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## TRANSCRIPTOMIC AND METABOLOMIC STUDY OF YELLOW-FLESHED CASSAVA CULTIVAR CARVITA-25, A MUTANT OF ADIRA-4

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### SUMMARY

Cassava tuberous roots are rich in starch but deficient in micronutrients such as provitamin A. The cassava cultivar Carvita-25 is a yellow variant of a white-fleshed cassava genotype (Adira-4) obtained through friable embryogenic callus. The following study aimed to ascertain substantial disparities in metabolite profiles and gene transcripts associated with carotenoid-related characteristics. Genotype Carvita-25 contains  $\beta$ -carotene and its derivative apocarotenoid, responsible for the yellow coloration of the tubers. The metabolite profile exhibited discrepancies in metabolite composition between the cultivars Adira-4 and Carvita-25. Genotype Adira-4 contains the highest levels of amino acid compounds, peptides, and their derivatives, while genotype Carvita-25 contains more amine-type compounds. Differential transcription levels were notable among the genes responsible for carotenoid biosynthesis, Manes.02G081700.1 (*PSY1*). An enhanced transcription level of *PSY1* was evident in Carvita-25 compared with Adira-4, while the lower transcription level of *PSY2* resulted in Carvita-25 compared with Adira-4. The GO (gene ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) analyses revealed the upregulation of 2,000 genes and downregulation of 1,772 genes in

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Carvita-25 compared with Adira-4. These comprise cytochrome P450 (*CYP82D47*), and bHLH family transcription factors were the candidate regulators of carotenoid-related genes in root tubers. This information can be further applicable to developing strategies for improving the quality of cassava plants rich in carotenoid compounds.

**Keywords:** Cassava genotypes, apocarotenoids, metabolites, phytoene synthase, transcriptome, yellow-fleshed cassava

**Key findings:** Carvita-25, a mutant cassava, has appeared to contain beta carotene and apocarotenoid metabolites with provitamin-A functions. The discrepancies in phytoene synthase genes and *PSY1* and cytochrome 450 (*CYP82D47*) have been the identified primary factors contributing to variances in the carotenoid profile of Carvita-25 from Adira-4.

## INTRODUCTION

Cassava is a tropical root crop playing an influential role in food security due to its ability to grow well in suboptimal soil conditions and retain its productivity even during prolonged periods of drought (Imakumbili *et al.*, 2021). Cassava tubers serve as the predominant nutrient reserve within plants that are rich in carbohydrates (Wang *et al.*, 2022). The tuber flesh is typically white, although other colors also exist in minor quantities within the cassava germplasm. However, in tubers the yellow shade is a general indication of a higher carotenoid content that is beneficial for human health (Carvalho *et al.*, 2016). Therefore, various efforts have been progressing to obtain cassava with better nutritional quality, especially carotenoid content. In cassava tubers, the improvement in nutritional quality is imperative given the substantial global prevalence of malnutrition, particularly in the community exclusively dependent on cassava as a food source (Kumar *et al.*, 2024).

Somatic embryogenesis induction, an approach to plant breeding, can be helpful to improve genetic quality through the application of biotechnology. It is one of the *in vitro* propagation methods that has the potential to cause variation in the genome or epigenome, resulting in somaclones (somaclonal variation) (Krishna *et al.*, 2016). The induction of friable embryogenic callus (FEC) is a frequently employed method for genetic engineering transformation in cassava (Elegba *et al.*,

2021). The FEC induction has become a potential inducer of somaclonal variation. In fact, it involves the use of high growth regulators, controlled photoperiods, and repeated embryogenic callus growth cycles (Ma *et al.*, 2015). Mutation and genetic variation in tissue culture occur due to various factors. One is stress caused by injury, exposure during the sterilization process, and incomplete tissue. Others are hormonal imbalance in the growth medium (such as high concentrations of auxins and cytokinins), sugar use as a substitute for photosynthesis in leaves, lighting, and the balance between humidity and the transpiration process (Bednarek and Orłowska, 2020). The development of the somaclonal variant, Carvita-25, has resulted from the cultivar Adira-4 through the application of the FEC induction technique (FEC line 25). Its characteristics comprised the yellow flesh and a distinctive flavor profile, which differentiates it from the cassava parental genotype Adira-4, with the white flesh and a slightly bitter taste (Hartati *et al.*, 2018).

