Expression Analysis of Anthocyanin Biosynthesis Pathway Genes in *Indosasa hispida* MeClure cv. 'Rainbow'

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Keywords: Indosasa Hispida MeClure cv. 'Rainbow', Anthocyanin Biosynthesis, Gene Expression.

Abstract: Anthocyanins existed in diverse plants have many health beneficial functions such as anti-inflammatory, anti-oxidation and antihypertensive. Studies have demonstrated that anthocyanin accumulation in plant organs is related to gene expression in anthocyanin biosynthesis pathway. In order to probe which gene or genes have correlation with the anthocyanin biosynthesis in bamboo species, the expression level of some genes involved in anthocyanin biosynthesis pathway was determined by real time PCR semi-quantitatively at seven different growth stages of *Indosasa hispida* cv. 'Rainbow'. Results showed that all of the genes studied were expressed with similar patterns in most tissues, and which also showed a positive correlation between the level of CHS, F3H, DFR, UFGT gene expression and anthocyanin accumulation.

1 INTRODUCTION

Anthocyanins are accumulated in cell vacuoles and produce a diverse pigmentation from orange to red, purple and blue in flowers, fruits and vegetables (Oancea, Oprean, 2011, Horbowicz et al, 2008). Anti-oxidant effects, protecting DNA and the photosynthetic machinery from high radiation fluxes, resistance to cold and drought stress, anti-aging and anti-cancer properties and recruitment of pollinators are some well-known roles of anthocyanins (Oancea, Oprean, 2011, Horbowicz et al, 2008). Studies have demonstrated that anthocyanin biosynthesis is mediated by the anthocyanin pathway some genes/transcription factors (Li et al, 2021).

Indosasa hispida MeClure cv. 'Rainbow' (Y. M. Yang et J. Wang) with high ornamental value, a new variety of *Indosasa hispida*, exhibits different degree of purplish red at different stages of its growth, and the purplish red material was ascertained as anthocyanin after isolation and analysis (Wang et al, 2012, Miao et al, 2014, Wang et al, 2014). Although the anthocyanin pathway has been thoroughly characterized and the correlation between gene expression level and anthocyanin accumulation in many plants having been identified (Yonekura-Sakakibara et al, 2019, Boss et al, 1996, Jenog et al, 2004), the question about how their biosynthesis is regulated in bamboo species is rarely known at present. In this study, ten structural genes related to anthocyanin biosynthesis of *I. hispida* MeClure cv. 'Rainbow' were determined by real time PCR semi-quantitatively at seven different growth stages to interpret a correlation between anthocyanin biosynthesis and gene expression level.

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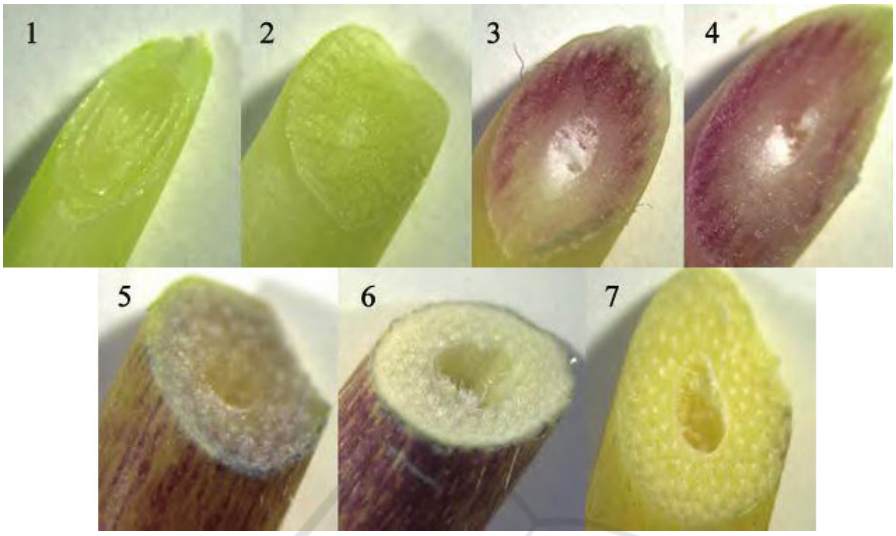
2 MATERIALS AND METHODS

2.1 Plant Materials

I. hispida MeClure cv. 'Rainbow' was obtained from greenhouse of Yunnan Academy of Forestry, and which was divided into seven growth stages according to colour differences (Figure 1). The culm tissues for RNA isolation were collected directly into liquid nitrogen and were stored at -80℃ until used.



Description of the growth stages: 1, 2: Tender apex without visible anthocyanin pigments; 3: Intermediate stem with purplish red tissues and colourless skin; 4: Intermediate stem with purplish red tissues and skin; 5: Intermediate stem with little purplish red tissues and purplish red skin; 6: Mature stem with colourless tissues and purplish red skin; 7: Mature stem without visible anthocyanin pigments.

