



Full Length Article

Molecular Response of Banana Plantlets to Drought Stress: A Transcriptomic Study on the Bioprocesses of Morphogenesis and Organ Development

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Abstract

Drought stress is a significant environmental challenge that reduces banana productivity by inducing morphological and physiological changes. Understanding these responses at a molecular level is essential but remains limited. This study analysed the transcriptomic responses of banana (*Musa acuminata* L. cv. Barangan Merah) plantlets to drought stress, focusing on morphogenesis and organ development. *In vitro* cultures were treated with polyethylene glycol (PEG) at 2.5, 7.5 and 10% concentrations to simulate drought conditions. Transcriptomic profiling was conducted using DAVID and DESeq2, with qPCR validation for selected genes, including MaSOB3, MaPAT1, MaPIN6 and MaARF10. These genes exhibited dynamic expression changes, reflecting the plant's molecular adjustments to drought stress. Photomorphogenesis-related genes were primarily affected under low and moderate stress, while growth-related genes showed significant changes under severe stress. For example, MaSOB3 was upregulated under mild stress but downregulated as stress intensified. MaARF10 showed increased expression under higher PEG concentrations, highlighting its role in auxin-mediated responses. Comparative analysis between RNA-seq and qPCR confirmed these findings, despite minor technical discrepancies. This study advances the understanding of drought-responsive genes in bananas, offering potential targets for developing drought-tolerant cultivars through genetic engineering or breeding. It also underscores the importance of molecular approaches in addressing climate-induced challenges in agriculture.

Keywords: Barangan merah cultivar; Drought stress; Morphogenesis; Polyethylene glycol; *Musa acuminata*

Introduction

Banana is commonly found in Southeast Asia, including Indonesia with tropical lowlands and highlands suitable for growing this fruit (Langhe *et al.* 2009). However, the worsening global warming has led to unpredictable climate changes, disrupting banana growth. Environmental changes can produce detrimental effects, such as the initiation of drought stress in cultivated plants. Drought stress occurs when there is insufficient water for root absorption, excessive transpiration due to increased environmental temperatures or high soil salinity (Farooq *et al.* 2015; Hussain *et al.* 2018). In some cases, drought conditions arise due to low soil moisture or the inability of plants to absorb water.

A significant challenge is posed by water stress to the banana cultivation system because banana plants lacking enough water may have delayed harvest cycles and smaller fruits (Panigrahi *et al.* 2021). Bananas grown in tropical and subtropical regions, characterized by shallow root systems and high water demands, are highly vulnerable to drought stress, which can reduce yields by as much as 65% under insufficient rainfall (Thingnam *et al.* 2023). Early signs of the changes comprise wilting and curling of leaves which tend to originate from decreased cell turgor pressure (Surendar *et al.* 2013a). Additionally, drought stress can be identified by a reduction in the greenness of leaves (Surendar *et al.* 2013b). As drought stress intensifies, leaves may fall prematurely, which affects the total number left and decreases the photosynthetic area, thereby hindering optimal growth.

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Another morphological parameter signifying drought stress in banana is height considering that plant growth depends on cell growth and development, including cell division, enlargement, and differentiation (Farooq *et al.* 2009). Physiologically, drought stress triggers biochemical responses such as the accumulation of osmoprotectants like proline, helping plants to mitigate oxidative damage and maintain cell stability (Kumar *et al.* 2021). Advances in molecular biology need to be conducted to understand the dynamics of gene expression changes in banana response to drought stress.

The described morphological changes have been recently identified using a molecular method through transcriptomic analysis. The genomes of a doubled haploid banana (*Musa acuminata* cv. Pahang) and *Musa balbisiana* were published by D'Hont *et al.* (2012) and Davey *et al.* (2013), respectively. Furthermore, Backiyarani *et al.* (2015) conducted a transcriptomic analysis on *Musa balbisiana* presenting affected biological processes, particularly DNA-dependent transcription (3,235 unigenes), defense (2,434 unigenes), and salt stress responses (1,798 unigenes). Another transcriptomic study was performed by Muthusamy *et al.* (2016) on the drought-tolerant ABB genome cultivar Saba and the drought-intolerant AAA genome cultivar Grand Naine. The results showed that drought stress altered the expression of ABA hormones crucial for signal transduction, cell wall or membrane components including cellulose, homogalacturonan, lignin and wax as well as cellular organelle constituents (chlorophyll and GDP-mannose).

