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RNA-Seq Reveals Transcription Factors Involved in Temperature-mediated Anthocyanin Accumulation and Biosynthesis in Purple Pakchoi (*Brassica campestris* ssp. *chinensis* Makino.)

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Abstract Temperature is a main environmental factor that affects anthocyanin biosynthesis and accumulation in purple pakchoi (*Brassica campestris* ssp. *Chinensis Makino.*). Purple pakchoi is one of the most popular vegetables with high content of anthocyanin in China. Recently, we found that the purple color of purple pakchoi cultivar, "ziyi", deepened after 10-day low temperature(5°C, LT) treatment with increased anthocyanin content compared to plants after 20°C (normal temperature, NT, control) treatment. Contrarily, the color of pakchoi treated with 10-day heat temperature (35°C, HT) became lighter with decreased anthocyanin content than that of control. The transcriptom analysis revealed a total of 51 008 unigenes from plants treated with NT，LT, and HT by RNA-seq. A total of 4 321 and 8 455 differentially expressed genes (DEGs) were identified from HT and LT compared to NT, respectively. Among these DEGs, 173 unigenes were downregulated in LT and upregulated in HT compared to NT. 218 unigenes were upregulated in LT and downregulated in HT. Further Gene Ontology enrichment analysis revealed a series of candidate genes that may be involve in temperature-mediated anthocyanin accumulation, including structural genes and 20 transcription factors. Collectively, our study provides a global view of transcriptiomic resources in response to temperature-induced anthocyanin accumulation in purple pakchoi.
Keywords Purple pakchoi; Anthocyanin; Temperature; RNA-seq

Anthocyanins, a kind of secondary metabolites, impart vivid colors of fruits, flowers and vegetative tissues of higher plants ((Bradshaw and Schemske, 2003; Hatier and Gould, 2009; Speciale et al., 2010). Anthocyanins possess antioxidant, anti-carcinogenic, anti-inflammatory, and anti-microbial properties (Hatier and Gould, 2009; Speciale et al., 2010). Anthocyanins were reported to increase plant resistance to abotic stresses and diseases (Hatier and Gould, 2009; Li et al., 2017), serve as attractants for insects and animals(Gould and Lister, 2006), and prevent people suffering from some diseases (Jing et al., 2008; Speciale et al., 2010; Fairlie-Jones et al., 2017), making anthocyanin biosynthesis and accumulation of important.

Anthocyanin is a member of flavonoid family and its biosynthesis pathway *thaliana*, has been well determined in *Arabidopsis*. The key enzymes mainly are: pheylalanine ammonialyase (PAL), cinnamate 4-hydroxylase (C4H), 4-coumarate CoA-ligase (4CL), 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), leucoanthocyanidin dioxygenase (LDOX), and UDP-glucose:flavonoid 3-*O*-glucosyltransferase (UFGT) (Winkel-Shirley, 2001; Ai and Zhu, 2018). After a series enzymatic reactions catalyzed by different enzymes, anthocyanins finally are synthesized from L-phenylalanine (L-Phe) (Winkel-Shirley, 2001). Similar flavonoid pathway is also observed in *Zea mays*, *Solanum melongena*, and *Petunia hydrida* etc. (Hloton and Cornish, 1995; Winkel-Shirley, 2001; Zhang et al., 2014).

Generally, anthocyanin biosynthesis is regulated by genetical and environmental factors, including structural genes, transcription factors (TFs) (Stracke et al., 2007; Lloyd et al., 2017), plant hormones (Carvalho et al., 2010), temperature (de Rosas et al., 2017), and light (Ma et al., 2019). Structural genes encode enzymes directly

participating in anthocyanin biosynthesis, such as PAL and C4H (Davies and Schwinn, 2003; Takos and Walker, 2006). TFs directly activate or repress the expression of structural genes to modulate flavonoid metabolites, including basic helix-loop-helix(bHLH), WD40, WRYK, Zinc finger, and R2R3 MYB families (Hichri et al., 2011; Fairlie-Jones et al., 2017). R2R3MYB, bHLH, and WD40 forming MBW complex are considerate as major regulators though the potential mechanisms of this complex are still unknow (Hichri et al., 2011).

Among environmental factors, both light and temperature have been identified to affect anthocyanin biosynthesis. Researches have revealed that light contributes to anthocyanin biosynthesis in *Lc* petunia and apple (Takos et al., 2006; Albert et al., 2009). It has been verified that low temperature (LT) promotes anthocyanin production in maize (Christie et al., 1994), red oranges (Lo Piero et al., 2005), and apples (Ubi et al., 2006), while high temperature inhibits anthocyanin accumulation (Mori et al., 2007). LT positively regulates *MdMYBA* expression, encoding an apple TF, which binds to *ANS* promoter and activates *ANS* expression, resulting in anthocyanin accumulation in apple skin (Ban et al., 2007). A PA1 -type MYB TF MdMYBPA1, was identified to response to low temperature and anthocyanin in red-fleshed apple (Wang et al., 2018). In potato (*Solanum tuberosum L.*), StMYB44 was characterized to repress anthocyanin accumulation under high temperature (Liu et al., 2019).

