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Physiological and Transcriptomic Analysis of Oat Seedlings in Response to Drought Stress

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Abstract. This study used oat (Avena sativa) as material to systematically analyze physiological responses and transcriptomic changes under simulated drought stress. Physiological results showed that drought stress significantly reduced leaf relative water content (RWC) and increased relative electrical conductivity (REC), while inducing dynamic changes in antioxidant enzyme activities (superoxide dismutase [SOD], peroxidase [POD], and catalase [CAT]). Transcriptome sequencing (RNA-Seq) revealed extensive gene expression changes, with 369 common differentially expressed genes (DEGs) across all stress time points—these were enriched in plant hormone signal transduction, mitogen-activated protein kinase (MAPK) signaling pathway, and starch/sucrose metabolism. Weighted gene co-expression network analysis (WGCNA) further identified one core module positively correlated with phenotypic traits, from which 9 hub genes were screened. These genes are involved in cell wall reinforcement, osmotic protection, and metabolic regulation—playing core roles in oat's drought response. This study provides a theoretical basis for understanding the molecular mechanisms of oat drought resistance.

Keywords: *Cellulose; Polyvinyl alcohol;Ionic gel; High-voltage window; Integrated flexible supercapacitors*

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1 Introduction

Oat (Avena sativaL.) is a vital annual cereal crop belonging to the Poaceae family, widely cultivated for both human consumption and animal husbandry. Due to its tender stems, high palatability, and rich nutritional value, oat has been recognized as an ideal forage grass, particularly for green feeding or hay production [1]. However, oat production is increasingly constrained by drought stress, a major environmental factor limiting crop yields worldwide. Across China, drought impacts roughly 38.3% of the national terrestrial area, with hyper-arid zones comprising approximately 10% [3]. This predicament is especially pronounced in the semi-arid and arid territories spanning northern, northeastern, northwestern, and southwestern regions, where insufficient moisture availability recurrently precipitates substantial crop productivity reductions [2].

Under water-deficit conditions, vegetation has developed intricate adaptive strategies encompassing physiological, biochemical, and molecular modifications. Physiologically, moisture limitation triggers detrimental responses including diminished foliar hydration status (quantified via relative water content), elevated membrane leakage (manifested as increased electrolyte efflux), and uncontrolled generation of reactive oxygen intermediates capable of inflicting cellular injury [5–8]. To counteract such oxidative insult, plants mobilize protective enzymatic machinery involving superoxide dismutase, peroxidase, and catalase, while concurrently synthesizing compatible solutes including proline and soluble carbohydrates to preserve intracellular equilibrium [9–12]. Concurrently, at the molecular level, drought stress triggers complex signaling cascades. Key phytohormones, particularly abscisic acid (ABA), jasmonic acid (JA), and salicylic acid (SA), act as central regulators, mediating stomatal closure and activating downstream stress-responsive genes [13]. Furthermore, calcium signaling and Mitogen-Activated Protein Kinase (MAPK) cascades serve as critical secondary messengers that transduce extracellular signals into intracellular responses, ultimately activating transcription factors (TFs) such as bZIP, MYB, NAC, and WRKY to orchestrate the expression of protective genes [13–15].

Despite these advances, the specific molecular regulatory networks governing drought resistance in oats remain largely unexplored. Previous studies have primarily focused on physiological indices or single-gene analyses. For instance, RNA-Seq analyses have identified DEGs enriched in alkaloid and flavonoid biosynthesis during germination under water stress [15], while other reports highlighted the role of wax and cell wall synthesis genes in tolerant varieties [16]. However, a systematic view of gene co-expression dynamics throughout the entire seedling stress response is still lacking.

To bridge this gap, this study employed an integrated approach combining physiological phenotyping with multi-time-point transcriptomic analysis. Employing the drought-tolerant oat genotype 'Gunman', progressive water deficit was simulated via 20% PEG-6000 treatment. Weighted Gene Co-expression Network Analysis was applied to transcend single-gene differential expression patterns, enabling identification of functionally coherent transcriptional modules and central regulatory genes linked to critical phenotypic attributes. This systems biology approach aims to:

Characterize the dynamic physiological responses (RWC, REC, antioxidant enzymes) during early drought stress; Decode the temporal transcriptional landscape of oat seedlings under drought; Identify core gene modules and hub genes that serve as potential targets for molecular breeding. These findings furnish an integrative theoretical framework for elucidating the molecular underpinnings of drought tolerance in oats, while yielding actionable perspectives for enhancing forage crop adaptability amid climatic perturbations.

2 Materials and Methods

2.1 Experimental Materials and Drought Stress Treatment

The experimental genotype employed was 'Gunman' oat. Morphologically uniform, fully developed seeds underwent surface disinfection via 10% (v/v) sodium hypochlorite exposure for 5 min, followed by ultrapure water rinsing and sterile filter paper desiccation. Seeds were germinated in quartz sand-containing hydroponic vessels maintained under controlled environmental conditions (14 h photoperiod, 24°C/15°C thermocycle) with daily renewal of half-strength Hoagland medium. Upon reaching the two-leaf stage, water deficit was imposed through 20% (w/v) PEG-6000 supplementation to the nutrient solution; control vessels received unmodified medium. Specimens were harvested at 0 (baseline), 4, 12, 24, and 36 h post-treatment, with triplicate biological replicates per temporal point. All samples underwent immediate cryopreservation in liquid nitrogen and subsequent storage at −80°C.

2.2 Physiological Index Measurement

RWC was measured by weighing method; REC was calculated as $(EC_1/EC_2) \times 100\%$, where EC_1 = initial conductivity (deionized water, 24 h) and EC_2 = final conductivity (boiling water bath, 20 min) [19]. SOD activity was measured via nitroblue tetrazolium (NBT) method; POD via guaiacol method; CAT via ultraviolet spectrophotometry. Chlorophyll content was extracted with 80% acetone (dark, 24 h), centrifuged (5000 $r \cdot min^{-1}$, 10 min), and absorbance measured at 663 nm and 645 nm—calculated using Arnon's formula [20].

