Functional analysis of differentially expressed genes under drought conditions in the KM94 cassava variety

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Phân tích chức năng của một số nhóm gene có biểu hiện khác biệt trong điều kiện xử lý hạn ở giống sắn KM94

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https://doi.org/10.55250/jo.vnuf.10.2.2025.003-011

Article info:
Received: 07/04/2025
Revised: 28/04/2025
Accepted: 02/06/2025

Keywords:
Cassava, drought, gene expression, functional categorization, RNA-Seq.

ABSTRACT

Cassava (Manihot esculenta) is a vital food crop in tropical and subtropical regions, providing a major source of calories for over 800 million people. Although cassava shows considerable tolerance to environmental stress, drought can still reduce yields by up to 60%, posing a serious threat under the increasing frequency and severity of drought events driven by climate change. This study aimed to investigate the transcriptional response of cassava leaves to drought stress by reanalyzing publicly available RNA-Seq datasets. A total of 4,749 genes were found to be up-regulated and 2,270 genes down-regulated under drought conditions, while 17,126 genes remained non-differentially expressed. Functional categorization using the MAPMAN platform enabled systematic classification of these genes into biological processes. Notably, pathways related to secondary metabolism, particularly phenylpropanoid and flavonoid biosynthesis, were significantly enriched, suggesting a strong antioxidative and structural reinforcement response. In contrast, genes involved in photosynthesis and energy-intensive metabolic processes were largely down-regulated. Additionally, pathways associated with protein modification, RNA processing, and redox homeostasis were activated, indicating complex post-transcriptional and post-translational regulation. Hormonal signaling, especially through abscisic acid, and transcription factors such as MYB, NAC, and WRKY families also showed significant changes, highlighting their roles in coordinating drought responses. The integration of gene expression data with functional pathway analysis provided a comprehensive view of how cassava adjusts its metabolism and regulatory networks under water deficit. These findings offer valuable insights into the molecular basis of drought tolerance in cassava and identify candidate genes that could be leveraged in breeding programs or biotechnology applications to develop drought-resilient cassava varieties, thereby contributing to food security under climate change.

Từ khóa:
Biểu hiện gen, cây sắn, hạn hán, phân loại chức năng, RNA-Seq.

TÓM TẮT

Sắn (Manihot esculenta) là loại cây quan trọng ở vùng nhiệt đới và cận nhiệt đới, cung cấp nguồn calo chính cho hơn 800 triệu người. Mặc dù sắn có khả năng chịu đáng kể với các điều kiện bất thuận, hạn hán làm giảm năng suất tới 60%, đe dọa nghiêm trọng với tần suất và mức độ nghiêm trọng ngày càng tăng do biến đổi khí hậu. Nghiên cứu phân tích dữ liệu RNA-Seq để khảo sát hệ gen phiên mã của lá sắn dưới điều kiện hạn. Kết quả cho thấy 4.749 gen tăng cường biểu hiện và 2.270 gen kìm hãm biểu hiện, trong khi 17.126 gen không thay đổi đáng kể. Phân loại chức năng sử dụng MAPMAN đã sắp xếp có hệ thống vào các quá trình sinh học khác nhau. Các con đường liên quan đến chuyển hóa thứ cấp, như sinh tổng hợp phenylpropanoid và flavonoid, được tăng cường, cho thấy vai trò quan trọng trong cơ chế chống oxy hóa và củng cố cấu trúc tế bào. Ngược lại, các gen liên quan đến quang hợp và quá trình chuyển hóa tiêu tốn năng *lượng chủ yếu bị giảm. Các con đường liên quan đến biến đổi protein, xử lý RNA và cân bằng oxi hóa-khử cũng được kích hoạt, phản ánh cơ chế điều hòa sau phiên mã và sau dịch mã. Sự thay đổi của các tín hiệu hormone, đặc biệt là abscisic acid, cùng các yếu tố phiên mã như MYB, NAC và WRKY nhấn mạnh vai trò điều phối phản ứng sinh lý với hạn. Việc tích hợp dữ liệu biểu hiện gen với phân tích chức năng đã cung cấp cái nhìn toàn diện về cách sắn điều chỉnh mạng lưới trao đổi chất và điều hòa dưới điều kiện thiếu nước. Kết quả này cung cấp những hiểu biết có giá trị về cơ sở phân tử của khả năng chịu hạn ở sắn và xác định các gen ứng viên sử dụng trong chương trình lai tạo hoặc ứng dụng công nghệ sinh học để phát triển giống sắn có khả năng chịu hạn, đảm bảo an ninh lương thực trong bối cảnh biến đổi khí hậu.*