Purple pakchoi (*Brassica rapa* ssp, *B.rapa*, *Chinensis* Makion) , rich of anthocyanins, is one of the most popular vegetables (Simone et al., 2006). In purple pakchoi, LT was reported to induce distinct anthocyanin metabolic pathways (Christie et al., 1994; Chalker-Scott, 1999) [10,9] [10,9]. In this study, we presented a purple pakchoi cultivar, 'ziyi', whose purple leaf color started to deepen at day 10 after LT treatment. To further study the mechanism of color change, we detected anthocyanin content in leaves. Results showed that the darker color leaves were of higher anthocyanin content. Leaves maintained at 5°C for 10 d were collected to extract total RNA for RNA sequencing (RNA-Seq). RNA-Seq results showed that anthocyanin biosynthesis related genes were differentially expressed in leaves at 5°C than that of control. qRT-PCR further confirmed that the expression levels of *C4H*, *CHS*, *F3H*, *HY5*, *UGT75C1*, and *FLS*, were increased after 5°C. Collectively, low temperature promotes leaf color deepening through anthocyanin biosynthesis pathway, causing anthocyanin accumulation in purple pakchoi.

1 Results

1.1 Temperature affects leaf color and anthocyanin content

To study the effect of temperature on leaf color, a purple pakchoi cultivar “yizi” was treated with LT, HT and NT conditions for 2 d, 4 d, 6 d, 8 d, 10 d, and 12 d, respectively. Results showed that leaf color of plants started to deepen after 2d-LT treatment and positively correlated with the time expose to LT (Figure 1A; Figure 1B). Contrarily, the leaf color of plants treated with HT turned to lighter at day 2 compared to that of plants treated with NT (Figure 1A). As leaf color associates with anthocyanin content, we measured the anthocyanin content in plant leaves exposed to different temperature conditions. It revealed that anthocyanin level gradually increased after LT from 3.79 mg/g to 13.41 mg/g at 5°C and gradually decreased after HT from 3.78 mg/g to 1.52 mg/g from day 2 to day 12 (Figure 1B). These data reveal that LT plays a positive regulator and HT is a negative regulator during anthocyanin biosynthesis and accumulation.

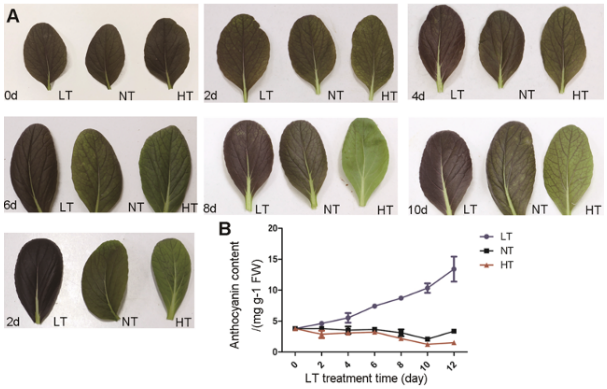

Figure 1 Anthocyanin content and the color of purple pakchoi at LT (5°C), NT (20°C), and HT (35°C). (A) Change of leaf color treated with LT, NT and HT for indicated days. (B) Anthocyanin content in purple pakchoi treated with different temperatures from 0 d to 12 d

1.2 High-through put sequencing and *de novo* assembly

To further investigate the mechanism of changed anthocyanin content after different temperature treatment, transcriptome profiles of pakchoi at day 10 under NT, LT, and HT conditions were analyzed, respectively. After RNA-Seq, a total of 148 517 620 135 058 374 and 132 198 886 raw reads were obtained from sample of NT, LT, and HT, respectively. After filtering reads with low quality, a total of 146 935 754 of NT 133 743 948 of LT and 130 788 732 of HTclean reads (a base quality greater than 20) were obtained (Table 1). All clean reads were *de nove* assembled by Trinity software, after which 51008 unigenes were obtained with an average length of 762.96 bp, a maximum length of 7 750 bp, a minimum length of 201 bp, an N50 of 1 074 bp, and an E90N50 of 1 333 bp (Table 1).

Table 1 Statics of high-through put sequencing.

Sample	Raw reads	Raw bases	Clean reads	Clean bases	Error rate (%)	Q20 (%)	Q30 (%)	GC content (%)
COLD1	46341400	6951210000	45899300	6.71E+09	0.0114	98.71	96.1	52.15
COLD2	47250524	7087578600	46795718	6.85E+09	0.0114	98.69	96.04	52.18
COLD3	41466450	6219967500	41048930	6E+09	0.0114	98.72	96.12	52.12
HEAT1	46072536	6910880400	45521712	6.65E+09	0.0117	98.58	95.74	52.24
HEAT2	44536370	6680455500	44097602	6.48E+09	0.0117	98.57	95.7	52.21
HEAT3	41589980	6238497000	41169418	6.03E+09	0.0116	98.62	95.84	52.1
NT1	62621216	9393182400	61906522	9.04E+09	0.0119	98.46	95.42	52.32
NT2	41923862	6288579300	41508494	6.05E+09	0.0117	98.58	95.74	52.25
NT3	43972542	6595881300	43520738	6.33E+09	0.0118	98.53	95.58	52.76

1.3 Functional annotation and classification of assembled transcripts

To further annotate these unigenes and understand the overall expression profiles of 'ziyi', all unigenes were searched against the NR, GO, KEGG, Pfam, COG, and Swiss-Prot. Totally, 51 008 unigenes had homologous sequences in at least one of the six mentioned databases. Among them, 42 828 (83.96%), 33 883 (66.43%), 30 426 (59.65%), 13 358 (26.19%), 25 998 (50.97%), and 20 882 (40.82%) unigenes were found in the NR, SwissProt, Pfam, COG, GO, and KEGG databases, respectively (Figure 2).

