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*Review*

# Anthocyanin biosynthesis in fruit tree crops: Genes and their regulation

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**The anthocyanin biosynthesis pathway is a little complex with branches responsible for the synthesis of a variety of metabolites. In fruit tree crops, during the past decade, many structural genes encoding enzymes in the anthocyanin biosynthetic pathway and various regulatory genes encoding transcription factors that regulate the expression of structural genes have been cloned and then functionally characterized in detail. In general, the structural genes involved in anthocyanin synthesis were coordinately expressed and their levels of expression were positively related to the degree of anthocyanin concentration; while, the coordinated expression pattern is striking a diverse among fruit crop species. Regulatory genes regulate spatiotemporally the structural genes and then form complicated metabolic network. Anthocyanin biosynthesis can be affected by external and internal factors, such as light, UV-B, low temperature and ABA through changes in expression of structural and regulatory genes.**

**Key words:** Anthocyanin, regulatory genes, structural genes, fruit tree crops, factors.

## INTRODUCTION

Fruits, such as apple, pear, peach, citrus, Chinese bayberry, play an important role in nutrient and health benefits for its rich sources of vitamin C, flavonoids, carotenoids (provitamin A), and other nutraceutical compounds. Of these healthful materials, anthocyanins belonging to the flavonoids compound family are water solvable pigments and have been suggested to reduce certain cancers, coronary heart diseases, oxidative stress and other age-related diseases (Ross and Kasum, 2002). Therefore, they are usually components of human diet and are not only considered exclusively as food products but also as therapeutic agents (Piero et al., 2005). A large number of fruits contain substantial anthocyanins, making their flesh and/or skin pink, red or purple as well as blue. Color is an important quality trait for fruit because consumers generally prefer fruits with red, blue or purple skin and flesh, and then obviously increase their marketability. For these reasons, anthocyanin biosynthesis in fruits becomes one aspect of currently active research area nowadays, which is helpful to understand the mechanism better and develop novel fruit cultivars with higher anthocyanin content.

In fruit tree crops, during the past decade, many structural genes encoding enzymes in the anthocyanin biosynthetic pathway and various regulatory genes encoding transcription factors that regulate the expression of structural genes have been cloned and then functionally characterized in detail (Boss et al., 1996; Honda et al., 2002; Cultrone et al., 2010; Borsani et al., 2010). In apple and grape, having a better knowledge of anthocyanin biosynthetic mechanisms compared to other fruit tree species, whereas many questions are yet to be determined (Matus et al., 2008), because some genes implicated in anthocyanin biosynthesis have pleiotropy and are affected by many internal and external factors. More recently, many new regulatory genes have been continually identified in grape, apple and other fruit trees (Hugueney et al., 2009; Cultrone et al., 2010; Feng et al., 2010). Some of these genes are sensitive to environmental stimuli, for example temperature, light, nutrition and hormones (Jeong et al., 2004; Piero et al., 2005; Ubi et al., 2006; Guo et al., 2008; Bureau et al., 2009; Moreno

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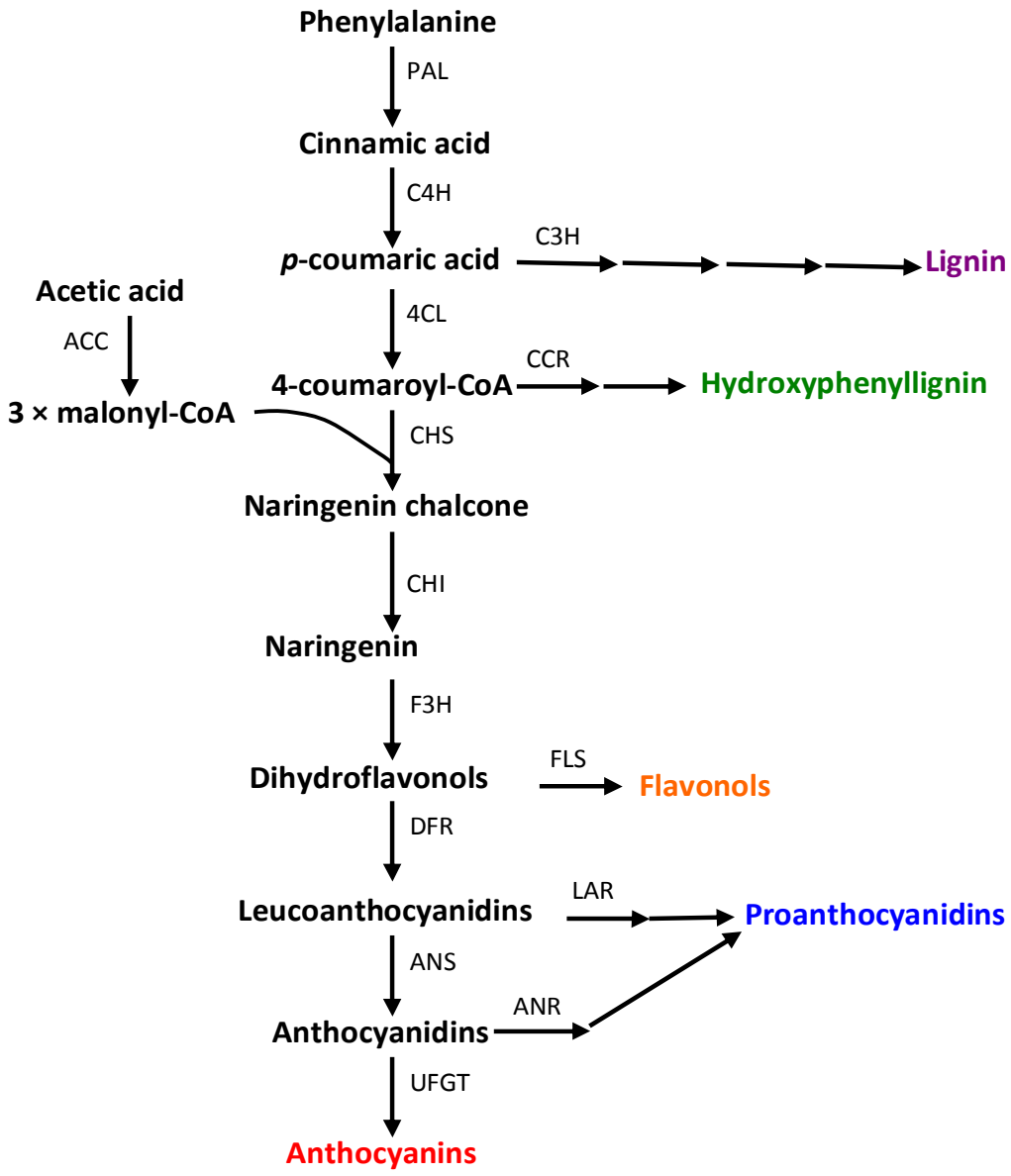


