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Bulk RNA-seq datasets analysis integration identifies robust drought-responsive genes and functional networks in *Eucalyptus grandis*

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ABSTRACT

Eucalyptus grandis is a cornerstone of global forestry, yet its productivity is increasingly threatened by drought. To elucidate the core molecular mechanisms of its drought response, we conducted a meta-analysis of three independent RNA-Seq datasets. Using a rigorous bioinformatic pipeline that included a leave-one-out Jackknife validation to ensure robustness and mitigate single-study bias, we identified a high-confidence set of drought-responsive genes. Our analysis pinpointed a core set of 472 robust differentially expressed genes (DEGs), with 274 being consistently upregulated and 198 downregulated. Functional analysis revealed a clear “growth-defense” tradeoff, considering that upregulated genes were predominantly involved in protective functions, including protein folding (heat shock proteins), the synthesis of osmolytes like inositol and trehalose, and abscisic acid (ABA) signaling, while genes related to cell division, DNA replication, and cell wall biosynthesis were significantly repressed, indicating a strategic diversion of resources away from growth. Also, protein-protein interaction network analysis provided a systems-level view of this strategy, visualizing a highly interconnected module where the induction of protein repair enzymes was linked to the coordinated shutdown of the DNA replication machinery. Finally, this work provides a reliable catalog of high-priority candidate genes, including many previously uncharacterized proteins, for future functional studies and for the development of molecular markers. Specially, these findings are crucial for breeding programs aimed at enhancing drought resilience, thereby supporting the sustainability of eucalyptus forestry under climate change.

Introduction

The escalating climate crisis presents unprecedented challenges to global ecosystems and forestry, with projections indicating continued increases in temperature and the frequency of extreme weather events such as droughts. Since pre-industrial times, the Earth’s surface temperature has risen by 1.1 °C and could surpass 4 °C by the end of this century. Plant populations existing outside their optimal climate conditions are likely to experience widespread die-offs, leading to reductions in their geographic distribution, a trend that poses a risk to one in every six species worldwide¹.

It is also known that plants have evolved complex molecular and physiological responses to cope with such stresses, including osmotic adjustment, antioxidant defense, and the modulation of growth and development, often orchestrated by intricate gene regulatory networks^{2–5}. The *Myrtaceae* family, to which the *Eucalyptus* genus belongs, encompasses a wide range of species adapted to diverse environments, offering a rich genetic resource for understanding stress tolerance mechanisms; however, the specific responses of key commercial species like *E. grandis* to intensifying drought require deeper investigation⁶. *Eucalyptus grandis* is a pillar of the global forestry economy, primarily due to its rapid growth and versatile wood, which is extensively used in pulp, paper, timber, and bioenergy production⁷. Brazil stands as one of the world’s leading producers of eucalyptus, along with India, China, and Australia, with extensive cultivated areas significantly contributing to its internal domestic product and export revenue, particularly through the pulp, paper, and bioenergy industries^{8,9}. While these cultivations are widespread, their expansion and sustainability, particularly in regions transitioning or bordering sensitive biomes like the Brazilian Cerrado, face increasing environmental scrutiny and the need for adaptive management practices, especially concerning water use and climatic resilience^{10,11}. The Cerrado, a global biodiversity hotspot, faces significant anthropogenic pressures, especially due to overfarming and agricultural land expansion¹². In this context, the study of and improvement of

drought-tolerant tree species like *E. grandis* becomes crucial for both economic and ecological perspectives¹³.

Among possible strategies for *E. grandis* improvement, RNA sequencing (RNA-Seq) represents a powerful and widely adopted tool for comprehensive gene expression analysis (transcriptomics)¹⁴. Various studies have used this approach to quantify gene expression levels, identify novel transcripts, and alternative splicing events in plant species, offering deep insights into the dynamic molecular responses of organisms to various stimuli, including abiotic stresses like drought^{3,15–21}.

In this context, we performed a meta-analysis integrating data from multiple independent RNA-Seq studies of *Eucalyptus grandis* to assess the consistency of findings related to drought response²². This approach allows us to overcome the limitations inherent in individual experiments, such as small sample sizes or specific conditions, thereby addressing a critical gap in the literature regarding a comprehensive view of drought response. By increasing the statistical power, we present a reliable and comprehensive catalog of essential genes and pathways for developing targeted strategies to enhance drought resilience in this economically important species.