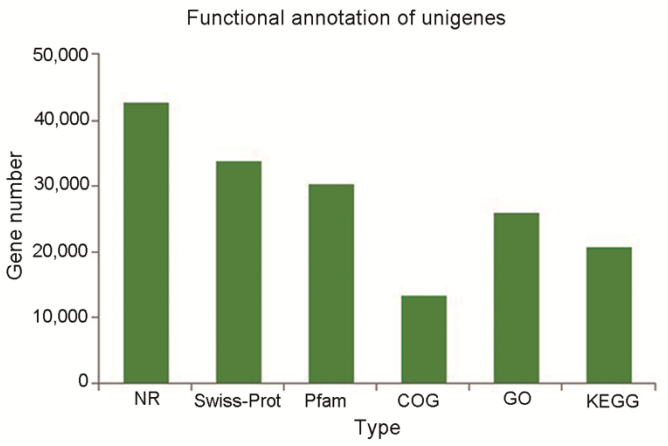

Figure 2 Functional annotation of unigenes

1.4 Temperature affects anthocyanin homeostatic genes

To calculate the expression levels of unigenes, we employed expected number of Fragments Per Kilobase of transcript sequence per Millions base pairs sequenced (FPKM) method (Trapnell et al., 2010). The DEGs were defined with $P \leq 0.05$ and fold change ≥ 2. Totally, 8 455 and 4 321unigenes were differentially expressed in LT and HT treatment, respectively (Figure 3). Among these DEG, a total of 391 unigens responded to LT and HT simultaneously of which 173 unigenes were upregulated in HT and downregulated in LT whereas 218 were downregulated in HT and upregulated in LT. These data indicated that the 391 unigenes might involve in temperature-associated anthocyanin biosynthesis and accumulation.

To further understand the 391 unigenes' functions, GO functional enrichment and KEGG pathway analyses were performed. The 391 unigenes were categorized into 44 functional groups in three main categories of "biologica process", "cellular component", and "molecular function". A total of 132 unigenes were assigned to binding, 129 were to catalytic activity, 98 were to metabolic process, and 97 were to cell (Figure 4). Ninety-five unigenes were categorized to cellular process, membrane, and cell part, respectively (Figure 4). These data revealed that unigenes involved in anthocyanin accumulation were functional diverse.

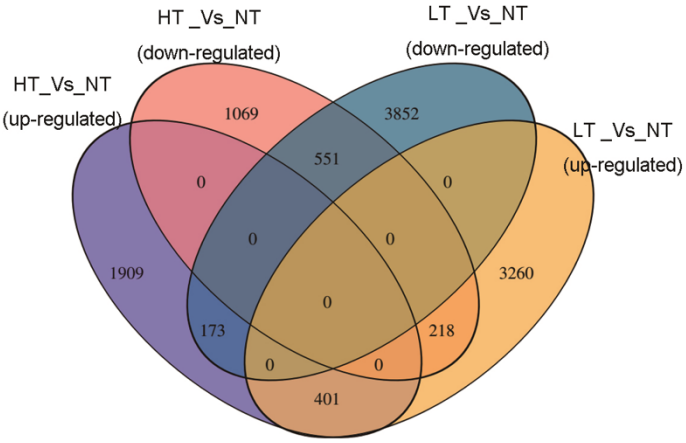

Figure 3 Venn analysis of differentially expressed unigenes

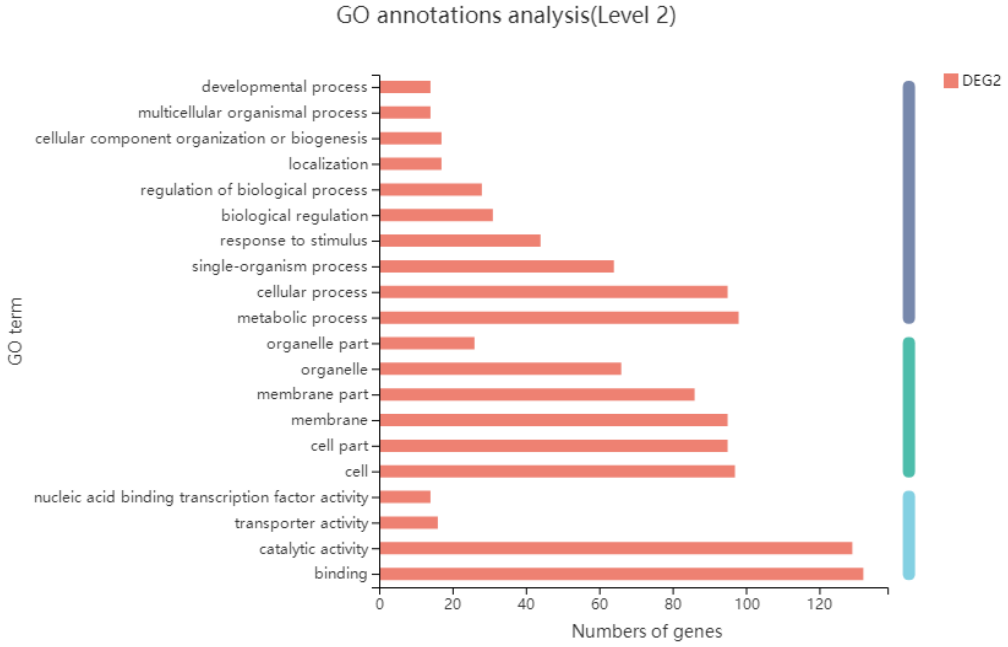

Figure 4 GO enrichment analysis of DEGs in response to LT and HT

By analyzing the KEGG pathways related to the DEGs, it was revealed that 295 unigenes were mapped to KEGG pathways to reveal the biological pathways that unigenes were involved. The largest group was pheylpropanoid biosynthesis which 10 unigenes were mapped to (Figure 5). Phenylalaninsurves as the substrate for flavonoids biosynthesis via phenylpropanoid pathway (Hichri et al., 2011). Eleven unigenes were mapped to anthocyanin biosynthesis or related pathways (Figure 5).