Carotenoids are the secondary metabolites that play a pivotal role in various organisms with diverse functions. In humans, carotenoids, such as  $\beta$ -carotene and  $\alpha$ -carotene, act as antioxidants and provitamin A to maintain ocular health. Moreover, carotenoids have a positive effect on degenerative diseases and the immune system (Kaulmann and Bohn, 2014). It is noteworthy that vitamin A deficiency can be lethal, with symptoms including blindness and death. In

plants, carotenoids perform an integral role in growth and development. These compounds function as photosystem components and precursors for signaling molecules and phytohormone synthesis (Sun *et al.*, 2022).

Although a positive association exists between the color of cassava storage root parenchyma and the total carotenoid content, the latter is unquantifiable through visual observations only. Identification and measurement of metabolite components is an approach for assessing the overall nutritional quality in cassava tubers (Rosado-Souza *et al.*, 2019). Moreover, the advent of omics has facilitated comprehensive investigation for the correlation between the metabolome and transcriptome in plants (Upton *et al.*, 2023). These two approaches in combination have proven to be a potent method to identify the specific genes involved in the accumulation of carotenoid compounds in diverse plant species. Such studies have been applicable in various plant species, including coffee beans (Hu *et al.*, 2022), melons (Diao *et al.*, 2023), sweet potatoes (Jia *et al.*, 2022), and citrus fruits (Chen *et al.*, 2023). Although in cassava, several genes associated with carotenoid biosynthesis have been notable, the underlying mechanisms, particularly those involved in accumulation in the root tubers, remain elusive and require continuous studies (Olayide *et al.*, 2023).

This study comprised a comparative analysis of the white cassava cultivar Adira-4 and the yellow mutant Carvita-25 to ascertain the differences in compound content and gene regulation in both cultivars. The presented findings will enhance the comprehension about the regulatory network of carotenoid biosynthesis in cassava tubers and facilitate the advancement of cassava varieties with augmented carotenoid content. The characterized cassava cultivar Carvita-25 will be significantly beneficial for the dissemination and consumption of food and for health purposes.

## MATERIALS AND METHODS

### Plant material

In this experiment, the two cassava cultivars, viz., the white-fleshed cassava cultivar 'Adira-4' and its yellow-fleshed mutant 'Carvita-25,' were specimens used, hereafter referred to as A-4 and C-25. Both cultivars underwent clonal cultivation in an experimental field at the Soekarno Science and Technology Area, National Research and Innovation Agency (BRIN), Cibinong-Bogor, Indonesia. It is evident that Carvita-25 has demonstrated consistent stability across a minimum of five planting cycles. Cassava tubers' successful harvesting occurred from the plants nine months after planting (MAP) with three biological replications. Following harvesting, the tubers (starchy flesh) received immediate freezing in liquid nitrogen before storing at -80 °C until further analysis.

### Metabolome analysis

The  $\beta$ -carotene content determination proceeded by HPLC (high-performance liquid chromatography), following the methodology of Wada *et al.* (2013). Untargeted metabolite profiling employed liquid chromatography-high resolution mass spectrometry (LC-HRMS). Cassava fresh tubers sustaining thorough grinding had 100 mg of the resulting powder transferred to a centrifugation tube. Then, the sample underwent extraction by vortexing with 1 ml of absolute methanol at room temperature for 30 minutes. The filtering of the supernatant through a 0.22  $\mu$ m PTFE filter reached its collection in a vial. The extraction process entailed three repeats. The collected supernatant then incurred desiccation in an oven at 27 °C. All the aforementioned processes proceeded without light. For each sample, the processes took place three times. The obtained sample extracts received analysis using a liquid chromatography system (Thermo

Scientific™ Vanquish™ UHPLC Binary Pump) on an Orbitrap high-resolution mass spectrometry instrument (Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap™ High-Resolution Mass Spectrometer), according to the methodology described by Suratno *et al.* (2023).

The obtained raw data from the total ion chromatograms (TICs) entailed extraction to reveal the metabolite content in cultivars A-4 and C-25 tubers. Performing the analysis used the Compound Discoverer® software (Thermo Scientific, USA) with an untargeted metabolomics workflow. The analysis encompassed all the raw data, including blanks (methanol), with the results filtered by name, focusing on the best matches with MzCloud and MS2 for DDA for preferred ions. The KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis succeeded in using MetaboAnalyst 6.0 (Pang *et al.*, 2024).