2.3 RNA-Seq

Total RNA was isolated using TRIzol methodology, with quality assessed via spectrophotometric quantification (NanoDrop 2000) and fragment analysis (Agilent 5300). Sequencing libraries were prepared and subjected to paired-end sequencing on the Illumina NovaSeq X Plus platform. Raw reads underwent quality trimming using fastp, followed by alignment to the oat reference genome (OT3098) via HISAT2 [21]. Transcript reconstruction and abundance estimation (FPKM normalization) were executed using StringTie [22]. Differential expression analysis was performed with DESeq2 [23], applying significance thresholds of $|\log_2(\text{fold change})| > 1$ and adjusted $P < 0.05$; resultant gene sets were subjected to functional enrichment characterization.

2.4 Real-Time Quantitative PCR (qRT-PCR) Validation

To validate RNA-Seq reliability, 5 genes were randomly selected for qRT-PCR. Total RNA was extracted using a plant RNA kit (BIOFIT), reverse-transcribed to cDNA using Exon Script RT Mix (Baoguang Biotech), and amplified with 2× SP qPCR Mix (Baoguang Biotech). Primer sequences are listed in Table 1. The internal reference gene was GLO1(stable expression across treatments). Amplification conditions: 94°C for 20 s, followed by 40 cycles of 94°C (10 s), 60°C (20 s), 72°C (10 s), and a melt curve analysis (65–95°C). Three technical/biological replicates were set; relative expression was calculated via the 2^{-}ΔΔCt method [24].

2.5 Gene Co-Expression Network and Hub Gene Analysis

Transcripts exhibiting FPKM > 1 across all specimens were selected for network construction. The WGCNA R package was employed to determine the optimal soft-thresholding power (β), ensuring scale-free topology fit index exceeded 0.8, with module merging height set at 0.25 (remaining parameters at default values). Module-trait correlations were calculated to identify key modules associated with stress; GO/KEGG annotations were performed for module genes. Hub genes were screened using Cytoscape's CytoHubba plugin (12 algorithms)—genes identified by ≥6 algorithms were selected as candidates. Final hub genes were visualized via Cytoscape.

2.6 Data Analysis

All experiments had 3 biological replicates. Data were processed via Excel 2019 and SPSS 19.0 (one-way ANOVA, LSD test); transcriptomic statistics (bubble plots, heatmaps, correlation plots) were generated via R.

Table 1 qRT-PCR Primer Information

Gene ID	Forward Primer (5'-3')	Reverse Primer (5'-3')
Pepsicol_Contig20547.path1	CTGCTCCTCCGGTATCAACA	TCATCTGTCCCCACCTTGTC
Pepsico2_Contig20137.path1	TGATAAGGCGTGGAAGTGGAC	GTACGCGAAGAAGAACCTCC
Pepsico2_Contig19896.path1	CCTGCGCCTTCTTCTTCTTC	CGTGGTCCATCTGCTCGTA
Gene ID	GGCGCATTCCTCTGTTCTTG	GAGGACTCTGCCGGCTT
Pepsicol_Contig11487.path1	ACCTTTACTCACGCTGGGAA	TTTGGATTAACGGCTCCCCT
AsGLO1	CGGCAAGATGGACCAGGCTAATG	GCGTCTTCAGCGGTGATGACTC

3 Results and Analysis

3.1 Physiological Responses of Oat Seedlings to Drought Stress

To evaluate drought-induced physiological changes, key indices were measured (Figure 1). Drought directly caused water loss: leaf RWC decreased continuously and significantly ($P < 0.05$), reaching the lowest point at 36 h (Figure 1A). Conversely, REC (reflecting membrane damage) increased continuously (Figure 1B). Photosynthetic systems were also impaired: chlorophyll content decreased overall but showed a transient, slight rebound at 12 h (Figure 1C). To mitigate oxidative damage, the antioxidant enzyme system was activated: SOD/POD activities increased first, then decreased ($P < 0.05$)—SOD peaked at 12 h, POD at 24 h (Figures 1D, 1E). CAT activity was more complex: it peaked at 1 h, declined, then rebounded significantly at 24–36 h ($P < 0.05$) (Figure 1F).

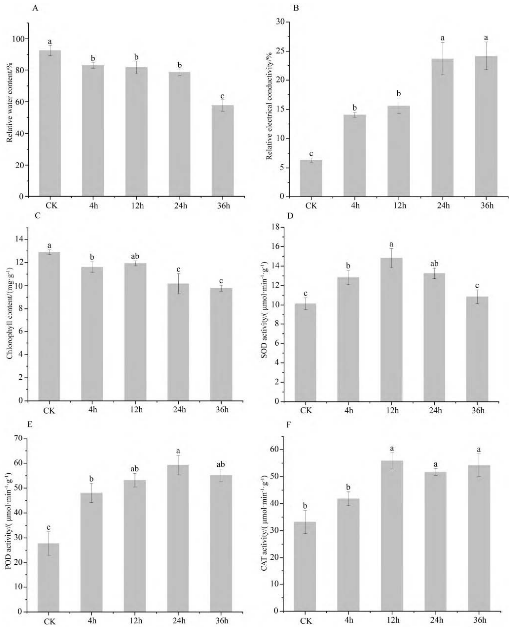


Figure 1 Effects of drought stress on physiological indices in leaves of oat seedlings

3.2 Effects of Drought on Gene Expression in Oat Leaves

RNA-Seq of 5 time points yielded 96.9 Gb clean data; Q30 bases accounted for ≥95.82%, and alignment rates to the reference genome ranged from 91.96% to 93.25%. PCA showed clear separation of samples by treatment time (PC1 = 20.13%, PC2 = 16.55% variance explained), confirming high data quality (Figure 2A). DEGs increased with stress duration: 2211 DEGs (1277 up, 984 down) at 4 h; 4035 (2587 up, 1448 down) at 12 h; 4779 (2333 up, 2446 down) at 24 h; 9318 (4858 up, 4360 down) at 36 h (Figure 2B).

