Journal of Oil Palm Research Vol. 37 (3) September 2025 p. 461-475
DOI: https://doi.org/10.21894/jopr.2024.0036

DISTINCT GENE CLUSTERS ARE EXPRESSED IN OIL PALM LEAF AND ROOT TISSUES IN RESPONSE TO DROUGHT STRESS

TISHA MELIA[1]*; FATAYAT[1]; SUKAMTO[1] and RIKI ARIO NUGROHO[1]

ABSTRACT

Elaeis guineensis *is a significant source of vegetable oil, yet it does not thrive in dry conditions. Thus, it is essential to understand the molecular response to drought in multiple oil palm tissues. This study analysed publicly available transcriptomic data from the leaves and roots of oil palms following drought. The data identified ~5,000 genes with significant gene expression changes, further clustered based on their expression patterns into 12 groups. The gene clusters showed distinct expression patterns in the two tissues examined, highlighting the different biological mechanisms. The involvement of the phytohormone abscisic acid in drought is implicated in both tissues by the differential expression of the activating transcription factor (TF) families homodomain-leucine zipper (HD-ZIP), ethylene response element binding factors (ERF) and no apical meristem (NAM),* Arabidopsis thaliana *activating factor (ATAF) and cup-shaped cotyledon (CUC) NAC. Gene clusters uniquely upregulated in leaves are enriched for TF family DNA binding one zinc finger (DOF) and lateral organ boundaries domain (LBD), which regulate hormone homeostasis and stomatal closure. Photosynthetic-related genes are downregulated in leaves, hypothesised to reduce photosynthesis activity, whereas heat shock proteins are enriched in upregulated genes found in roots. Finally, this study assembled ribonucleic acid (RNA) sequencing reads to discover ~6,200 novel genes whose expression are sensitive to drought. Our results expand the knowledge of molecular response following drought and demonstrate how identifying gene clusters can help to form hypotheses of molecular mechanisms.*

Keywords: bioinformatics, clustering, drought, sequencing, transcriptome.

Received: 31 August 2023**; Accepted:** 7 April 2024**; Published online:** 21 June 2024.

INTRODUCTION

The African oil palm (*Elaeis guineensis*) is an essential source of edible oil, providing an average yield of 3.5 t ha^{-1}. It is one of the most efficient species to produce oil while occupying a small land footprint (Barcelos *et al.*, 2015). Global oil palm producers are Indonesia and Malaysia, which boast a climate that enables oil palm to meet its water requirement of 2,000 mm yr^{-1} (Corley *et al.*, 2017). Palms subjected to water-deficit stress for more than 90 days exhibited a decrease in oil yield ranging from 25%-35% (Afandi *et al*., 2022; Woittiez *et al*., 2017). Drought caused physical changes in *E. guineensis*, including lower female/male flower ratio, higher floral abortion, and lower photosynthetic rate (Afandi *et al*., 2022; Suharyanti *et al*., 2020), all of which directly reduce oil yield. Therefore, it is crucial to understand how oil palm tolerate water deficits, particularly in the face of climate change. *El Niño* brings anomalous weather conditions in Southeast Asia, resulting in elevated temperatures, reduced precipitation and increased aridity (Khor *et al*., 2021).

Understanding molecular responses to drought stress is an active area of research in plants, particularly in *Arabidopsis thaliana* and *Zea mays* L. (maize). When exposed to drought stress, maize activates numerous phytohormones, alters ion fluxes, and accumulates reactive oxygen species (ROS) as stress signals (Gupta *et al*., 2020; Leng & Zhao, 2020). These stress signals activate transcription factors (TF) that regulate a complex system of stress-responsive genes. Known TFs that have been shown to regulate plants' response to drought stress are basic region / leucine zipper (ZIP),

[1] Computer Science Department,
Faculty of Mathematics and Natural Sciences,
Universitas Riau, Pekanbaru, 28293 Riau, Indonesia.

* *Corresponding author e-mail: tisha.melia@lecturer.unri.ac.id*

AP2/ERF, No apical meristem (NAM), *A. thaliana* activating factor (ATAF) and Cup-shaped cotyledon (CUC) NAC, WRKY and nuclear transcription factor Y (NF-Y) (Leng & Zhao, 2020). One of the main phytohormones used in drought stress is abscisic acid (ABA), which was shown to regulate multiple TFs (Jiao *et al*., 2022b; Wu *et al*., 2022; Yang *et al*., 2022; Zhao *et al*., 2014). TFs that were triggered by ABA (ABA-dependent) and other mechanisms (ABA-independent) regulate the transcription of downstream stress-related genes. Many of these genes coordinate molecular mechanisms to reduce water loss by closing the stomatal aperture, decreasing the photosynthesis rate, and altering root systems (Hong *et al*., 2020; Jiao *et al*., 2022a; Leng & Zhao, 2020). Furthermore, plants that undergo water-deficit stress must control the accumulation of ROS since its highly reactive hydroxyl radical may damage photosynthesis-related proteins and thylakoidal membranes (De Carvalho, 2008).

Transcriptomic changes following drought treatment have been reported in oil palm roots (Wang *et al*., 2020) and leaves (Salgado *et al*., 2022), with the latter focusing only on microRNA changes. Root transcriptome analyses confirmed the involvement of hormone regulation and ABC transporters. In terms of cellular functions, Wang *et al*. (2020) found an enrichment of cell wall biogenesis, phenylpropanoid biosynthesis, cellular ketone metabolism, ion transport and homeostasis as well as small molecule biosynthesis. Many of these functional categories have previously been identified in other plants as playing a role in phytohormone signalling mechanisms, altered growth rates and maintaining ROS homeostasis. The same study reported water deficit regulates several ABA-dependent TFs. In a separate study, Salgado *et al.* (2022) discovered miRNA affected by drought in oil palm leaves, which were predicted to target TFs, including myeloblastosis (MYB), homeobox (HOX) and NF-Y. These findings emphasise the importance of noncoding RNA in understanding the molecular response to drought. A recent study by Leão *et al.* (2022) showed that drought stress has a significant impact on protein phosphorylation and carbohydrate metabolism in leaves. This highlights the importance of phosphorylation in protein modification and the role of carbohydrates in signal transduction and energy production, which can enhance drought tolerance in plants.

Despite previous research, it is unclear how protein-coding genes in oil palm leaves respond to drought and how their gene expression patterns differ from the root transcriptome. This information is essential for identifying ways to improve oil palm genetics and increase drought tolerance. The present study addresses this knowledge gap by collecting publicly available RNA-sequencing datasets from the roots and leaves of oil palms following drought treatment. Water deficit stress regulates approximately 5,000 genes, which we cluster into 12 groups. Various gene clusters exhibited distinct patterns of gene expression profiles and were enriched with unique functional categories. Common transcription factors, namely homeodomain-leucine zipper (HD-ZIP), ERF and NAC, were identified as significantly activated in both tissues, alongside TFs that were uniquely enriched and upregulated in leaves, DNA binding one zinc finger (DOF) and LBD. Many of these TFs regulate stress-related genes to improve drought tolerance. Furthermore, we identified approximately 6,000 novel transcripts displaying characteristics commonly found in protein-coding genes. The majority of these transcripts exhibited significant expression alterations in response to drought. Thus, the present study enhances our understanding of how oil palm responds to drought stress, with significant implications for crop improvement and molecular-based drought tolerance phenotyping.

MATERIALS AND METHODS

RNA Sequencing Datasets

We obtained RNA sequencing data from 15 young *tenera E. guineensis* samples subjected to drought treatment from published studies (SRA accession PRJNA573093 and PRJDB9517, *Table 1*) (Ferreira *et al*., 2022; Salgado *et al*., 2022; Wang *et al*., 2020). Apical leaves were collected from the first nine palms after either no drought treatment (controls) or withholding water for seven and 14 days; the following six samples were taken from the roots of control palms and palms that were not given water for 14 days. Leaf samples were taken from clones, while root samples were from seedlings. All raw reads showed good sequencing quality as assessed by FastQC (Andrews, 2010). We mapped raw reads to the oil palm genome version EG 5.1 (Sanusi *et al*., 2018) using spliced transcripts aligment to a reference (STAR) (Dobin & Gingeras, 2015). To visualise the mapped reads, we created normalised bigwig tracks using DeepTools (Ramírez *et al*., 2016), excluding any reads that were mapped into multiple places in the genome. We used normalisation factors extracted from the trimmed mean of the M-values (TMM) method in edgeR (McCarthy *et al*., 2012) to normalise bigwig files.