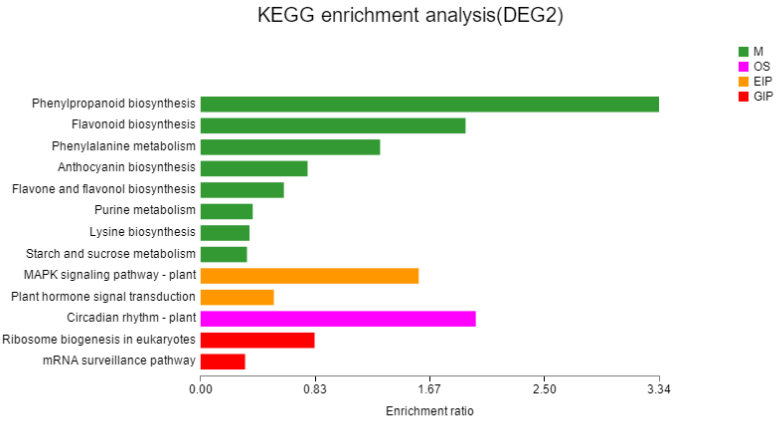

Figure 5 Analysis of KEGG pathway of the 391 DEGs

1.5 Discovery of anthocyanin-associated genes

As anthocyanin biosynthesis is affected by structural genes encoding diversity enzymes were analyzed. Structural genes, including PAL2 (TRINITY_DN16735_c1_g7), *CHS* (TRINITY_DN18090_c2_g1), 4CL (TRINITY_DN13099__c0__g2), *F3H* (TRINITY_DN11496_c0_g2), CHI (TRINITY_DN 18244_c0_g1), and *UGT75C1* (TRINITY_DN6415_c0_g1), were found to be up-regulated in LT and down-regulated in up-regulated, (Figure 6A). Additionally, transcription factors play vital roles in anthocyanin homeostatics, therefore we also analyzed the differentially expressed transcription factors. Among DEGs, 20 unigenes might encode transcription factors based GO and KEGG analyses. Three unigenes, *MYB6* (TRINITY_DN17146_c0_g5), *bZIP1* (TRINITY_DN12420_c0_g1), and *COL2* (*COSTANs-LIKE 2*), were up-regulated after HT and down-regulated after LT (Figure 6B). Contrarily, fourteen unigenes, encoding MYB4, bZIP22, bHLH44, bHLH128, ERF9, DREB1B, ERF043, ERF023, ERF72, TOE1, TOE3, WRKY44, GATA7, and GATA22, were down-regulated after HT and up-regulated in LT (Figure 6C). These results indicated that these transcription factors might be involved in anthocyanin biosynthesis or accumulation to response to temperature stress.

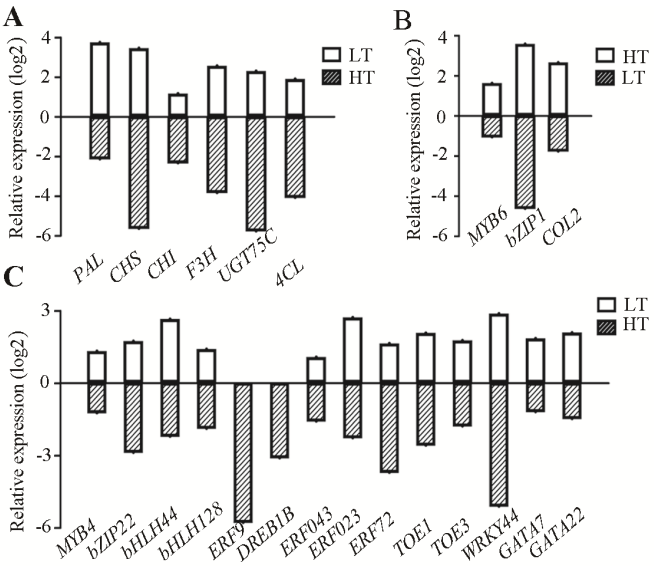

Figure 6 Relative expression (log2) of structural genes (A) and transcription factors (B) and (C) in LT and HT in RNA-Seq. (A-C) Gene expression level was analyzed by RSEM, P -adjust <0.05

2 Discussion

Low temperature has been described to be important for increasing anthocyanin production. In *Arabidopsis*, apples, and pears, anthocyanins was reported to be induced by LT (Leyva et al., 1995). LT increased anthocyanin concentration through promoting the expression of anthocyanin biosynthesis-related genes (Lo Piero et al., 2005;

Steyn et al., 2009; Ubi et al., 2006). In our study, anthocyanin concentration of purple pakchoi gradually increased over time from day 0 to day 12 following LT treatment and decreased following HT treatment (Figure 1). Similar anthocyanin concentration alteration was found in *Brasscica rapa*, indicating temperature plays a vital role in enhancing anthocyanin concentration in these plants (Ahmed et al., 2015).

Because of the development of next generation sequencing (NGS), transcriptome sequencing has become an effective research tool with low cost and high throughput (Galla et al., 2009; Kang et al., 2013). Through RNA-Seq analysis, we observed lots of genes altered their expression levels after LT treatment. Among DEGs, *PAL*, *CHS*, *CHI*, *F3H*, *4CL,* and *UGT75C* expression levels, involved in anthocyanin biosynthesis, were identified to be induced by LT and inhibited by HT (Figure 6). These results suggested that these genes play important roles in temperature-mediated anthocyanin biosynthesis in pakchoi, consistent with the findings of Qu et al. (2015). Previous researches revealed that *CHS* were differentially expressed between red and yellow flesh sweet cherry (*Prunus avium* L.) (Wei et al., 2015). Similar results were obtained in arctic mustard and purple lettuce transcriptomes (Butler et al., 2014; Zhang et al., 2016). In our study, we proved that most enzymes were also involved in anthocyanin biosynthesis in purple pakchoi.