1. INTRODUCTION

Cassava (*Manihot esculenta*) is a staple root crop believed to have originated in South America and is now extensively cultivated in tropical and subtropical regions worldwide. Cassava's starchy tubers make it a crucial source of carbohydrates and energy in many developing countries [1]. This crop plays a significant socio-economic role in Vietnam, contributing approximately one billion U.S. dollars annually [2]. Despite being recognized for its inherent drought tolerance [3], cassava cultivation in Vietnam is increasingly affected by climate change and prolonged and severe droughts [4]. These adverse environmental conditions can reduce yield and crop quality [3, 4]. Understanding the molecular and physiological mechanisms underlying cassava's drought response is therefore of great importance, as it may enable the development of improved crop management and breeding strategies.

Plants, including cassava, employ various physiological and molecular mechanisms to cope with water deficit stress in their leaves [5]. At the physiological level, one of the most critical responses is the regulation of the stomatal aperture to minimize water loss through transpiration [6]. Cassava has been reported to maintain relatively efficient stomatal control, which aids in conserving water under drought conditions. In parallel, plants often accumulate osmoprotectants in their leaf tissues to help maintain cell turgor and protect cellular components [5]. Antioxidant enzymes further mitigate the damaging effects of reactive oxygen species (ROS) generated by drought stress. On the molecular level, abscisic acid plays a central role in signaling pathways that trigger drought-responsive genes [7]. Regulatory proteins, such as transcription factors, bind to specific *cis*-regulatory elements in gene promoters, activating cascades of downstream effectors that enhance stress tolerance [8]. These regulatory components act with protein kinases, phosphatases, and other signaling intermediates. Additionally, functional proteins involved in this response include enzymes responsible for synthesizing osmoprotectants, structural proteins that reinforce cell walls, and transporters that mediate ion and water movement [9]. Thus, these physiological and molecular adaptations underscore identifying which gene families and regulatory networks are most crucial to drought tolerance in cassava.

The aim of this study was to reanalyze publicly available RNA-Seq datasets to investigate the transcriptional responses of cassava leaves under drought stress. Specifically, the study identified differentially expressed genes (DEGs), categorized their functions using MAPMAN, and explored key metabolic and regulatory pathways involved in drought tolerance. By integrating gene expression profiling with pathway analysis, the study aimed to provide insights into the molecular mechanisms underlying cassava's drought response and to identify candidate genes for future functional validation and crop improvement.

2. RESEARCH METHODS

2.1. Data collection

The recent assembly of the cassava genome (NCBI RefSeq assembly: GCF_001659605.2) [10] was used as a template in this study. A recent publicly available RNA-Seq dataset (accession: GSE98537) was obtained from the GEO NCBI. In this dataset, drought stress was applied by withholding water for 12 days until visible wilting symptoms appeared. Leaf tissues were collected from fully expanded leaves at the apex of plants at the six-week-old vegetative growth stage.

2.2. Methods

- *Reanalysis of transcriptome dataset:* For transcriptome reanalysis, raw sequencing data were first retrieved from the corresponding GEO accessions and processed using standard RNA-Seq analytical pipelines. Briefly, FastQC was used to assess the quality of raw sequencing reads. Adapter sequences and low-quality bases were trimmed using Trimmomatic with a sliding window approach and a minimum read length of 50 bp. The cleaned reads were then aligned to the cassava reference genome using HISAT2 with default parameters. Read counts per gene were quantified with featureCounts using the GTF annotation file. Differential gene expression analysis was performed using DESeq2 in an R script, applying a threshold of |fold change| ≥ 2 and an adjusted p-value < 0.05 for significance. DEGs were visualized using the heatmap package in an R script.