Figure 1: The cross profile of *I. hispida* MeClure cv. 'Rainbow' at different growth stages.

2.2 Main Reagents

DEPC-treated Water; UltraPower Nucleic Acid Stain; 75% ethanol; isopropyl alcohol; chloroform; Takara RT-PCR Kit; PCR mix Kit; TRNzol® Reagent.

2.3 RNA Extraction and cDNA Synthesis

Total RNA was isolated from culm tissues using TRNzol® Reagent (TIANGEN Biotech (Beijing) Co., Ltd.). Purity and concentration of isolated RNA was determined by protein nucleic acid analyzer, and integrity of which was verified by electrophoresis on 1.2% agarose gel, respectively. RNA was reverse transcribed to cDNA with the first strand cDNA synthesis kit (TaKaRa Biotechnology (Dalian) Co., Ltd.) according to the manufacture's protocols. The reverse transcription system and conditions are as follows: Oligo (dT) Primer 1 Ml, dNTP Mixture 1 μL, Total RNA 3 μL, RNase-free dH2O up to 10 μL; 65℃, 5 min annealing response, ice chill; 5×PrimeScript® Buffer 4 μL, RNase Inhibitor 0.5 μL, PrimeScript® RTase 1μL, RNase Free dH2O 4.5 μL; 30℃ 10 min, 42℃ 60 min, 95℃ 5min.

2.4 PCR Cloning and Analysis of the Products

Primers were designed on the basis of transcriptome data acquired by our group earlier, and the designed primers for amplifying gene are as follows (Tab. 1). Conditions for PCR of genes involved are as follows: 95℃ for 5 min followed by 35 cycles at 95℃ for 45s, at annealing temperature listed below for 30 s, and at 72℃ for 5 min, with a final extension at 72℃ for 8 min. The PCR products were assessed by agarose gel electrophoresis, taking actin primer as a reference gene.

Table 1: PCR primer and reaction condition for PCR.

Gene name	Primer sequences (5'-3')	Size (bp)	Temp (°C)
actin	F:ATGGCTGAAGAGGATATCCAGC (22) R:TYCCATGCCAATAAAAGATGGCTG (24)		51
PAL	F:CTCTCGTTTTCTTCTCCC (18) R:CCGTTCATCATGCTGTTC (18)	339	52
4CL	F:GACCACCTCCCGCTCCACGACT (22) R:TCGACGGAGTTAGGTAAAAGCA (22)	200	57
CHS	F:GAAGGCGATCAAGGAGTGGG (20) R:TTGAGGAGGTGGAAGGTGAGAC (22)	474	57
CHI	F:GCCCTTGTTTCTGCTCCT (18) R:CTGCCACGTTCGTGTTCT (18)	304	60
F3H	F:GCTCTCGGAGGCAATG(16) R:TGGGCTCGTCCAGTAT (16)	427	53
F3'5'H	F:CCAGACCATCACCGA (15) R:ACTGCACGAACACCA (15)	514	58
DFR	F:GGGAGAGGAGGTGAA (15) R:CGGGGTCTTTGGATT (15)	304	57
ANS	F:AAGCTGCTCGCCATCCTG (18) R:TTCGTCCGTGACCAACTCC (19)	447	58
LDOX	F:CCTGAGCCTGAGCGGTCTGT (20) R:GGCACCTGGGGTAGTAGTTG (20)	649	57
UFGT	F:TCAGATGGGTGTCAAAT (17) R:GGAGTACCACAGCAAAG (17)	236	60

Abbreviations: PAL, phenylalanine ammonia lyase; 4CL, coenzyme A ligase; CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone-3-hydroxylase; F3'5'H, flavonoid-3',5'-hydroxylase; LDOX, leucoanthocyan-idin dioxygenase; DFR, dihydroflavonol 4-reductase; ANS, Anthocyanidin Synthase; UFGT, UDP glucose-flavonoid 3-*O*-glucosyl transferase.

3 RESULTS

3.1 Analytical Results of Isolated Total RNA

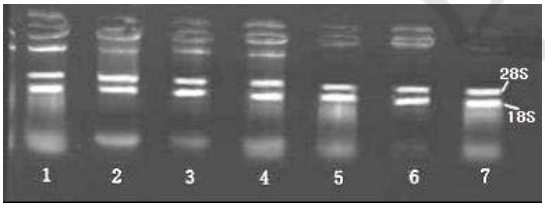


Figure 2: Electrophoresis Image of total RNA at different stages.

The agarose gel electrophoresis of total RNA at different stages was shown in figure 2. The data of A260/A280 ratio and concentration of total RNA listed Tab. 2 shows that total RNA isolated for study can be used for cDNA synthesis.

