pearson correlation analysis among the screened DAMs, hub genes, phytohormone levels, physiological traits, and SWC. Strong correlations were observed, notably between hub genes and DAMs, as well as between DAMs and key physiological traits (Fig. 10, **Table S9**). Furthermore, SWC exhibited a significant negative correlation with Pro, zeatin, dihydrozeatin, and indole-3-acetate ($p < 0.05$), but a significant positive correlation with ST ($p < 0.05$). This pattern underscores the profound influence of soil water content on the physiological status of *T. ramosissima*. MDA content was strongly positively correlated with Pro ($p < 0.01$). It strongly negatively correlated with SS ($p < 0.01$), suggesting that increased membrane lipid peroxidation triggers Pro accumulation and SS consumption to alleviate oxidative damage. In addition, NSC content was strongly

negatively correlated with SP, Pro, and MDA ($p < 0.01$). It strongly positively correlated with SS and ST ($p < 0.01$), indicating that there was a tight metabolic coupling between NSC and other stress-responsive compounds. Notably, ST content exhibited a significant negative correlation with zeatin, dihydrozeatin, and indole-3-acetate ($p < 0.05$), suggesting a potential trade-off between starch consumption and the signaling or action of these growth-related hormones during adaptation.

4 Discussion

4.1. Physiological metabolic changes in *T. ramosissima* during coppice dune development

The formation and development of coppice dunes are mainly regulated by soil water content [32]. Our data show that SWC decreased gradually as coppice dunes developed (**Table S1**). Correspondingly, SP and Pro contents were lowest at the initial and growth stages of coppice dunes and highest at the decline stage of coppice dunes. Notably, NSC exhibited highly significant negative correlations with SP and Pro. These findings suggest that *T. ramosissima* reallocates limited NSC resources away from growth and toward defense to adapt to the development of coppice dunes, prioritizing amino acid synthesis and transport, which in turn drives the accumulation of SP and Pro, thereby preventing water loss and maintaining osmotic balance [19]. In contrast, SS content was lowest at the decline stage of coppice dunes, a finding that differs from reports in *Calligonum leucocladum* [33]. This discrepancy may reflect a species-specific strategy in which SS in *T. ramosissima* shifts from osmotic regulation to energy provision as dune development progresses and drought intensifies [34].

Changes in NSC content can reflect plants' carbon supply status and their adaptive strategies to environmental changes [23, 35, 36]. Specifically, ST is an important component of NSC and serves as a long-term energy storage mechanism [23]. In this study, we found that NSC content was lowest at the decline stage of coppice dunes. This result may stem from multiple factors: (i) stomatal closure under severe drought limiting photosynthetic carbon fixation [37]; (ii) NSC consumption for drought defense mechanisms [38]; and (iii) prioritization of resource allocation to the construction and storage of below-ground traits, which serve as a mobilizable energy reserve for growth and development after the alleviation of drought stress [39]. Correspondingly, field observations confirmed that *T. ramosissima* failed to flower at the decline stage of coppice dunes (Fig. 11). This observed impaired reproductive capacity is likely due to insufficient NSC reserves, resulting from reduced photochemical efficiency under stress [23]. Finally, we also found that ST was significantly positively correlated with SWC, indicating that a decrease in SWC promoted the consumption and decomposition of ST to meet plants' energy demand.

Abiotic stress typically triggers excessive ROS accumulation, leading to oxidative damage to cellular membranes, proteins, and nucleic acids [40]. MDA, a product of membrane lipid peroxidation, serves as a key indicator of the extent of ROS-induced damage, while antioxidant enzymes function to prevent

excessive ROS accumulation ^[41]. In this study, the highest MDA content was observed in *T. ramosissima* at the decline stage of coppice dunes. This elevation is likely attributed to two main reasons. On the one hand, SWC was at its lowest during this stage, which induced ROS production in leaves and subsequently triggered lipid peroxidation of membrane lipid ^[42]. On the other hand, the extensive dieback of *T. ramosissima* on dunes led to the lowest canopy density, rendering the plants more susceptible to photoinhibition and resulting in the highest MDA content ^[43]. To reduce oxidative damage, *T. ramosissima* enhanced CAT activity, contributing to ROS homeostasis. However, a strong positive correlation was observed between Pro and MDA, with a negative correlation observed with SWC (Fig. 10), suggesting that the enzymatic antioxidant system alone may be insufficient for timely ROS scavenging. Meanwhile, the concurrent increase in Pro likely serves dual purposes: osmotic adjustment and direct antioxidant defense, thereby helping stabilize ROS levels ^[44]. Nevertheless, persistent drought stress ultimately overwhelms these protective mechanisms, leading to significant cellular damage, as reflected by elevated MDA content ^[45].

4.2. Endogenous hormone changes in *T. ramosissima* during coppice dune development

Zeatin, dihydrozeatin, and indole-3-acetate play crucial roles in regulating plant growth and development ^[46, 47]. Among cytokinins, zeatin is the most active and ubiquitous ^[48]. Zeatin and dihydrozeatin have both been shown to promote the division and differentiation of plant cells ^[49, 50], whereas indole-3-acetate enhances plant height, increases branch number, and promotes biomass accumulation ^[46]. Consistent with their known regulatory roles, our study revealed that *T. ramosissima* dynamically regulated the levels of zeatin, dihydrozeatin, and indole-3-acetate throughout the development of coppice dunes. Specifically, during the transition from the initial to the growth stage of coppice dunes, these hormones accumulated (Fig. 11). It is likely due to improvements in the rhizosphere microenvironment, such as the increase in coppice dunes volume and height, which provides extensive growth space for plant root systems, enhances water and nutrient uptake, and supports the development of larger aboveground biomass. Simultaneously, facilitates the temporary retention of additional moisture, while the formation of a biological soil crust reduces soil water evaporation ^[51]. In addition, the "fertile island" effect promotes soil nutrient enrichment ^[52]. These changes promote elevated hormone levels and increase aboveground biomass ^[35]. Conversely, from the growth to the stable stage of coppice dunes, the contents of zeatin and indole-3-acetate in *T. ramosissima* decreased (Fig. 11). This shift coincides with continuously declining SWC and may reflect a strategic reallocation of resources from vigorous vegetative growth towards maintenance and defense ^[10]. Notably, from the stable to the decline stage of coppice dunes, the contents of zeatin, dihydrozeatin, and indole-3-acetate in *T. ramosissima* increased. This may be closely related to the dwarfism and withered branches caused by drought stress in *T. ramosissima*. These morphological adjustments reduce overall water demand and may alter internal hormone homeostasis or signaling, ultimately serving as a survival mechanism to prolong viability under extreme stress. In addition, both SWC and ST content showed significant negative correlations with zeatin, dihydrozeatin, and indole-3-acetate, indicating that decreased SWC can act as a stress signal to

promote hormone accumulation and trigger plant stress resistance mechanisms. Meanwhile, the growth-promoting effects of these endogenous hormones require ST consumption to meet energy demands.