### Transcriptome analysis

Total RNA extraction from the cassava tubers used the NucleoSpin RNA Plant and Fungi Kit (Macherey-Nagel) following the manufacturer's protocol. High-quality RNA with an RIN value greater than six progressed in the construction of cDNA libraries, which subsequently entailed sequencing on the Illumina paired-end sequencing platform with a read length of 150 base pairs by Novogene Bioinformatics Technology Co., Ltd., Singapore. The quality of the reads, as evaluated, utilized the FastQC software (version 0.12.1) (Vashishtha *et al.*, 2022). The raw data then underwent filtration using the Adapter Removal command (Schubert *et al.*, 2016) and Super Deduper (Petersen *et al.*, 2015), with the objective of removing adapters and discarding PCR sequence duplicates until the obvious reads occurred. The clean reads reached subsequent alignment with the cassava reference transcript, version 8.1, downloaded from the Phytozome database (<https://phytozome.jgi.doe.gov/>). The process of alignment and quantification of total reads achieved summarization at the gene level using the Salmon method (Patro *et al.*, 2017).

In comparing the gene expression differences between cassava cultivars A-4 and C-25, differentially expressed gene (DEG) analysis continued using the DESeq2 software (Bioconductor) with RStudio version 4.2.0 (Love *et al.*, 2014). Gene Ontology (GO) enrichment analysis commenced on the DEGs obtained using the DAVID program (Huang *et al.*, 2009).

### qPCR analysis

The performed qPCR engaged the CFX Connect Real-Time PCR Detector (Bio-Rad) and SYBR Green I master mix, following the manufacturer's instructions. In validating the transcriptomic data, primers for four genes, namely, *phytoene synthase-1* (*PSY1*), *phytoene synthase-2* (*PSY2*), *lycopene-β* (*Lyc-β*), and *ζ-carotene desaturase* (*ZDS*), succeeded in developing using the Primer-3 program. Three biological replicates employed helped conduct relative expression analysis of target genes. The *actin* gene was the option as the internal reference gene for qPCR data normalization. Obtaining relative expression level values relied on the formula  $2^{-\Delta\Delta Ct}$ , with the Ct value of each gene sample normalized to the Ct value of the reference gene (Livak and Schmittgen, 2001).

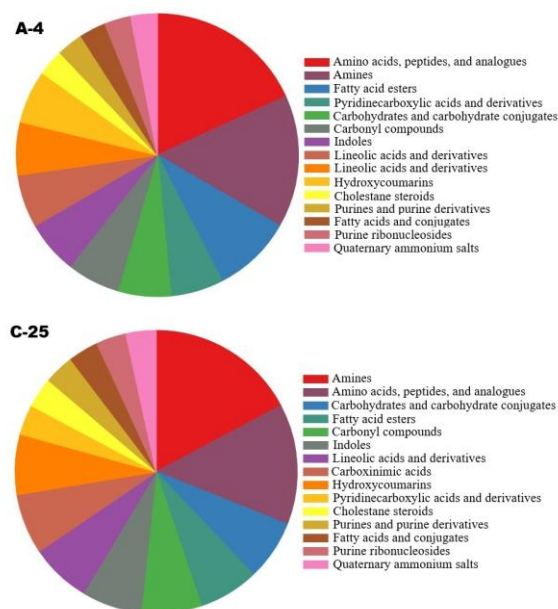
## RESULTS

### Phenotypic characterization and metabolites' analysis

The said study employed two cassava cultivars, designated A-4 and C-25, which exhibited diverse leaf and tuber pigmentation traits (Table 1). Cultivar A-4 displayed yellowish-green leaves and white tubers, whereas the genotype C-25 revealed reddish-brown leaves and yellow tubers. The HPLC analysis revealed the cultivar A-4 had negligible β-carotenoid content and was below the limit of detection. In contrast, cultivar C-25 exhibited considerably higher β-carotenoid content. The TIC chromatogram analysis displayed the presence of 216 diverse chemical compounds

**Table 1.** Leaf and tuber characteristics of Adira-4 and Carvita-25.

Genotype	Code	Leaf color	Root color	$\beta$ -carotene ( $\mu\text{g.g}$ )
Adira-4	A-4	Yellowish-green	White	<0,02
Carvita-25	C-25	Reddish-brown	Yellow	22,6-23,7

**Figure 1.** A pie chart displaying the chemical composition of the tubers of the cassava genotypes Adira-4 (A-4) and Carvita-25 (C-25).

