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CONSERVED TRANSCRIPTIONAL RESPONSES TO SALT AND WATER STRESS IN OIL PALM (*Elaeis guineensis* Jacq.) LEAF TISSUES

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ABSTRACT

*Abiotic stresses negatively impact oil palm (*Elaeis guineensis *Jacq.) cultivation. Transcription factors (TFs) are essential proteins regulating gene expression in plants. While numerous TF gene families are implicated in plant responses to abiotic stress, their specific roles in oil palm's drought and salinity responses remain largely unexplored. This study aimed to get insights into the TFs' involvement in the oil palm's response to these stresses. Twenty TF genes, classified into four families (MYB, HD-ZIP, NF-Y and HSFF) and exhibiting strong co-expression under both salinity and drought conditions, were selected for in-depth analysis. Such behaviour suggests a shared regulatory mechanism for abiotic stress responses. Promoter analysis revealed the presence of known stress-responsive *cis*-regulatory elements, while sequence homology analysis indicated functional conservation with stress-tolerance genes in other plants. Quantitative real-time PCR (qRT-PCR) validated RNA-Seq expression profiles for 19 of the 20 selected genes. While these findings offer promising avenues for genetic modification, a cautious approach is necessary. Understanding the complex regulatory networks of these TFs and their potential side effects is crucial to avoid unintended consequences. Future study should focus on the functional validation of these TFs and integrate this knowledge with potential inherent tolerance mechanisms to develop resilient oil palm cultivars.*

Keywords: abiotic stress, drought, salinity, tolerance, transcriptome.

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INTRODUCTION

Palm oil reigns supreme as the world's leading vegetable oil, with consumption in 2022-2023 reaching a staggering 85 million tonnes. This dwarfs the output of soybean (60.0 million tonnes), rapeseed (31.0 million tonnes) and sunflower seed oil (18.5 million tonnes) (Statista, 2024). Despite possessing millions of hectares suitable for oil palm (*Elaeis guineensis* Jacq.) cultivation, Brazil's 2022 harvest only reached around 200,000 ha, ranking it 10th globally and contributing less than 1% of the world's production (Foreign Agricultural Series-U.S. Department of Agriculture [FAS-USDA], 2024). A whopping 93% of its plantations are concentrated within the Legal Amazon Area, primarily in the Northeastern region of the Pará State (Instituto Brasileiro de Georgrafia e Estatistica [IBGE], 2024). Expanding oil palm cultivation in Brazil presents a dilemma: Continue operating within the Amazon Biome, with its abundant year-round rainfall, or venture into drier climates that present significant challenges.

Oil palm thrives in constant moisture, but Brazil's Midwest and Northeast regions experience extended droughts. Therefore, irrigation is

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required for oil palm to succeed in these regions. These trees require around 2,000 mm of water annually and become vulnerable after just 90 days without water (Corley *et al.*, 2018). The Cerrado Savanna in Brazil's Midwest have dry winters, and the Northeast is semi-arid, leading to water scarcity and a concerning trend: Soil salinisation. As water evaporates, salts accumulate in the soil, harming plants and threatening sustainability. This isn't unique to Brazil; globally, 30% of irrigated land suffers from salinity, with projections reaching over 50% by 2050 (Ivushkin *et al.*, 2019; Machado & Serralheiro, 2017; Shahid *et al.*, 2018; Zaman *et al.*, 2018).

Plants exhibit remarkable adaptive capabilities in response to abiotic stresses such as drought and salinity, primarily mediated by the intricate gene regulatory networks operating at the transcriptional and post-transcriptional levels (Angon *et al*., 2022; Wang *et al*., 2022). Transcription factors (TFs), constituting a substantial portion of the plant genome (7%-10%) (Wang *et al*., 2019), function as pivotal regulators in orchestrating plant stress responses (Zhang *et al*., 2022). These proteins, organized into diverse families, regulate the expression of downstream genes to enhance stress tolerance (Javed *et al*., 2020; Wang *et al*., 2022). Specifically, stress-inducible transcription factors from the MYB, WRKY, bZIP and AP2/ERF families are known to be pivotal for plant resilience against various environmental stresses (Chaudhari *et al*., 2023; Hajyzadeh *et al*., 2015; He *et al*., 2018;). Notably, the HD-Zip family, a large and plant-specific group of TFs, has been implicated in both plant growth and stress responses (Belamkar *et al*., 2014; Bhattacharjee *et al*., 2016; Gong *et al*., 2019; Sharif *et al*., 2021). Overexpression of HD-Zip TFs has been shown to enhance drought tolerance (Dezar *et al*., 2005; Gong *et al*., 2019; Jiao *et al*., 2022) and confer resistance to combined drought and salt stress conditions (Zhao *et al*., 2014).

Building on the work of Vieira *et al.* (2020) and Salgado *et al.* (2021, 2022), this study further investigates the transcriptomic response of young oil palms to abiotic stresses. Their large-scale analyses using miRNA and RNA-Seq elucidated responses to high salinity and water deprivation, respectively. This study focuses on the crucial role of TF in early stress responses. By comprehensively analysing all differentially expressed TF genes, not just those targeted by miRNAs, it expands on these prior works. This approach provides a more complete understanding of TF families and their constituent genes commonly affected by both stresses. Twenty TF genes representing distinct families were chosen for structural and functional annotation, along with their promoter sequences. Subsequent qRT-PCR analysis validates their expression patterns under both salinity and water deficit conditions.

MATERIALS AND METHODS

RNA-Seq Data Generation

The raw RNA sequencing (RNA-Seq) data used in this study are publicly available in the Sequence Read Archive (SRA) database at the National Center for Biotechnology Information (NCBI) under BioProject ID PRJNA573093 and BioSample ID SAMN12799239.

For details on plant material, growth conditions and stress treatments, refer to Bittencourt *et al.* (2022), Leão *et al.* (2022), Salgado *et al.* (2021, 2022) and Vieira *et al.* (2020). In brief, experiments were conducted in a greenhouse at Embrapa Agroenergia, Brasília, DF, Brazil (S-15.732°, W-47.900°). Young bifid saplings received the treatments. Oil palm clones, regenerated from embryogenic callus derived from adult AM33 genotype leaves, were used. Apical leaves from control and stressed plants were collected after 12 days of salinity stress or 14 days of drought stress. Salinity treatments included a control (0.0 g NaCl/100 g substrate) and a stressed group (2.0 g NaCl/100 g substrate). Drought treatments consisted of a control (field capacity) and a stressed group (water withheld). Three replicates were collected per treatment. Plant tissue was immediately frozen in liquid nitrogen and stored at -80°C until RNA extraction, library preparation, and sequencing. Total RNA isolation, quality and quantity analysis followed the methods described by Salgado *et al.* (2021, 2022). RNA sequencing (RNA-Seq) was outsourced to GenOne Company (Rio de Janeiro, RJ, Brazil) using an Illumina HiSeq platform with a paired-end strategy.

RNA-Seq Data Analysis

RNA-Seq analyses, performed using OmicsBox version 1.3, are detailed in Salgado *et al.* (2021, 2022). Briefly, high-quality RNA-Seq reads were mapped to the oil palm reference genome (Singh *et al.*, 2013) downloaded from NCBI (BioProject PRJNA192219; BioSample SAMN02981535) in March and October 2020. The mapping used the default settings of STAR software (Dobin *et al.*, 2013) within OmicsBox version 1.3. Gene expression levels were quantified using HTSeq v0.9.0 (Anders *et al.*, 2015) with default parameters within OmicsBox v1.3. Differential expression analysis between control and stressed samples was performed using edgeR v3.28.0 (Robinson *et al.*, 2010). This study employed a simple experimental design with an exact statistical test in edgeR. No filters for low-count genes were applied.