Results

The RNA sequencing data sets of the three independent studies, designated Study A (SRP178354), Study B (SRP458400) and Study C (SRP406292), were processed to assess data quality and quantify gene expression, as shown in Table 1. Study A produced an average of 22.27 ($\pm$ 4.60) million reads per sample, Study B averaged 29.31 ($\pm$ 3.11) million reads, and Study C produced the highest average count at 81.13 ($\pm$ 12.91) million reads. The transcription alignment rates using Salmon were consistently high in all studies, with Study A showing 89.35% ($\pm$ 0.96%), Study B 88.28% ($\pm$ 0.86%), and Study C 88.22% ($\pm$ 0.57%) mean alignment percentages. Following transcript-to-gene mapping and initial filtering for low expression, a total of 26,821 genes were retained for downstream differential expression analyses.

Venn diagram (Figure 1A) revealed a core set of 46 genes identified by all three individual analyses and the overall meta-analysis when comparing the DEGs from each study with the Significant Meta-DEGs. The Jackknife resampling procedure culminated in the identification of a core set of highly robust DEGs. As depicted in the Venn diagram (Figure 1B), a total of 472 genes consistently met the significance criteria (meta-analysis FDR < 0.05 and |weighted LFC| $\geq$ 1) across the full meta-analysis and all three leave-one-out Jackknife iterations. Within this robust set, 274 genes were consistently up-regulated and 198 were consistently down-regulated.

Gene Ontology (GO) enrichment analysis was performed on the Meta-DEGs. For the up-regulated genes (Figure 2A), highly significant terms included response to heat, protein folding, response to abiotic stimulus, and stress response. For the down-regulated genes (Figure 2B), enriched GO terms were dominated by categories such as cell wall organization or biogenesis, cell cycle, mitotic cell cycle, and various polysaccharides metabolic processes linked to cell wall composition.

The Protein-Protein Interaction (PPI) network (Figure 3) revealed a prominent, highly interconnected cluster of up-regulated proteins. This central group included heat shock proteins (SHSP20, HSP70), ClpA/ClpB proteases, and proteins involved in osmolyte synthesis (INO1, TPP). The network also showed down-regulation of proteins essential for DNA replication (MCM, SLD5). Other distinct clusters highlighted proteins involved in antioxidant defense (TRX), cell wall dynamics (PMEI), and hormonal signaling. Numerous uncharacterized proteins showed substantial changes in expression levels within these functional groups. The symbols mapping addressed to each protein are available in Supplementary Information 1.

Discussion

Methodological robustness and the core drought-responsive gene set

This meta-analysis enabled a more comprehensive characterization of the drought response in *Eucalyptus grandis* than any single study alone, initially identifying a broad set of differentially expressed genes. This was followed by a rigorous validation of these findings using a Jackknife analysis. This procedure acted as a crucial filter to test the stability of the results. The analysis revealed that the exclusion of Study B (SRP458400) had a profound impact on the overall gene list, evidenced by a high Jaccard dissimilarity of 0.637. This value indicates a significant divergence, suggesting that Study B contributed a strong, unique transcriptional signature, likely stemming from specific experimental conditions or genotypes, that was not consistently corroborated by the other datasets.

By successfully identifying and quantifying this influence, the Jackknife analysis demonstrated its power to detect study-specific biases. Consequently, the 472 DEGs that remained significant regardless of any single study's exclusion represent a core set of robust drought-responsive genes. Their biological signals make them high-priority candidates for subsequent functional investigation and potential targets for breeding programs aimed at enhancing drought resilience.

Drought induces a growth-defense tradeoff in *Eucalyptus grandis*

For the up-regulated genes (Figure 2A), a strong enrichment of processes related to stress response and protein metabolism (especially folding) was observed. This aligns with contemporary understanding that plants under drought activate a complex

network of protective mechanisms to maintain cellular homeostasis¹³. Highly significant terms included response to heat, response to temperature stimulus, protein folding, response to abiotic stimulus, and stress response. Drought frequently induces secondary oxidative and heat stresses, necessitating such multifaceted responses^{6,23}. The molecular basis for these observations in our eucalyptus analysis involves several key gene families whose roles in stress adaptation are well-documented in recent literature^{24–26}.

The significant up-regulation of genes encoding small heat shock proteins (SHSP20), various chaperones of the ClpA/ClpB family, and numerous Heat Shock Protein 70 (HSP70) members is crucial. These molecular chaperones are essential for preventing protein misfolding and aggregation, and for refolding denatured proteins, thereby safeguarding cellular functions under stress. This role is consistently highlighted in plant stress physiology, including the studies on *Eucalyptus grandis* referenced for this meta-analysis(2019)²⁴, which also reported the induction of these protective gene families. Nonetheless, the authors further revealed the upregulation of genes involved in ROS detoxification, secondary metabolism, and the biosynthesis of protective compounds like polyamines and stachyose. Our results indicate the co-induction of Heat Shock Factors (HSF), which are master regulators of HSP expression, further emphasizing the activation of this chaperone-based defense^{27,28}. Additionally, BAG domain-containing proteins and J domain-containing proteins (JD), which function as co-chaperones that modulate HSP70 activity, were also prominent in our results, indicating a sophisticated regulation of protein quality control systems²⁹.