Novel Gene Discovery and Characterisation

Mapped sequencing reads from each sample were used to assemble transcripts using StringTie (Shumate *et al*., 2022) based on the EG5.1 annotation as the starting point. We merged assembled

TABLE 1. RNA-SEQUENCING SAMPLES USED IN THIS STUDY

Sample	Tissue	Treatment	Accession #	Uniquely mapped reads	Citation
1	Leaf	Control replicate 1	SRR10219438	20,576,526	Ferreira *et al*. (2022); Salgado *et al*. (2022)
2	Leaf	Control replicate 2	SRR10219439	27,106,414	
3	Leaf	Control replicate 3	SRR10219424	22,015,317	
4	Leaf	Drought (7 days) replicate 1	SRR10219435	33,554,595	
5	Leaf	Drought (7 days) replicate 2	SRR10219436	21,634,316	
6	Leaf	Drought (7 days) replicate 3	SRR10219437	20,702,478	
7	Leaf	Drought (14 days) replicate 1	SRR10219432	30,894,589	
8	Leaf	Drought (14 days) replicate 2	SRR10219433	26,408,182	
9	Leaf	Drought (14 days) replicate 3	SRR10219434	25,008,000	
10	Root	Control replicate 1	DRR286073	29,891,464	Wang *et al.* (2020)
11	Root	Control replicate 2	DRR286072	45,118,907	
12	Root	Control replicate 3	DRR286077	35,989,393	
13	Root	Drought (14 days) replicate 1	DRR286076	16,035,703	
14	Root	Drought (14 days) replicate 2	DRR286075	11,582,438	
15	Root	Drought (14 days) replicate 3	DRR286074	25,066,789	

transcripts from all samples into one final assembly. Assembled transcripts were filtered to exclude genes that are <200 nucleotides in length and overlapping with known gene annotations. Novel gene structures are provided as a Gene Transfer Format (GTF) file available to be downloaded from https://my.unri.ac.id/8cjruY.

We produced mature transcripts for each gene by concatenating exon sequences defined by our gene structures. We used the mature mRNA sequences to find all open reading frames (ORFs) in the six reading frames (three reading frames in the sense direction and another three in the antisense direction). For each reading frame, we search for the occurrence of a Pfam domain using the Perl web service provided by the European Bioinformatics Institute. We only consider a match for an E-value less than 0.05. Mature mRNA transcripts were also used to identify TF sequences using the search feature in PlantTFDB (Jin *et al*., 2017).

Gene Expression Quantification

Gene expression levels for annotated and novel genes were quantified using featureCounts (Liao *et al*., 2014), counting only uniquely mapped reads at exons. A gene is considered expressed when the total reads across conditions is at least 15, with at least one sample having a minimum of 10 reads. The edgeR package (McCarthy *et al*., 2012) was used to normalise and perform differential expression analyses. To quantify gene expression level changes between two conditions, we directly compared the normalised gene expression counts between the two conditions (fold-changes). The statistical significance of fold-changes is assessed by edgeR with false discovery rate (FDR) correction applied. A gene is said to be differentially expressed if the $|\log2(\text{fold-changes})| > 2$ at FDR <0.05. Negative log2(fold-changes) indicates downregulation of gene expression patterns, while upregulation of gene expression will have positive log2(fold-changes).

Gene Clustering

We used consensus clustering (Monti *et al*., 2003) to identify a robust grouping of genes based on fold-changes of the four differential expression analyses described above. Briefly, we used the k-means method to cluster genes into 2-15 clusters, where the clustering is repeated fifty times in each choice of cluster number. We randomly subsampled (80%) of the data in each repeat of the k-means clustering. Cluster robustness is assessed in the form of a consensus matrix, whose value at row i and column j is the frequency of genes i and j clustered together across all repetitions. To aid in choosing the appropriate number of clusters, a cumulative distribution function (CDF) graph of the values in the consensus matrix is created. The changes in the area under the CDF curves (delta K) were also provided to delineate small differences between cluster number choices.

Functional Enrichment

To understand the significance of the identified gene clusters, we performed functional enrichment analyses to determine if there is a greater prevalence of genes with known functions in a cluster, as compared to by chance. The four types of functional enrichment we performed are detailed below. For each functional enrichment type,

p-values of the enrichment strength were adjusted with the Benjamini-Hochberg method, setting the significance level at <0.05.

Kyoto* Encyclopedia *of Genes and Genomes Orthology (KO) and Gene Ontology (GO) enrichment. We downloaded EG5.1 coding sequences (CDS) from PalmXplore (Sanusi *et al*., 2018), which were then used to identify Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and GO categories for each gene using EggNOG (Huerta-Cepas *et al*., 2019). The enrichment was done by comparing the number of genes found in a specific KEGG pathway/GO category to all genes that are differentially expressed at any point during drought treatments (5,168 genes). The enrichment analyses were done using the enricher function in clusterProfiler (Yu *et al*., 2012).

Transcription factor (TF) enrichment. We downloaded 2910 TF sequences found in oil palm from PlantTFDB (Jin *et al*., 2017) comprising 58 TF families. We used these annotated TFs to identify TFs in the *E. guineensis* CDS by BLAST. The enrichment score of having TF family *f* in cluster *c* was calculated as Equation (1):

$$\frac{g_{f,c}}{g_{f',c}} \Big/ \frac{g_{f,c'}}{g_{f',c'}} \quad (1)$$

where, $g_{f,c}$ is the number of genes that are TF family *f* in cluster *c*; $g_{f',c}$ is the number of genes that are NOT TF family *f* in cluster *c*; $g_{f,c'}$ is the number of genes that are TF family *f* in the cluster other than *c* and $g_{f',c'}$ is the number of genes that are NOT TF family *f* in the cluster other than *c*. Enrichment significance was determined using Fisher's exact test.

Fatty acid biosynthesis genes enrichment. A total of 42 fatty acid biosynthesis genes were collected from PalmXplore (Sanusi *et al*., 2018), which were then used to perform enrichment in the same way we performed TF enrichment.

RESULTS AND DISCUSSION

Massive Transcriptomic Changes Occurred in Response to Drought

We collated RNA-sequencing data that had been generated from 15 young *E. guineensis* palms that had been subjected to drought treatments or served as controls (*Table 1*). Drought treatments consisted of withholding water for seven or 14 days. Leaves were collected from nine palms (three palms each for controls, seven day drought and 14 day drought) (Wang *et al*., 2020) and roots were collected from the remaining palms (three palms each for controls and 14 day drought) (Ferreira *et al*., 2022; Salgado *et al*., 2022). The average number of uniquely mapped reads to the EG5.1 genome per sample is 25.3 and 27.2 million for leaf and root samples, respectively.

The transcriptomic data from these samples showed the expected grouping, as shown in the PCA plot in *Figure 1a*. The first principal component, accounting for 48% of the total variance, clearly separates the samples by the tissue of origin. The second principal component (16% of total variance) correlates well with drought severity from controls (bottom) to 14 day drought (top). All but one of the control samples do not overlap with the seven day drought samples, suggesting that the drought response is well underway by day seven. All 14 day drought samples are clustered together at the top of the PCA plot, indicating massive transcriptomic changes occurred in response to drought.

To quantify the extent of the gene expression response to aridity, we conducted differential expression analyses on the 23,539 genes expressed in these samples. As leaf samples cover three time points, we compared Day 7 drought with control samples (D7/Control), Day 14 with Day 7 samples (D14/D7), and Day 14 with control samples (D14/Control); however, only the D14/Control comparison was possible for root samples. For all comparisons, a greater number of genes exhibited downregulation of expression than upregulation (on average of 62% *vs.* 38%, *Table 2*) at a minimum of four fold-change. This pattern suggests that young palms responded to drought stress by significantly reducing their transcription activity. The prevalence of downregulated expression was also reported for miRNAs after exposure to drought and genes affected by high salinity stress (Ferreira *et al*., 2022; Salgado *et al*., 2022). A total of 5168 genes were identified as differentially expressed in at least one of the four comparative analyses (*Figure 1b* and *1c*, *Table S1*), which corresponds to slightly less than a quarter (~22%) of all expressed genes.

We examined the 10 most differentially expressed genes at each time point (Day 7 and 14 in leaf samples, as well as Day 14 in root samples) to better understand the transcriptomic changes brought about by drought (*Table 3*). Three out of 10 genes whose expression significantly changed on Day 7 following drought in leaves are TFs, which may be involved in regulating the early response to drought stress. Two of these, namely p5.00_sc00016_p0198 and p5.00_sc00135_p0003, showed strong upregulation. The former is a HD-ZIP TF family member. Members of this TF family are involved in the initiation of drought response in maize (Jiao *et al*., 2022a), while p5.00_sc00135_p0003 is an auxin-responsive IAA protein, which has been associated with enhanced drought tolerance in *A. thaliana* (Shani *et al*., 2017). At Day 14 post