4.3. Key metabolic pathways in *T. ramosissima*'s adaptation to coppice dune development

This study provides the first integrated transcriptomic and metabolomic profile of *T. ramosissima* across the developmental stages of coppice dunes, generating 72.52 Gb of high-quality sequencing data that constitutes a valuable resource for *Tamarix* functional genomics. Our analysis found that glutathione metabolism, ABC transporters, and glycerophospholipid metabolism are key pathways co-enriched with both DEGs and DAMs, highlighting their central role in *T. ramosissima*'s adaptation to coppice dune development (Fig. 11). Notably, these pathways are highly conserved, aligning with core mechanisms reported in other plant species under drought stress^[53, 54, 55]. This suggests a fundamental adaptive strategy across aridland plants.

During the transition from the initial to the growth stage of coppice dunes, SWC began to decline. In response, *T. ramosissima* upregulated most genes in the glutathione metabolism pathway (e.g., encoding glutathione S-transferase, dehydroascorbate reductase, and glutathione peroxidase) to enhance redox homeostasis, stabilize the mitochondrial electron transport chain, and mitigate early oxidative stress. Furthermore, it activated the ABC transporters pathway (particularly ABCB, ABCC, and ABCG subfamilies), promoting the transport of secondary metabolites and hormones to support shoot and leaf morphogenesis^[54, 55]. We also found that levels of L-proline and hydroxyproline associated with the ABC transporters pathway were upregulated, further corroborating their vital role in maintaining osmotic balance under drought stress (**Table S8**). In contrast, deoxyguanosine and D-ribose were downregulated, indicating that drought exacerbates the risk of ROS-induced DNA damage (**Table S8**). The glycerophospholipid metabolism pathway was also induced, leading to upregulation of genes that facilitate cell division, membrane biosynthesis, and signal transduction, thereby supporting rapid growth and enhancing drought tolerance^[56]. As dunes progressed from the growth to the stable stage of coppice dunes, SWC continued to decrease. Reflecting a strategic shift, most DEGs in the three core pathways were downregulated. This may be because *T. ramosissima* can preferentially allocate resources to other defense mechanisms that better ensure its survival. From the stable to the decline stage of coppice dunes, both SWC and NSC content decreased to their minimum levels, accompanied by substantial accumulation of MDA and an elevated GSH/GSSG ratio. Under these conditions, the plants upregulated the three metabolic pathways to repair membrane damage, enhance antioxidant capacity, and transport stress-related substances, thereby resisting drought stress.

In summary, *T. ramosissima* dynamically regulated the expression of key genes and metabolites in core metabolic pathways across different developmental stages of coppice dunes, ultimately enabling adaptation to microhabitat changes in arid environments.

4.4. Hub genes mediating *T. ramosissima*'s drought response

Four hub genes associated with *T. ramosissima*'s response to coppice dune development were identified via WGCNA (Fig. 9). The *TRINITY_DN2662_c2_g1* (*MAPKK5-like*) and *TRINITY_DN4872_c1_g2* (*bHLH47-like*) were identified in the MEturquoise module. Investigations in potato have revealed that overexpression of *StMAPKK5* significantly increases relative water content, antioxidant enzyme activity, and Pro accumulation, while reducing MDA content, thereby enhancing drought tolerance^[57]. Furthermore, virus-induced gene silencing (VIGS)-mediated silencing of *GhMAPKK5* in cotton accelerates wilting under salt and drought stress. In contrast, overexpression of *GhMAPKK5* in *A. thaliana* promotes root growth and seed germination^[58]. These findings suggest that *MAPKK5-like* and *bHLH47-like* may also contribute to improved drought tolerance in *T. ramosissima*. Similarly, *TRINITY_DN1805_c0_g1* (*SAP4-like*) and *TRINITY_DN740_c1_g1* (*EXO70B1*) were identified from the MEyellow module. Prior research has demonstrated that overexpression of *ZmSAP8* and *MdSAP15* enhances drought stress tolerance in *Arabidopsis thaliana*^[25, 59]. Consistently, in *A. thaliana*, *Exo70B1* and *Exo70B2* positively regulate abscisic acid (ABA)- and mannitol-induced stomatal closure; overexpression of *VviExo70B* in *Vitis vinifera* callus and *A. thaliana* improves tolerance to drought and NaCl stress while increasing ABA sensitivity^[60]. Accordingly, we hypothesize that *SAP4-like* and *EXO70B1* may exert analogous functions in *T. ramosissima*'s adaptation to water deficit. These results indicate that the four identified hub genes hold promise as candidate genes for enhancing drought tolerance in *T. ramosissima*.

5 Limitations

This study has several limitations that need to be explicitly acknowledged. First, plant roots play a key role in water and nutrient uptake under drought stress. However, this study mainly focused on leaf samples of *T. ramosissima* and did not focus on root samples. Second, although WGCNA has revealed the potential role of hub genes in drought resistance regulation in depth, the present study did not conduct in situ functional validation experiments (e.g., gene overexpression, silencing, or knockout) in *T. ramosissima*, nor did it perform heterologous expression validation in model plants (e.g., *A. thaliana*), which limits the translational applicability of the research results. To address these limitations, future studies should integrate root transcriptome and metabolome analyses to provide a more comprehensive understanding of whole-plant adaptive strategies; second, overexpress or silence the identified hub genes and explore their effects on drought-related phenotypes.

