Transcriptome analyses reveal anthocyanin biosynthesis in eggplants

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Transcriptome analyses reveal anthocyanins biosynthesis in eggplant (*Solanum melongena* L.)

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ABSTRACT

We obtained a white-peel eggplant (L6-5) by EMS mutation in our previous study, whose total anthocyanin content was significantly decreased as compared with that of wild-type (WT). To analyse the anthocyanin biosynthesis mechanism in eggplants, we analysed the eggplant peel by RNA-seq in this study. The transcript results revealed upregulation of 465 genes and downregulation of 525 genes in L6-5 as compared with the WT eggplant. A total of 11 anthocyanin biosynthesis structure genes were significantly downregulated in L6-5 as compared with that in WT. Meanwhile, on the basis of the RT-PCR results of four natural eggplant cultivars, the expression pattern of 11 anthocyanin biosynthesis structure genes was consistent with the anthocyanin content. Thus, we speculated the anthocyanin biosynthesis pathway in eggplant peel. The transcript and RT-PCR results suggested positive regulation of *MYB1*, *MYB108* and *TTG8* and negative regulation of *bHLH36* in anthocyanin biosynthesis. This study enhanced our cumulative knowledge about anthocyanin biosynthesis in eggplant peels.

Key words: Anthocyanin biosynthesis pathway; Eggplant; EMS mutant; Transcriptomics.

INTRODUCTION

An eggplant (*Solanum melongena* L.) is an important vegetable across the world. Several researches have indicated that the colour of an eggplant is determined by the type and content of anthocyanin in it (Nothmann et al. 1976). Anthocyanin makes the eggplant not only colourful (Niño-Medina et al. 2017) but also beneficial for human health when consumed (Jing et al. 2015).

There are six types of anthocyanin in plants, namely, pelargonidin, cyanidin, delphinidin, peonidin, petunidin and malvidin (Chaves-Silva et al. 2018). However, the anthocyanin biosynthesis pathway is conserved. The key anthocyanin biosynthesis structure gene had been identified in several plants such as potato (Liu et al. 2015b; Zhang et al. 2017) and litchi (Zhang et al. 2016a). After the catalysis of phenylalanine by phenylalanine ammonia lyase (*PAL*), the biosynthesis was catalysed by chalcone synthase (*CHS*), chalcone isomerase (*CHI*), flavanone 3-hydroxylase (*F3H*), flavonoid 3′-hydroxylase (*F3′H*), flavonoid 3′,5′-hydroxylase (*F3′5′H*), dihydroflavonol 4-reductase (*DFR*), anthocyanidin synthase (*ANS*) and 3-O-glycosyltransferases (*UFGT*) step-wise in the cytoplasm (Bowles et al. 2005; Chaves-Silva et al. 2018; Tanaka & Ohmiya 2008; Tanaka et al. 2008). Finally, anthocyanin is transported into the vacuole by GST-ABC transport (Goodman et al. 2004), vesicle-mediated mass transport (Zhang et al. 2006) or toxic compound extrusion transport (Marinova et al. 2007).

The anthocyanin biosynthesis structure genes were regulated by the MBW ternary complex (R2R3-MYB, bHLH and WD40) (Liu et al. 2015a). In Arabidopsis, most of the R2R3-MYB positive molecules regulated the structure gene expression. *AtMYB75* regulated the expression of *PAL*, *C4H* and *4 CL*. *AtMYB12*, *AtMYB11* and *AtMYB111* regulated the expression of *AtCHS*, *AtCHI and AtF3H* (Liu et al. 2015a; Stracke et al. 2007). However, several MYBs were reported to negatively regulate or repress the expression of anthocyanin biosynthesis structure genes. In Arabidopsis, the MYBs can negatively regulate the *DFR* and *UFGT* expression (Matsui et al. 2008). The other way in which MYBs negatively regulate the expression of biosynthesis structure genes was through the inhibition of the formation of the MBW complex (Matsui et al. 2008).

Delphinidin-3-rutinoside was identified as the major anthocyanin in eggplant peels (Li et al. 2017; Todaro et al. 2009; Zhang et al. 2014). Several researches have indicated that the anthocyanin biosynthesis structure genes *CHS*, *DFR* and *ANS* were involved in eggplant peel anthocyanin biosynthesis. However, *PLA* may not be involved in anthocyanin biosynthesis (Xi-Ou et al. 2017; Zhang et al. 2014). A total of 73 *R2R3MYB* genes were identified in eggplant genome, and only *SmMYB1* and *SmMYB6* positively regulated anthocyanin biosynthesis (Wang et al. 2016). The overexpression of *SmMYB1* resulted in the significant accumulation of anthocyanin, with most anthocyanin structural genes being dramatically upregulated (Zhang et al. 2016b). A recent study suggested that light can positively regulate the anthocyanin structural genes and the R2R3-MYB expression and lead to anthocyanin biosynthesis (Li et al. 2018; Li et al. 2017).

In our previous study, we obtained a white eggplant (L6-5) by EMS mutation (Xi-Ou et al. 2017). Meanwhile, the EMS-induced mutation in the L6-5 genome was analysed, with the results showing that the Sme2.5_06210.1_g00004.1 (UFGT) nonsynonymous mutations may result in a decrease in eggplant anthocyanin content (unpublished). In this study, the EMS mutant L6-5 (white) and the WT eggplant (purple) were used to analyse the anthocyanin biosynthesis mechanism through transcriptome analysis. Our results showed that the anthocyanin structural genes were dramatically downregulated in L6-5 and that the MYB and bHLH transcription factors were also involved in the regulation of anthocyanin biosynthesis. This study identified the key structure gene and regulation factors involved in eggplant peel anthocyanin biosynthesis and provided a good foundation for breeding anthocyanin-rich eggplant cultivar.

MATERIAL AND METHODS

Plant material

The L6-5 eggplant was an EMS-induced mutant showing a white peel, and the WT eggplant had a purple peel (Figure 1a). The four natural eggplant cultivar included one white peel (WS), two purple peels (PS1 and PS2) and one purple black peel (PBS) (Figure 2a). The eggplants were transplanted in the field located in South Subtropical Crop Research Institute, Chinese Academy of Tropical Agricultural Sciences.

Analysis of the total anthocyanin content

Total anthocyanin was detected by UV–Visible Spectroscopy according to a method described elsewhere (Xi-Ou et al. 2017).

RNA extraction, library construction and transcriptome sequencing

The eggplant peel was collected at 15 days after self-pollination and then immediately frozen in liquid nitrogen and stored at −80°C. Then, the total RNA was extracted using the Column Plant RNAout2.0 Kit (Tian Enze Beijing). A total amount of 1 μg qualified RNA per sample was used as the input material for the library preparation. The sequencing libraries were generated using the VAHTS mRNA-seq v2 Library Prep Kit for Illumina® (Vazyme, NR601) following the manufacturer's recommendations. Clustering of the index-coded samples was performed on the cBot Cluster Generation System (Illumina) according to the manufacturer's instructions. After cluster generation, the library preparations were sequenced on the Illumina Hiseq X Ten Platform and 150-bp paired-end module.

Function annotation

After the low-quality reads were filtered, the clean reads were aligned to the eggplant reference genome (Hirakawa et al. 2014) (http://eggplant.kazusa.or.jp/index.html) using TopHat (v2.1.1) (Kim et al. 2013). The FPKM was calculated using Cuffdiff (v1.3.0) (Trapnell et al. 2012) to normalise the gene expression. Genes with the corrected p values of <0.05 and the absolute value of log2 (fold change) <1 were assigned to be significantly differentially expressed.

The GO terms and KEGG pathway with corrected p values of <0.05 were considered to be significantly enriched among the differentially expressed genes.

RT-PCR analysis

Approximately 1 μg of RNA was synthesised into cDNA with Oligo dT18 according to the manufacturer's instructions (Takara Dalian). Gene expression was analysed using Roche

LightCycler 480 Thermal Cycler. About 10 μL of the reaction mixture contained 5 μL of 2X Maxima SYBR Green RT-PCR Master Mix (ThermoFisher), 2 μL of primers, 1 μL of cDNA and 2 μL of RNase-free water. The amplification programme was as follows: 95°C for 3 min, 95°C for 15 s, 60°C for 30 s and 72°C for 15 s, 45 cycles. The primers used in this study were suggested by Zhang et al. (2014b), which are listed in Table S1. qRT-PCR analyses were performed in three biological and two technical replications.

RESULTS

Anthocyanin content decreased significantly in the L6-5 mutant

The L6-5 mutant fruit is white, and WT is purple. The total anthocyanin content in the L6-5 mutant fruit peel was significantly decreased in comparison with that in the WT eggplant (Figure 1b). In addition, the total anthocyanin content of four natural eggplant cultivar was PBS > PS1 = PS2 > WS (Figure 2b).

Differential expression genes (DEGs) between WT and L6-5

About 44–54 million clean reads of each sample were obtained, and 88%–90% clean reads were mapped to the reference genome (Table S2). A total of 465 genes were upregulated, and 525 genes were downregulated in L6-5 in comparison with that in WT (Figure 3 and Table S3). There were a total of 381, 496 and 146 DEGs classified as 'biological process', 'molecular function' and 'cellular component' (Figure 4). In 'biological process', the largest subcategory was single-organism metabolic process (146 genes). In 'molecular function', the largest subcategory was catalytic activity (275 genes). In 'cellular component', the largest subcategory was extracellular region (30 genes).