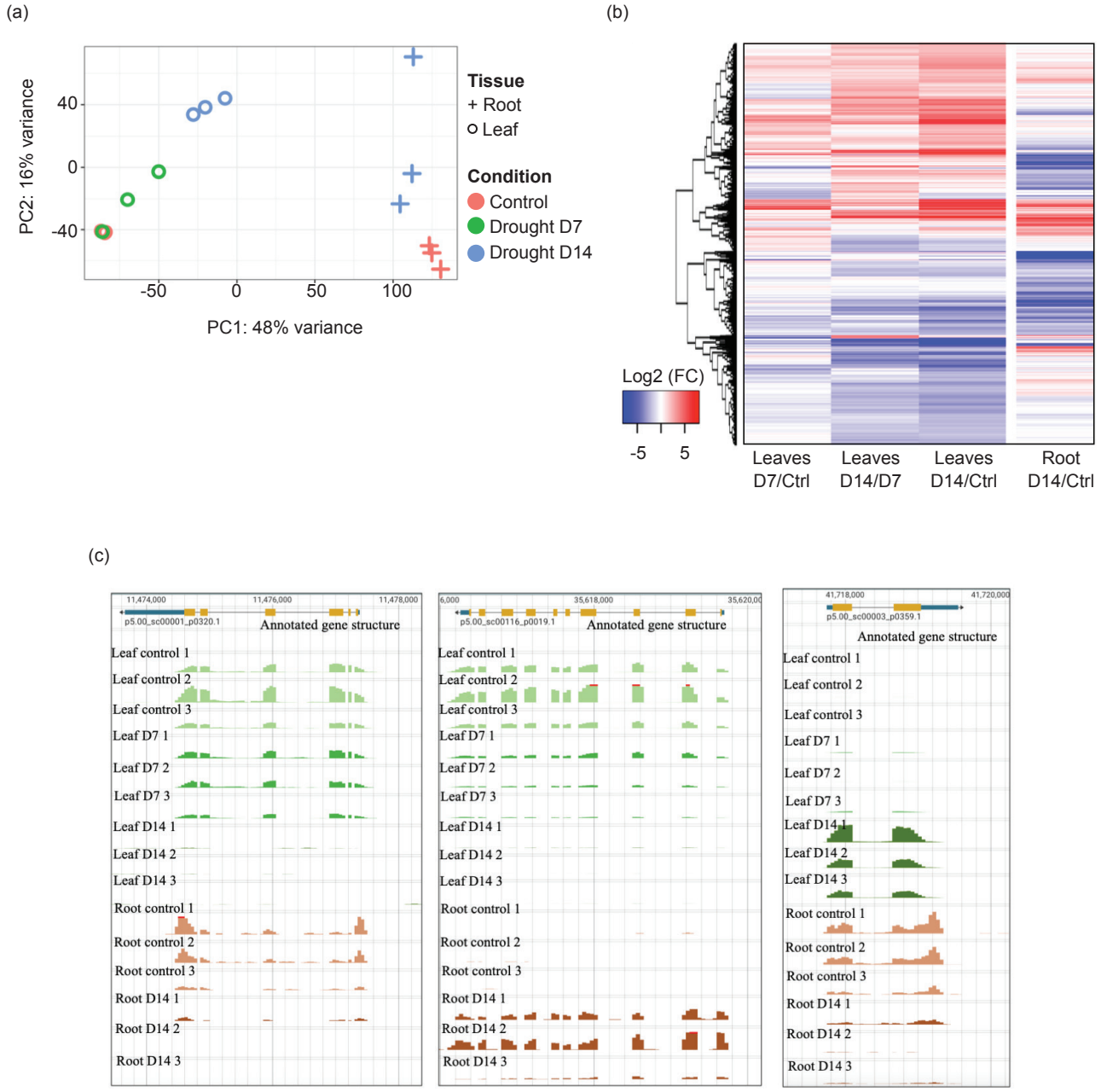

Figure 1. Transcriptomic response to drought treatment. (a). PCA of the 15 RNA-seq samples. (b) Heatmap depicting gene expression level changes for 5,168 regulated genes in response to drought treatment. The rows correspond to genes and columns correspond to the four treatment comparisons discussed in the text. The colours indicate the log2(fold-changes) of gene expression patterns. (c) Genome browser screenshots for three genes that are differentially expressed following drought treatment. The top track presents gene structures, followed by gene expression tracks showing read pileups in every sample.

drought treatment, eight of the 10 most regulated genes in leaves showed robust downregulation. Genes that were downregulated include two known transcriptional regulators, whereby the maize orthologue of p5.00_sc00127_p0068 is involved in kernel storage (Zhan *et al*., 2018), while orthologues of p5.00_sc00034_p0162 are involved in plant response to stress (Qian *et al*., 2021). The p5.00_sc00034_p0162 gene exhibited a significant 3-fold increase in expression on Day 7 of the drought treatment, followed by downregulation by Day 14, suggesting that its expression may only be essential during the early stages of the stress response. In roots of oil palm seedlings subjected to 14 days of drought treatment, the top 10 differentially expressed genes were downregulated except for a heat shock protein gene, p5.00_sc00001_p0173. The heat shock protein family are molecular chaperones in distressed plants. Members of this family were found to be significantly upregulated in the roots of *Canavalia rosea* following drought treatment (Zhao *et al*., 2018).

TABLE 2. NUMBER OF GENES THAT ARE DIFFERENTIALLY EXPRESSED IN RESPONSE TO DROUGHT

	D7/control	D14/D7	D14/control	
	Leaves	Leaves	Leaves	Root
Fold change <-4	261	1,319	1,936	1,114
Fold change >4	218	1,079	1,619	344
Total	**479**	**2,398**	**3,555**	**1,458**

TABLE 3. TOP DIFFERENTIALLY EXPRESSED GENES IN EACH TIME POINT

Comparison	Gene name	Top 10 differentially expressed genes	
		Direction of regulation	Annotation
D7/control leaves	*p5.00_sc00001_p0582*	Down	-
	p5.00_sc00016_p0198	Up	TF from HD-ZIP family; a homeobox-leucine zipper protein
	p5.00_sc00018_p0081	Up	-
	p5.00_sc00087_p0033	Up	-
	p5.00_sc00089_p0007	Down	-
	p5.00_sc00107_p0001	Down	Isoprene synthase
	p5.00_sc00127_p0028	Up	Remorin (C-terminal region) protein
	p5.00_sc00135_p0003	Up	Aux IAA protein, known as a TF
	p5.00_sc00360_p0022 (CCA1)	Down	TF belonging to MyB Family; a protein LHY-like isoform X1
	p5.00_sc15516_p0001	Down	-
D14/d7 leaves	*p5.00_sc00001_p0732*	Down	-
	p5.00_sc00006_p0051	Down	Protoporphyrinogen oxidase
	p5.00_sc00044_p0061	Up	Alpha beta hydrolase domain-containing protein
	p5.00_sc00104_p0041	Down	UAA transporter family
	p5.00_sc00107_p0001	Down	Isoprene synthase
	p5.00_sc00127_p0068	Down	TF from bZIP family; regulatory protein opaque-2
	p5.00_sc00135_p0039	Down	-
	p5.00_sc00389_p0013 (PARP3)	Up	Poly (ADP-ribose) polymerase
	p5.00_sc00034_p0162	Down	TF from the bHLH family
	p5.00_sc00059_p0119	Down	-
D14/control root	*p5.00_sc00001_p0173*	Up	Heat shock protein
	p5.00_sc00006_p0166	Down	Glycosyl hydrolase family 9
	p5.00_sc00018_p0026	Down	Glycosyltransferase 2 family
	p5.00_sc00019_p0112	Down	-
	p5.00_sc00038_p0180	Down	Plastocyanin-like domain
	p5.00_sc00058_p0071	Down	-
	p5.00_sc00078_p0012	Down	Microtubule associated protein (MAP65/ASE1 family)
	p5.00_sc00123_p0044	Down	-
	p5.00_sc00271_p0007	Down	-
	p5.00_sc00063_p0036	Down	Soluble NSF attachment protein, SP

Note: The symbol "-" indicates that no annotation was found for the gene.

Distinct Clusters of Differential Expression Profiles were Observed in Response to Drought

To discern the biological mechanisms of drought response, we clustered all differentially expressed genes (5,168) based on fold-change profiles obtained from the four differential expression analyses discussed earlier (*Figure 1b*). A consensus clustering method was employed to identify robust clusters. This method repeats the clustering algorithm multiple times and identifies consistent groupings across iterations. We determined the optimal number of clusters by evaluating the consensus matrix (*Figure 2a*), which displays how frequently pairs

of genes are clustered together across repetitions. Consistent clustering in the consensus matrix (*Figure 2a*) established twelve distinct groups.

Increasing the number of clusters did not enhance the stability of the identified groups, as observed by the negligible change in the consensus matrix. Distinct gene expression patterns were evident for the 12 identified clusters in the four differential expression analyses (*Figure 2b* and *2c*). Of these, five clusters (cluster 4, 9, 11, 8 and 2) exhibited common regulation patterns across both examined tissues (1438 genes, 27.8% of regulated genes, *Figure 2b* and *2c*). Five additional clusters (clusters 1, 3, 5, 7, and 12) showed expression regulation occurring exclusively in either roots or leaves, indicating the likely tissue-specificity of these genes' function. The bulk of the regulated genes (3,283, 63.5%) belong to these five clusters. Cluster 6 and 10, comprising 8.6% of the genes (447), exhibited contrasting expression patterns.