6 Conclusions

(1) From the initial to the growth stage of coppice dunes, SWC gradually decreased, while the MDA content in *T. ramosissima* increased. In response, *T. ramosissima* resisted drought stress by enhancing SP and Pro contents and CAT activity.

(2) The decrease in SWC can promote the accumulation of zeatin, dihydrozeatin, and indole-3-acetate in *T. ramosissima*, thereby accelerating starch consumption to meet plant growth demands.

(3) *T. ramosissima* mainly coordinates gene expression and metabolite accumulation in the ABC transporters, glycerophospholipid metabolism, and glutathione metabolism pathways to adapt to coppice dune development.

Abbreviations

All abbreviations are defined upon first use in the manuscript.

Declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Author Contributions: W.G. and H.Z. carried out experiments. Z.W. and J.Z. developed the research ideas and designed the experiments. Z.W. acquired funding and revised the manuscript. Y.X. and B.Z. contributed to the field data collection. W.G. wrote the manuscript with contributions from all other authors. The authors declare no conflicts of interest.

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Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon request.

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Figures

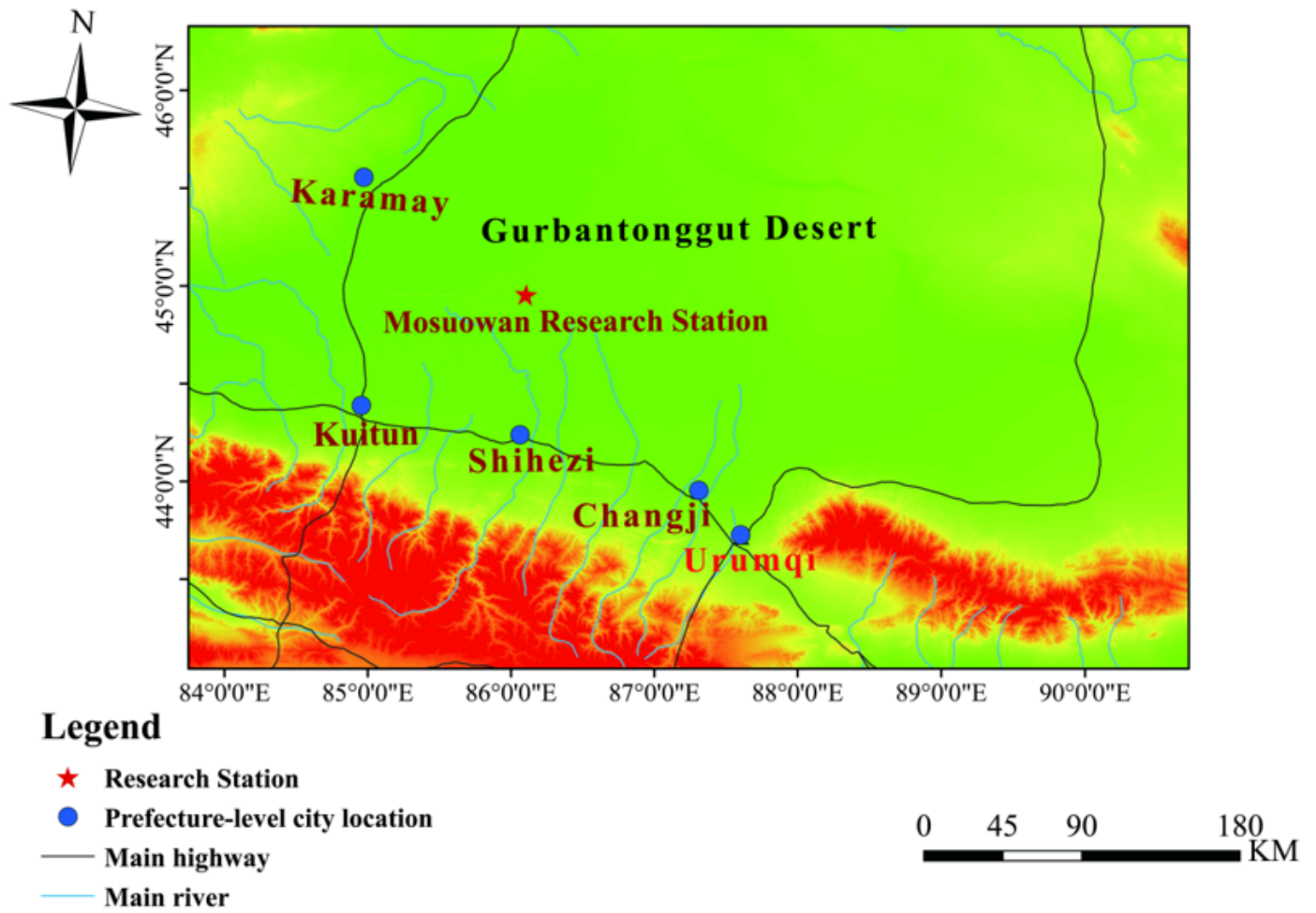


Figure 1

The sample plot of the study area ^[23].

