Scientia Horticulturae 235 (2018) 9–20

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Comparative transcriptomic analysis of white and red Chinese bayberry (*Myrica rubra*) fruits reveals flavonoid biosynthesis regulation

Liyu Shi[a], Xin Chen[b], Wei Chen[b], Yonghua Zheng[a,*], Zhenfeng Yang[b,*]

[a] College of Food Science and Technology, Nanjing Agricultural University, Nanjing, 210095, People's Republic of China
[b] College of Biological and Environmental Sciences, Zhejiang Wanli University, Ningbo, 315100, People's Republic of China

ARTICLE INFO

Keywords:
Chinese bayberry
Transcriptome
RNA-seq
Flavonoid
Proanthocyanidin
Transcription factors

ABSTRACT

To fully elucidate molecular mechanisms of flavonoid biosynthesis in Chinese bayberry, transcriptomes of two genotypes with different colours, white cultivar (Shuijing, SJ) and red cultivar (Biqi, BQ), were compared. During fruit development, 'BQ' exhibited substantial increase of total anthocyanins content together with dramatic decrease of total soluble proanthocyanidins (PAs) content, while 'SJ' displayed decrease of total soluble PAs levels but failed to accumulate anthocyanin. Based on the sequencing results, 124,265 unigenes were generated with an average length of 708 bp. All genes involved in anthocyanin biosynthesis and glycosylation were identified and their expression patterns were in accordance with total anthocyanin accumulation in developing fruits of 'BQ' and 'SJ'. Expression levels of leucoanthocyanidin reductase (LAR) and anthocyanidin reductase (ANR) were down-regulated in 'SJ' in agreement with the decrease in PAs, while an opposite trend was observed in 'BQ'. The higher expression levels of LAR and ANR in 'BQ' may be an important reason for the higher levels of total soluble PAs as compared to 'SJ'. Phylogenetic analysis showed that seven MYBs could be identified as putative homologues of PA-specific regulator and exhibited considerable genotypic and temporal specificity of expression. In addition, three WD40 genes in Chinese bayberry clustered with the WD40s related to flavonoid biosynthesis in other species. Finally, RNA-Seq data was validated by using qRT-PCR analysis with a high correlation, suggesting that the RNA-Seq data here are credible. These results provide new insights into the regulation of the complex branching pathway leading to various flavonoid compounds biosynthesis in bayberries.

1. Introduction

Chinese bayberry (*Myrica rubra* Sieb. & Zucc.) is a subtropical fruit native to southern China and other Asian countries with high commercial value for its flavor and appealing color. The fruit is rich in anthocyanins as well as other flavonoids compounds and recognized as a good source of natural antioxidants (Bao et al., 2005). Flavonoids are major plant secondary metabolites that contribute in many ways to the growth and survival of plants and also serve as antioxidant and anticancer agents in the human diet (Winkel-Shirley, 2001; Tohge et al., 2005). Anthocyanins, proanthocyanidins (PAs) and flavonols are the predominant flavonoids in fruit (Saito et al., 2013). Since anthocyanin accumulation is responsible for fruit coloration, the genetics and biochemistry of the anthocyanin biosynthetic pathway has been well studied. Recently, increasing interest has been attracted to PAs because of their bioactive functions. Indeed, PAs contribute to the quality of many important plant products, such as some berries, tea, and wine and also benefit human health by protecting against free radical injury and cardiovascular disease (Bagchi et al., 2000; Cos et al., 2004; Aron and Kennedy, 2008). However, to our knowledge, there are no published studies on PA biosynthesis in Chinese bayberry fruit.

The flavonoids metabolism pathway has been well characterized over the past several decades (Supplementary Fig. S1). The biosynthesis of anthocyanins, PAs and flavonols shares a series of common enzymatic steps, which are catalyzed by phenylalanine ammonia lyase (PAL), cinnamate 4-hydroxylase (C4H), 4-coumarate CoA ligase (4CL), chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H) and flavanone 3′-hydroxylase (F3′H). Activities of