Functional Enrichment of Gene Clusters

To identify the biological mechanisms associated with each cluster, we assess whether any enrichment exists for specific functional gene groups. A significant enrichment for a particular functional group may indicate the biological mechanisms involved. Initially, we assessed the significant enrichment of genes from KEGG Orthology (KO) (Kanehisa *et al.*, 2023) in any of the 12 clusters. KO is a manually curated database of molecular functions derived from pathways, modules and BRITE hierarchies. We found that seven clusters

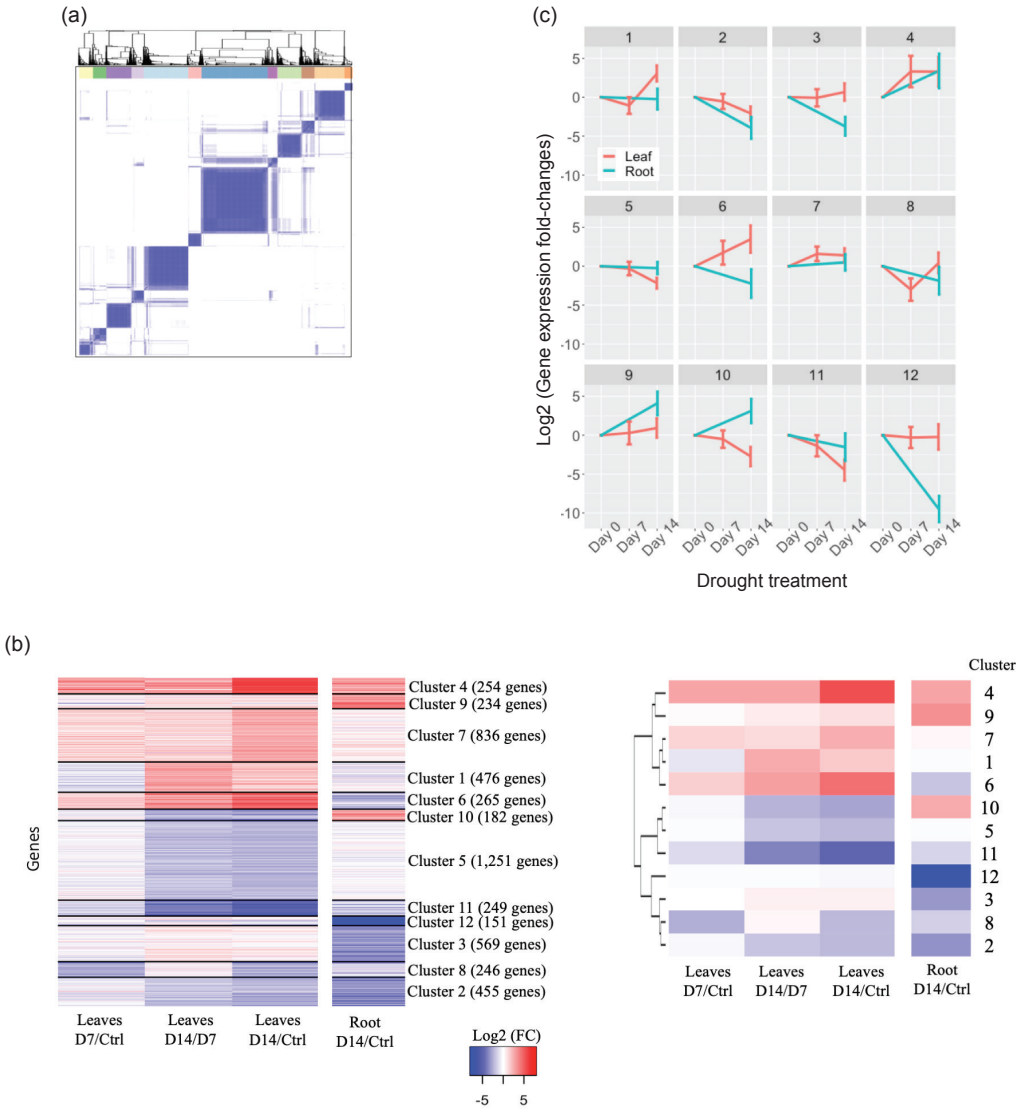

Figure 2. (a) A heatmap of the consensus matrix showing the frequency genes being clustered together in a specific row and column. The darker the blue colour, the more frequently the genes cluster together. (b) Left: expression heatmap of the four differential expression analyses (columns). Colour corresponds to the magnitude of changes in expression (log2(fold-changes)) in each comparison. Genes are arranged into the 12 identified clusters. Right: a similar heatmap on the left, but it depicts the average log2(fold-change) per cluster instead of the fold changes of individual genes. (c) The same information as in (b, right) is plotted as line graphs. Error bars are standard deviations.

exhibited significant enrichment for nine distinct KOs at an adjusted *p*-value <0.05 (*Figure 3a*). Next, we analysed GO terms, which are a collection of controlled vocabularies explaining gene products' functions and locations (Carbon *et al*., 2009), where we found five distinct clusters enriched for 22 GO terms (*Figure 3b*). We identified five unique clusters with 23 enriched GO terms (*Figure 3b*). Subsequently, we examined whether any of the 56 TF families defined in PlantTFDB (Jin *et al*., 2017) were significantly present in any of the clusters, which led to the discovery of four such clusters (*Figure 3c*). Finally, we assessed significant enrichment for the biosynthesis of fatty acids, whereby we identified that only cluster 3 was enriched.

Drought-induced Gene Expression Responses that are Common in Leaves and Roots

Genes in cluster 4 and 9 are upregulated in both tissues, with genes in cluster 4 exhibiting a stronger upregulation pattern as early as Day 7 of drought (*Figure 2b*). Cluster 4 is enriched for HD-ZIP, auxin-responsive IAA proteins (*Figure 3a*), Ethylene Response Element Binding Factors (ERF) TF family (*Figure 3c*) and cellulase activity (*Figure 3b*). The first three enriched functional categories, *i.e*., HD-ZIP, auxin-responsive IAA proteins and ERF, are known transcriptional regulators. HD-ZIP are TFs that regulate responses to abiotic stress and plant growth (Elhiti & Stasolla, 2009; Li *et al*., 2022), including initiating drought response by activating downstream stress-related genes in maize, rice and *A. thaliana* through ROS signaling and/or ABA-dependent pathways (Jiao *et al*., 2022a; Leng & Zhao, 2020; Li *et al*., 2022; Zhao *et al*., 2014). Similarly, the ERF TF family has been implicated in regulating plant tolerance in response to stress mediated by both ABA-dependent and ABA-independent pathways (Leng & Zhao, 2020; Qin *et al*., 2007; Wu *et al*., 2022; Xie *et al*., 2019). ERF was also shown to activate a subset of auxin-responsive proteins (Shani *et al*., 2017), which are known as transcriptional repressors hypothesised to regulate organogenesis, growth and environmental responses through auxin-signaling pathways (Shani *et al*., 2017; Wang *et al*., 2010). Auxin-responsive proteins are regulated following drought in oil palms (Ferreira *et al*., 2022; Wang *et al*., 2020), potentially coordinating growth rate and direction during such stressors.

Cluster 9 is enriched with the TF family NAC (NAM, ATAF1/2, CUC2; *Figure 3c*), which is known to respond to stress through ABA-dependent signaling pathways in maize (Leng and Zhao, 2020). Our results confirm previous observations in young oil palms, where the three ABA-dependent TFs (HD-ZIP, ERF and NAC) and pathways (hormone regulation and cell wall biogenesis) are regulated following drought (Wang *et al*., 2020). Taken together, common drought stress responses in both leaves and roots of oil palms are similar to the responses observed on model plants (Leng & Zhao, 2020; Shani *et al*., 2017; Xie *et al*., 2019). In summary, water scarcity triggers the production of diverse phytohormones including ABA and others, evidenced through the activation of ABA-dependent TFs (HD-ZIP, ERF and NAC) (Leng & Zhao, 2020; Li *et al*., 2022) along with ABA-independent pathways (Xie *et al*., 2019).

Upregulated Gene Expression Patterns in Responses to Drought Uniquely Found in Leaves

Clusters with marked differences in the direction of gene regulation between leaf and root samples are particularly interesting, characterised by unique stress responses in the two tissues. Cluster 1, 6 and 7 showed robust upregulation of gene expression patterns in leaves, with contrasting downregulation (cluster 6) or no regulation (cluster 1 and 7) of gene expression levels in roots (*Figure 2b* and *2c*). Cluster 6 is uniquely enriched for β-glucosidases (*Figure 3a* and *3b*), which have been shown to regulate the activation of phytohormones, cell wall lignification and stress response (Kongdin *et al*., 2021; Opassiri *et al*., 2006). β-glucosidases are classified into multiple glycoside hydrolase families (Cairns & Esen, 2010), which were reported to be essential in the activation of the phytohormone ABA by hydrolysing inactive ABA in *A. thaliana* (Jiao *et al*., 2022a). ABA, in turn, triggers different signaling pathways to increase stress tolerance (Leng & Zhao, 2020; Xie *et al*., 2019) (*Figure 3d*).