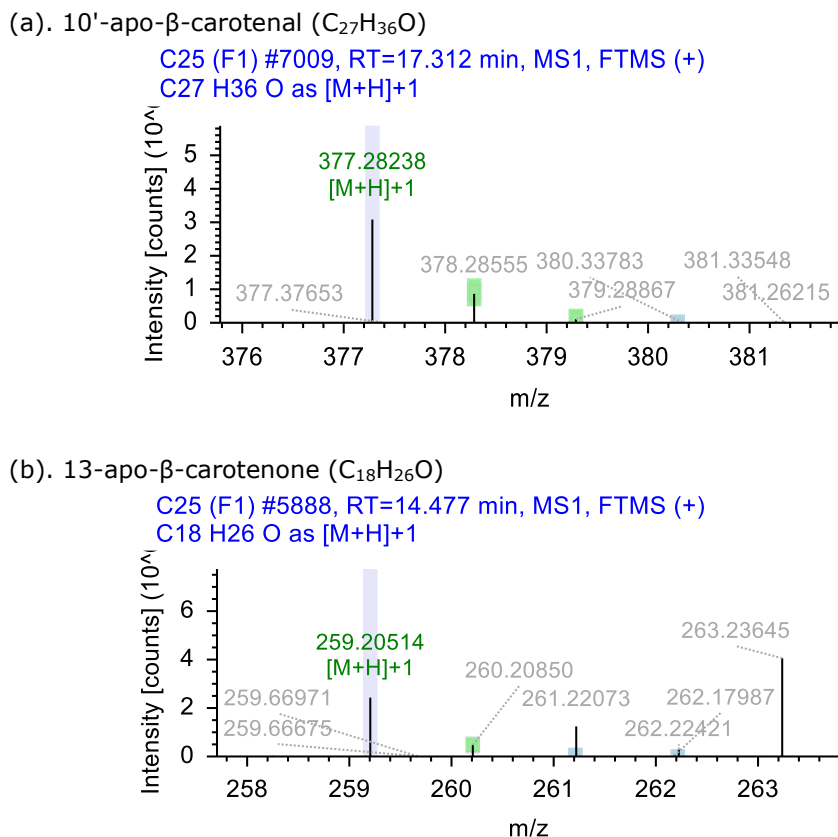
in both cultivars, with compounds totaling 137 identified in cultivar A-4 and 178 compounds identified in cultivar C-25. A total of 38 chemical compounds were exclusively distinct in the cultivar A-4, with 79 detected only in cultivar C-25 and 99 detected collectively in both cassava genotypes.

The GO enrichment analysis of the metabolite components carried out in cassava cultivars A-4 and C-25 revealed a predominance of amino acid compounds and their derivatives, amine compounds, and fatty acid esters (Figure 1). Amino acids isoleucine, histidine, L-arginine, and gamma-aminobutyric acid (GABA) were also present in both cultivars A-4 and C-25. Amino acid isoleucine appeared to be more abundant in A-4, while GABA was more dominant in C-25. Cultivar A-4 also contained amino acids not found in C-25, such as L-alanine and glutamic acid (Glu), while cultivar C-25 contained the amino acid L-valine. In this study, untargeted metabolite

analysis of the cultivar C-25 tuberous root disclosed the presence of two compounds: 10'-apo- $\beta$ -carotenal ( $\text{C}_{27}\text{H}_{36}\text{O}$ ) and 13-apo- $\beta$ -carotenone ( $\text{C}_{18}\text{H}_{26}\text{O}$ ). These compounds achieved detection at retention times (RT) of 17.31 and 14.47, with molecular weights of 377,282 and 259,205, respectively (Figure 2).

### Transcriptome analysis

The transcriptomes found in the cassava cultivars A-4 and C-25 tubers bore comparison to further examine the underlying molecular mechanisms that regulate carotenoid variations in these tubers. Sequencing data totaling 75.7 GB succeeded in generating from six sets of cDNA libraries. Following the quality control check and removal of adapters and repetitive sequences from PCR, 336,650,187 raw reads and 330,812,218 clean reads resulted. The average Q30 value exceeds 94.06%. The average GC content was 43.21%, with a



**Figure 2.** Apocarotenoid compounds detected in cultivar Carvita-25 tuber samples. 10'-apo- $\beta$ -carotenal (C<sub>27</sub>H<sub>36</sub>O) –(a) and 13-apo- $\beta$ -carotenone (C<sub>18</sub>H<sub>26</sub>O) –(b).

sequencing error of 0.025% (less than 0.1%) (Table 2). These statistics indicate that the quality of the sequencing data was adequate for further analysis.