Table 2: Purity and concentration of total RNA from culm tissues.

Sample	1	2	3	4
A260	10.98	27.06	29.56	30.41
A280	11.05	22.40	21.25	22.25
A260/A280	0.991	1.286	1.459	1.484
Concentration (ng/μL)	295.6	839	1057	1000
Sample	5	6	7	
A260	12.98	13.90	19.04	
A280	8.67	10.28	14.38	
A260/A280	1.531	1.342	1.376	
Concentration (ng/μL)	496.8	567.6	682.4	

3.2 Expression of the Anthocyanin Biosynthesis Pathway Genes

As depicted in figure 3, the target genes with the exception of F3H, DFR and UFGT were all expressed with different levels at the first two stages. No anthocyanin pigments were seen in young tissues, presumably because UFGT is missing. In the pigmented tissues, the expression levels of several genes increased dramatically, but the expression levels were gradually decreased toward the end of ripening. Half of genes were not expressed in the

ripening tissues without visible anthocyanin pigments compared with the first two stages, in which 4CL and CHI still showed higher levels.

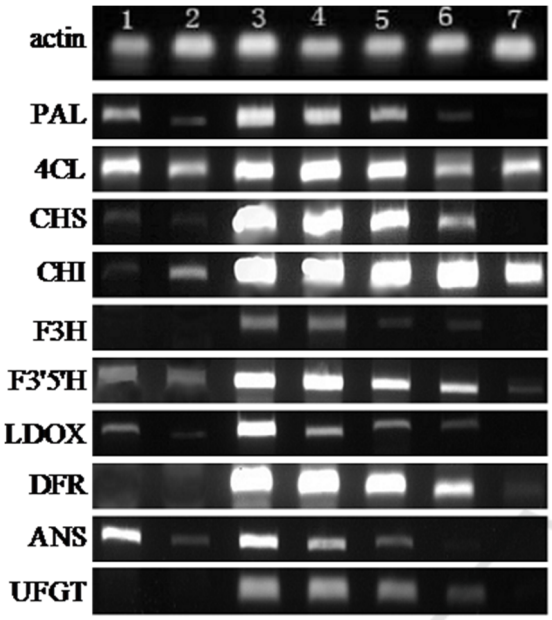


Figure 3: Electrophoresis Image of PCR products at different growth stages.

4 DISCUSSION

It was evident that anthocyanin accumulation in plant is associated with gene expression in anthocyanin biosynthesis pathway (Rouholamin et al, 2015). Ten structure genes related to anthocyanin biosynthesis pathway were investigated at seven different stages of a new variety of *Indosasa hispida*. Similar expression patterns of PAL and 4CL were observed at the whole growth stages, and the difference between them is that there was no expression of PAL in the unpigmented ripening tissue. The results indicated that they are not only the precursor genes of anthocyanin biosynthesis pathway, but also related to the accumulation of anthocyanin.

Only weak expression of CHS and CHI was detected at the first two stages while the expression of the two genes increased dramatically to a very high level subsequently. Notably, the high levels of expression of CHI lasted to the unpigmented ripening stage. Therefore, there is a significant positive correlation between the accumulation of anthocyanin and the expression of CHS and CHI, especially CHS gene.

Previous study showed that the colour of grape skins is blue or red was determined by the ratio of F3'5'H/F3H (Simone, Gabriele, 2007). In the study, both F3'5'H and F3H were expressed in culm tissues contained visible anthocyanin pigments, where levels of F3'5'H was higher. It can be deduced that F3'5H and F3H might commonly regulate anthocyanin biosynthesis of *I. hispida* MeClure cv. 'Rainbow' to account for the fact of purplish red.

The expression of LDOX and ANS showed same trends, both of them had no expression in the unpigmented ripening tissue. DFR and UFGT were only expressed obviously in the pigmented tissues, which demonstrate that DFR and UFGT are two key encoding enzyme genes to regulate anthocyanin biosynthesis of *I. hispida* MeClure cv. 'Rainbow', and DFR expression was consistent to the study on other species (Hasegawa, 2001).

5 CONCLUSIONS

For preliminary study the expression of genes involved in anthocyanin biosynthesis pathway of *I. hispida* MeClure cv. 'Rainbow', ten genes was determined at seven different growth stages. It can be seen from the results that the appearance of anthocyanin at the onset of ripening coincides with increased expression of each of the genes encoding biosynthetic enzymes in this pathway, and which suggested that the induction of anthocyanin synthesis is triggered by regulatory genes in *I. hispida* MeClure cv. 'Rainbow'. Further, the content analysis of anthocyanin in various tissues is currently being undertaken by our group to understand the deeper correlation between anthocyanin biosynthesis and gene expression level.

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