Among these genes, MYB TFs have been identified as key determinants of the color differences among cultivated plant varieties. In *Arabidopsis*, AtMYB11/PFG2, AtMYB12/PFG1, AtMYB111/PFG3, AtMYB75/PAP1, AtMYB90/PAP2, AtMYB113, and AtMYB114 have been identified to have the ability to activate the expression of anthocyanin biosynthetic genes, while AtMYB3, AtMYB4, AtMYB7, and AtMYB32 repress the expression of these genes (Dubos et al., 2010). In horticultural plants, many MYB TFs, such as Chinese cabbage BrMYB2, *Tricyrtis* sp. TrMYB1, and purple cauliflower BoMYB2, were identified to be involved in anthocyanin biosynthesis (He et al., 2016; Kanemaki et al., 2018). In our study, we found 6 unigenes were differentially expressed, which annotated to MYB or MYB-like family, including *MYB4* and *MYB6*. These data suggested that MYB4 and MYB6 might be also involved in temperature-mediated anthocyanin biosynthesis in pakchoi, which are deserved to be further studied.

Collectively, using a high-throughput sequencing platform, the transcriptomes of purple pakchoi at 5°C, 20°C, and 35°C were surveyed. 8 571 differentially expressed unigenes were observed between two treatments. We revealed the mechanism of anthocyanin biosynthesis and accumulation under different temperature. Purple pakchoi adjusts gene expression levels to accumulate anthocyanin to response to temperature stress. These findings provide the first global insights into the mechanisms of anthocyanin metabolism in purple pakchoi. In addition, this study involves pakchoi sequencing data, for which there is no sequenced genome, for future investigations of the molecular biological mechanisms of this species.

3 Materials and Methods

3.1 Plant material and growth conditions

The purple pakchoi, 'ziyi', seeds were supplied by Protected Horticultural Research Institute, Shanghai Academy of Agricultural Sciences (China). The seeds were sown in plastic plates containing peat: vermiculite (2 : 1, v/v) in a growth chamber for temperature treatment or harvest seeds.

3.2 Temperature treatment

Four-week-old leaves were used after germination in the growth chamber (PGX-350A, Shenxian thermostatic equipment factory, Shanghai). The growth chamber was set to ~60% relative humidity and the photo period was 6-h light/8-h darkness using a photosynthetic photon flux density (PPFD) of 800~1 000 μmol m-2s-1. Plants were treated with LT (5°C, LT) or NT (control) for 2, 4, 6, 8, 10 and 12 d and each group had 60 plants. After treatment, leaves were harvested to detect anthocyanin content and extract total RNA for RNA-Seq or detecting gene expression.

3.3 Anthocyanin content measurement

Total anthocyanin content was measured with the modified pH differential method (AOAC office method 2005.2)

(Sukwattanasinit et al., 2007; Jungmin et al., 2008). Briefly, a total of 20 mg of leaf samples was used to extract with 10 mL of extraction buffer [95% ethanol and 1.5 mol·L^{-1}HCl (V/V=85:15)] at room temperature in the dark for 24 h. The extracted solution (1mL) was added with 2 mL of 0.025 M KCl buffer at pH 1.0 and 0.4 M NaAc at pH 4.5, adjusted to a pH value of 1.0 or 4.5 with hydrochloride. Absorbance was measured with a nucleic acid/protein analyzer (Beckman Coulter, Inc. USA) at 536 nm and 700 nm in the buffers with pH 1.0 and pH 4.5 buffers, respectively. The total absorbance (A) was calculated as follows:

$$A = \left(A_{536} - A_{700}\right)\ \text{pH}\ 1.0 - \left(A_{536} - A_{700}\right)\ \text{pH}\ 4.5$$

The TAC was derived using cyanidin-3-glucoside, which has a molar extinction coefficient of 26 900 $Lcm^{-1}mol^{-1}$ and a molecular weight of 449.2 $gmol^{-1}$. The results are expressed as milligrams of cyanidin-3-glucoside equivalent per gram of the fresh sample weight.

3.4 RNA extraction and gene expression analysis

Total RNA was isolated from control and LT samples using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. RNA quality and quantity were detected using an Agilent 2100 Bioanalyzer (Agilent, MN，US). The qualified total RNA was used to synthesize first strand cDNA, which used as templates for qRT-PCR. The qRT-PCR was conducted and SYBR Green PCR Kit (ABI7300) in a MicroAmp™ 96-well plate were measured on a StepOnePlus™ Real-Time PCR System (ABI, US). The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression levels of the target genes. Primers used are listed in Table 1.

3.5 RNA-Seq

The extracted total RNA was isolated from plants treated with NT, 10-day LTand 10-day HT. RNA concentration and quality were detected using Agilent 2 100 Bioanalyzer. The synthesized cDNA libraries were sequenced by Illumina NextSeq 500 instrument (Personalbio, China). The transcriptome sequence processing was previously described (Jia et al., 2015). Briefly, raw data with low quality (reads with a base quality less than 20) were filtered to obtain clean data, followed by *de novo* assembly with Trinity software (version: r20140717, k-mer 25 bp, http://trinityrnaseq.sf.net). Transcipt abundance was normalized using a k-mer value of 25 reads per kilobase of exon model per million mapped reads (RPKM) (Wagner et al., 2012). Differentially expressed genes (DEGs) were defined with fold change≥2.00 and $P \leq 0.05$.