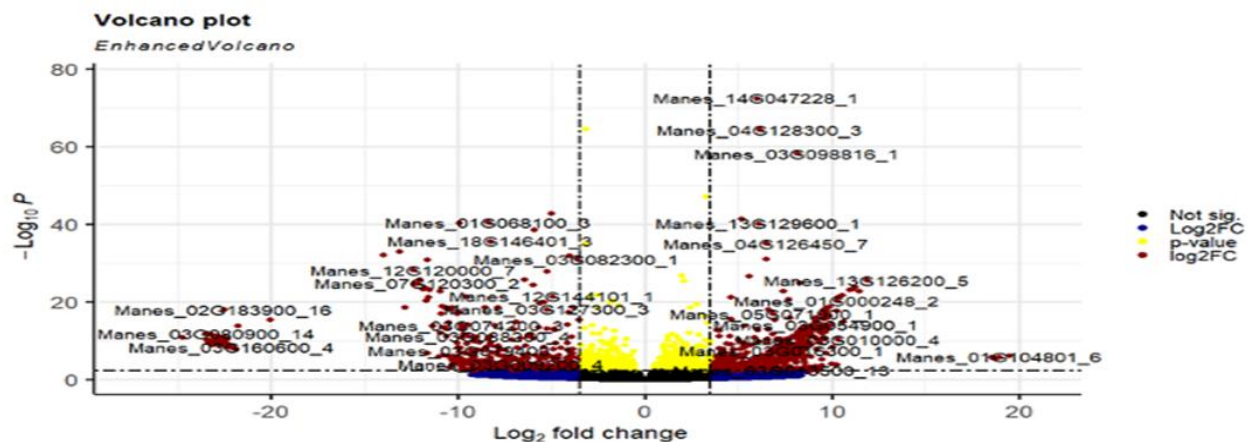
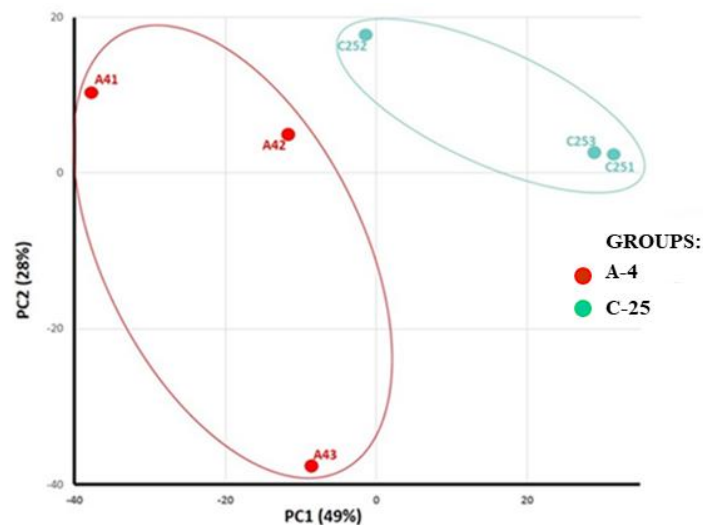
The identified unigenes totaled 42,360, with 2,000 exhibiting increased expression and 1,772 displaying decreased expression in the cassava cultivar C-25 compared with the genotype A-4 (Figure 3). The PCA analysis further demonstrated the first principal component (PC1) discriminated the expression pattern of the cultivar C-25 from A-4 by 49% of the genes' variation. Meanwhile, the second principal component (PC2) explained an additional 28% of the variation and separated the genes' expression patterns of cultivar C-25 from A-4 (Figure 4). Three plants of cultivar Adira-4, utilized as biological replicates, had exhibited substantial variations in the gene expression, as evidenced by their failure to cluster. In contrast, the cultivar C-25 replicates

demonstrated a tendency to cluster, suggesting similarities in their expression patterns.

A GO-based gene enrichment analysis progressed to elucidate the functions and processes associated with gene transcripts in the two cassava cultivars exhibiting differential expressions in the cultivar C-25 compared with A-4. The data revealed 32 GO terms that exhibited a statistically significant increase in C-25 relative to A-4, entailing classification into 13 'biological processes,' eight 'molecular functions,' and six 'cellular components.' Conversely, the cultivar C-25 transcripts that decreased in comparison with A-4 resulted in one category of 'molecular function' and two categories of 'biological process' and 'cellular component.' The genes that play a significant positive role in biological process, cellular component, and molecular function in yellow-fleshed cultivar C-25 were notable, including