Genes Selection and Annotation

This study unveils TF potentially critical for stress response in young oil palm. Building upon the work of Salgado *et al.* (2021, 2022) who identified differentially expressed genes (DEGs) through functional annotation using GhostKOALA (Kanehisa *et al*., 2016), this study employed a multi-step approach to pinpoint TF potentially involved in the young oil palm's stress response: a) Relevant scientific literature was reviewed to identify key plant transcription factor families associated with stress responses; b) the DEGs were filtered to retain only those belonging to the pre-selected TF families; c) this list was further refined by selecting DEGs that exhibited differential expression under both salinity and drought stress conditions; and d) finally, genes displaying the most significant positive or negative regulation in response to stress were prioritised for further analysis.

Intron and exon data were retrieved from National Center for Biotechnology Information (NCBI) (Sayers *et al*., 2022). Promoter sequences (1,432 bp upstream of ATG) for each gene were extracted from the oil palm reference genome (Singh *et al*., 2013). The PlantCARE database (Lescot, 2022) identified known *cis*-elements with a significance threshold (Score ≤ 5). Additionally, MEME software (Bailey *et al*., 2015) identified potential TF binding motifs within promoters, retaining only those with a statistically significant E-value ≤ 0.05.

Protein sequences were retrieved from the oil palm reference genome (Singh *et al*., 2013). Only statistically significant domains (E-value ≤ 1) were retained after analysis using NCBI Conserved Domain Search. Functional annotation was performed using EggNOG (Huerta-Cepas *et al*., 2019). Multiple sequence alignments (protein and promoter regions) were generated with ClustalW (Thompson *et al*., 1994). Phylogenetic trees were constructed using the maximum likelihood method with 1,000 bootstrap replicates in MEGA11 (Tamura *et al*., 2021). DNA Features Viewer (Zulkower & Rosser, 2020) generated figures for cis-element analysis results (motifs), protein domains and intron/exon regions.

RNA Extraction, Reverse Transcriptase-PCR and Quantitative Real-Time PCR Analyses

Total RNA isolation, quality and quantity analyses were performed as described by Salgado *et al.* (2021, 2022). Following quantification, the isolated RNA was used as a template for cDNA synthesis using the SuperScript® IV Reverse Transcriptase kit (Invitrogen) according to the manufacturer's instructions. The positive control gene was *EgEfMPOB00119*, encoding the constitutive 40S ribosomal protein S23 mRNA in *E. guineensis*.

FASTA sequences for the selected genes were downloaded from NCBI and imported into PerlPrimer software for primer design. qRT-PCR reactions were performed in 96-well plates on a 7,500 real-time PCR system (Applied Biosystems) using PowerUp™ SYBR™ Green Master Mix (Thermo Fisher Scientific) according to the manufacturer's protocol. The reaction conditions were: 50°C for 2 min, 95°C for 2 min, followed by 40 cycles of 95°C for 5 s and 60°C for 30 s. A melting curve analysis was performed to confirm amplicon specificity. Three biological replicates were performed for each sample. The expression level of the untreated control sample was set to 1 for normalisation. The qRT-PCR data analysis was done using the $2^{-\Delta\Delta CT}$ method (Rao *et al*., 2013).

Pair-by-Pair Comparison and Correlation Analysis

A pairwise correlation analysis was performed to compare gene expression changes identified by RNA-Seq and qRT-PCR for DEGs under both salinity and drought stress conditions. Log$_2$ (fold change) values from each method were used as input data for the analysis.

RESULTS AND DISCUSSION

Although roots are the primary sensors of salinity and drought stress, this study prioritised leaf tissue for molecular analysis due to the challenges associated with root sample preparation and the initial focus on morphophysiological parameters. Despite the potential for distinct responses in roots, the presented findings offer valuable insights into the molecular mechanisms underlying leaf adaptation to these abiotic stresses.

Selection of Putative Transcription Factors Responsive to Drought and Salinity Stresses

Employing the GhostKOALA annotation platform (Kanehisa *et al*., 2016), the current study identified 36 and 19 TF families exhibiting differential expression in response to drought and salinity stress, respectively. Eighteen of these families were commonly affected by both stress conditions (data not shown). Building on previous identification of stress-responsive miRNAs potentially regulating TFs (Salgado *et al*., 2021, 2022), as well as in the transcriptome profiles of oil palm under both stresses (Bittencourt *et al*., 2022; Leão *et al*., 2022), a subset of 20 DEGs from four TF families was selected for further analysis (*Table 1*).

TABLE 1. PROFILE OF TRANSCRIPTION FACTOR (TF) GENES FROM THE LEAF OF OIL PALM PLANTS DIFFERENTIALLY EXPRESSED UNDER SALT AND WATER STRESS ACCORDINGLY TO RNA-Seq AND qPCR ANALYSES, FOLD CHANGE (FC), LOG$_2$ (FC) AND FALSE DISCOVERY RATE (FDR)

Gene #	Gene ID	Protein ID	TF family	Salt stress			Drought stress		
				RNA-Seq		qPCR	RNA-Seq		qPCR
				Log_2 (FC)	FDR	Log_2 (FC)	Log_2 (FC)	FDR	Log_2 (FC)
01	LOC105032141	XP_010904805.1	MYB	2,064	0,000	1,895	5,650	0,000	3,113
02	LOC105033230	XP_010906239.1	MYB	-3,259	0,000	-5,059	-4,404	0,000	-5,059
03	LOC105038205	XP_010912232.1	HSF	-2,665	0,000	-1,184	-2,762	0,000	-0,971
04	LOC105039680	NP_001306836.1	NFY-C	2,121	0,000	-0,943	4,228	0,000	-1,690
05	LOC105040563	XP_010915439.1	HSF	1,213	0,000	1,459	3,530	0,000	1,803
06	LOC105040719	XP_010915677.1	MYB	-3,141	0,000	-1,358	-2,490	0,000	-2,059
07	LOC105041192	XP_010916352.1	MYB	-2,226	0,000	-2,556	-1,629	0,000	-1,152
08	LOC105044046	XP_010920127.1	MYB	-2,454	0,000	-3,322	-2,430	0,000	-2,322
09	LOC105048425	XP_010926024.1	MYB	2,817	0,000	3,276	4,536	0,000	2,759
10	LOC105048648	XP_010926338.1	HD-ZIP	-0,832	0,016	-0,737	-3,068	0,000	-2,396
11	LOC105048799	XP_010926549.1	MYB	0,831	0,029	1,485	3,214	0,000	2,373
12	LOC105053150	XP_019708739.1	HD-ZIP	1,405	0,027	1,070	-1,798	0,007	-1,690
13	LOC105053200	XP_010932583.1	MYB	1,823	0,001	1,844	-1,552	0,001	-1,737
14	LOC105053827	XP_010933430.1	MYB	1,455	0,022	0,774	3,319	0,000	0,748
15	LOC105053827	XP_010933626.2	HD-ZIP	1,127	0,024	1,736	2,140	0,000	1,118
16	LOC105055678	XP_010935898.1	MYB	3,378	0,000	2,134	3,974	0,000	1,915
17	LOC105056442	XP_010936941.1	MYB	-4,104	0,000	-5,059	-2,942	0,000	-4,059
18	LOC105056443	XP_010936942.1	MYB	-1,926	0,002	-2,252	-2,513	0,000	-3,644
19	LOC105058320	XP_010939500.1	MYB	1,904	0,000	1,220	2,387	0,000	1,480
20	LOC105058124	XP_010939223.1	NFY-B	-0,746	0,002	-0,059	-0,908	0,000	-0,644

Note: Protein (Super)Families: MYB - Myb transcription factor, plants (IPR015495); HD-ZIP - Homeobox-leucine zipper protein, class I, plant (IPR045224); NFY - Histone Fold (IPR009072) - Transcription factor CBF/NF-Y/archaeal histone domain (IPR003958); HSF - Heat Shock Transcrition Factor (PTHR10015).