3.6 Gene annotation and ontology

The functional annotations were performed by comparing the sequences with those in public databases, including NCBI nonredundant protein database (NR), Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), Cluster of Orthologous Groups of proteins (COG), Pfam, and Swiss-prot database. According to GO term, unigenes were classified within molecular functions, cellular components, and biological processes (Conesa et al., 2005). To investigate metabolic and cellular pathways, unigenes were also assigned to special biochemical pathways based on KEGG database.

Authors' contributions

Hongfang Zhu conducted detected the anthocyanin contents under LT and HT, harvested samples for RNA-Seq and analysis the results of RNA-Seq. Dandan Xi prepared this manuscript. Xiaofeng Li and Lu Gao helped Hongfang Zhu to carry out these experiments. Yuying Zhu designed all experiments and directed experiments. All authors read and approved the final manuscript.

Acknowledgments

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References

Ahmed N.U., Park J., Jung H., Hur Y., and Nou I., 2015, Anthocyanin biosynthesis for cold and freezing stress tolerance and desirable color in Brassica rapa, Funct Integr Genomic, 15(4): 383-394
https://doi.org/10.1007/s10142-014-0427-7
PMid:25504198

Ai Y., and Zhu Z., 2018, Melatonin antagonizes jasmonate-triggered anthocyanin biosynthesis in Arabidopsis thaliana, J Agr Food Chem, 66(21): 5392-5400
https://doi.org/10.1021/acs.jafc.8b01795
PMid:29758982

Albert N.W., Lewis D.H., Zhang H., Irving L.J., Jameson P.E., and Davies K.M., 2009, Light-induced vegetative anthocyanin pigmentation in Petunia, J Exp Bot, 60(7): 2191-2202
https://doi.org/10.1093/jxb/erp097
PMid:19380423 PMCid:PMC2682507

Ban Y., Honda C., Hatsuyama Y., Igarashi M., Bessho H., and Moriguchi T., 2007, Isolation and functional analysis of a MYB transcription factor gene that is a key regulator for the development of red coloration in apple skin, Plant Cell Physiol, 48(7): 958-970
https://doi.org/10.1093/pcp/pcm066
PMid:17526919

Bradshaw H.D., and Schemske D.W., 2003, Allele substitution at a flower color locus produces a pollinator shift in monkeyflowers, Nature. 426: 176-178
https://doi.org/10.1038/nature02106
PMid:14614505

Butler T., Dick C., Carlson M.L., and Whittall J.B., 2014, Transcriptome analysis of a petal anthocyanin polymorphism in the arctic mustard, Parrya nudicaulis, PLos One, 9: e101338
https://doi.org/10.1371/journal.pone.0101338
PMid:25033465 PMCid:PMC4102464

Carvalho R.R.F., Quecini V., and La Zaro E.Q.P.P., 2010, Hormonal modulation of photomorphogenesis-controlled anthocyanin, Plant Sci, 178(3): 258-264
https://doi.org/10.1016/j.plantsci.2010.01.013

Chalker-Scott L., 1999, Environmental significance of anthocyanins in plant stress responses, Photochem Photobiol, 70(1): 1-9
https://doi.org/10.1111/j.1751-1097.1999.tb01944.x

Christie P.J., Alfenito M.R., and Walbot V., 1994, Impact of low-temperature stress on general phenylpropanoid and anthocyanin pathways: Enhancement of transcript abundance and anthocyanin pigmentation in maize seedlings, Planta, 194(4): 541-549
https://doi.org/10.1007/BF00714468

Conesa A., Götz S., García-Gómez J.M., Terol J., Talón M., and Robles M., 2005, Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research, Bioinformatics, 21(18): 3674-3676
https://doi.org/10.1093/bioinformatics/bti610
PMid:16081474

Davies K.M., and Schwinn K.E., 2003, Transcriptional regulation of secondary metabolism, Funct Plant Biol, 30(9): 913
https://doi.org/10.1071/FP03062
PMid:32689076

De Rosas I., Ponce M.T., Malovini E., Deis L., Cavagnaro B., and Cavagnaro P., 2017, Loss of anthocyanins and modification of the anthocyanin profiles in grape berries of malbec and bonarda grown under high temperature conditions, Plant Sci, 258: 137-145
https://doi.org/10.1016/j.plantsci.2017.01.015
PMid:28330557

Dubos C., Stracke R., Grotewold E., Weisshaar B., Martin C., and Lepiniec L., 2010, MYB transcription factors in Arabidopsis, Trends Plant Sci, 15(10): 573-581
https://doi.org/10.1016/j.tplants.2010.06.005
PMid:20674465

Fairlie-Jones L., Davison K., Fromentin E., and Hill A., 2017, The effect of anthocyanin-rich foods or extracts on vascular function in adults: a systematic review and meta-analysis of Randomised controlled trials, Nutrients, 9(8): 908
https://doi.org/10.3390/nu9080908
PMid:28825651 PMCid:PMC5579701

Galla G., Barcaccia G., Ramina A., Collani S., Alagna F., Baldoni L., Cultrera N.G., Martinelli F., Sebastiani L., and Tonutti P., 2009, Computational annotation of genes differentially expressed along olive fruit development, BMC Plant Biol, 9(1): 128
https://doi.org/10.1186/1471-2229-9-128
PMid:19852839 PMCid:PMC2774695

Gould K.S., and Lister C., 2006, Flavonoid functions in plants. In: Andersen, OM., Markham,KR, eds. Flavonoids:chemistry, biochemtry and applications, Boca Ration: CRC Press, 397-411
https://doi.org/10.1201/9781420039443.ch8
PMid:15786806

