Expression analysis of correlative regulatory factors of anthocyanin in 'Cuihongli' and 'Qiangcuili'

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Abstract. MYB10, bHLH and WD40 are correlative regulatory factors of anthocyanin. In this paper, the expression of MYB10, bHLH and WD40 in the peel and the pulp of two plum cultivars were analyzed with the materials of 'Cuihongli' and 'Qiangcuili'. The results showed that, with the development of the fruit of 'Cuihongli', the expression of MYB10 in the peel was downregulated firstly then upregulated, and the expression of MYB10 was not detected in the flesh of 'Cuihongli's pulp and the peel and the pulp of the 'Qiangcuili'. The expression of bHLH and WD40 showed that they had no significant correlation with the accumulation of anthocyanin in the two cultivars. It is suggested that the deletion of MYB10 gene expression result in the lack of anthocyanin in the flesh of 'Qiangcuili'.

1. Introduction

The color is not only the core index in the quality of the fruit's appearance, but also closely related to the quality of the fruit, and it has an important influence on the commodity value of the fruit and its processed products. Anthocyanin is a kind of the flavonoid produced in the secondary metabolism of plants[1]. As a kind of water-soluble pigment, it is widely present in the vacuole of the epidermal cells of the angiosperm, and show red, purple, blue and other colors in plants. [2]. Genes controlling the synthesis of anthocyanin are divided into structural genes and regulatory genes, the structural genes directly encode the enzyme which synthesize and metabolism of anthocyanin, and the expression of these genes are regulated by the transcription factors[3]. In the metabolic pathway, at least three transcription factors regulate the synthesis of anthocyanin, including the R2R3-MYB transcription factor, the bHLH (helix-ring-helix) transcription factor, and the WD40 repeat protein. Richard et al. found that the MdMYB10 isolated from apple and positively influence the formation of the color of the fruit[4]. It is shown that the MYB transcription factor form MBW (MYB-bHLH-WD40) protein complex with bHLH and WD40 to regulate the expression of structural genes in the metabolism pathway of flavonoids[5]; PpMYB10 and PpbHLH3 effect the expression of DFR and UFGT genes in the peel of peach, then the anthocyanin in the peel is gradually accumulated[6]. 'Cuihongli' and 'Qiangcuili' are two plum cultivars extensively planted in Sichuan. 'Cuihongli' is selected from the Chinese plum (*Prunus*

salicina Lindl). It is named because the purple peel when the fruit is ripe[7]. 'Qiangcuili' is one of the best plum varieties in Maoxian, Sichuan Province. From mid-July to late August, the fruit is ripe with green peel and yellow pulp. It is shown that the red and purple-black color of the varieties is mainly determined by the content of anthocyanin[8,9]. At present, the research on the 'Cuihongli' and 'Qiangcuili' in China has concentrated on its high-quality and high-yield cultivation technology[10], but little research on the molecular mechanism of fruit coloring. This research focus on the expression analysis of MYB10, bHLH and WD40 of the fruit in 'Cuihongli' and 'Qiangcuili'. To explore the molecular mechanism of the difference color of the two cultivars.

2. Materials and Methods

2.1. Materials

5-year-old 'Cuihongli' (purple-red peel and yellow pulp) and 'Qiangcuili' (yellow-green peel and pulp) were planted in Yanmen Township, Wenchuan County, Aba Tibetan Qiang Autonomous Prefecture, Sichuan Province (latitude 31°28'34"N, longitude 103°37'18"E, altitude 1460 m). The row spacing of the plant is 3 m × 3 m, the conventional cultivation and management are basically the same. 'Cuihongli' was sampled on June 2nd (Young fruit period), June 22nd (the initial stage of expansion), July 12nd (the rapid expansion period), August 1st (the initial stage of coloring), August 21st (the half-red stage), August 31st (the full-red stage) and September 10th (the maturation period), respectively.

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'Qiangcuili' was sampled on June 2st(Young fruit period), June 22nd(the initial stage of expansion), July 12nd(the rapid expansion period), August 1st(initial stage of maturation) and August 21st(the maturation period), respectively. The experiment was carried out by randomized blocks design, fruits at the same height in the east, south, west and north of the tree was selected and brought back to the laboratory in the ice box. The peel was separated from the pulp. After the liquid nitrogen treatment, fruits were stored in the cryogenic refrigerator at -80 °C for total RNA extraction and expression analysis.

2.2. Methods

Real-time Quantitative PCR used PsActin as reference and design primers according to *Prunus avium* and other related species gene sequence. Primers were synthesised by Bioengineering (Shanghai) Co., Ltd., as shown in Table 1.

