Transcriptional reprogramming during recovery from drought stress in *Eucalyptus grandis*

Demissew Tesfaye Teshome[1], Godfrey Elijah Zharare[2], Raphael Ployet[3], and Sanushka Naidoo[1,4]

[1]Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa, [2]Department of Agriculture, University of Zululand, KwaDlangezwa, South Africa, [3]Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA, [4]Corresponding author: sanushka.naidoo@fabi.up.ac.za

Keywords

Water deficit; *Eucalyptus*; Forest tree; Drought release; RNA-seq; Signaling pathways

Running head

Recovery from drought stress in *Eucalyptus grandis*

Abstract

The importance of drought as a constraint to agriculture and forestry is increasing with climate change. Genetic improvement of plants' resilience is one of the mitigation strategies to curb this threat. Although recovery from drought stress is important to long-term drought adaptation and has been considered as an indicator of dehydration tolerance in annual crops, this has not been well explored in forest trees. Thus, we aimed to investigate the physiological and transcriptional changes during drought stress and rewatering in *Eucalyptus grandis* W. Hill ex Maiden. We set up a greenhouse experiment where we imposed drought stress on 2-year-old seedlings and rewatered the recovery group after 17 days of drought. Our measurement of leaf stomatal conductance (gs) showed that, while gs was reduced by drought stress, it fully recovered after 5 days of rewatering. The RNA-seq analysis from stem samples revealed that genes related to known stress responses such as phytohormone and reactive oxygen species signaling were upregulated, while genes involved in metabolism and growth were downregulated due to drought stress. We observed reprogramming of signal transduction pathways and metabolic processes at 1 day of rewatering, indicating a quick response to rewatering. Our results suggest that recovery from drought stress may entail alterations in the jasmonic acid, salicylic acid, ethylene, and brassinosteroid signaling pathways. Using co-expression network analysis, we identified hub genes, including the putative orthologs of *ABI1*, *ABF2*, *ABF3*, *HAI2, BAM1*, *GolS2*, and *SIP1* during drought and *CAT2*, *G6PD1*, *ADG1*, and *FD-1* during recovery. Taken together, by highlighting the molecular processes and identifying key genes, this study gives an overview of the mechanisms underlying the response of *E. grandis* to drought stress and recovery that trees may face repeatedly throughout their long life cycle. This provides a useful reference to the identification and further investigation of signaling pathways and target genes for future tree improvement.

Keywords: drought release; *Eucalyptus*; forest tree; RNA-seq; signaling pathways; water deficit

Introduction

Drought stress is among the most important abiotic stress factors that constrain agriculture and forestry. For example, it causes as high as 50% estimated yield loss in annual crops (Toreti et al., 2019). In forestry, the losses caused by drought stress can be in the form of seedling and mature tree mortality (Breshears et al., 2005; Beloiu et al., 2022) and reduction of tree growth (Lévesque et al., 2014; Dang et al., 2019). In recent years, we have witnessed frequent episodes of unprecedented droughts affecting different areas of the world (Toreti et al., 2019; Williams et al., 2022). Such observations attest to climate predictions that forecast extended and more frequent extreme weather events due to global warming (IPCC, 2007; 2021). Thus, in addition to the worldwide effort to slow down global warming by reducing anthropogenic emissions, sector-based mitigation strategies should be equally urgently undertaken.

Planting drought-resistant species and genotypes is one of the sustainable strategies of drought mitigation, particularly in plantation forestry. Drought resistance in plants may involve the strategies of dehydration escape, avoidance, tolerance, and recovery (Fang and Xiong, 2015; Volaire, 2018). Plants' ability to quickly recover from drought stress is not only one of the strategies of resistance, but also is an indicator of dehydration tolerance and/or avoidance. For example, the recovery from a drought event has been established as an indicator of dehydration tolerance in annual crops such as maize (Hayano-Kanashiro et al., 2009; Chen et al., 2016), wheat (Vassileva et al., 2011; Abid et al., 2016), and rice (Kamoshita et al., 2004; Efisue et al., 2009). It can be hypothesized that recovery from drought could be even more important in forest trees, owing to their long life cycle, which

implies an exposure to recurring cycles of drought and rewatering. Indeed, the ability to recover from drought has been found to be a key trait of saplings that survive and adapt to weather anomalies (Beloiu et al., 2022), and it has been reported to be correlated to dehydration tolerance of different woody species (Blackman et al., 2009). More specifically in *Eucalyptus* L'Hér., exposure to recurrent drought was found to improve the speed of recovery, emphasizing its contribution to long-term adaptation (Nóia Júnior et al., 2020). However, despite its importance, relatively less research attention has been devoted to investigating recovery from drought stress in woody plants.

In plants, morpho-physiological traits vary in the time they take to recover, as well as the rate and extent of recovery compared with a pre-drought status depending on their sensitivity to and severity of drought (Miyashita et al., 2005; Montwé et al., 2014; Gessler et al., 2020). These variations may be related to diverse mechanisms of recovery employed by different plant species (Frosi et al., 2017) depending on the extent of drought-induced damages (Gauthey et al., 2022). Recovery of xylem hydraulic capacity, which might be achieved either by refilling or growth of new tissues, is one example. Severe damage to the vascular system means that recovery can only be achieved by growth of new tissues resulting in delayed recovery (Secchi et al., 2021; Gauthey et al., 2022). This indicates that understanding the underlying mechanisms of recovery is key to deploy this trait as a potential tree breeding target.

Transcriptomic analysis has been established as a valuable technique that enables the investigation of the genome-wide molecular mechanisms underlying the response of plants to biotic and abiotic stresses, while reducing the genome size as only a portion of the genome is expressed (Agarwal et al., 2014; Lowe et al., 2017; Wang et al., 2020). High-throughput RNA sequencing (RNA-seq) technologies have emerged as the preferred methods for transcriptome sequencing (Conesa et al., 2016; Lowe et al., 2017), and this was matched by

the availability of bioinformatics tools and computational resources that facilitated the efficient handling and analysis of big data. Such developments allowed the identification of metabolic pathways, gene regulatory networks, and key genes governing plant responses to biotic and abiotic stresses, thereby facilitating genetic improvement. Previous studies employed these techniques to explore the transcriptomic changes underlying the drought stress responses of *Eucalyptus* spp. including *Eucalyptus camaldulensis* Dehnh. (Thumma et al., 2012), *Eucalyptus globulus* Labill. (Spokevicius et al., 2017; Ulloa et al., 2022), and *Eucalyptus cladocalyx* F. Muell. (Spokevicius et al., 2017), as well as hybrids of *Eucalyptus alba* Reinw. ex Blume, *Eucalyptus urophylla* S. T. Blake, and *Eucalyptus grandis* W. Hill ex Maiden (Villar et al., 2011). Similarly, transcriptomic analysis can give a good insight into the molecular changes in trees during recovery from drought stress and may be used to identify key signaling pathways and genes that govern these responses. However, such studies are limited in forest trees (Fox et al., 2018; Pervaiz et al., 2021) and no study has been reported for *Eucalyptus* spp.

Angiosperms such as *Eucalyptus* are largely more susceptible to drought than gymnosperms such as pine (Mitchell et al., 2013; Moran et al., 2017). A recent report showed that tree mortality due to drought in Eucalypt plantations in Brazil have reached up to 22%, with an estimated economic loss of $13 million over 3 years in the studied 1400 hectare plantation (Tupinambá-Simões et al., 2022). Yet, angiosperms including *Eucalyptus* generally have a better ability to recover from drought than conifers owing to their relatively more efficient recovery of hydraulic conductivity (Johnson et al., 2012; Mitchell et al., 2013). While *E. grandis* is one of the most widely used species in planted forests for its fast growth and good wood properties (Acosta et al., 2008; Brockerhoff et al., 2008), it is relatively more susceptible to drought stress as compared with other species such as *E. camaldulensis* (de Moraes Goncalves et al., 2013; du Toit et al., 2017). Thus, genetic improvement of *E. grandis*

for current and future climate should consider recovery from drought as one of its targets. Given that *E. grandis* is mainly used for its wood products, it is important to understand the trade-off between drought response, growth, and downstream wood formation. In this regard, the stem responses are a good proxy for how the trees respond to and recover from drought stress.

In the current study, we investigated the molecular mechanisms underlying both the response to and the recovery from drought stress in *E. grandis*. We analyzed the expression profile of genes using transcriptome sequencing and found important gene categories and main signaling pathways triggered by drought stress and rewatering. We further identified key genes that are highly correlated with drought stress and recovery using gene co-expression network analysis. Our study gives an overview of the molecular changes associated with drought stress and recovery that trees face recurrently throughout their long life cycle and provides a useful reference to the identification of pathways and target genes for tree improvement.

Materials and methods

Plant material and treatment conditions

Drought stress and recovery greenhouse pot experiments with three replications were laid down in a randomized complete block design using each phytotron as a block. Two-year-old *E. grandis* clone TAG5 (Mondi, South Africa) seedlings were transplanted into 10 L pots with a 4:2:1 (v/v) mixture of potting mix, river sand, and vermiculite. The plants were acclimatized to the phytotron conditions for 15 days by watering them every day. The pots were randomly assigned into well-watered control (WW), water-stressed (WS), and rewatered (WR) treatments. Drought stress was imposed on the WS and WR treatments by withholding water until a target pot weight corresponding to 25% plant available water

(PAW), equivalent to ~22% container capacity, was achieved. Subsequently, water lost via evapotranspiration was replaced to maintain the PAW levels in the pots between 25 and 30%. Soil moisture content was monitored gravimetrically by measuring pot weight. Recovery treatments (WR) were rewatered after 17 days of withholding water, whereas WW plants were watered every day. The plants were kept under 16 h of light and 8 h of dark throughout the experiment using supplementary light. The mean daily minimum and maximum temperatures across the three phytotrons were 24.15 ± 1.51 °C and 29.27 ± 3.62 °C, respectively. Slow-release fertilizer was applied at a rate of 10 g per pot (Viera et al., 2016).

Measurements and tissue harvest were conducted at the peak of the drought in the day, between 2 h before and after solar noon. Stomatal conductance of leaves was measured using AP4 porometer (Delta-T Devices Ltd, Cambridge, UK) from one fully developed leaf exposed to full sunlight from each of six ramets at 7, 17, 18 and 22 days after withholding water. In the case of the WR group, 17, 18, and 22 days correspond to 1 day before and 1 and 5 days after rewatering, respectively. Stem samples were harvested from two ramets in each of the three blocks at 7 and 22 days of withholding water for the drought experiment as well as 1 and 5 days of rewatering for the recovery experiment. Stem segments of 2 cm length were cut at a height of 30 cm above ground. Each segment was ~1 cm in diameter. The tissue samples were immediately flash-frozen in liquid nitrogen, ground with a prechilled IKA A11 basic analytical mills (IKA-Werke GmbH & Co. KG, Germany) followed by grinding into fine powder in liquid nitrogen using mortar and pestle and kept at –80 °C until use for RNA-seq analysis.

Transcriptome data analysis

Quality assessment, read mapping, and transcript quantification

Stem samples from 22 days of drought as well as 1 and 5 days of rewatering with the respective WW controls, each of which had three biological replicates, were sent to BGI Tech Solutions (Hong Kong) Co., Limited, Hong Kong for RNA sequencing on the Illumina HiSeq platform (Illumina, San Diego, CA, USA). Paired-end RNA-seq reads of 150 and 100 base pairs were obtained from three rounds of sequencing. The quality of the RNA-seq data was assessed using FASTQC version 0.11.7 and quality trimming was conducted using Trimmomatic version 0.36 (Bolger et al., 2014). Quality assessment was repeated after trimming and concatenation of sequence data from the three rounds of sequencing. This was followed by mapping the preprocessed RNA-seq data to the *E. grandis* reference genome v2.0 (Myburg et al., 2014) using Spliced Transcripts Alignment to a Reference (STAR) software (Dobin et al., 2013) and transcript quantification was conducted using StringTie version 1.3.4d (Pertea et al., 2015).

Differential gene expression and Gene Ontology enrichment analyses

Differential gene expression analysis of WS and WR treatments compared with the respective WW controls was conducted using the R package DESeq2 version 1.32.0 (Love et al., 2014). A $\log_2$ fold change cut-off point of 0.5 and an adjusted *P*-value < 0.05 were used to determine differential expression of genes. Significantly enriched biological process Gene Ontology (GO) terms for the up and downregulated differentially expressed genes (DEG) were identified against the set of expressed genes using the R package GO-seq version 3.14 (Young et al., 2010) with a Benjamini–Hochberg adjusted *P*-value < 0.1. The enriched GO terms were then visualized using GO-Figure! (Reijnders and Waterhouse, 2021).

Co-expression network analysis

The RNA-seq data were checked for outliers by clustering the samples, and genes expressed in at least 25% of the samples, with transcripts per million (TPM) values higher than or equal

to 1, were retained for co-expression analysis. This resulted in 16,764 genes whose expression profiles were standardized across the 15 conditions. These genes were used for co-expression network analysis using Pearson correlation coefficient and the Weighted Gene Co-expression Network Analysis (WGCNA) R package version 1.70–3 (Langfelder and Horvath, 2008) for co-expression modules detection. An unsigned adjacency matrix, created with a soft thresholding power of 9 corresponding to a scale-free topology fit R^2 of 0.8, was converted to a topological overlap matrix. Module detection was then conducted by hierarchical clustering followed by dynamic tree cutting using the flashClust and cutreeDynamic functions of the WGCNA package, respectively, with a minimum module size of 50 genes. Module eigengenes were then calculated and close modules were merged with a cut height of 0.1.