The up-regulation of Fe(II)/2-oxoglutarate-dependent dioxygenases (Fe2OGD) is also significant. This vast enzyme superfamily is involved in critical plant processes, including the biosynthesis of hormones like abscisic acid (ABA), gibberellins, and ethylene, and the production of protective secondary metabolites such as flavonoids and other phenolics^{30,31}. Enhanced ABA levels, for instance, are pivotal for drought response, promoting stomatal closure and inducing wide-ranging transcriptional changes³². Flavonoids, widely produced by eucalyptus, can act as potent antioxidants, mitigating oxidative damage, which is a common secondary effect of drought stress. The enrichment of non-specific serine/threonine protein kinases (STK) underscores the activation of crucial signaling pathways^{2,33}. These kinases are key components in perceiving stress signals and transducing them to initiate downstream adaptive responses, including the regulation of gene expression and physiological adjustment³⁴.

Genes associated with maintaining genome integrity, such as DNA helicases (MCM) and Replication Protein A subunits (RPA), were also up-regulated. Abiotic stresses like drought can lead to increased DNA damage, and DNA repair mechanisms are essential for plant survival and recovery^{35,36}. The involvement of F-box domain-containing proteins, which are specificity factors for E3 ubiquitin ligases in the ubiquitin-proteasome system, points to the importance of regulated protein turnover. This system selectively degrades damaged or regulatory proteins, facilitating cellular reprogramming and resource reallocation under stress conditions³⁷. Finally, the enrichment of cellular carbohydrate metabolic processes, with genes like starch synthase (SS), trehalose-6-phosphate phosphatase (TPP), and phosphofructokinase (PFK), highlights metabolic reprogramming. Accumulation of soluble sugars such as sucrose, trehalose, raffinose family oligosaccharides is a common plant strategy during drought, serving as osmolytes for osmotic adjustment, osmoprotectants for cellular structures, and signaling molecules³⁸. Studies on eucalyptus species under drought have shown significant alterations in carbohydrate profiles, supporting their role in stress adaptation³⁹. Collectively, these up-regulated gene categories in *Eucalyptus grandis* reflect a comprehensive and evolutionarily conserved strategy to defend against drought, encompassing protein protection, detoxification, signaling, genome stability, and metabolic adjustments, all of which are actively researched areas in current plant stress biology.

Conversely, the down-regulated genes (Figure 2B) revealed a significant suppression of processes primarily associated with cell growth, division, and cell wall biosynthesis. The enriched GO terms were dominated by categories such as Cell wall organization (GO:0071555) or biogenesis (GO:004254), Plant-type cell wall organization or biogenesis (GO:0071669), and various polysaccharide and carbohydrate metabolic processes linked to cell wall components (e.g., Hemicellulose metabolic process (GO:0010410), Xylan biosynthetic process (GO:0045492), Xyloglucan metabolic process (GO:0010411), Beta-glucan metabolic process (GO:0051273). Furthermore, a clear down-regulation of Cell cycle (GO:0007049), Mitotic cell cycle (GO:0000278) and DNA replication initiation (GO:000627) was evident. The suppression of Transmembrane transport (GO:0055085) and Cellular component organization (GO:0016043) also points towards a general reduction in cellular growth and expansion activities. This pattern suggests a strategy of resource conservation under drought, where energy and resources are diverted away from growth-related processes to prioritize survival mechanisms, as highlighted by the up-regulated gene functions^{40–42}.

Protein interaction network reveals key functional modules driving drought adaptation in *Eucalyptus grandis*

The Protein-Protein Interaction (PPI) network depicted in Figure 3 provides a visual representation of the functional relationships among differentially expressed genes (DEGs) in *Eucalyptus grandis* subjected to drought stress. A prominent and highly interconnected assemblage of proteins within the network suggests a pivotal role in orchestrating the plant's response to drought. A dominant theme emerging from this group is the maintenance of protein homeostasis. While this includes the expected

upregulation of multiple heat shock proteins (HSPs) and their Heat Shock Factor (HSF) activators, the network's value lies in detailing the system's complexity. It shows these core chaperones functioning alongside specialized co-chaperones like BAG domain (logFC +4.22) and J domain-containing proteins and, notably, reveals the strong induction of a protein-L-isoaspartate O-methyltransferase (PIMT, logFC +1.80). The co-regulation of PIMT, an enzyme that directly repairs isomerized amino acids in damaged proteins, provides a more granular insight than the GO analysis, pointing to a comprehensive strategy that combines damage prevention with active enzymatic repair to preserve the proteome^{27,28,43}.