- *Functional annotation of gene:* MAPMAN software [11] was employed to classify and visualize DEGs within known functional pathways and categories as previously described [12]. First, gene annotations were mapped to the corresponding bin classes using the MapMan mapping files. Once the DEGs were assigned to their respective functional bins, the software generated graphical representations, or "maps," of metabolic and regulatory pathways.

- *Analysis of metabolic pathways*: To analyze metabolic pathways using MAPMAN, DEGs identified from RNA-Seq data are first mapped to known functional categories using species-specific annotation files. These gene-to-bin mappings allow the software to assign each gene to a biological process, such as photosynthesis, lipid metabolism, or amino acid biosynthesis [11]. Expression values (e.g., $\log_2$ fold change) are then imported into MAPMAN, which visualizes the data as colored maps where up- and down-regulated genes appear in red and green, respectively.

- *Analysis of secondary metabolic pathways:* To analyze secondary metabolism pathways using MAPMAN, DEGs from RNA-Seq data are first annotated and assigned to MAPMAN "bins" that represent specific branches of secondary metabolism, such as phenylpropanoids, flavonoids, terpenoids, and glucosinolates. These annotations are made using pre-built mapping files for cassava or by customizing mappings based on orthologous genes from model species. The gene expression data, typically in the form of $\log_2$ fold change values, are then uploaded to MAPMAN [11]. The software visualizes the data in pathway diagrams where each gene is shown as a colored square, red for up-regulated and green for down-regulated.

- *Analysis of regulatory pathways:* To analyze regulatory pathways using MAPMAN, DEGs from transcriptome data are mapped to specific regulatory bins. These mappings are based on annotated reference files that link gene IDs to known regulatory functions [11]. After importing expression data into MAPMAN, the software displays regulatory networks with color-coded markers, typically red for up-regulated and green for down-regulated genes.

3. RESULTS AND DISCUSSION

3.1. Reanalysis of transcriptome datasets of cassava leaves under drought treatment

To characterize the transcriptional response of cassava leaves under drought stress, we reanalyzed the publicly available RNA-Seq dataset and quantified gene expression changes using fold-change values. Genes were classified as up-regulated when expression increased ≥ 2-fold and down-regulated if expression decreased by at least 2-fold (≤ -2-fold) (Figure 1A). This analysis revealed a total of 4,749 up-regulated genes and 2,270 down-regulated genes, whereas 17,126 genes were identified as non-DEGs (Figure 1B).

Among the DEGs, *Manes_06G147200* showed the highest up-regulation with a fold change of 672.10-fold, whereas *Manes_07G050400* was the most strongly down-regulated, with a fold change of -1109.53-fold. These results suggested that cassava exhibits extensive transcriptional reprogramming in response to drought.

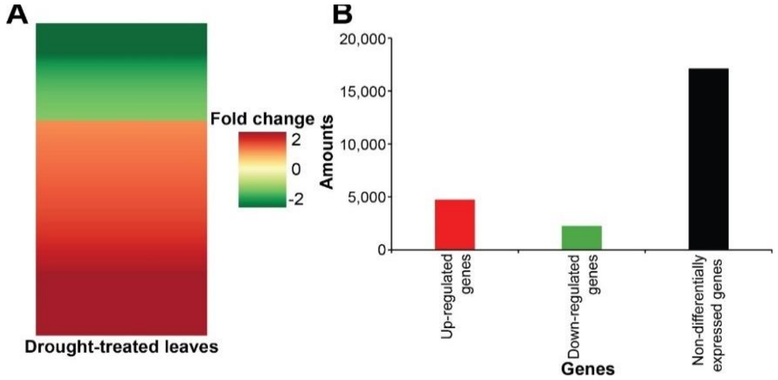

Figure 1. Transcriptome profiling of cassava leaves under drought stress
(A) Heatmap showing fold-change in gene expression;
(B) Bar chart displaying the number of differentially expressed genes.