A total of 657 DEGs were mapped to 107 pathways. The largest pathway was a metabolic pathway containing 211 genes (Figure 5), followed by the biosynthesis of secondary metabolites pathway (149 genes). The anthocyanin biosynthesis pathway contained four genes, the flavone and flavonol biosynthesis pathway contained 19 genes, and ABC transporters pathway contained eight genes.

The anthocyanin biosynthesis structure genes were downregulated in the L6-5 mutants

A total of 11 structure genes were identified in the DESs, including *4 CL*, *CHI*, *CHS(2)*, *F3H*, *F3'5'H*, *DFR*, *ANS* and *UFGT*(2) (Table 1). The 11 structure gene expressions were all downregulated in L6-5 as compared with that in WT. Moreover, the expression level was validated by RT-PCR (Figure 6). However, except that the 4 CL expression was not significantly downregulated at $P < 0.05$, the other 10 gene expressions were significantly downregulated.

Furthermore, the 11 structure gene expression levels were analysed in the four natural eggplant cultivars. The results revealed that those 11 structure gene expression levels were PBS > PS1 = PS2 > WS (Figure 7).

Transcription factors

The expression level of 45 transcription factors changed in L6-5 than in WT (Table 2). In addition, the expressions of 19 of the 45 transcription factors were downregulated in L6-5, whereas that of the other 26 transcription factors were upregulated when compared with that in WT. The largest transcription factor was the ethylene-responsive transcription family, which contained 11 numbers, followed by the bHLH transcription factor family, which contained eight genes. There were a total of seven MYB transcription factors involved.

MYB transcription factor

A total of seven MYB transcription factors were up- or downregulated in L6-5 in comparison with that in WT. The *MYB1* (Sme2.5_05099.1_g00002.1), *MYB108* (Sme2.5_00155.1_g00001.1), *EFM* (Sme2.5_06702.1_g00005.1) and *MYB5* (Sme2.5_07055.1_g00007.1) expressions were downregulated in L6-5. However, the expressions of *APL* (Sme2.5_03242.1_g00007.1), *MYB 330* (Sme2.5_00805.1_g00004.1) and *MYB12* (Sme2.5_11221.1_g00002.1) were upregulated in L6-5 than in WT (Figure 8).

The seven MYB protein sequences were aligned to the known MYBs, which were related to anthocyanin synthesis (Liu et al. 2015a). The results showed that *EFM*, *APL* and *MYB12* were classified as negative regulators. However, *MYB1*, *MYB108*, *MYB 330* and *MYB5* were classified as positive regulators (Figure 9).

To further analyse the MYB factor function in anthocyanin biosynthesis. The MYB expression levels were detected in four natural eggplant cultivars of different peel colours. The results showed that the expressions of *MYB1*, *MYB108*, *EFM* and *MYB12* were upregulated in coloured eggplants (PS1, PS2 and PBS) than in colourless eggplants (WS). The *MYB330* expression was PBS > PS2 = WS > PS1. The MYB expression was PBS > WS > PS1 = PS2. All seven MYB expressions in PBS were higher than those in PS1, PS2 and WT (Figure 10).

bHLH transcription factor

There were eight bHLH transcription factors in the DEGs (Table 2). However, the RT-PCR results revealed that only *TT8* (Sme2.5_29845.1_g00001.1) and *TTG1* (Sme2.5_00747.1_g00013.1) significantly decreased in L6-5 than in WT (Figure 11).

The expression of TT8 was upregulated in coloured eggplants when compared with that in colourless eggplants. However, the expressions of *TTG1* and *bHLH36* (Sme2.5_00735.1_g00008.1) in coloured eggplants were downregulated as compared with that in colourless eggplants. The *bHLH18* (Sme2.5_00407.1_g00003.1), *bHLH93* (Sme2.5_13712.1_g00001.1) and *bHLH49* (Sme2.5_01808.1_g00003.1) expressions were WS = PBS > PS1 = PS2. The expression of *bHLH94* (Sme2.5_04383.1_g00001.1) was PBS > WS = PBS2 > PBS1, whereas that of *bHLH7*1 was PBS > WS > PS1 > PS2 (Figure 12).

DISCUSSIONS

In the present study, the EMS mutant L6-5 (white) and the WT eggplants (purple) were used to analyse the eggplant anthocyanin biosynthesis pathway. As compared with the natural cultivar or other treatments, such as bagging, the eggplant material used in this study could significantly eliminate the influence of the genetic background and environmental factors.

The anthocyanin biosynthesis pathway in plants is known (Tanaka & Ohmiya 2008; Tanaka et al. 2008), and the structure gene and regulation factor had been cloned and analysed, respectively (Dubos et al. 2010; Liu et al. 2015a). In the present study, 11 anthocyanin biosynthesis structure genes were identified, including *CHS* (Sme2.5_01077.1_g00016.1 and Sme2.5_02154.1_g00001.1), *CHI* (Sme2.5_01193.1_g00009.1), *F3H*

(Sme2.5_00015.1_g00020.1), *F3'5'H* (Sme2.5_04313.1_g0000 1.1), *DFR* (Sme2.5_01401.1_g00004.1), *ANS* (Sme2.5_01638.1_g00005.1) and *UFGT* (Sme2.5_06210.1_g00004.1 and Sme2.5_00228.1_g00013.1). The RNA-seq and RT-PCR results revealed significantly decreased expressions in L6-5 than in WT. The RT-PCR of the natural eggplant cultivar also revealed that these gene expression levels were on the order of purple black > purple > white. Although upregulation of 11 structure genes was noted in coloured eggplants than in colourless eggplants, the expressions of *F3'5'H*, *DFR*, *ANS* and *UFGT1* (Sme2.5_06210.1_g00004.1) in coloured eggplants were strongly upregulated. This result suggested that *F3'5'H*, *DFR*, *ANS* and *UFGT* (Sme2.5_06210.1_g00004.1) played more important roles in the anthocyanin biosynthesis pathway. In our previous study, the SNP between L6-5 and WT was analysed by whole genome re-sequencing, and the results revealed that only *UFGT* (Sme2.5_06210.1_g00004.1) possessed a nonsynonymous mutation among the abovementioned 11 structure genes (data unpublished). Thus, we speculated that the nonsynonymous mutation of Sme2.5_06210.1_g00004.1 may result in decreased production of anthocyanin.

Furthermore, anthocyanin biosynthesis of eggplants is reportedly affected by several environmental factors, such as light (Li et al. 2018; Li et al. 2017). The RNA-seq results showed that the 11 abovementioned anthocyanin biosynthesis structure genes were upregulated during light-induced anthocyanin biosynthesis in eggplants (Li et al. 2018; Li et al. 2017). In conclusion, we speculated the anthocyanin biosynthesis pathways in eggplants as shown in Figure 13 based on literature review.

A total of 73 R2R3MYB genes were identified in the eggplant genome (Wang et al. 2016). The MYBs played a key role in the regulation of anthocyanin biosynthesis (Dubos et al. 2010). In the present study, only seven MYB genes were differentially expressed between WT and L6-5, which may regulate eggplant anthocyanin biosynthesis. The RT-PCR results of the mutant and four natural eggplant cultivars suggested upregulation of only *MYB1* (Sme2.5_05099.1_g00002.1) and *MYB108* (Sme2.5_00155.1_g00001.1) in coloured eggplants as compared with that in colourless eggplants. These results suggested that *MYB1* and *MYB108* may positively regulate anthocyanin biosynthesis. MYB1 positively regulates anthocyanin biosynthesis, which was identified by the overexpression experiment (Jiang et al. 2016; Li et al. 2017; Zhang et al. 2016b; Zhang et al. 2014). Moreover, *MYB1* positively regulated the expression of anthocyanin biosynthesis structural genes (Zhang et al. 2016b), and further research showed that MYB1 binds to the promoters of CHS and DFR (Jiang et al. 2016). However, the function of *MYB108* in the regulation of anthocyanin biosynthesis has not yet been reported.

BHLH is another key transcription factor that regulates anthocyanin biosynthesis. MYBs-TT8-TTG1 is well-known to form MBW for regulating the expression of anthocyanin biosynthesis structure gene (Chaves-Silva et al. 2018). In the eight *bHLH* genes, only the expression of *TT8* (Sme2.5_29845.1_g00001.1) was found to be upregulated in coloured eggplants as compared with that in colourless eggplants. Moreover, TT8 was upregulated during light-induced eggplant anthocyanin biosynthesis (Li et al. 2017). However, overexpression anther *TT8-like* (Sme2.5_00592.1_g00005.1) gene in tobacco leaves cannot accumulate anthocyanin (Li et al. 2017). It has been indicated that *TT8-like* (Sme2.5_00592.1_g00005.1) may not be involved in eggplant anthocyanin biosynthesis. These results suggest that *TT8* (Sme2.5_29845.1_g00001.1) may positively regulate eggplant anthocyanin biosynthesis. Interestingly, the expressions of *TTG1* (Sme2.5_00747.1_g00013.1) and *bHLH36* (Sme2.5_00735.1_g00008.1) were downregulated in coloured eggplants than in colourless eggplants of four natural eggplant cultivars. Moreover, *TTG1* was upregulated in the transcript date. Therefore, *bHLH36* may negatively regulate anthocyanin biosynthesis.

CONCLUSIONS

The total anthocyanin content of L6-5 was significantly decreased when compared with that of WT. A total of 465 genes were upregulated, and 525 were downregulated in L6-5 as compared with that in WT. According to the transcript date and the RT-PCR results, we believe that the anthocyanin biosynthesis pathway in eggplant peel and the *MYB1*, *MYB108* and *TTG8* transcription factors positively regulate anthocyanin biosynthesis and that *bHLH36* negatively regulates anthocyanin biosynthesis.