This subset comprised 13 MYB (Ambawat *et al.*, 2013), three HD-ZIP (Chen *et al.*, 2014), two HSFs (Guo *et al.*, 2016), two NF-Y from Histone Fold Motif family (IPR009072) members (Stephenson *et al.*, 2007). Salinity stress significantly upregulated eleven genes and downregulated nine, while drought stress upregulated nine genes and downregulated eleven (*Table 1*). A total of 18 of the 20 genes exhibited similar qualitative responses to both stresses. However, gene 13 and 14 displayed contrasting qualitative responses, being upregulated under salt stress and downregulated under drought stress.

Soil salinity and water deficit, imposing severe constraints on crop growth, constitute significant environmental challenges (Qin *et al.*, 2011). In response, plants activate intricate signalling cascades, encompassing both abscisic acid (ABA)-dependent and -independent pathways (Yamaguchi-Shinozaki *et al.*, 2007). Transcription factors serve as pivotal regulators within these pathways, modulating stress tolerance. These proteins orchestrate the expression of genes involved in osmotic homeostasis, ionic balance and other stress mitigation processes, thereby contributing to overall plant resilience (Ni *et al.*, 2013; Xu *et al.*, 2015).

Differential Expression and Correlation Analyses

To further characterise the response of the 20 selected genes to abiotic stress, their expression levels were quantified using qRT-PCR. Primer sequences are provided in *Table 2*. qRT-PCR analysis revealed significant upregulation of gene 1, 5, 9 and 11 under water deprivation stress, with the remaining genes showing downregulation (*Figure 1*). Similarly, under salt stress, genes 1, 5, 9, 11-16 and 19 exhibited upregulation, while genes 2-4, 6-8, 10, 17, 18 and 20 displayed downregulation (*Figure 1*).

Gene expression patterns showed a high correlation ($R^2 = 0.7317$) between salt and drought stress (*Figure 2*), indicating similar responses for most genes. However, gene 12 and 13 displayed contrasting regulation. These findings align with prior studies (Hazzouri *et al.*, 2020; Li *et al.*, 2019;

TABLE 2. PAIRS OF PRIMERS USED FOR DIFFERENTIAL EXPRESSION ANALYSIS USING THE qRT-PCR TECHNIQUE OF THE 20 PROSPECTED GENES

Gene #	Forward primer	Reverse primer
01	5′-GATAAGAATGGTCTGAAGAAGGG-3′	5′-CAATTGCTGACCACTTGTTCC-3′
02	5′-GGAGGACCAGAAACTTGTAGAC-3′	5′-ATTAGAGACCATCTGTTGCCC-3′
03	5′-TTTTCGCAAGATTGATGCTG-3′	5′-TCATGAGATGCTCCTGTTGC-3′
04	5′-GGTAGACCTCTCTGCATCG-3′	5′-CCTACCACCAGCTGAAAATACC-3′
05	5′-GCTCAACACCTATGGGTTTCG-3′	5′-ACCTTCTCTTCGATCTTCCTCTG-3′
06	5′-CACCACTTAAGATCCGTTGCC-3′	5′-GCTATCAAATCCCACCTGTCAC-3′
07	5′-ACGAGAAGCTCATTAACTACATCC-3′	5′-CTATCAATGACCACCGATTGCC-3′
08	5′-GGACCAAGGAGGAAGATGAC-3′	5′-AGAGACCATTTGTTGCCGAG-3′
09	5′-CTAAGTGCTCAGGCTTGAAGAG-3′	5′-GTCCTCCAGTAGTTCTTTATCTCG-3′
10	5′-TCGAGCGTGGTGTTTAATG-3′	5′-CGGAGCAGTACCAAGAGAGG-3′
11	5′-TTTAGGCAATAGGTGGGCAG-3′	5′-TATTGAGCTTCTCCACAGACAG-3′
12	5′-AGTCACAGTTCACAAGAGCAC-3′	5′-CATTCAGAGCACGAAGGAGAG-3′
13	5′-AGCTAGCAGGATTGCTTAGG-3′	5′-CTGGTCAACAGTTTCAGCG-3′
14	5′-CAAGGGTTCATGGACACCAG-3′	5′-TTCGACCATTTGTTTCCAAGC-3′
15	5′-GACACAAGCCATGATTGAAG-3′	5′-TAGCGAGCAACCCTTTTATCTC-3′
16	5′-CAAGCAAGCTGGTCTATTGAGG-3′	5′-CGGCTGGTTTGGATTCACTC-3′
17	5′-CTTCACTCTAGCGATATCTACCG-3′	5′-AACGTTTCAACCCTGCTCTC-3′
18	5′-CCATGTTGTTCAAAGGAGGG-3′	5′-GCAATTAGAGACCATCTGTTTCC-3′
19	5′-CCTACTAATACTGGGCTGATGAG-3′	5′-GGTGTTCCAATAGTTCTTGATGTC-3′
20	5′-GGTGGGAATTCTTCCTCTTC-3′	5′-TAATTCAGAGACCAACCCAACC-3′
ICG	5′-CCAGGGTTCAGCTGATTAAG-3′	5′-TCGTCCAAATCCAGCAATC-3′

Note: ICG - Internal control gene: *EgEfMPOB00119*.

Rasmussen *et al*., 2013; Zhu, 2002) suggesting a core set of genes involved in early responses to both stresses in oil palm.

Gene expression profiles (*Figure 3*) largely agreed between RNA-Seq and qRT-PCR, with one exception: Gene 4 showed positive regulation under both stresses in RNA-Seq but negative in qRT-PCR. This discrepancy highlights potential technique-specific variations. Further analysis using alternative methods or replicates may be needed to confirm gene 4's expression pattern under stress.

Annotation of the Promoter Region from the Genes Coding for the Putative TFs

Phylogenetic analysis based on the 1,432-nucleotide promoter sequences upstream of the start codon for each gene revealed four distinct groups among the 20 TFs (data not shown). However, these groupings did not correlate with the expression profiles of the genes under drought and salinity stress (*Table 1*). MEME analysis identified three conserved promoter motifs present in all 20 genes. Similar to the phylogenetic analysis, the distribution of these motifs did not exhibit any significant pattern within the four previously identified phylogenetic groups.

Analysis of the promoter regions using the PlantCARE database revealed the presence of seven putative cis-acting elements potentially associated with stress responses in the 20 selected TF genes. These elements included the ABA-responsive element (ABRE), stress-responsive element (STRE), motifs responsive to methyl jasmonate (MeJA) signalling (CGTCA-motif and TGACG-motif), the core promoter elements TATA-box and CAAT-box and the salicylic acid and auxin-responsive element as-1.

Consistent with their regulatory role, all 20 TF gene promoters contained key cis-elements. Notably, both CAAT-box and TATA-box elements were ubiquitous, suggesting their potential role in stress response. The CAAT-box, known for enhancing promoter activity, might contribute to increased expression under stress (Song *et al*., 2018). The TATA-box, often found in stress-responsive genes (Roelofs *et al*., 2010), could facilitate rapid and variable regulation under stress conditions (Yu *et al*., 2012).