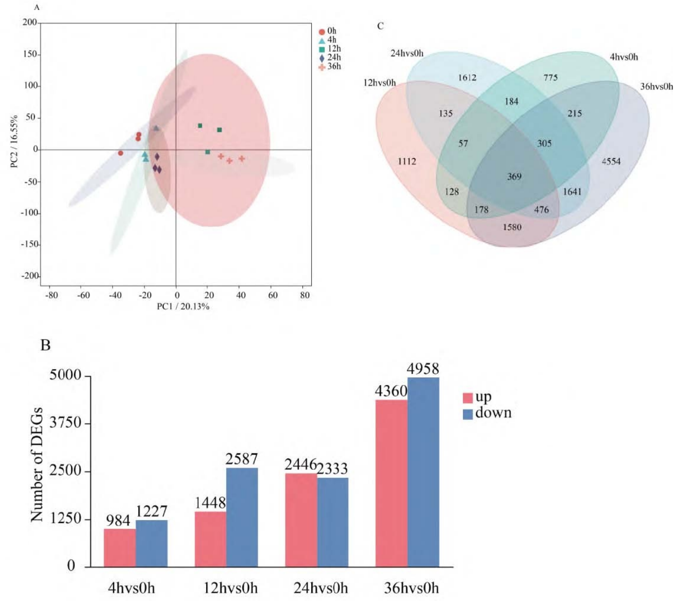


Figure 2 RNA-Seq result analysis (A) Principal component analysis; (B) Statistics of differentially expressed genes in each group; (C) Venn diagram

3.3 GO and KEGG Enrichment Analysis of Core DEGs

To explore functions of persistent drought-response genes, GO/KEGG enrichment was performed on 369 common DEGs. GO analysis (Figure 3A) showed significant enrichment in: (1) Biological Process: trehalose biosynthesis/metabolism, disaccharide/oligosaccharide biosynthesis/metabolism, ABA-activated signaling; (2) Molecular Function: phosphatase activity, phosphoester hydrolase activity. KEGG analysis (Figure 3B) revealed enrichment in plant hormone signal transduction, starch/sucrose metabolism, and MAPK signaling pathway.

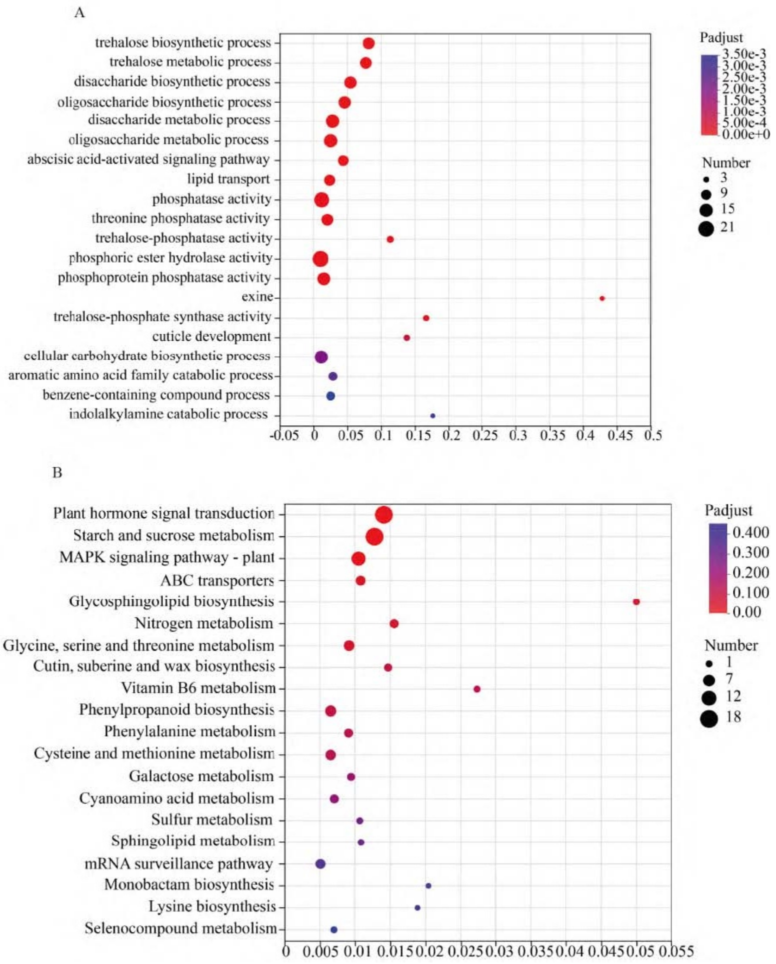


Figure 3 GO (A) and KEGG (B) enrichment analysis of 369 common DEGs under drought stress

3.4 qRT-PCR Validation of RNA-Seq Results

Five genes were randomly selected for qRT-PCR validation (internal reference: GLO1). Results showed consistent expression trends between qRT-PCR and RNA-Seq, confirming data reliability (Figure 4).

3.5 Weighted Gene Co-Expression Network Analysis (WGCNA)

WGCNA was performed on 8577 high-quality genes. With the optimal soft threshold $\beta = 7$ ($R^2 > 0.80$), 11 co-expression modules were constructed (Figure 5; Figure 6A). The gray module (464 genes, 5.4%) contained unclustered genes. Module-trait correlation analysis identified a brown module positively correlated with stress time, POD, and CAT activities (Figure 6B). Enrichment analysis of the brown module showed: (1) KEGG: plant hormone signal transduction, MAPK signaling, oxidative phosphorylation (Figure 7A); (2) GO: positive regulation of cytoplasmic Ca^{2+} concentration, Ca^{2+} transmembrane transport, Ca^{2+} sequestration, oxidoreductase activity (Figure 7B). Nine hub genes were screened from the brown module (Table 2), including: (1) Pepsico2_Contig7993.path1: Late Embryogenesis Abundant (LEA) protein; (2) Pepsico2_Contig19792.path1: responsive to water deprivation/RNA binding; (3) Pepsico2_Contig2150.path2: protein tyrosine/serine/threonine kinase; (4) Pepsico2_Contig12416.path1: cellulose synthase. These genes cover stress protection, signal transduction, and cell structure synthesis.

