Plant Molecular Biology (2018) 98:1–18
https://doi.org/10.1007/s11103-018-0771-4

REVIEW

Roles of R2R3-MYB transcription factors in transcriptional regulation of anthocyanin biosynthesis in horticultural plants

Aung Htay Naing[1] · **Chang Kil Kim**[1]

Received: 6 June 2018 / Accepted: 23 August 2018 / Published online: 30 August 2018

Abstract

Key message **This review contains functional roles of MYB transcription factors in the transcriptional regulation of anthocyanin biosynthesis in horticultural plants. This review describes potential uses of MYB TFs as tools for metabolic engineering for anthocyanin production.**

Abstract Anthocyanins (ranging from red to blue) are controlled by specific branches of the anthocyanin biosynthetic pathway and are mostly visible in ornamentals, fruits, and vegetables. In the present review, we describe which R2R3-MYB transcription factors (TFs) control the transcriptional regulation of anthocyanin structural genes involved in the specific branches of the anthocyanin biosynthetic pathway in various horticultural plants (e.g., ornamentals, fruits, and vegetables). In addition, some MYBs responsible for anthocyanin accumulation in specific tissues are described. Moreover, we highlight the phylogenetic relationships of the MYBs that suppress or promote anthocyanin synthesis in horticultural crops. Enhancement of anthocyanin synthesis via metabolic genetic engineering of anthocyanin MYBs, which is described in the review, is indicative of the potential use of the mentioned anthocyanin-related MYBs as tools for anthocyanin production. Therefore, the MYBs would be suitable for metabolic genetic engineering for improvement of flower colors, fruit quality, and vegetable nutrients.

Keywords Anthocyanins · Ornamental plants · Fruits · Gene expression · R2R3-MYB · Vegetables

Introduction

Anthocyanins are flavonoid compounds found in plants. Their diverse colors, ranging from red to blue, can be observed in horticultural crops, such as ornamentals, fruits, and vegetables. Anthocyanins play substantial roles in the functions of horticultural plants, and their presence determines fruit quality and flower colors as well as providing nutritional and pharmaceutical properties in vegetables, features which are of interest to flower enthusiasts and plant breeders.

Generally, anthocyanins (cyanidin, pelargonidin, and delphinidin derivatives) are synthesized via three main branches of the biosynthetic pathway (Fig. 1), and transcriptional regulation of the structural genes [such as chalcone synthase (*CHS*), chalcone isomerase (*CHI*), flavanone 3-hydroxylase (*F3H*), flavonoid 3′-hydroxylase (*F3′H*), dihydroflavonol 4-reductase (*DFR*), and anthocyanidin synthase (*ANS*)] involved in the three main branches leads to the production of the different flavonoid pigments in many plant species.

Over the past decade, the roles of transcription factors (TFs) in the regulation of transcript levels of structural genes have been identified in many plant species (Allan et al. 2008). Based on their DNA binding domains, TFs are generally classified into three main families, namely, R2R3-MYB domains, basic helix-loop-helix (bHLH) domains, and conserved WD40 repeats (WDR) (Ramsay and Glover 2005). R2R3-MYBs have been shown to be involved in phenylpropanoid metabolism (Hichri et al. 2011) and activate the structural genes involved in the anthocyanin biosynthetic pathway in many plants, including model and horticultural crops (Albert et al. 2014; Xie et al. 2014; Zhang et al. 2017). Metabolic genetic engineering via anthocyanin regulatory MYBs can change cell *metabolism* by altering its pathway enzyme(s) or regulatory protein(s) using recombinant DNA technology; thus, metabolic genetic engineering of MYBs has generated

✉ Chang Kil Kim
ckkim@knu.ac.kr

[1] Department of Horticultural Science, Kyungpook National University, Daegu 41566, Republic of Korea