Also, PIMT is linked to a downregulated protein containing a WD40 repeats region (WDRR, logFC -1.81). This protein is connected to a highly coordinated cluster of other down-regulated proteins essential for DNA replication, including multiple MCM DNA helicases (logFCs -1.64, -1.57, and -1.46), Sld5 (logFC -1.76), and a Replication Protein A (RPA) subunit (logFC -1.84). The coordinated downregulation of this group is a molecular signature of cell cycle arrest, a critical energy conservation strategy under drought. Since WD repeats are known to form scaffolds for protein complexes, the suppression of this specific protein likely helps arrange the shutdown of DNA replication^{44,45}. Then, the connection between the upregulated protein repair enzyme (PIMT) and this downregulated replication group visually represents a key strategic shift in the plant's drought response of actively suppressing costly cell division while simultaneously boosting the maintenance and repair of existing cellular machinery to ensure survival⁴⁶⁻⁴⁸.

This central group of interacting proteins also revealed significant metabolic adjustments and osmoprotective strategies. The upregulation of an Inos-1-P-synth domain-containing protein (INO1, logFC +2.32), responsible for myo-inositol-1-phosphate synthesis, points to the accumulation of inositol-derived osmolytes. This finding is consistent with literature indicating that INO1 upregulation and subsequent inositol accumulation contribute to drought and salinity tolerance in various plant species by helping maintain cell turgor⁴⁹⁻⁵¹. Similarly, trehalose 6-phosphate phosphatase (TPP, logFC +1.72) was shown as upregulated, suggesting the synthesis of trehalose, another key osmoprotectant and signaling molecule. Carbohydrate metabolism showed complex readjustments, with Starch synthase (SS, one instance at logFC +2.35) being upregulated, while Sucrose synthase (SUS, logFC -1.95) and a Glycosyl hydrolase 32 family member (GH32, logFC -3.19) were downregulated, indicating a significant redirection of carbon resources^{38,39,52,53}. These findings align with a conserved metabolic strategy in plants under abiotic stress, characterized by the accumulation of osmoprotectants and a significant redirection of carbon resources, a pattern where the upregulation of INO1 and the downregulation of growth-related genes like Sucrose Synthase (SUS) suggest a trade-off diverting carbon from growth to survival. Such a strategy is corroborated by recent studies; for instance, one of them⁵⁴ demonstrated in upland cotton (*Gossypium hirsutum*) that the positive regulation of GhIPS1-A, an ortholog of myo-inositol-1-phosphate synthase, is directly linked to maintaining fibre yield under drought, underscoring the critical role of the inositol pathway in preserving agronomic traits during stress. This focus on specific protective pathways is complemented by the findings of one of the works²⁵ selected for this pipeline, which showed a general downregulation of metabolism and growth genes under drought, confirming a broader strategy of resource conservation that prioritizes survival mechanisms over growth, mirroring the specific gene expression changes presented in your data.

Beyond these central responses, other distinct clusters of interacting proteins highlighted other adaptive strategies. Enhanced antioxidant defense mechanisms were suggested by the upregulation of thioredoxin (TRX, logFC +2.62), crucial for redox homeostasis^{52,55-57}. Alterations in cell wall dynamics were implied by the differential expression of proteins such as a Pectin Methylesterase Inhibitor⁵⁸ (PMEI, logFC -2.28), which influences cell wall rigidity, and various glycosyl hydrolases (e.g., GH5, logFC -1.69; GH18, logFC +1.75)⁵⁹. Hormonal signaling adjustments also extended to auxin pathways, as evidenced by the downregulation of an auxin-responsive PIN protein (logFC -2.69)⁶⁰, consistent with stress-induced growth inhibition. In contrast, a protein containing an AAI domain (logFC +5.63), often associated with alpha-amylase inhibitors or stress responses, was strongly upregulated^{61,62}. Signaling and regulatory processes were also strongly represented within this major functional module. Several PPM-type phosphatase domain-containing proteins (PPM/PP2C, with consistently positive logFCs: +2.14, +2.32, +2.35, +2.67) were upregulated. These PP2C phosphatases are critical, often negative, regulators in abscisic acid (ABA) signaling, a cornerstone of plant drought response. Their upregulation in eucalyptus aligns with their documented involvement in stress tolerance across diverse plant systems. While the precise outcome of PP2C upregulation can be context-dependent, it signifies an active modulation of the ABA-mediated stress response⁶³⁻⁶⁸. Other signaling components, including Glycosyltransferase 1 family proteins (GST1, logFCs +1.24, +2.18)⁶⁹, a putative UDP-glycosyltransferase^{16,52} (UGT, logFC +2.82), a non-specific serine/threonine protein kinase (STK, logFC +1.26), and a tyrosine-protein phosphatase (PTP, logFC +1.65), were also differentially regulated, indicating broad involvement of phosphorylation cascades and conjugation pathways^{2,33,70}.