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Figure 1

Figure 1 The phenotype and total anthocyanin content of WT and L6-5

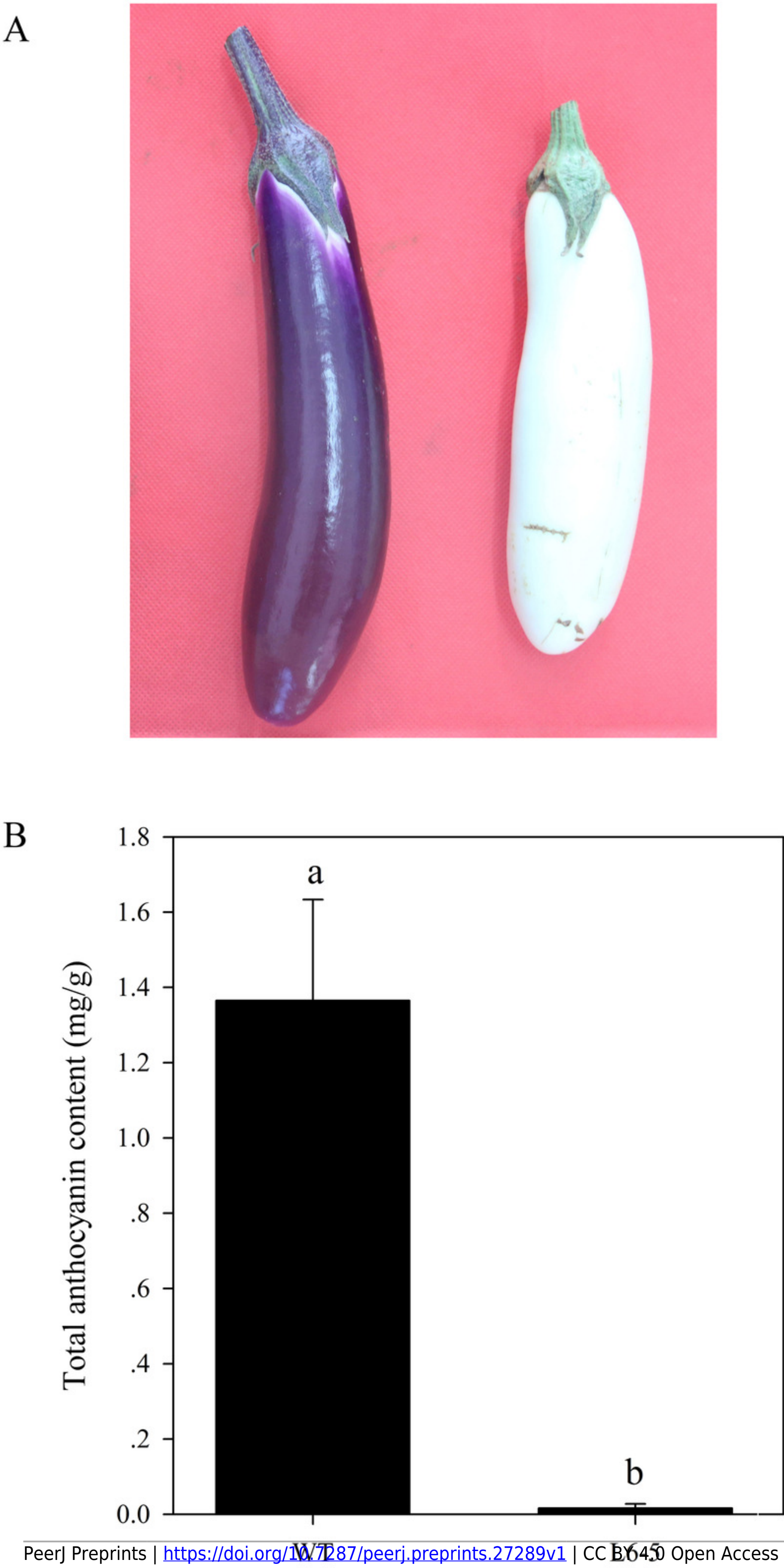
A
B
1.8
1.6
1.4
1.2
1.0
.8
.6
.4
.2
0.0
Total anthocyanin content (mg/g)
a
b
WT
E6-5

Figure 2

Figure 2 The phenotype and total anthocyanin content of four natural eggplant cultivars

A) The fruit colour of four natural eggplant cultivars and B) the total anthocyanin content of four natural eggplant cultivars