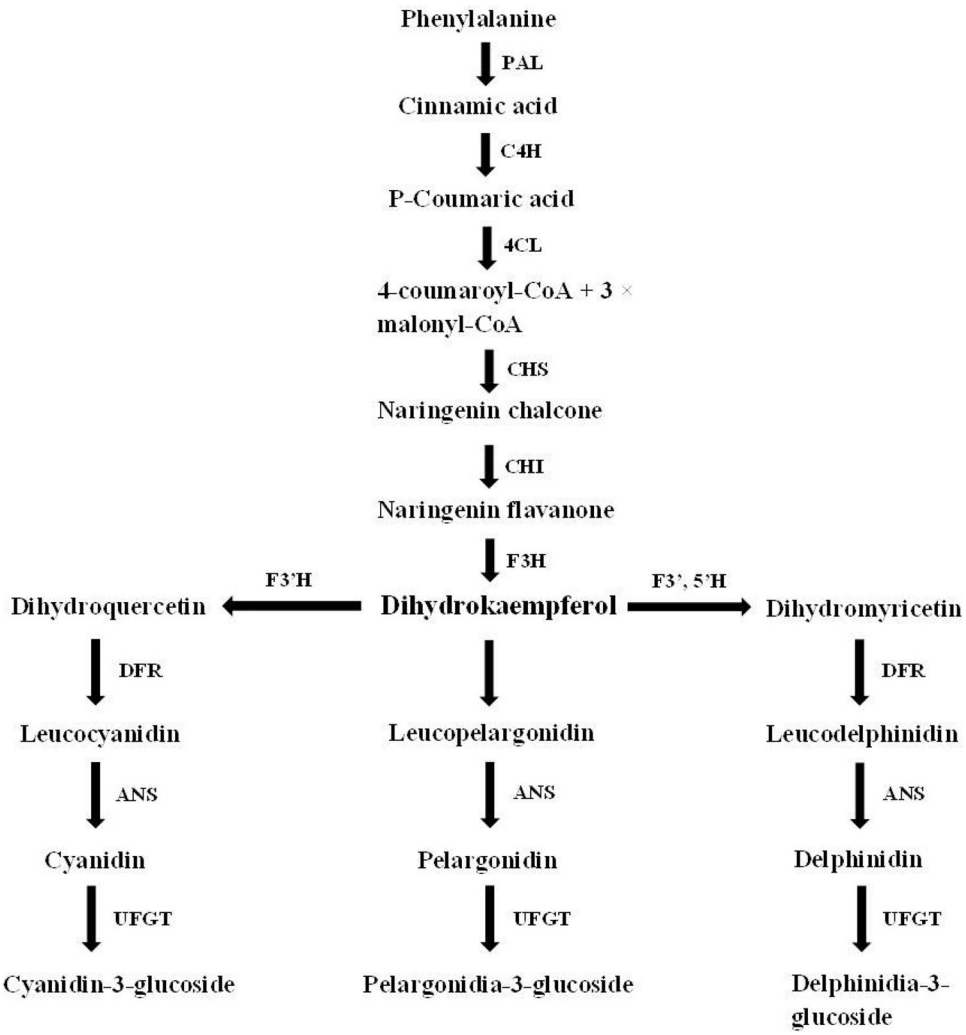


Fig. 1 Three main branches of anthocyanin biosynthetic pathway in plants. *CHS* chalcone synthase enzyme, *CHI* chalcone isomerase, *F3H* flavanone 3-hydroxylase, *F3′H* flavanone 3′-hydroxylase, *F3′5′H* flavanone 3′,5′-hydroxylase, *DFR* Dihydroflavonol 4-reductase, *ANS* anthocyanidin synthase, *UFGT* UDP-glucose: flavonoid 3-*O*-glucosyltransferase

diverse colors and color patterns in several ornamental plants and produced anthocyanin-rich fruits and vegetables with high nutritional and pharmaceutical properties (Laitinen et al. 2008; Hichri et al. 2011). However, MYBs that suppress the structural genes in some plant species have also been identified. In horticultural plants, differential roles of MYBs regarding transcriptional control of the structural genes have not been thoroughly reviewed. In addition, amino acid sequences of MYBs that exhibit different functional roles have not been phylogenetically analyzed.

In the present review, we describe which R2R3-MYBs promote or suppress the expression of the structural genes involved in the anthocyanin biosynthesis pathway of ornamental plants, fruits, and vegetables and analyze the phylogenetic relationships of the MYBs. In addition, we highlight studies that show the enhancement of anthocyanin contents in horticultural crops by metabolic genetic engineering of anthocyanin regulatory MYBs.