The GO enrichment analyses were conducted against the set of expressed genes for all the modules using the R package GOseq version 3.14 (Young et al., 2010) to have an overview of the functional enrichment in each module. The correlation of module eigengenes with the experimental treatments was computed using biweight midcorrelation analysis to select modules of biological interest. Potential hub genes were identified from the selected modules using gene trait significance (GS) and module membership (kME). To include at least 10% of the genes in a module as potential hub genes, a threshold of the absolute values of GS > 0.82 and kME > 0.9 were used. From these genes, those which were also differentially expressed for the respective treatments ($\log_2$ fold change cut-off point of 0.5 and an adjusted *P*-value < 0.05) were used for further analysis. The GO enrichment analysis was conducted on the resulting sets of genes using GOseq R package.

Results

Stomatal conductance decreases during drought stress but does not quickly recover upon rewatering

We set up a greenhouse experiment where 2-year-old *E. grandis* seedlings were exposed to 17 days of drought and subsequently rewatered to monitor their recovery from drought stress. Measurement of leaf stomatal conductance (gs) at 17 and 22 days of withholding water revealed a significant reduction in gs compared with the well-watered control (Figure 1A), indicating that withholding water induced drought stress response in the plants. Stomatal conductance of leaves did not fully recover after 1 day of rewatering as there was still a significant difference in gs between rewatered and well-watered treatments (Figure 1B). However, there was no significant difference in gs between these treatments at 5 days of rewatering, showing that gs fully recovered (Figure 1B). These results indicate that *E. grandis* fine tunes stomatal aperture to minimize water loss during drought stress and reopen them during recovery to return to and maintain their normal physiology.

Transcriptome sequencing reveals the expression profile of *E. grandis* during short-term drought stress and drought recovery

We obtained 100 and 150 bp paired-end RNA-seq reads sequenced on the Illumina HiSeq platform for stem samples taken after 22 days of drought stress as well as 1 and 5 days of rewatering. Differential gene expression analysis comparing these treatments with the respective well-watered controls resulted in a total of 4446 DEGs. The number of downregulated DEGs was slightly higher than the upregulated DEGs during drought stress, which may be an indication of a compromised physiology (Figure 2A). A similar trend was observed during recovery at 1 day of rewatering (Figure 2A), while the number of up and downregulated DEGs at 5 days of rewatering were equivalent, with a lower total number of

DEGs than that at 1 day of rewatering (Figure 2A). These quick changes in the transcriptome imply a quick recovery of several traits upon rewatering.

A search for *E. grandis* transcription factors (TFs) annotated by the Plant Transcription Factor Database (Jin et al., 2016) identified a total of 291 TF DEGs belonging to 40 TF families during both drought stress and recovery (Figure 2B, Table S1). The TF families such as NAC, bHLH, ERF, and MYB, which are well known for their involvement in regulating abiotic stress responses in plants (Nakashima et al., 2012; Balti et al., 2020), were represented by both up- and downregulated genes during drought as well as recovery at 1 and 5 days of rewatering (Figure 2C, Table S1). Notably, the ERF/DREB2 transcription factor EgrDREB2.5, previously shown to be related to drought response in *Eucalyptus* vascular tissues (Nguyen et al., 2017), was significantly induced during drought stress. A putative ortholog of the *Arabidopsis* Heynh. Carbon starvation inducible TF *ATAF1/ANAC002* (Garapati et al., 2015), previously reported to be induced in response to drought in *Eucalyptus* (Ployet et al., 2019), was upregulated during drought stress. Conversely, several putative orthologs of this TF were downregulated during recovery at 1 day of rewatering. These results suggest a variation in the cellular carbon status of plants during drought stress and recovery. Furthermore, some TF families were only represented by DEGs unique to either drought stress or recovery at 1 day of rewatering (Figure 2C, Table S1). This included an upregulated gene in the NF-YB TF family as well as several downregulated genes in the RAV, E2F/DP, NF-X1, Nin-like, and TCP TF families, which were differentially expressed particularly during drought stress. The TF families FAR1, Trihelix, LFY, HB, BBR-BPC, and GATA were represented by upregulated genes, while SRS and CAMTA TF families were represented by downregulated genes, which were specifically differentially expressed during recovery at 1 day of rewatering.

The change in the expression of some of the TF genes during both drought stress and recovery was relatively high (Table S1). This included the putative orthologs of *WRKY41* and

MYB80, which are known to be involved in defense against pathogens (Higashi et al., 2008) and pollen development (Phan et al., 2011) in *Arabidopsis*, respectively. While *WRKY41* was downregulated during drought stress, *MYB80* was upregulated. The putative orthologs of *WRKY33*, a gene involved in defense against pathogens (Tao et al., 2022), and *CBF4/DREB1D*, a negative regulator of dehydration tolerance (Vonapartis et al., 2022) were downregulated during recovery at 1 day of rewatering. The putative orthologs of *NAC010*, which regulates secondary cell wall biosynthesis in *Arabidopsis* and poplar (Grant et al., 2010; Zhong et al., 2021) was among the genes downregulated during recovery at 5 days of rewatering, while that of *AGL16*, which was previously shown to be a negative regulator of salt response (Zhao et al., 2021) in *Arabidopsis*, was upregulated.

Functional enrichment analysis of DEGs indicates contrasting effects of drought stress and rewatering on phytohormone and ROS signaling pathways, photosynthesis, carbohydrate metabolism, and growth

To get a general overview of the molecular processes affected by drought stress and rewatering, we conducted GO enrichment analysis for the respective up- and downregulated DEGs (Figure S1). Several biological processes related to the physiology, growth and development, and stress response of *E. grandis* were reprogrammed during drought stress and recovery. Protein modification processes such as protein phosphorylation, meristem maintenance, primary meristem development, and response to biotic stimulus were significantly enriched in the downregulated category of DEGs during drought stress (Figure 3A).

The GO terms enriched in the upregulated category of DEGs during drought were mainly related to abiotic stress responses and signaling including response to heat, osmotic stress, and water deprivation (Figure 3B). Abscisic acid (ABA) and reactive oxygen species (ROS)

signaling as well as protein folding, which are commonly associated with stress responses in general and drought response in particular (Ortbauer, 2013; Noctor et al., 2014; Yoshida et al., 2014), were also enriched (Figure 3B). The GO term galactose metabolic processes (Figure 3B) was enriched in the upregulated DEGs along with starch biosynthetic and metabolic processes (Table S2).

In line with the higher number of downregulated DEGs during recovery at 1 day of rewatering (Figure 2A), there were several GO terms enriched in this category. Some of these were related to stress responses and included response to oxygen containing compounds, oxidative stress (Figure 3C), ABA signaling, osmotic stress, and water deprivation (Table S2). In addition to these, jasmonic acid (JA)-mediated signaling pathway, regulation of salicylic acid (SA) biosynthetic processes, negative regulation of ethylene (ET)-activated signaling pathway, and response to wounding were also enriched in the downregulated category of DEGs during recovery at 1 day of rewatering (Table S2, Figure 3C).

The main GO terms enriched in the category of DEGs that were upregulated at 1 day of rewatering are reminiscent of a reversal to normal physiology (Figure 3D). Protein targeting to chloroplast, photosynthesis, photosystem *I* assembly, plastid and thylakoid membrane organization were enriched in this category, suggesting that recovery of photosynthesis is key to recovery from drought stress in *E. grandis*. In support of this, terms related to porphyrin-containing compounds, tetrapyrrole, and chlorophyl biosynthetic processes were also enriched (Fig 3D, Table S2). Furthermore, the GO term response to nutrient levels was upregulated during recovery at 1 day of rewatering. On the other hand, the GO term ROS metabolic processes was enriched in the upregulated DEGs, suggesting that, in addition to the downregulation of ROS production and signaling (Figure 3C), the removal of residual ROS and their reduced intermediates is important during the recovery of plants from drought stress.

Co-expression network analysis identifies key drivers of response to drought stress and rewatering

To identify key molecular processes and genes that modulate response to and recovery from drought stress, we conducted weighted gene co-expression network analysis (Figure S1) for 16,764 genes expressed across the 15 conditions. This resulted in 43 co-expression modules with sizes ranging from 43 to 724 genes after retention of genes with the absolute value of Pearson correlation > 0.9 and false-discovery rate adjusted *P*-value < 0.05. Modules of particular interest with regard to drought stress and rewatering were identified based on the correlation of module eigengenes with the experimental conditions (Figure S2). Accordingly, the top three modules with significant correlations were selected for further analysis. The modules *yellow* ($r = 0.87$, $P = 2e{-}05$), *darkorange2* ($r = 0.84$, $P = 7e{-}05$) and *grey60* ($r = 0.79$, $P = 4e{-}04$) were selected for drought stress at 22 days after withholding water, while *salmon* ($r = 0.88$, $P = 2e{-}05$), *steelblue* ($r = 0.75$, $P = 0.001$) and *blue* ($r = 0.66$, $P = 0.008$) modules were selected for recovery at 1 day of rewatering. The *floralwhite* module was selected as the only module with a lower correlation ($r = 0.53$, $P = 0.04$) with recovery at 5 days of rewatering. The GO enrichment analysis on the genes in each module (Table S3) and intersection of these genes with the up- and downregulated DEGs for the respective correlated treatments (Figure 4A and B) indicated that the selected modules are indeed strongly related to the experimental conditions considered.

A total of 304 potential hub genes were selected based on the absolute values of kME and GS from each of the selected modules except the *floralwhite* module for which no genes qualified the selection threshold. From each set of potential hub genes, those which were differentially expressed ($\log_2$ fold change ≥ 0.5 and adjusted *P*-value < 0.05) for the respective experimental treatments that correlate with the respective module were selected for further analysis (Figure S1). This resulted in a total of 196 genes (hereafter hub-DEGs) identified

from six of the seven modules of interest (Table S4). Reassessment of GO enrichment on each set of hub-DEGs indicated that these genes are involved in key biological processes governing the response of trees to drought stress and rewatering (Figure 5).

In the sets of hub-DEGs from the drought-related modules, i.e., *yellow*, *darkorange2*, and *grey60*, GO terms related to response to drought such as responses to water deprivation, ABA, H_2O_2, and heat as well as galactose metabolic processes and protein folding were enriched (Figure 5). The known importance of these processes to drought resistance in plants suggests that the identified hub-DEGs (Table S4) could be key drivers of drought resistance in *E. grandis*. Additionally, TF genes in the TF families known to regulate stress responses such as HSF, MYB, bZIP, and ERF were among the hub-DEGs of these modules (Table S4). This included *EgrDREB2–5* and several potential homologs of *AtHSFA2*, a hub regulator of stress response in *Arabidopsis* (Nishizawa et al., 2006). Overall, these results suggest that the induction of ABA signaling pathways and changes in non-structural carbohydrates (NSC) metabolism could be among the key biological processes involved in the response of *E. grandis* to drought stress and that key genes related to these processes such as the putative orthologs of *ABI1*, *ABF2*, *ABF3*, *HAI2*, *GolS2*, *BAM1*, *STS*, and *SIP1* could be among the central hubs of drought response (Figure 6A, Table S4).

In the sets of hub DEGs selected from the *salmon* and *blue* modules, which were associated with recovery at 1 day of rewatering, response to oxidative stress, stomatal complex morphogenesis, and maltose and starch metabolic processes were enriched (Figure 5). A putative ortholog of the TF *AtbZIP14* in the *salmon* module was upregulated during recovery at 1 day of rewatering and was the only TF hub-DEG identified from the recovery related modules (Table S4). Several genes related to primary metabolic processes such as a potential ortholog of the glucose-6-phosphate dehydrogenase 1, *AtG6PD1*, a key enzyme of the oxidative pentose phosphate pathway in non-photosynthetic tissues in *Arabidopsis* (Wakao and

Benning, 2005), were detected as hub-DEGs in the recovery related modules. Putative orthologs of *AtCAT2*, which encodes the antioxidant enzyme catalase (CAT) in *Arabidopsis*, were also among the hub-DEGs in the recovery related modules. Altogether, these results suggest a readjustment of metabolic pathways upon rewatering and show the importance of maintaining the ROS homeostasis during this process (Figure 6B).

Discussion

In the present study, we investigated the molecular mechanisms that may contribute to dehydration avoidance and tolerance as well as recovery from drought in *E. grandis*. Our study mainly focused on woody stem tissues which have received relatively less research attention despite their significant contribution to drought response as being essential vascular tissues (Spokevicius et al., 2017) and their critical role during recovery since wood is a major carbon sink (Johnson et al., 2012).

One of the early responses of plants during drought stress is minimizing water loss by fine tuning the stomatal aperture through which evapotranspirational loss of water and photosynthetic gas exchange occur. Stomatal conductance, which measures the level of gas and water vapor exchange, is an important indicator of stomatal regulation and a proxy to evaluate photosynthesis under drought stress conditions (Medrano et al., 2002). Eucalypts have been reported to control water loss through closing their stomata upon drought stress and reopening them upon the availability of water relatively quickly compared with the stomatal response during the stress period (Eksteen et al., 2013; Correia et al., 2014b). Our results support such behavior and reveal some underlying molecular changes that could relate to this process such as components of the ABA signaling pathways.