**Table 2.** Summary of the raw and clean reads of the transcriptome data for cassava cultivars Adira-4 and Carvita-25.

Samples	Raw data	Raw reads	Error (%)	Q30 (%)	GC (%)	Clean reads	Low quality	Adapter related
						Count	Count	Count
A41	12.7	42422380	0.02	94.47	43.45	41661180	0	760665
A42	12.8	42654087	0.02	94.41	46.20	40314985	23	2338403
A43	12.9	86155262	0.03	93.42	41.78	85666432	0	488830
C251	11.9	39544453	0.03	93.88	42.60	38591373	3	950960
C252	13.1	43633581	0.02	94.19	42.56	42815560	3	815824
C253	12.3	82240424	0.03	93.97	42.64	81762688	0	477736

**Figure 3.** The volcano plot provides a visual representation of the cassava genotype Carvita-25 tuber dataset in comparison with the Adira-4 genotype dataset, highlighting the presence of differentially expressed genes (DEGs) in the former.**Figure 4.** Principal component analysis (PCA) showed transcriptome relationships between genotypes A-4 and C-25 based on transcripts detected in cassava tuber samples (FPKM>1). Clustering based on genotype replication and separation between genotypes, as visualized by PC1 vs PC2.

Manes.05G168100, which is a *Cytochrome P450 (CYP82D47)* superfamily gene, *PHYB*, and several transcription factors, such as Manes.04G131000 (*bHLH68*), Manes.02G197900 (*bHLH81*), *bHLH96*, TFIID subunit 14b, heat shock factor protein (*HSF30*), heat stress transcription factor (*HSA-3*), *HSA-6b*, and general transcription factor IIH subunit 2.

Transcript differences were evident in several genes involved in the accumulation of  $\beta$ -carotene in cassava tubers and the genes encoding *phytoene synthase (PSY)*. Manes.02G081700.1 (*PSY1*) and Manes.16G099600.2 (*LYCe*) appeared to have the highest transcripts in yellow-fleshed cultivar C-25 compared with transcripts in white-fleshed genotype A-4, with differences of 3.24 ( $p < 0.05$ ) and 4.82 ( $p < 0.05$ ), respectively. In contrast, Manes.01G124200.4 (*PSY2*) showed a negative correlation with a lower transcript abundance in yellow-fleshed C-25 than in white-fleshed cultivar A-4 by -1.00 ( $p < 0.05$ ). Other transcripts, such as Manes.01G001200.1 (*zeta-carotene isomerase / 15-cis-zeta-carotene isomerase*), also showed higher transcripts in the cultivar C-25 than in A-4, with a difference of 1.33 (Figure 5a). In validating the accuracy of the RNAseq analysis, in the carotenoid biosynthesis pathway, four functional genes obtained selection for abundance measurement by qPCR analysis. The expression patterns of four genes in cassava cultivars A-4 and C-25 were consistent with the patterns shown by the RNA sequencing analysis. Similarly, the Pearson correlation coefficient between qPCR quantification and the transcriptomes reached up to 0.69 ( $p < 0.05$ ), indicating the transcriptome results were reliable (Figure 5b).

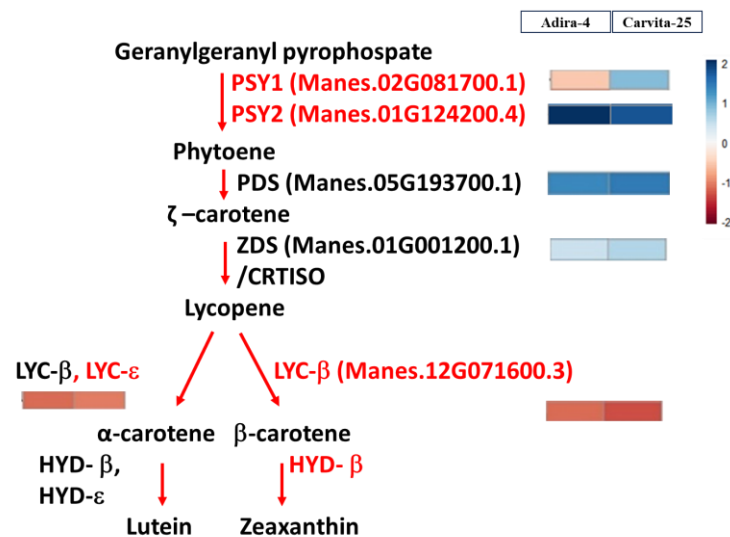
## DISCUSSION

Recent studies have shown a marked shift in consumer preferences toward yellow-fleshed cassava over white-fleshed ones. This might be due to the higher nutritional values and health benefits associated with yellow-fleshed cassava (Bechoff *et al.*, 2018). Moreover, it is noteworthy that yellow-fleshed varieties