Despite ubiquitous CAAT-box and TATA-box elements, TF promoters exhibited variation. Notably, *HSF* genes (3 & 5) possessed ABRE, as-1, and stress-related motifs (CGTCA, TGACG) alongside core elements. Interestingly, genes 2, 6-10 displayed all identified cis-elements (ABRE, STRE, as-1, CGTCA,

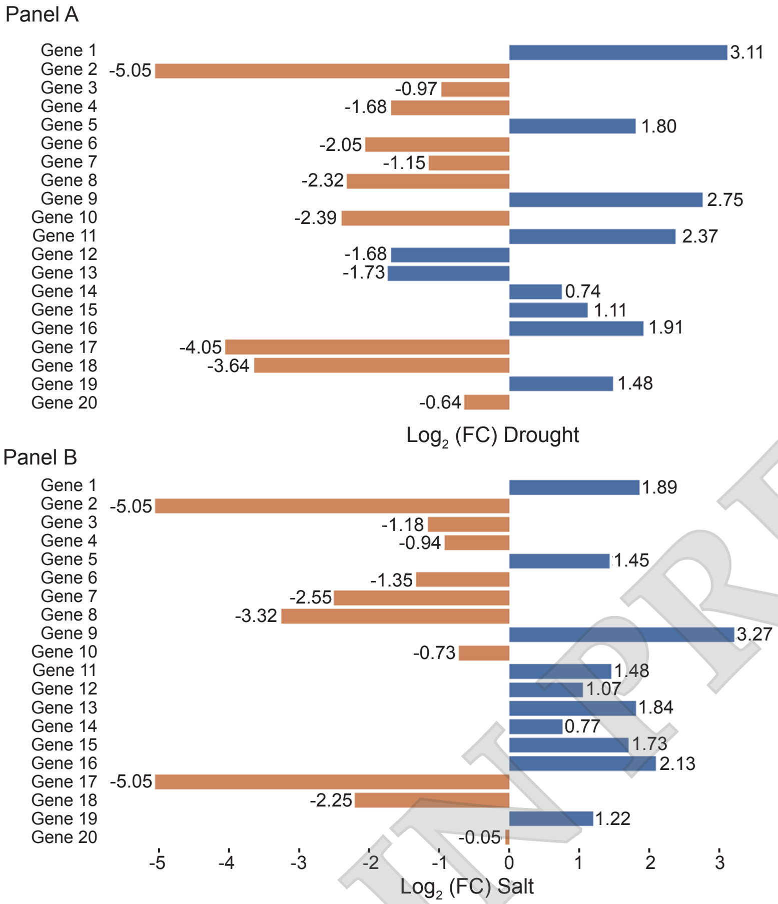

Figure 1. Log$_2$ (FC) in transcript abundance of 20 selected genes measured using qRT-PCR. Panel A depicts the results for young oil palm plants exposed to drought stress and panel B shows the results for plants exposed to saline stress. FC denotes fold change.

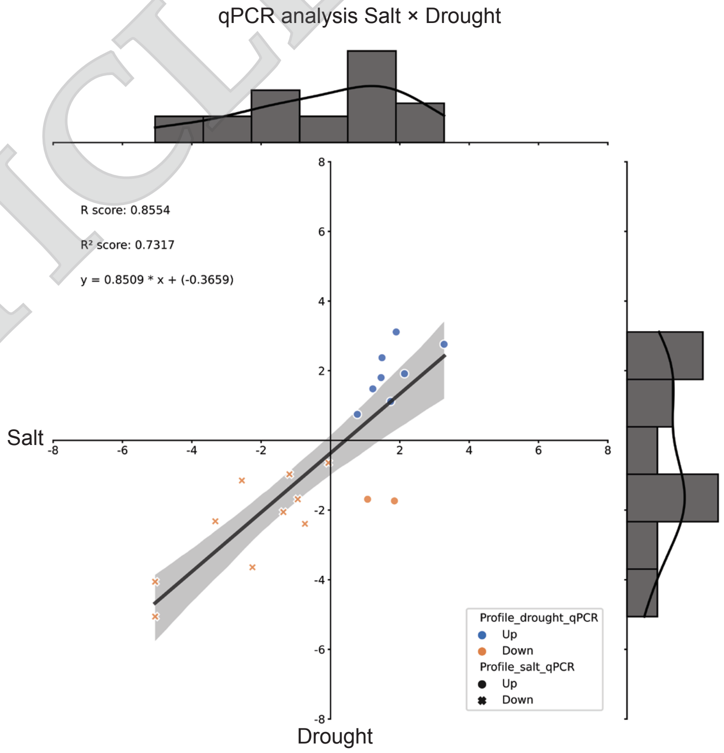

Figure 2. Log$_2$ (FC) correlation analysis of transcript abundance changes for the 20 selected genes identified through qRT-PCR. The analysis compares gene expression profiles under saline stress ("salt") and water stress ("drought"). FC denotes fold change.

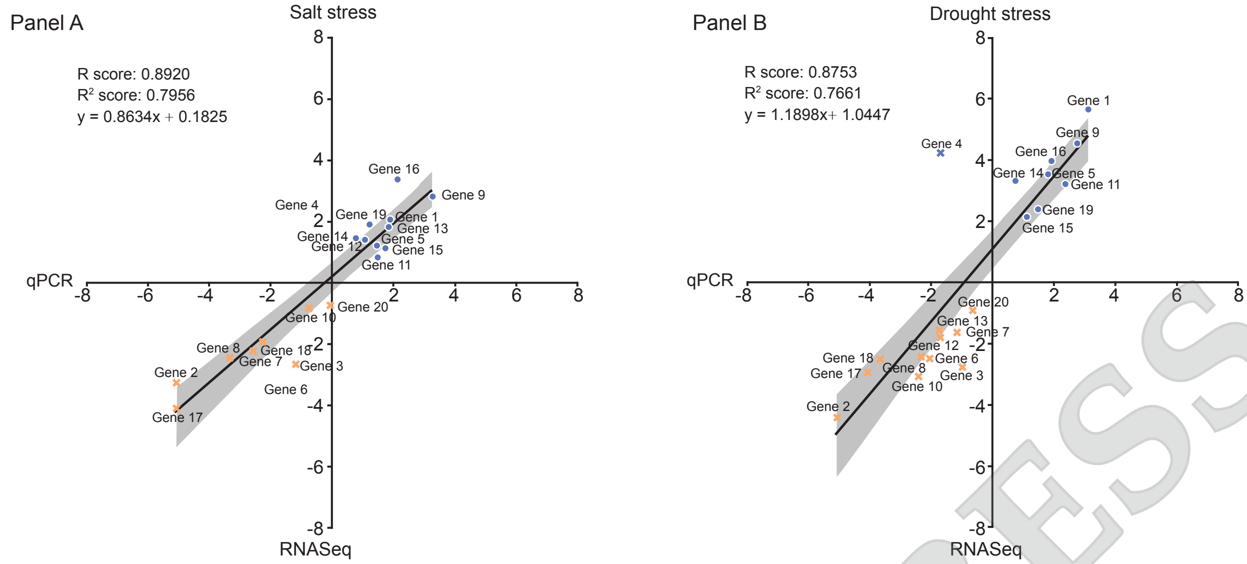

Figure 3. Correlation analysis of the Log$_2$(FC) for the 20 selected genes. Panel A compares FC in gene expression under saline stress and panel B compares under drought stress, as measured by qRT-PCR and RNA-Seq. FC denotes fold change.

TGACG) associated with ABA, heat/osmotic, salicylic acid and methyl jasmonate responses. While 18/20 genes showed similar expression under both stresses (validated by RNA-Seq and qRT-PCR), no correlation between cis-element number/position and expression patterns was observed.

Structural and Functional Annotation of the Coding Region of the Putative TFs

The 20 TF genes displayed coding sequence lengths varying up to 7,500 nucleotides (Gene 4) and intron numbers ranging from zero (Gene 20) to nine (Gene 12), with distinct intron-exon patterns for each TF family - egMYB: 2 introns; egHSFF: 1 intron; egNFY: 0-1 introns; egHD-ZIP: 2-9 introns (data not shown). Phylogenetic analysis based on protein sequences identified three clades (*Figure 4a*), with Gene 9 (*MYB*) forming a solitary clade, eight MYB proteins in a second clade and the remaining proteins from all TF families clustering within the third clade.

Arabidopsis studies revealed contrasting stress responses upon HSFA4A activation (tolerance) *vs.* inactivation (hypersensitivity) under salt stress (Pérez-Salamó *et al.*, 2014). Similarly, over-expression of lily *HSFA3* genes in *Arabidopsis led* to salt hypersensitivity (Wu *et al.*, 2018). The present study found a *HSFA3* (Gene 3) was negatively-regulated, while *HSFC2b* (Gene 5) was positively-regulated under drought and salinity stresses. This suggests potentially distinct roles for these HSFs in oil palm stress responses.

Analysis of conserved protein domains revealed 23 distinct types among the 19 stress-responsive proteins (excluding gene 6). Notably, PLN03091 and PLN03212 domains were consistently present in all *MYB TFs* (Genes 1, 2, 7-9, 11, 13, 14, 16-19; *Figure 4b*). REB1, Myb and SANT domains were also identified within these MYB proteins, but their distribution varied.