Abscisic acid acts as an essential signal during drought response in plants (Yoshida et al., 2014). In the current study, the ABA signaling pathway was enriched in the upregulated DEGs during drought stress (Figure 3B) and putative orthologs of genes in the pathway such as *ABI1*, *ABF2*, *ABF3*, and *HAI2* were identified as hub-DEGs in the drought related modules (Figure 6A, Table S4). While *ABI1* forms ABA receptor complex under the presence of ABA, it has also been shown to directly deactivate *SLAC1*, through which stomatal regulation is governed (Brandt et al., 2012). The ABF TFs regulate the expression of ABA-induced drought-responsive genes (Yoshida et al., 2010) and have been linked to improved dehydration tolerance in several annual and perennial plants including poplar (Yang et al., 2020). The genes connected to these ABFs in the drought-related modules included known drought response genes such as those encoding heat shock proteins and dehydrins (Yoshida et al., 2010; Thumma et al., 2012) as well as genes not yet related to drought response or of unknown function (Table S4). The latter included the putative orthologs of *FAB1*, a gene involved in pollen tube growth in *Arabidopsis* (Whitley et al., 2009; Serrazina et al., 2014), *RSP31*, a splice factor known to be induced by stress (Lopato et al., 1996), *CAP1*, a gene involved in cell division and elongation (Barrero et al., 2002), as well as uncharacterized genes such as the putative orthologs of the *Arabidopsis* genes *AT3G55140* and *AT5G14500*, which are predicted to encode proteins in the pectin lyase-like and aldose 1-epimerase families, respectively. The HAI protein phosphatases, known to be induced by drought stress in *Arabidopsis* (Fujita et al., 2009), have been considered as negative feedback regulators of ABA signaling and were also associated with negative regulation of osmotic adjustment (Bhaskara et al., 2012). In our conditions, putative orthologs of these genes were upregulated during drought stress, consistent with the activation of the ABA signaling pathways and the relatively lower osmotic adjustment previously reported in *E. grandis* compared with that of other *Eucalyptus* spp. (Lemcoff et al., 1994). These results confirm the pivotal role of ABA

signaling in modulating drought stress response genes observed in previous studies in *Eucalyptus* (Spokevicius et al., 2017; Ployet et al., 2019) as well as several genes that were not yet related to drought response in these tree spp. and could be part of a species-specific response. Furthermore, the induction in the stem tissues of the ABA signaling pathways along with ABA-induced genes such as *ANAC002*, previously shown to be related to secondary cell wall formation in *E. grandis* (Ployet et al., 2019), suggests that ABA could play a critical role in the formation of the vascular system under reduced water availability as recently proposed in poplar (Yu et al., 2021).

During recovery, though ABA signaling pathway was enriched in the downregulated DEGs at 1 day of rewatering (Table S2), gs did not fully recover (Figure 1B). In *E. globulus*, ABA concentration was found to decrease within 24 h of rewatering in the root and stem. However, it did not decrease to the control level in the leaf up to 7 days (Correia et al., 2014a). Thus, in our conditions, residual ABA accumulated during the drought period may have contributed to the relatively slow recovery of gs. However, we did not measure ABA levels in our study. Additionally, a recent study showed that the accumulation of ET during drought stress could be more important in delaying stomatal reopening during recovery than residual ABA (Yao et al., 2021). Here, we found that key genes putatively involved in ET biosynthesis (*ACS1* and *ACO4*, data not shown) and signaling (*EBF1*, *EBF2,* and *ERF4*, Table S1) were downregulated during recovery at 1 day of rewatering. Thus, a slow reduction of ET emission following drought may have also contributed to the relative delay in the recovery of gs.

While ABA has been known to be the main phytohormone involved in drought response, there is increasing evidence for the involvement of other phytohormones. Brassinosteroid (BR) biosynthesis was enriched in one of the drought modules (Table S3), where three putative orthologs of the *AtBEN1* gene, which is known to regulate the biosynthesis and

metabolism of different BRs in *Arabidopsis* (Yuan et al., 2007), were upregulated. Interestingly, a putative ortholog of a *BZR1*/*BES1* TF acting downstream of the BR signaling pathway was upregulated during both drought stress and recovery at 1 day of rewatering, while its putative paralog *BEH4* was upregulated during recovery (Table S1). The BZR1/BES1 TFs are known to regulate many genes related to growth and stress response in *Arabidopsis* (Guo et al., 2013). Our results suggest that BR could regulate drought response and growth through the *BZR1/BES1*-induced and repressed genes during drought and vice versa during recovery in *Eucalyptus*. The role of BR with regard to drought response could include ROS detoxification and improvement of photosynthetic efficiency as has been reported for different plant species including *E. urophylla* (Barros Junior et al., 2021). We also found that genes related to SA and JA signaling were downregulated during recovery from drought. In agreement with our result, Correia et al. (2014a) reported a slight decrease in xylem sap JA content of *E. globulus* during recovery from drought. Previous studies indicated the involvement of SA in detoxification of ROS during drought stress (Munne-Bosch and Penuelas, 2003), and repression of SA-related genes during recovery (Fox et al., 2018). However, the role of both JA and SA during recovery from drought remains unclear. Given the important role of these phytohormones in biotic stress responses of plants including *E. grandis* (Mangwanda et al., 2015), this might indicate a possible predisposition to biotic attack in the early period of recovery from drought.

Drought stress increases ROS accumulation as a result of photorespiration, which is enhanced by a limited CO_2 supply to the photosynthesis system, along with drought-induced changes in the mitochondria, cell wall, plasma membrane, and the apoplast (Noctor et al., 2014). While ROS are important signaling molecules, plants have developed enzymatic and non-enzymatic detoxification mechanisms as they also damage cells. As can be expected, ROS signaling was enriched in the upregulated DEGs during drought stress (Figure 3B), which included

EgCu/ZnSOD1, a gene previously shown to be a marker of drought induced oxidative stress in *Eucalyptus* (Hodecker et al., 2018). During recovery, GO terms related to ROS signaling were enriched in the downregulated DEGS while the term ROS metabolic processes was enriched in the upregulated DEGs (Figure 3C), which might indicate a need for removal of residual ROS and their intermediates. This is consistent with previous studies which reported that protection from oxidative stress is important during recovery from drought (Munne-Bosch and Penuelas, 2003; Mafakheri et al., 2011). For example, a higher activity of CAT was reported during recovery from drought (Mafakheri et al., 2011). However, this might not be enough to detoxify the ROS accumulated during the drought period and/or possibly continues to accumulate during recovery. Thus, increased expression of genes which encode antioxidative enzymes might be required. In line with this, we identified upregulated genes putatively encoding CAT and peroxidase (POX) as hub-DEGs in the recovery-related modules (Figure 6B, Table S4). The CAT-encoding gene *AtCAT2* was reported to modulate response to long-term heat stress in *Arabidopsis* (Ono et al., 2021) and could play a similar role in drought adaptation via its contribution to the recovery process. However, CAT-encoding genes were downregulated during recovery from drought in *Pinus halepensis* Miller (Fox et al., 2018). This may indicate a difference in either the source of ROS or the mechanism of detoxification among tree species.

Sugars play a vital role in plant metabolism and serve as osmolytes as well as signaling molecules during drought stress (Mitchell et al., 2013; Kaur et al., 2021). In *Eucalyptus obliqua* L'Hér., it has been shown that the stem contributes the highest share of overall NSC content, while a high level of seasonal variation in NSC content, particularly starch, was also observed in the stem (Smith et al., 2018). Thus, it can be hypothesized that trees could employ stricter NSC regulation mechanisms in their stems upon exposure to drought stress given the pivotal role of NSC for growth and stress response. During drought stress, plant

responses may involve starch breakdown and increase in the concentration of soluble sugars (Rodríguez-Calcerrada et al., 2011). For example, the concentration of soluble sugars in the stem has been shown to increase under drought stress conditions in *E. globulus* (Duan et al., 2013) and *E. urophylla* (Chen et al., 2020). Our results suggest substantial involvement of NSC metabolism during both drought stress and recovery. We identified potential key genes involved in starch degradation and galactose metabolism including beta-amylase 1, glycosyl hydrolase, galactinol synthase, raffinose synthase, and stachyose synthase genes, which were among the hub-DEGs in the drought related modules (Figure 6A, Table S4). These genes have been reported to contribute to improving dehydration tolerance in *Arabidopsis* (Taji et al., 2002; Nishizawa et al., 2008; Zanella et al., 2016; Li et al., 2020).

The importance of sugar metabolism during drought stress has been considered to continue during recovery from drought (Rodríguez-Calcerrada et al., 2011). The role of sugars during recovery may include ROS detoxification (Nishizawa et al., 2008), recovery of xylem hydraulic function (Savi et al., 2016; Tomasella et al., 2017; Secchi et al., 2021), primary and secondary metabolism (Wedeking et al., 2018), and compensatory growth (Guo et al., 2020). However, it has been shown that plants may prioritize sugar storage during recovery, which indicates 'stress memory' (Galiano et al., 2017). In line with this, we have identified putative genes involved in the biosynthesis of starch including ADP glucose pyrophosphorylase 1 (*ADG1*) and three unknown genes as hub-DEGs in the recovery related modules (Figure 6B, Table S4). Conforming to the role of sugars in primary and secondary metabolism, *G6PD1* which encodes a key enzyme in the oxidative pentose phosphate pathway was among the hub-DEGs in the recovery related modules (Figure 6B, Table S4). *G6PD1* was reported to be associated with dehydration tolerance (Liu et al., 2013; Long et al., 2016) but has not been implicated in recovery from drought. These results suggest a crucial role of soluble sugars

during recovery from drought, and merits further investigation on the regulation of specific NSCs involved in the different functions.

The known functions of some of the hub-DEGs identified in the recovery related modules were not directly related to stress response. This included four unknown genes putatively involved in stomatal complex morphogenesis and a putative bZIP TF gene, *FD-1* (Table S4), which is known to regulate flowering in *Arabidopsis* (Abe et al., 2005). Recent studies in *Arabidopsis* showed that this TF has a broader role in meristem maintenance (Gorham et al., 2018) and that it also regulates the expression of genes in phytohormone and sugar signaling pathways (Collani et al., 2019; Zhu et al., 2020). Thus, future study may investigate its role during recovery from drought. These results indicate that recovery from drought stress might involve regulation of different biological processes not necessarily related to stress response.

In conclusion, this study investigated the molecular mechanisms underlying the response to and recovery from drought stress (Figure 6A and B) in woody stem tissues of a tree species. Our results not only confirmed the role of known drought response mechanisms such as ABA and ROS signaling pathways in *E. grandis*, but also identified previously unreported or unknown potential key genes in these pathways, some of which have been associated with drought resistance in other species. By analyzing the transcriptomic changes underlying recovery from short-term drought stress for the first time in *Eucalyptus*, our results showed that recovery from drought stress includes reprogramming of signal transduction pathways and metabolic processes. We found transcriptomic evidence indicating alterations in the JA, SA, ET, and BR signaling pathways also during recovery from drought stress. Pertaining to previous studies that showed that plants face oxidative stress during recovery from drought stress, we have found, at the molecular level, that considerable detoxification mechanisms were in place. Sugar metabolism and soluble sugar signaling were also detected as molecular markers during drought stress and recovery from drought. Altogether, this suggests a central

role of these signaling pathways in the trade-off between stress response and growth in *Eucalyptus*, providing new targets for future genetic improvement.

Data and materials availability

The data sets supporting the results of this manuscript are available on the National Centre for Biotechnology Information (NCBI) repository with the BioProject accession number of PRJNA896601 (https://www.ncbi.nlm.nih.gov/sra/PRJNA896601).

Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

Conflict of interest

None declared.

Funding

This work was supported by the Technology Innovation Agency, Department of Science and Innovation of South Africa through the Forest Bioeconomy Innovation Cluster Program 1, Woldia University through the Woldia-UP PhD Scholarship Program, and the University of Pretoria Doctoral Research Bursary to D.T.T.

Authors' contributions

D.T.T. and S.N. conceived and designed the experiment; D.T.T. executed the experiment and collected the data; D.T.T. and R.P. analyzed the data; D.T.T. drafted the manuscript; G.E.Z., R.P., and S.N. contributed critically to the interpretation of results and drafts of the manuscript. All authors revised the final draft and gave final approval for publication.

References

Abe, M., Kobayashi, Y., Yamamoto, S., Daimon, Y., Yamaguchi, A., Ikeda, Y., et al. (2005). FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. *Science* 309(5737)**,** 1052-1056.

Abid, M., Tian, Z., Ata-Ul-Karim, S.T., Wang, F., Liu, Y., Zahoor, R., et al. (2016). Adaptation to and recovery from drought stress at vegetative stages in wheat (*Triticum aestivum*) cultivars. *Funct. Plant Biol.* 43(12)**,** 1159-1169.

Acosta, M.S., Mastrandrea, C., and Lima, J.T. (Year). "Wood technologies and uses of *Eucalyptus* wood from fast grown plantations for solid products", in: *Proceedings of the 51st International Convention of Society of Wood Science and Technology, Concepción, Chile*), 10-12.

Agarwal, P., Parida, S.K., Mahto, A., Das, S., Mathew, I.E., Malik, N., et al. (2014). Expanding frontiers in plant transcriptomics in aid of functional genomics and molecular breeding. *Biotechnol J* 9(12)**,** 1480-1492.

Balti, I., Benny, J., Perrone, A., Caruso, T., Abdallah, D., Salhi-Hannachi, A., et al. (2020). Identification of conserved genes linked to responses to abiotic stresses in leaves among different plant species. *Funct. Plant Biol.* 48(1)**,** 54-71.

Barrero, R.A., Umeda, M., Yamamura, S., and Uchimiya, H. (2002). *Arabidopsis* CAP regulates the actin cytoskeleton necessary for plant cell elongation and division. *The Plant Cell* 14(1)**,** 149-163.

Barros Junior, U.O., Lima, M.D., Alsahli, A.A., and Lobato, A.K. (2021). Unraveling the roles of brassinosteroids in alleviating drought stress in young *Eucalyptus urophylla* plants: implications on redox homeostasis and photosynthetic apparatus. *Physiol. Plant.* 172(2)**,** 748-761.