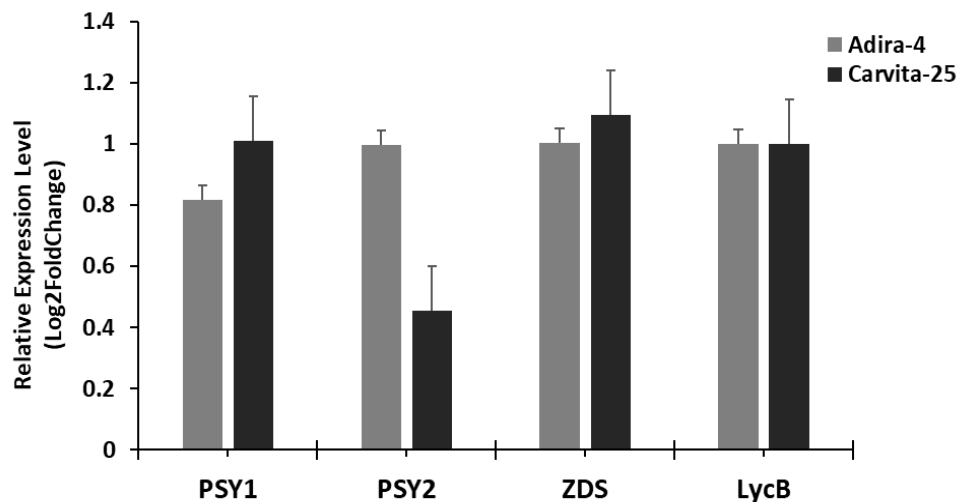
possess a sweet taste, while white-fleshed varieties can be sweet or bitter. The bitter taste of these varieties has been referable to the high content of toxic cyanogenic glycosides ( $\text{HCN} > 100 \text{ mg/kg}$ ) and their high antioxidant levels. It is evident that yellow-fleshed varieties are favorable for local food preparation (Ayetigbo *et al.*, 2018). The yellow-fleshed cassava cultivar C-25 with carotenoid content and other metabolite compounds that can act as bioactive compounds seemed to provide more health benefits, although the total number of metabolites identified in this study was fewer than in previous reports (Xiao *et al.*, 2021). Drapal *et al.* (2019) evaluated the biochemical diversity of cassava germplasm and detected more than 100 metabolites using a targeted metabolite detection approach. White-fleshed cassava tubers contain elevated levels of primary amino acid metabolites, such as asparagine, Glu, isoleucine, lysine, methionine, serine, and threonine, versus yellow-fleshed tubers (Xiao *et al.*, 2021; Olayide *et al.*, 2023). Conversely, the colored tubers were dominant with secondary metabolites, such as flavones, phenylpropanoids, and alkaloids with higher concentrations (Xiao *et al.*, 2021).

The amino acid GABA, identified in the yellow-fleshed C-25, seemed to play a positive role in plant response to various abiotic stimuli, such as drought, salt, cold, and heat tolerance (Jin *et al.*, 2023). Tomatoes with elevated GABA levels have surfaced to preserve the nutritional quality of the fruit (Tilahun *et al.*, 2021). In the presented study, the compounds  $\beta$ -apo-10'-carotenal and  $\beta$ -apo-13, which are the derivatives of  $\beta$ -carotene, were also noticeable in the yellow-fleshed cultivar C-25 (Imtiaz *et al.*, 2023). APOs represent a heterogeneous class of essential metabolites derived from the oxidative cleavage of carotenoids, including  $\beta$ -apo-8'-carotenal,  $\beta$ -apo-10'-carotenal, and  $\beta$ -apo-13-carotenone. The oxidative cleavage of the carotenoids to APO can occur via an enzymatic process, mediated by carotenoid cleavage dioxygenases (CCDs), or a non-enzymatic process (not site-specific) involving ROS and an enzymatic process (site-specific) involving lipoxygenases

(a).



(b).