Hatier J.B., and Gould K.S., 2009, Anthocyanin function in vegetative organs. In: Gould KS, Davies K, Winefield C, eds. Anthocyanins: biosythesis, functions, and applications, New York:Spring, pp.1-19
https://doi.org/10.1007/978-0-387-77335-3_1

He Q., Zhang Z.F., and Zhang L.G., 2016, Anthocyanin accumulation, antioxidant ability and stability, and a transcriptional analysis of anthocyanin biosynthesis in purple heading chinese cabbage (Brassica rapa L. ssp.pekinensis), J Agr Food Chem, 64(1): 132-145
https://doi.org/10.1021/acs.jafc.5b04674
PMid:26709726

Hichri I.N., Barrieu F., Bogs J., Kappel C., Delrot S., and Lauvergeat V., 2011, Recent advances in the transcriptional regulation of the flavonoid biosynthetic pathway, J Exp Bot, 62(8): 2465-2483
https://doi.org/10.1093/jxb/erq442
PMid:21278228

Hloton T.A., and Cornish E.C., 1995, Genetics and biochemistry of ant hocyanin biosynthesis, Plant Cell, 7: 1071-1083
https://doi.org/10.1105/tpc.7.7.1071
PMid:12242398 PMCid:PMC160913

Jia X.L., Wang G.L., Xiong F., Yu X.R., Xu Z.S., Wang F., and Xiong A.S., 2015, De novo assembly, transcriptome characterization, lignin accumulation and anatomic characteristics: novel insights into lignin biosynthesis during celery leaf development, SCI REP-UK, 5(5): 8259
https://doi.org/10.1038/srep08259
PMid:25651889 PMCid:PMC4317703

Jing P., Bomser J.A., Schwartz S.J., He J., Magnuson B.A., and Giusti M.M., 2008, Structure-function relationships of anthocyanins from various anthocyanin-rich extracts on the inhibition of Colon cancer cell growth, J Agr Food Chem, 56(20): 9391-9398
https://doi.org/10.1021/jf8005917
PMid:18800807

Jungmin L., Christopher R., and Ronalde W.E., 2008, Correlation of two anthocyanin quantification methods: HPLC and spectrophotometric methods, Food Chem, 110(3): 782-786
https://doi.org/10.1016/j.foodchem.2008.03.010

Kanemaki A., Otani M., Takano M., Fujimoto T., Okuhara H., Nomizu T., Kondo M., Kobayashi H., Tatsuzawa F., and Nakano M., 2018, Ectopic expression of the R2R3-MYB gene from Tricyrtis sp. results in leaf color alteration in transgenic Pelargonium crispum, Sci Hortic-Amsterdam, 240: 411-416
https://doi.org/10.1016/j.scienta.2018.06.029

Kang C., Darwish O., Geretz A., Shahan R., Alkharouf N., and Liu Z., 2013, Genome-scale transcriptomic insights into early-stage fruit development in woodland strawberry fragaria vesca, Plant Cell, 25(1): 1960-1978
https://doi.org/10.1105/tpc.113.111732
PMid:23898027 PMCid:PMC3723606

Leyva A., Jarillo J.A., Salinas J., and Martinez-Zapater J.M., 1995, Low temperature lnduces the accumulation of phenylalanine ammonia-lyase and chalcone synthase mrnas of, Plant Physiol, 108(1): 39-46
https://doi.org/10.1104/pp.108.1.39
PMid:12228452 PMCid:PMC157303

Li P., Li Y.J., Zhang F.J., Zhang G.Z., Jiang X.Y., Yu H.M., and Hou B.K., 2017, The Arabidopsis UDP-glycosyltransferases UGT79B2 and UGT79B3, contribute to cold, salt and drought stress tolerance via modulating anthocyanin accumulation, Plant J, 89: 85-103
https://doi.org/10.1111/tpj.13324
PMid:27599367

Liu Y., Lin-Wang K., Espley R.V., Wang L., Li Y., Liu Z., Zhou P., Zeng L., Zhang X., Zhang J., and Allan A.C., 2019, StMYB44 negatively regulates anthocyanin biosynthesis at high temperatures in tuber flesh of potato, J Exp Bot, 70(15): 3809-3824
https://doi.org/10.1093/jxb/erz194
PMid:31020330 PMCid:PMC6685667

Lloyd A., Brockman A., Aguirre L., Campbell A., Bean A., Cantero A., and Gonzalez A., 2017, Advances in the MYB-bHLH-WD Repeat (MBW) pigment regulatory model: addition of a WRKY factor and co-option of an anthocyanin MYB for betalain regulation, Plant Cell Physiol, 58(9): 1431-1441
https://doi.org/10.1093/pcp/pcx075
PMid:28575507 PMCid:PMC5914458

Lo Piero A.R., Puglisi I., Rapisarda P., and Petrone G., 2005, Anthocyanins accumulation and related gene expression in red orange fruit induced by low temperature storage, J Agr Food Chem, 53(23): 9083-9088
https://doi.org/10.1021/jf051609s
PMid:16277406

Ma Z.H., Li W.F., Mao J., Li W., Zuo C.W., Zhao X., Dawuda M.M., Shi X.Y., and Chen B.H., 2019, Synthesis of light-inducible and light-independent anthocyanins regulated by specific genes in grape 'Marselan' (V. vinifera L.), PEERJ, 7: e6521
https://doi.org/10.7717/peerj.6521
PMid:30842905 PMCid:PMC6398381

Mori K., Goto-Yamamoto N., Kitayama M., and Hashizume K., 2007, Effect of high temperature on anthocyanin composition and transcription of flavonoid hydroxylase genes in 'Pinot noir' grapes (Vitis vinifera), J Hortic Sci Biotech, 82(2): 199-206
https://doi.org/10.1080/14620316.2007.11512220