Beloiu, M., Stahlmann, R., and Beierkuhnlein, C. (2022). Drought impacts in forest canopy and deciduous tree saplings in Central European forests. *For Ecol Manag* 509**,** 120075.

Bhaskara, G.B., Nguyen, T.T., and Verslues, P.E. (2012). Unique drought resistance functions of the highly ABA-induced clade A protein phosphatase 2Cs. *Plant Physiol.* 160(1)**,** 379-395.

Blackman, C.J., Brodribb, T.J., and Jordan, G.J. (2009). Leaf hydraulics and drought stress: response, recovery and survivorship in four woody temperate plant species. *Plant, Cell Environ.* 32(11)**,** 1584-1595.

Bolger, A.M., Lohse, M., and Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30(15)**,** 2114-2120.

Brandt, B., Brodsky, D.E., Xue, S., Negi, J., Iba, K., Kangasjärvi, J., et al. (2012). Reconstitution of abscisic acid activation of SLAC1 anion channel by CPK6 and OST1 kinases and branched ABI1 PP2C phosphatase action. *Proc. Natl. Acad. Sci. USA* 109(26)**,** 10593-10598.

Breshears, D.D., Cobb, N.S., Rich, P.M., Price, K.P., Allen, C.D., Balice, R.G., et al. (2005). Regional vegetation die-off in response to global-change-type drought. *Proc. Natl. Acad. Sci. USA* 102(42)**,** 15144-15148.

Brockerhoff, E.G., Jactel, H., Parrotta, J.A., Quine, C.P., and Sayer, J. (2008). Plantation forests and biodiversity: oxymoron or opportunity? *Biodivers. Conserv.* 17(5)**,** 925-951.

Chen, D., Wang, S., Cao, B., Cao, D., Leng, G., Li, H., et al. (2016). Genotypic variation in growth and physiological response to drought stress and re-watering reveals the

critical role of recovery in drought adaptation in maize seedlings. *Front Plant Sci* 6**,** 1241.
Chen, X., Zhao, P., Ouyang, L., Zhu, L., Ni, G., and Schäfer, K.V. (2020). Whole-plant water hydraulic integrity to predict drought-induced *Eucalyptus urophylla* mortality under drought stress. *For. Ecol. Manage.* 468**,** 118179.
Collani, S., Neumann, M., Yant, L., and Schmid, M. (2019). FT modulates genome-wide DNA-binding of the bZIP transcription factor FD. *Plant Physiol.* 180(1)**,** 367-380.
Conesa, A., Madrigal, P., Tarazona, S., Gomez-Cabrero, D., Cervera, A., McPherson, A., et al. (2016). A survey of best practices for RNA-seq data analysis. *Genome Biol* 17(1)**,** 1-19.
Correia, B., Pintó-Marijuan, M., Castro, B.B., Brossa, R., López-Carbonell, M., and Pinto, G. (2014a). Hormonal dynamics during recovery from drought in two *Eucalyptus globulus* genotypes: from root to leaf. *Plant Physiol. Biochem.* 82**,** 151-160.
Correia, B., Pintó-Marijuan, M., Neves, L., Brossa, R., Dias, M.C., Costa, A., et al. (2014b). Water stress and recovery in the performance of two *Eucalyptus globulus* clones: physiological and biochemical profiles. *Physiol. Plant.* 150(4)**,** 580-592.
Dang, H., Lu, P., Yang, W., Han, H., and Zhang, J. (2019). Drought-induced reductions and limited recovery in the radial growth, transpiration, and canopy stomatal conductance of Mongolian Scots pine (*Pinus sylvestris* var. *mongolica* Litv): a five-year observation. *Forests* 10(12)**,** 1143.
de Moraes Goncalves, J.L., Alvares, C.A., Higa, A.R., Silva, L.D., Alfenas, A.C., Stahl, J., et al. (2013). Integrating genetic and silvicultural strategies to minimize abiotic and biotic constraints in Brazilian eucalypt plantations. *For Ecol Manag* 301**,** 6-27.
Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., et al. (2013). STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 29(1)**,** 15-21.
du Toit, B., Malherbe, G.F., Kunneke, A., Seifert, T., and Wessels, C.B. (2017). Survival and long-term growth of eucalypts on semi-arid sites in a Mediterranean climate, South Africa. *Southern For* 79(3)**,** 235-249.
Duan, H., Amthor, J.S., Duursma, R.A., O'Grady, A.P., Choat, B., and Tissue, D.T. (2013). Carbon dynamics of eucalypt seedlings exposed to progressive drought in elevated [CO2] and elevated temperature. *Tree Physiology* 33(8)**,** 779-792.
Efisue, A., Tongoona, P., Derera, J., and Ubi, B. (2009). Screening early-generation progenies of interspecific rice genotypes for drought-stress tolerance during vegetative phase. *J Crop Improv* 23(2)**,** 174-193.
Eksteen, A.B., Grzeskowiak, V., Jones, N.B., and Pammenter, N.W. (2013). Stomatal characteristics of *Eucalyptus grandis* clonal hybrids in response to water stress. *Southern For* 75(3)**,** 105-111.
Fang, Y., and Xiong, L. (2015). General mechanisms of drought response and their application in drought resistance improvement in plants. *Cell. Mol. Life Sci.* 72(4)**,** 673-689.
Fox, H., Doron-Faigenboim, A., Kelly, G., Bourstein, R., Attia, Z., Zhou, J., et al. (2018). Transcriptome analysis of *Pinus halepensis* under drought stress and during recovery. *Tree Physiol* 38(3)**,** 423-441.
Frosi, G., Harand, W., Oliveira, M.T.d., Pereira, S., Cabral, S.P., Montenegro, A.A.d.A., et al. (2017). Different physiological responses under drought stress result in different recovery abilities of two tropical woody evergreen species. *Acta Bot Brasilica* 31**,** 153-160.
Fujita, Y., Nakashima, K., Yoshida, T., Katagiri, T., Kidokoro, S., Kanamori, N., et al. (2009). Three SnRK2 protein kinases are the main positive regulators of abscisic acid

signaling in response to water stress in *Arabidopsis*. *Plant Cell Physiol.* 50(12)**,** 2123-2132.
Galiano, L., Timofeeva, G., Saurer, M., Siegwolf, R., Martínez-Vilalta, J., Hommel, R., et al. (2017). The fate of recently fixed carbon after drought release: towards unravelling C storage regulation in *Tilia platyphyllos* and *Pinus sylvestris*. *Plant, Cell Environ.* 40(9)**,** 1711-1724.
Garapati, P., Feil, R., Lunn, J.E., Van Dijck, P., Balazadeh, S., and Mueller-Roeber, B. (2015). Transcription factor *Arabidopsis* activating factor1 integrates carbon starvation responses with trehalose metabolism. *Plant Physiol.* 169(1)**,** 379-390.
Gauthey, A., Peters, J.M., Lòpez, R., Carins-Murphy, M.R., Rodriguez-Dominguez, C.M., Tissue, D.T., et al. (2022). Mechanisms of xylem hydraulic recovery after drought in *Eucalyptus saligna*. *Plant, Cell Environ.* 45(4)**,** 1216-1228.
Gessler, A., Bottero, A., Marshall, J., and Arend, M. (2020). The way back: recovery of trees from drought and its implication for acclimation. *New Phytol.* 228(6)**,** 1704-1709.
Gorham, S.R., Weiner, A.I., Yamadi, M., and Krogan, N.T. (2018). HISTONE DEACETYLASE 19 and the flowering time gene FD maintain reproductive meristem identity in an age-dependent manner. *J. Exp. Bot.* 69(20)**,** 4757-4771.
Grant, E.H., Fujino, T., Beers, E.P., and Brunner, A.M. (2010). Characterization of NAC domain transcription factors implicated in control of vascular cell differentiation in *Arabidopsis* and *Populus*. *Planta* 232(2)**,** 337-352.
Guo, H., Li, L., Aluru, M., Aluru, S., and Yin, Y. (2013). Mechanisms and networks for brassinosteroid regulated gene expression. *Curr. Opin. Plant Biol.* 16(5)**,** 545-553.
Guo, T., Tian, C., Chen, C., Duan, Z., Zhu, Q., and Sun, L.Z. (2020). Growth and carbohydrate dynamic of perennial ryegrass seedlings during PEG-simulated drought and subsequent recovery. *Plant Physiol. Biochem.* 154**,** 85-93.
Hayano-Kanashiro, C., Calderón-Vázquez, C., Ibarra-Laclette, E., Herrera-Estrella, L., and Simpson, J. (2009). Analysis of gene expression and physiological responses in three Mexican maize landraces under drought stress and recovery irrigation. *PLoS one* 4(10)**,** e7531.
Higashi, K., Ishiga, Y., Inagaki, Y., Toyoda, K., Shiraishi, T., and Ichinose, Y. (2008). Modulation of defense signal transduction by flagellin-induced WRKY41 transcription factor in *Arabidopsis thaliana*. *Mol. Genet. Genomics* 279(3)**,** 303-312.
Hodecker, B.E.R., Pita-Barbosa, A., de Barros, N.F., and Merchant, A. (2018). Water availability preceding long-term drought defines the tolerance of *Eucalyptus* to water restriction. *New forests* 49(2)**,** 173-195.
IPCC (2007). "Summary for policymakers," in *Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change,* ed. M. Parry, Canziani O, Palutikof J, Van der Linden P, Hanson C. (Cambridge, UK: Cambridge University Press), 976pp.
IPCC (2021). Summary for Policymakers. In: Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S. L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M. I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T. K. Maycock, T. Waterfield, O. Yelekçi, R. Yu and B. Zhou (eds.)].
Jin, J., Tian, F., Yang, D.-C., Meng, Y.-Q., Kong, L., Luo, J., et al. (2016). PlantTFDB 4.0: toward a central hub for transcription factors and regulatory interactions in plants. *Nucleic Acids Res.***,** gkw982.

Johnson, D.M., McCulloh, K.A., Woodruff, D.R., and Meinzer, F.C. (2012). Hydraulic safety margins and embolism reversal in stems and leaves: why are conifers and angiosperms so different? *Plant Sci.* 195**,** 48-53.

Kamoshita, A., Rodriguez, R., Yamauchi, A., and Wade, L. (2004). Genotypic variation in response of rainfed lowland rice to prolonged drought and rewatering. *Plant Prod Sci* 7(4)**,** 406-420.

Kaur, H., Manna, M., Thakur, T., Gautam, V., and Salvi, P. (2021). Imperative role of sugar signaling and transport during drought stress responses in plants. *Physiol. Plant.* 171(4)**,** 833-848.

Langfelder, P., and Horvath, S. (2008). WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics* 9(1)**,** 1-13.

Lemcoff, J.H., Guarnaschelli, A.B., Garau, A.M., Bascialli, M.E., and Ghersa, C.M. (1994). Osmotic adjustment and its use as a selection criterion in *Eucalyptus* seedlings. *Can J For Res* 24(12)**,** 2404-2408.

Lévesque, M., Rigling, A., Bugmann, H., Weber, P., and Brang, P. (2014). Growth response of five co-occurring conifers to drought across a wide climatic gradient in Central Europe. *Agric For Meteorol* 197**,** 1-12.

Li, T., Zhang, Y., Liu, Y., Li, X., Hao, G., Han, Q., et al. (2020). Raffinose synthase enhances drought tolerance through raffinose synthesis or galactinol hydrolysis in maize and *Arabidopsis* plants. *J. Biol. Chem.* 295(23)**,** 8064-8077.

Liu, J., Wang, X., Hu, Y., Hu, W., and Bi, Y. (2013). Glucose-6-phosphate dehydrogenase plays a pivotal role in tolerance to drought stress in soybean roots. *Plant Cell Rep.* 32(3)**,** 415-429.

Long, X., He, B., Fang, Y., and Tang, C. (2016). Identification and characterization of the glucose-6-phosphate dehydrogenase gene family in the para rubber tree, *Hevea brasiliensis*. *Front Plant Sci* 7**,** 215.

Lopato, S., Waigmann, E., and Barta, A. (1996). Characterization of a novel arginine/serine-rich splicing factor in *Arabidopsis*. *The plant cell* 8(12)**,** 2255-2264.

Love, M.I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 15(12)**,** 1-21.

Lowe, R., Shirley, N., Bleackley, M., Dolan, S., and Shafee, T. (2017). Transcriptomics technologies. *PLoS Comp. Biol.* 13(5)**,** e1005457.

Mafakheri, A., Siosemardeh, A., Bahramnejad, B., Struik, P., and Sohrabi, Y. (2011). Effect of drought stress and subsequent recovery on protein, carbohydrate contents, catalase and peroxidase activities in three chickpea (*Cicer arietinum*) cultivars. *Aust J Crop Sci* 5(10)**,** 1255-1260.

Mangwanda, R., Myburg, A.A., and Naidoo, S. (2015). Transcriptome and hormone profiling reveals *Eucalyptus grandis* defence responses against *Chrysoporthe austroafricana*. *BMC Genomics* 16(1)**,** 1-13.

Medrano, H., Escalona, J.M., Bota, J., Gulías, J., and Flexas, J. (2002). Regulation of photosynthesis of C3 plants in response to progressive drought: stomatal conductance as a reference parameter. *Ann. Bot.* 89(7)**,** 895-905.

Mitchell, P.J., O'Grady, A.P., Tissue, D.T., White, D.A., Ottenschlaeger, M.L., and Pinkard, E.A. (2013). Drought response strategies define the relative contributions of hydraulic dysfunction and carbohydrate depletion during tree mortality. *New Phytol.* 197(3)**,** 862-872.

