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Transcriptome analysis of purple pigment formation in *Colocasia esculenta*

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Abstract: Taro (*Colocasia esculenta* (L.) Schott) is an important crop in Africa, Southeast Asia, and subtropics and is used as a food and medicine. The purple color pigmentation is an appealing character in taro. We sampled taro corms of the cultivar 'Lipu Taro' at four developmental stages, including LPYS1 (without purple pigment, 50 days of development (DOD)), LPYS2 (very few purple pigments, 75 DOD), LPYS3 (moderate purple pigments, 115 DOD) and LPYS4 (high purple pigments, 205 DOD). The purpose of our study was to identify the key genes underpinning the purple pigmentation in taro based on RNA-sequencing. Through RNA-Seq, 6453 differentially expressed transcripts (DETs) were identified between purple and non-purple pigmented samples. We identified 41 and 12 flavonoid and anthocyanin related DETs transcripts, respectively. These DETs were upregulated at LPYS2, LPYS3, and LPYS4 as compared to LPYS1, indicating their positive contribution to the color formation in taro. Moreover, we identified several DETs encoding for transcription factors, including MYB and bHLH, known to be major regulators of structural genes involved in the flavonoid-anthocyanin pathway. Finally, we reported several plant hormones (ethylene, auxin, gibberellin, jasmonic acid, and cytokinin) related DETs, which are predicted to play important roles in the corm coloration. Different regulation of transcripts representing the flavonoid-anthocyanin biosynthesis pathway, plant hormone transduction pathway, and transcription factors may have key roles in purple pigmentation in taro. Our findings will facilitate future research on improving the quality and appeal of taro.

Introduction

The bulb plants are important in the balanced human diet as sources of starch, vitamins, and other nutrients. Flowering bulbs have an important advantage over regular plants. They can store energy from one growing season to the next. When a bulb starts to grow, it utilizes the stored energy reserves to develop roots, shoots, and leaves (Rees and Hanks, 1979; Abdissa *et al.*, 2011). Taro is an eminent root crop belonging to the family Araceae (common name: Aroids) and cultivated in all agricultural lands. Southeast Asia and subtropics are the main production regions. Taro contains high starch and numerous active ingredients with wide pharmacological actions (Lebot *et al.*, 2004; Caillon *et al.*, 2006). This has fostered its cultivation in China for more than 2300 years for food and Chinese herbal medicine (Lebot *et al.*, 2004). Taro is an economically important crop, and efforts are ongoing to breed high quality and high yielding cultivars (Ebert and Waqainabete, 2018). Some taro cultivars at maturity have a purple coloring pattern that has important aesthetical and medicinal values. The color pattern in taro has been linked to anthocyanin compounds (Champagne *et al.*, 2013), which are the most common plant pigments. Variation in anthocyanin content in plant tissues leads to different coloring patterns (Wilbert *et al.*, 1997). Anthocyanin is produced in land plants during many seasons and ontogenetic stages, expressly during high-intensity light coupled with environmental stress (Hughes *et al.*, 2014). Anthocyanins are found to be synthesized in evergreen species during low-temperature conditions and in response to abiotic stress, i.e., drought and salinity, etc. (Lee and Collins, 2001; Eryılmaz, 2006). They protect the

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photosynthetic responsive tissues from high-intensity light via attenuating green light and antioxidant activity (Close and Beadle, 2003; Hughes *et al*., 2010).

The anthocyanin biosynthesis is the result of secondary metabolic pathways in plants, highly conserved in regulatory components (Feller *et al*., 2011). Plant hormones are closely linked with the genes important for organ coloration (Seymour *et al*., 2013). Significant anthocyanin accumulation in particular plant tissues is regulated by the response of different hormones such as ethylene and abscisic acid (ABA) (McAtee *et al*., 2013). Previous studies showed that auxin, ABA, gibberellins (GA), cytokinin, and jasmonic acid (JA) interact in controlling the anthocyanin biosynthesis (Jeong *et al*., 2004; Loreti *et al*., 2008). Also, several transcription factors (TFs) have been found in apples, strawberries, and grapes, etc., which have the functions in regulating the anthocyanin biosynthesis structural genes (Century *et al*., 2008). The identification and functional characterizations of MYB and bHLH TFs revealed to be vital in the transcription of the anthocyanin biosynthesis structural genes including, chalcone isomerase, chalcone synthase, and anthocyanin reductase (Deluc *et al*., 2008; Gonzalez *et al*., 2008).

Recent advances in sequencing technologies have facilitated large-scale transcriptome sequencing, which assists us in gene expression and functional genomic studies (Wang *et al*., 2009). Whole transcriptome research provides a fast, high throughput means to obtain plant gene function and insight into the biosynthesis of active ingredients and their regulatory networks (Saito, 2013). Transcriptome analysis has been widely employed in plants, i.e., *Arabidopsis*, rice, tomato, sorghum, and potato, to divulge key genes regulating different biological processes (Bai *et al*., 2014; Ma *et al*., 2016; Wang *et al*., 2016). In this study, transcriptome sequencing was employed to investigate the dynamic expression changes of genes at four developmental stages in taro corm. We retrieved differentially expressed transcripts related to TFs, plant hormones, flavonoid-anthocyanin pathways, having a key role in developing purple color in taro corm.

Materials and Methods

Plant material and growth conditions

In our study, the taro cultivar 'Lipu Taro' was used as plant material. Lipu Taro belongs to the betel nut taro species, originating in Lipu County in Guangxi, China. Lipu Taro is grown mostly in the tropical regions at 18°N–20°N and can tolerate shade and withstand prolonged flooding. The corms were planted in the greenhouse under a 16/8 photoperiod at 28°C (day) and 22°C (night) at Xiuren Town, Guilin City, Guangxi Province, China. Corm samples were collected at four developmental stages, i.e., LPYS1, LPYS2, LPYS3, and LPYS4 were collected at 50, 75, 115, and 205 days of development (DOD), respectively. LPYS3 and LPYS4 had high purple color pigments in the corm; LPYS2 had very few purple color pigments while LPYS1 had no purple color pigment. The samples were selected from upper to middle parts of the corm in three biological replicates, snap-frozen in liquid nitrogen, and stored at −80°C till further use.

RNA extraction and cDNA library construction

Total RNA from the selected samples was extracted using the TRIzol reagent. Afterward, the extracted total RNA was digested using DNaseI and purified by Micropoly (A) PurisTM mRNA purification kit (Ambion, USA). The mRNA was converted into cDNA fragments using reverse transcriptase (High-Capacity cDNA Reverse Transcription Kit, ThermoFisher, USA) and a high concentration of random hexamer primers. The synthesized cDNA was spliced to the range of 300–500 bp using ultrasound and purified by Ampure beads. Subsequently, purified cDNA was prepared with a library using TruSeqTM DNA prep kit–Set A (Illumina, USA) and amplified by the TruSeq PE Cluster kit (Illumina, USA).