Indications of significant shifts in secondary metabolism or hormone biosynthesis pathways were observed through the strong upregulation of several Fe(II)- and 2-oxoglutarate-dependent dioxygenases (Fe2OGD, logFCs +2.24, +3.20, +4.36). However, one member of this Fe2OGD family (logFC -3.03) and a Cytochrome P450 (CYP, logFC -3.45) were downregulated, suggesting specific modulations within these broad enzyme families^{30,31}. Furthermore, classic indicators of robust drought adaptation were prominently featured, most notably the very strong upregulation of a dehydrin (DHN, logFC +6.93), a

protein well-known for its role in protecting cellular structures from dehydration damage^{71–73}. Also, a homeobox domain-containing protein (logFC +3.24), a generic type of transcription factor, was upregulated^{74–76}. The upregulation of a DOG1 domain-containing protein (logFC +2.20), linked to ABA signaling and abiotic stress tolerance, further underscored the plant's comprehensive adaptive measures⁷⁷.

A significant aspect of this network is the presence of numerous uncharacterized/hypothetical proteins (UNK) that showed substantial changes in expression levels (e.g., with logFCs such as +5.66, +4.20, and -1.57) across these interconnected functional groups. These proteins did not present any recognizable domains or protein families. Such uncharacterized proteins represent a reservoir of potentially novel genes that are crucial for the drought response in *Eucalyptus grandis* and are candidates for future functional characterization.

In summary, the PPI network analysis paints a comprehensive picture of a multifaceted and highly coordinated known response of *E. grandis* to drought conditions. The findings emphasize the critical roles of protein protection and quality control, osmotic adjustment through the synthesis of compatible solutes like inositol and trehalose, active modulation of ABA signaling pathways, and a strategic inhibition of growth. These primary responses are complemented by adjustments in redox homeostasis, cell wall modifications, altered secondary metabolism, and the induction of well-established stress-protective proteins like dehydrins.

Methods

RNA-Seq datasets

Three independent RNA-seq studies were selected from the NCBI SRA database⁷⁸, adhering to strict inclusion criteria: paired-end Illumina data from *Eucalyptus grandis* experiments comparing exclusively well-watered controls against drought-stressed samples (Table 2). The list of selected studies, samples and associated metadata are provided in Supplementary Information 2.

Raw sequencing data were retrieved via in-house bash scripts. The script used the fasterq-dump module of SRA Toolkit v3.2.1⁷⁹ for data download, with GNU Parallel 20250422⁸⁰ being employed to execute simultaneous download and processing operations, significantly accelerating the retrieval of all samples. Following the download, paired-end FASTQ files were compressed and renamed according to a predefined mapping scheme to ensure consistency. The entire process featured comprehensive logging and error handling to guarantee data integrity.

Quality control

Quality control was implemented using a multi-tool approach for initial FASTQ validation and FastQC v0.12.1⁸¹ for comprehensive per-sample quality metrics assessment. Adapter trimming and read filtering were performed with bbduk from BBTools v39.23⁸², applying parameters including k-mer trimming (k=23, mink=11, hdist=1), quality-based trimming ($Q \geq 10$), and removal of reads shorter than 50 bp. Paired-end consistency was enforced (tpe, tbo flags), and Illumina adapters were trimmed. Quality control reports were aggregated using MultiQC v1.28⁸³ for comprehensive visualization of dataset characteristics.

Salmon quantification

Transcript quantification was executed with Salmon v1.10.3⁸⁴ in alignment-based mode, correcting for GC content and sequence-specific biases. The index was constructed from the *E. grandis* reference transcriptome (GenBank accession code GCF_016545825.1). Salmon output files were organized into study-specific directories matching the original SRA project identifiers for systematic downstream analysis.