Miyashita, K., Tanakamaru, S., Maitani, T., and Kimura, K. (2005). Recovery responses of photosynthesis, transpiration, and stomatal conductance in kidney bean following drought stress. *Environ. Exp. Bot.* 53(2)**,** 205-214.

Montwé, D., Spiecker, H., and Hamann, A. (2014). An experimentally controlled extreme drought in a Norway spruce forest reveals fast hydraulic response and subsequent recovery of growth rates. *Trees* 28(3)**,** 891-900.
Moran, E., Lauder, J., Musser, C., Stathos, A., and Shu, M. (2017). The genetics of drought tolerance in conifers. *New Phytol.* 216(4)**,** 1034-1048.
Munne-Bosch, S., and Penuelas, J. (2003). Photo-and antioxidative protection, and a role for salicylic acid during drought and recovery in field-grown *Phillyrea angustifolia* plants. *Planta* 217(5)**,** 758-766.
Myburg, A.A., Grattapaglia, D., Tuskan, G.A., Hellsten, U., Hayes, R.D., Grimwood, J., et al. (2014). The genome of *Eucalyptus grandis*. *Nature* 510(7505)**,** 356-362.
Nakashima, K., Takasaki, H., Mizoi, J., Shinozaki, K., and Yamaguchi-Shinozaki, K. (2012). NAC transcription factors in plant abiotic stress responses. *Biochim Biophys Acta Gene Regul Mech* 1819(2)**,** 97-103.
Nguyen, H.C., Cao, P.B., San Clemente, H., Ployet, R., Mounet, F., Ladouce, N., et al. (2017). Special trends in CBF and DREB2 groups in *Eucalyptus gunnii* vs *Eucalyptus grandis* suggest that CBF are master players in the trade-off between growth and stress resistance. *Physiol. Plant.* 159(4)**,** 445-467.
Nishizawa, A., Yabuta, Y., and Shigeoka, S. (2008). Galactinol and raffinose constitute a novel function to protect plants from oxidative damage. *Plant Physiol.* 147(3)**,** 1251-1263.
Nishizawa, A., Yabuta, Y., Yoshida, E., Maruta, T., Yoshimura, K., and Shigeoka, S. (2006). *Arabidopsis* heat shock transcription factor A2 as a key regulator in response to several types of environmental stress. *Plant J.* 48(4)**,** 535-547.
Noctor, G., Mhamdi, A., and Foyer, C.H. (2014). The roles of reactive oxygen metabolism in drought: not so cut and dried. *Plant Physiol.* 164(4)**,** 1636-1648.
Nóia Júnior, R.d.S., Amaral, G.C., Pezzopane, J.E.M., Fonseca, M.D.S., Câmara da Silva, A.P., and Xavier, T.M.T. (2020). Ecophysiological acclimatization to cyclic water stress in *Eucalyptus*. *J For Res* 31(3)**,** 797-806.
Ono, M., Isono, K., Sakata, Y., and Taji, T. (2021). CATALASE2 plays a crucial role in long-term heat tolerance of *Arabidopsis thaliana*. *Biochem. Biophys. Res. Commun.* 534**,** 747-751.
Ortbauer, M. (2013). "Abiotic stress adaptation: protein folding stability and dynamics," in *Abiotic Stress -Plant Responses and Applications in Agriculture,* eds. K. Vahdati & C. Leslie. (Rijeka: InTech), 3-25.
Pertea, M., Pertea, G.M., Antonescu, C.M., Chang, T.-C., Mendell, J.T., and Salzberg, S.L. (2015). StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. *Nat. Biotechnol.* 33(3)**,** 290-295.
Pervaiz, T., Liu, S.-W., Uddin, S., Amjid, M.W., Niu, S.-H., and Wu, H.X. (2021). The transcriptional landscape and hub genes associated with physiological responses to drought stress in *Pinus tabuliformis*. *Int J Mol Sci* 22(17)**,** 9604.
Phan, H.A., Iacuone, S., Li, S.F., and Parish, R.W. (2011). The MYB80 transcription factor is required for pollen development and the regulation of tapetal programmed cell death in *Arabidopsis thaliana*. *The Plant Cell* 23(6)**,** 2209-2224.
Ployet, R., Veneziano Labate, M.T., Regiani Cataldi, T., Christina, M., Morel, M., San Clemente, H., et al. (2019). A systems biology view of wood formation in *Eucalyptus grandis* trees submitted to different potassium and water regimes. *New Phytol.* 223(2)**,** 766-782.
Reijnders, M.J., and Waterhouse, R.M. (2021). Summary visualizations of gene ontology terms with GO-Figure! *Front Bioinform* 1**,** 638255.

Rodríguez-Calcerrada, J., Shahin, O., del Rey, M.d.C., and Rambal, S. (2011). Opposite changes in leaf dark respiration and soluble sugars with drought in two Mediterranean oaks. *Funct. Plant Biol.* 38(12)**,** 1004-1015.

Savi, T., Casolo, V., Luglio, J., Bertuzzi, S., Gullo, M.A.L., and Nardini, A. (2016). Species-specific reversal of stem xylem embolism after a prolonged drought correlates to endpoint concentration of soluble sugars. *Plant Physiol. Biochem.* 106**,** 198-207.

Secchi, F., Pagliarani, C., Cavalletto, S., Petruzzellis, F., Tonel, G., Savi, T., et al. (2021). Chemical inhibition of xylem cellular activity impedes the removal of drought-induced embolisms in poplar stems–new insights from micro-CT analysis. *New Phytol.* 229(2)**,** 820-830.

Serrazina, S., Dias, F.V., and Malhó, R. (2014). Characterization of FAB 1 phosphatidylinositol kinases in *Arabidopsis* pollen tube growth and fertilization. *New Phytol.* 203(3)**,** 784-793.

Smith, M.G., Miller, R.E., Arndt, S.K., Kasel, S., and Bennett, L.T. (2018). Whole-tree distribution and temporal variation of non-structural carbohydrates in broadleaf evergreen trees. *Tree Physiology* 38(4)**,** 570-581.

Spokevicius, A.V., Tibbits, J., Rigault, P., Nolin, M.-A., Müller, C., and Merchant, A. (2017). Medium term water deficit elicits distinct transcriptome responses in *Eucalyptus* species of contrasting environmental origin. *BMC Genomics* 18(1)**,** 1-17.

Taji, T., Ohsumi, C., Iuchi, S., Seki, M., Kasuga, M., Kobayashi, M., et al. (2002). Important roles of drought-and cold-inducible genes for galactinol synthase in stress tolerance in *Arabidopsis thaliana*. *Plant J.* 29(4)**,** 417-426.

Tao, H., Miao, H., Chen, L., Wang, M., Xia, C., Zeng, W., et al. (2022). WRKY33-mediated indolic glucosinolate metabolic pathway confers resistance against *Alternaria brassicicola* in *Arabidopsis* and *Brassica* crops. *J Integr Plant Biol* 64(5)**,** 1007-1019.

Thumma, B.R., Sharma, N., and Southerton, S.G. (2012). Transcriptome sequencing of *Eucalyptus camaldulensis* seedlings subjected to water stress reveals functional single nucleotide polymorphisms and genes under selection. *BMC Genomics* 13(1)**,** 1-21.

Tomasella, M., Häberle, K.-H., Nardini, A., Hesse, B., Machlet, A., and Matyssek, R. (2017). Post-drought hydraulic recovery is accompanied by non-structural carbohydrate depletion in the stem wood of Norway spruce saplings. *Sci Rep* 7(1)**,** 1-13.

Toreti, A., Belward, A., Perez-Dominguez, I., Naumann, G., Luterbacher, J., Cronie, O., et al. (2019). The exceptional 2018 European water seesaw calls for action on adaptation. *Earth's Future* 7(6)**,** 652-663.

Tupinambá-Simões, F., Bravo, F., Guerra-Hernández, J., and Pascual, A. (2022). Assessment of drought effects on survival and growth dynamics in eucalypt commercial forestry using remote sensing photogrammetry. A showcase in Mato Grosso, Brazil. *For Ecol Manag* 505**,** 119930.

Ulloa, J.L., Aguayo, P., Conejera, D., Rubilar, R., Balocchi, C., and Valenzuela, S. (2022). Transcriptomic response in foliar and root tissues of a drought-tolerant *Eucalyptus globulus* genotype under drought stress. *Trees* 36(2)**,** 697-709.

Vassileva, V., Signarbieux, C., Anders, I., and Feller, U. (2011). Genotypic variation in drought stress response and subsequent recovery of wheat (*Triticum aestivum* L.). *J. Plant Res.* 124(1)**,** 147-154.

Viera, M., Ruíz Fernández, F., and Rodríguez-Soalleiro, R. (2016). Nutritional prescriptions for *Eucalyptus* plantations: lessons learned from Spain. *Forests* 7(4)**,** 84.

Villar, E., Klopp, C., Noirot, C., Novaes, E., Kirst, M., Plomion, C., et al. (2011). RNA-Seq reveals genotype-specific molecular responses to water deficit in *Eucalyptus*. *BMC Genomics* 12(1)**,** 1-18.

Volaire, F. (2018). A unified framework of plant adaptive strategies to drought: crossing scales and disciplines. *Global Change Biol.* 24(7)**,** 2929-2938.
Vonapartis, E., Mohamed, D., Li, J., Pan, W., Wu, J., and Gazzarrini, S. (2022). CBF4/DREB1D represses XERICO to attenuate ABA, osmotic and drought stress responses in *Arabidopsis*. *Plant J.* 110(4)**,** 961-977. doi: https://doi.org/10.1111/tpj.15713.
Wakao, S., and Benning, C. (2005). Genome-wide analysis of glucose-6-phosphate dehydrogenases in *Arabidopsis*. *Plant J.* 41(2)**,** 243-256.
Wang, X., Li, N., Li, W., Gao, X., Cha, M., Qin, L., et al. (2020). Advances in Transcriptomics in the Response to Stress in Plants. *Glob Med Genet* 7(02)**,** 030-034.
Wedeking, R., Maucourt, M., Deborde, C., Moing, A., Gibon, Y., Goldbach, H.E., et al. (2018). 1H-NMR metabolomic profiling reveals a distinct metabolic recovery response in shoots and roots of temporarily drought-stressed sugar beets. *PloS one* 13(5)**,** e0196102.
Whitley, P., Hinz, S., and Doughty, J. (2009). *Arabidopsis* FAB1/PIKfyve proteins are essential for development of viable pollen. *Plant Physiol.* 151(4)**,** 1812-1822.
Williams, A.P., Cook, B.I., and Smerdon, J.E. (2022). Rapid intensification of the emerging southwestern North American megadrought in 2020–2021. *Nat Clim Chang***,** 1-3.
Yang, Y., Li, H.-G., Wang, J., Wang, H.-L., He, F., Su, Y., et al. (2020). ABF3 enhances drought tolerance via promoting ABA-induced stomatal closure by directly regulating ADF5 in *Populus euphratica*. *J. Exp. Bot.* 71(22)**,** 7270-7285.
Yao, G.Q., Li, F.P., Nie, Z.F., Bi, M.H., Jiang, H., Liu, X.D., et al. (2021). Ethylene, not ABA, is closely linked to the recovery of gas exchange after drought in four Caragana species. *Plant, Cell Environ.* 44(2)**,** 399-411.
Yoshida, T., Fujita, Y., Sayama, H., Kidokoro, S., Maruyama, K., Mizoi, J., et al. (2010). AREB1, AREB2, and ABF3 are master transcription factors that cooperatively regulate ABRE-dependent ABA signaling involved in drought stress tolerance and require ABA for full activation. *Plant J.* 61(4)**,** 672-685.
Yoshida, T., Mogami, J., and Yamaguchi-Shinozaki, K. (2014). ABA-dependent and ABA-independent signaling in response to osmotic stress in plants. *Curr. Opin. Plant Biol.* 21**,** 133-139.
Young, M.D., Wakefield, M.J., Smyth, G.K., and Oshlack, A. (2010). Gene ontology analysis for RNA-seq: accounting for selection bias. *Genome Biol* 11(2)**,** 1-12.
Yu, D., Janz, D., Zienkiewicz, K., Herrfurth, C., Feussner, I., Chen, S., et al. (2021). Wood formation under severe drought invokes adjustment of the hormonal and transcriptional landscape in Poplar. *International journal of molecular sciences* 22(18)**,** 9899.
Yuan, T., Fujioka, S., Takatsuto, S., Matsumoto, S., Gou, X., He, K., et al. (2007). BEN1, a gene encoding a dihydroflavonol 4-reductase (DFR)-like protein, regulates the levels of brassinosteroids in *Arabidopsis thaliana*. *Plant J.* 51(2)**,** 220-233.
Zanella, M., Borghi, G.L., Pirone, C., Thalmann, M., Pazmino, D., Costa, A., et al. (2016). β-amylase 1 (BAM1) degrades transitory starch to sustain proline biosynthesis during drought stress. *J. Exp. Bot.* 67(6)**,** 1819-1826.
Zhao, P.X., Zhang, J., Chen, S.Y., Wu, J., Xia, J.Q., Sun, L.Q., et al. (2021). *Arabidopsis* MADS-box factor AGL16 is a negative regulator of plant response to salt stress by downregulating salt-responsive genes. *New Phytol.* 232(6)**,** 2418-2439.
Zhong, R., Lee, C., Haghighat, M., and Ye, Z.-H. (2021). Xylem vessel-specific SND5 and its homologs regulate secondary wall biosynthesis through activating secondary wall NAC binding elements. *New Phytol.* 231(4)**,** 1496-1509.