Qu Y.T., Zhou A.M., Zhang X., Tang H.W., Liang M., Han H., and Zuo Y.H., 2015, De novo transcriptome sequencing of low temperature-treated phlox subulata and analysis of the genes involved in cold stress, Int J Mol Sci, 16: 9732-9748
https://doi.org/10.3390/ijms16059732

Simone J.R., Michael I., Rod J., And V.C.T., and Tomkins B., 2006, Characterization of flavonol conjugates in immature leaves of Pak Choi [Brassica rapa L. Ssp. chinensis L. (Hanelt.)] by HPLC-DAD and LC-MS/MS, J Agr Food Chem, 54(13): 4855-4860
https://doi.org/10.1021/jf060154j

Speciale A., Canali R., Chirafisi J., Saija A., Virgili F., and Cimino F., 2010, Cyanidin-3-O-glucoside protection against TNF-α-induced endothelial dysfunction: involvement of nuclear Factor-κB signaling, J Agr Food Chem, 58(22): 12048-12054
https://doi.org/10.1021/jf1029515
PMid:20958056

Steyn W.J., Wand S.J.E., Jacobs G., Rosecrance R.C., and Roberts S.C., 2009, Evidence for a photoprotective function of low-temperature-induced anthocyanin accumulation in apple and pear peel, Physiol Plantarum, 136: 461-472
https://doi.org/10.1111/j.1399-3054.2009.01246.x
PMid:19493306

Stracke R., Ishihara H., Huep G., Barsch A., Mehrtens F., Niehaus K., and Weisshaar B., 2007, Differential regulation of closely related R2R3-MYB transcription factors controls flavonol accumulation in different parts of the Arabidopsis thaliana seedling, Plant J, 50(4): 660-677
https://doi.org/10.1111/j.1365-313X.2007.03078.x
PMid:17419845 PMCid:PMC1976380

Sukwattanasinit T., Burana-Osot J., and Sotanaphun U., 2007, Spectrophotometric method for quantitative determination of total anthocyanins and quality characteristics of roselle (Hibiscus sabdariffa), Planta Med, 73(14): 1517-1522
https://doi.org/10.1055/s-2007-990262
PMid:17992627

Takos A.M., Jaffe F.W., Jacob S.R., Bogs J., Robinson S.P., and Walker A.R., 2006, Light-Induced expression of a MYB gene regulates anthocyanin biosynthesis in red apples, Plant Physiol, 142: 1216-1232
https://doi.org/10.1104/pp.106.088104
PMid:17012405 PMCid:PMC1630764

Takos A.M., and Walker A.R., 2006, Light-induced expression of a MYB gene regulates anthocyanin biosynthesis in red apples, Plant Physiol, 142: 1216-1232
https://doi.org/10.1104/pp.106.088104
PMid:17012405 PMCid:PMC1630764

Trapnell C., Williams B.A., Pertea G., Mortazavi A., Kwan G., van Baren M.J., Salzberg S.L., Wold B.J., and Pachter L., 2010, Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation, Nat Biotechnol, 28: 511-515
https://doi.org/10.1038/nbt.1621
PMid:20436464 PMCid:PMC3146043

Ubi B.E., Honda C., Bessho H., Kondo S., Wada M., Kobayashi S., and Moriguchi T., 2006, Expression analysis of anthocyanin biosynthetic genes in apple skin: effect of UV-B and temperature, Plant Sci, 170(3): 571-578
https://doi.org/10.1016/j.plantsci.2005.10.009

Wagner G.P., Kin K., and Lynch V.J., 2012, Measurement of mRNA abundance using RNA-seq data: RPKM measure is inconsistent among samples, THeor Biosci, 131(4): 281-285
https://doi.org/10.1007/s12064-012-0162-3
PMid:22872506

Wang N., Qu C.Z., Jiang S.H., Chen Z.J., Xu H., Fang H.F., Fang H.C., Su M.Y., Zhang J., Wang Y.C., and Liu W.J., and Zhang Z.Y., 2018, The proanthocyanidin-specific transcription factor MdMYBPA1 initiates anthocyanin synthesis under low-temperature conditions in red-fleshed apples, Plant J, 96(1): 39-55
https://doi.org/10.1111/tpj.14013
PMid:29978604

Wei H.R., Chen X., Zong X.J., Shu H.R., Gao D.S., and Liu Q.Z., 2015, Comparative transcriptome analysis of genes involved in anthocyanin biosynthesis in the red and yellow fruits of sweet cherry (Prunus avium L.), Plos One, 10(3): e121164
https://doi.org/10.1371/journal.pone.0121164
PMid:25799516 PMCid:PMC4370391

Winkel-Shirley B., 2001, Flavonoid Biosynthesis: A colorful model for genetics, biochemistry, Cell biology, and biotechnology, Plant Physiol, 126(2): 485-493
https://doi.org/10.1104/pp.126.2.485
PMid:11402179 PMCid:PMC1540115

Zhang Y.J., Hu Z.L., Chu G.H., Huang C., Tian S.B., Zhao Z.P., and Chen G.P., 2014, Anthocyanin accumulation and molecular analysis of anthocyanin biosynthesis-associated genes in eggplant (Solanum melongena L.), J Agr Food Chem, 62(13): 2906-2912
https://doi.org/10.1021/jf404574c

Zhang Y.Z., Xu S.Z., Cheng Y.W., Ya H.Y., and Han J.M., 2016, Transcriptome analysis and anthocyanin-related genes in red leaf lettuce. Genet Mol Res, 15(1)
https://doi.org/10.4238/gmr.15017023