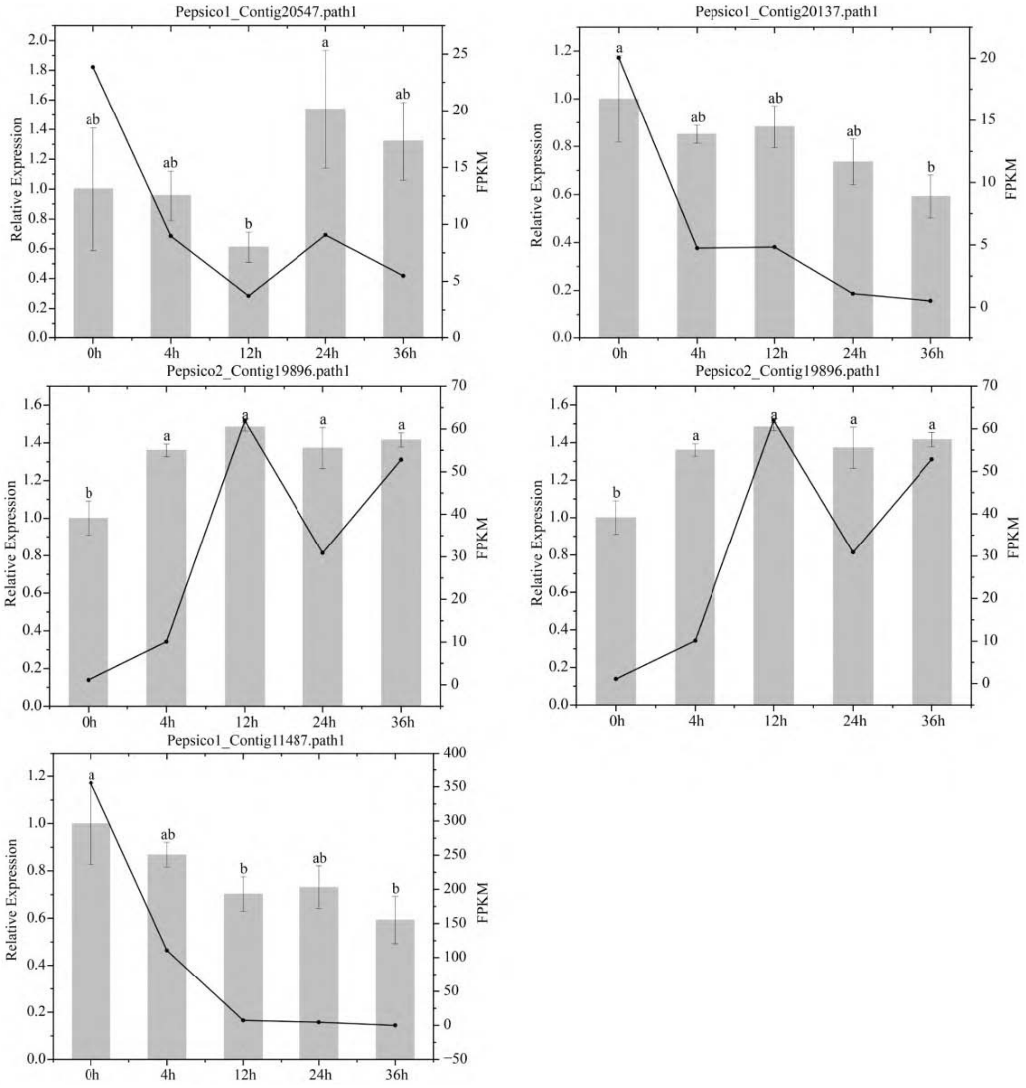


Figure 4 Validation of transcriptome data by quantitative real-time PCR

Table 2 Drought resistance-related hub genes identified in the Brown module and their functional annotations

Module	Hub Gene ID	Gene Description
Brown	Pepsico1_Contig16031.path2	START domain (lipid/sterol binding/transport)
Brown	Pepsicol_Contig3693.path2	Ceramidase
Brown	Pepsico1_Contig5749.path2	Zinc ion binding/DNA binding
Brown	Pepsico1_Contig7903.path1	Unknown
Brown	Pepsico2_Contig12416.path1	Cellulose synthase
Brown	Pepsico2_Contig19792.path1	Response to water deprivation/RNA binding
Brown	Pepsico2_Contig2150.path2	Protein tyrosine and serine/threonine kinase
Brown	Pepsico2_Contig7993.path1	Late embryogenesis abundant (LEA) protein
Brown	Pepsico2_Contig9135.path1	Reticulon (endoplasmic reticulum membrane protein)