Zhu, Y., Klasfeld, S., Jeong, C.W., Jin, R., Goto, K., Yamaguchi, N., et al. (2020). TERMINAL FLOWER 1-FD complex target genes and competition with FLOWERING LOCUS T. *Nat Commun* 11(1), 1-12.

Figures

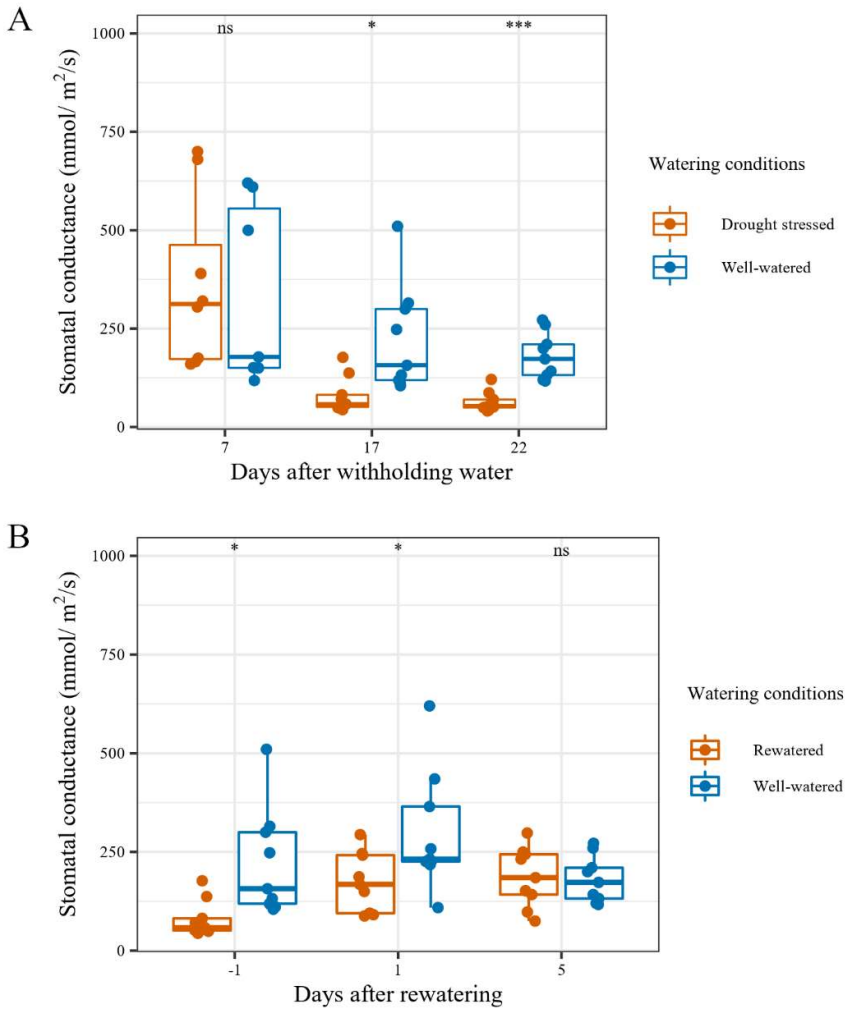

Figure 1 Leaf stomatal conductance during drought stress and recovery in *E. grandis*. (A) Stomatal conductance measured during drought stress. (B) Stomatal conductance measured during recovery from drought stress. Negative and positive numbers in the *x*-axis in B indicate measurements before and after rewatering, respectively. Pairwise *t*-test, ns = non-significant at $P < 0.05$, * = $P < 0.05$, *** = $P < 0.001$.

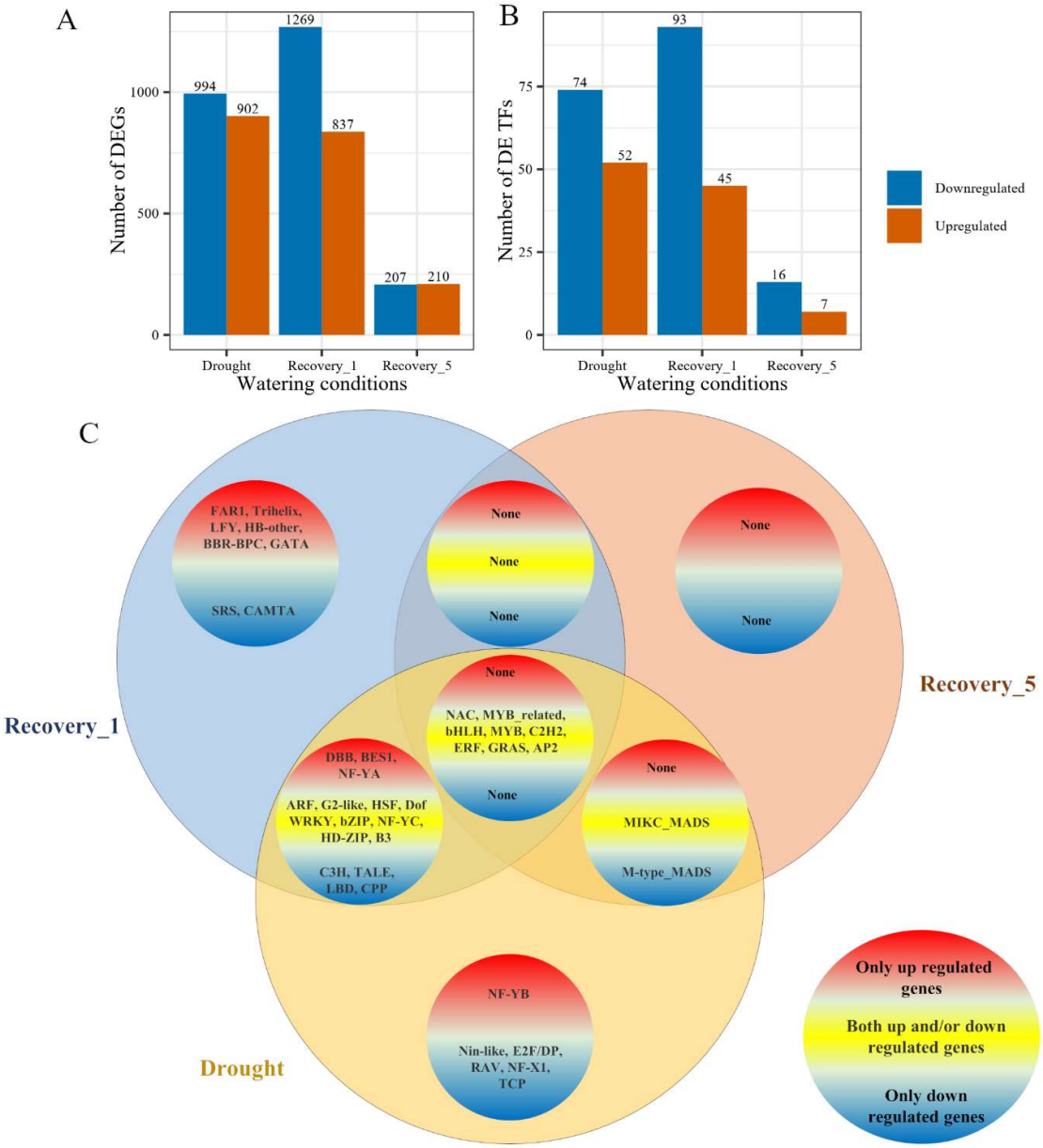

Figure 2 Differentially expressed genes during drought stress and recovery in *E. grandis*. (A) All differentially expressed genes. (B) Differentially expressed transcription factor (TF) genes. (B) Representation of TF families in different experimental treatments and time points. Differential expression analysis was conducted using DESeq2 R package using a Log2 fold change cut-off point of 0.5 and an adjusted *P*-value <0.05.

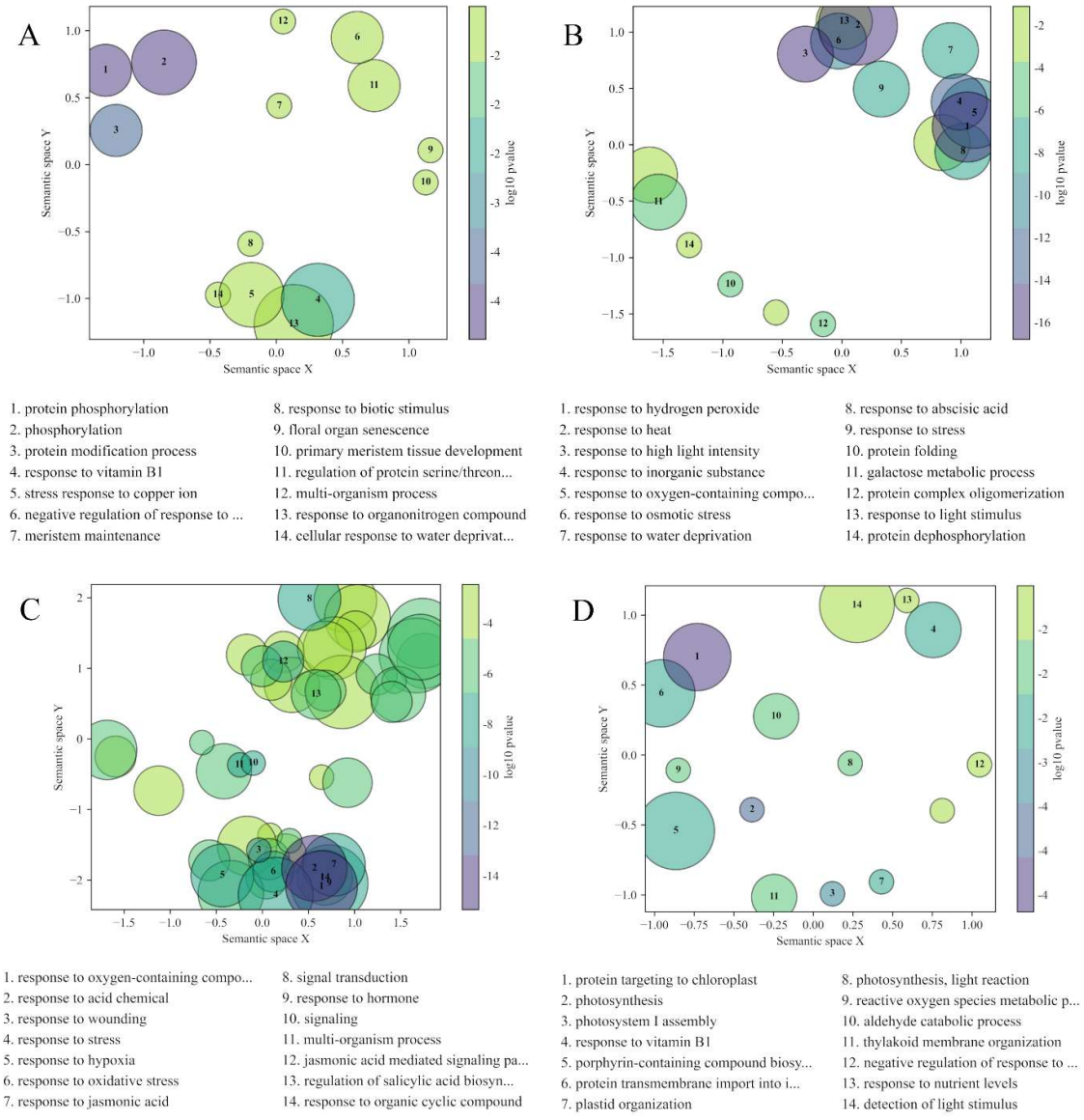

Figure 3 Biological process Gene Ontology (GO) enrichment for differentially expressed genes during drought stress and recovery in *E. grandis*. (A) Downregulated during drought. (B) Upregulated during drought. (C) Downregulated during recovery at 1 day of rewatering. (D) Upregulated during recovery at 1 day of rewatering. The GO enrichment analysis was conducted using the R package GO-seq with a Benjamini–Hochberg adjusted *P*-value <0.1. The enriched GO terms were visualized using GO-Figure! The size of bubbles shows the

number of significantly enriched GO terms. The details of enriched GO terms are given in Table S2.