Illumina sequencing and assembly

The cDNA library was sequenced from 5′ and 3′ ends on an Illumina genome analyzer following the manufacturer's instruction and paired-end reads were obtained. The Illumina-based sequencing was done by Metware Biotechnology Co., Ltd., Wuhan, China. The raw reads were analyzed and checked for good quality using >20 Phred score based on the forward and reverse reads for the entire run. Paired-end reads were aligned to the available reference genome (Yin *et al*., 2021) using HISAT2 (2.1.0) (Kim *et al*., 2015). StringTie (1.3.4) (Pertea *et al*., 2015) was employed to count the number of reads mapped to each gene and quantification of gene expression as the number of fragments per kilobase of the transcript sequence per million base pairs sequenced (FPKM). The differential expression analysis was performed using the DESeq2 R package (2.11.38). Transcripts with p-value < 0.05 were considered as significantly differentially expressed in comparative analysis.

Functional annotation and classification

The transcripts identified are processed through the similarity-based analyses pipeline for annotation and functional classification using GetORF and EMBOSS software. Protein coding sequences were found from different contigs. Blastp was used to compare the predicted protein-coding sequences in GenBank non-redundant protein sequence (NR), gene ontology (GO), Kyoto encyclopedia of genes and genomes (KEGG), and eukaryotic orthologous groups of proteins (KOG) database. The best matching one was chosen as the annotation of each transcript.

The GO enrichment functional annotations were retrieved using Blast2GO software that classified the transcripts into biological process, cellular component, and molecular function. Furthermore, the KEGG database was employed to categorize the transcripts in different pathways according to the KO numbers.

RNA-Seq data evaluation

The expression pattern of nine transcripts randomly selected differentially expressed genes was done by qRT-PCR to validate the RNA-Seq data. The primers of the nominated genes were designed using AmplifX 1.5.4. The *Actin* gene was used as an internal control in qRT-PCR (Jyothi Lekshmi *et al*., 2020); the reaction was performed in a 96-wells plate on an ABI prism 7500 Real-Time PCR system

(Applied Biosystem, Foster City, CA, USA) using SYBR Green Master ROX (TaKaRa). The relative expression level of the selected DETs was calculated with the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Results

Plant samples and preliminary analysis of sequencing data

In this study, we sampled taro corms at four developmental stages, including LPYS1 (50 DOD), LPYS2 (75 DOD), LPYS3 (115 DOD), and LPYS4 (205 DOD). The corms at the early developmental stages (LPYS1 and LPYS2) had no purple or very few color patterns in contrast to samples at the late developmental stages (Fig. 1), indicating that the color formation starts at early developmental stages.

A total of 12 libraries (three biological replicates of each sample) sequencing data generated approximately 50.32 million raw reads. The raw reads were cleaned using Trimmomatic (Bolger *et al.*, 2014). After stringent quality and data cleaning, 50.13 million clean reads were obtained with an average of 53% GC contents (Tab. S1). The Illumina sequencing generated 150–250 bp read size for three biological repeats of each sample. Correlation plots were developed between three biological repeats of respective taro samples (Fig. 2). Given plot showed that there is no significant variability among replicates of the respective sample. Thus, it is supposed that there is no influence of the sampling and sequencing in the following secondary analysis of the respective taro samples.

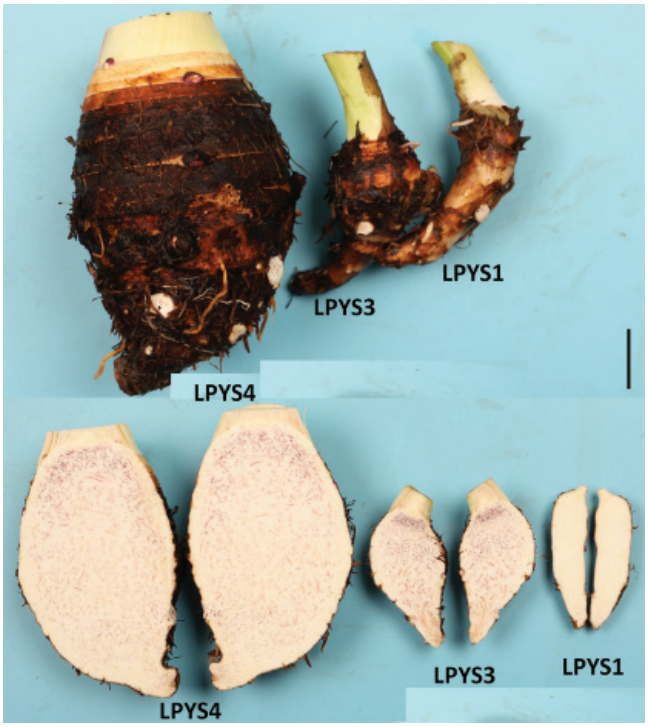

FIGURE 1. Phenotypic comparison of taro corms based on the purple pigmentation.
LPYS1 sample is without the purple pigmentation whereas LPYS3 and LPYS4 have purple color patterns. LPYS2 picture is not included due to very low purple color that made the camera impossible to differentiate the sample between purple and non-purple pigmentation.

Functional annotation and differential transcript expression patterns

In total, 5968 detected transcripts had blast matches to known proteins in the Nr database; 5249 transcripts were assigned to GO terms, including biological process, cellular component, and molecular functions (Tab. S2). A total of 2633 transcripts showing significant homology were assigned to the appropriate KOG clusters. Moreover, transcripts were mapped to the KEGG database, and 2540 transcripts were classified into different KEGG terms.

For differential expressed transcript (DET) analysis, three pairwise comparisons, i.e., LPYS1 *vs.* LPYS2, LPYS1 *vs.* LPYS3, and LPYS1 *vs.* LPYS4 were made. A total of 3445 DETs were identified between LPYS1 *vs.* LPYS2 samples, of which 1341 transcripts were upregulated, and 2104 transcripts were identified to be downregulated in LPYS1 compared to LPYS2. In LPYS1 *vs.* LPYS3, 351 transcripts were upregulated and 1512 were downregulated in LPYS1 relative to the LPYS3, while 419 and 2484 transcripts were up and downregulated, respectively, in LYPS1 compared to the LYPS4. A Venn diagram of all pairwise comparisons revealed that 609 DETs were overlapped in all the given comparisons, representing key genes associated with color formation (Fig. 3; Tab. S3).