**Figure 1.** Simplified schematic of anthocyanin biosynthesis pathway and its branches. PAL, Phe ammonia-lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate-CoA ligase; CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; DFR, dihydroflavonol 4-reductase; ANS, anthocyanidin synthase; UFGT, UDPG-flavonoid-3-*O*-glucosyltransferase; ANR, anthocyanidin reductase; LAR, leucoanthocyanidin reductase; FLS, flavonol synthase; ACC, acetyl CoA carboxylase; CCR, cinnamyl-CoA reductase; C3H, 4-coumarate 3-hydroxylase.

et al., 2010; Crifó et al., 2011), which generally can lead to change in accumulation of anthocyanins in fruit. In practice, some cultural methods could be taken to increase anthocyanins content to satisfy the consumers.

Previous reviews in detail, described different aspects of anthocyanins biosynthesis, regulation, and their functions in plants and humans (Liu et al., 2006; Guo et al., 2008; Li et al., 2010). Herein, we will confer an overview of anthocyanins research, with a major focus on updating the recent progress on genes involved in anthocyanins biosynthetic pathway in fruit tree crops.

## ANTHOCYANINS BIOSYNTHESIS PATHWAY

The anthocyanin biosynthesis pathway (Figure 1) is a little complex with branches responsible for the synthesis of a variety of metabolites and has been, nowadays, almost completely elucidated (Boss et al., 1996). There are a wide range of constructive genes, such as phenylalanine ammonia lyase (PAL), chalcone synthase (CHS), anthocyanidin synthase (ANS) involved in anthocyanin biosynthesis. Phenylalanine is a direct precursor for the synthesis of anthocyanidins. The conversion from

phenylalanine to anthocyanines requires a series of enzyme-catalyzed reaction. First of all, phenylalanine transformed to trans-cinnamic acid through trans-elimination of ammonia catalyzed by PAL. After that trans-cinnamic acid mediated by C4H and 4CL changes into 4-coumaroyl-CoA. One molecular of 4-coumaroyl-CoA together with three molecules of malonyl CoA were catalyzed by CHS form naringenin chalcone which is rapidly and stereospecifically isomerized to Naringenin by CHI. After hydroxylated at the 3-position by F3H, naringenin converts to dihydroflavonols which, subsequently, is reduced to leucoanthocyanidin by DFR. ANS catalyzes the synthesis of corresponding colored anthocyanidins using leucoanthocyanidin as substrate. At last, the hydroxyl group at C3 of anthocyanidins is glycosylated by glycosyl moieties from UDP-activated sugar donor molecule by the action of UFGT to yielded corresponding anthocyanins stored finally in the vacuole, a subcellular compartment with an acidic environment. Anthocyanidins are modified by glycosyl moieties in various ways in a family- or species-specific manner, and their color greatly depends on the number of hydroxyl groups on the B-ring; the larger the number of groups, the darker the blue color (Tanaka et al., 2008).

## STRUCTURAL GENES

Structural genes encoding the enzymes of the anthocyanin biosynthetic pathway are conserved in plant kingdom (Holton and Cornish, 1995). In fruit crops, most of them have been isolated and well characterized (Boss et al.,1996; Piero et al., 2005; Ubi et al., 2006; Borsani et al., 2010).

A large number of studies pointed out that the structural genes involve in anthocyanin synthesis were coordinately expressed and their levels of expression were positively related to the degree of anthocyanin concentration (Honda et al., 2002; Piero et al., 2005; Borsani et al., 2010; Crifó et al., 2011), but there is some variability in the specific steps involved. In apple, as an example, coordinated expression of *MdCHS, MdF3H, MdANS, pDFR and pUFGluT* together was correlated well with anthocyanin synthesis (Honda et al., 2002). While, the transcript levels of *UFGT* were higher in red fruits compared to white in grape (Kobayashi et al., 2001) and *CHS, ANS* and *UFGT* in citrus (Cotroneo et al., 2006). From the aforementioned information, the coordinated expression pattern of structural genes related to improving anthocyanin synthesis is dependent on fruit crop species.

Among structural genes, PAL is the first gene involved in anthocyanin synthesis, while its role has been controversial. Some previous works (Zhao et al., 1994; Wang et al., 2000) supposed that PAL activity was closely related to anthocyanin synthesis; while others (Ju et al., 1995; Lo Piero et al., 2005; Feng et al., 2008) point to that PAL may not be a key enzyme for anthocyanin development.