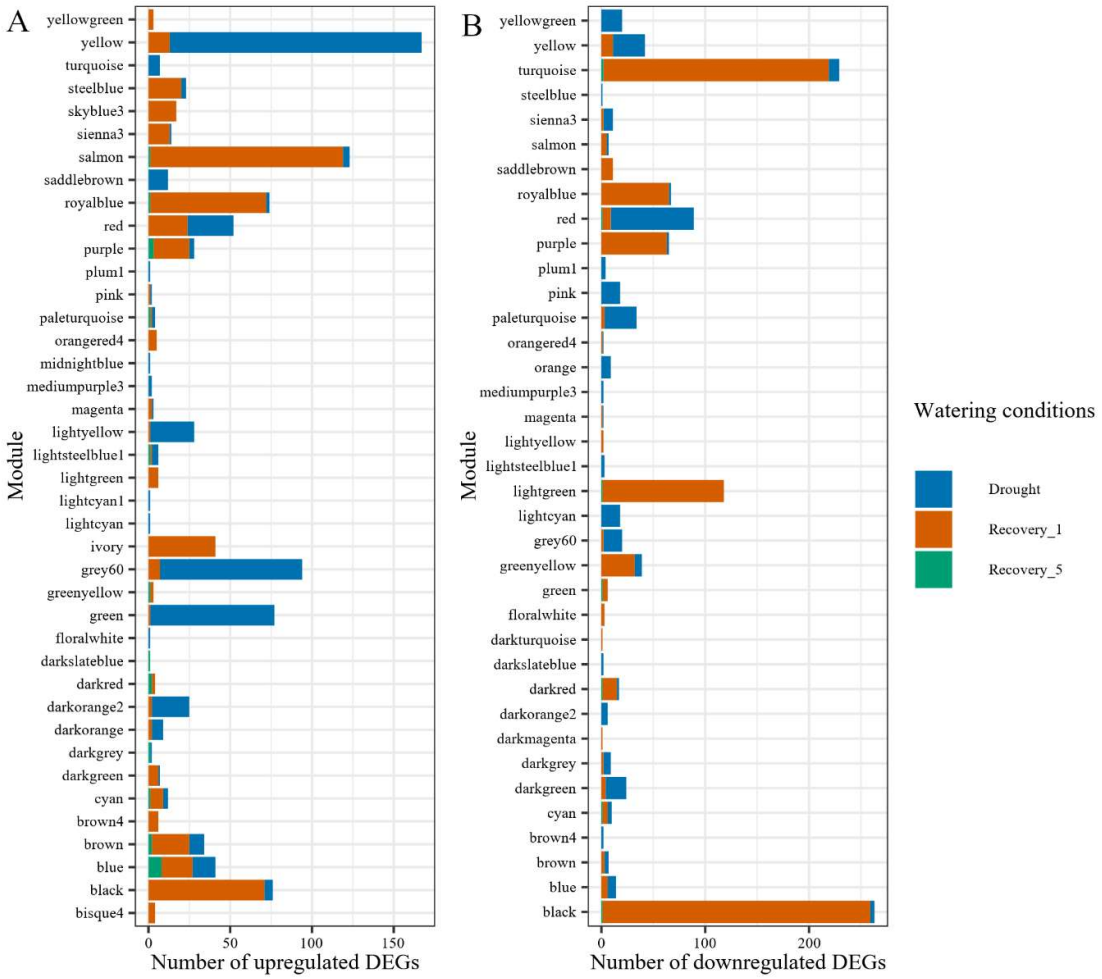

Figure 4 Differentially expressed genes in the co-expression network. (A) Upregulated DEGs in the network. (B) Downregulated DEGs in the network. Differential expression analysis was conducted using DESeq2 R package using a Log2 fold change cut-off point of 0.5 and an adjusted *P*-value <0.05.

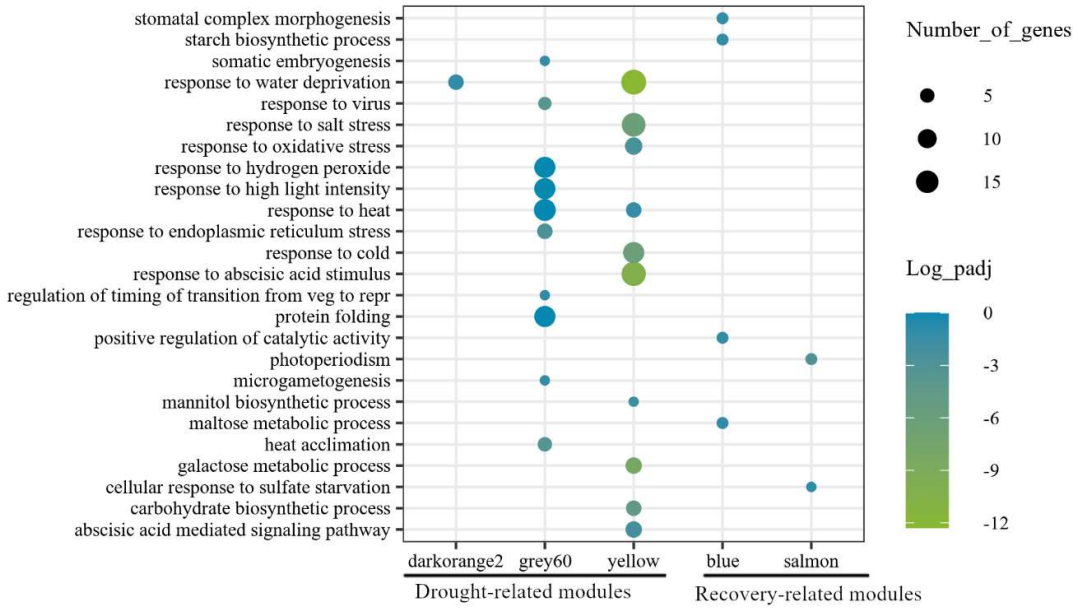

Figure 5 Biological process Gene Ontology (GO) enrichment for differentially expressed hub genes in the selected co-expression modules. The GO enrichment analysis was conducted using the R package GO-seq with a Benjamini-Hochberg adjusted *P*-value <0.1. The enriched GO terms were visualized using dot plot in R. The size of dots represent the number of differentially expressed hub genes in the enriched GO term.

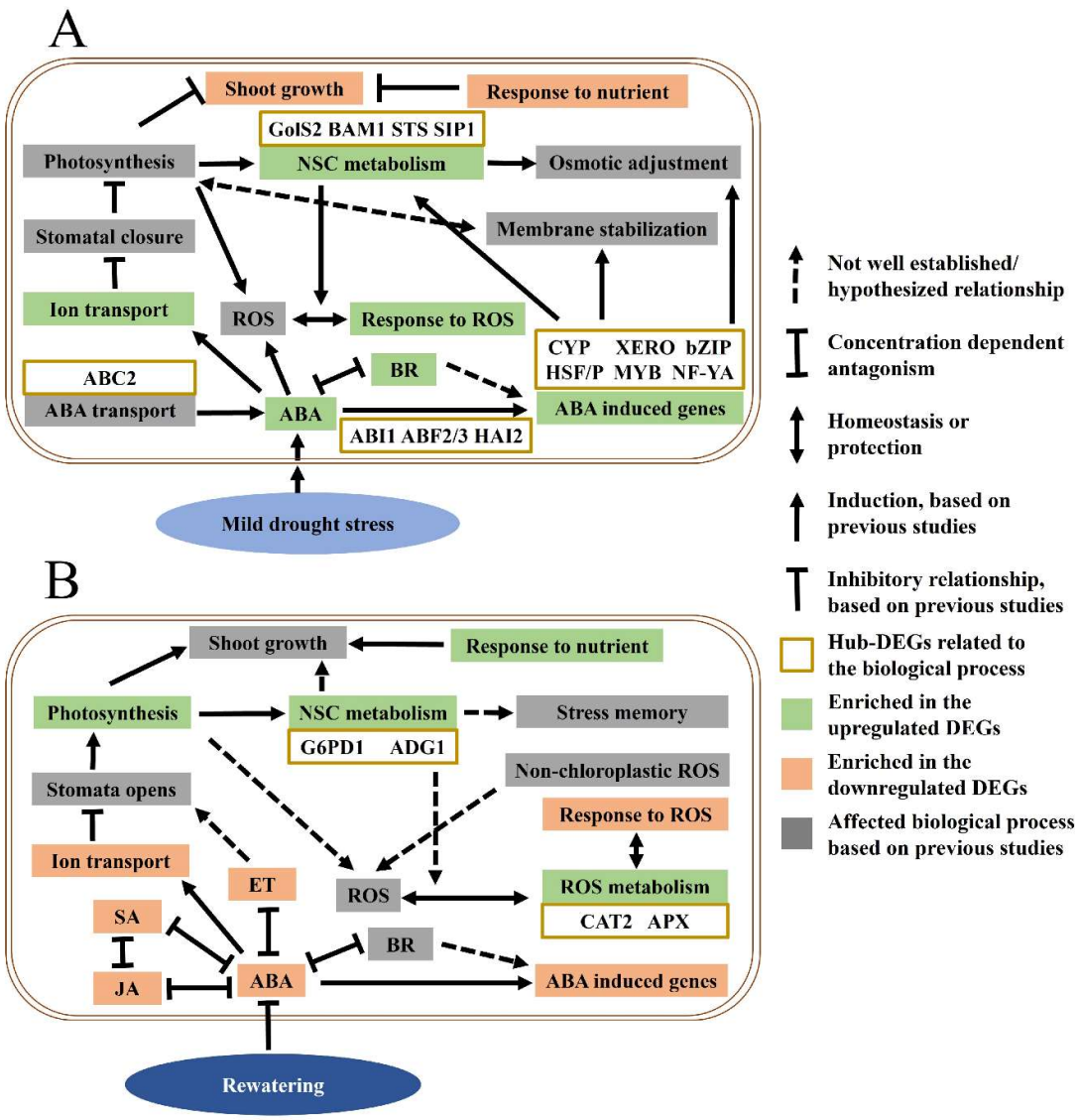

Figure 6 A simplified model for the transcriptomic responses to drought stress and rewatering in *E. grandis*. (A) During drought stress, ABA signaling pathway was activated which causes stomatal closure, among other changes. This might affect photosynthesis and contribute to reduction of shoot growth. Abscisic acid also induces the expression of drought stress responsive genes such as heat shock proteins and dehydrins. Drought increases the accumulation of ROS, which are scavenged by enzymatic and non-enzymatic antioxidants. Soluble sugars, dehydrins and others may also promote accumulation of solutes for osmotic

adjustment. (B) During recovery, genes related to ROS and ABA signaling were mostly downregulated. As a result, stomata opens and photosynthesis and shoot growth increase. Genes related to non-structural carbohydrate metabolism, catalase and ascorbate peroxidase were upregulated. This might be used in the detoxification of ROS resulting from increased respiration and photosynthesis as opposed to photorespiration, which is one of the main sources of ROS during the drought period. Carbohydrates may be used for recovery of hydraulic conductance, shoot growth, or may be stored in the form of starch indicating short-term stress memory. Abbreviations are found in the text.