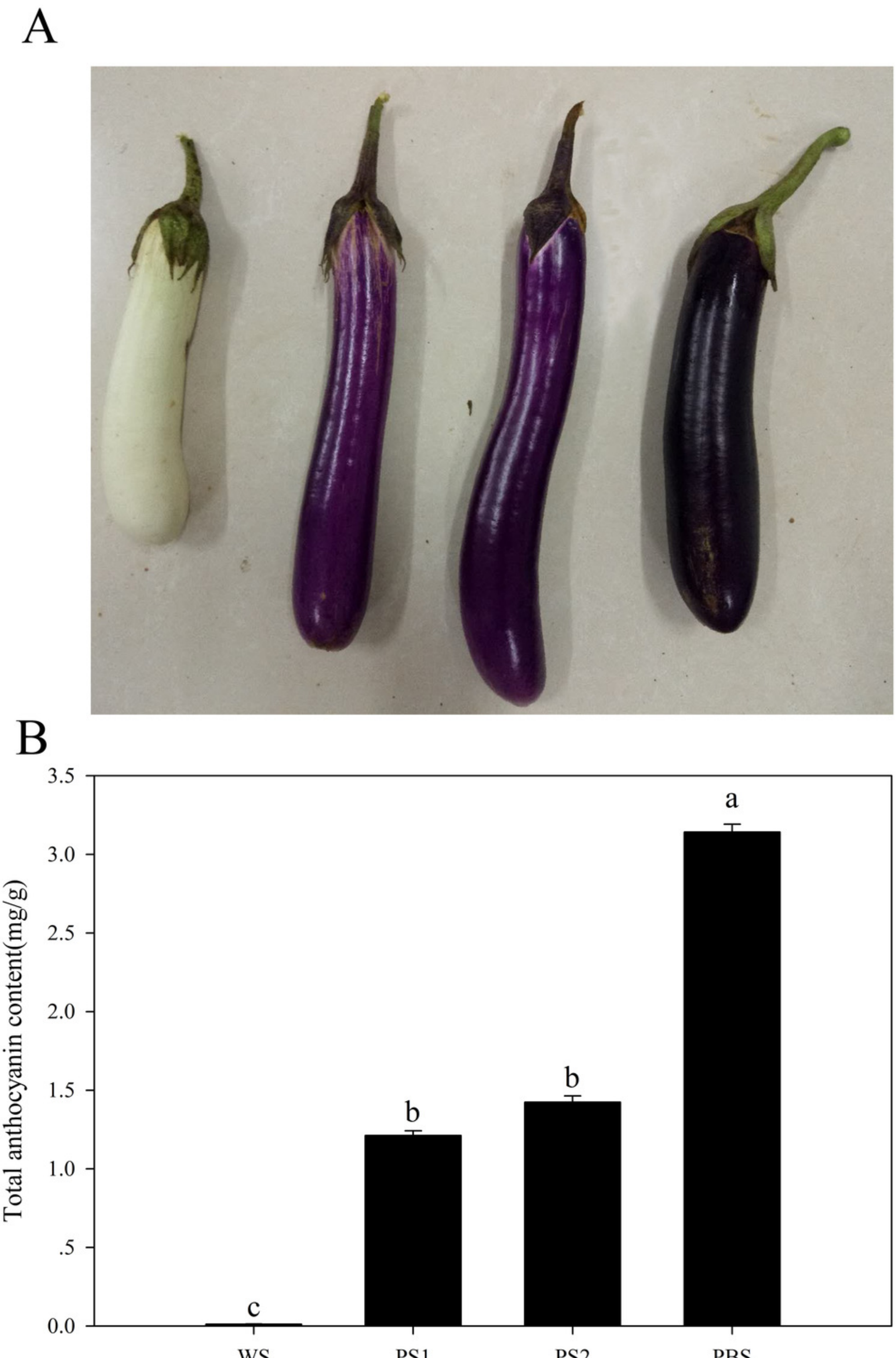

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Figure 3

Figure 3 The volcano figure of a differentially expressed gene

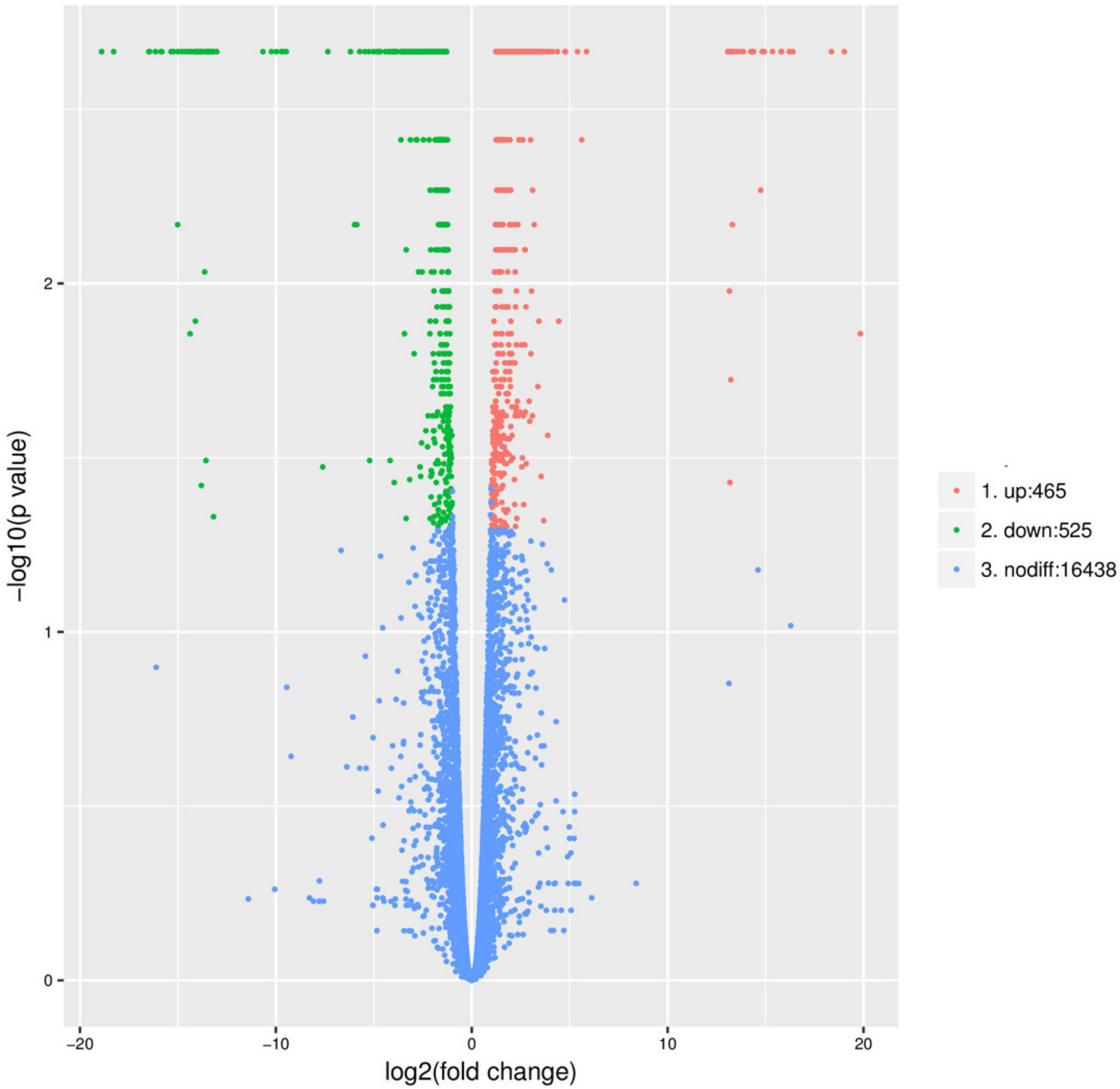

Figure 4

Figure 4 The GO analysis of a differentially expressed gene

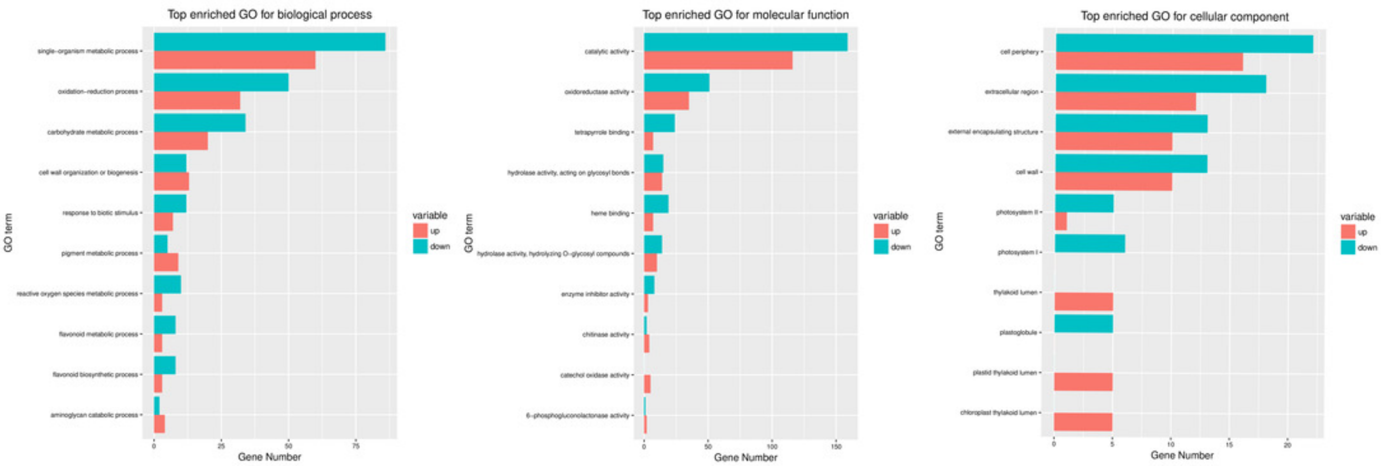

Figure 5

Figure 5 The KEGG pathway analysis of a differentially expressed gene

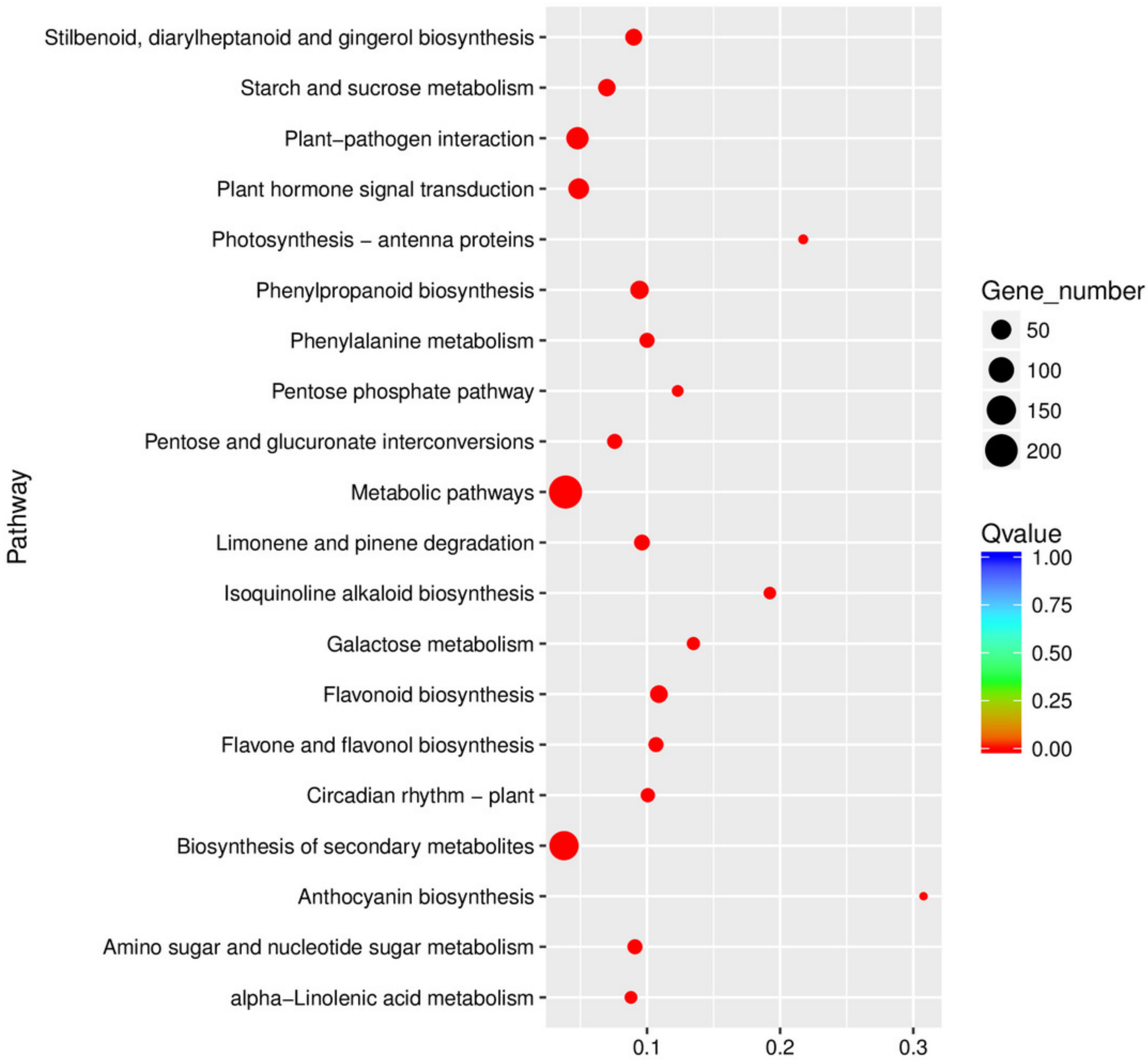

Figure 6

Figure 6 The QT-PCR results of 11 anthocyanin synthesis structure genes between WT and L6-5