Cluster 1 is enriched for the DOF TF family (*Figure 3a)* (Bhaskara *et al*., 2012; Liu *et al*., 2021), which is a plant-specific transcriptional regulator involved in seed development, hormone regulation and abiotic stress response (Leng & Zhao, 2020; Zou & Sun, 2023). The DOF TF family was shown to be sensitive to ABA under water-deficit conditions and was implicated in increased drought tolerance in *A. thaliana* (Corrales *et al*., 2017), apple (Chen *et al*., 2020) and tomato (Corrales *et al*., 2014) by enhancing ROS scavenging ability (Zang *et al*., 2017; Zou & Sun, 2023). In *A. thaliana*, the DOF gene named CDF3 regulates genes involved in protecting stressed plants from osmotic and oxidative stress (Corrales *et al*., 2017). Through conducting metabolite profiling, the same study revealed that protective metabolites were present in a higher concentration in plants with overexpressed CDF3.

Cluster 7 showed significant enrichment for the LBD TF family, which has been demonstrated to enhance drought tolerance in maize (Jiao *et al*., 2022b; Wu *et al*., 2023) and *A. thaliana* (Guo *et al*., 2020b). It was suggested that *Zm*LBD, an LBD in maize, interacts with the IAA5 protein to regulate

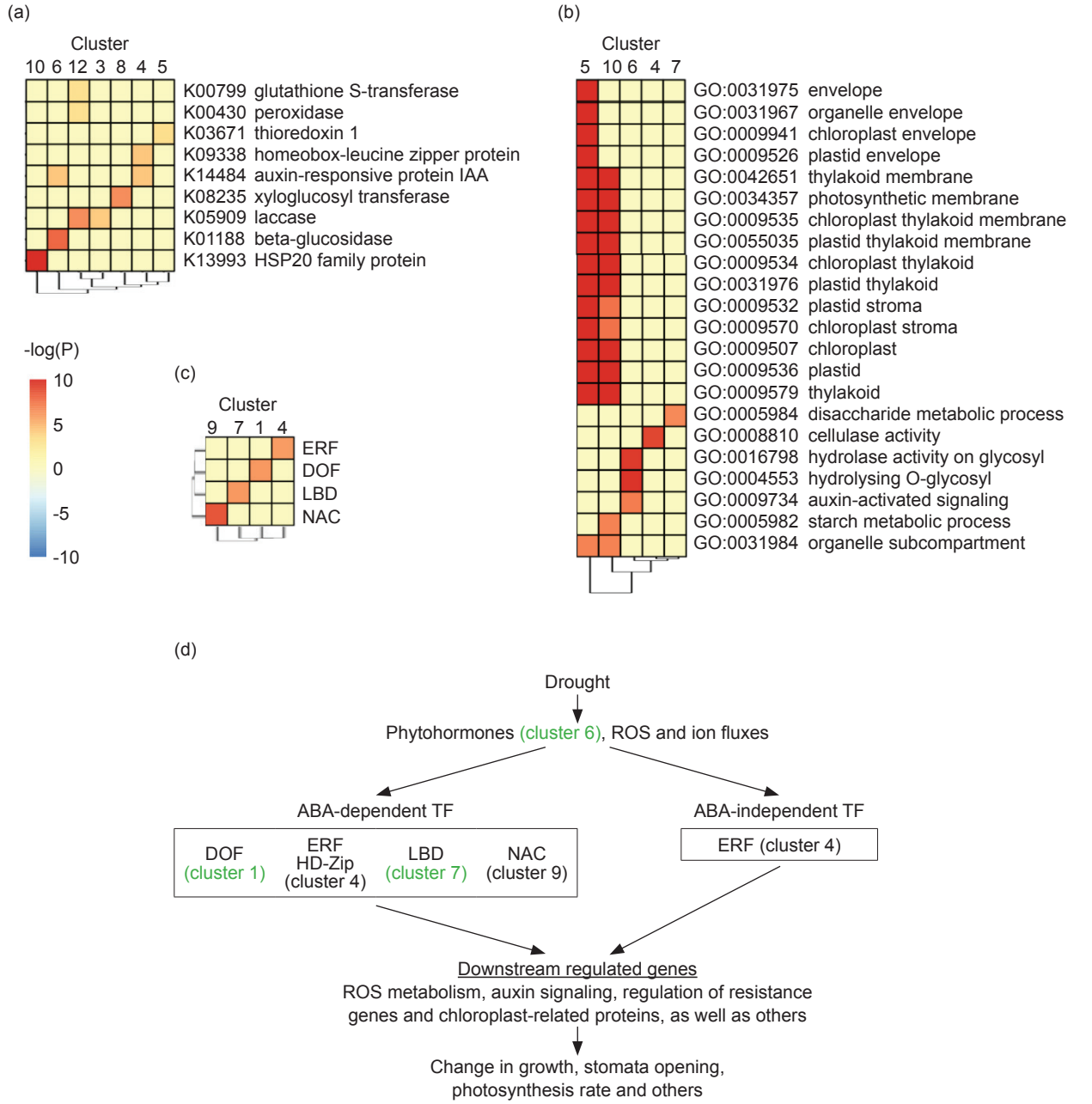

Figure 3. (a) Functional enrichments of the identified gene clusters for KEGG Orthology, (b) GO terms and (c) TF Family. Colours represent the –log(P value) for each enrichment analysis. (d) Hypothesised molecular mechanisms following drought. Cluster names were added next to/under each molecular mechanism involved. Clusters that were specific to leaves were written in green.

auxin biosynthesis genes, modulating H_2O_2 levels and increasing resistance to drought (Jiao *et al.*, 2022a). Additionally, LBD15 in *A. thaliana* was shown to bind directly to the promoter of ABI4, a gene in the ABA signaling pathway, resulting in the closure of stomata. In conclusion, clusters with strong upregulation patterns, exclusively in leaves, are enriched for ABA-responsive transcription factors DOF and LBD. These factors were shown to regulate genes to enhance drought tolerance by facilitating the biosynthesis of protective metabolites and limiting water loss through stomatal closure (*Figure 3d*).

Downregulated Gene Expression Patterns Found in Leaves During Water Deficit

Cluster 5 and 10 exhibit significant reduction in gene expression patterns in leaves, with cluster 10 presenting upregulation and cluster 5 showing no regulation in roots (*Figure 2b* and *2c*). Both clusters show enrichment in chloroplast-related proteins (*Figure 3b*). These proteins were markedly suppressed in drought-stressed *A. thaliana*, cassava and potato plants (Chang *et al.*, 2019; Hong *et al.*, 2020; Tamburino *et al.*, 2017). Moreover, chloroplast-related proteins were shown intimately linked with

photosynthetic activity and stomatal closure to retain water during stress (Hong *et al*., 2020). Cluster 10 exhibits a unique enrichment for heat-shock protein HSP20 (*Figure 3a*). The HSP20 protein family acts as molecular chaperones, contributing to a tolerance of biotic and abiotic stresses by stabilising cell structure and function and folding and transporting auxiliary proteins (Chini *et al*., 2004; Guo *et al*., 2020a).

Root-specific Gene Expression Changes in Response to Drought

Genes in cluster 3 and 12 exhibited a marked decrease in gene expression levels in roots resulting from drought treatment (*Figure 2b* and *2c*). Both clusters are enriched for laccase genes (*Figure 3a*), and cluster 3 exhibits additional enrichment in genes related to fatty acid biosynthesis. Laccase genes are known to play a role in lignin biosynthesis (Liu *et al*., 2017; Zhu *et al*., 2023), while fatty acid biosynthesis is implicated in forming callus and lateral root development. Tea plants subjected to water-deficit stress exhibited significant downregulation of genes associated with lignin and long-chain fatty acid biosynthesis. This led to reduced accumulation of necessary enzymes in the respective biosynthesis pathways during drought. As a result, the plants could better adapt to challenging conditions (Gu *et al*., 2020).

Many Drought-responsive Genes are Novel

Many genes are only transcribed following a stimulus, which reduces their likelihood of being discovered and included in published annotations. We assembled genome-wide transcriptomic data to catalogue all drought-responsive genes, a subset of which may be novel. This effort uncovered 9,329 transcribed regions that do not overlap with annotated genes in the EG5.1 genome; 2,475 of these transcripts contain multiple exons (multi-exonic, *Figure 4a*). We further analysed these uncharacterised transcript regions by examining features commonly present in known genes, including an open reading frame (ORF) of 150 amino acids or more, splicing evidence, a protein family (PFAM) domain, sequence similarity to known TFs, and significant changes in gene expression levels following exposure to drought stress. We identified 6,209 transcripts exhibiting at least one of the five properties we evaluated (*Table 3*). We designated these 6,209 transcripts as novel genes (*Table 4*, *Figure 4a* and *4b*). The data highlights that a considerable proportion of the novel genes (73.8% or 4,583) possess a PFAM domain, with a subset of 247 (equivalent to 3.9% of the novel genes) being identified as TFs. Furthermore, 2,121 new genes are significantly differentially expressed following water deficit stress (*Table 5*), which account for 41% of all differentially expressed genes. Given the sizable number of novel drought-responsive genes, our discovery of new genes offers a valuable resource for understanding oil palm's response to drought stress. Overall, we have demonstrated that our 6,209 new genes are likely to function as protein-coding genes.