Supplementary figures

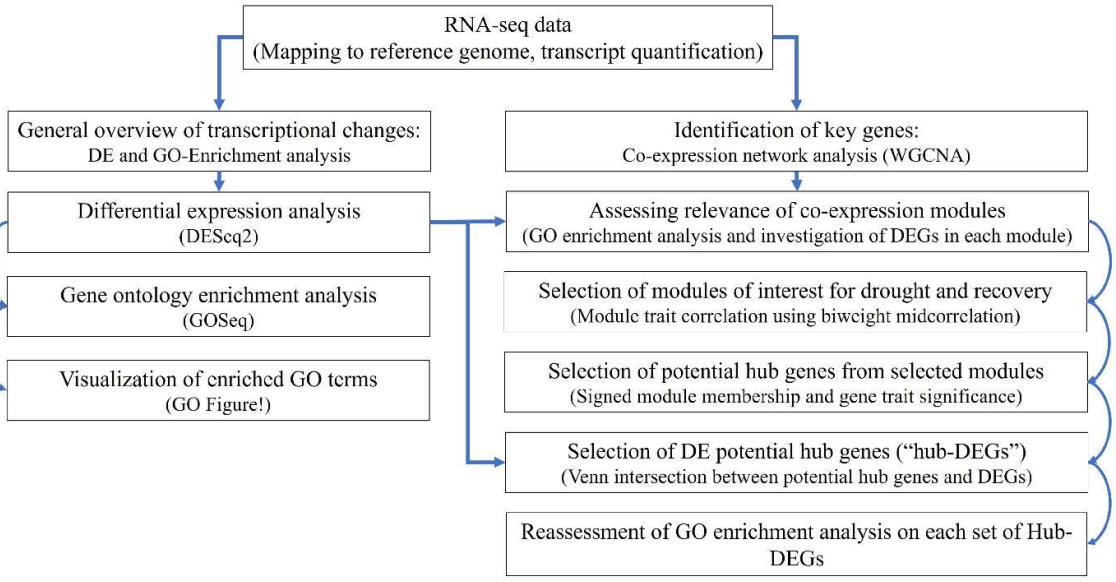

Figure S1. A flow diagram showing bioinformatic analysis of transcriptome data

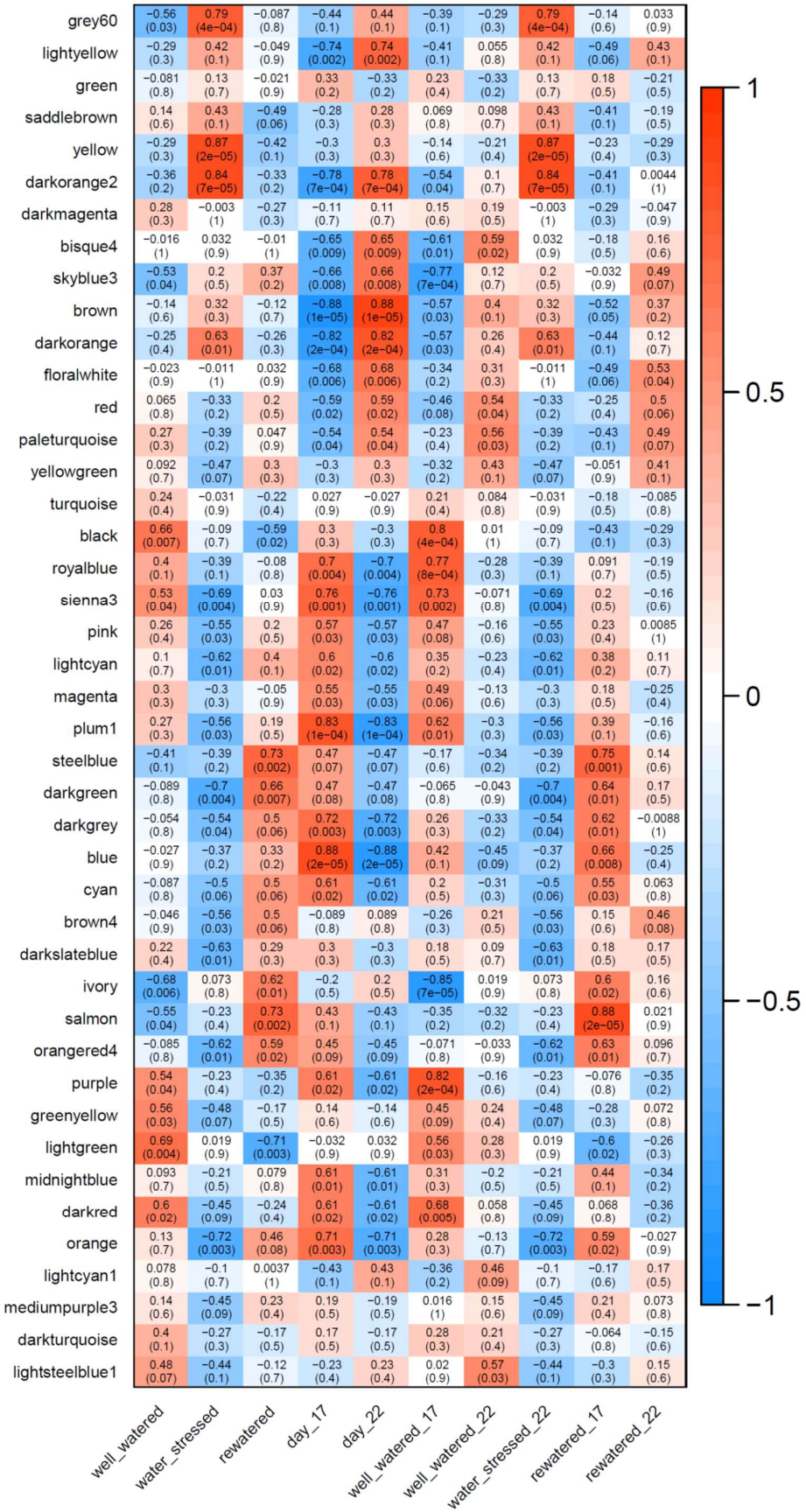
grey60 −0.56 (0.03) 0.79 (4e−04) −0.087 (0.8) −0.44 (0.1) 0.44 (0.1) −0.39 (0.1) −0.29 (0.3) 0.79 (4e−04) −0.14 (0.6) 0.033 (0.9)
lightyellow −0.29 (0.3) 0.42 (0.1) −0.049 (0.9) −0.74 (0.002) 0.74 (0.002) −0.41 (0.1) 0.055 (0.8) 0.42 (0.1) −0.49 (0.06) 0.43 (0.1)
green −0.081 (0.8) 0.13 (0.7) −0.021 (0.9) 0.33 (0.2) −0.33 (0.2) 0.23 (0.4) −0.33 (0.2) 0.13 (0.7) 0.18 (0.5) −0.21 (0.5)
saddlebrown 0.14 (0.6) 0.43 (0.1) −0.49 (0.06) −0.28 (0.3) 0.28 (0.3) 0.069 (0.8) 0.098 (0.7) 0.43 (0.1) −0.41 (0.1) −0.19 (0.5)
yellow −0.29 (0.3) 0.87 (2e−05) −0.42 (0.1) −0.3 (0.3) 0.3 (0.3) −0.14 (0.6) −0.21 (0.4) 0.87 (2e−05) −0.23 (0.4) −0.29 (0.3)
darkorange2 −0.36 (0.2) 0.84 (7e−05) −0.33 (0.2) −0.78 (7e−04) 0.78 (7e−04) −0.54 (0.04) 0.1 (0.7) 0.84 (7e−05) −0.41 (0.1) 0.0044 (1)
darkmagenta 0.28 (0.3) −0.003 (1) −0.27 (0.3) −0.11 (0.7) 0.11 (0.7) 0.15 (0.6) 0.19 (0.5) −0.003 (1) −0.29 (0.3) −0.047 (0.9)
bisque4 −0.016 (1) 0.032 (0.9) −0.01 (1) −0.65 (0.009) 0.65 (0.009) −0.61 (0.01) 0.59 (0.02) 0.032 (0.9) −0.18 (0.5) 0.16 (0.6)
skyblue3 −0.53 (0.04) 0.2 (0.5) 0.37 (0.2) −0.66 (0.008) 0.66 (0.008) −0.77 (7e−04) 0.12 (0.7) 0.2 (0.5) −0.032 (0.9) 0.49 (0.07)
brown −0.14 (0.6) 0.32 (0.3) −0.12 (0.7) −0.88 (1e−05) 0.88 (1e−05) −0.57 (0.03) 0.4 (0.1) 0.32 (0.3) −0.52 (0.05) 0.37 (0.2)
darkorange −0.25 (0.4) 0.63 (0.01) −0.26 (0.3) −0.82 (2e−04) 0.82 (2e−04) −0.57 (0.03) 0.26 (0.4) 0.63 (0.01) −0.44 (0.1) 0.12 (0.7)
floralwhite −0.023 (0.9) −0.011 (1) 0.032 (0.9) −0.68 (0.006) 0.68 (0.006) −0.34 (0.2) 0.31 (0.3) −0.011 (1) −0.49 (0.06) 0.53 (0.04)
red 0.065 (0.8) −0.33 (0.2) 0.2 (0.5) −0.59 (0.02) 0.59 (0.02) −0.46 (0.08) 0.54 (0.04) −0.33 (0.2) −0.25 (0.4) 0.5 (0.06)
paleturquoise 0.27 (0.3) −0.39 (0.2) 0.047 (0.9) −0.54 (0.04) 0.54 (0.04) −0.23 (0.4) 0.56 (0.03) −0.39 (0.2) −0.43 (0.1) 0.49 (0.07)
yellowgreen 0.092 (0.7) −0.47 (0.07) 0.3 (0.3) −0.3 (0.3) 0.3 (0.3) −0.32 (0.2) 0.43 (0.1) −0.47 (0.07) −0.051 (0.9) 0.41 (0.1)
turquoise 0.24 (0.4) −0.031 (0.9) −0.22 (0.4) 0.027 (0.9) −0.027 (0.9) 0.21 (0.4) 0.084 (0.8) −0.031 (0.9) −0.18 (0.5) −0.085 (0.8)
black 0.66 (0.007) −0.09 (0.7) −0.59 (0.02) 0.3 (0.3) −0.3 (0.3) 0.8 (4e−04) 0.01 (1) −0.09 (0.7) −0.43 (0.1) −0.29 (0.3)
royalblue 0.4 (0.1) −0.39 (0.1) −0.08 (0.8) 0.7 (0.004) −0.7 (0.004) 0.77 (8e−04) −0.28 (0.3) −0.39 (0.1) 0.091 (0.7) −0.19 (0.5)
sienna3 0.53 (0.04) −0.69 (0.004) 0.03 (0.9) 0.76 (0.001) −0.76 (0.001) 0.73 (0.002) −0.071 (0.8) −0.69 (0.004) 0.2 (0.5) −0.16 (0.6)
pink 0.26 (0.4) −0.55 (0.03) 0.2 (0.5) 0.57 (0.03) −0.57 (0.03) 0.47 (0.08) −0.16 (0.6) −0.55 (0.03) 0.23 (0.4) 0.0085 (1)
lightcyan 0.1 (0.7) −0.62 (0.01) 0.4 (0.1) 0.6 (0.02) −0.6 (0.02) 0.35 (0.2) −0.23 (0.4) −0.62 (0.01) 0.38 (0.2) 0.11 (0.7)
magenta 0.3 (0.3) −0.3 (0.3) −0.05 (0.9) 0.55 (0.03) −0.55 (0.03) 0.49 (0.06) −0.13 (0.6) −0.3 (0.3) 0.18 (0.5) −0.25 (0.4)
plum1 0.27 (0.3) −0.56 (0.03) 0.19 (0.5) 0.83 (1e−04) −0.83 (1e−04) 0.62 (0.01) −0.3 (0.3) −0.56 (0.03) 0.39 (0.1) −0.16 (0.6)
steelblue −0.41 (0.1) −0.39 (0.2) 0.73 (0.002) 0.47 (0.07) −0.47 (0.07) −0.17 (0.6) −0.34 (0.2) −0.39 (0.2) 0.75 (0.001) 0.14 (0.6)
darkgreen −0.089 (0.8) −0.7 (0.004) 0.66 (0.007) 0.47 (0.08) −0.47 (0.08) −0.065 (0.8) −0.043 (0.9) −0.7 (0.004) 0.64 (0.01) 0.17 (0.5)
darkgrey −0.054 (0.8) −0.54 (0.04) 0.5 (0.06) 0.72 (0.003) −0.72 (0.003) 0.26 (0.3) −0.33 (0.2) −0.54 (0.04) 0.62 (0.01) −0.0088 (1)
blue −0.027 (0.9) −0.37 (0.2) 0.33 (0.2) 0.88 (2e−05) −0.88 (2e−05) 0.42 (0.1) −0.45 (0.09) −0.37 (0.2) 0.66 (0.008) −0.25 (0.4)
cyan −0.087 (0.8) −0.5 (0.06) 0.5 (0.06) 0.61 (0.02) −0.61 (0.02) 0.2 (0.5) −0.31 (0.3) −0.5 (0.06) 0.55 (0.03) 0.063 (0.8)
brown4 −0.046 (0.9) −0.56 (0.03) 0.5 (0.06) −0.089 (0.8) 0.089 (0.8) −0.26 (0.3) 0.21 (0.5) −0.56 (0.03) 0.15 (0.6) 0.46 (0.08)
darkslateblue 0.22 (0.4) −0.63 (0.01) 0.29 (0.3) 0.3 (0.3) −0.3 (0.3) 0.18 (0.5) 0.09 (0.7) −0.63 (0.01) 0.18 (0.5) 0.17 (0.5)
ivory −0.68 (0.006) 0.073 (0.8) 0.62 (0.01) −0.2 (0.5) 0.2 (0.5) −0.85 (7e−05) 0.019 (0.9) 0.073 (0.8) 0.6 (0.02) 0.16 (0.6)
salmon −0.55 (0.04) −0.23 (0.4) 0.73 (0.002) 0.43 (0.1) −0.43 (0.1) −0.35 (0.2) −0.32 (0.2) −0.23 (0.4) 0.88 (2e−05) 0.021 (0.9)
orangered4 −0.085 (0.8) −0.62 (0.01) 0.59 (0.02) 0.45 (0.09) −0.45 (0.09) −0.071 (0.8) −0.033 (0.9) −0.62 (0.01) 0.63 (0.01) 0.096 (0.7)
purple 0.54 (0.04) −0.23 (0.4) −0.35 (0.2) 0.61 (0.02) −0.61 (0.02) 0.82 (2e−04) −0.16 (0.6) −0.23 (0.4) −0.076 (0.8) −0.35 (0.2)
greenyellow 0.56 (0.03) −0.48 (0.07) −0.17 (0.5) 0.14 (0.6) −0.14 (0.6) 0.45 (0.09) 0.24 (0.4) −0.48 (0.07) −0.28 (0.3) 0.072 (0.8)
lightgreen 0.69 (0.004) 0.019 (0.9) −0.71 (0.003) −0.032 (0.9) 0.032 (0.9) 0.56 (0.03) 0.28 (0.3) 0.019 (0.9) −0.6 (0.02) −0.26 (0.3)
midnightblue 0.093 (0.7) −0.21 (0.5) 0.079 (0.8) 0.61 (0.01) −0.61 (0.01) 0.31 (0.3) −0.2 (0.5) −0.21 (0.5) 0.44 (0.1) −0.34 (0.2)
darkred 0.6 (0.02) −0.45 (0.09) −0.24 (0.4) 0.61 (0.02) −0.61 (0.02) 0.68 (0.005) 0.058 (0.8) −0.45 (0.09) 0.068 (0.8) −0.36 (0.2)
orange 0.13 (0.7) −0.72 (0.003) 0.46 (0.08) 0.71 (0.003) −0.71 (0.003) 0.28 (0.3) −0.13 (0.7) −0.72 (0.003) 0.59 (0.02) −0.027 (0.9)
lightcyan1 0.078 (0.8) −0.1 (0.7) 0.0037 (1) −0.43 (0.1) 0.43 (0.1) −0.36 (0.2) 0.46 (0.09) −0.1 (0.7) −0.17 (0.6) 0.17 (0.5)
mediumpurple3 0.14 (0.6) −0.45 (0.09) 0.23 (0.4) 0.19 (0.5) −0.19 (0.5) 0.016 (1) 0.15 (0.6) −0.45 (0.09) 0.21 (0.4) 0.073 (0.8)
darkturquoise 0.4 (0.1) −0.27 (0.3) −0.17 (0.5) 0.17 (0.5) −0.17 (0.5) 0.28 (0.3) 0.21 (0.4) −0.27 (0.3) −0.064 (0.8) −0.15 (0.6)
lightsteelblue1 0.48 (0.07) −0.44 (0.1) −0.12 (0.7) −0.23 (0.4) 0.23 (0.4) 0.02 (0.9) 0.57 (0.03) −0.44 (0.1) −0.3 (0.3) 0.15 (0.6)
well_watered
water_stressed
rewatered
day_17
day_22
well_watered_17
well_watered_22
water_stressed_22
rewatered_17
rewatered_22
1
0.5
0
−0.5
−1

Figure S2. The correlation between module eigengenes and experimental conditions computed using biweight midcorrelation analysi

Supplementary tables

Table S1. List of differentially expressed transcription factor genes during drought stress and recovery in *E. grandis*

Table S2. Details of biological process gene ontology enrichment summary using GO-figure for differentially expressed genes during drought stress and recovery *E. grandis*

Table S3. Summary of biological process gene ontology enrichment analysis for all co-expression modules

Table S4. Potential hub genes and differentially expressed hub genes identified from six modules of interest