These seemingly contradictory results can be elucidated by the hypothesis supposed by Ju et al. (1995) that only when anthocyanin was synthesized beginning with the deamination of Phenylalanine would PAL be critical for anthocyanin synthesis; on the other hand, when precursors were sufficient, anthocyanin synthesis would not depend on PAL activity. PAL, not a tissue-specific gene, could express in flower, leaf, roots and seeds (Boss et al., 1996; Salvatierra et al., 2010), and was enhanced by light (Feng et al., 2010; Niu et al., 2010), low temperature (Lo Piero et al., 2005; Crifó et al., 2011) and abscisic acid (ABA) (Jeong et al., 2004), while suppressed by 1-methylcyclopropene (Jiang et al., 2001), a-naphthaleneaceticacid (NAA) and shading (Jeong et al., 2004). Interestingly, Feng et al. (2008) found that the effect of bagging treatment toward PAL activity was not obvious, indicating PAL was likely to be regulated by other genes rather than light directly. In grapevine, PAL has been shown to be encoded by multigene families (Sparvoli et al., 1994), but no one member of these multigene families, to date, is identified in fruit tree crops. In this case, PAL maybe has no other members indeed, just more likely post-translational processing results in PAL functional diversity.

C4H, 4CL sequentially convert cinnamic acid to phenolic precursors for anthocyanin biosynthesis. In strawberry, these two structural genes do not seem to have a determining role in the differences of fruit pigmentation, but had a closely related to synthesis of lignin monomers (Salvatierra et al., 2010). Compared to other structural genes, the information about C4H, 4CL enhancing anthocyanin synthesis is relatively poor. Consequently, further investigations are necessary for the role of these two genes in anthocyanin synthesis.

CHS (Chalcone synthases), the well-known representatives of the type III polyketide synthase (PKS) superfamily, catalyze the condensation of 4-hydroxycinnamoyl-CoA and three malonyl-CoA molecules to form the chalcone derivative, naringenin chalcone, which is the first committed step in the phenylpropanoid pathway of plants, leading to the biosynthesis of flavonoids, isoflavonoids, and anthocyanins (Ferrer et al., 1999). The genes encoding CHS constitute a multigene family. In grape, three *CHS* members, to date have been identified referred to as CHS1, CHS2, CHS3, respectively (Jeong et al., 2008). On the amino acid level, the homology between CHS1 and CHS2 was 96%, and between CHS3 and CHS1, and CHS3 and CHS2 both were 89%, indicating very high homologies among CHSs. CHS3 was predominantly expressed in the berry skin of red cultivars during coloration, while CHS1 and CHS2 mainly in the leaves and berry skin of both white and red cultivars (Yamamoto et al., 2002) implicating that CHS3 was mainly responsible for anthocyanin synthesis, and CHS1 and CHS2 for other metabolites. More recently, it has been demonstrated that the expression of CHS1 and

CHS2 coincided with flavonol: and CHS2 and CHS3 with anthocyanin biosynthesis (Jeong et al., 2008). From these two studies, CHS2 can be considered as a multi-function CHS member. In citrus, two CHS cDNAs were obtained (Moriguchi et al., 1999; Lu et al., 2009), and shared 86.6% identity each other at the amino-acid level, but their detailed function remains unknown.

The CHS members in other fruit tree species are necessary to be identified. Numerous studies, not including Boss et al. (1996), have demonstrated that the expression of the *CHS* in fruit tissue was passively associated with fruit coloring as shown in apple (Honda et al., 2002), citrus (Cotroneo et al., 2006; Bernardi et al., 2010; Wang et al., 2010 ), pear (Zhang et al., 2011), grape (Yamamoto et al., 2002), bilberry (Jaakola et al., 2002), etc; and was up-regulated by light (Feng et al., 2010), low temperature (Lo piero et al. 2005; Crifó et al., 2011), and UV-B (Ubi et al., 2006), and was inhibited or unchanged by the ethanol treatment (Kereamy et al., 2002). Thus, the *CHSs* in fruit tree crops seemed to be under different transcription controls, respectively.

Like CHS genes, *CHI*s also constitute a multigene family. Thus far, two CHI members (CHI1 and CHI2) were isolated and exhaustively analyzed (Jeong et al., 2008), indicating that CHI1 strongly associated with anthocyanin synthesis (Jeong et al., 2004), while CHI2 with flavan-3-ol (Jeong et al., 2008). In apple, two sequences similar to MdCHI in the apple genome have not been isolated as yet (Takos et al., 2006a, b). Some studies suggested expression of CHI was related to anthocyanin synthesis (Feng et al., 2008, 2010), others showed not (Niu et al., 2010). Thus far, incongruence can be reasonably explained through leaning from PAL as earlier discussed. The level of transcript of CHS gene can be affected by environmental stimuli, such as light.

A wide body of studies suggested that F3H was related to anthocyanin synthesis (Honda et al., 2002; Castellarin et al., 2007a, b, c; Palapol et al. 2009; Feng et al., 2010; Niu et al. 2010 ); and enhanced by light (Kim et al., 2003), exogenous ethylene (Ashraf et al., 2003), UV-B (Ubi et al. 2006), water deficit (Castellarin et al., 2007b) and low temperature (Ban et al., 2007), etc., but not affected by ethanol (Kereamy et al., 2002). Sparvoli et al. (1994) pointed out that F3H has been shown to be encoded by multi-gene families. In grape, two F3H members, to date were isolated and subsequently analyzed (Jeong et al., 2004, 2008), indicating that the transcription of CHS1, CHS2, F3H1, and F3H 2 coincided with flavonol; and CHS2, CHS3, and F3H2 with anthocyanin biosynthesis. While, in other fruit tree species, no one F3H members has been reported as yet.