Analysis of conserved protein domains in the coding regions revealed distinct domain compositions for the identified stress-responsive proteins (*Figure 4b*). HSF proteins (Gene 3 & 5) possess HSF and DNA-binding domains, suggesting roles in heat stress response and gene activation. Proteins from Gene 4, 10, 12, 15 and 20 exhibited domains HOX, homeodomain, homeodomain superfamily, Homeobox, HHT1, HALZ, and H4, suggesting a potential role in developmental regulation. Additionally, the presence of domains like Histone superfamily, START, HAP5, HD-ZIP_N, START_Argleabra2_like and CBFD_NFYB_HMF highlights potential interactions with chromatin remodelling and other transcription factors. Notably, the HD-ZIP_N domain suggests membership in the HD-Zip family.

MEME analysis revealed seven motifs (8-50 amino acids) in 18/20 proteins (*Figure 4c*). The number of motifs per protein varied between one and five, with the proteins from genes 11 and 19 exhibiting the highest number of motifs. Motif 1, 2 and 4 consistently occurred within PLN03091/PLN03212 domains (Gene 1, 2, 7-9, *etc.*). Motif 5 was unique to HSFF proteins (Gene 3 & 5), while motif 6 was specific to potential HD-Zip family members (Gene 10, 12 & 15). Motif 3 had the broadest presence (9 proteins), while motif 7 was identified in only 3 (Gene 10, 11 & 19).

Analysis of conserved domains and motifs (*Figure 4b, 4c*) aligned with GhostKOALA's functional predictions (KO0300 – Transcription Factor group). Proteins within the same TF family

Description of motifs

Symbol	Motif Consensus
🟥	NYLRPDIKRGNFSEEEEDLIIKLHSLLGNRWSLIAGRLPGRTDNEIKNYW
🟦	KVGLKKGPWTPEEDKILISYIQAHGEGNWRALPKNAGLLRCGKSCRLRWT
🟩	MGRAPCCD
🟪	NTHJKKKLLKMGIDPKTH
🟨	FVVWDPFEFARALLPRNFKHRNFSSFVRQLNTYGFRKIDPDRWEFANEDF
🟩	DQIKTLEENFKEHNKLDPKQKLRLARDLGLQPRQVEIWFQNRRARWKAKQ
🟦	KGRWERRLQTDIDGAKQALCE

Note: HSF - Heat Shock Transcrition Factor; HD-ZIP_N - Homeodomain-leucine zipper; HOX - homeobox; START - StAR-related Lipid Transfer; HALZ - Homeobox associated leucine zipper; SANT - SWI3, ADA2, N-CoR and TFIIIB; REB1 - RNA polymerase I Enhancer Binding protein; HAP5 - Heme Activator Protein 5 subunit; SRPBCC - START/RHO_alpha_C/PITP/Bet_v1/CoxG/CalC; CBFD_NFYB_HMF - Histone-like transcription factor (CBF/NF-Y) and archaeal histone.

Figure 4. In silico Analysis of Stress-Responsive Protein Sequences. (A) Phylogenetic tree constructed using MEGA11 software. (B) Protein domain architecture. (C) Conserved motifs were identified within the amino acid sequences. Different colours represent distinct domains or motifs.

shared similar domain architectures and motif compositions. This consistency reinforces the *in-silico* annotation and suggests family-specific sequence features relevant to their regulatory roles.

GO term enrichment analysis revealed diverse functions for the 20 identified TFs. The associated GO terms spanned biological processes (87 terms), molecular functions (nine terms), and cellular components (four terms). Notably, some TFs had more assigned GO terms, suggesting greater functional complexity (*e.g.*, gene 14: 29 terms, Genes 9, 11, 19: 23 terms each). This highlights the potential for varied roles of these TFs in the oil palm's stress response.

Thirteen DEGs encode MYB TFs, known for diverse stress responses (Dubos *et al.*, 2010; Roy, 2016; Wang *et al.*, 2021). *MYB20* (Gene 13) showed contrasting expression: Salinity-induced (positive) *vs.* drought-repressed (negative). Gao *et al.* (2014) reported AtMYB20's negative role in ABA-mediated drought response, while Wang *et al.* (2021) linked its salinity tolerance to MYB TFs repressing ABA repressors (PP2Cs). *MYB30* (Gene 16) exhibited increased expression under both stresses. This TF regulates salt tolerance via AOX1s (mitochondrial alternative oxidase) and wax synthesis genes for cuticle reinforcement, protecting leaves from stress (Gong *et al.*, 2020; Lee & Suh, 2015; Wang *et al.*, 2021).

Two DEGs encode NF-Y family members. These hetero-trimeric TFs (NF-YA, NF-YB, NF-YC) bind to CCAAT boxes in promoters, regulating gene expression (Nelson *et al.*, 2007). Studies link NF-Y to abiotic stress responses (Leyva-González *et al.*, 2012; Li *et al.*, 2013; Kumar, 2014; Zhao *et al.*, 2014). Notably, NF-YA, a target of miR169, enhances salt tolerance in *Arabidopsis* (Li *et al.*, 2013).

The present study revealed differential expression of *NF-Y* subunit genes (NF-YB3, NF-

YC2) under both stresses. *NF-YB3* (Gene 20) showed negative regulation, while *NF-YC2* (gene 4) displayed positive regulation. Our prior work linked miR169 to NF-Y regulation in oil palms under stress (Salgado *et al.*, 2021, 2022). miR169 was down-regulated, while NF-YA protein increased only under salinity stress.

Leyva-González *et al.* (2012) proposed a model for NF-Y regulation under stress, postulating that stress induces NF-YA activation while concurrently decreasing miR169 levels. The findings of Salgado *et al.* (2021), demonstrating negative regulation of miR169 and positive regulation of NF-YA protein in oil palm leaves under salinity stress, align with this proposed model. Elevated NF-YA might form inactive complexes with NF-YB/YC subunits, potentially repressing stress response genes and acting as a negative feedback loop during prolonged stress. However, this model seems salinity-specific. Drought stress also reduces miR169 (Salgado *et al.*, 2022) but lacks the NF-YA protein increase, suggesting additional drought-specific regulatory mechanisms requiring further investigation.

Previous studies have indicated stress-specific functions for NF-Y subunits. *At*NF-YB1 in *Arabidopsis* and its maize orthologous *Zm*NF-YB2 have been shown to enhance drought tolerance (Nelson *et al.*, 2007), establishing them as potential markers for stress resilience. The present study revealed the upregulation of *NF-YC2* (Gene 4) under both salinity and drought conditions. This expression pattern aligns with the overexpression of *Pw*HAP5 (NF-YC2 homologue) in *Picea wilsonii*, which has been associated with increased stress tolerance (Li *et al.*, 2013), suggesting a potential positive role for NF-YC2 in oil palm stress responses.

Three DEGs (10, 12 and 15) encode HD-Zip TFs in the present study. HD-Zip TFs belong to a diverse plant superfamily involved in development and stress responses (Belamkar *et al.*, 2014; Gong *et al.*, 2019; Li et al., 2022; Sharif *et al.*, 2021). These Genes displayed contrasting expression: Gene 15 (positive under both stresses), Gene 10 (negative under both) and Gene 12 (positive under salinity, negative under drought). This suggests potential subfamily- or gene-specific roles for HD-Zip TFs in oil palm's abiotic stress responses.

Class I HD-Zip TFs are known for their positive roles in plant stress responses (Dezar *et al.*, 2005; Gong *et al.*, 2019; Jiao *et al.*, 2022; Zhao *et al.*, 2014). This study identified three DEGs from distinct subfamilies: HD-Zip I (Gene 10), HD-Zip II (Gene 15) and HD-Zip IV (Gene 12). Their contrasting expression patterns (Gene 10: Negative under both stresses, Gene 15: Positive under both, Gene 12: Positive under salinity, negative under drought) suggest potential subfamily-specific roles in oil palm's abiotic stress responses.