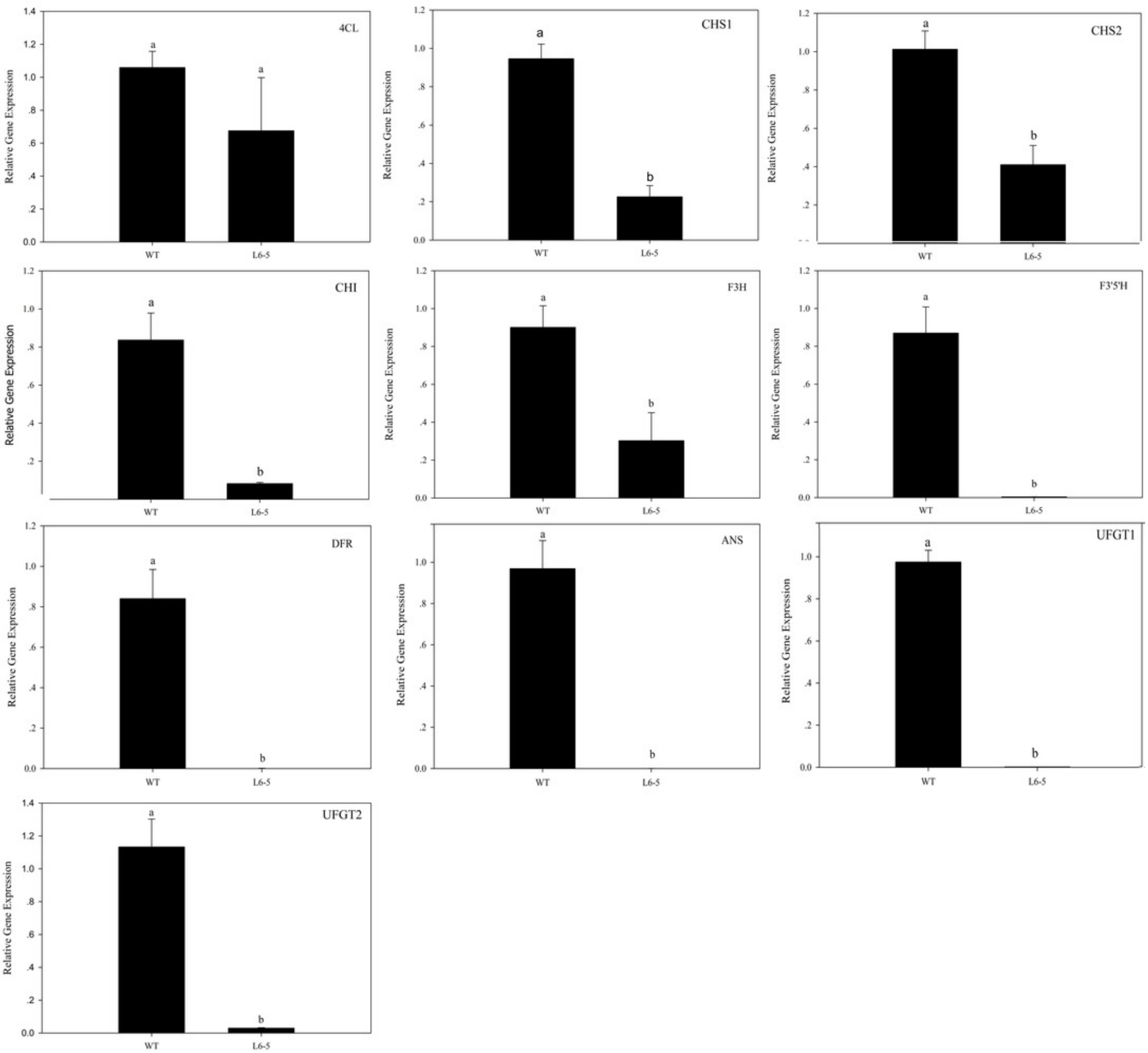

Figure 7

Figure 7 The QT-PCR results of 11 anthocyanin synthesis structure genes in four natural eggplant cultivars