Morphogenesis and organ development are central to plant adaptation under drought stress, as they directly affect growth and survival. In a study by Rymaszewski *et al.* (2017), *Arabidopsis thaliana* showed decreased expression of genes related to leaf formation due to drought stress. Studies on *Arabidopsis* and rice have shown that drought alters key developmental pathways, such as auxin signaling and cell expansion, which are critical for root and shoot architecture (Roosjen *et al.* 2018; Zhang *et al.* 2021). In maize, drought stress impacts trichome branching and root hair elongation, enhancing water uptake efficiency (Lan *et al.* 2018a). These findings emphasize the importance of exploring molecular pathways in different crops to identify conserved mechanisms. Morphological observations of banana plant pinnae, which were supported by preliminary transcriptomic analysis, identified gene expression changes in the bioprocesses (Widiyanto *et al.* 2024). Genes related to root and leaf development in banana plantlets may have expression changes as drought tolerance responses that require confirmation through transcriptomic data analysis.

For banana plants, limited studies have investigated molecular responses to drought, particularly those involving morphogenesis and organ development. Advancements in transcriptomic technologies offer an opportunity to bridge this gap by identifying key genetic regulators and pathways involved in stress tolerance. This study investigates the transcriptomic responses of *Musa acuminata* L. cv. Barangan Merah plantlets under varying drought stress

levels induced by polyethylene glycol (PEG). By integrating bioinformatics tools such as DAVID and DESeq2 with qPCR validation, the research aims to elucidate the genetic basis of drought stress responses and identify potential gene targets for developing drought-resilient banana cultivars.

Materials and Methods

Experimental materials and treatments

Transcriptome data analysis was conducted in this study to investigate how banana plantlets responded to drought stress, particularly in the bioprocesses of morphogenesis and organ development. Furthermore, all mRNA sequencing (mRNA-seq) data were sourced from the NCBI BioProject database (BioProject ID: PRJNA970186). The data represented transcriptome profiles from *in vitro* cultures of *Musa acuminata* cv. Barangan Merah subjected to water stress through polyethylene glycol (PEG) addition at varying concentrations of 0% (code BK, control), 2.5% (code BP2), 7.5% (code BP7) and 10% (code BP10) PEG6000 (Widiyanto *et al.* 2024). The relative expression of genes in the banana plantlets affected by drought stress was confirmed using qPCR. During this process, total RNA was isolated from *in vitro* cultures of banana plantlets obtained from SEAMEO BIOTROP, Bogor. The re-trial was conducted using the same method and conditions applied to the transcriptome data samples. Each treatment included five biological replicates, which were combined to produce the total RNA source. Four RNA samples were used for gene expression confirmation, with three technical replicates for relative gene expression measurement using qPCR.

Transcriptomic analysis for GO enrichment and differentially expressed genes (DEG)

The processing of sequencing data was conducted using a transcriptomic method to identify and analyze gene expression modulation in bioprocesses related to stress responses and the included transcription factors. The software used for GO analysis was the Database for Annotation, Visualization, and Integrated Discovery (DAVID) v. 6.8 (<https://david.ncifcrf.gov/>). This software provides annotations across various categories to examine the interrelationships among genes in the bioprocesses (Huang *et al.* 2009). To identify the relative expressions of annotated genes, the DESeq package in R software was used to evaluate the read-count data associated with genes participating in morphogenesis and organ development (Love *et al.* 2014). Each unigene was categorized according to the transcripts per million (TPM) ratios compared to the control with a cut-off ratio of $\log_{10} > 2$ and P -value < 0.001 .

Confirmation of gene expression using qPCR

Primers were designed for housekeeping and target genes using the Primer3Plus software (<http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi>). The results were compared with the available banana genome at CIRAD and cross-checked using Primer Blaster software (https://banana-genomchub.southgreen.fr/primer_blaster) to ensure that the designed primers hit the *Musa acuminata* AAA genome, with Table 1 presenting the list of designed primers.