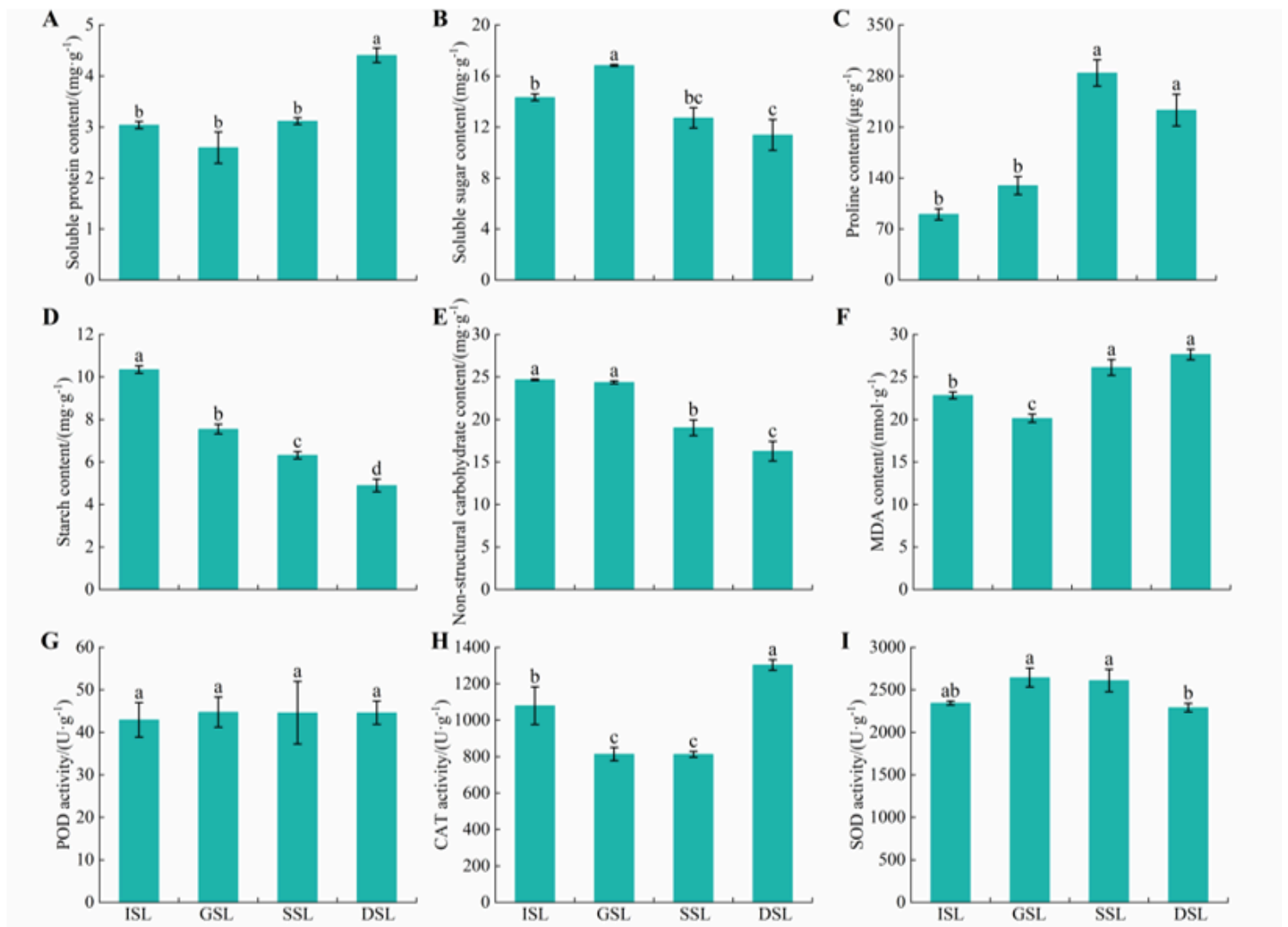


Figure 2

Changes in osmotic adjustment substances, non-structural carbohydrates (NSC), and antioxidant enzyme activities of *T. ramosissima* on coppice dunes at different developmental stages. (A) Soluble protein (SP) content. (B) Soluble sugar (SS) content. (C) Proline (Pro) content. (D) Starch (ST) content. (E) Non-structural carbohydrates (NSC) content. (F) Malondialdehyde (MDA) content. (G) Peroxidase (POD) activity. (H) Catalase (CAT) activity. (I) Superoxide dismutase (SOD) activity. ISL, GSL, SSL, and DSL represent the leaves of *T. ramosissima* growing on coppice dunes at the initial, growth, stable, and decline stages, respectively. Different lowercase letters (a, b, c, d) indicate statistically significant differences among different stages at $p < 0.05$.

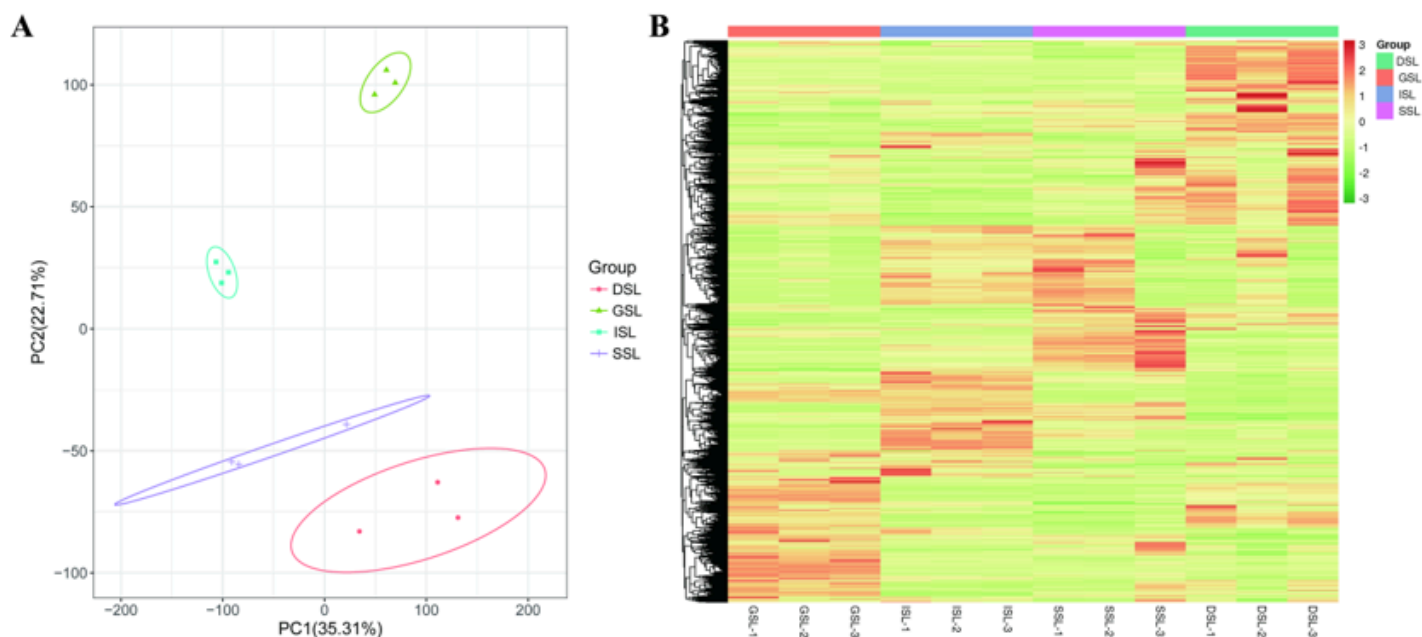


Figure 3

Quality control of metabolomics data and metabolite abundance across different samples. (A) Principal component analysis (PCA) of metabolomic samples. (B) Expression patterns of metabolites in different samples. ISL, GSL, SSL, and DSL represent the leaves of *T. ramosissima* growing on coppice dunes at the initial, growth, stable, and decline stages, respectively.