KEGG pathways and Gene Ontology enrichment analysis

All the significantly expressed DETs were mapped on the KEGG pathways to uncover the significantly enriched ones. There were ten pathways retrieved having mostly enriched genes. Apart from metabolic and biosynthesis of secondary metabolites pathways, flavonoid–anthocyanin biosynthesis, plant hormone transduction, carbon metabolism, and biosynthesis of amino acids, were observed to be significantly enriched at different developmental stages of taro (Fig. 4a). According to the previously published literature, the flavonoid–anthocyanin biosynthesis pathway has a major contribution in the different color pigments accumulation in different plant species (Lee and Collins, 2001; Eryılmaz, 2006). We identified 57 DETs enriched in the flavonoid–anthocyanin biosynthesis pathway, having different expression patterns in the taro samples. Likewise, in the current study, flavonoid–anthocyanin biosynthesis responsive transcripts were revealed to be significantly enriched in biological processes, including biosynthesis processes, cellular localization, phenylpropanoid metabolic process, and flavonoid biosynthetic process (Fig. 4b). We deduced that the 57 DETs enriched in the flavonoid-anthocyanin biosynthesis pathway may have a key role in the purple pigment biosynthesis in taro.

RNA-Seq data validation

We randomly selected nine transcripts (Tab. S8) for qRT-PCR to evaluate the validity of the RNA-Seq data (Fig. 5). The qRT-PCR expression patterns of the nine DETs in the four samples followed the same expression pattern as per RNA-Seq data. This result confirms that our RNA-seq expression data and subsequent interpretations are reliable.

Differentially expressed transcripts related to flavonoid-anthocyanin pathway

The flavonoid-anthocyanin biosynthesis pathway has been well characterized in plants (Jaakola, 2013). Anthocyanins

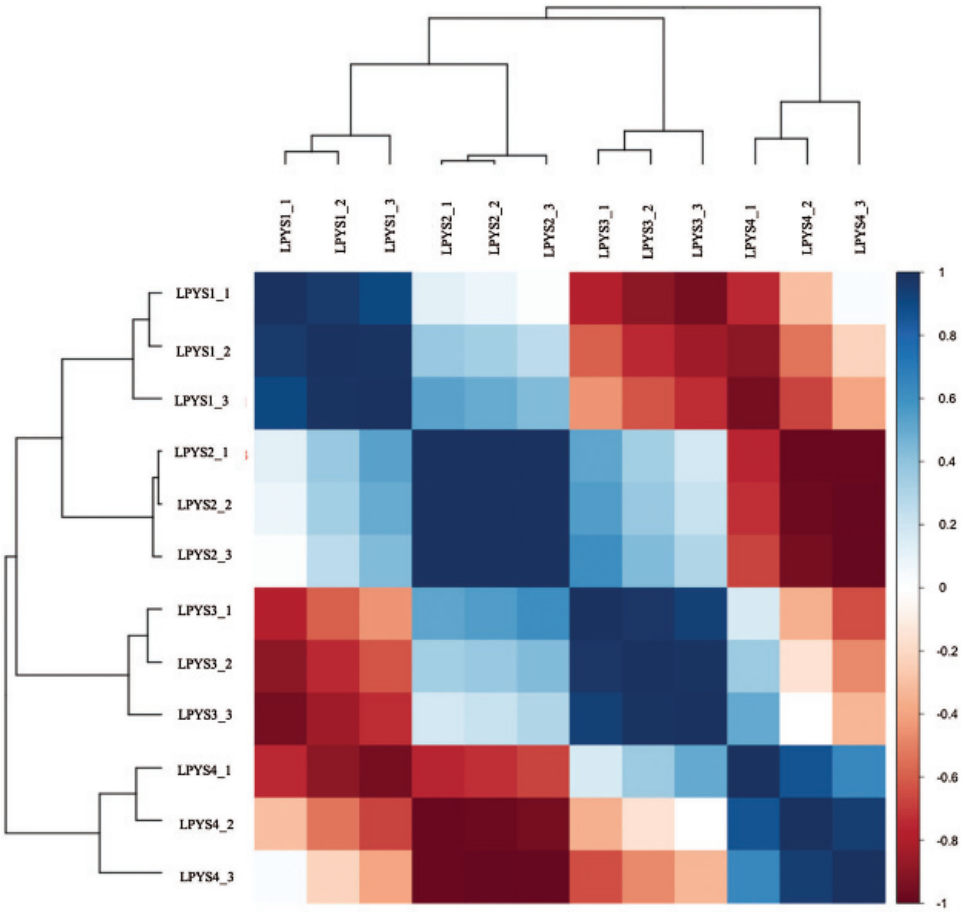

FIGURE 2. Heatmap illustrating the correlation among biological replicates of different taro samples collected at different developmental stages. Blue to red color ribbon exhibiting the range of positive to negative correlation among replicates and samples, respectively.

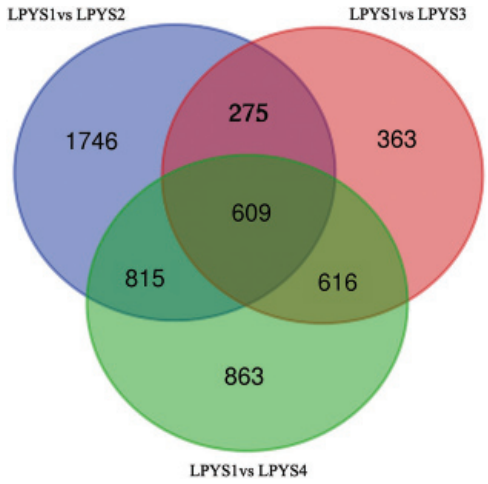

FIGURE 3. Venn diagram showing the overlapping of DETs among different pairwise comparisons of taro samples.

are the main coloring pigments in plants (Spelt *et al.*, 2002). To predict the molecular mechanisms participating in purple color pigmentation in taro, we mapped the DETs in the flavonoid-anthocyanin pathway and retrieved their expression patterns in different compartments of the pathway. In total, 41 transcripts were identified to be differentially regulated in this pathway (Fig. 6). The transcripts participating in flavonoid biosynthesis belong to the chalcone synthase, chalcone isomerase, flavonol synthase, shikimate O-hydroxycinnamoyl transferase, caffeoyl-CoA O-methyltransferase, trans-cinnamate 4-monooxygenase, flavonoid 3'-monooxygenase, coumaroylquinate (coumaroylshikimate) 3'-monooxygenase, and dihydroflavonol 4-reductase/flavanone 4-reductase families. Among these 41 DETs, most of the transcripts were upregulated in LPYS2, LPYS3 and LPYS4 as compared to LPYS1 samples, suggesting that these DETs positively contribute to the purple pigment formation in taro. Moreover, we identified 12 different transcripts belonging to the anthocyanin specific pathway, having up and down-regulation in LPYS2, LPYS3 and LPYS4 samples comparative to LPYS1 samples. Distinctly, in LPYS1 *vs.* LPYS4, we observed that 10 out of the 12 DETs were upregulated in LPYS4, indicating a high activity of anthocyanin related genes in mature taro corm, which may be responsible for purple coloration (Fig. 4c; Tab. S4).