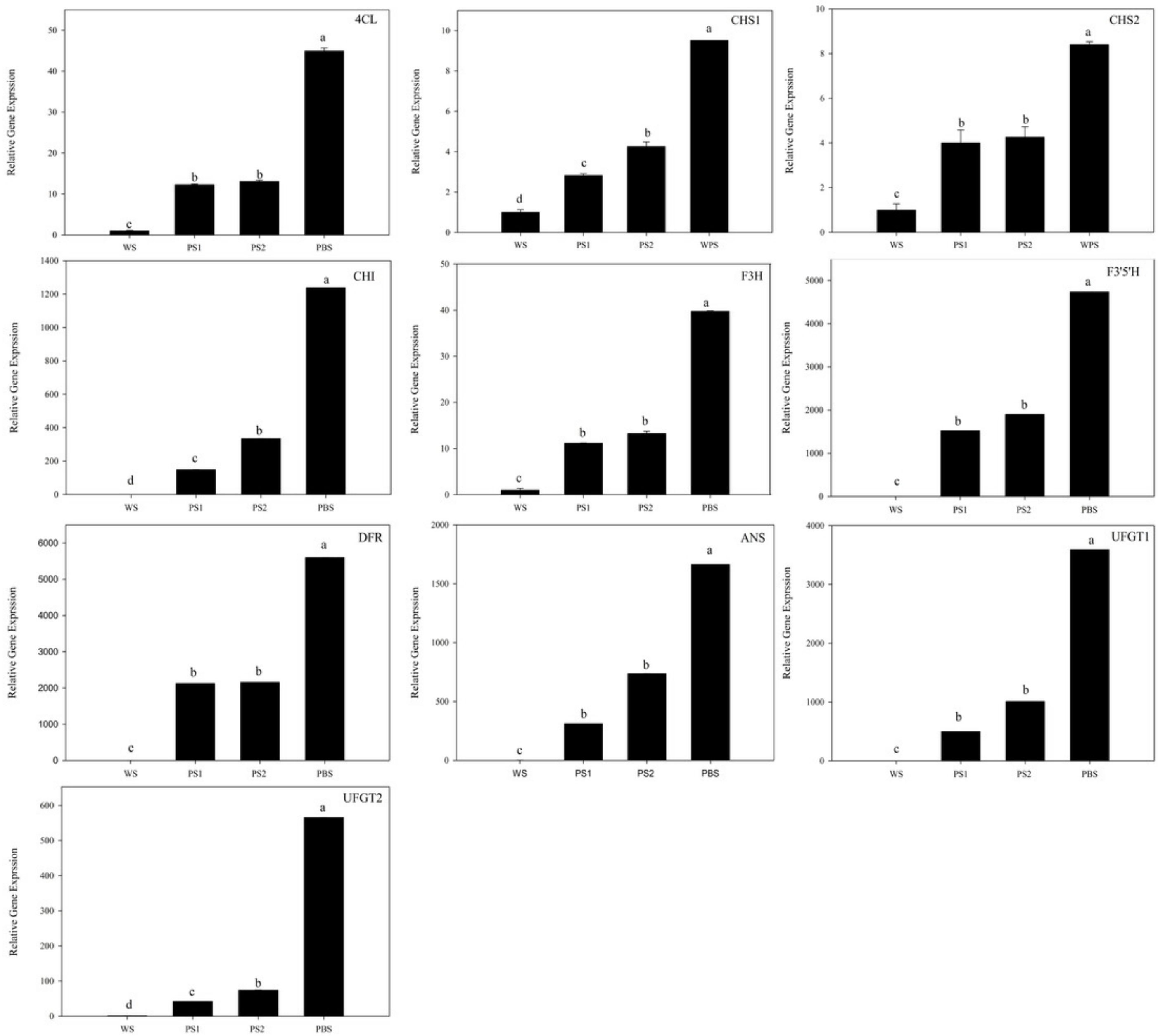

Figure 8

Figure 8 The QT-PCR results of the MYB transcription factor between WT and L6-5

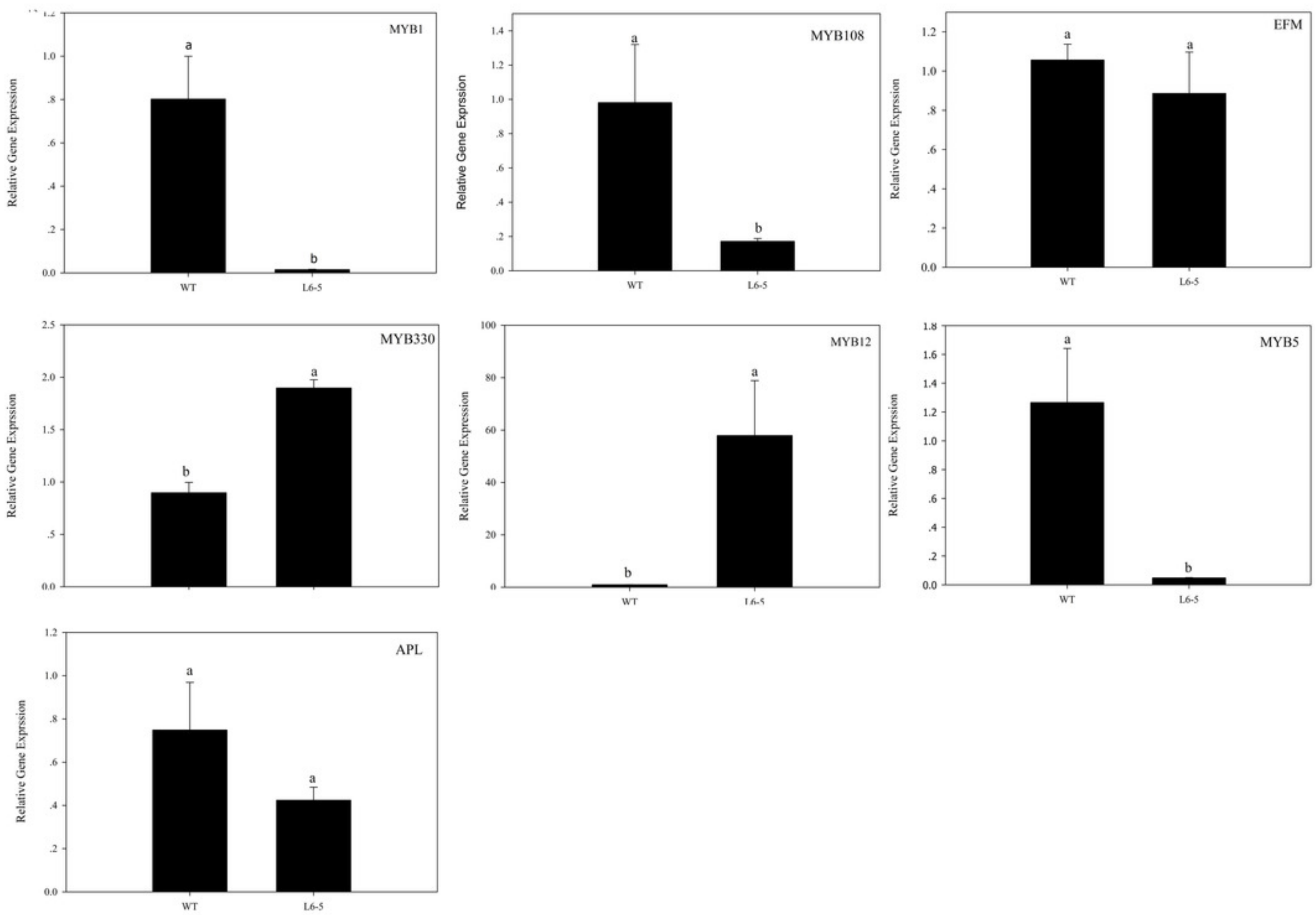

Figure 9

Figure 9 The polygenetic tree of MYBs involved in anthocyanin biosynthesis

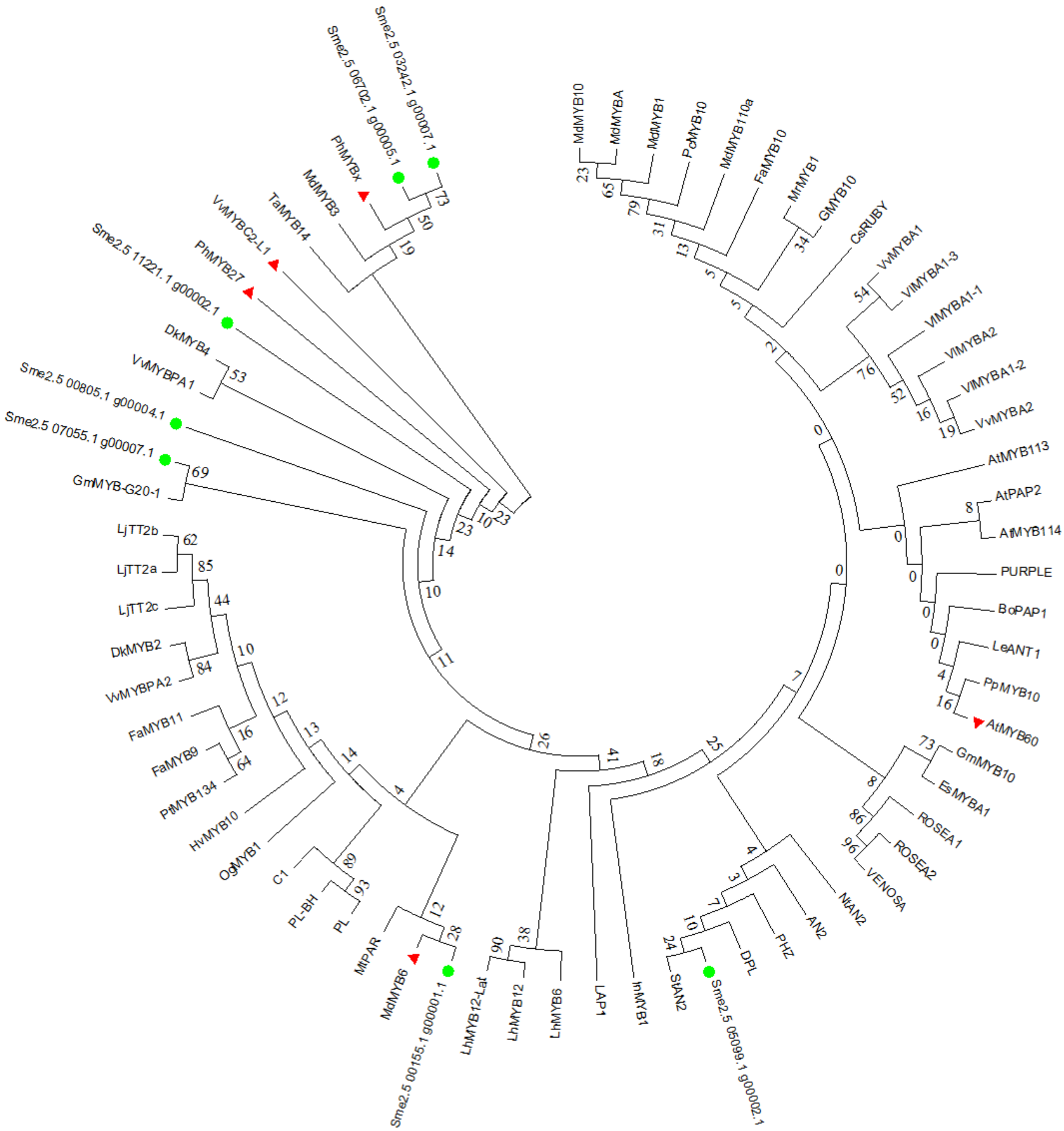

Figure 10

Figure 10 The QT-PCR results of the MYB transcription factor in four natural eggplant cultivars

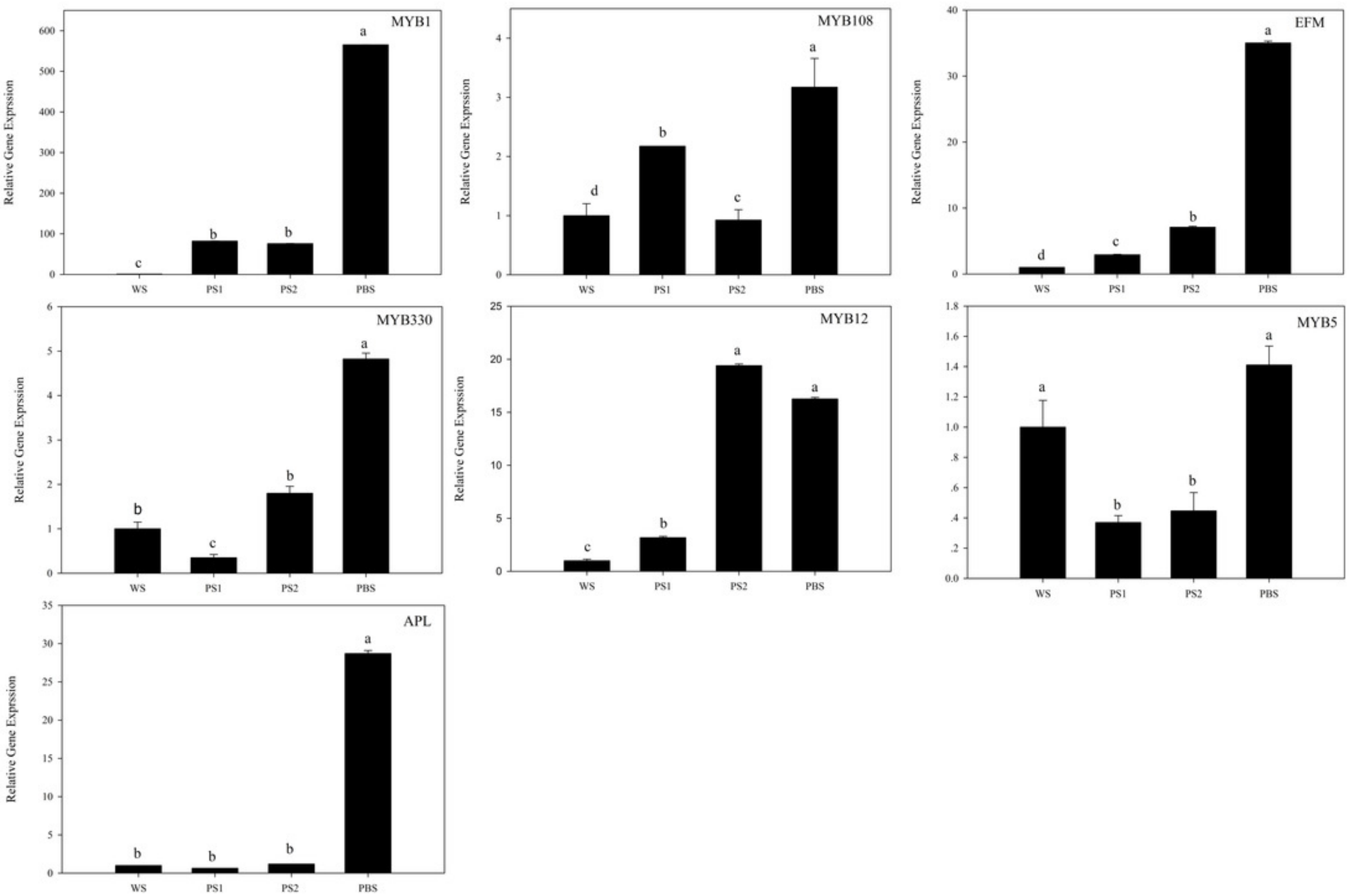

Figure 11

Figure 11 The QT-PCR results of the bHLH transcription factor between WT and L6-5