This pattern agrees with previous transcriptomic studies in cassava under various abiotic stress treatments, which also reported widespread changes in gene expression. Based on the polyethylene glycol 6000 (PEG 6000) RNA-Seq study by Fu et al. (2016), DEGs in cassava under PEG-induced dehydration stress were identified and characterized across various tissues and time points [13]. The results showed that PEG treatment significantly altered the transcriptomic landscape of cassava, with 2,785 DEGs detected across the folded leaves, fully expanded leaves, bottom leaves, and root tissues [13]. Among these DEGs, 1,111 up-regulated and 1,073 down-regulated transcripts were recorded [13]. Roots responded most rapidly, with 1,792 DEGs identified, followed by bottom leaves (675), fully expanded leaves (512), and folded leaves (485) [13]. Additionally, at least 18,166 expressed sequence tags from drought-treated and well-watered cassava tissues, identifying 8,577 unigenes [14]. Notably, numerous drought-responsive genes were only found in dehydration-stressed tissues, including *RD22*, *RD19*, *LEA5*, and several heat shock proteins, homeobox transcription factors (*ATHB-12*), and oxidative stress protectants, such as glutathione peroxidases and monodehydroascorbate reductases [14]. These data build a solid foundation for understanding drought tolerance mechanisms in cassava.

3.2. Categorization of differentially expressed genes in cassava leaves under drought treatment

In this study, the functional categorization of DEGs in cassava leaves under drought stress provides a comprehensive view of the molecular responses activated during water-deficit conditions. Using the MAPMAN , DEGs were sorted into biological categories ("bins") based on functional annotation (Table 1). As a result, 6,796 (out of 7,019) DEGs were assigned to MAPMAN bin categories (Figure 2).

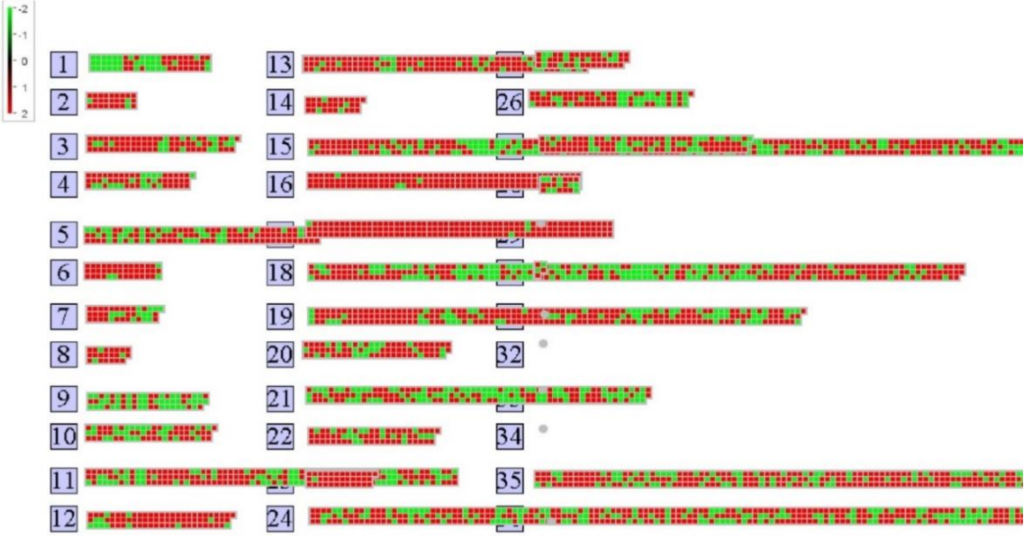

Figure 2. Functional overview of differentially expressed genes in cassava leaves under drought stress using MAPMAN visualization

Among the significantly enriched categories, secondary metabolism (Bin 9) was notably represented, with 64 DEGs (*p*-value < 0.05). This category includes genes involved in the biosynthesis of secondary metabolites such as flavonoids, phenylpropanoids, and other aromatic compounds, which are widely recognized for their roles in antioxidative defense and osmoprotection under drought. The induction of these pathways suggests that cassava actively mobilizes biochemical defenses to counteract oxidative damage caused by drought-induced stress.