Differentially expressed phytohormones

In this study, we identified differentially expressed transcripts related to phytohormones including abscisic acid (ABA), auxin, gibberellin (GA), ethylene, jasmonic acid (JA), salicylic acid (SA), and cytokinin (Tab. S5). We retrieved 57 ethylene-responsive transcripts, 54 auxin-responsive DETs, six different ABA-responsive transcripts, 18 different GA responsive transcripts, two JA, one SA responsive transcripts, 14 cytokinin transcripts differentially expressed among taro samples (Fig. 7). These DETs display varying patterns of expression changes across developmental stages, implying a complex mechanism involving phytohormones in color formation. Nonetheless, between LYPS1 and LYPS4, except for the SA gene, most of the phytohormone-related

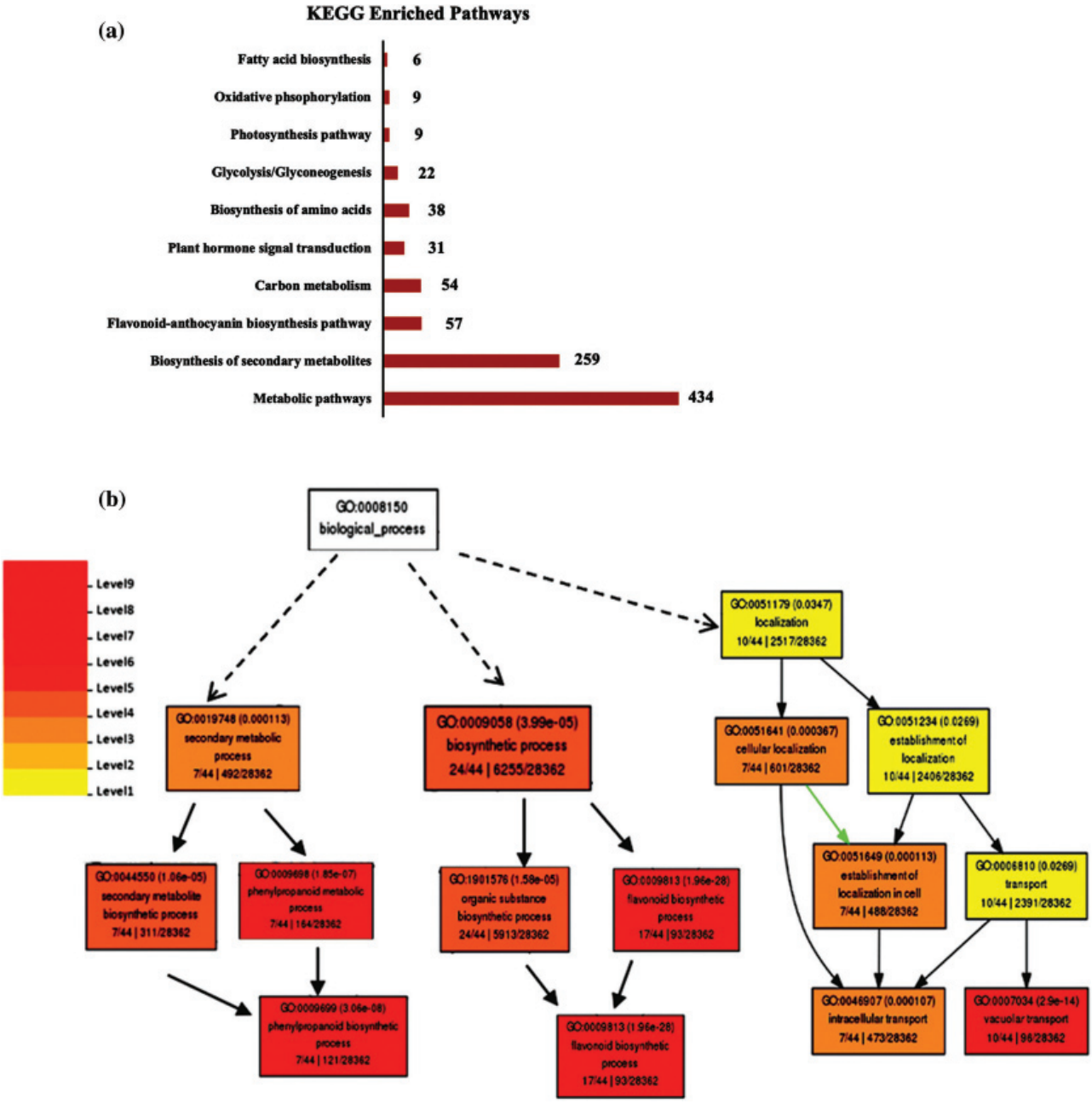

FIGURE 4. Significant enrichment of DETs in KEGG pathways and GO terms.
(a) Significant top putative KEGG pathways exhibiting the regulation of DETs in different biological pathways. **(b)** DETs enriched in flavonoid-anthocyanin biosynthesis pathway are categorized into different classes underlying the biological process related GO term.

DETs were strongly upregulated in LYPS4, globally indicating a positive contribution to the color formation in taro corm.

Differential expression pattern of transcription factors

Transcription factors (TFs) are major regulators of gene expression. We extended our study on the major TF families differentially expressed in different taro samples. We identified 198 TFs distributed into different families (Fig. 8), including MYB (40), bHLH (35), AP2-ERF (23), bZIP (7), GATA (9), NAC (2), TGA (4) and WRKY (10). More details of the TFs expression pattern are provided in the Tab. S6. We further performed a K-Means clustering analysis that delineates transcripts with similar patterns of expression. In total, ten clusters of genes were identified (Fig. 9). Many flavonoid–anthocyanin-related structural genes were grouped in Clusters 10 and 7 (Tab. S7). Further investigation of co-clustered TFs with these structural genes may facilitate the identification of potential TFs that regulate purple color formation in taro.