DFR and ANS reported previously were enhanced by light (Kim et al., 2003; Feng et al., 2010), low temperature (Piero et al., 2005), UV-B (Ubi et al., 2006), but unaffected by ethanol (Kereamy et al., 2002) and 2-chloroethyphosphonic acid (Kereamy et al., 2003) and had a major role in enhancing anthocyanin synthesis (Honda et al., 2002; Cultrone et al., 2010; Jaalola et al., 2010; Niu et al., 2010; Zhang et al., 2011).

Of these two genes, only two *DFR* members have been isolated and analyzed in apple, referred to as MdDFR1 and DdDFR2, and in Chinese bayberry, referred to as MrDFR1 and MrDFR2, respectively (Espley et al., 2007; Niu et al., 2010). In apple, MdDFR1 were shown to be up-regulated in pigmented fruit tissue but not to any great extent in leaf. In contrast, MdDFR2 showed little or no activity in fruit but was highly elevated in the leaves of 'Red Field'.

In Chinese bayberry, MrDFR1 was clear-cut associated with anthocyanin content, but not MrDFR2, indicating that DFR members each are generally responsible for different function in fruit tree crops.

UFGT, last step gene of anthocyanin synthesis, could be a key enzyme in coloration during anthocyanin accumulation in grape (Kobayashi et al., 2001), apple (Ubi et al., 2006), mangosteen (Palapol et al. 2009), strawberry (Salvatierra et al., 2010) and Chinese bayberry (Niu et al., 2010) and so forth. However, this gene was expressed in all of the citrus leaves tested, including the non-pigmented and thus was not related to anthocyanin synthesis, but *ANS* or *DFR* could be (Lo Piero et al., 2005, 2006; Cultrone et al., 2010).

Cultrone et al. (2010) believed that UFGT is a family of enzymes that exhibits broad substrate specificity for flavonoids and anthocyanidins with 3-hydroxyl groups. Based on this hypothesis, it is reasonable that some of *UFGT* members were not related to anthocyanin synthesis. To our knowledge, this case is true to apple, in which had two *UFGT* members, referred to as UFGluT and UFGalT, respectively, and UFGluT expression is strong related to anthocyanin synthesis but UFGalT not (Honda et al., 2002). UFGT could be enhanced by environmental stimuli, such as ethanol (Kereamy et al., 2002), UV-B, low temperature (Ubi et al., 2006; Lo Piero et al., 2005), ethylene (Umphon et al., 2007), water deficiency (Castellarin et al., 2007b).

Collectively, the structural genes involved in anthocyanin synthesis are coordinately expressed and regulated by a wide variety of environmental factors, which control the anthocyanin synthesis.

The multigene family phenomenon make anthocyanin synthesis pathway more complex. To date, for grape and apple, the mechanism of anthocyanin synthesis is relatively clear. However, in other fruit tree species, such pear, Chinese bayberry and Citrus, etc; more investigations are required to elucidate the mechanism of anthocyanin synthesis.

## REGULATED GENES

In fruit tree crops just as other plant species, the structural genes of the anthocyanin synthesis were largely regulated by a complex of MYB transcription

**Table1.** Regulatory genes identified in fruit tree crops and its targeting structural genes.

| Fruit species | MYB | bHLH | Structural genes | Fruit tissue | Reference |
|---|---|---|---|---|---|
| Apple | MdMYB1 | MdHLH3, | ANS | peel | Takos et al., 2006a |
| | MdMYBA | HLH33 | | peel | Ban et al., 2007 |
| | MdMYB10 | | | Peel/flesh/leaf | Espley et al., 2009 |
| Grape | VvMYB5b | MYCA1 | VvLAR and VvANR | Peel | Deluc et al., 2008 |
| | VvMYB5a | | UFGT, C4H, ANS, F3H, | Peel | Deluc et al., 2006 |
| | VvMYBA1 | | CHI, CHS, 4CL, DFR | Peel | Walker et al., 2007 |
| | VvMYBA2 | | UFGT | | Walker et al., 2007 |
| | | | UFGT | | |
| | | | ANR, UFGT | | |
| Pear | PyMYB10, | | DFR | Peel | Feng et al., 2010 |
| | PcMYB10 | | | Peel | Pierantoni et al., 2010 |
| | PpyMYB10 | | | | Wang et al., 2010a |
| | PbMYB10 | | | | Wang et al., 2010a |
| mangosteen | GmMYB10 | | GmDFR, GmUFGT, GmLDOX | pericarp | Palapol et al., 2009 |
| strawberry | FaMYB1 | | ANS, UFGT | flesh | Aharoni et al., 2001 |
| | FaMYB10 | | DFR | | Wang et al., 2010a |
| | FvMYB10 | | DFR | | Wang et al., 2010a |
| biberry | VmTDR4 | | VmMYB2 | Peel/flesh | Jaakola et al., 2010 |
| Chinese bayberry | MrMYB1 | | AtDFR | flesh | Niu et al., 2010 |
| Loquat | EjMYB10 | | | Unknown | Wang et al., 2010a |
| Medlar | MgMYB10 | | | Unknown | Wang et al., 2010a |
| Quince | CoMYB10 | | | Unknown | Wang et al., 2010a |
| Apricot | ParMYB10 | | DFR | Unknown | Wang et al., 2010a |
| Plum | PiMYB10 | | | Unknown | Wang et al., 2010a |
| | PdmMYB10 | | | Unknown | Wang et al., 2010a |
| | PsMYB10 | | | Unknown | Wang et al., 2010a |
| Cherry | PavMYB10 | | DFR | Unknown | Wang et al., 2010a |
| | PcrMYB10 | | DFR | Unknown | Wang et al., 2010a |
| | PcfMYB10 | | | Unknown | Wang et al., 2010a |
| Almond | PdMYB10 | | DFR | Unknown | Wang et al., 2010a |
| Peach | PprMYB10 | | DFR | Unknown | Wang et al., 2010a |
| Red raspberry | RiMYB10 | | DFR | Unknown | Wang et al., 2010a |