TABLE 4. PROPERTIES OF THE NOVEL TRANSCRIBED REGIONS

Item	Novel transcripts	Novel genes
Number of transcripts/genes	9,329	6,209
Average #exons/gene	2.4	3.0
Average width (bp)	1,979.4	2,624.0
Average longest ORF (aa)	160.6	207.4
% Genes with PFAM domains	49.1%	73.8%
% Genes that are putative TF	2.6%	3.9%

TABLE 5. NUMBER OF NOVEL GENES THAT ARE DIFFERENTIALLY EXPRESSED IN RESPONSE TO DROUGHT

Item	D7/control	D14/D7	D14/control	
	Leaves		Leaves	Root
Fold change <-4	118	631	900	334
Fold change >4	89	426	635	129
Total	**207**	**1,057**	**1,535**	**463**

CONCLUSION

Elaeis guineensis is a crucial source of vegetable oils. However, arid conditions markedly lower the oil output, emphasising the necessity of breeding drought-resistant oil palms. Accordingly, understanding how oil palms respond to drought stress is essential. Here, we present the first report to compare transcriptomic changes in two oil palm tissues, including roots and leaves, under drought conditions. This study comprehensively compares the transcriptomic profiles and aims to pinpoint any tissue-specific changes caused by drought stress. A total of 6,209 genes were identified as differentially expressed following water-deficit stress. These genes were then organised into 12 robust gene clusters, with several clusters showing significant enrichments for specific functional categories. Gene clusters upregulated in both tissues are enriched for three ABA-dependent TFs: HD-ZIP, ERF and NAC, known regulators of stress-related genes. The leaf transcriptome exhibited a unique enrichment for upregulated LBD and DOF TFs, previously implicated in facilitating protective metabolite production and stomatal

closure. Furthermore, chloroplast-related proteins were strongly downregulated in leaves, indicating a potential reduction in photosynthesis rate. Genes involved in lignin and fatty acid biosynthesis were enriched in clusters downregulated solely in roots, suggesting the cessation of non-essential activities under stressful conditions. Finally, we discovered and characterised 6,209 novel transcripts with features commonly found in genes, such as protein domains, long ORF, sequence similarity to known TFs and splicing evidence. A notable 41% of differentially expressed genes responding to drought treatment were novel, emphasising their significant contribution to drought tolerance. Our findings provide essential resources in comprehending oil palm's response to drought, revealing distinct biological mechanisms induced in various tissues.

ACKNOWLEDGEMENT

Computational resources to complete this work were provided by MAHAMERU BRIN HPC, National Research and Innovation Agency of Indonesia (BRIN).

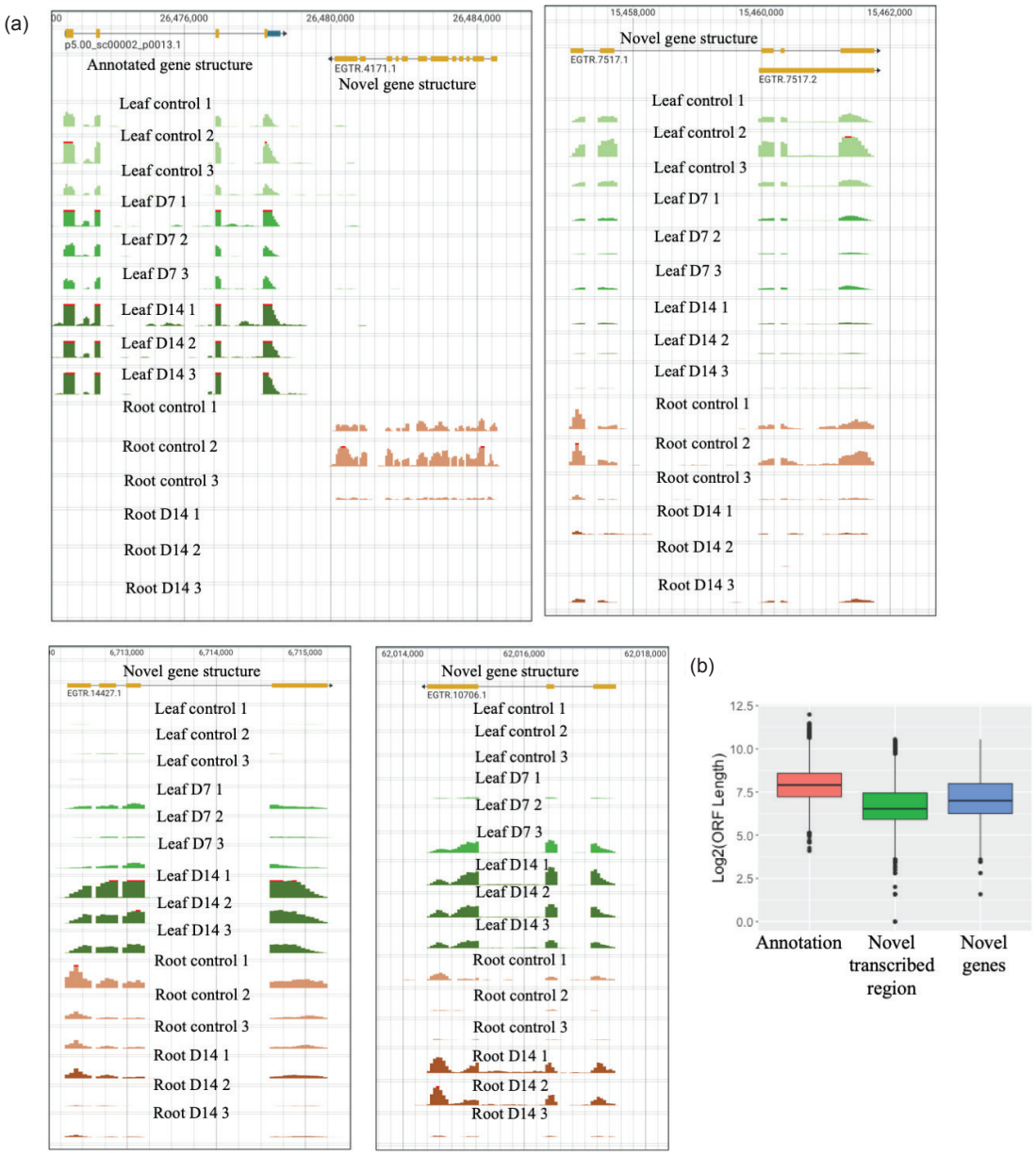

Figure 4. (a) Genome browser screenshots for four novel genes that are differentially expressed following drought treatment. The top track illustrates gene structures, followed by gene expression tracks depicting read pileups in each samples. These novel genes demonstrate significant downregulation in either roots (top-left) or both tissues; upregulation in leaves, but downregulation in roots (bottom-left); and upregulation in both tissues (bottom-right). (b) The distribution of ORFs (as log2) for these three categories is shown. In this study, we discovered annotated genes to have the longest open reading frame (ORF), followed by novel genes and then transcribed regions.

REFERENCES

Afandi, A. M., Zulkifli, H., Nur Zuhaili, H. A. Z. A., Norliyana, Z. Z., Hisham, H., Saharul, A. M., Dzulhelmi, M. N., & Vu Thanh, T. A. (2022). Oil palm water requirement and the need for irrigation in dry Malaysian areas. *Journal of Oil Palm Research*, *35*(2), 391–405. https://doi.org/10.21894/jopr.2022.0052

Andrews, S. (2010). *FastQC: A quality control tool for high throughput sequence data*. http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Barcelos, E., Rios, S. D. A., Cunha, R. N. V., Lopes, R., Motoike, S. Y., Babiychuk, E., Skirycz, A., & Kushnir, S. (2015). Oil palm natural diversity and the potential for yield improvement. *Frontiers in Plant Science*, *6*, 190. https://doi.org/10.3389/fpls.2015.00190

Bhaskara, G. B., Nguyen, T. T., & Verslues, P. E. (2012). Unique drought resistance functions of the highly ABA-induced clade A protein phosphatase 2Cs. *Plant Physiology*, *160*(1), 379–395. https://doi.org/10.1104/pp.112.202408

Cairns, J. R., K., & Esen, A. (2010). β-Glucosidases. *Cellular and Molecular Life Sciences*, *67*(20), 3389–3405. https://doi.org/10.1007/s00018-010-0399-2

Carbon, S., Ireland, A., Mungall, C. J., Shu, S., Marshall, B., & Lewis, S. (2009). The AmiGO hub and the web presence working group: AmiGO: Online access to ontology and annotation data. *Bioinformatics*, *25*(2), 288–289. https://doi.org/10.1093/bioinformatics/btn615

Chang, L., Wang, L., Peng, C., Tong, Z., Wang, D., Ding, G., Xiao, J., Guo, A., & Wang, X. (2019). The chloroplast proteome response to drought stress in cassava leaves. *Plant Physiology and Biochemistry*, *142*, 351–362. https://doi.org/10.1016/j.plaphy.2019.07.025