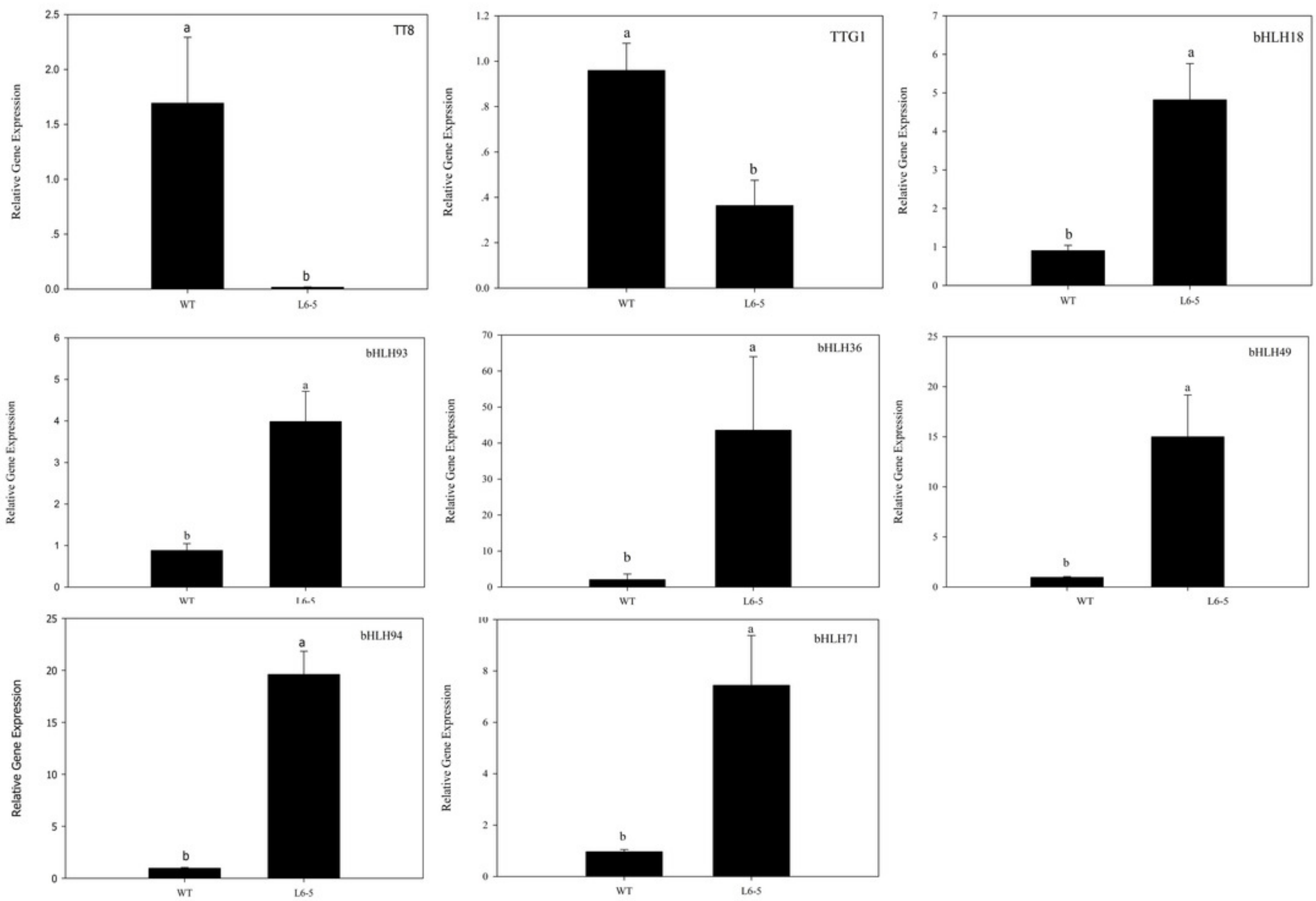

Figure 12

Figure 12 The QT-PCR results of the bHLH transcription factor in four natural eggplant cultivars

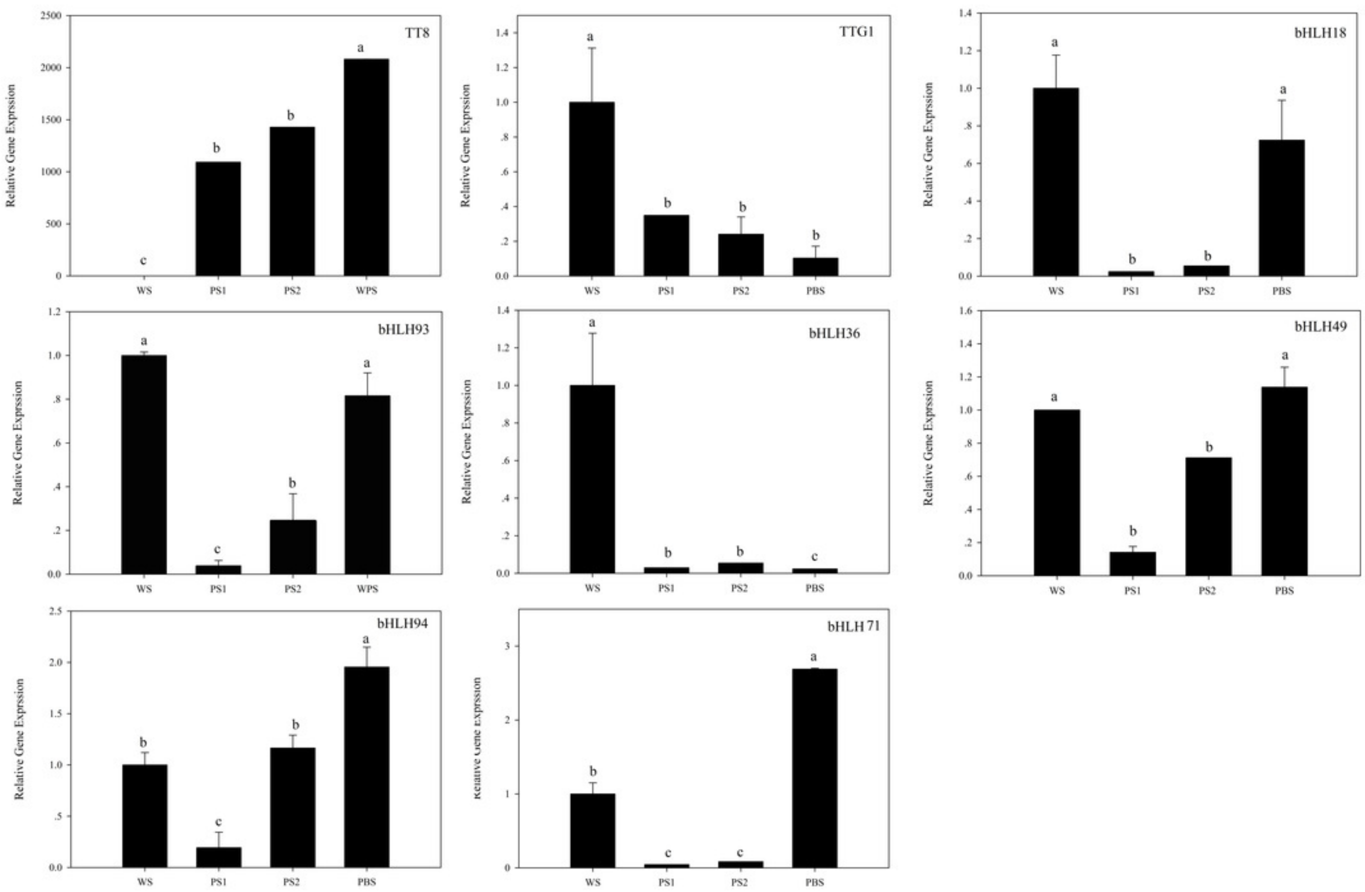

Figure 13

Figure 13 Simplified scheme related to anthocyanin biosynthesis in eggplant peels