Abbreviations: PAs, proanthocyanidins; ANR, anthocyanidin reductase; MYB, Myeloblastosis proto-oncogene; bHLH, basic helix-loop-helix; PAL, phenylalanine ammonia lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate CoA ligase; CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavonoid 3-hydroxylase; F3′H, flavonoid 3′-hydroxylase; DFR, dihydroflavonol 4-reductase; ANS, anthocyanidin synthase; UFGT, UDP-glucose flavonoid 3-*O*-glucosyl transferase; OMT, *O*-methyltransferase; ACT, anthocyanin acyltransferase; FLS, flavonol synthase; LAR, leucoanthocyanidin reductase; UGT72L1, uridine diphosphate glycosyl transferase; FPKM, fragments per kilobase of exon per million fragments; RNA-seq, RNA sequencing; qRT-PCR, quantitative real-time PCR

* Corresponding authors.
E-mail addresses: zhengyh@njau.edu.cn (Y. Zheng), yangzf@zwu.edu.cn (Z. Yang).

https://doi.org/10.1016/j.scienta.2018.02.076
Received 29 October 2017; Received in revised form 22 February 2018; Accepted 26 February 2018

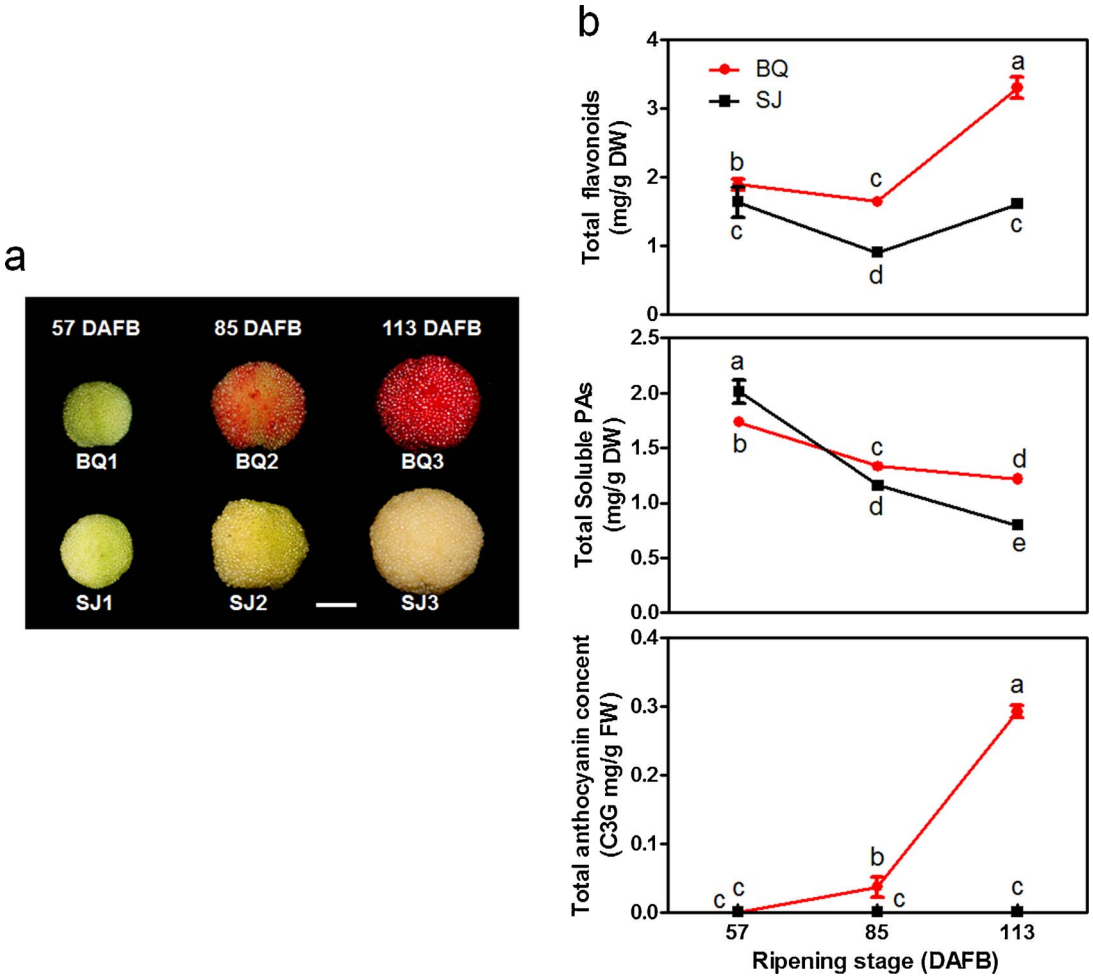

Fig. 1. Images and flavonoids contents of Chinese bayberry fruits. (a) Images of 57 DAFB 'Biqi' (BQ1), 85 DAFB 'Biqi' (BQ2), 113 DAFB 'Biqi' (BQ3), 57 DAFB 'Shuijin' (SJ1), 85 DAFB 'Shuijin' (SJ2) and 113 DAFB 'Shuijin' (SJ3). The white bar represents 1 cm. (b) Contents of total flavonoids, soluble PAs and anthocyanins in BQ and SJ cultivars of three stages. Bars indicate standard errors; different letters indicate statistically significant differences at $P < 0.05$ according to Duncan's multiple range test.

these enzymes lead to the synthesis of dihydroflavonols, which are subsequently either converted by flavonol synthase (FLS) to the flavonols or reduced by dihydroflavonol 4-reductase (DFR) to the leucocyanidins. Anthocyanin synthase (ANS) then catalyses the last common step shared by the anthocyanin and PA biosynthesis pathways. The final modification steps in the anthocyanin pathway include glycosylation by UDP-flavonoid glucosyltransferase (UFGT), methylation by *O*-methyltransferase (OMT) and acylation by anthocyanin acyltransferase (ACT). While, leucoanthocyanidin reductase (LAR) and anthocyanidin reductase (ANR) are specific to the PA branch pathway and convert leucocyanidin and cyanidin to catechin and epicatechin, respectively (Abrahams et al., 2003; Xie et al., 2003). Subsequently epicatechin can be converted to epicatechin 3′-*O*-glucoside by the action of UGT72L1, which is an epicatechin-specific glycosyltransferase identified in Medicago (Pang et al., 2013).