factors (TF), basic helix-loop-helix (bHLH) TFs and WD-repeat proteins (MYB-bHLH-WD40: MBW ) at the level of transcription. Apple and grape were the best studied species in terms of regulation of anthocyanin synthesis by TF, extending our knowledge about anthocyanin synthesis metabolism. Many IFs have been isolated and analyzed (Table 1), because TF was highly conserved in fruit tree species.

In grapevine, the VvMYBA1 and VvMYBA2 regulate specifically the expression of the UFGT gene and thereby

control fruit coloration. The skin color mutation from black to white/green arise from a retrotransponson insertion inside the promoter sequence of MYBA1 and several nonconservative substitutions in coding sequence of MYBA2 (Kobayashi et al., 2002; Walker et al., 2007). These two regulated genes locate on a single bacterial artificial chromosome and either of them can regulate colour in the grape berry, so mutational events taking place in two these adjacent regulated genes were essential for the genesis of the white grapes, but did not know which one first (Walker et al., 2007). Other MYBs (VvMYB5a and VvMYB5b) are involved in the control of different branches of the phenylpropanoid pathway, including anthocyanin synthesis pathway. In tobacco transformed with VvMYB5a, the biosynthesis of condensed tannins was enhanced, and lignin metabolism altered (Deluc et al., 2006). Like VvMYB5a, VvMYB5b regulate particularly the anthocyanin and the pro-anthocyanidin pathways (Deluc et al., 2008). In grapevine, to date, two WD32 ((VvWDR1, VvWDR2) and two bHLH proteins (VvMYCA1, VvMYC1) have been identified, respectively. However, only VvMYCA1 and VvWDR1 are closely related to anthocyanin synthesis. VvMYCA1 may regulate UFGT and ANR through combining with similar elements in promoters of these two structure genes, whereas VvWDR1 may be part of a MYB/bHLH complex, making them both affect positively the synthesis of anthocyanins. *CsMYB8* and *CsMYC2* identified from *Citrus Sinensis* were not related to anthocyanin biosynthesis, while *CsMYC2* may be involved in the regulation of the flavonoid biosynthetic pathway (Cultrone et al., 2010).

In apple, three MYB genes, that is, MdMYB1, MdMYBA, MdMYB10, have been identified to belong to the R2R3 class and are responsible for anthocyanin accumulation (Takos et al., 2006a; Espley et al., 2007). At the deduced amino acid level, MdMYB1 and MdMYBA are identical and differ from MdMYB10 in three amino acids (Ban et al., 2007). Wang et al. (2010) believed that these three MYB genes were likely alleles of each other. MdMYB1 and MdMYBA expression are correlated with anthocyanin synthesis in the fruit skin (Takos et al. 2006a) and MdMYB10 controlled anthocyanin production in fruit (peel and flesh) and leaves (Espley et al., 2007). The methylation within MdMYB10 promoter seemingly decides apple skin patterning (blushed and striped) (Telias et al., 2011). Interestingly, MdMYB10 can activate its own promoter via an upstream MYB-binding motif for its own protein product (Espley et al., 2009). From previous studies, MYBs needs a bHLH partner to enhance anthocyanin accumulation. Thus far, two bHLH genes, that is, MdbHLH3 and MdbHLH33, have been identified in apple and MdbHLH3 is more suitable to MdMYB10 than MdbHLH33.

Besides apple and grapevine, MYBs has been studied intensively in other fruit tree crops, such as pear (Feng et al., 2010), mangosteen (Palapol et al., 2009), Chinese bayberry (Niu et al., 2010) and strawberry (Aharoni et al., 2001). In general, MYBs are helpful in enhancing anthocyanin accumulation, but some repressing anthocyanin production were also identified, including MdMYB17 in apple (Wang et al., 2011), and FaMYB1 in strawberry (Aharoni et al., 2001). External and internal factors, such as light (Feng et al., 2010; Niu et al., 2010), UV-B, low temperature (Ban et al., 2007) and ABA (Jeong et al., 2004) affect IF expression, and then regulate spatio-temporally the structural genes. More recently, an IF, VmTDR4, regulating MYBs related to anthocyanin synthesis has been identified from Bilberry (Jaakola et al., 2010), indicating that a complex regulating network is present in fruit tree crops regulating the anthocyanin biosynthesis pathway. Furthermore, it were these suchlike IFs that, perhaps, make anthocyanin biosynthesis pathway organically linked with other metabolic pathways such soluble sugar, organic acid, and hormone.

## FUTURE DEVELOPMENT

Anthocyanins biosynthesis in fruit tree crops is one aspect of currently active research area not only due to visual appeal but nutritional value. To develop an anthocyanin-rich cultivar, a better understanding of the regulatory network controlling anthocyanin biosynthesis in fruit tree crops is a prerequisite. However, transcription factors identified in fruit tree crops are limited, and how to cooperatively respond to internal and external factors, and then regulate structural genes needs to further investigate. Furthermore, according to mutated gene controlling anthocyanin biosynthesis, we can develop functional molecular markers to carry out marker-assisted selection in fruit tree crops. This promising work needs numerous explorations in future. Another challenging aspect would be to understand fully the effects of internal and external factors on anthocyanin accumulation in different fruit tree crops. In this case, we can use some strategies to regulate anthocyanin accumulation in practice.