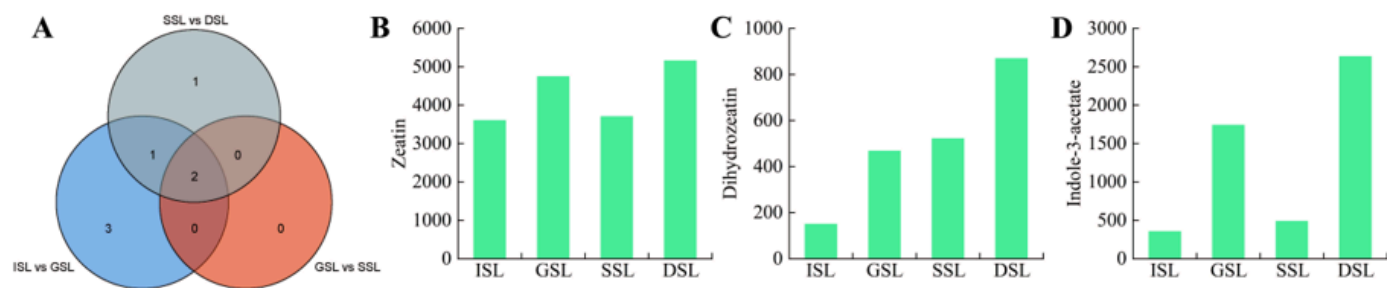


Figure 4

Changes in endogenous hormone contents of *T. ramosissima* under coppice dune development. Venn analysis of metabolites involved in the metabolic pathways of plant hormone signal transduction (A). Changes in zeatin (B), dihydrozeatin (C), and indole-3-acetate (D) abundances in different samples. ISL, GSL, SSL, and DSL represent the leaves of *T. ramosissima* growing on coppice dunes at the initial, growth, stable, and decline stages, respectively.

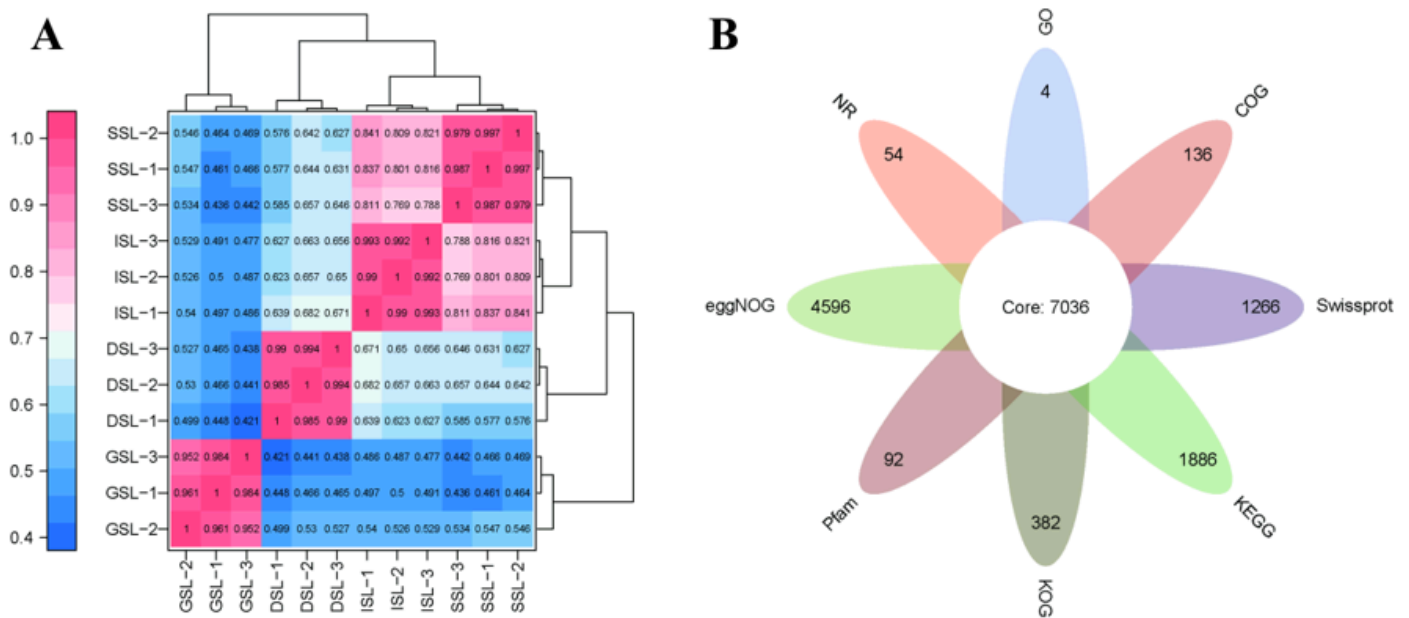


Figure 5

Sample quality control and unigene annotation of transcriptomics. (A) Correlation heatmap between samples. (B) Annotation status of unigenes in different databases.