Functional roles of R2R3-MYBs in anthocyanin production

Ornamental plants

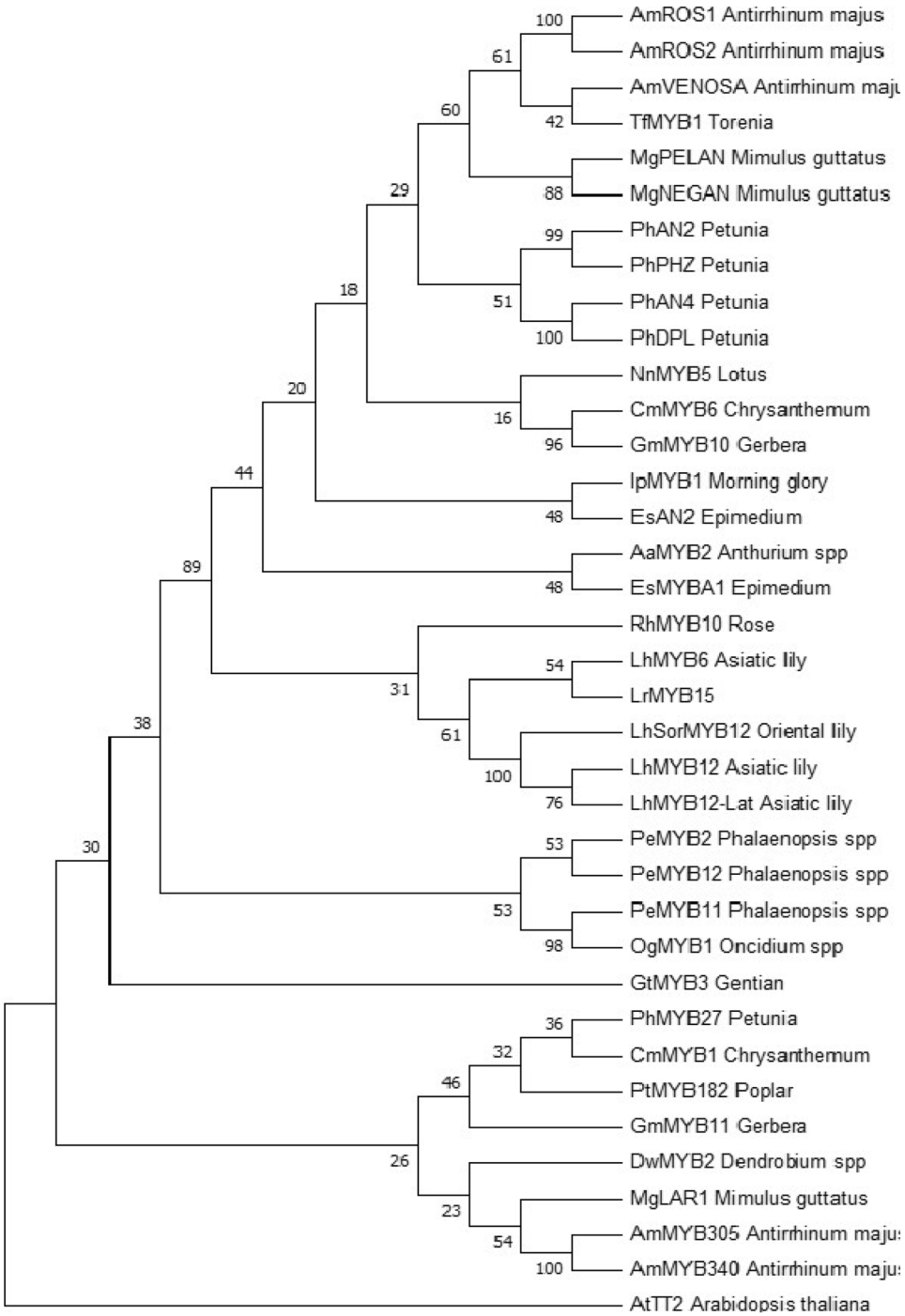
Diverse flower colors produced via different branches of the anthocyanin pathway are found in ornamental plants, in which TFs such as MYB, bHLH, and WDR individually or coordinately promote transcript levels of structural genes that determine flower colors. Although several R2R3-MYBs that are involved in color pigmentation of many ornamental plants have been identified, some MYBs that suppress anthocyanins have also been reported in some ornamental plants. Nevertheless, those MYBs that have been reported so far have not been thoroughly summarized to date. In the present review, MYBs that promote or suppress structural genes involved in the anthocyanin production pathway of ornamental plants are well summarized (Table 1). In addition, the phylogenetic relationships of the

Table 1 Identified R2R3-MYBs that promote or suppress anthocyanin structural genes in ornamental plants