Differential gene expression and meta-analysis integration

Adapting the approach of a referred⁸⁵ work, transcript-level quantifications were imported into R v4.4.3 using tximport v1.34.0⁸⁶. Gene-level counts were then filtered for low expression using the filterByExpr module of edgeR v4.4.2⁸⁷, and libraries were normalized using the Trimmed Mean of M-values (TMM) method. Subsequently, differential gene expression analysis was performed independently for each of the three studies using DESeq2 (v1.46.0)⁸⁸. To ensure stability, log2 fold changes (LFCs) were moderated using the lfcShrink function with the ashR estimator. Meta-analysis was then conducted on genes common across all studies. Raw p-values from individual analyses were combined using Fisher's method metaRNASeq v1.0.8⁸⁹, and a combined effect size was calculated as a weighted average LFC. Significant meta-DEGs were defined as genes with a combined FDR < 0.05 and an absolute weighted LFC ≥ 1 . The stability of these findings was assessed using a leave-one-out Jackknife resampling procedure; genes that remained significant in the full analysis and all Jackknife iterations were termed as robust DEGs. Finally, a highly rigorous common DEGs set was identified by further filtering these robust genes, requiring them to also be individually significant in all three studies (FDR < 0.05, $|LFC| \geq 1$) with a consistent direction of regulation. To evaluate the stability of the results and the influence of each dataset on the meta-analysis, the Jackknife validation was detailed through a quantitative analysis. Jaccard dissimilarity was calculated to measure the magnitude of change in the set

of differentially expressed genes (DEGs) upon the exclusion of each study. In addition to this index, the impact of each study was characterized by quantifying gene flux: "genes lost" (DEGs that ceased to be significant after a study's exclusion) and "genes gained" (genes that became significant in its absence) were tallied. This dual approach allowed measuring the magnitude of the change (dissimilarity), providing a more robust assessment of each study's contribution to the final meta-analysis result. The entire process was implemented in the R environment with custom scripts. Resulting plots were generated with ggplot2 v3.5.2⁹⁰ and ggVennDiagram v1.5.2⁹¹. Data manipulation was handled with the tidyverse suite v2.0.0⁹².

Functional enrichment and network analysis

To build the protein-protein interaction (PPI) networks, protein sequences of the consistently up- and down-regulated genes were retrieved for *Eucalyptus grandis* (egr) via KEGG REST API (KEGGREST (development version), n.d.). The PPI networks were then assembled using the STRING database v11.30⁹³, with *Eucalyptus grandis* as the reference organism. A medium confidence cutoff of 0.400 was adopted, following established thresholds for plant interactomes. Functional enrichment analysis was performed using STRING's built-in tools, with Gene Ontology (GO) terms clustered by semantic similarity (threshold ≥ 0.8) and sorted by gene count to prioritize biologically coherent pathways. The networks were visualized and annotated in Cytoscape v3.10.3⁹⁴, integrating mean Fold Change (FC) values and node-specific enriched GO terms for enhanced interpretability, considering an FDR ≤ 0.004 . All graphs were annotated and personalized with Inkscape v1.4.

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Author contributions statement

J.V.A.T. executed the pipelines and designed the study under the supervision of G.Q.P and K.K.P.O. N.M.P.L. and R.A.S. contributed to the review and supervision of the manuscript and the work. All authors reviewed the manuscript.

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Data availability statement

The raw sequencing data analyzed in this study are available in the NCBI SRA repository under accession numbers SRP178354, SRP458400, and SRP406292. The complete analysis scripts are archived and available on GitHub at <https://github.com/jvtarss/euca-2025>.

Competing interests statement

The authors declare no conflict of interest.