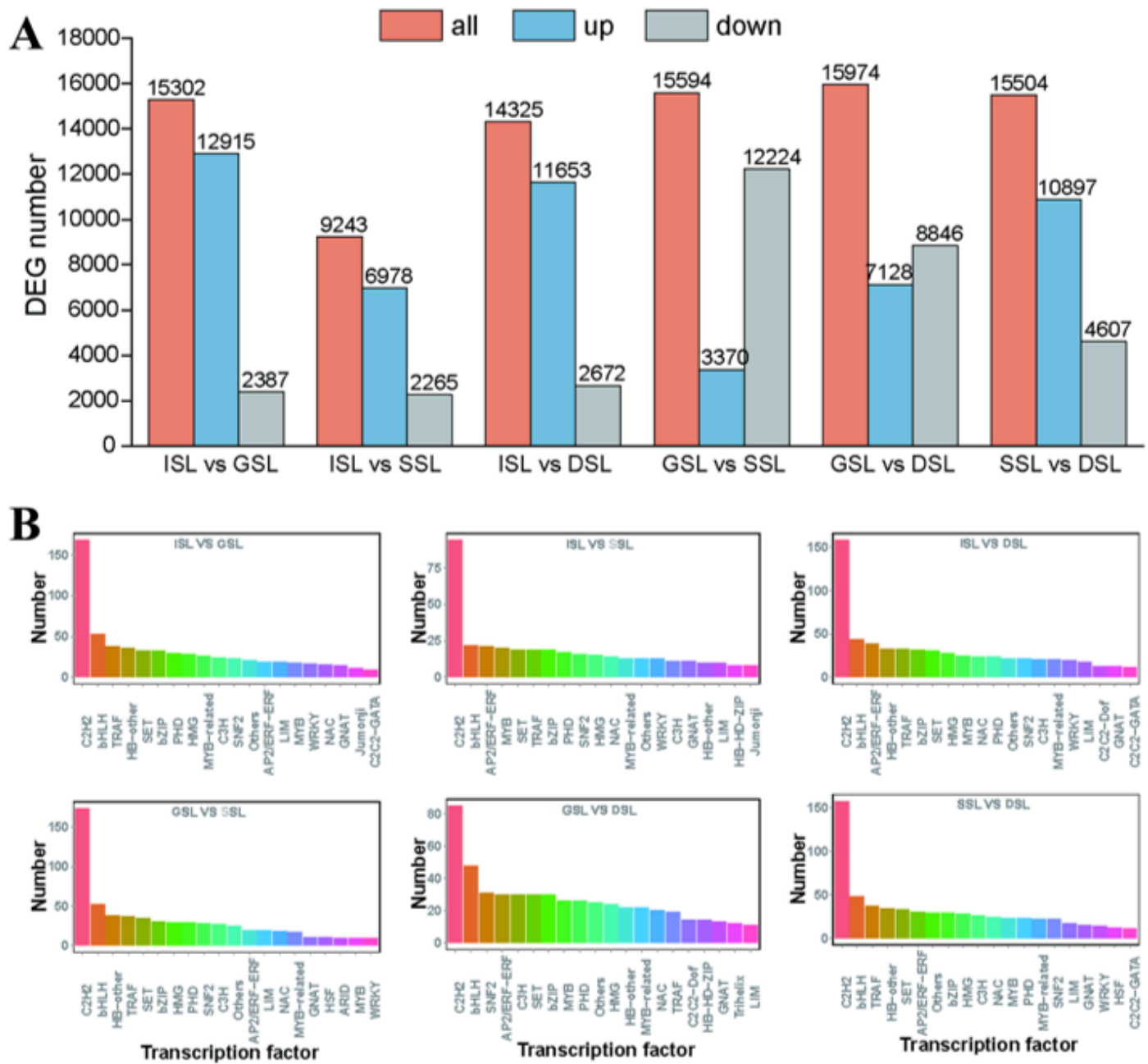


Figure 6

Statistics of the number of DEGs (A) and transcription factors (B) in different comparison groups.