Discussion

Color is one of the most attractive sceneries in nature and it confers diverse functions (Schiestl and Johnson, 2013). In most plant species, coloration is mostly caused by flavonoids, particularly anthocyanins (Mol *et al.*, 1998; Wessinger and Rausher, 2012). Anthocyanins are water-soluble plant pigments responsible for the red, purple, and blue coloring of many plant tissues (Tohge *et al.*, 2017). The anthocyanin biosynthesis is highly conserved in structural

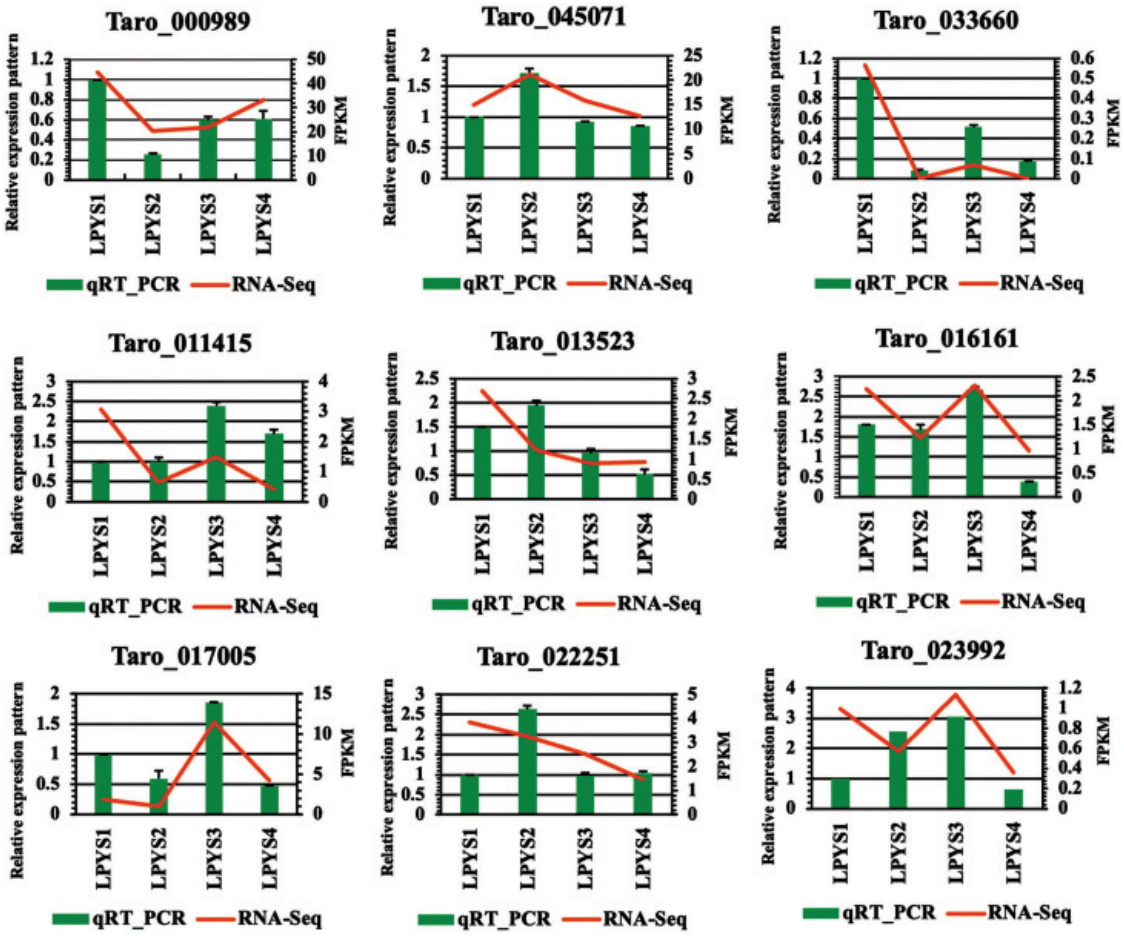

FIGURE 5. qRT-PCR validation of nine selected transcripts.
Gene expression analysis based on qRT-PCR between taro samples collected at four developmental stages (LPYS1, LPYS2, LPYS3 and LPYS4). The *Actin* gene was used as an endogenous gene. The bars show standard deviation.

and regulatory components (Feller *et al.*, 2011; Tohge *et al.*, 2017). In our study, genes, i.e., chalcone synthase, anthocyanin synthase, flavonol synthase, were upregulated in the LPYS2, LPYS3, and LPYS4 taro samples relative to the LPYS1 sample. The regulatory mechanisms of anthocyanin production have been characterized in many plants, e.g., petunia, snapdragon, *Arabidopsis*, and maize (Scalzo *et al.*, 2008; Tanaka *et al.*, 2008; Gao *et al.*, 2016; Ma *et al.*, 2019). It has been proposed that either increasing the functional activity of the pathway or activating the regulators can increase the anthocyanin intensity in petunia flowers and apple color (Spelt *et al.*, 2002; Liu *et al.*, 2020). We conclude that the high activity of flavonoid–anthocyanin structural genes contributes to the purple color formation.

It has been reported that the combined action of R2R3-MYB and bHLH TFs played an important role in regulating the anthocyanin genes in plants (Heim *et al.*, 2003). *MdMYB1* was proposed to be involved in the anthocyanin pigment accumulation in apples (Li *et al.*, 2012). In a previous study, *RsMYB1* was verified as a positive regulator to transcriptionally activate the anthocyanin biosynthesis pathway in red radish (Lim *et al.*, 2016). *MYB34* was identified to be downregulated in purple-colored cabbage leaves and upregulated in white cabbage at all growth stages (Jin *et al.*, 2018). In this study, unlike LPYS1 and LPYS2, *MYB34* was downregulated in LPYS3. These findings illustrate that *MYB34* TF may be negatively induced in colored pigmented genotypes. Similarly, AP2 TFs were identified as involved in the different coloring patterns in fruits (Gu *et al.*, 2017). Given the upregulation expression pattern of different AP2 TFs in LPYS3 and LPYS4, we can illustrate that AP2 might be regulating the accumulation of purple color pigment in LPYS3 and LPYS4. Apart from AP2 TFs, NAC TFs seem to be important for the carotenoids pigment production, important for different coloring patterns in plants. It was found that overexpression of *NAC25* results in reduced carotenoid levels and inhibition of fruit ripening by changing the carotenoid pathway flux and downregulating the ethylene-responsive genes in tomato (Zhu *et al.*, 2014). In our study, purple pigmented samples, LPYS3 and LPYS4, showed downregulation of *NAC25* TF, and these results indicate that *NAC25* may be playing role in inhibiting the color pigment production.

Likewise, members of bHLH and MYB TF families were the most differentially expressed between colored and non-colored taro samples, indicating that they may play preponderant regulatory roles in color formation in taro. The upregulation of several WRKY TFs such as *WRKY4*, *WRKY17*, *WRKY21*, and *WRKY48* in LPYS2, LPYS3, and LPYS4, may be linked to the anthocyanin production through activation of genes involved in the flavonoid-anthocyanin biosynthesis. In *Arabidopsis*, it