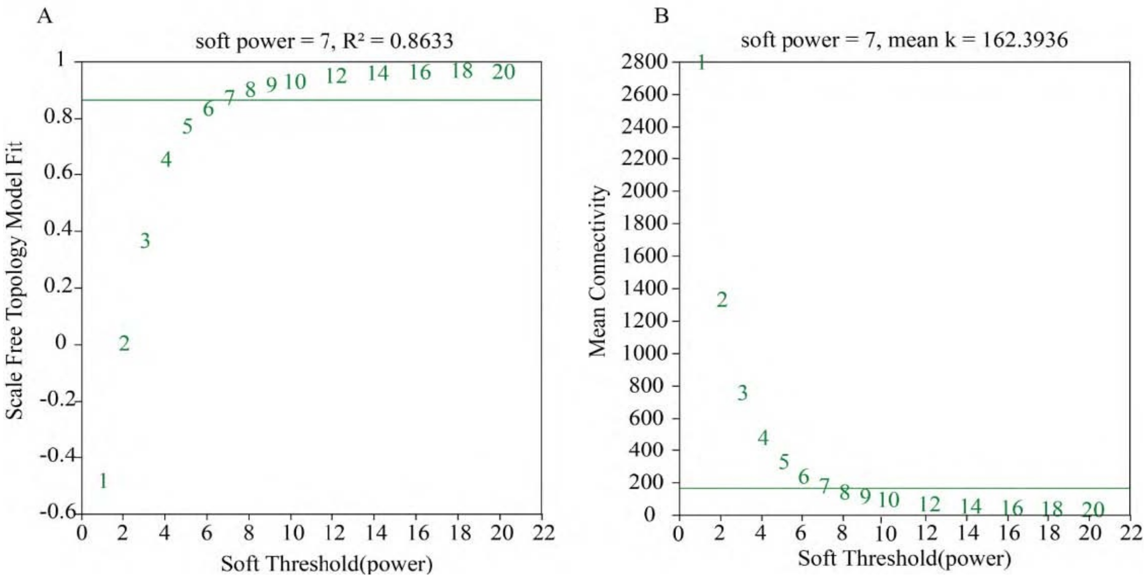
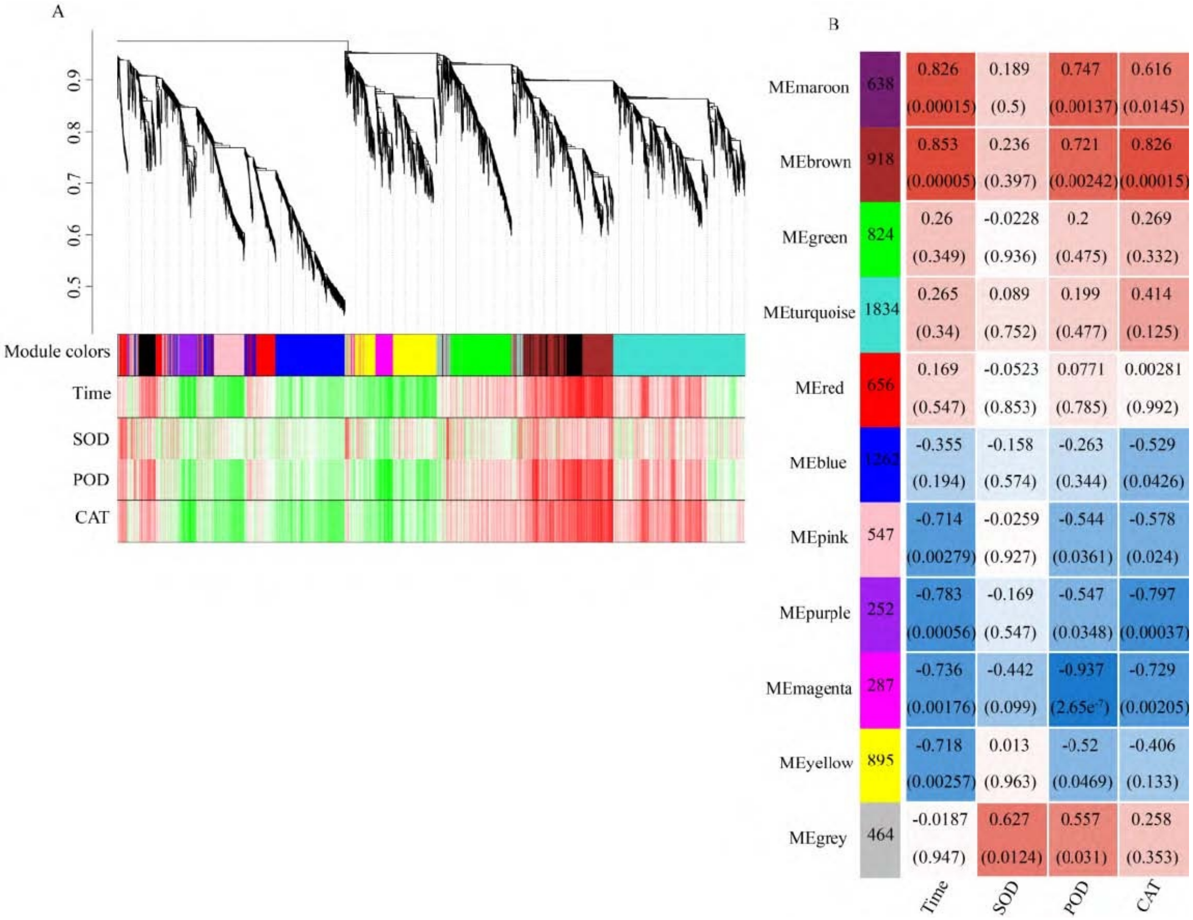


Figure 5 Determination of the soft-thresholding power (β)



		Time	SOD	POD	CAT
MEmaroon	638	0.826 (0.00015)	0.189 (0.5)	0.747 (0.00137)	0.616 (0.0145)
MEbrown	918	0.853 (0.00005)	0.236 (0.397)	0.721 (0.00242)	0.826 (0.00015)
MEgreen	824	0.26 (0.349)	-0.0228 (0.936)	0.2 (0.475)	0.269 (0.332)
MEturquoise	1834	0.265 (0.34)	0.089 (0.752)	0.199 (0.477)	0.414 (0.125)
MEred	656	0.169 (0.547)	-0.0523 (0.853)	0.0771 (0.785)	0.00281 (0.992)
MEblue	1262	-0.355 (0.194)	-0.158 (0.574)	-0.263 (0.344)	-0.529 (0.0426)
MEpink	547	-0.714 (0.00279)	-0.0259 (0.927)	-0.544 (0.0361)	-0.578 (0.024)
MEpurple	252	-0.783 (0.00056)	-0.169 (0.547)	-0.547 (0.0348)	-0.797 (0.00037)
MEmagenta	287	-0.736 (0.00176)	-0.442 (0.099)	-0.937 ($2.65e^{-7}$)	-0.729 (0.00205)
MEyellow	895	-0.718 (0.00257)	0.013 (0.963)	-0.52 (0.0469)	-0.406 (0.133)
MEgrey	464	-0.0187 (0.947)	0.627 (0.0124)	0.557 (0.031)	0.258 (0.353)

Figure 6 Systematic clustering tree and module division diagram (A) Module-trait correlation heatmap; (B) Correlation between module eigengenes and traits