Chen, P., Yan, M., Li, L., He, J., Zhou, S., Li, Z., Niu, C., Bao, C., Zhi, F., Ma, F., & Guan, Q. (2020). The apple DNA-binding one zinc-finger protein *MdDof54* promotes drought resistance. *Horticulture Research*, *7*(1), 195. https://doi.org/10.1038/s41438-020-00419-5

Chini, A., Grant, J. J., Seki, M., Shinozaki, K., & Loake, G. J. (2004). Drought tolerance established by enhanced expression of the CC-NBS-LRR gene, *ADR1*, requires salicylic acid, *EDS1* and *ABI1*. *The Plant Journal*, *38*(5), 810–822. https://doi.org/10.1111/j.1365-313X.2004.02086.x

Corley, R. H. V., Rao, V., Palat, T., & Praiwan, T. (2017). Breeding for drought tolerance in oil palm. *Journal of Oil Palm Research*, *30*, 26–35. https://doi.org/10.21894/jopr.2017.0011

Corrales, A., Carrillo, L., Lasierra, P., Nebauer, S. G., Dominguez-Figueroa, J., Renau-Morata, B., Pollmann, S., Granell, A., Molina, R., Vicente-Carbajosa, J., & Medina, J. (2017). Multifaceted role of cycling DOF factor 3 (*CDF3*) in the regulation of flowering time and abiotic stress responses in *Arabidopsis*. *Plant Cell & Environment*, *40*(5), 748–764. https://doi.org/10.1111/pce.12894

Corrales, A., Nebauer, S. G., Carrillo, L., Fernández-Nohales, P., Marqués, J., Renau-Morata, B., Granell, A., Pollmann, S., Vicente-Carbajosa, J., Molina, R. V., & Medina, J. (2014). Characterization of tomato cycling Dof factors reveals conserved and new functions in the control of flowering time and abiotic stress responses. *Journal of Experimental Botany*, *65*(4), 995–1012. https://doi.org/10.1093/jxb/ert451

De Carvalho, M. H. C. (2008). Drought stress and reactive oxygen species: Production, scavenging and signaling. *Plant Signaling & Behavior*, *3*(3), 156–165. https://doi.org/10.4161/psb.3.3.5536

Dobin, A., & Gingeras, T. R. (2015). Mapping RNA-seq reads with STAR. *Current Protocols in Bioinformatics*, *51*(1), 1–19. https://doi.org/10.1002/0471250953.bi1114s51

Elhiti, M., & Stasolla, C. (2009). Structure and function of homodomain-leucine zipper (HD-Zip) proteins. *Plant Signaling & Behavior*, *4*(2), 86–88. https://doi.org/10.4161/psb.4.2.7692

Ferreira, T. M. M., Filho, J. A. F., Leão, A. P., De Sousa, C. A. F., & Souza, M. T. (2022). Structural and functional analysis of stress-inducible genes and their promoters selected from young oil palm (*Elaeis guineensis*) under salt stress. *BMC Genomics*, *23*(1), 735. https://doi.org/10.1186/s12864-022-08926-6

Gu, H., Wang, Y., Xie, H., Qiu, C., Zhang, S., Xiao, J., Li, H., Chen, L., Li, X., & Ding, Z. (2020). Drought stress triggers proteomic changes involving lignin, flavonoids and fatty acids in tea plants. *Scientific Reports*, *10*(1), 15504. https://doi.org/10.1038/s41598-020-72596-1

Guo, L. M., Li, J., He, J., Liu, H., & Zhang, H. M. (2020a). A class I cytosolic HSP20 of rice enhances

heat and salt tolerance in different organisms. *Scientific Reports*, *10*(1), 1383. https://doi.org/10.1038/s41598-020-58395-8

Guo, Z., Xu, H., Lei, Q., Du, J., Li, C., Wang, C., Yang, Y., Yang, Y., & Sun, X. (2020b). The *Arabidopsis* transcription factor *LBD15* mediates ABA signaling and tolerance of water-deficit stress by regulating *ABI4* expression. *The Plant Journal*, *104*(2), 510–521. https://doi.org/10.1111/tpj.14942

Gupta, A., Rico-Medina, A., & Caño-Delgado, A. I. (2020). The physiology of plant responses to drought. *Science*, *368*(6488), 266–269. https://doi.org/10.1126/science.aaz7614

Hong, Y., Wang, Z., Liu, X., Yao, J., Kong, X., Shi, H., & Zhu, J. K. (2020). Two chloroplast proteins negatively regulate plant drought resistance through separate pathways. *Plant Physiology*, *182*(2), 1007–1021. https://doi.org/10.1104/pp.19.01106

Huerta-Cepas, J., Szklarczyk, D., Heller, D., Hernández-Plaza, A., Forslund, S. K., Cook, H., Mende, D. R., Letunic, I., Rattei, T., Jensen, L. J., von Mering, C., & Bork, P. (2019). eggNOG 5.0: A hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. *Nucleic Acids Research*, *47*(D1), D309–D314. https://doi.org/10.1093/nar/gky1085

Jiao, P., Jiang, Z., Wei, X., Liu, S., Qu, J., Guan, S., & Ma, Y. (2022a). Overexpression of the homeobox-leucine zipper protein *ATHB-6* improves the drought tolerance of maize (*Zea mays* L.). *Plant Science*, *316*, 111159. https://doi.org/10.1016/j.plantsci.2021.111159

Jiao, P., Wei, X., Jiang, Z., Liu, S., Guan, S., & Ma, Y. (2022b). *ZmLBD2* a maize (*Zea mays* L.) lateral organ boundaries domain (*LBD*) transcription factor enhances drought tolerance in transgenic *Arabidopsis thaliana*. *Frontiers in Plant Science*, *13*, 1000149. https://doi.org/10.3389/fpls.2022.1000149

Jin, J., Tian, F., Yang, D-C., Meng, Y-Q., Kong, L., Luo, J., & Gao, G. (2017). Plant TFDB 4.0: Toward a central hub for transcription factors and regulatory interactions in plants. *Nucleic Acids Research*, *45*(D1), D1040–D1045. https://doi.org/10.1093/nar/gkw982

Kanehisa, M., Furumichi, M., Sato, Y., Kawashima, M., & Ishiguro-Watanabe, M. (2023). KEGG for taxonomy-based analysis of pathways and genomes. *Nucleic Acids Research*, *51*(D1), D587–D592. https://doi.org/10.1093/nar/gkac963

Khor, J. F., Ling, L., Yusop, Z., Tan, W. L., Ling, J. L., & Soo, E. Z. X. (2021). Impact of *El Niño* on oil palm yield in Malaysia. *Agronomy*, *11*(11), 2189. https://doi.org/10.3390/agronomy11112189

Kongdin, M., Mahong, B., Lee, S-K., Shim, S-H., Jeon, J-S., & Ketudat Cairns, J. R. (2021). Action of multiple rice β-glucosidases on abscisic acid glucose ester. *International Journal of Molecular Sciences*, *22*(14), 7593. https://doi.org/10.3390/ijms22147593

Leão, A. P., Bittencourt, C. B., Da Silva, T. L. C., Neto, J. C. R., De Oliveira Braga, Í., Vieira, L. R., De Aquino Ribeiro, J. A., Abdelnur, P. V., De Sousa, C. A. F., & Júnior, M. T. S. (2022). Insights from a multi-omics integration (MOI) study in oil palm (*Elaeis guineensis* Jacq.) response to abiotic stresses: Part Two – Drought. *Plants*, *11*(20), 2786. https://doi.org/10.3390/plants11202786

Leng, P., & Zhao, J. (2020). Transcription factors as molecular switches to regulate drought adaptation in maize. *Theoretical and Applied Genetics*, *133*(5), 1455–1465. https://doi.org/10.1007/s00122-019-03494-y

Li, Y., Yang, Z., Zhang, Y., Guo, J., Liu, L., Wang, C., Wang, B., & Han, G. (2022). The roles of HD-ZIP proteins in plant abiotic stress tolerance. *Frontiers in Plant Science*, *13*, 1027071. https://doi.org/10.3389/fpls.2022.1027071

Liao, Y., Smyth, G. K., & Shi, W. (2014). featureCounts: An efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics*, *30*(7), 923–930. https://doi.org/10.1093/bioinformatics/btt656

Liu, Q., Dong, G., Ma, Y., Zhao, S., Liu, X., Li, X., Li, Y., & Hou, B. (2021). Rice glycosyltransferase gene *UGT85E1* is involved in drought stress tolerance through enhancing abscisic acid response. *Frontiers in Plant Science*, *12*, 790195. https://doi.org/10.3389/fpls.2021.790195

Liu, Q., Luo, L., Wang, X., Shen, Z., & Zheng, L. (2017). Comprehensive analysis of rice laccase gene (*OsLAC*) family and ectopic expression of *OsLAC10* enhances tolerance to copper stress in *Arabidopsis*. *International Journal of Molecular Sciences*, *18*(2), 209. https://doi.org/10.3390/ijms18020209

McCarthy, D. J., Chen, Y., & Smyth, G. K. (2012). Differential expression analysis of multifactor