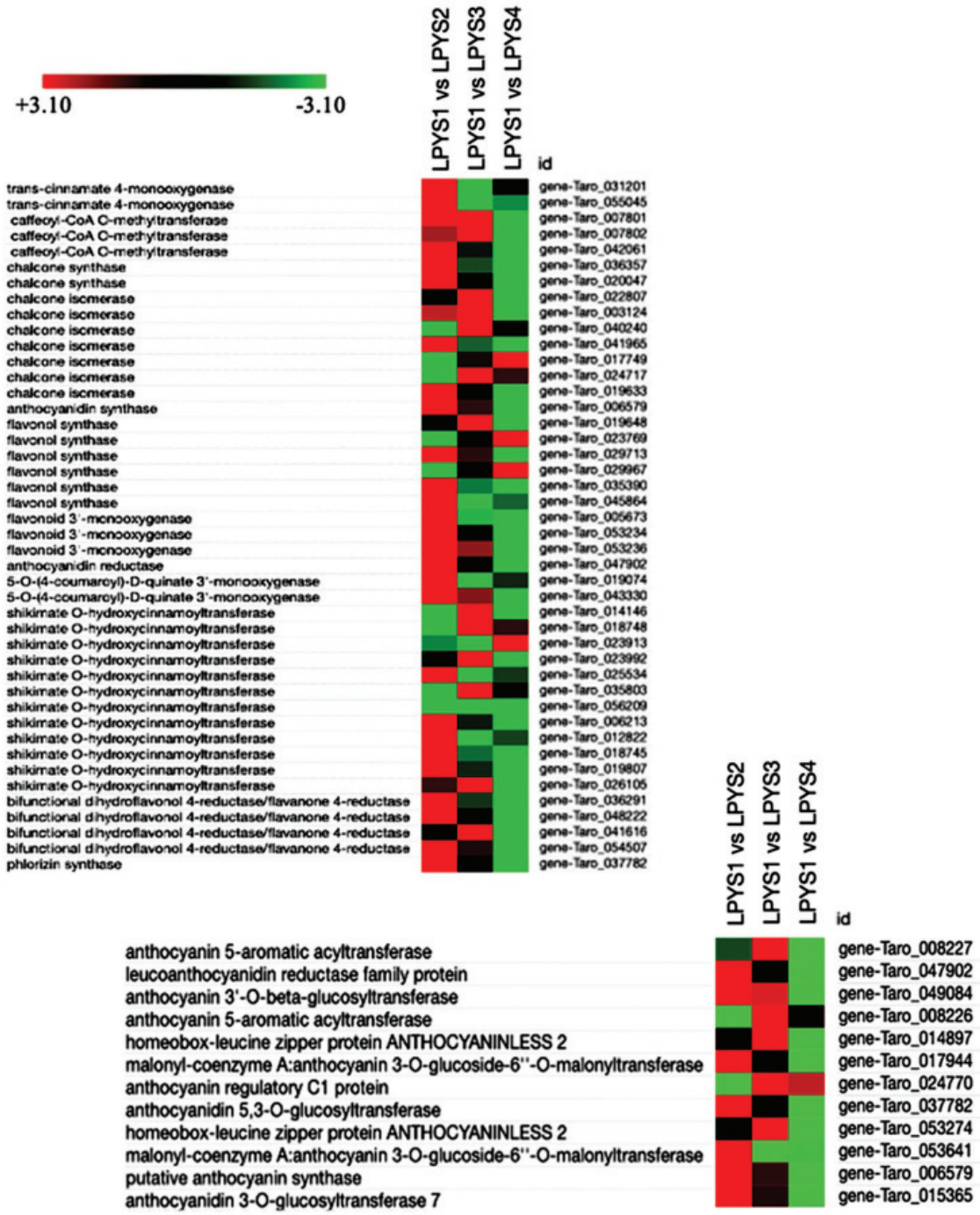

FIGURE 6. Flavonoid-anthocyanin pathway elaborating the flavonoid and anthocyanin responsive transcripts. Heatmap showing the expression pattern of the differentially expressed transcripts involved in the flavonoid pathway.

has been revealed that *WRKY48* stimulates the regulation of genes participating in the anthocyanin biosynthesis (Rushton *et al.*, 2010). *AtWRKY14* regulates the production of proanthocyanins, a subset of tannin compounds in the flavonoid biosynthesis pathway (Mao *et al.*, 2011).

Hormones are important factors inducing anthocyanin accumulation (Wang *et al.*, 2015). Auxin has been shown to regulate anthocyanin biosynthesis (Schiestl and Johnson, 2013). Endogenous overexpression of auxin was found to impede the anthocyanin accumulation in strawberries and grapes (Deluc *et al.*, 2008). Herein, we observed several auxin-responsive transcripts, i.e., *gene-Taro-029783*, *gene-Taro-030371*, *gene-Taro-010281*, and *gene-Taro-047186*, upregulated in LPYS4 and predicted to play a key role in color formation in taro. ABA related transcripts were also upregulated in the LPYS3 and LPYS4 compared to LPYS1 and LPYS2 samples. The previous literature demonstrated that ABA treatment increased the anthocyanin accumulation in cherry (Shen *et al.*, 2014), grape (Sandhu *et al.*, 2011), and strawberry (Li *et al.*, 2014). Moreover, the expressions of PYRs/PYLs and PP2Cs and SnRK2 were increased by the ABA treatment that ultimately enhanced the anthocyanin accumulation in apple (Ma *et al.*, 2019). Similarly, cytokinins are positive regulators of anthocyanin biosynthesis (Solfanelli *et al.*, 2006; Ma *et al.*, 2019). We retrieved 14 different cytokinin responsive transcripts, highly expressed in purple