Species	R2R3-MYBs	Proven target genes	References
Petunia	PhAN2	*CHS, DFR*	Quattrocchio et al. (1993)
	PhAN4	*CHS, DFR*	Albert et al. (2009)
	PhDPL	*Flavonoid genes except FLS, 3GT, AT*	Spelt et al. (2000)
	PhPHZ	*Flavonoid genes except F3'5'H*	Albert et al. 2011
	PhMYB27	*Supress flavonoid genes*	Albert et al. (2011)
Rose	RhMYB10	*DFR*	Lin-Wang et al. (2010)
Gentian	GtMYB3	*CHS, 5AT*	Nakatsuka et al. (2008)
Mimulus guttatus	MgPLA1	*Flavonoid genes*	Lowry et al. (2012)
	MgPELAN	*Flavonoid genes*	Yuan et al. (2014)
	MgNEGAN	*Flavonoid genes*	Yuan et al. (2016)
	MgLAR1	*FLS*	
Morning glory	IpMYB1	*CHS, CHI, F3H, F3'H, DFR, ANS, GT, GST*	Morita et al. (2006)
Asiatic lily	*LhMYB6*	*DFR*	Nakatsuka et al. (2009)
	LhMYB12	*CHS, DFR*	Yamagishi et al. (2010, 2012, 2014)
	MYB12-Lat	*CHS, DFR*	
Oriental lily	*LhMYB12*	*CHS, F3H, DFR*	Yamagishi (2011)
Lilium regale	LrMYB15	*CHSa, CHSb, DFR, ANS*	Yamagishi (2016)
Antirrhinum majus	AmROS1	*F3H, F3'H, DFR, FLS, UFGT*	Martin et al. (1991)
	AmROS2	*CHI, F3H, F3'H, FLS, ANS, UFGT, and AT*	Moyano et al. (1996)
	AmVENOSA	*CHI, F3H, F3'H, FLS, ANS, UFGT, and AT*	Schwinn et al. (2006)
	AmMYB305	*CHS, CHI, F3H, DFR, ANS*	Shang et al. (2011)
	AmMYB340	*CHS, CHI, F3H, DFR, AS*	
Torenia	TfMYB1	*CHS, F3H,fDFR, ANS, UFGT*	Nishijima et al. (2013)
Anthurium spp.	AaMYB2	*F3H* and *ANS*	Li et al. (2016)
Chrysanthemum	CmMYB6	*DFR*	Liu et al. (2015)
	CmMYB1	*Supress flavonoid genes*	Zhu et al. (2013)
Gerbera	GmMYB10	*Late flavonoid genes*	Elomaa et al. (2003)
Phalaenopsis spp.	PeMYB2	*F3'5'H, DFR1, ANS3*	Hsu et al. (2015)
	PeMYB11	*F3'5'H, DFR1, ANS3*	
	PeMYB12	*F3'5'H, DFR1, ANS3*	
Oncidium spp.	OgMYB1	*CHI, DFR*	Chiou et al. (2008)
Dendrobium spp.	DhMYB2	*DFR, ANS*	Wu et al. (2003)
Lotus	MnMYB5	*GST*	Sun et al. (2016)
Epimedium	*EsMYBA1*	*DFR, ANS*	Haung et al. (2013, 2016)
	EsAN2	*CHS, CHI, ANS*	
Poplar	*PtMYB182*	*Supress DFR, ANS*	Yoshida et al. (2015)

MYBs associated with the anthocyanin production or suppression in ornamental plants are also presented (Fig. 2).

Several MYBs [anthocyanin 2 (PhAN2), anthocyanin 4 (PhAN4), purple haze (PhPHZ), and deep purple (PhDPL)] determine the flower color of petunias (Quattrocchio et al. 1993; Spelt et al. 2000; Albert et al. 2009, 2011), with PhAN2 being responsible for the red coloration in the corolla and PhAN4 inducing the same in the anthers and corolla tubes by interacting with bHLH TF anthocyanin 1 (PhAN1). However, their activation in specific tissues depends on the light conditions because they induce weak pigmentation in the flower bud surface, but strong pigmentation in the leaves, under high light conditions (Albert et al. 2009, 2011). PhDPL and PhPHZ also activate the transcript levels of the same structural genes; however, the former determines the color pattern venation in the corolla tubes, whereas the latter is responsible for blushing of the flower bud (Albert et al. 2011) and induces pigmentation under light conditions where PhAN1 is highly expressed. Albert et al. (2011) suggested that both MYBs have the amino acid signature motif ([DE]Lx2[RK]x3Lx6Lx3R), which has been identified as being important for interaction with another co-regulator, bHLH, in *Arabidopsis* (Zimmermann et al. 2004). Thus, in the petunia, the