The RNA isolation was performed using the CTAB method according to Diningrat *et al.* (2015) and the quality was confirmed with a nanodrop. Total RNA samples were synthesized into cDNA using reverse transcriptase with the GoScript™ Reverse Transcription System Promega kit. Subsequently, qPCR analysis was conducted using GoTaq® qPCR Master Mix Promega reagents and a qRT-PCR thermal cycler (QuantStudio 1 ThermoFisher). The PCR cycle complied with Diningrat *et al.* (2015)'s protocol, which involved activating the polymerase for 15 min at 95°C, then polymerizing 40 times for 15 s at 95°C, 30 s at 60°C and 30 s at 72°C. Housekeeping genes were used to normalize target gene expression data, and the comparative $2^{-\Delta\Delta CT}$ method was used to determine relative gene expression levels. The formula $\Delta\Delta CT = (CT_{\text{target gene}} - CT_{\text{housekeeping gene}})_{\text{treatment}} - (CT_{\text{target gene}} - CT_{\text{housekeeping gene}})_{\text{control}}$ was used in this calculation (Livak and Schmittgen 2001).

Statistical analysis

All statistical analyses were conducted using R software (v. 4.2). One-way ANOVA was used to compare gene expression levels across treatments, followed by Tukey's HSD test for pairwise comparisons. Statistical significance was set at $P < 0.05$.

Results

The transcriptomic analysis performed using DAVID and DESeq software showed changes in the expression of different groups of genes at each level of stress. Generally, the highest number of genes identified with expression changes were found in the BP10 sample (28 genes), followed by the BP2 sample (21 genes) and the BP7 sample (15 genes). The genes identified in the BP2 sample were associated with photomorphogenesis, fruit development, and leaf development. The genes in BP7 were associated with photomorphogenesis, trichome branching, and root hair elongation, while those in BP10 were associated with photomorphogenesis, growth, trichome morphogenesis and pollen development. Moreover, photomorphogenesis was observed in all samples (Table 2).

The results of transcriptomic data analysis conducted using DESeq showed changes in the expression of genes identified during morphogenesis and organ development (Table 3). In leaf development, among the genes identified in the BP2 sample, six were upregulated, while four were

downregulated. Genes related to trichome branching and morphogenesis were downregulated, while those associated with pollen development in BP10 tended to present an increase in gene expression. In photomorphogenesis, a total of six, six, and five genes showed gene expression changes in the BP2, BP7 and BP10 samples, respectively.

To confirm analysis accuracy and robustness, selected genes were validated using the qPCR method. Therefore, an independent experiment was set up with three different PEG concentrations and control treatments. In general, relative gene expression values were similar with mRNA-seq (Fig. 1). The qPCR approach was used to validate some of the selected genes to verify the robustness and accuracy of the study. As a result, three distinct PEG concentrations and control treatments were used in a separate experiment. As seen in Fig. 1, relative gene expression values were often comparable with mRNA-seq. The MaSOB3 gene showed increased expression in the BP2 sample, followed by a decreased expression in the BP7 and BP10. The expression of the MaPAT1 gene shows a decreased expression in BP2, followed by increased expression in BP7 and BP10. The MaPIN6 gene decreased, while MaARF10 tended to increase in expression. Moreover, all these genes could be amplified, showing the potential to validate the sequences.

Discussion

Tolerance responses can include morphological changes, alterations in physiological activities (metabolism), biochemical changes, and molecular-level modifications. Some of the morphological responses observed in banana plants under drought stress include alterations in root dry weight as well as leaf shape and color (Surendar *et al.* 2013a). A study conducted by Rymaszewski *et al.* (2017) found that drought stress with a soil water capacity of 45-12% led to a decreased expression of genes related to leaf development in *Arabidopsis thaliana* plants. Various genes are induced in response to drought at the transcriptional level, and the products of these genes function in drought tolerance. In this situation, changes in gene expression (up and down regulation) may occur. We suspect that differences in drought stress levels will result in different defense responses. Based on our research findings, we observed that there is a dynamic change in gene expression, which is also marked by differences in gene ontology affected by varying levels of drought stress. Gene expression analysis revealed that genes, such as MaSOB3, MaPAT1, MaPIN6 and MaARF10, displayed dynamic expression patterns under varying drought stress levels. These results align with recent studies that demonstrate the role of auxin signaling in regulating root and shoot development under water-deficit conditions (Lan *et al.* 2018a; Zhang *et al.* 2021). For example, in maize, drought stress was found to affect trichome branching and root hair elongation, improving water absorption efficiency, similar to the role of MaPIN6 in auxin-mediated root development in bananas.