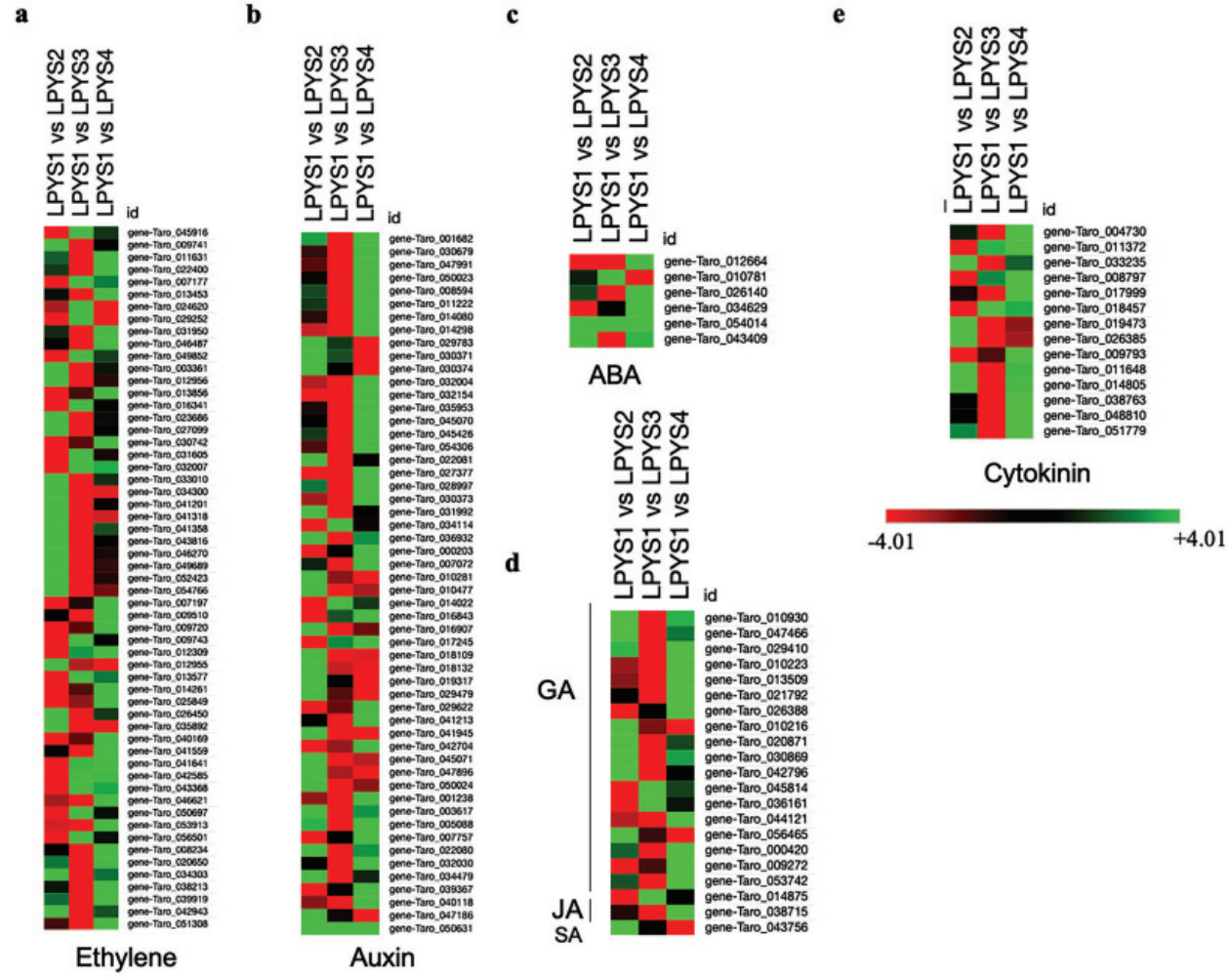

FIGURE 7. Heatmap showing the fold change-based expression of identified phytohormones expressed differentially in taro samples. The identified a) ethylene, b) auxin, c) ABA, d) GA, JA and SA, and e) cytokinin are shown in different heatmap clusters.

pigmented taro genotypes. Gibberellins (GAs) and Jasmonic acids (JAs) are two important phytohormones taking part in growth and defense via physical interactions with DELLA and JAZs (Wild *et al.*, 2012; Hou *et al.*, 2013). The GAs were predicted to modulate the anthocyanin accumulation through the activity of DELLA proteins. We retrieved the GAs transcripts, having high expression patterns in LPYS1 and LPYS2 genotypes than LPYS4. According to the literature, the presence of GA, MYBL2 and JAZs complex suppress the anthocyanin biosynthesis, while in the absence of GA, DELLA proteins accumulate and sequestrate MYBL2 and JZs complex, resulting in the activation of anthocyanin biosynthesis in *Arabidopsis* (Das *et al.*, 2012). Thus in our study, the MYB TFs and JAZs expression patterns set a threshold for GA signaling, determine the switch off or switch on the anthocyanin biosynthesis. Apart from GA signaling, JA stimulates anthocyanin accumulation in many plant systems (Balbi and Devoto, 2008). In *Arabidopsis*, JA in the presence of sucrose has an effect to induce the expression of genes coding for enzymes, involved in the anthocyanin biosynthesis (Chen *et al.*, 2007). In this study, we observed various expression patterns for the phytohormone genes over taro corm growth stages. More in-depth studies of highly induced or repressed phytohormone-related DETs will be needed to better clarify the roles of each phytohormone in color formation in taro.

Overall, RNA-Seq data sketched out the genome-wide regulation of different transcripts related to TFs, phytohormones, and flavonoid-anthocyanin pathway in purple pigmented and non-purple pigmented taro samples. Many transcripts could be useful for researchers to reveal their functions and relation with anthocyanin biosynthesis.

Conclusion

In this study, taro samples were collected at different growth stages to uncover the transcriptome changes underlying the formation of purple coloration. Particularly, the flavonoid–anthocyanin biosynthesis pathway was found to be a key pathway for color formation in taro. Differentially expressed genes related to this pathway were identified, and the link with the purple pigmentation was established. Similarly, TFs may play a major role in the regulation of plant hormones and flavonoid–anthocyanin biosynthesis pathway-related genes, important for purple color pigmentation in LPYS3 and LPYS4. Collectively, our results provide a foundation for breeding efforts towards purple-colored taro.

Availability of Data and Materials: The RNA-seq datasets generated in the current study were submitted to NCBI SRA: PRJNA639211.