production of anthocyanin by anthocyanin MYBs would occur via interaction with the co-transcription factor bHLH. Although the abovementioned MYBs are grouped in a clade and participate in the induction of anthocyanin biosynthesis (Fig. 2), PhMYB27, which is not related to the above MYBs, appeared to be an anthocyanin suppressor because it was highly expressed under non-anthocyanin-inductive shade conditions and was suppressed during anthocyanin-inductive light conditions (Albert et al. 2011, 2014). In addition, its homologous expression in the petunia also resulted in the reduction of anthocyanin in the flowers (Fig. 3) (Albert et al. 2014). Moreover, PhMYB27 has strong sequence similarity to that of CmMYB1 from chrysanthemums and PtMYB182 from poplars, which also appeared to be MYB suppressors because they have been shown to downregulate structural genes involved in anthocyanin pathway (Zhu et al. 2013; Yoshida et al. 2015). In addition, the suppressor CmMYB1 is not grouped in the same clade as the anthocyanin regulator

◂**Fig. 2** Phylogenetic analysis of protein sequences of R2R3-MYB transcription factors associated with regulation or suppression of anthocyanin in ornamental plants. The evolutionary history was inferred using the Neighbor-joining method. The bootstrap consensus tree inferred from 1500 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% of the bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1500 replicates) are shown next to the branches (Felsenstein 1985). The evolutionary distances were computed using the p-distance method and are in units of the number of amino acid differences per site. The analysis included 37 amino acid sequences. All ambiguous positions were removed for each sequence pair. There were a total of 434 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al. 2016). All R2R3-MYB sequences were retrieved from the GenBank database and accession numbers are as follows: Petunias PhAN2 (ABO21074), PhAN4 (ADQ00392), PhDPL (ADW94950), PhPHZ (ADW94951), PhMYB27 (AHX24372); Rose RhMYB10 (ABX79949); *Gentian triflora* GtMYB3 (BAF96933); *Mimulus guttatus* MgPELAN (AHJ80987), MgNEGAN (AHJ80988), MgLAR1 (ALP48587); Morning glory IpMYB1 (BAE94388); *Asiatic lily* LbMYB6 (BAJ05399), LbMYB12 (BAJ05398), LbMYB12-Lat (BAO04194); Oriental lily LhMYB12 (BAJ22983), LrMYB15 (BAU2993); *Antirrhinum majus* AmROSEA1 (ABB83826), AmROSEA2 (ABB83827), AmVENOSA (ABB83828), AmMYB305 (P81391), AmMYB340 (P81396); *Torenia fournieri* TfMYB1 (BAN17385); *Anthurium andraeanum* AaMYB2 (AML84515); Chrysanthemum CmMYB6 (AKP06190), CmMYB1 (AEO27497); Gerbera GmMYB10 (CAD87010), GmMYB11 (ACG69457); *Phalaenopsis equestription* PeMYB2 (AIS35919); *Phalaenopsis equestris* PeMYB11 (AIS35928), PeMYB12 (AIS35929); *Oncidium grower ramsey* OgMYB12 (ABS58501); *Dendrobium* spp. DwMYB2 (AAO49411); Lotus *Nelumbo nucifera* NnMYB5 (ALU11263); *Epimedium sagittatum* EsMYBA1 (AGT39060), EsAN2 (ALO24363); Poplar MYB182 (AJI76863); *Arabidopsis thaliana* AtTT2 (NP_198405.1), which is involved in regulation of proanthocyanidin biosynthesis, is included as an outgroup

CmMYB6 (Liu et al. 2015). Similarly, GmMYB10 from the gerbera, which is a homolog of CmMYB6 and produces pelargonidin in vegetative and flower tissues by interacting with GmMYC1 (Laitinen et al. 2008), is not grouped in the same clade as GmMYB11, which is not involved in anthocyanin production (Elomaa et al. 2003). In fact, GmMYB11 has been found to be a homolog of the suppressor PhMYB27. This indicates that the MYBs associated with anthocyanin production or suppression in flowers can be identified by phylogenetic analysis.