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### REFERENCES

Aharoni A, Ric De Vos CH, Wein M, Sun ZK, Greco R, Kroon A, Mol, JMN, Connell APO (2001). The strawberry FaMYB1 transcription factor suppresses anthocyanin and flavonol accumulation in transgenic tobacco. Plant J. 28(3): 319-332.

Ashraf EK, Chervin C, Roustan JP, Cheynier V, Souquet JM, Moutounet M, Raynal J, Ford C, Latché A, Pech JC, Bouzayen M (2003).

Exogenous ethylene stimulates the long-term expression of genes related to anthocyanin biosynthesis in grape berries. Physiol. Plantarum, 119: 175-182.
Ban Y, Honda C, Hatsuyama Y, Igarashi M, Bessho H, Moriguchi T (2007). Isolation and functional analysisis 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.
Bernardi J, Licciardello C, Russo MP, Chiusano ML, Carletti G, Recupero GR, Marocco A (2010). Use of a custom array to study differentially expressed genes during blood orange (*citrus sinensis* L. Osbeck) ripening. J. Plant Physiol. 167: 301-310.
Boss P, Davies C, Robinson S (1996). Analysis of the expression of anthocyanin pathway genes in developing *Vitis vinifera* L.cv. Shiraz grape berries and the implication for pathway regulation. Plant Physiol. 111: 1059-1066.
Borsani O, Gonzalez-Neves G, Ferrer M, Monza (2010). Anthocyanins accumulation and genes-related expression in berries of cv. Tannat (*Vitis vinifera* L.). J. Appl. Hortic. 12(1): 3-9.
Bureau S, Renard CM, Reich M, Ginies C, Audergon J (2009). Change in anthocyanin concentrations in red apricot fruits during ripening. Food Sci. Technol. pp. 372-377.
Castellarin SD, Matthews MA, Gaspero GD, Gambetta GA (2007a). Water deficits accelerate ripening and induce changes in gene expression regulating flavonoid biosynthesis in grape berries. Planta, 227: 101-112.
Castellarin SD, Pfeiffer A, Sibilotti P, Degan M, Peterlunger E, Gaspero GD (2007b). Transcriptional regulation of anthocyanin biosynthesis in ripening fruits of grapevine under seasonal water deficit. Plant Cell Environ. 30: 1381-1399.
Castellarin SD, Gaspero GD (2007c). Transcriptional control of anthocyanin biosynthetic genes in extreme phenotypes for berry pigmentation of naturally occurring grapevines. BMC Plant Biol. 7: p. 46.
Crifó T, Puglisi I, Petrone G, Recupero R, Piero ARL (2011). Expression analysis in response to low temperature stress in blood oranges: implication of the flavonoid biosynthetic pathway. Gene, 476: 1-9.
Cotroneo PS, Russo MP, Ciuni M, Reforgiato RG, Lo PAR (2006). Quantitative real time reverse transcriptase-PCR profiling of anthocyanin biosynthetic genes during orange fruit ripening. J. Am. Soc. Hort. Sci. 13: 537-543.
Cultrone A, Cotroneo PS, Reforgiato Recupero G (2010). Cloning and molecular characterization of R2R3-MYB and bBHLH-MYC transcription factors from *Citrus sinensis*. Tree Genet. Genomes, 6: 101-112.
Deluc L, Barrieu F, Marchive C, Lauvergeat V, Decendit A, Richard T, Carde JP, Mérillon JM, Hamdi S (2006). Characterization of a grapevine R2R3-MYB transcription factor that regulates the phenylpropanoid pathway. Plant Physiol. 140: 499-511.
Deluc L, Bogs J, Walker AR, Ferrier T, Decendit A, Merillon JM, Robinson SP, Barrieu F (2008). The transcription factor VvMYB5b contributes to the regulation of anthocyanin and proanthocyanidin biosynthesis in developing grape berries. Plant Physiol. 147: 2041-2053.
Espley RV, Hellens RP, Putterill J, Stevenson D, Kutty-Amma S, Allan AC (2007). Red colouration in apple fruit is due to the activity of the MYB transcription factor, MdMYB10. Plant J. 49: 414-427.
Espley RV, Brendolise C, Chagné D, Amma SK, Green S, Volz R, Putterill J, Schouten HJ, Gardiner SE, Hellens RP, Allan AC (2009). Multiple repeats of a promoter segment causes transcription factor autoregulation in red apples. Plant Cell, 21: 168-183.
Feng SQ, Chen XS, Zhang CY, Liu XJ, liu ZC, Wang HB, Wang YL, Zhou CH (2008). Relationship between anthocyanin biosynthesis and related enzymes activity in *Pyrus pyrifolia* mantianhong and its bud sports aoguan. Agric. Sci. China, 7(11): 1318-1323 .
Feng SQ, Wang YL, Yang S, Xu YT, Chen XS (2010). Anthocyanin biosynthesis in pears is regulated by a R2R3-MYB transcription factor PyMYB10. Planta, 232: 245-255.
Ferrer JL, Jez EM, Bowman ME, Dixon RA, Noel JP (1999). Structure of chalcone synthase and the molecular basis of plant polyketide biosynthesis. Nat. Struct. Biol. 6: 775-784.