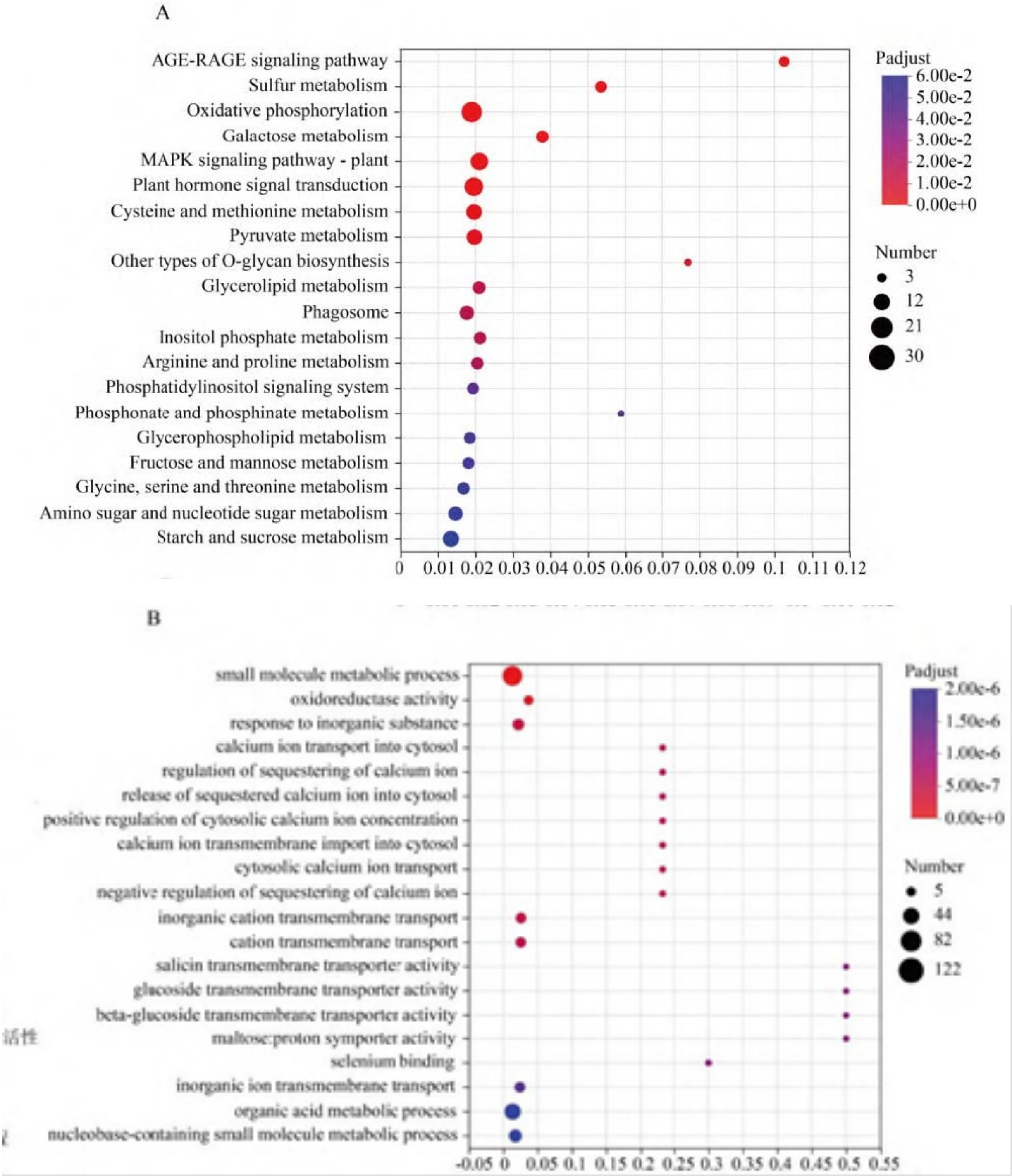


Figure 7 KEGG (A) and GO (B) enrichment analysis of genes in the Brown module

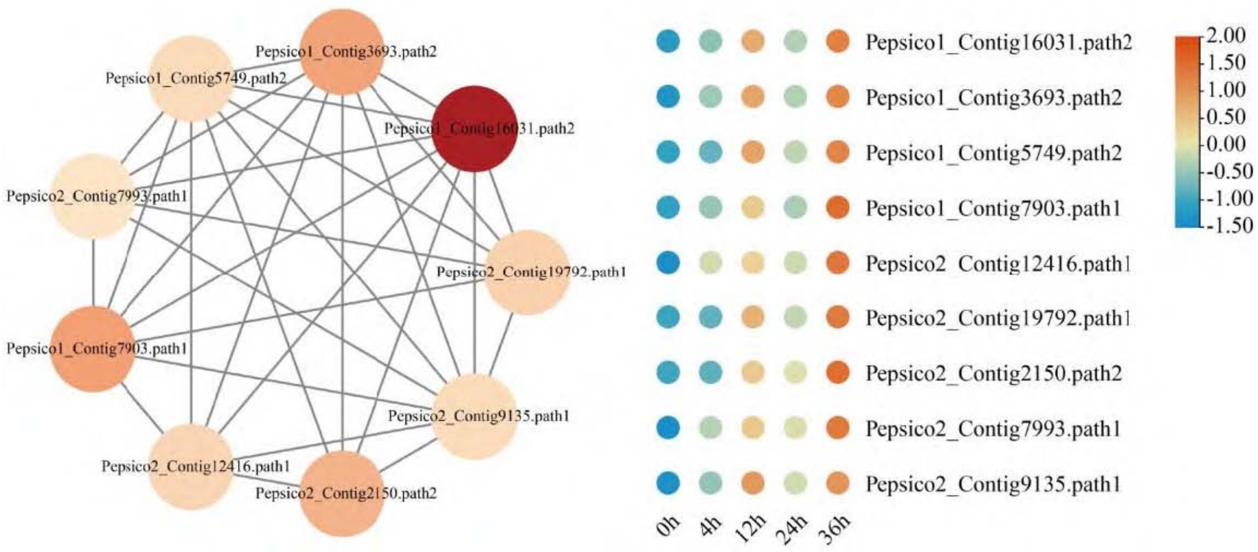


Figure 8 Interaction network and expression patterns of hub genes in the Brown module

4. Discussion

Climate change–driven intensification of drought episodes presents an existential challenge to global agricultural output and nutritional security. Deciphering the physiological and molecular foundations of crop desiccation tolerance transcends theoretical interest, constituting an imperative for sustainable agronomic systems. Oat (*Avena sativa* L.), a temperate-region staple cereal and foundational forage component across China's semi-arid and arid zones, represents an exemplary system for probing these adaptive mechanisms. While previous studies have extensively documented the physiological responses of oats to drought, such as alterations in photosynthetic efficiency and osmotic adjustment [6–8], the systemic transcriptional regulatory networks governing these responses remain largely uncharted. This study bridges this critical knowledge gap by integrating time-series physiological phenotyping with high-throughput transcriptomic sequencing and Weighted Gene Co-expression Network Analysis (WGCNA). Our findings present a comprehensive, dynamic portrait of the oat seedling's response to progressive drought stress, revealing a sophisticated interplay between cellular homeostasis maintenance, signal transduction, and metabolic reprogramming.