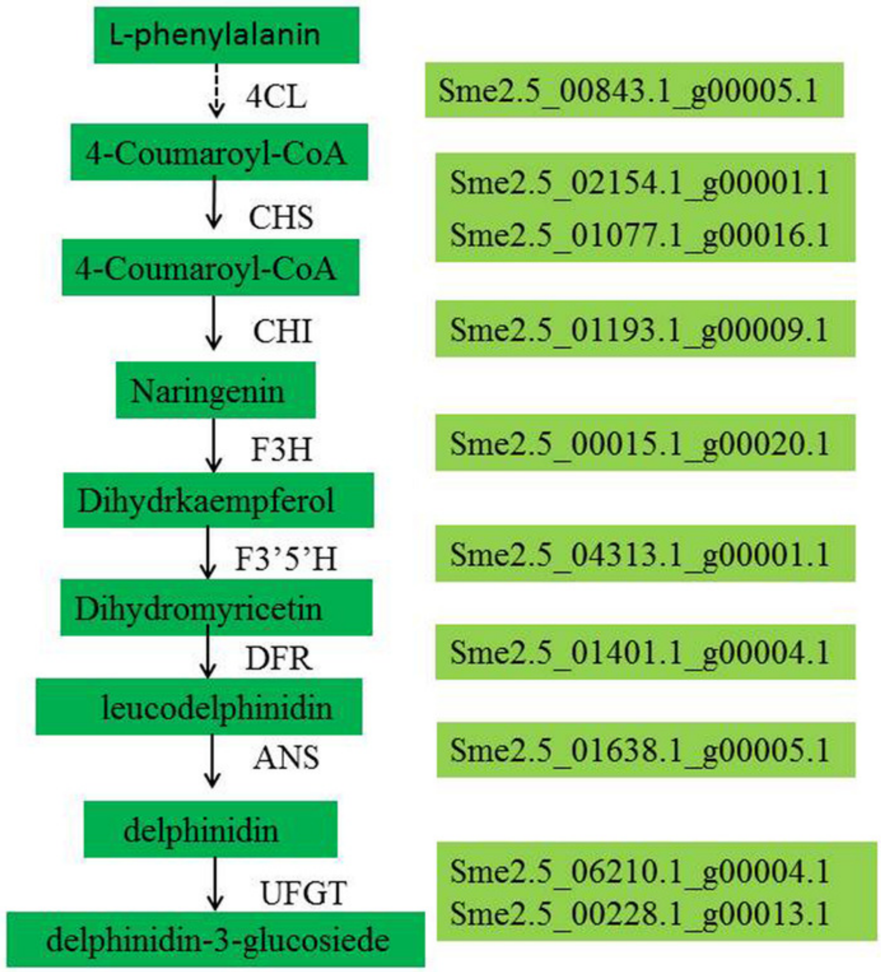

Table 1(on next page)

Table 1 The anthocyanin biosynthesis structure genes

Table 1 The anthocyanin biosynthesis structure genes

Gene ID	Log2(fold change)	Description	Symbol
Sme2.5_00843.1_g00005.1	-1.71241	4-coumarate--CoA ligase 2-like [Solanum pennellii]	4CL
Sme2.5_01077.1_g00016.1	-2.33431	chalcone synthase [Iochroma cyaneum]	CHS1
Sme2.5_02154.1_g00001.1	-2.17678	chalcone synthase [Iochroma cyaneum]	CHS2
Sme2.5_01193.1_g00009.1	-5.72024	chalcone--flavonone isomerase 2-like [Nelumbo nucifera]	CHI
Sme2.5_00015.1_g00020.1	-2.08458	flavanone 3-hydroxylase [Solanum melongena]	F3H
Sme2.5_04313.1_g00001.1	-9.46718	Flavonoid3',5'-hydroxylase; Short=F3'5'H;	F3'5'H
Sme2.5_01401.1_g00004.1	-9.71117	dihydroflavonol 4-reductase [Solanum melongena]	DFR
Sme2.5_01638.1_g00005.1	-9.97277	anthocyanin synthase [Solanum melongena]	ANS
Sme2.5_06210.1_g00004.1	-10.6575	anthocyanidin 3-O-glucosyltransferase [Solanum tuberosum]	UFGT1
Sme2.5_00228.1_g00013.1	-7.34178	Anthocyanidin 3-O-glucosyltransferase;	UFGT2

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Table 2 (on next page)

Table 2 Transcription factor of the DEGs

1 **Table 2 Transcription factor of the DEGs**

Gene ID	Log2(fold change)	Description
Sme2.5_29845.1_g00001.1	0	anthocyanin-related transcription factor TT8 [Solanum melongena]
Sme2.5_00747.1_g00013.1	-1.82426	anthocyanin-related transcription factor TTG1 [Solanum melongena]
Sme2.5_12868.1_g00001.1	1.56121	ethylene-responsive transcription factor RAP2-3 [Solanum tuberosum]
Sme2.5_00641.1_g00006.1	-1.41013	NAC domain transcription factor [Solanum lycopersicum]
Sme2.5_05942.1_g00005.1	1.94131	PREDICTED: bZIP transcription factor 11-like [Nicotiana attenuata]
Sme2.5_03302.1_g00002.1	-1.92613	PREDICTED: bZIP transcription factor 27-like [Capsicum annuum]
Sme2.5_00211.1_g00009.1	-2.1073	PREDICTED: ethylene-responsive transcription factor 1B-like [Solanum tuberosum]
Sme2.5_00787.1_g00017.1	1.24563	PREDICTED: ethylene-responsive transcription factor 8-like [Solanum tuberosum]
Sme2.5_06802.1_g00002.1	1.20428	PREDICTED: ethylene-responsive transcription factor ERF011-like [Solanum tuberosum]
Sme2.5_00250.1_g00015.1	1.28785	PREDICTED: ethylene-responsive transcription factor ERF027-like [Solanum tuberosum]
Sme2.5_03441.1_g00007.1	1.388	PREDICTED: ethylene-responsive transcription factor ERF038-like [Solanum lycopersicum]
Sme2.5_00346.1_g00021.1	1.70497	PREDICTED: ethylene-responsive transcription factor ERF038-like [Solanum

		pennellii]
Sme2.5_00713.1_g00012.1	2.15341	PREDICTED: ethylene-responsive transcription factor ERF110-like isoform X1 [Solanum tuberosum]
Sme2.5_07921.1_g00003.1	1.57856	PREDICTED: ethylene-responsive transcription factor WIN1 [Solanum pennellii]
Sme2.5_03438.1_g00003.1	1.28349	PREDICTED: ethylene-responsive transcription factor WIN1-like [Solanum tuberosum]
Sme2.5_01314.1_g00005.1	-1.72305	PREDICTED: heat stress transcription factor A-4c-like [Solanum tuberosum]
Sme2.5_00159.1_g00006.1	-1.34469	PREDICTED: heat stress transcription factor B-3 [Solanum tuberosum]
Sme2.5_03242.1_g00007.1	1.36091	PREDICTED: myb family transcription factor APL isoform X1 [Solanum tuberosum]
Sme2.5_06702.1_g00005.1	-1.06999	PREDICTED: myb family transcription factor EFM [Solanum lycopersicum]
Sme2.5_00514.1_g00007.1	1.00619	PREDICTED: nuclear transcription factor Y subunit A-10 isoform X2 [Solanum tuberosum]
Sme2.5_11618.1_g00002.1	-1.17939	PREDICTED: nuclear transcription factor Y subunit B-3-like [Solanum pennellii]
Sme2.5_00556.1_g00018.1	2.96067	PREDICTED: probable WRKY transcription factor 40 [Solanum tuberosum]
Sme2.5_00009.1_g00017.1	-1.25591	PREDICTED: probable WRKY transcription factor 53 [Solanum tuberosum]
Sme2.5_00708.1_g00006.1	2.63227	PREDICTED: probable WRKY transcription factor 70 [Solanum tuberosum]
Sme2.5_00407.1_g00003.1	-1.71587	PREDICTED: transcription factor bHLH18-like [Solanum pennellii]
Sme2.5_00735.1_g00008.1	1.37478	PREDICTED: transcription factor bHLH36-like [Capsicum annuum]

Sme2.5_01808.1_g00003.1	1.23941	PREDICTED: transcription factor bHLH49-like [Solanum tuberosum]
Sme2.5_05426.1_g00001.1	1.65182	PREDICTED: transcription factor bHLH71-like [Capsicum annuum]
Sme2.5_13712.1_g00001.1	1.7215	PREDICTED: transcription factor bHLH93-like [Solanum pennellii]
Sme2.5_04383.1_g00001.1	2.81124	PREDICTED: transcription factor bHLH94-like [Solanum lycopersicum]
Sme2.5_02186.1_g00004.1	-2.00871	PREDICTED: transcription factor CYCLOIDEA-like isoform X1 [Solanum pennellii]
Sme2.5_00584.1_g00015.1	-1.62938	PREDICTED: transcription factor DIVARICATA-like [Capsicum annuum]
Sme2.5_29204.1_g00001.1	inf	PREDICTED: transcription factor DIVARICATA-like [Solanum pennellii]
Sme2.5_04464.1_g00002.1	-1.3253	PREDICTED: transcription factor JUNGBRUNNEN 1 [Solanum tuberosum]
Sme2.5_02639.1_g00008.1	-2.83798	PREDICTED: transcription factor JUNGBRUNNEN 1-like isoform X2 [Solanum tuberosum]
Sme2.5_03969.1_g00004.1	1.54828	PREDICTED: transcription factor LUX [Solanum tuberosum]
Sme2.5_00155.1_g00001.1	-1.82995	PREDICTED: transcription factor MYB108-like [Solanum lycopersicum]
Sme2.5_11221.1_g00002.1	2.50601	PREDICTED: transcription factor MYB12 [Solanum tuberosum]
Sme2.5_02073.1_g00002.1	1.32049	PREDICTED: transcription factor MYC2-like [Solanum tuberosum]
Sme2.5_03511.1_g00006.1	1.10684	PREDICTED: transcription factor MYC2-like [Solanum tuberosum]
Sme2.5_05245.1_g00004.1	1.93606	PREDICTED: transcription factor PCL1-like [Solanum tuberosum]
Sme2.5_08464.1_g00002.1	-1.72959	PREDICTED: transcription factor TGA4-like isoform X1 [Solanum lycopersicum]
Sme2.5_07178.1_g00001.1	1.68574	PREDICTED: transcription factor WER-like [Solanum pennellii]

Sme2.5_02680.1_g00006.1	-4.67357	PREDICTED: WRKY transcription factor 44 [Solanum tuberosum]
Sme2.5_11773.1_g00001.1	1.70179	WRKY transcription factor 6 [Solanum tuberosum]

2 Inf,