Table 1. Primer sequences for Real-time Quantitative PCR

Primer	Nucleotide of sequences (5'-3')
PsActinF	GCAGACAGGATGAGCAAGGAGATTAC
PsActinR	TCTGTTGGAAGTACTGAGGGATG
MYB10F	AGGTGGTCATTGATTGCTC

MYB10R	GGTCTTTATTGTTTCTTGGG
bHLHF	CGCCGAGGATAACAACAG
bHLHR	CCCGATTTACGACACC
WD40F	ATTGGGACTTCAGCATC
WD40R	CAGGCAATGTCGTAAACC

3. Results and Discussion

3.1. Expression of MYB10 during fruit development of 'Cuihongli'

The expression of MYB10 gene during the fruit development of 'Cuihongli' was analyzed. It was found that the expression of MYB10 was only detected in the peel of 'Cuihongli'. The expression of MYB10 was higher at June 2nd, decreased at the early stage of fruit expansion, upregulated at the rapid expansion stage(July 12nd), then decreased gradually, and suddenly increased at September 10th. On the whole, the relative expression of MYB10 decreased at first and then increased, and reached the lowest in August 31st, the highest in stages of fruit ripening, which was 9.54 times as high as that in the whole red stage of fruit (Fig. 1).

Fig. 1 Expression of the MYB10 gene in the peel of 'Cuihongli'

3.2. Expression of bHLH during fruit development of 'Cuihongli' and 'Qiangcuili'

bHLH has different expression patterns of 'Cuihongli' and 'Qiangcuili'. On the whole, the expression of bHLH was decreased in the peel, but the expression of bHLH increased significantly and reached the highest during the rapid expansion period of 'Cuihongli', while the expression of bHLH was the lowest in the whole red period. It is worth noting that the expression of bHLH gene in peel is always higher than that of pulp during the

development of 'Cuihongli'. In the peel of 'Qiangcuili', the expression of bHLH was the highest in the young fruit stage, decreased significantly in the early stage of fruit expansion, reached the lowest in the rapid expansion period, and then gradually increased. In pulp, the expression of bHLH showed the tendency of upregulation firstly and then downregulation, and peaked in the rapid expansion period. The expression of pulp were less than that in peel almost during all the stages(Fig. 2).

Fig. 2 Expression of the bHLH gene in the peel and pulp of 'Cuihongli' and 'Qiangcuili'
Note: CHP, QCP, CHR and QCR in the figure represent the peel of 'Cuihongli', 'Qiangcuili' and the pulp of them respectively. Similarly hereinafter

3.3. Expression of WD40 during fruit development of 'Cuihongli' and 'Qiangcuili'

In the peel of 'Cuihongli', the expression of WD40 reached the highest level at the early stage of fruit expansion, and then decreased gradually; the expression of WD40 decreased gradually in pulp and slightly

increased in semi-red stage. In the peel of 'Qiangcuili', the expression pattern of WD40 was similar to that in 'Cuihongli' peel, which was up-regulated and then down-regulated, but the expression of WD40 was the highest at the initial ripening stage, while in pulp, the expression pattern increased gradually and peaked at fruit ripening stage(Fig. 3).

Fig. 3 Expression of the WD40 gene in the peel and pulp of 'Cuihongli' and 'Qiangcuili'

4. Conclusions

Through Real-time Quantitative PCR analysis, the relative expression of MYB10 showing a pattern of first decreasing and then rising, which was consistent with that of Pham Anh Tuan[11] on the MYB10 of peach. The expression of bHLH and WD40 was detected in both the peel and the pulp of the whole fruit development period of the two plum varieties. The WD40 showed a similar expression pattern in the peel of two varieties, and the

expression of WD40 decreased after up-regulation, but in the two cultivars, the expression of WD40 showed a completely opposite tendency. According to the content of anthocyanin in previous study[12], the results showed that the expression of bHLH and WD40 had no significant correlation with the accumulation of anthocyanin, but the expression of MYB10 was not detected in the 'Qiangcuili' and the pulp of 'Cuihongli'. The accumulation of anthocyanin was affected by the expression of MYB10, which was consistent with the results of the study on the peel of the apple. The

expression of MYB10 was extremely low, resulting in no accumulation of anthocyanin in the peel and pulp of ‘Qiangcuili’ and the pulp of ‘Cuihongli’. The results provide a theoretical basis for the future study on the color difference between ‘Cuihongli’ and ‘Qiangcuili’.

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