Guo J, Han W, Wang MH (2008). Ultraviolet and environmental stresses involved in the induction and regulation of anthocyanin biosynthesis: A review. Afr. J. Biotechnol. 7(25): 4966-4972.
Holton T, Cornish E (1995). Genetics and biochemistry of anthocyanin biosynthesis. Plant Cell, 7: 1071-1083.
Honda C, Kotoda N, Wada M, Kondo S, Kobayashi S, Soejima J, Zhang Z, Tsuda T, Moriguchi T (2002). Anthocyanin biosynthetic genes are coordinately expressed during red coloration in apple skin. Plant Physiol. Bioch. 40: 955-962.
Hugueney P, Provenzano S, Verriès C, Ferrandino A, Meudec E, Batelli G, Merdinoglu D, Cheynier V, Schubert A, Ageorges A (2009). A novel cation-dependent *O*-methyltransferase involved in anthocyanin methylation in grapevine. Plant Physiol. 150: 2057-2070.
Jaakola L, Maatta K, Pirttila AM, Torronen R, Karenlampi S, Hohtola A (2002). Expression of genes involved in anthocyanin biosynthesis in relation to anthocyanin, proanthocyanidin, and flavonol levels during bilberry fruit development. Plant Physiol. 130: 729-739.
Jaakola L, Poole M, Jones MO, Karppinen TK, Koskimmäki JJ, Hohtola A, Häggman H, Fraser PD, Manning K, King GJ, Thomson H, Seymour GB (2010). A Squamosa MADS box gene involved in the regulation of anthocyanin accumulation in Bilberry fruit. Plant Physol. 153: 1619-1629.
Jeong ST, Goto-Yamanoto G, Kobayashi S, Eaka M (2004). Effects of plant hormones and shading on the accumulation of anthocyanins and expression of anthocyanin biosynthetic genes in grape berry skins. Plant Sci. 167: 247-252.
Jeong ST, Yamamoto NG, Hashizume K, Esaka M (2008). Expression of multi-copy glavonoid pathway genes coincides with anthocyanin, flavonol and flavan-3-ol accumulation, grapevine. Vitis, 47(3): 135-140.
Jiang YJ, Joyce DC, Terry LA (2001).1-methylcyclopropene treatment affects strawberry fruit decay. Postharvest Biol. Tec. 23: 227-232.
Ju ZG, Liu CL, Yuan YB (1995). Activities of chalcome synthase and UDPGal: flavonoid-3-o-glycosyltransferase in relation to anthocyanin synthesis in apple. Sci. Hortic. 63: 175-185.
Kereamy AE, Chervin C, Souquet JM, Moutounet M, Monje MC, Nepveu F, Mondies H, Ford CM, Heeswijck RV, Roustan JR (2002). Ethanol triggers grape gene expression leading to anthocyanin accumulation during berry ripening. Plant Sci. 163: 449-454.
Kereamy AE, Chervin C, Roustan JP, Cheynier B, Souquet JM, Moutounet M, Raynal J, Ford C, Latché A, Pech JC, Bouzayen M (2003). Exogenous ethylene stimulates the long-term expression of genes related to anthocyanin biosynthesis in grape berries. Physol. Plantarum, 119: 175-182.
Kim SH, Lee JR, Hong ST, Yoo YK, An GH, Kim SR (2003). Molecular cloning and analysis of anthocyanin biosynthesis genes preferentially expressed in apple skin. Plant Sci. 165: 403-413.
Kobayashi S, Ishimaru M, Ding CK, Yakushiji H, Goto N (2001). Comparison of UDP-glucose: flavonoid 3-O-glucosyltransferase (UFGT) gene sequences between white grapes (*Vitis vinifera*) and their sports with red skin. Plant Sci. 160: 543-550.
Kobayashi S, Ishimaru M, Hiraoka K, Honda C (2002). MYB-related genes of the Kyoho grape (*Vitis labruscana*) regulate anthocyanin biosynthesis. Planta, 215: 924-933.
Li M, Zheng QQ, Dou ZJ, Guo SJ, Jiang JY, Su XD, Yu SL (2010). Control mechanism of anthocyanins synthesis metabolism in fruit and its influence factors. J. Anhui Agric. Sci. 38(16): 8381-8384 (in Chinese).
Liu SY, Huang YF, Zhang SZ (2006). Regulatory gene of anthocyanin biosynthesis in plant. Plant Physiol. J. 42(4): 747-754.
Lo Piero AR, Consoli A, Puglisi I, Orestano G, Reforgiato RG, Petrone G (2005). Anthocyaninless cultivars of sweet orange lack to express the UDP-glucose flavonoid 3-*O*-glucosyl transferase. J. Plant. Biochem. Biotechnol. 14: 1–6.
Lo Piero AR, Puglisi I, Petrone G (2006). Gene characterization, analysis of expression and in vitro synthesis of dihydroflavonol 4-reductase from [*Citrus sinensis* (L.) Osbeck]. Phytochemis. 67(7): 684-695.
Lu X, Zhou W, Gao F (2009). Cloning, characterization and localization of *CHS* gene from blood orang, *Citrus sinensis* (L.) Osbeck cv. Ruby. Mol. Biol. Rep. 36: 1983-1990.
Matus JT, Aquea F, Arce-Johnson P (2008). Analysis of the grape MYB R2R3 subfamily reveals expanded wine quality-related clades and conserved gene structure organization across *Vitis* and *Arabidopsis*