RNA-Seq experiments with respect to biological variation. *Nucleic Acids Research*, *40*(10), 4288–4297. https://doi.org/10.1093/nar/gks042

Monti, S., Tamayo, P., Mesirov, J., & Golub, T. (2003). Consensus clustering: A resampling-based method for class discovery and visualization of gene expression microarray data. *Machine Learning*, *52*(1/2), 91–118. https://doi.org/10.1023/A:1023949509487

Opassiri, R., Pomthong, B., Onkoksoong, T., Akiyama, T., Esen, A., & Cairns, J. R. K. (2006). Analysis of rice glycosyl hydrolase family 1 and expression of *Os4bglu12* β-glucosidase. *BMC Plant Biology*, *6*(1), 33. https://doi.org/10.1186/1471-2229-6-33

Qian, Y., Zhang, T., Yu, Y., Gou, L., Yang, J., Xu, J., & Pi, E. (2021). Regulatory mechanisms of bHLH transcription factors in plant adaptive responses to various abiotic stresses. *Frontiers in Plant Science*, *12*, 677611. https://doi.org/10.3389/fpls.2021.677611

Qin, F., Kakimoto, M., Sakuma, Y., Maruyama, K., Osakabe, Y., Tran, L-S. P., Shinozaki, K., & Yamaguchi-Shinozaki, K. (2007). Regulation and functional analysis of *ZmDREB2A* in response to drought and heat stresses in *Zea mays* L.: *ZmDREB2A* in drought and heat stress response. *The Plant Journal*, *50*(1), 54–69. https://doi.org/10.1111/j.1365-313X.2007.03034.x

Ramírez, F., Ryan, D. P., Grüning, B., Bhardwaj, V., Kilpert, F., Richter, A. S., Heyne, S., Dündar, F., & Manke, T. (2016). deepTools2: A next generation web server for deep-sequencing data analysis. *Nucleic Acids Research*, *44*(W1), W160–W165. https://doi.org/10.1093/nar/gkw257

Salgado, F. F., Da Silva, T. L. C., Vieira, L. R., Silva, V. N. B., Leão, A. P., Costa, M. M. D. C., Togawa, R. C., De Sousa, C. A. F., Grynberg, P., & Souza, M. T. (2022). The early response of oil palm (*Elaeis guineensis* Jacq.) plants to water deprivation: Expression analysis of miRNAs and their putative target genes, and similarities with the response to salinity stress. *Frontiers in Plant Science*, *13*, 970113. https://doi.org/10.3389/fpls.2022.970113

Sanusi, N. S. N. M., Rosli, R., Halim, M. A. A., Chan, K. L., Nagappan, J., Azizi, N., Amiruddin, N., Tatarinova, T. V., & Low, E-T. L. (2018). PalmXplore: Oil palm gene database. *Database*, *2018*, bay095. https://doi.org/10.1093/database/bay095

Shani, E., Salehin, M., Zhang, Y., Sanchez, S. E., Doherty, C., Wang, R., Mangado, C. C., Song, L., Tal, I., Pisanty, O., Ecker, J. R., Kay, S. A., Pruneda-Paz, J., & Estelle, M. (2017). Plant stress tolerance requires auxin-sensitive Aux/IAA transcriptional repressors. *Current Biology*, *27*(3), 437–444. https://doi.org/10.1016/j.cub.2016.12.016

Shumate, A., Wong, B., Pertea, G., & Pertea, M. (2022). Improved transcriptome assembly using a hybrid of long and short reads with StringTie. *PLOS Computational Biology*, *18*(6), e1009730. https://doi.org/10.1371/journal.pcbi.1009730

Suharyanti, N. A., Mizuno, K., & Sodri, A. (2020). The effect of water deficit on inflorescence period at palm oil productivity on peatland. *E3S Web of Conferences*, *211*, 05005. https://doi.org/10.1051/e3sconf/202021105005

Tamburino, R., Vitale, M., Ruggiero, A., Sassi, M., Sannino, L., Arena, S., Costa, A., Batelli, G., Zambrano, N., Scaloni, A., Grillo, S., & Scotti, N. (2017). Chloroplast proteome response to drought stress and recovery in tomato (*Solanum lycopersicum* L.). *BMC Plant Biology*, *17*(1), 40. https://doi.org/10.1186/s12870-017-0971-0

Wang, L., Lee, M., Ye, B., & Yue, G. H. (2020). Genes, pathways and networks responding to drought stress in oil palm roots. *Scientific Reports*, *10*(1), 21303. https://doi.org/10.1038/s41598-020-78297-z

Wang, S., Bai, Y., Shen, C., Wu, Y., Zhang, S., Jiang, D., Guilfoyle, T. J., Chen, M., & Qi, Y. (2010). Auxin-related gene families in abiotic stress response in *Sorghum bicolor*. *Functional & Integrative Genomics*, *10*(4), 533–546. https://doi.org/10.1007/s10142-010-0174-3

Woittiez, L. S., Van Wijk, M. T., Slingerland, M., Van Noordwijk, M., & Giller, K. E. (2017). Yield gaps in oil palm: A quantitative review of contributing factors. *European Journal of Agronomy*, *83*, 57–77. https://doi.org/10.1016/j.eja.2016.11.002

Wu, M., He, W., Wang, L., Zhang, X., Wang, K., & Xiang, Y. (2023). *PheLBD29*, an *LBD* transcription factor from Moso bamboo, causes leaf curvature and enhances tolerance to drought stress in transgenic *Arabidopsis*. *Journal of Plant Physiology*, *280*, 153865. https://doi.org/10.1016/j.jplph.2022.153865

Wu, Y., Li, X., Zhang, J., Zhao, H., Tan, S., Xu, W., Pan, J., Yang, F., & Pi, E. (2022). ERF subfamily transcription factors and their function in plant responses to abiotic stresses. *Frontiers in Plant*

Science, *13*, 1042084. https://doi.org/10.3389/fpls.2022.1042084

Xie, Z., Nolan, T. M., Jiang, H., & Yin, Y. (2019). AP2/ERF Transcription factor regulatory networks in hormone and abiotic stress responses in *Arabidopsis*. *Frontiers in Plant Science*, *10*, 228. https://doi.org/10.3389/fpls.2019.00228

Yang, C., Huang, Y., Lv, P., Antwi-Boasiako, A., Begum, N., Zhao, T., & Zhao, J. (2022). NAC transcription factor *GmNAC12* improved drought stress tolerance in soybean. *International Journal of Molecular Sciences*, *23*(19), 12029. https://doi.org/10.3390/ijms231912029

Yu, G., Wang, L-G., Han, Y., & He, Q-Y. (2012). clusterProfiler: An R package for comparing biological themes among gene clusters. *OMICS: A Journal of Integrative Biology*, *16*(5), 284–287. https://doi.org/10.1089/omi.2011.0118

Zang, D., Wang, L., Zhang, Y., Zhao, H., & Wang, Y. (2017). *ThDof1.4* and *ThZFP1* constitute a transcriptional regulatory cascade involved in salt or osmotic stress in *Tamarix hispida*. *Plant Molecular Biology*, *94*(4-5), 495–507. https://doi.org/10.1007/s11103-017-0620-x

Zhan, J., Li, G., Ryu, C-H., Ma, C., Zhang, S., Lloyd, A., Hunter, B. G., Larkins, B. A., Drews, G. N., Wang, X., & Yadegari, R. (2018). Opaque-2 regulates a complex gene network associated with cell differentiation and storage functions of maize endosperm. *Plant Cell*, *30*(10), 2425–2446. https://doi.org/10.1105/tpc.18.00392

Zhao, P., Wang, D., Wang, R., Kong, N., Zhang, C., Yang, C., Wu, W., Ma, H., & Chen, Q. (2018). Genome-wide analysis of the potato Hsp20 gene family: Identification, genomic organization and expression profiles in response to heat stress. *BMC Genomics*, *19*(1), 61. https://doi.org/10.1186/s12864-018-4443-1

Zhao, Y., Ma, Q., Jin, X., Peng, X., Liu, J., Deng, L., Yan, H., Sheng, L., Jiang, H., & Cheng, B. (2014). A novel maize homeodomain-leucine zipper (HD-Zip)I gene, *Zmhdz10*, positively regulates drought and salt tolerance in both rice and *Arabidopsis*. *Plant and Cell Physiology*, *55*(6), 1142–1156. https://doi.org/10.1093/pcp/pcu054

Zhu, J., Zhang, H., Huang, K., Guo, R., Zhao, J., Xie, H., Zhu, J., Gu, H., Chen, H., Li, G., Wei, C., & Liu, S. (2023). Comprehensive analysis of the laccase gene family in tea plant highlights its roles in development and stress responses. *BMC Plant Biology*, *23*(1), 129. https://doi.org/10.1186/s12870-023-04134-w

Zou, X., & Sun, H. (2023). DOF transcription factors: Specific regulators of plant biological processes. *Frontiers in Plant Science*, *14*, 1044918. https://doi.org/10.3389/fpls.2023.1044918