Another highly enriched category was cell wall organization (Bin 21), which comprised 188 DEGs (*p*-value < 0.05). Enriching genes related to cell wall organization reflects cassava's strategy to maintain cellular integrity under drought stress. As a root crop, cassava relies on flexible yet leaf and stem structures to withstand dehydration without irreversible collapse. Remodeling the cell wall, through modulation of cellulose, hemicellulose, lignin, and expansin activity, helps reduce cell expansion, limit water loss, and preserve turgor pressure. Strengthened cell walls may protect vascular tissues, ensuring continued water transport to critical tissues during prolonged drought periods. Such structural adjustments are crucial for cassava's survival in semi-arid environments where soil moisture fluctuations are common. Genes involved in polyamine metabolism (Bin 8) were significantly enriched, which highlights the potential role of small polycationic molecules in drought adaptation. Polyamines are known to stabilize membranes, scavenge ROS, and modulate stress signaling pathways. Their accumulation during drought may help cassava mitigate cellular damage and maintain growth under stress conditions. This adaptive strategy is consistent with previous studies in other plant species that demonstrate cell wall adjustments as a common drought tolerance mechanism [15].

Table 1. Functional classification of differentially expressed genes in cassava leaves under drought stress according to MAPMAN categories

#	Bin	Name	Amount of genes	*p*-value
1	1	Photosynthesis	66	0.025744078085466
2	2	Cellular respiration	27	0.040326422943699
3	3	Carbohydrate metabolism	82	0.324510892147123
4	4	Amino acid metabolism	56	0.478990222417836
5	5	Lipid metabolism	129	0.871375067421655
6	6	Nucleotide metabolism	42	0.148549864822544
7	7	Coenzyme metabolism	40	0.469770231225937
8	8	Polyamine metabolism	23	0.004543562368506
9	9	Secondary metabolism	64	0.000001325973296
10	10	Redox homeostasis	70	0.250700830430775
11	11	Phytohormone action	204	0.216560294813246
12	12	Chromatin organisation	78	0.566261732360305
13	13	Cell division	155	0.096848835028473
14	14	DNA damage response	31	0.687320872360191
15	15	RNA biosynthesis	576	0.941103994296522
16	16	RNA processing	145	0.000085319279382
17	17	Protein biosynthesis	166	0.050007034018298
18	18	Protein modification	358	0.000060327436547
19	19	Protein homeostasis	268	0.003853173000796
20	20	Cytoskeleton organisation	79	0.826087664081563
21	21	Cell wall organisation	188	0.000009436844085
22	22	Vesicle trafficking	69	0.211616779098572
23	23	Protein translocation	37	0.370244157787179
24	24	Solute transport	405	0.213118777817012

#	Bin	Name	Amount of genes	*p*-value
25	25	Nutrient uptake	50	0.680122553361687
26	26	External stimuli response	88	0.984055499224743
27	27	Multi-process regulation	112	0.803343049079233
28	28	Plant reproduction	20	0.649140458429430
29	30	Clade-specific metabolism	5	0.131154687962860
30	35	Not assigned	3163	0.001217606440060

The protein modification category (Bin 18) showed the highest number of assigned DEGs, with 358 genes significantly enriched (*p*-value < 0.05). The significant activation of protein modification pathways highlights the need for dynamic regulation of protein activity during drought in cassava. Post-translational modifications, such as phosphorylation and ubiquitination, enable cassava cells to rapidly activate or deactivate stress-responsive proteins without requiring new gene transcription. This flexibility is vital under drought, where cellular signaling, osmotic regulation, and metabolic reprogramming must adjust quickly to avoid damage. Given cassava's relatively slow shoot growth compared to cereals, efficient protein turnover and regulation likely enhance its resilience by prioritizing survival functions over growth under water-limited conditions. The enrichment of genes associated with protein homeostasis (Bin 19) suggested that drought stress triggers active mechanisms to maintain protein quality and stability. Under water-deficit conditions, misfolded or damaged proteins accumulate due to oxidative stress. Genes encoding chaperones, heat shock proteins, and components of the ubiquitin-proteasome system were differentially expressed, indicating enhanced protein folding, repair, and degradation processes to protect cellular function during drought. In addition, RNA processing (Bin 16) was also significantly enriched, with 145 genes involved (*p*-value < 0.05). This suggested that drought stress triggers extensive modulation at the post-transcriptional level, including mRNA splicing, editing, and degradation. Such processes are critical for fine-tuning gene expression in response to fluctuating stress intensity and ensuring transcriptome flexibility.