Two DEGs encode HSFs, key regulators in plant stress responses (Guo *et al.*, 2016). They induce heat-shock proteins (HSPs) and activate stress-responsive genes (Ohama *et al.*, 2017). HSFs respond to various stresses, including heat, drought, cold and salt (Li *et al.*, 2019). There are three HSF classes: A (activators), B (repressors) and C (unknown function) (Ohama *et al.*, 2017; Wu *et al.*, 2018).

At last, understanding a gene's regulatory influence is crucial. For example, consider Gene 15, an oil palm HD-Zip II gene, positively regulated under both stresses, which silencing using techniques like RNAi or CRISPR-Cas9 could be a promising strategy for engineering tolerance. Does it have a widespread regulatory role, affecting dozens or hundreds of genes under stress conditions, or is its influence limited to just a few target genes? Additionally, how many regulatory factors control Gene 15 itself? These questions are vital because manipulating genes with extensive regulatory roles carries a higher risk of unintended consequences. Future research should focus on functionally validating these TFs and identifying those that enhance stress tolerance without disrupting other crucial plant functions. Integrating these findings with existing knowledge of inherent tolerance mechanisms will be vital for developing robust stress-tolerant oil palm cultivars.

A Gene Regulatory Network (GRN), named EG-Net (Carvalho da Silva *et al.*, not published), was constructed for *Elaeis guineensis* to address some of the aforementioned questions. EG-Net, a GRN derived from 428 oil palm transcriptomes (comprising 21,852 target genes and 3,069 regulators), indicates that this oil palm HD-Zip II gene regulates 3,925 genes (235 downregulated and 165 upregulated by both salinity and drought stresses). Conversely, it is regulated by 58 regulators, including 49 transcription factors, seven long non-coding RNAs (lncRNAs) and two epigenetic regulators (EpRs). Based on these findings, silencing this gene could directly impact 400 genes exhibiting similar qualitative responses to both abiotic stresses. Notably, 25 of these genes encode transcription factors that, in turn, regulate a substantial number of genes.

CONCLUSION

The present study investigated the potential for engineering stress-tolerant oil palm cultivars by identifying TFs involved in the response to salinity and drought stress. To the best of our knowledge, there were no prior reports to suggest inherent tolerance in oil palms to such stresses. Transcriptome data was collected from young oil palm leaves subjected to severe stress (high salinity and water deprivation) for two weeks (this study, Salgado *et al*. 2021, 2022). This induced visible signs of stress, such as browning and wilting. The data reflects the plants' responses to these extreme conditions, rather than their inherent tolerance mechanisms. It's important to note that prolonged exposure to such severe stress would likely be detrimental to the plants.

This study identified 20 TFs from four distinct families that appear to play a crucial role in the response to both salinity and drought stress. The consistency observed in the behaviour of these TFs suggests the existence of shared molecular pathways. This overlap presents a valuable opportunity to pinpoint genes capable of conferring tolerance to both stresses. However, further consideration is required before selecting candidate TF genes for genetic engineering or editing.

Stress-responsive TFs offer significant potential for engineering plants with enhanced stress tolerance. A systematic approach involving the identification, characterisation and functional validation of TFs can accelerate the development of stress-resilient crops. While TF research has been extensive, it often neglects the broader context of GRNs. The development and incorporation of such GRN analysis into this process might, as shown for an oil palm HD-Zip II gene in the present study, certainly provide valuable insights into the complex regulatory mechanisms underlying stress responses, leading to more effective and targeted genetic engineering strategies.

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REFERENCES

Ambawat, S., Sharma, P., Yadav, N. R., & Yadav, R. C. (2013). MYB transcription factor genes as regulators for plant responses: An overview. *Physiology and Molecular Biology of Plants*, *19*(3), 307–321. https://doi.org/10.1007/s12298-013-0179-1

Anders, S., Pyl, P. T., & Huber, W. (2015). HTSeq–A Python framework to work with high–throughput sequencing data. *Bioinformatics*, *31*(2), 166–169. https://doi.org/10.1093/bioinformatics/btu638

Angon, P. B., Tahjib-Ul-Arif, M., Samin, S. I., Habiba, U., Hossain, M. A., & Brestic, M. (2022). How do plants respond to combined drought and salinity stress-A systematic review. *Plants*, *11*(21), 2884. https://doi.org/10.3390/plants11212884

Bailey, T. L., Johnson, J., Grant, C. E., & Noble, W. S. (2015). The MEME Suite. *Nucleic Acids Research*, *43*(W1), W39–W49. https://doi.org/10.1093/nar/gkv416

Belamkar, V., Weeks, N. T., Bharti, A. K., Farmer, A. D., Graham, M. A., & Cannon, S. B. (2014). Comprehensive characterization and RNA-Seq profiling of the HD-Zip transcription factor family in soybean (*Glycine max*) during dehydration and salt stress. *BMC Genomics*, *15*, 950. https://doi.org/10.1186/1471-2164-15-950

Bhattacharjee, A., Khurana, J. P., & Jain, M. (2016). Characterization of rice homeobox genes, *oshox22* and *oshox24*, and over-expression of *oshox24* in transgenic *Arabidopsis* suggest their role in abiotic stress response. *Frontiers in Plant Science*, *7*, 627. https://doi.org/10.3389/fpls.2016.00627

Bittencourt, C. B., Carvalho da Silva, T. L., Rodrigues Neto, J. C., Vieira, L. R., Leão, A. P., De Aquino Ribeiro, J. A., Abdelnur, P. V., De Sousa, C. A. F., & Souza, M. T., Jr. (2022). Insights from a multi-omics integration (MOI) study in oil palm (*Elaeis guineensis* Jacq.) response to abiotic stresses: Part one–Salinity. *Plants*, *11*(13), 1755. https://doi.org/10.3390/plants11131755

Chaudhari, R. S., Jangale, B. L., Krishna, B., & Sane, P. V. (2023). Improved abiotic stress tolerance in *Arabidopsis* by constitutive active form of a banana DREB2 type transcription factor, *MaDREB20.CA*, than its native form, *MaDREB20*. *Protoplasma*, *260*(3), 671–690. https://doi.org/10.1007/s00709-022-01805-7

Chen, X., Chen, Z., Zhao, H., Zhao, Y., Cheng, B., & Xiang, Y. (2014). Genome-wide analysis of soybean HD-Zip gene family and expression profiling under salinity and drought treatments. *PLoS ONE*, *9*(2), e87156. https://doi.org/10.1371/journal.pone.0087156

Corley, R. H. V., Rao, V., Palat, T., & Praiwan, T. (2018). Breeding for drought tolerance in oil palm. *Journal of Oil Palm Research*, *30*, 26–35.

Dezar, C. A., Gago, G. M., González, D. H., & Chan, R. L. (2005). *Hahb-4*, a sunflower homeobox-leucine zipper gene, is a developmental regulator and confers drought tolerance to *Arabidopsis thaliana* plants. *Transgenic Research*, *14*(4), 429–440. https://doi.org/10.1007/s11248-005-5076-0

Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., & Gingeras, T. R. (2013). STAR: Ultrafast universal RNA-seq aligner. *Bioinformatics*, *29*(1), 15–21. https://doi.org/10.1093/bioinformatics/bts635

Dubos, C., Stracke, R., Grotewold, E., Weisshaar, B., Martin, C., & Lepiniec, L. (2010). MYB transcription factors in *Arabidopsis*. *Trends in Plant Science*, *15*(10), 573–581. https://doi.org/10.1016/j.tplants.2010.06.005

Foreign Agricultural Service – U.S. Department of Agriculture (FAS-USDA). (2024). *https://apps.fas.usda.gov/psdonline/app/index.html#/app/downloads* (Accessed April 10, 2024).