4.1 Dynamic Physiological Responses: A Tale of Two Phases

The physiological data obtained in this study delineate a clear biphasic response of oat seedlings to PEG-simulated drought stress, characterized by an initial "resistance phase" followed by a "damage phase." The continuous decline in Relative Water Content (RWC) and the concomitant increase in Relative Electrical Conductivity (REC) are unequivocal indicators of cellular dehydration and plasma membrane system impairment [26]. Interestingly, the kinetic profiles of antioxidant enzymes (SOD, POD) revealed a nuanced regulatory pattern. The initial surge in SOD and POD activities (peaking around 12–24 h) represents a successful activation of the cellular antioxidant defense system. SOD functions as the primary defensive barrier, catalyzing the conversion of superoxide anions ($O_2\bullet^-$) into hydrogen peroxide, which is subsequently neutralized by POD and CAT [9]. This synchronized enzymatic cascade efficiently eliminates ROS accumulated during initial stress phases, thereby safeguarding cellular macromolecules—including lipids, proteins, and nucleic acids—from oxidative deterioration.

However, the subsequent decline in enzyme activities at the 36 h time point suggests a transition to the damage phase. This phenomenon, consistent with observations in other cereals under severe stress [27], implies that the biosynthetic capacity or the stability of these enzymes becomes compromised under prolonged dehydration. The complexity of CAT activity, which exhibited a secondary peak at 24–36 h, hints at a distinct regulatory mechanism or the involvement of different CAT isoforms with varying sensitivities to H_2O_2 accumulation. Collectively, these physiological data establish a robust temporal framework, confirming that our experimental setup successfully induced a progressive stress response, which served as the foundation for the subsequent transcriptomic analysis.

4.2 Transcriptional Reprogramming: Conserved Pathways and Oat-Specific Signatures

The RNA-Seq data revealed a massive-scale transcriptional reprogramming, with the number of Differentially Expressed Genes (DEGs) increasing exponentially from 2,211 at 4 h to 9,318 at 36 h. This escalation underscores the systemic nature of the drought response, transitioning from the activation of early signaling components to widespread changes in metabolic and structural genes. The functional enrichment analysis of the 369 core DEGs—those consistently dysregulated across all time points—provides profound insights into the molecular priorities of the oat seedling.

First, the significant enrichment of trehalose biosynthesis and starch/sucrose metabolism highlights the plant's immediate priority to maintain osmotic homeostasis. Trehalose, a non-reducing disaccharide, functions as a compatible solute, protecting cellular structures from dehydration-induced damage [29]. The upregulation of genes in this pathway suggests a metabolic shift away from growth and towards survival, a strategy also observed in sweet sorghum where exogenous trehalose alleviated drought symptoms [30]. Second, the prominence of Abscisic Acid (ABA) signaling and Mitogen-Activated Protein Kinase (MAPK) cascades confirms their status as evolutionarily conserved central hubs in the plant stress response network [13]. These pathways likely act upstream of the observed physiological changes, sensing the initial water deficit and transducing the signal to activate downstream effectors. The identification of genes related to calcium signaling further supports this, as Ca^{2+} influx is often the earliest cellular event following stress perception [31].

4.3 Systems-Level Insights from WGCNA: Unveiling the Regulatory Architecture

While traditional DEG analysis provides a list of candidate genes, it often fails to capture the synergistic relationships between genes. Our application of WGCNA addressed this limitation by identifying functionally coherent gene modules. The identification of the Brown module—which was positively correlated with stress duration, POD, and CAT activities—represents a significant conceptual advancement. Unlike previous studies that focused on static comparisons (e.g., control vs. stressed) [15], our module-trait correlation analysis pinpointed a network specifically associated with the plant's adaptive capacity (i.e., antioxidant enzyme activity).

The functional annotation of this Brown module was particularly illuminating. The convergence of calcium signaling, MAPK cascades, and plant hormone signal transduction within this single module suggests a highly integrated regulatory circuit. Specifically, the GO terms related to "cytoplasmic calcium ion concentration" and "calcium transmembrane transport" indicate that calcium acts as a pivotal second messenger in this process. Upon drought perception, calcium channels are likely activated, leading to a cytosolic Ca^{2+} spike that is sensed by Calcineurin B-Like (CBL) proteins and Calcium-Dependent Protein Kinases (CDPKs). These kinases, in turn, activate the MAPK cascade and phosphorylate downstream transcription factors, creating a multi-layered signaling web. This systems-level view demonstrates that drought tolerance is not governed by isolated genes but by robust, interconnected networks.

4.4 Hub Genes: From Networks to Functional Candidates

Within the Brown module, we employed a rigorous computational approach (CytoHubba with 12 algorithms) to identify nine hub genes, representing the most highly connected and potentially most influential nodes in the network. The functional characterization of these genes provides a shortlist of prime candidates for future functional validation:

Cellulose Synthase (Pepsico2_Contig12416.path1): The upregulation of this gene suggests that reinforcing cell wall architecture is a key strategy for oat seedlings to withstand dehydration-induced mechanical stress. Cellulose, the primary structural component of plant cell walls, provides tensile strength. Under drought, the synthesis of a more robust cell wall may help maintain cell turgor and prevent collapse. This finding is strongly supported by evidence in cotton (Gossypium hirsutum), where silencing of CesA genes led to compromised fiber integrity and reduced cellulose content [32].

Late Embryogenesis Abundant (LEA) Protein (Pepsico2_Contig7993.path1): LEAs are hydrophilic proteins that accumulate during the late stages of embryogenesis and in response to desiccation stress. They function as "molecular shields," stabilizing proteins and membranes in the absence of water. The identification of LEAas a hub gene reinforces its fundamental role in protecting cellular structures during drought. Studies in perennial ryegrass (Lolium perenne) have shown that overexpression of LEA genes enhances drought tolerance by reducing membrane damage [33].