Table 1: Primer pairs for the selected and housekeeping genes. MaACT and MaBT are housekeeping genes. MaSOB3, MaPAT1, MaPIN6 and MaARF10 are genes selected for qPCR validation

No.	Musa ID	Gene Name	Abbreviation	Forward	Reverse	Product size
1	Ma10_p01140.1	<i>Actin histone-lysine N-methyltransferase setd3</i>	<i>MaACT</i>	CTGACTGGCAGCAGGACATA	CCAAATCGTGCCCTTTGAACT	162
2	Ma01_p13380.1	<i>Beta tubulin</i>	<i>MaBT</i>	AGTCCGGAGCTTCAACCTTT	ACGCTGACGATGGAGAAGAC	221
3	Ma02_p18200.1	<i>Putative AT-hook DNA-binding family protein</i>	<i>MaSOB3</i>	TCGCAGCCACATTCTTGAAC	GCAATCCTTCTGTGACCAATCG	96
4	Ma09_p11650.1	<i>GRAS family transcription factor</i>	<i>MaPAT1</i>	ATCGTCAATCCCTGTGATCCG	TGACTTCCAGATTGCTCAAGGG	103
5	Ma03_p22930.1	<i>Auxin efflux carrier family protein</i>	<i>MaPIN6</i>	GGATCTCACAGTTTCCTTCGTTG	ACATCACGGGTAGAAAGTCCTC	71
6	Ma04_p18800.1	<i>Auxin response factor 10</i>	<i>MaARF10</i>	AATGTGAACCGTGTGAACCC	AGAAGGGAGCAAGATGGATAGC	73

Table 2: Gene ontologies related to morphogenesis and organ development affected by drought stress. BP2, BP7 and BP10 refer to banana plantlets subjected to 2.5, 7.5 and 10% PEG treatments, respectively. BK is the control group without PEG treatment

GO Term	Number of DEG		
	BP2	BP7	BP10
Photomorphogenesis	6	6	5
Fruit development	5		
Leaf development	10		
Trichome branching		4	
Root hair elongation		5	
Growth			5
Trichome morphogenesis			5
Pollen development			13

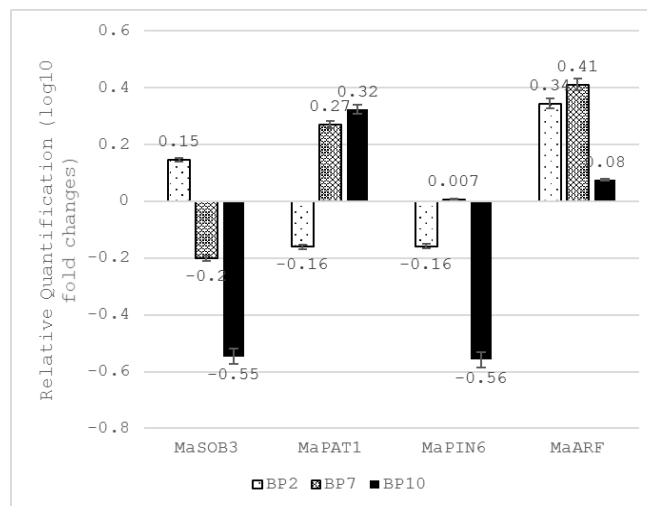


Fig. 1: Relative expression values of MaSOB3, MaPAT1, MaPIN6, and MaARF genes in BP2, BP7 and BP10 samples based on qRT-PCR. BP2, BP7 and BP10 represent 2.5, 7.5 and 10% PEG treatments, respectively. BK serves as the control

Genes experiencing changes in expression during the bioprocesses are related to the growth and development of vegetative and reproductive organs. Some identified genes, such as the MaSOB3, encode AT-hook domain binding proteins that play a role in inhibiting cell elongation (Xiao *et al.* 2009). These proteins interact with AT-rich DNA sequences to regulate the expression of genes. Increased expression of the SOB3 gene in *Arabidopsis thaliana* plants inhibits the production of the signaling hormone auxin by binding to the YUC8 promoter and suppressing the expression (Favero *et al.* 2016).