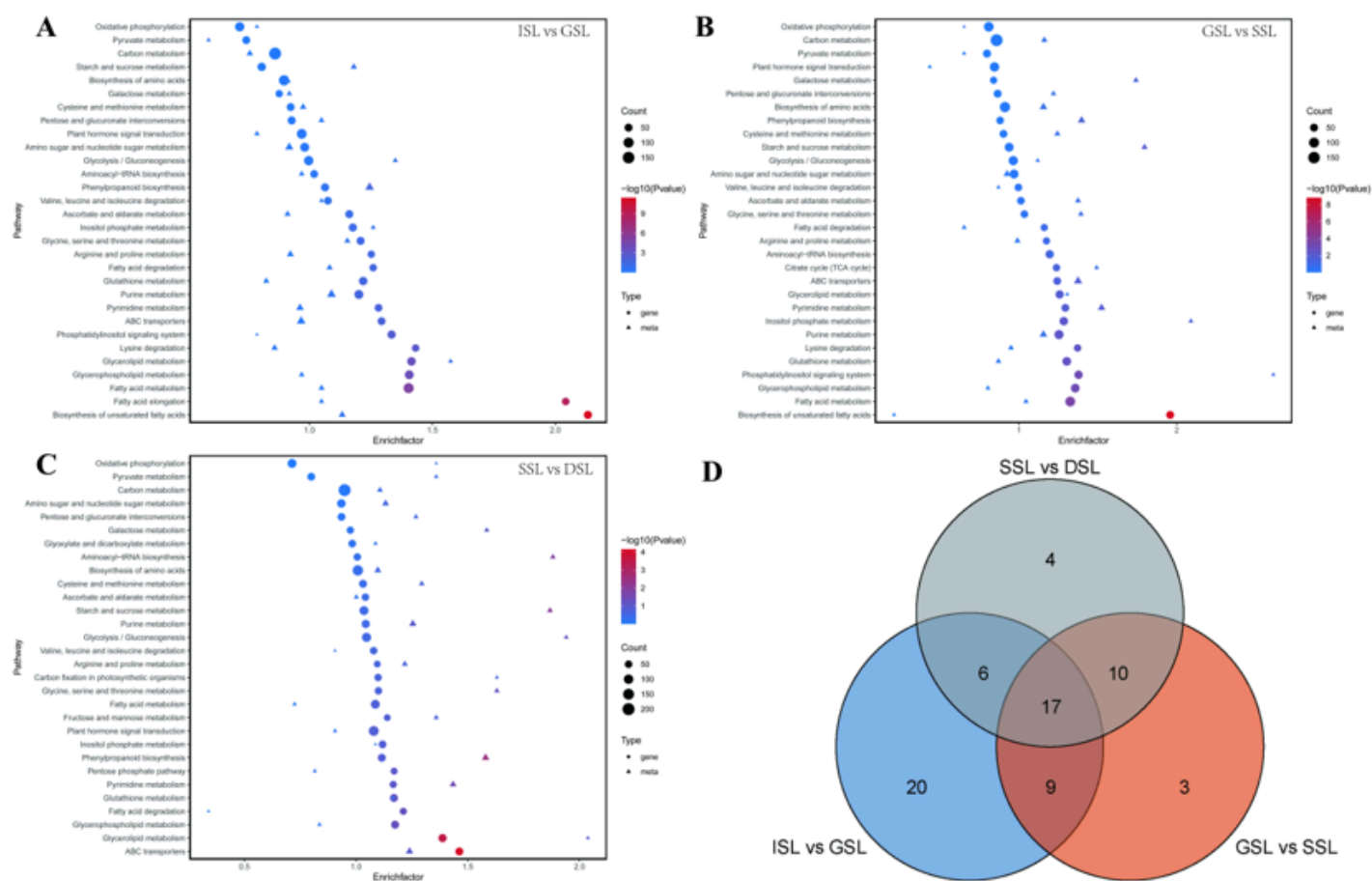


Figure 7

Co-enrichment of KEGG pathways in transcriptome and metabolome. KEGG co-enrichment analysis of transcriptome and metabolome in ISL vs GSL (A), GSL vs SSL (B), and SSL vs DSL (C). (D) Screening of key differential metabolites.

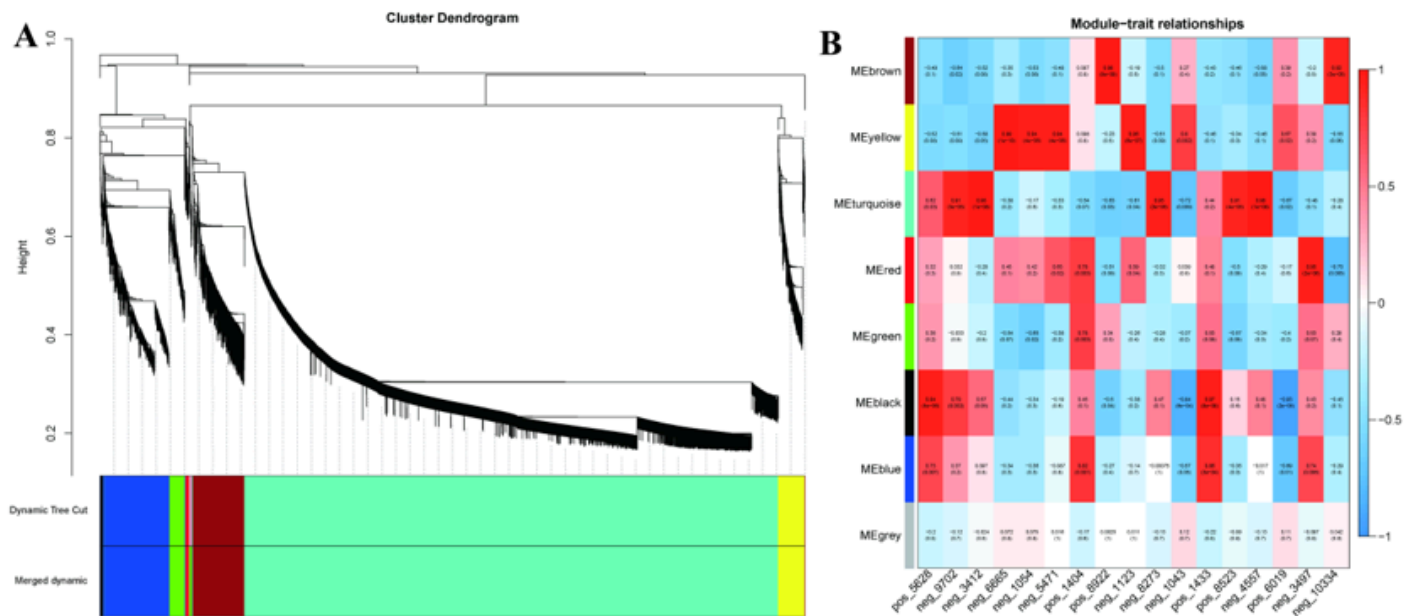


Figure 8

Cluster dendrogram (A) and module division of DEGs (B).