Gao, S., Zhang, Y. L., Yang, L., Song, J. B., & Yang, Z. M. (2014). *AtMYB20* is negatively involved in plant adaptive response to drought stress. *Plant and Soil*, *376*(1–2), 433–443. https://doi.org/10.1007/s11104-013-1992-6

Gong, Q., Li, S., Zheng, Y., Duan, H., Xiao, F., Zhuang, Y., He, J., Wu, G., Zhao, S., Zhou, H., & Lin, H. (2020). SUMOylation of *MYB30* enhances salt tolerance by elevating alternative respiration via transcriptionally upregulating *AOX1a* in *Arabidopsis*. *The Plant Journal*, *102*(6), 1157–1171. https://doi.org/10.1111/tpj.14689

Gong, S., Ding, Y., Hu, S., Ding, L., Chen, Z., & Zhu, C. (2019). The role of HD-Zip class I transcription factors in plant response to abiotic stresses. *Physiologia Plantarum*, *167*(4), 516–525. https://doi.org/10.1111/ppl.12965

Guo, M., Liu, J.-H., Ma, X., Luo, D.-X., Gong, Z.-H., & Lu, M.-H. (2016). The plant heat stress transcription factors (Hsfs): Structure, regulation, and function in response to abiotic stresses. *Frontiers in Plant Science*, *7*, 114. https://doi.org/10.3389/fpls.2016.00114

Hajyzadeh, M., Turktas, M., Khawar, K. M., & Unver, T. (2015). *miR408* overexpression causes increased drought tolerance in chickpea. *Gene*, *555*(2), 186–193. https://doi.org/10.1016/j.gene.2014.11.002

Hazzouri, K. M., Flowers, J. M., Nelson, D., Lemansour, A., Masmoudi, K., & Amiri, K. M. A. (2020). Prospects for the study and improvement of abiotic stress tolerance in date palms in the post-genomics era. *Frontiers in Plant Science*, *11*, 293. https://doi.org/10.3389/fpls.2020.00293

He, M., He, C.-Q., & Ding, N.-Z. (2018). Abiotic stresses: General defenses of land plants and chances for engineering multistress tolerance. *Frontiers in Plant Science*, *9*, 1771. https://doi.org/10.3389/fpls.2018.01771

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

Instituto Brasileiro de Geografia e Estatística (IBGE). (2024). *https://www.ibge.gov.br/explica/producao-agropecuaria/dende/br* (Accessed April 10, 2024).

Ivushkin, K., Bartholomeus, H., Bregt, A. K., Pulatov, A., Kempen, B., & De Sousa, L. (2019). Global mapping of soil salinity change. *Remote Sensing of Environment*, *231*, 111260. https://doi.org/10.1016/j.rse.2019.111260

Javed, T., Shabbir, R., Ali, A., Afzal, I., Zaheer, U., & Gao, S.-J. (2020). Transcription factors in plant stress responses: Challenges and potential for sugarcane improvement. *Plants*, *9*(4), 491. https://doi.org/10.3390/plants9040491

Jiao, P., Jiang, Z., Wei, X., Liu, S., Qu, J., Guan, S., & Ma, Y. (2022). 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

Kanehisa, M., Sato, Y., & Morishima, K. (2016). BlastKOALA and GhostKOALA:

KEGG tools for functional characterization of genome and metagenome sequences. *Journal of Molecular Biology*, *428*(4), 726–731. https://doi.org/10.1016/j.jmb.2015.11.006

Leão, A. P., Bittencourt, C. B., Carvalho Da Silva, T. L., Rodrigues Neto, J. C., Braga, Í. O., Vieira, L. R., De Aquino Ribeiro, J. A., Abdelnur, P. V., De Sousa, C. A. F., & Souza Júnior, M. T. (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

Lee, S. B., & Suh, M. C. (2015). Advances in the understanding of cuticular waxes in *Arabidopsis thaliana* and crop species. *Plant Cell Reports*, *34*(4), 557–572. https://doi.org/10.1007/s00299-015-1772-2

Lescot, M. (2002). PlantCARE, a database of plant *cis*-acting regulatory elements and a portal to tools for *in silico* analysis of promoter sequences. *Nucleic Acids Research*, *30*(1), 325–327. https://doi.org/10.1093/nar/30.1.325

Leyva-González, M. A., Ibarra-Laclette, E., Cruz-Ramírez, A., & Herrera-Estrella, L. (2012). Functional and transcriptome analysis reveals an acclimatization strategy for abiotic stress tolerance mediated by *Arabidopsis* NF-YA family members. *PLoS ONE*, *7*(10), e48138. https://doi.org/10.1371/journal.pone.0048138

Li, L., Yu, Y., Wei, J., Huang, G., Zhang, D., Liu, Y., & Zhang, L. (2013). Homologous HAP5 subunit from *Picea wilsonii* improved tolerance to salt and decreased sensitivity to ABA in transformed *Arabidopsis*. *Planta*, *238*(2), 345–356. https://doi.org/10.1007/s00425-013-1894-0

Li, W., Wan, X.-L., Yu, J.-Y., Wang, K.-L., & Zhang, J. (2019). Genome-wide identification, classification, and expression analysis of the Hsf gene family in carnation (*Dianthus caryophyllus*). *International Journal of Molecular Sciences*, *20*(20), 5233. https://doi.org/10.3390/ijms20205233

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

Machado, R., & Serralheiro, R. (2017). Soil salinity: Effect on vegetable crop growth. Management practices to prevent and mitigate soil salinization. *Horticulturae*, *3*(2), 30. https://doi.org/10.3390/horticulturae3020030

Nelson, D. E., Repetti, P. P., Adams, T. R., Creelman, R. A., Wu, J., Warner, D. C., Anstrom, D. C., Bensen, R. J., Castiglioni, P. P., Donnarummo, M. G., Hinchey, B. S., Kumimoto, R. W., Maszle, D. R., Canales, R. D., Krolikowski, K. A., Dotson, S. B., Gutterson, N., Ratcliffe, O. J., & Heard, J. E. (2007). Plant nuclear factor Y (NF-Y) B subunits confer drought tolerance and lead to improved corn yields on water-limited acres. *Proceedings of the National Academy of Sciences*, *104*(42), 16450–16455. https://doi.org/10.1073/pnas.0707193104

Ni, Z., Hu, Z., Jiang, Q., & Zhang, H. (2013). *GmNFYA3*, a target gene of miR169, is a positive regulator of plant tolerance to drought stress. *Plant Molecular Biology*, *82*(1–2), 113–129. https://doi.org/10.1007/s11103-013-0040-5

Ohama, N., Sato, H., Shinozaki, K., & Yamaguchi-Shinozaki, K. (2017). Transcriptional regulatory network of plant heat stress response. *Trends in Plant Science*, *22*(1), 53–65. https://doi.org/10.1016/j.tplants.2016.08.015

OmicsBox – Bioinformatics Made Easy. (2019). BioBam Bioinformatics. https://www.biobam.com/omicsbox

Pérez-Salamó, I., Papdi, C., Rigó, G., Zsigmond, L., Vilela, B., Lumbreras, V., Nagy, I., Horváth, B., Domoki, M., Darula, Z., Medzihradszky, K., Bögre, L., Koncz, C., & Szabados, L. (2014). The heat shock factor A4A confers salt tolerance and is regulated by oxidative stress and the mitogen-activated protein kinases MPK3 and MPK6. *Plant Physiology*, *165*(1), 319–334. https://doi.org/10.1104/pp.114.237891

Qin, F., Shinozaki, K., & Yamaguchi-Shinozaki, K. (2011). Achievements and challenges in understanding plant abiotic stress responses and tolerance. *Plant and Cell Physiology*, *52*(9), 1569–1582. https://doi.org/10.1093/pcp/pcr106

Rao, X., Huang, X., Zhou, Z., & Lin, X. (2013). An improvement of the 2^(-ΔΔCT) method for quantitative real-time polymerase chain reaction data analysis. *Biostatistics, Bioinformatics and Biomathematics*, *3*(3), 71–85.