Another gene participating in the photomorphogenesis process is MaPAT1, which encodes phytochrome A signal transduction 1 protein. This protein belongs to the GRAS

protein family and functions as the first signal transducer from the phytochrome A receptor, playing a role in the processes of de-etiolation and hypocotyl elongation (Bolle *et al.* 2000). In leaf and root development, the MaPIN6 and MaARF10 genes encode the auxin efflux carrier 6 and auxin response factor 10 proteins. These genes are included in the regulation of gene expression mediated by auxin content. PIN6 plays a role in auxin transport and regulates auxin homeostasis during plant development. The loss of PIN6 function can disrupt primary root growth and lateral root development (Cazzonelli *et al.* 2013). ARF10 acts as a transcription factor that regulates gene expression based on auxin availability. Under drought stress conditions, banana pseudostems show an increase in MaARF10 gene

Table 3: Relative gene expression in the bioprocesses of morphogenesis and organ development under drought stress based on mRNA-seq. Variations in stress levels were found to initiate significant changes in the expression of genes. BP2, BP7 and BP10 represent 2.5%, 7.5%, and 10% PEG treatments, respectively. BK serves as the control

GO Term	Gene Name	DEG (Log ₁₀ fold changes)		
		BP2	BP7	BP10
Fruit development	auxin response factor 10(ARF10)	1.0998		
	auxin response factor 8(ARF8)	-1.0502		
	cullin4(CUL4)	-0.9456		
	glycine-rich protein 2B(GRP2B)	-1.1875		
	myosin family protein with Dil(XIK)	-1.0325		
Leaf development	AP2/B3-like transcriptional factor family protein(NGA1)	-0.9351		
	Inorganic H pyrophosphatase family protein(AVP1)	-0.9955		
	Pentatricopeptide repeat (PPR) superfamily protein(DOT4)	1.1647		
	aldehyde dehydrogenase 5F1(ALDH5F1)	1.0083		
	auxin response factor 10(ARF10)	1.0998		
	breast cancer associated RING 1(BARD1)	1.3012		
	cullin4(CUL4)	-0.9456		
	cytochrome P450, family 90, subfamily D, polypeptide 1(CYP90D1)	-1.1875		
	growth-regulating factor 5(GRF5)	0.9709		
	high-affinity nickel-transport family protein(AT2G16800)	1.0348		
Photomorphogenesis	ATP binding cassette subfamily B19(ABCB19)	-0.9858		-1.1998
	casein kinase 1-like 3(ckl3)	0.9513		
	cullin4(CUL4)	-0.9456		
	SPA1-related 3(SPA3)	-0.9456	-0.9044	
	GRAS family transcription factor(PAT1)	-1.1232	-1.0749	
	protochlorophyllide oxidoreductase A(PORA)	-1.7257	-0.6169	
	Putative AT-hook DNA-binding family protein(SOB3)		0.9046	1.2635
	alpha/beta-Hydrolases superfamily protein(KAI2)		1.0033	0.9479
	auxin transport protein (BIG)(BIG)		1.1714	
	homeobox protein ATH1(ATH1)			-1.0994
Root hair elongation	Auxin efflux carrier family protein(PIN1)			0.9373
	Auxin efflux carrier family protein(PIN6)		-0.9430	
	RAC-like GTP binding protein 5(ARAC5)		0.7548	
	actin 7(ACT7)		0.8007	
	myosin family protein with Dil(XIK)		-0.9879	
Trichome branching	plasma membrane intrinsic protein 2;4(PIP2;4)		-0.8522	
	Calcium-binding EF-hand family protein(AT2G46600)		-0.7776	
	HECT ubiquitin protein ligase family protein KAK(KAK)		0.9561	
	NAD(P)-binding Rossmann-fold superfamily protein(AN)		-1.0372	
Trichome morphogenesis	myosin family protein with Dil(XIK)		-0.9879	
	ABI-1-like 1(ABIL1)			-1.1402
	Actin-like ATPase superfamily protein(DIS1)			1.3465
	myosin family protein with Dil(XIK)			-1.0554
	plant/protein (Protein of unknown function, DUF538)(SVB)			-1.1998
Growth	retinoblastoma-related 1(RBR1)			0.9983
	HOPM interactor 7(ATMIN7)			-0.9770
	Ribosomal protein S6e(EMB3010)			-1.1927
	cinnamate-4-hydroxylase(C4H)			1.5032
	lipoxygenase 1(LOX1)			-0.9352
	ribosomal protein S6(RPS6)			1.0175
Pollen development	BPS1-like protein (DUF793)(AT1G74450)			-1.0909
	K ⁺ uptake permease 11(KUP11)			1.0358
	Leucine-rich repeat protein kinase family protein(AT2G01820)			0.9583
	Phosphoglycerate mutase, 2,3-bisphosphoglycerate-independent(iPGAM1)			0.9885
	Transducin/WD40 repeat-like superfamily protein(NEDD1)			0.9786
	WRKY DNA-binding protein 34(WRKY34)			-1.0822
	autoinhibited Ca(2+)-ATPase 9(ACA9)			1.0878
	cinnamate-4-hydroxylase(C4H)			1.5032
	galacturonosyltransferase 13(GAUT13)			1.0710
	glyceraldehyde-3-phosphate dehydrogenase of plastid 1(GAPCP-1)			0.9685
retinoblastoma-related 1(RBR1)			0.9983	
root and pollen arfgap(RPA)			1.0537	
tubby like protein 6(TLP6)			0.9786	