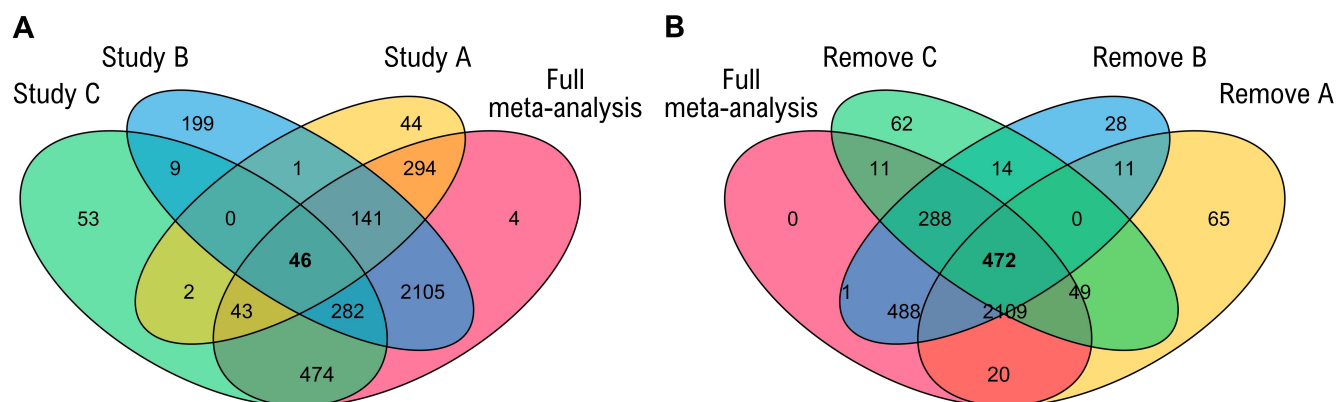


Figure 1. Venn diagrams showing (A) the number of common DEGs shared among the individual studies and meta-analysis, and (B) the number of common DEGs among different Jackknife tests and meta-analysis results.

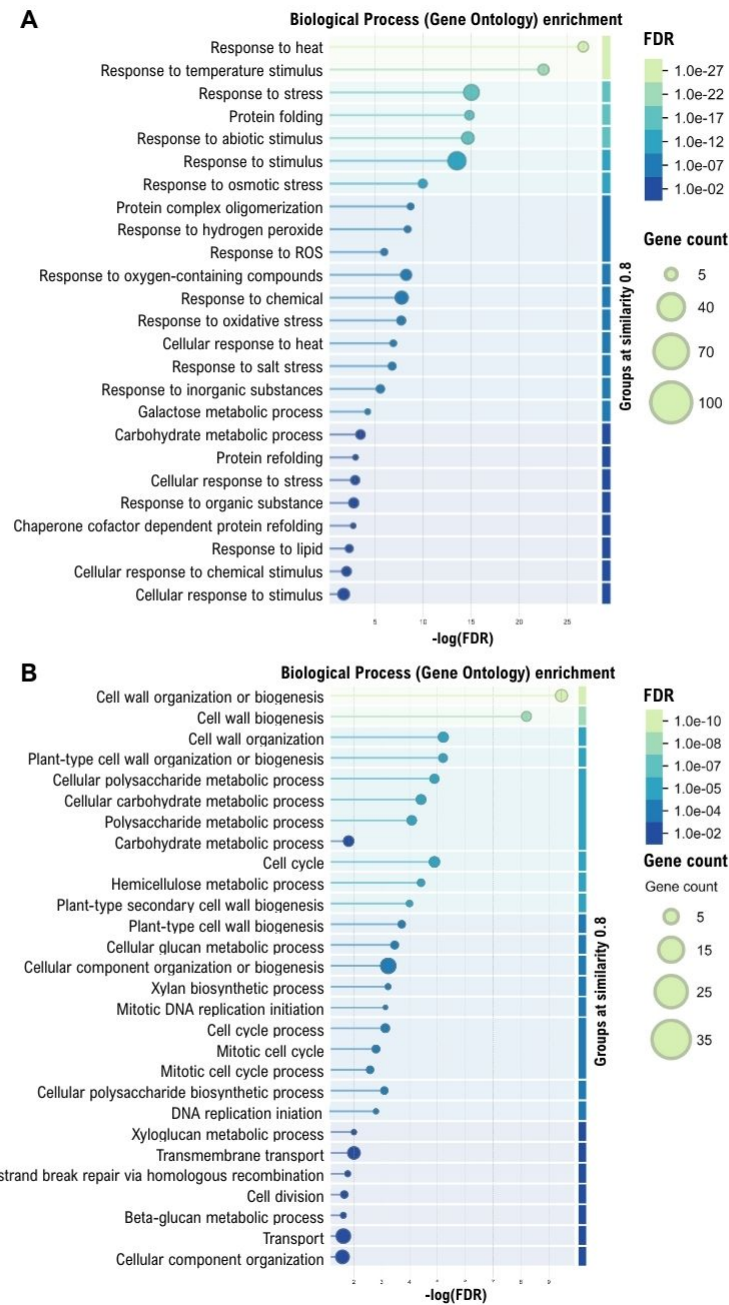


Figure 2. Gene Ontology (GO) enrichment analysis of Biological Processes (BP) for differentially expressed genes, with term networks generated by STRING. (A) Enriched GO BP terms for up-regulated genes. The displayed terms have False Discovery Rates (FDR) ranging from 1×10^{-27} to 1×10^{-2} . (B) Enriched GO BP terms for down-regulated genes, with displayed terms having FDRs in the range of $1 \times 10^{-10} < \text{FDR} < 1 \times 10^{-2}$. In both panels, significance is visualized on a $-\log(\text{FDR})$ scale, and terms were functionally grouped based on a similarity coefficient of 0.8.