**Figure 5.** Gene expression profile detected by RNA-seq and RT-qPCR. Heat map representation of the abundance of carotenoids biosynthesis-related gene transcripts in cassava tubers of the cultivars Adira-4 and Carvita-25, based on (a) RNA sequencing data and (b) Validation of the RNA sequencing and RT-qPCR results in target genes. Three replicates were used for each gene, and the error bars represent the mean  $\pm$  SE.

and peroxidases (Imtiaz *et al.*, 2023). Moreno *et al.* (2020) reported APO metabolic processes occur spontaneously because of ROS reactions, or alternatively, they may undergo catalyzation by enzymes that generally belong to the carotenoid's dioxygenase family. These enzymes include  $\beta$ -carotene 15,15'-oxygenase (BCO1) and  $\beta$ -carotene 9',10'-oxygenase (BCO2) (Durojaye *et al.*, 2019). Furthermore,  $\beta$ -carotene compounds can undergo oxidative

cleavage reactions, resulting in the formation of  $C_{27}$   $\beta$ -apo-10'-carotenal and its alcohol  $C_{18}$   $\beta$ -apo-13-carotenone. This is a product of specific enzymatic reactions by CDDs, particularly CDD7 and CDD8 (Imtiaz *et al.*, 2023). Similarly, the latest results indicated the formation of 10'-apo- $\beta$ -carotenal ( $C_{27}H_{36}O$ ) and 13-apo- $\beta$ -carotenone ( $C_{18}H_{26}O$ ) compounds, which can occur due to enzymatic and non-enzymatic reactions.

The analysis of gene transcript differences in the carotenoid biosynthesis pathway revealed an association with variations in the carotenoids' level of the cassava cultivars exhibiting pale yellow and deep yellow tuber colors. However, these findings have not yet produced definitive conclusions (Xiao *et al.*, 2021; Olayide *et al.*, 2023). The biosynthesis of  $\beta$ -carotene can gain regulation from other genes and regulatory factors both inside and outside the carotenoid biosynthetic pathway (Olayide, 2022). For example, the genes *phytoene synthase*, *Z-isomerase*, *zeta-carotene desaturase*, *carotenoid isomerase*, *CCD4*, *VDE1*, and *NCED2* have high correlations with the fruit carotenoid biosynthetic pathway in the melon (Diao *et al.*, 2023). Similarly, in citrus, gene expression analysis was unable to fully determine the variations in carotenoid content and composition, indicating the potential involvement of other genes and mechanisms that need further verification (Wei *et al.*, 2017). In cassava genotypes, the expression of the *psy1/psy2* genes does not generally exhibit an obvious pattern (Olayide *et al.*, 2023). However, in the case of the white-fleshed cultivar A-4 and the yellow-fleshed cultivar C-25, different transcript abundances were notable in the *psy1/psy2* genes. Therefore, in color characteristics and carotenoid content levels, the role of the *psy1/psy2* genes remains to be elucidated. Although the results showed a high expression of *psy1* in yellow-fleshed cassava, with no corresponding increase in psy protein (Jaramillo *et al.*, 2022).

The APO compounds detected in the cassava cultivar C-25 were assumably the result of enzymatic processes involving cytochrome P450 enzymes (CYP82D47) with higher transcript abundance in the genotype C-25 than in A-4. Transgenic sweet potato storage roots with overexpressed *IbCYP82D47* exhibited higher carotenoid concentrations (Xing *et al.*, 2022). The gene *CitCYP97B*, which hydroxylates the  $\beta$ -ring of  $\beta$ -cryptoxanthin, has been demonstrated to exert a negative regulatory effect on  $\beta$ -cryptoxanthin content in

citrus fruits (Zhang *et al.*, 2024). The enzymes CYP450 play a pivotal role in facilitating metabolic steps that provide structural diversity during the biosynthesis of carotenoids and oxidative cleavage of APO products. The CYP450 superfamily is one of the most abundant protein families in plants, with an expression across a wide range of plant species (Alagoz *et al.*, 2022). Members of this superfamily also perform various functions in primary and secondary metabolism (Li and Wei, 2020). Consequently, additional investigation into the function of *CYP82D47* is necessary to clarify the underlying mechanisms of carotenoid formation and accumulation in cassava tubers.

## CONCLUSIONS

Analysis of metabolite compounds and gene transcript abundance in white-fleshed cassava cultivar A-4 and yellow-fleshed cultivar C-25 revealed diverse profiles. The metabolomic analysis disclosed the yellow-fleshed cultivar C-25 contains metabolite compounds that correlate with the color of the tubers and other important compounds beneficial for health. Furthermore, the varied abundance of functional gene transcripts in the carotenoid biosynthesis pathway and several other transcription factors suggested a potential association with the color of yellow-fleshed cassava cultivar C-25.

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