Note:
Upregulated
Downregulated

expression, indicating that the regulation of auxin-activated genes is being suppressed. When auxin levels are low, the Aux/IAA transcriptional co-repressor prevents ARF from controlling auxin-regulated genes (Roosjen *et al.* 2018). At

high levels, auxin acts as 'molecular glue' between the TIR1/AFB receptor and Aux/IAA proteins, leading to the ubiquitination and degradation of Aux/IAA, thereby releasing ARF from the inhibition.

Four genes were detected with changes in expression regarding trichome branching, particularly in the BP7 sample. Based on GO analysis, the identified genes belonged to the trichome development group. However, a deeper examination showed that these identified genes, including MaKAK and MaAN, functioned in drought stress regulation. The MaKAK gene, or HECT ubiquitin-protein ligase, plays a major role in the degradation of several short-lived proteins in eukaryotes and influences gene and protein expression regulation (Al-Saharin *et al.* 2022). Studies on the HECT domain are crucial for a better understanding of ubiquitin-protein ligases in plants. In a previous investigation, the UPL3 gene was associated with trichome growth in *Arabidopsis thaliana* and found to be highly expressed in leaves (Lan *et al.* 2018b). The increased MaKAK expression based on DEG data suggests the possibility of leaf senescence processes which can occur due to the ubiquitination of certain related proteins, thereby regulating leaf aging under drought stress conditions.

The polarized growth of leaf cells and trichome branching through the microtubule cytoskeleton are known to be controlled by the angustifolia (AN) gene. Gachomo *et al.* (2013) claim that the AN gene in higher plants negatively regulates genes that cause stress and controls stress reactions. This gene regulates plant stress responses at the transcriptional level by controlling the production of genes associated with ROS and stress response. Based on transcriptomic data from banana pseudostems, the MaAN gene had decreased expression compared to the control, signifying the role of MaAN in controlling drought stress responses in banana plants. Elimination of the AN gene in *Arabidopsis thaliana* shows higher DREB2 gene expression compared to the control when subjected to drought stress (Gachomo *et al.* 2013). Further investigations in rice (*Oryza sativa*) have also implicated AN orthologs in modulating abiotic stress responses, particularly by influencing the antioxidant machinery and maintaining cellular homeostasis during adverse environmental conditions (Zhu *et al.* 2017; Li *et al.* 2020). These findings collectively suggest that AN gene functions are highly conserved and integral to plant adaptive responses to abiotic stresses.

The qPCR analysis was conducted on four genes selected in the bioprocesses of morphogenesis and organ development where two housekeeping genes (MaACT and MaBT) were used for data normalization. Total RNA for qPCR analysis was isolated from banana shoot sections of BP2, BP7 and BP10 samples, while BK was applied as the control treatment. The results showed diverse gene expression, and the confirmation performed with the four DEG showed similar expression trends between qPCR and RNA-seq data. In some test samples, there were discrepancies between the transcriptomic analysis and qPCR results, such as for MaGolS4, MaSOB3 and MaPAT1 genes. Similar results were reported in a banana analysis conducted by Hu *et al.* (2017) which showed some validated genes presenting different expressions compared

to the transcriptomic data. There are several possible reasons for the discrepancies between qPCR gene expression results and transcriptomic data. First, this condition may be due to the different alternative forms of genes, as RNA-seq analysis is capable of detecting gene expression from all alternative forms, while qPCR only detects from one alternative form. Second, RNA-seq tends to struggle with low-coverage (downregulated) genes because the resulting analysis shows low statistical power, but qPCR can detect downregulated genes more accurately and precisely (Hu *et al.* 2017).