Rasmussen, S., Barah, P., Suarez-Rodriguez, M. C., Bressendorff, S., Friis, P., Costantino, P., Bones, A. M., Nielsen, H. B., & Mundy, J. (2013). Transcriptome responses to combinations of stresses in *Arabidopsis*. *Plant Physiology*, *161*(4), 1783–1794. https://doi.org/10.1104/pp.112.210773

Roelofs, D., Morgan, J., & Stürzenbaum, S. (2010). The significance of genome-wide transcriptional regulation in the evolution of stress tolerance. *Evolutionary Ecology*, *24*(3), 527–539. https://doi.org/10.1007/s10682-009-9345-x

Roy, S. (2016). Function of MYB domain transcription factors in abiotic stress and epigenetic control of stress response in plant genome. *Plant Signaling & Behavior*, *11*(1), e1117723. https://doi.org/10.1080/15592324.2015.1117723

Salgado, F. F., Vieira, L. R., Silva, V. N. B., Leão, A. P., Grynberg, P., Do Carmo Costa, M. M., Togawa, R. C., De Sousa, C. A. F., & Souza Júnior, M. T. (2021). Expression analysis of miRNAs and their putative target genes confirm a preponderant role of transcription factors in the early response of oil palm plants to salinity stress. *BMC Plant Biology*, *21*(1), 518. https://doi.org/10.1186/s12870-021-03296-9

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 Júnior, 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

Sayers, E. W., Bolton, E. E., Brister, J. R., Canese, K., Chan, J., Comeau, D. C., Connor, R., Funk, K., Kelly, C., Kim, S., Madej, T., Marchler-Bauer, A., Lanczycki, C., Lathrop, S., Lu, Z., Thibaud-Nissen, F., Murphy, T., Phan, L., Skripchenko, Y., . . . Sherry, S. T. (2022). Database resources of the national center for biotechnology information. *Nucleic Acids Research*, *50*(D1), D20–D26. https://doi.org/10.1093/nar/gkab1112

Shahid, S. A., Zaman, M., & Heng, L. (2018). Soil salinity: Historical perspectives and a world overview of the problem. In M. Zaman, S. A. Shahid, & L. Heng (Eds.), *Guideline for salinity assessment, mitigation and adaptation using nuclear and related techniques* (pp. 43–53). Springer International Publishing. https://doi.org/10.1007/978-3-319-96190-3_2

Sharif, R., Raza, A., Chen, P., Li, Y., El-Ballat, E. M., Rauf, A., Hano, C., & El-Esawi, M. A. (2021). HD-ZIP gene family: Potential roles in improving plant growth and regulating stress-responsive mechanisms in plants. *Genes*, *12*(8), 1256. https://doi.org/10.3390/genes12081256

Singh, R., Ong-Abdullah, M., Low, E.-T. L., Manaf, M. A. A., Rosli, R., Nookiah, R., Ooi, L. C.-L., Ooi, S., Chan, K.-L., Halim, M. A., Azizi, N., Nagappan, J., Bacher, B., Lakey, N., Smith, S. W., He, D., Hogan, M., Budiman, M. A., Lee, E. K., … Sambanthamurthi, R. (2013). Oil palm genome sequence reveals divergence of interfertile species in Old and New Worlds. *Nature*, *500*(7462), 335–339. https://doi.org/10.1038/nature12309

Song, S., Xu, Y., Huang, D., Miao, H., Liu, J., Jia, C., Hu, W., Valarezo, A. V., Xu, B., & Jin, Z. (2018). Identification of a novel promoter from banana aquaporin family gene (*MaTIP1;2*) which responds to drought and salt-stress in transgenic *Arabidopsis thaliana*. *Plant Physiology and Biochemistry*, *128*, 163–169. https://doi.org/10.1016/j.plaphy.2018.05.003

Statista. (2024). https://www.statista.com/statistics/263937/vegetable-oils-global-consumption/ (Accessed on April 10, 2024).

Stephenson, T. J., McIntyre, C. L., Collet, C., & Xue, G.-P. (2007). Genome-wide identification and expression analysis of the NF-Y family of transcription factors in *Triticum aestivum*. *Plant Molecular Biology*, *65*(1–2), 77–92. https://doi.org/10.1007/s11103-007-9200-9

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, *38*(7), 3022–3027. https://doi.org/10.1093/molbev/msab120

Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, *22*(22), 4673–4680. https://doi.org/10.1093/nar/22.22.4673

Vieira, L. R., Silva, V. N. B., Casari, R. A. D. C. N., Carmona, P. A. O., Sousa, C. A. F. D., & Souza Junior, M. T. (2020). Morphophysiological responses of young oil palm plants to salinity stress. *Pesquisa Agropecuária Brasileira*, *55*, e01835. https://doi.org/10.1590/S1678-3921.pab2020.v55.01835

Wang, J., Ye, Y., Xu, M., Feng, L., & Xu, L. (2019). Roles of the *SPL* gene family and miR156 in the salt stress responses of tamarisk (*Tamarix chinensis*). *BMC Plant Biology*, *19*(1), 370. https://doi.org/10.1186/s12870-019-1977-6

Wang, W., Pang, J., Zhang, F., Sun, L., Yang, L., Fu, T., Guo, L., & Siddique, K. H. M. (2022). Saltresponsive transcriptome analysis of canola roots reveals candidate genes involved in the key metabolic pathway in response to salt stress. *Scientific Reports*, *12*(1), 1666. https://doi.org/10.1038/s41598-022-05700-2

Wang, X., Niu, Y., & Zheng, Y. (2021). Multiple functions of MYB transcription factors in abiotic stress responses. *International Journal of Molecular Sciences, 22*(11), 6125. https://doi.org/10.3390/ijms22116125

Wu, Z., Liang, J., Wang, C., Zhao, X., Zhong, X., Cao, X., Li, G., He, J., & Yi, M. (2018). Overexpression of lily HsfA3s in *Arabidopsis* confers increased thermotolerance and salt sensitivity via alterations in proline catabolism. *Journal of Experimental Botany, 69*(8), 2005–2021. https://doi.org/10.1093/jxb/ery035

Xu, Z., Gongbuzhaxi, Wang, C., Xue, F., Zhang, H., & Ji, W. (2015). Wheat NAC transcription factor *TaNAC29* is involved in response to salt stress. *Plant Physiology and Biochemistry*, *96*, 356–363. https://doi.org/10.1016/j.plaphy.2015.08.013

Yamaguchi-Shinozaki, K., & Shinozaki, K. (2007). Improving plant drought, salt and freezing tolerance by gene transfer of a single stress-inducible transcription factor. In J. A. Goode & D. Chadwick (Eds.), *Rice biotechnology: Improving yield, stress tolerance and grain quality* (Novartis Foundation Symposium 236, pp. 142–153). Wiley. https://doi.org/10.1002/9780470515778.ch13

Yu, Y., Xu, W., Wang, J., Wang, L., Yao, W., Xu, Y., Ding, J., & Wang, Y. (2012). A core functional region of the RFP1 promoter from Chinese wild grapevine is activated by powdery mildew pathogen and heat stress. *Planta, 237*(1), 293–303. https://doi.org/10.1007/s00425-012-1769-9

Zaman, M., Shahid, S. A., & Heng, L. (2018). *Guideline for salinity assessment, mitigation and adaptation using nuclear and related techniques*. Springer International Publishing. https://doi.org/10.1007/978-3-319-96190-3

Zhang, H., Zhu, J., Gong, Z., & Zhu, J.-K. (2022). Abiotic stress responses in plants. *Nature Reviews Genetics*, *23*(2), 104–119. https://doi.org/10.1038/s41576-021-00413-0

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.-K. (2002). Salt and drought stress signal transduction in plants. *Annual Review of Plant Biology*, *53*(1), 247–273. https://doi.org/10.1146/annurev.arplant.53.091401.143329

Zulkower, V., & Rosser, S. (2020). DNA Features Viewer: A sequence annotation formatting and plotting library for Python. *Bioinformatics*, *36*(15), 4350–4352. https://doi.org/10.1093/bioinformatics/btaa213