It has been reported that flavonoid biosynthesis can also be transcriptionally regulated by three types of transcription factors, including R2R3-MYB factors, basic helix–loop–helix (bHLH) proteins, and WD40 proteins (Ramsay and Glover, 2005). These regulators physically interact to form the MBW complex that binds to promoters and activates transcriptions of their target genes (Baudry et al., 2004). It is the MYB transcription factors that seem to impart specificity, and numerous MYB factors controlling different branches of the flavonoid pathway have been identified in many plant species. For instance, the biosynthesis of anthocyanins in grapevine is regulated by VvMYBA1 and VvMYBA2, while VvMYBPA1 is shown to control PAs accumulation (Bogs et al., 2007; Espley et al., 2007). Other MYB transcription factors, PtMYB134, AtTT2 and DkMYB2 specifically regulate PA biosynthesis in persimmon fruit, *Arabidopsis* seed and poplar leaves, respectively (Nesi et al., 2001; Akagi et al., 2010; Gesell et al., 2014). However, for Chinese bayberry, only two genes, *MrMYB1* and *MrMYB2*, have been isolated and reported to control fruit anthocyanin formation (Niu et al., 2010), while the specific MYB transcription factors involved in PA or flavonol regulation in Chinese bayberry remain unclear.

The interaction between bHLH and MYB proteins arises early during land plant evolution and the bHLH member is essential for the activity of the MYB partner (Feller et al., 2011). It has been showed that a given bHLH can interact with several different MYBs, providing multiple functional combinations. For example, the bHLH factor VvMYC1 from grapevine is shown to interact with different MYB proteins (VvMYB5a/5b, VvMYBA1/A2 and VvMYBPA1) for inducing promoters of flavonoid pathway genes involved in biosynthesis of anthocyanin and/or PA (Hichri et al., 2010). Likewise, the WD40 factor TTG1 can interact with TT8-TT2 and TT8-AtPAP1 complex to form different ternary transcription complexes that regulate PA and anthocyanin biosynthesis, respectively (Baudry et al., 2004; Shin et al., 2015). Thus, the WD40 cofactors are also adaptable and can control different branches of the flavonoid pathway.

The high-throughput RNA sequencing (RNA-Seq) based on next-generation sequencing (NGS) and de novo assembly have recently emerged as a powerful approach for gene discovery and expression profiling (Kalra et al., 2013; Chen et al., 2015). In this technique, there