Conclusion

In conclusion, the results of gene expression profiles presented in morphogenesis and organ development bioprocesses showed that roots and leaves as well as certain genes included in photomorphogenesis experienced dynamic expression changes due to drought stress. The decreased expression of growth-related genes such as MaPIN6 and the increased expression of MaARF10, which played a role in regulating auxin-related gene expression, were suspected to contribute to the reduced growth of banana plants under drought conditions. In other terms, genes participating in leaf senescence tended to show increased expression, further worsening the decline in growth performance. Based on the level of drought stress, genes related to photomorphogenesis and leaf development were identified under low drought stress. Subsequently, those associated with responses and expression regulation was identified under moderate drought stress, and growth-related genes were observed in high drought stress conditions. Future research should focus on the functional validation of these genes in field trials to assess their real-world applicability in enhancing drought tolerance. Additionally, integrating these gene targets into breeding programs using advanced molecular techniques could provide a pathway toward developing more resilient banana cultivars under water-limited conditions.

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Author Contributions

SNW: Conceptualization and research supervision; SD: Writing of original draft, methodology, data curation and formal analysis; EM: Validation, review and editing; HN:

Review and editing the manuscript; DSD: Visualization, review and editing; JEC: Conceptualization and research investigation.

Conflicts of Interest

The authors declare that they have no conflicts of interest in the research.

Data Availability

The transcriptome data were registered and can be accessed on NCBI BioProject database (ID PRJNA970186), under the Sequence Read Archive (SRA) ID No. SRR24677732 (for BK), SRR24677730 (for BP2) and SRR24677728 (for BP10).

Ethics Approval

This study did not involve human participants, animal subjects, or clinical trials and therefore, ethical approval was not required. All procedures conducted in this research complied with relevant institutional and national guidelines for scientific research integrity.

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References

- Al-Saharin R, H Hellmann, S Mooney (2022). Plant E3 ligases and their role in abiotic stress response. *Cells* 11:1–29
- Backiyarani S, S Uma, MS Saraswathi, AS Saravanakumar, A Chandrasekar (2015). Transcriptome analysis of banana (*Musa balbisiana*) based on next-generation sequencing technology. *Turk J Agric For* 39:705–717 <https://doi.org/10.3906/tar-1406-171>
- Bolle C, K Csaba, C Nam-Hai (2000). PAT1, a new member of the GRAS family, is involved in phytochrome A signal transduction. *Genes Dev* 14:1269–1278
- Cazzonelli CI, M Vanstraelen, S Simon, K Yin, A Carron-Arthur, N Nisar, G Tarle, AJ Cuttriss, IR Searle, E Benkova, U Mathesius, J Masle, J Friml, BJ Pogson (2013). Role of the *Arabidopsis* PIN6 Auxin transporter in auxin homeostasis and auxin-mediated development. *PLoS One* 8:1–14
- D'Hont A, F Denoeud, JM Aury, FC Baurens, F Carreel, O Garsmeur, B Noel, S Bocs, G Droc, M Rouard (2012). The banana (*Musa acuminata*) genome and the evolution of monocotyledonous plants. *Nature* 488:214–215
- Davey M, G Ranganath, JA Harikrishna, SW Lee, K Norzulaani, K Johan (2013). A draft *Musa balbisiana* genome sequence for molecular genetics in polyploid, inter- and intra-specific *Musa* hybrids. *BMC Gen* 14:1–20
- Diningrat DS, SM Widiyanto, A Pancoro, D Shim, B Panchangam, N Zembower, JE Carlson (2015). Transcriptome of teak (*Tectona grandis*, L.f) in vegetative to generative stages development. *J Plant Sci* 10:1–14 <https://doi.org/10.3923/jps.2015.1.14>
- Farooq M, M Hussain, A Wakeel, KHM Siddique (2015). Salt stress in maize: Effects, resistance mechanisms and management. A review. *Agron Sustain Dev* 35:461–481
- Farooq M, A Wahid, N Kobayashi, D Fujita, SMA Basra (2009). Plant drought stress: Effects, mechanisms, and management. *Agron Sustain Dev* 29:185–212
- Favero DS, CN Jacques, A Iwase, KN Le, J Zhao, K Sugimo, MM Neff (2016). Suppressor of phytochrome B4-#3 represses genes associated with auxin signaling to modulate hypocotyl growth. *Plant Physiol* 171:2701–2716
- Gachomo EW, JC Lopez, SR Smith, AB Cooksey, OM Oghoghohomeh, N Johnson, L Moussa, SO Kotchoni (2013). The cell morphogenesis angustifolia (AN) gene, a plant homolog of CtBP/BARS, is involved in abiotic and biotic stress response in higher plants. *BMC Plant Biol* 13:1–11
- Hu W, Z Ding, W Tie, Y Yan, Y Liu, C Wu, J Liu, J Wang, M Peng, B Xu, Z Jin (2017). Comparative physiological and transcriptomic analyses provide integrated insight into osmotic, cold, and salt stress tolerance mechanisms in banana. *Sci Rep* 7:1–12
- Huang DW, BT Sherman, RA Lempicki (2009). Systematic and integrative analysis of large gene lists using DAVID Bioinformatics Resources. *Nat Prot* 4:44–57
- Hussain M, S Farooq, W Hasan, S Ul-Allah, M Tanveer, M Farooq, A Nawaz (2018). Drought stress in sunflower: Physiological effects and its management through breeding and agronomic alternatives. *Agric Water Manage* 201:152–167
- Kumar M, MK Patel, N Kumar, AB Bajpal, KHM Siddique (2021). Metabolomics and molecular approaches reveal drought stress tolerance in plants. *Intl J Mol Sci* 22:9108–9130
- Lan W, W Ma, Y Miao (2018a). Role of auxin signaling and trichome branching in drought stress tolerance in maize. *Plant Biol Res* 18:113–124
- Lan W, W Ma, Y Miao (2018b). Role of HECT ubiquitin protein ligases in *Arabidopsis thaliana*. *J Plant Sci Phytopathol* 2:20–30
- Langhe ED, L Vyrdaghs, PD Maret, T Denham (2009). Why bananas matter: An introduction to the history of banana domestication. *Ethnobot Res Appl* 7:165–177
- Li H, Y Wang, X Yang, T Zhao (2020). Functional analysis of ANGSTIFOLIA gene orthologs in rice highlights their roles in regulating abiotic stress responses. *J Plant Res* 133:371–382
- Livak KJ, TD Schmittgen (2001). Analysis of relative gene expression data using real time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25:402–408
- Love MI, W Huber, S Anders (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Gen Biol* 15:1–21
- Muthusamy M, S Uma, S Backiyarani, MS Saraswathi, A Chandrasekar (2016). Transcriptomic changes of drought-tolerant and sensitive banana cultivars exposed to drought stress. *Front Plant Sci* 7:1–14
- Panigrahi N, AJ Thompson, S Zobelzu, JW Knox (2021). Identifying opportunities to improve management of water stress in banana production. *Sci Hortic* 276:1–9
- Roosjen M, S Paque, D Weijers (2018). Auxin response factors: Output control in auxin biology. *J Exp Bot* 69:179–188
- Rymaszewski W, D Vile, A Bediee, M Dauzat, N Luchaire, D Kamrowska, C Granier, J Hennig (2017). Stress-related gene expression reflects morphophysiological responses to water deficit. *Plant Physiol* 174:1913–1930
- Surendar KK, DD Durga, I Ravi, P Jeyakumar, K Velayudham (2013a). Studies on the impact of water deficit on morphological, physiological and yield of banana (*Musa spp.*) cultivars and hybrids.