Protein Kinase (Pepsico2_Contig2150.path2)**: This gene, annotated as a protein tyrosine and serine/threonine kinase, likely represents a key component of the signaling cascade. It may function upstream of the antioxidant response, phosphorylating and activating transcription factors or other enzymes involved in ROS detoxification.

Zinc Finger Protein (Pepsico1_Contig5749.path2)**: This transcriptional regulator, possessing dual zinc coordination and DNA-binding functionalities, represents a compelling candidate for modulating downstream stress-adaptive gene expression. Zinc finger proteins rank among the most expansive transcription factor superfamilies in plant genomes, with documented involvement in developmental programming and environmental stress adaptation [34].

The diversity of functions represented by these hub genes—spanning structural reinforcement (CESA), cellular protection (LEA), and signal transduction (Kinase, TF)—underscores the multifaceted nature of drought adaptation.

4.5 Integration of Physiology and Transcriptomics: A Proposed Model

Synthesizing our physiological and transcriptomic data, we propose a working model for the drought response in oat seedlings (Figure 9). Upon initial exposure to PEG-induced osmotic stress (Phase 1: Sensing & Signaling), the plant perceives water deficit, triggering an influx of Ca^{2+} ions. This activates CDPKs and MAPK cascades, which in turn stimulate the ABA signaling pathway. Concurrently, transcription factors (e.g., the identified Zinc Finger protein) are activated. These transcription factors coordinate the induction of rapid-response genes, thereby triggering trehalose biosynthesis and upregulating antioxidant enzymatic machinery (SOD, POD, CAT).This phase is characterized by physiological stability and effective ROS scavenging. If the stress persists (Phase 2: Acclimation & Damage), the plant shifts to a survival mode, upregulating structural genes like CESAto fortify cell walls and LEAto protect cellular components. However, if the stress exceeds the plant's adaptive capacity, the antioxidant system becomes overwhelmed, leading to membrane damage (increased REC) and a breakdown of homeostasis.

4.6 Comparative Analysis with Other Cereals

When compared to model cereals like rice and maize, the drought response in oat exhibits both conserved and unique features. The core signaling pathways (ABA, MAPK, Ca^{2+}) are universally conserved, highlighting their fundamental importance in plant stress biology [13, 17, 18]. However, the specific downstream targets and the kinetics of response appear to be species-specific. For instance, while rice and maize studies often highlight aquaporins and specific NAC TFs [17, 18], our study in oat placed greater emphasis on cell wall modification (via CESA) and specific metabolic adjustments (via trehalose). This divergence likely reflects the distinct evolutionary pressures faced by forage oats versus grain cereals, where the former prioritizes vegetative biomass and persistence under grazing or cutting.

This study delineates a temporally resolved, biphasic adaptive framework of oat (Avena sativa) seedlings under progressive drought stress, bridging dynamic physiological shifts with large-scale transcriptional reprogramming. The physiological trajectory clearly transitions from an initial resistance phase to a subsequent damage phase: the early surge in SOD and POD activities (peaking at 12–24 h) effectively scavenges reactive oxygen species to maintain cellular homeostasis, whereas the sustained dehydration at 36 h overwhelms the antioxidant system, as evidenced by rising relative electrical conductivity and declining relative water content. Transcriptomic profiling further reveals the escalating regulatory complexity of drought response—from 2,211 differentially expressed genes (DEGs) at 4 h to 9,318 at 36 h. Functional enrichment of 369 constitutively dysregulated core DEGs highlights evolutionarily conserved survival strategies, including trehalose-mediated osmotic adjustment, starch and sucrose metabolic reallocation, and the central role of ABA and MAPK signaling cascades in stress perception and transduction. These findings not only corroborate established drought response paradigms in cereals but also emphasize the metabolic priority of osmotic protection over growth in forage oats facing water limitation.

Integrating weighted gene co-expression network analysis (WGCNA) moves beyond individual DEG lists to map the hierarchical regulatory architecture of oat drought tolerance. The brown module, positively correlated with stress duration and antioxidant enzyme activities, emerges as a functional hub linking calcium signaling, hormone transduction, and structural adaptation. Nine screened hub genes span three core adaptive axes: CESA-mediated cellulose synthesis for cell wall reinforcement, LEA protein accumulation for macromolecular stabilization, and kinase/zinc finger transcription factors for upstream signal relay. This systems-level perspective reframes drought tolerance as a coordinated network output rather than a property of single genes. While the PEG-simulation and transcript-only scope represent limitations, the identified hub genes provide high-priority targets for future CRISPR or VIGS functional validation, as well as molecular marker development for breeding climate-resilient forage oats suited to semi-arid cultivation regions.

4.7 Limitations and Future Directions

While this study provides a comprehensive overview, several limitations warrant attention. First, the use of PEG-6000, while a standard laboratory technique, does not perfectly replicate the complex soil-root interactions and hydraulic properties of field drought. Second, transcriptomic data, while powerful, only infers potential protein activity; post-transcriptional and post-translational modifications (e.g., protein phosphorylation) are equally critical and require proteomic validation. Third, the identified hub genes are computational predictions; their causal roles must be validated through functional genomics approaches.

Future research should focus on: Functional Validation: Utilizing CRISPR-Cas9 gene editing or virus-induced gene silencing (VIGS) to knock down or knock out the identified hub genes (CESA, LEA, etc.) to confirm their roles in drought tolerance. Multi-Omics Integration: Combining transcriptomics with metabolomics and proteomics to build a more complete picture of the metabolic fluxes and regulatory events occurring during stress. Field Trials: Evaluating the performance of oat lines with manipulated expression of hub genes under natural drought conditions to assess their agronomic value.

5. Conclusions

Collectively, this investigation delivers the most exhaustive characterization presently available of both physiological and transcriptional adaptations in oat seedlings subjected to water deficit. Rather than relying solely on conventional differential expression catalogs, the construction of a gene co-expression network has enabled the pinpointing of pivotal regulatory modules and central hub genes that likely function as master controllers orchestrating the drought tolerance program. The identification of CESAand LEAas central players offers novel molecular targets for the genetic improvement of forage oats. This work not only advances our fundamental understanding of stress biology in a key forage crop but also lays the groundwork for developing climate-resilient cultivars capable of thriving in water-limited environments.

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