A substantial portion of DEGs, 3,163, were categorized under the "Not assigned" bin. This limitation highlights the challenges associated with functional genomics in non-model crops such as cassava, where many drought-responsive genes may lack characterized homologs in model species. Future efforts, including improved genome annotation through high-quality reference assemblies, expanded functional databases, and large-scale transcriptome studies across different conditions and tissues, could help assign functions to these currently unannotated genes.

3.3. Functional enrichment of metabolic and regulatory pathways reveals key drought-responsive mechanisms in cassava leaves

In this study, we investigated metabolic pathways affected by drought stress in cassava leaves. Our study revealed broad alterations across primary and secondary metabolic pathways (Figure 3). A considerable number of genes involved in photosynthesis, particularly those associated with light reactions, photophosphorylation, and photorespiration, were differentially expressed. Many of these genes showed reduced expression, which suggests that drought stress suppresses photosynthetic activity to minimize water loss and the accumulation of ROS. The downregulation of photosynthesis and other energy-intensive metabolic processes likely represents an adaptive strategy to conserve limited energy resources and reduce the generation of ROS, which can cause cellular damage under drought-induced oxidative stress. In contrast, several genes in the oxidative pentose phosphate and fermentation pathways showed increased expression, which suggests a shift in carbon flow away from energy-demanding processes and toward mechanisms that support stress adaptation. This shift appears to promote the generation of nicotinamide adenine dinucleotide phosphate and adenosine triphosphate under restricted photosynthetic activity, which helps maintain energy supply and redox balance within the cell. Genes related to mitochondrial electron transport and the tricarboxylic acid cycle also showed significant modulation, suggesting that mitochondrial respiration is actively regulated to meet energy demands under drought. Carbohydrate metabolism, including starch and sucrose metabolism, displayed mixed expression patterns, which suggest both energy mobilization and storage adjustments. Next, lipid metabolism, especially in pathways related to membrane lipid turnover, showed a substantial number of up-regulated genes. This may be linked to membrane remodeling and signaling under drought-induced cellular stress. Similarly, amino acid metabolism, including genes related to proline and branched-chain amino acids, was activated, proline being a well-known osmoprotectant under drought conditions. Additionally, nutrient assimilation and related metabolic components, including nitrogen (NO_3^-/NH_3) and sulfur (SO_4^{2-}) metabolism, were modulated, along with pathways for nucleotide biosynthesis, which suggests that essential macromolecular biosynthesis and turnover are tightly regulated during stress adaptation.