- Intl J Agric Sci* 3:473–482
- Surendar KK, DD Durga, I Ravi, S Krishnakumar, KS Ramesh, K Velayudham (2013b). Impact of water deficit on photosynthetic pigments and yield of banana cultivars and hybrids. *Plant Genet Trait* 4:17–24
- Thingnam SS, DS Lourebam, PS Tongbram, V Lokya, S Tiwari, MK Khan, A Pandey, M Hamurcu, R Thangjam(2023). A perspective review on understanding drought stress tolerance in wild banana genetic resources of Northeast India. *Genes* 14:370-390
- Widiyanto SN, FS Inabuy, T Nugraheni, S Duve, DS Diningrat, JE Carlson (2024). Studying the transcriptome profiling of banana plantlets exposed to water stress and the alteration in their major bioprocesses. *Jor J Biol Sci* 17:37–66
- Xiao C, C Fulu, Y Xuhong, L Chentao, F Yong (2009). Over-Expression of an AT-Hook Gene, AHL22, delays flowering and inhibits the elongation of the hypocotyl in *Arabidopsis thaliana*. *Plant Mol Biol* 71:39–50
- Zhang H, J Zhu, Z Gong, JK Zhu (2021). Abiotic stress responses in plants. *Nat Rev Genet* 22:104–120
- Zhu M, Y Xu, J Yu, J Shi, C Jiang (2017). Characterization of an ANGUSTIFOLIA-like gene associated with drought tolerance in rice (*Oryza sativa*). *Plant Physiol Biochem* 112:78–87