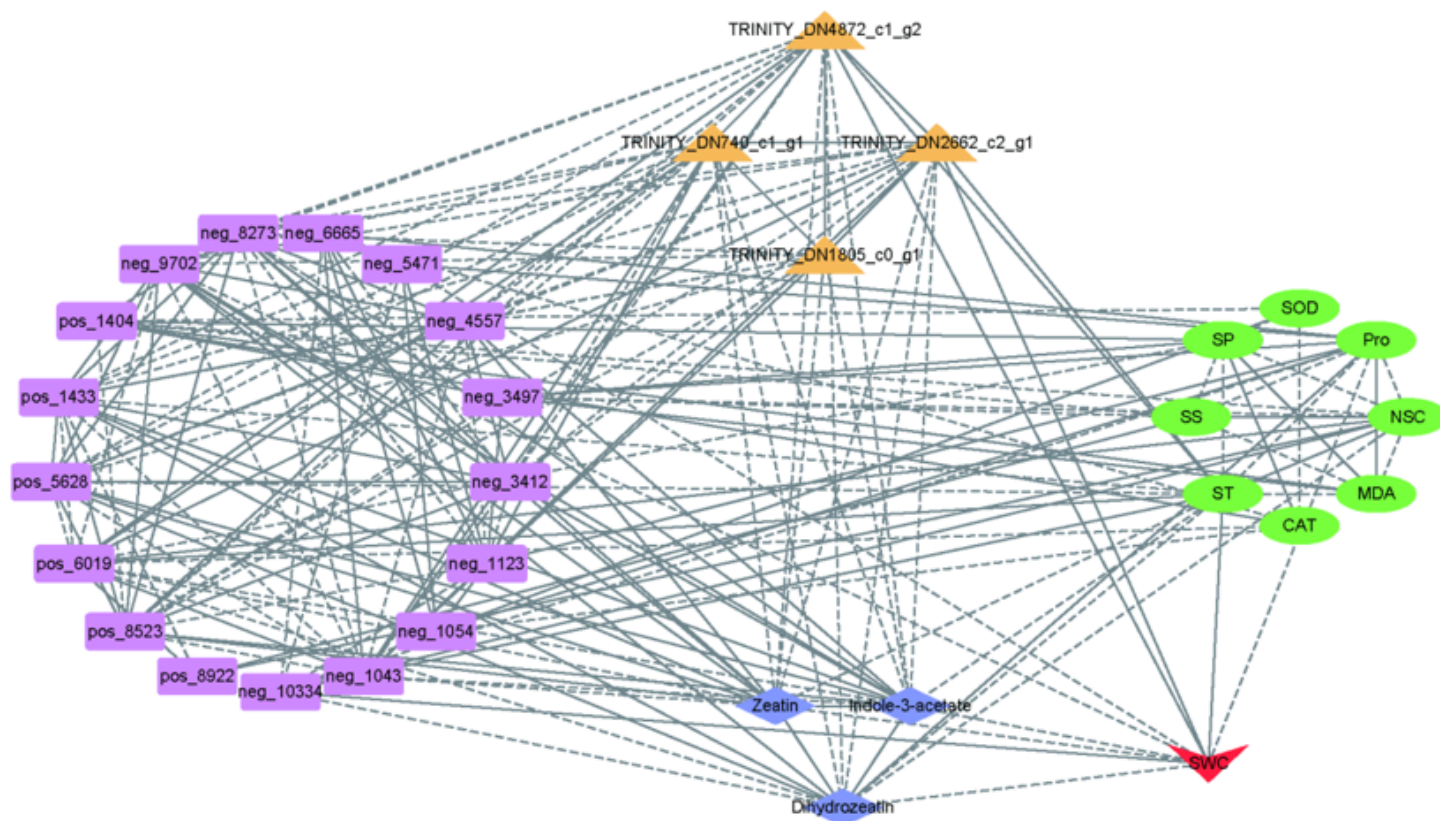


Figure 10

Co-expression network analysis of hub genes, DAMs, phytohormone levels, physiological traits, and SWC. Diamonds represent phytohormones, rectangles represent DAMs, triangles represent hub genes, ellipses represent physiological traits, and inverted triangles represent SWC. Solid lines indicate significantly positive correlations ($p < 0.05$), while dashed lines indicate significantly negative correlations ($p < 0.05$). MDA, malondialdehyde; NSC, non-structural carbohydrates; ST, starch; SS, soluble sugar; SOD, superoxide dismutase; CAT, catalase; SP, soluble protein; Pro, proline. For detailed information on the correlation network, refer to **Table S9**.

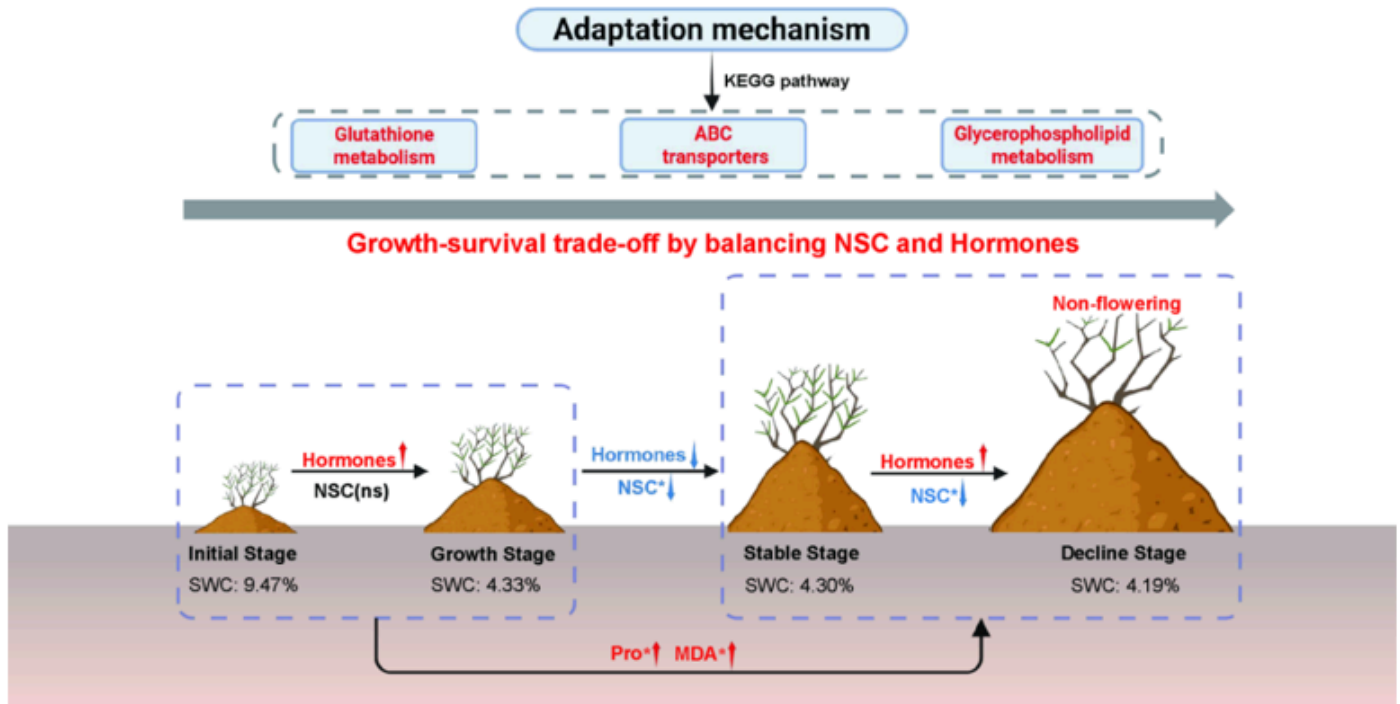


Figure 11

Adaptive strategies of *T. ramosissima* under coppice dune development. MDA, malondialdehyde; NSC, non-structural carbohydrates; Pro, proline; SWC, soil water content. *, significant difference ($p < 0.05$); ns, not significant ($p > 0.05$). Hormones included zeatin and indole-3-acetate in the leaves of *T. ramosissima*.

Supplementary Files

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