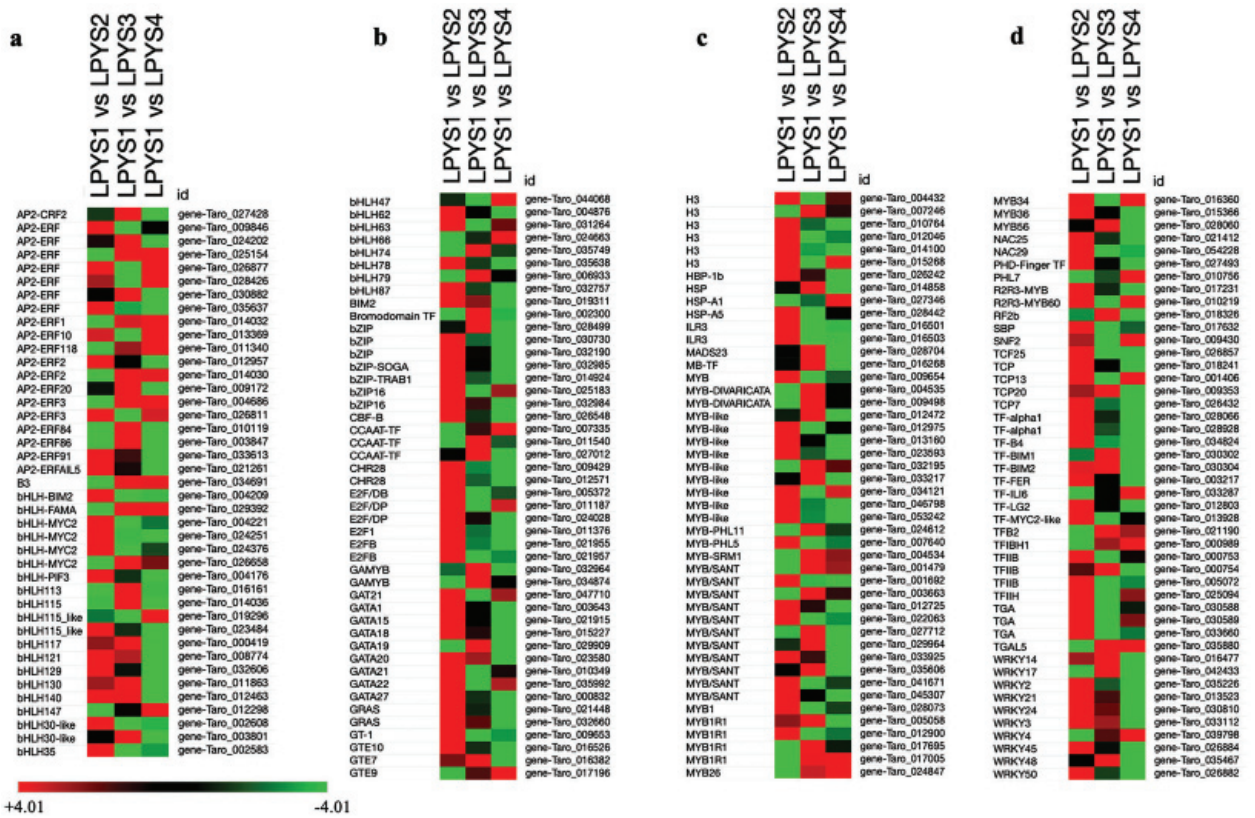

FIGURE 8. Heatmap illustrating the differentially expressed transcription factors from different families in taro samples. All the TFs are retrieved through BLASTX from the Plant transcription factor database.

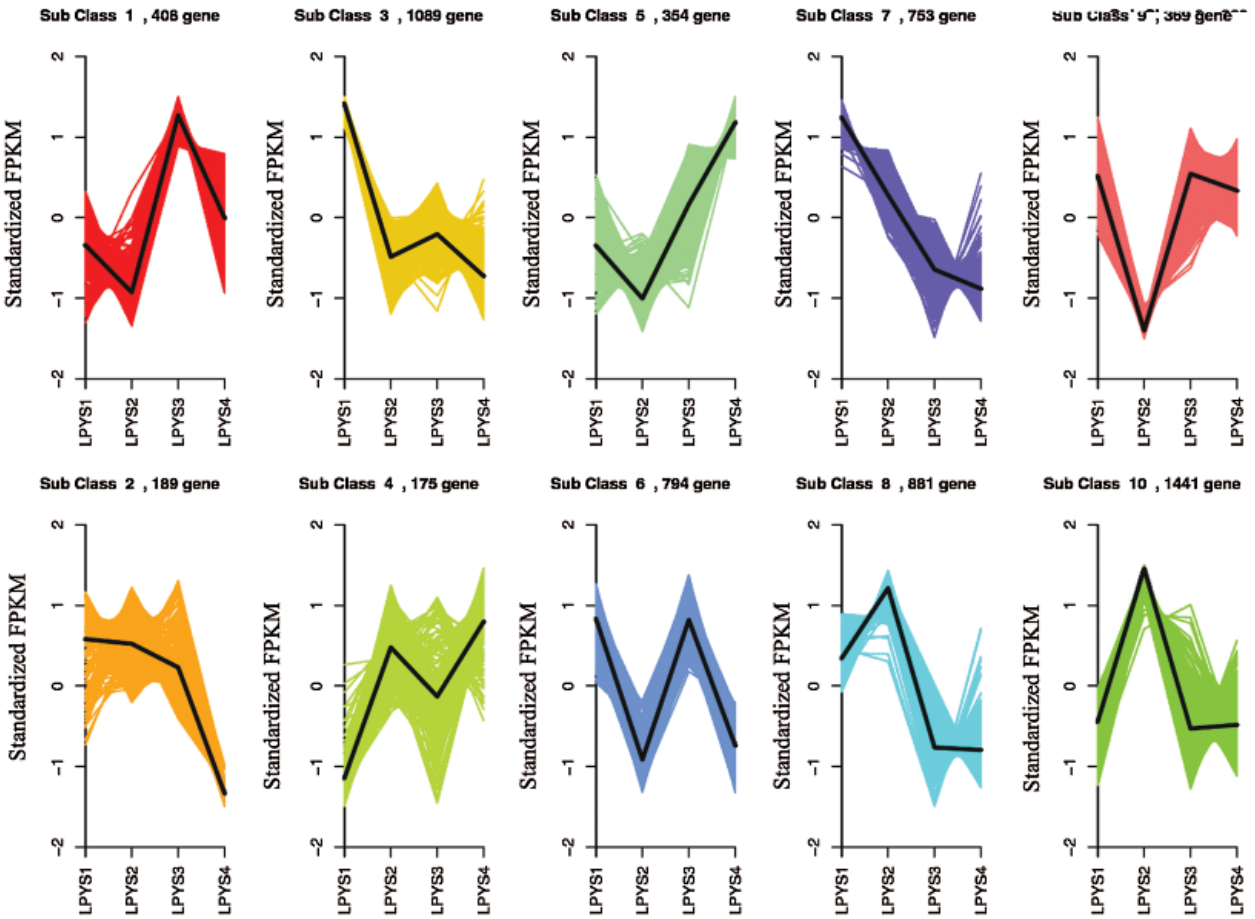

FIGURE 9. K-Means clustering of transcripts based on standardized FPKM values. The numbers of genes clustered in each subclass are mentioned.

Author Contributions: Conceptualization, FH, WD, and SW; methodology, FH, WD, JH, HJ, and ZQ; validation, FH, SH, and LL; formal analysis, FH, WD, JH, HJ, and ZQ; investigation, FH, SH, and LL; writing—original draft preparation, FH; writing—review and editing, WD and SW; supervision, WD and SW; project administration, WD and SW; funding acquisition, WD and SW. All the authors read and approved the manuscript.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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Appendix

Supplementary files

Supplementary Table S1. Overview of RNA-Seq paired-end data produced by Illumina Sequencing.

Supplementary Table S2. A list of differentially expressed transcripts in taro samples at different developmental stages. The differentially expressed transcripts were retrieved by FDR < 0.05 as a threshold level. The transcript IDs, FPKM values, GO terms and chromosomal position are mentioned in the table.

Supplementary Table S3. A detailed list of transcripts overlapping in pairwise comparisons of taro samples.

Supplementary Table S4. DETs involved in KEGG enriched flavonoid-anthocyanin biosynthesis pathway and their expression pattern. All the KEGG IDs and descriptions of each transcript related to the pathway are mentioned in the table.

Supplementary Table S5. A detailed list of phytohormones related transcripts. The phytohormone related transcripts are divided into separate clusters.

Supplementary Table S6. A detailed list of transcription factors expressed differentially in purple and non-purple pigmented taro samples.

Supplementary Table S7. K-Means clustering of genes based on standardized FPKM values.

Supplementary Table S8. A list of primers used for RNA-Seq data validation.