genomes. BMC Plant Biol. 8: p. 83.
Moreno FP, Monagas M, Blanch GP, Bartolomé, Castillo MLR (2010). Enhancement of anthocyanins and selected aroma compounds in strawberry fruits through methyl jasmonate vapor treatment. Eur. Food Res. Technol. 230: 989-999.
Moriguchi T, Kita M, Tomono Y, Endo-Inagaki T, Omura M (1999). One type of chalcone synthase gene expressed during embryogenesis regulates the flavonoid accumulation in citrus cell cultures. Plant Cell Physiol. 40(6): 651-655.
Niu SS, Xu CJ, Zhang WS, Zhang B, Li X, Wang KL, Ferguson IB, Allan AC, Chen KS (2010). Coordinated regulation of anthocyanin biosynthesis in Chinese bayberry (*Myrica rubra*) fruit by a R2R3 MYB transcription factor. Planta, 231: 887-899.
Palapol Y, Ketsa S, Wang KL, Ferguson BI, Allan AC (2009). A MYB transcription factor regulates anthocyanin biosynthesis in mangosteen (*Garcinia mangostana* L.) ftuit during ripening. Planta, 229: 1323-1334.
Pierantoni L, Dondini L, De Franceschi P, Musacchi S, Winkel BS, Sansavini S (2010). Mapping of an anthocyanin-regulating MYB transcription factor and its expression in red and green pear, *Pyrus communis*. Plant Physiol. Bioch. 48(12): 1020-1026.
Piero ARL, Puglisi I, Rapisarda P, Petrone G (2005). Anthocyanins accumulation and related gene expression in red orange fruit induced by low temperature storage. J. Agr. Food Chem. 53: 9083-9088.
Ross JA, Kasum CM (2002). Diertary flavonoids: Bioavailabiligy metabolic effects, and safegy. Annu. Rev. Nutr. 22: 19-34.
Salvatierra A, Pimentel P, Moya-Leon MA, Caligari PDS, Herrera R (2010). Comparison of transcriptional profiles of flavonoid genes and anthocyanin contents during fruit development of two botanical froms of *Fragaria chiloensis ssp. Chiloensis*. Phytochemistry, 71: 1893-1847.
Sparvoli F, Martin C, Scienza A, Gavazzi G, Tonelli C (1994). Cloning and molecular analysis of structural genes involved in flavonoid and stilbene biosynthesis in grape (*Vitis vinfera*). Plant Mo. Biol. 24: 743-755.
Takos AM, Jaffé FW, Jacob SR, Bogs J, Robinson SP, Walker AR (2006a). Light-induced expression of a MYB gene regulates anthocyanin biosynthesis in red apple. Plant Physiol. 142: 1216-1232.
Takos AM, Robinson SP, Walker AR (2006b). Condensed tannin biosynthesis genes are regulated separately from other falvonoid biosynthesis genes in apple fruit skin. Plant Sci. 170: 487-499.
Tanaka Y, Sasaki N, Ohmiya A (2008). Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. Plant J. 54: 733-749.
Telias A, Wang KL, Stevenson DE, Cooney JM, Hellens RP, Allan AC, Hoover EE, Bradeen JM (2011). Apple skin patterning is associated with defferential expression of MYB10. BMC Plant Biol. 11: p. 93.
Ubi BU, Honda C, Bessho H, Kondo S, Wada M, Kobayashi S, Moriguchi T (2006). Expression analysis of anthocyanin biosynthetic genes in apple skin: effect of UV-B and temperature. Plant Sci. 170: 571-578.
Umphon AT, Roustan JP, Chervin C (2007). The stimulation by ethylene of the UDP glucose-flavonoid 3-*O*-glucosyltransferase (UFGT) in grape tissues is independent from the MybA transcription factors. Vitis, 46(4): 210-211.
Walker AR, Elizabeth L, Jochen B, Debra AJ, Mc D, Mark RT, Simon PR (2007) White grapes arose through the mutation of two similar and adjacent regulatory genes. Plant J. 49: 772–785.
Wang H, Arakawa O, Motomura Y (2000). Influence of maturity and bagging on the relationship between anthocyanin accumulation and phenylalanine ammonia-lyase (PAL) activity in Jonathan apples. Postharvest Biol. Tech. 19: 123-128.
Wang Y, Li J, Xia RX (2010). Expression of chalcone synthase and chalcone isomerase genes and accumulation of corresponding flavonoids during fruit maturation of Guoqing No.4 satsuma mandadarin (Citrus *unshiu* Marcow). Sci. Hortic. 125: 110-116.
Wang KL, Bolitho K, Grafton K, Kortstee A, Karunairetnam S, McGhie TK, Espley RV, Hellens RP, Allan AC (2010a). An R2R3 MYB transcription factor associated with regulation of the anthocyanin biosynthetic pathway in Rosaceae. BMC Plant Biol.10: p. 50.
Wang KL, Micheletti D, Palmer J, Volz R, Lozano L, Espley R, Hellens RP, Chagne D, Rowan DD, Troggio M, Iglesias I, Allan AC (2011). High temperature reduces apple fruit colour via modulation of the anthocyanin regulatory complex. Plant Cell Environ. 34(7): 1176-1190.
Winkel-Shirley B (2001). Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. Plant physiol. 126(2): 485-493.
Yamamoto NG, Wan GH, Masaki K, Kobayashi (2002). Structure and transcription of three chalcone synthase genes of grapevine (v*itis vinifera*). Plant Sci. 162: 867-872.
Zhang XD, Allan AC, Yi Q, Chen LC, Li K, Shu Q, Su J (2011). Differential gene expression analysis of yunnan red pear, Pyrus Pyrifolia, during fruit skin coloration. Plant Mol. Biol. Rep. 29: 305-314.
Zhao ZF, Zhao Y, Wu GF (1994). Studies on relationship between anthocyanidin and PAL. Acta Horticult. Sinica chinese 21(2): 199-200.