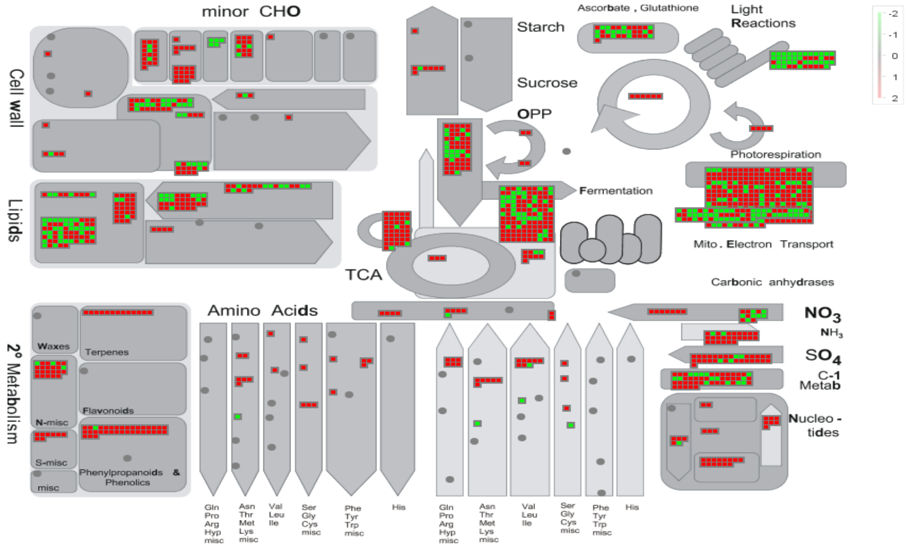

Figure 3. Overview of metabolic pathways affected by drought stress in cassava leaves

Of particular importance, the secondary metabolism pathways, especially phenylpropanoid and flavonoid biosynthesis, exhibited prominent transcriptional activation . These compounds play crucial roles in antioxidant defense and cell wall fortification. Furthermore, cell wall metabolism was also affected, with both up- and down-regulated genes involved in lignin biosynthesis, hemicellulose remodeling, and pectin modification, reflecting structural adjustments to limit water loss (Figure 4).

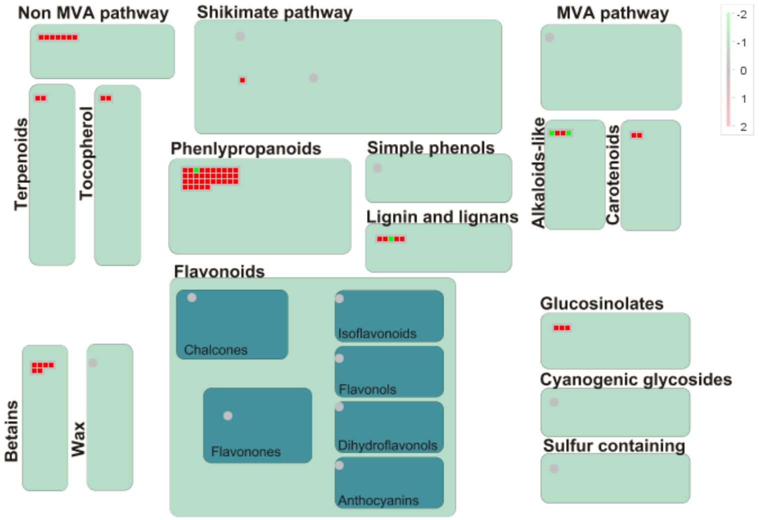

Figure 4. Secondary metabolism pathways are differentially regulated in cassava leaves under drought stress

The MAPMAN overview of regulatory pathways revealed extensive activation of signaling and stress-responsive genes in cassava leaves exposed to drought stress. One of the most prominent responses was observed in abscisic acid signaling, where a high number of genes showed up-regulation (Figure 5). The strong induction of abscisic acid signaling observed in drought-stressed cassava leaves reflects abscisic acid's central role as a master regulator of water stress responses. In contrast, genes related to auxin, gibberellin, and brassinosteroid pathways showed mixed patterns of up- and down-regulation, suggesting complex hormonal crosstalk. Generally, drought stress promotes antagonistic interactions between abscisic acid and growth-promoting hormones such as auxin and gibberellins, leading to growth inhibition and prioritization of survival pathways. Meanwhile, ethylene-related genes exhibited both activation and repression, consistent with ethylene's dual role in regulating senescence and stress adaptation. The observed patterns imply that in cassava under drought, abscisic acid signaling likely antagonizes auxin-mediated growth pathways to conserve resources, while partially synergizing with ethylene signaling to coordinate adaptive responses such as stomatal closure, oxidative stress mitigation, and controlled senescence. This hormonal balance may enable cassava to fine-tune its physiological adjustments under water-deficient conditions.