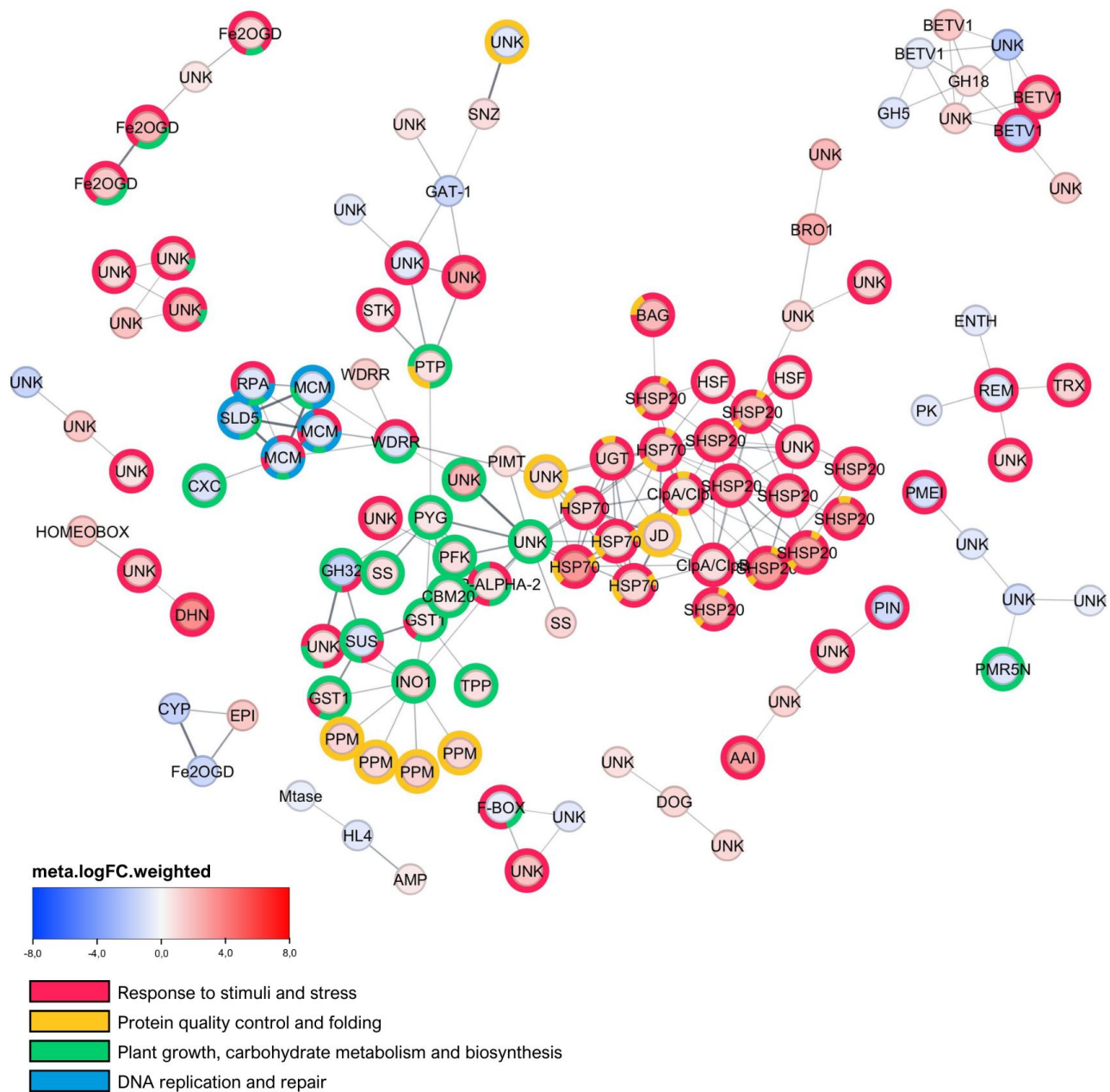


Figure 3. PPI network of differentially expressed genes (DEGs), generated using STRING and visualized with Cytoscape. The network includes both up- and down-regulated DEGs. Unique isolated nodes and clusters containing only two nodes were removed. Nodes are annotated with Gene Ontology (GO) terms using a flat donut label style, with terms grouped into broader categories. The GO terms displayed have False Discovery Rates (FDR) in the range of $4.17 \times 10^{-17} < \text{FDR} < 4.15 \times 10^{-2}$. The network was organized using the Prefuse Force Directed layout algorithm, weighted by STRING interaction scores.

Supplementary Files

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