The transcription factor category displayed strong transcriptional activation, with many up-regulated genes. These likely include members of drought-responsive families such as MYB, NAC, bZIP, and WRKY, which modulate downstream stress-related gene networks. The observed patterns indicate that cassava reprograms transcriptional activity to prioritize defense and survival over growth. Multiple genes encoding thioredoxins, glutaredoxins, ascorbate/glutathione enzymes, and catalases exhibited differential expression in the redox regulation section. Their activation underlines the importance of oxidative stress mitigation in cassava's drought response. Efficient control of oxidative stress is crucial for cassava's drought survival strategy, as minimizing ROS accumulation helps preserve cellular structures, maintain photosynthetic efficiency, and prevent premature senescence during prolonged water-deficient conditions. Other components of signal transduction, including calcium regulation, MAP kinases, receptor kinases, and G-proteins, showed minimal differential expression, which suggests early or transient activation of these pathways may have occurred before the sampling point or may play a tissue-specific role not captured in this analysis. This regulatory landscape highlights a tightly coordinated network of hormonal signals, redox adjustments, and transcriptional regulation that underpins cassava's physiological and molecular response to drought stress.

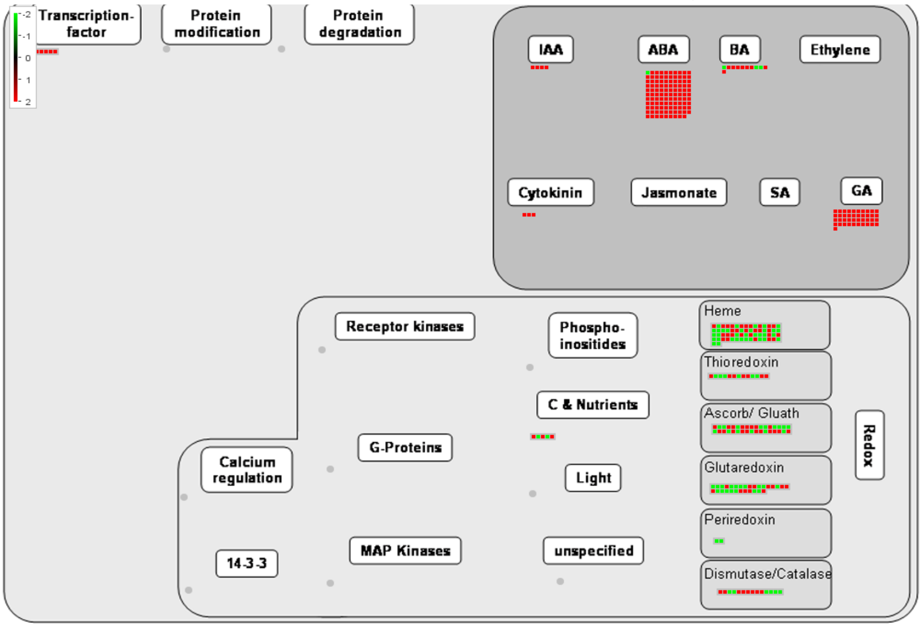

Figure 5. Overview of regulatory pathways activated in cassava leaves under drought stress

4. CONCLUSIONS

This study provided a reanalysis of cassava transcriptome data under drought stress. Through differential expression analysis, we identified 4,749 up-regulated and 2,270 down-regulated genes, with a substantial number of these genes associated with known drought-responsive functions. Functional categorization using MAPMAN highlighted several key metabolic and regulatory pathways involved in cassava's drought adaptation. Genes related to phenylpropanoid and flavonoid biosynthesis were strongly up-regulated. Photosynthesis-related genes showed broad down-regulation, while pathways related to oxidative pentose phosphate metabolism, fermentation, mitochondrial respiration, and amino acid synthesis were up-regulated to support cellular energy balance and stress tolerance. Regulatory networks also exhibited extensive transcriptional changes. Abscisic acid signaling emerged as a dominant pathway, accompanied by altered expression in other hormone-related genes, transcription factors, and redox-associated genes. These findings underscore the importance of hormonal and redox regulation in orchestrating the drought response.

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