Morita et al. (2006) suggested that MYB1 (a homolog of PhAN2) activated anthocyanin structural genes in the flower limbs and tubes of the morning glory by interacting with the co-TFs WDR1 and bHLH2. Similarly, in the gentian, GtMYB3 (a homolog of PhAN2) interacted with GtbHLH1 (a homolog of PhAN1) and produced anthocyanin via upregulation of the structural genes (Nakatsuka et al. 2008). A similar regulatory role of anthocyanin-related MYBs has been confirmed in tobacco. It is likely that these MYBs, which are homologs of PhAN2, regulate the structural genes by interacting with co-TFs in a similar way to PhAN2.

According to phylogenetic analysis, sequences of the anthocyanin regulatory MYBs (MgPELAN, MgNEGAN, AmVENOSA, AmROSEA1, and AmROSEA2) existing in *Mimulus guttatus* and *Antirrhinum majus* are related by divergent evolution from a common ancestor; however, the sequences within the same species are more homologous. In *Mimulus guttatus*, MgPELAN is responsible for pigmentation in the petal lobe, whereas MgNEGAN induces anthocyanin spots in the nectar guide (Lowry et al. 2012; Yuan et al. 2014, 2016). In addition, the functional role of MgPELAN can be confirmed by its low transcript level in yellow flowers (Yuan et al. 2014). In *A. majus*, AmROSEA1 induced strong red coloration in both epidermal layers of the corolla by activating the transcript levels of *F3H, F3'H, DFR, FLS*, and *UFGT*; AmROSEA2 induced weak pigmentation in the inner epidermal layer of the corolla by increasing the expression level of *F3'H*; and AmVENOSA influenced the venation color patterns in the petals by enhancing the transcript levels of *CHI, F3H, F3'H, FLS, ANS, UFGT*, and *AT* (Schwinn et al. 2006; Shang et al. 2011). Interestingly, AmMYB305 and AmMYB340 are not phylogenetically related to AmVENOSA, AmROSEA1, and AmROSEA2; however, they are related to MgLAR1 from *Mimulus guttatus* and participate in anthocyanin regulation, in which MgLAR1 regulates the flavonoid synthase that determines corolla patterning (Lowry et al. 2012; Yuan et al. 2014, 2016), whereas AmMYB305 and AmMYB340 promote anthocyanin production (Moyano et al. 1996) by activating a subset of anthocyanin structural genes. Although the MYBs participated in anthocyanin production, the reasons why they are not grouped in the same clade as other MYBs from the same species is still unclear. TfMYB1 from *Torenia fournieri* controls the red pigmentation in the torenia flower by elevating the transcript levels of early (*TfCHS* and *TfF3H*) and late (*TfDFR, TfANS*, and *TfUFGT*) biosynthetic genes (Nishijima et al. 2013), and is a member of the clade containing the MYBs MgPELAN, MgNEGAN, AmVENOSA, AmROSEA1, and AmROSEA2.

As for the abovementioned ornamental plants, color pattern variations (such as red, pink, and purple) in *Anthurium andraeanum* (Hort.) were determined by AaMYB2 and were associated with its differential expression (Li et al. 2016), in which AaMYB2 mainly upregulated the transcript levels of *AaF3H* and *AaANS*. The same functional role of AaMYB2 has been shown in tobacco by the induction of anthocyanin via transcriptional activation of the same structural genes. In fact, most of the sequences of anthocyanin regulatory MYBs are variations of the AaMYB2 sequence, which is synonymous with EsMYBA1 and EsAN1 from *Epimedium*, which influence pigmentation in the leaves, flowers, and flower buds (Huang et al. 2013, 2016). In addition, NnMYB5 from the lotus (*Nelumbo* Adans; Nelumbonaceae), which has strong sequence similarity with EsAN2, induced attractive

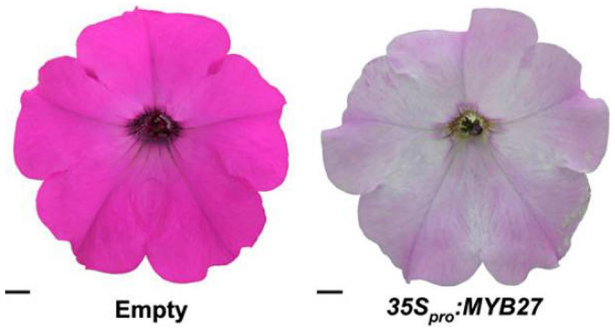


Fig. 3 PhMYB27 is a suppressor of anthocyanin pigmentation. This image was taken from a publication by Albert et al. (2014) and is copyrighted by the American Society of Plant Biologists

flower color (Sun et al. 2016). An identical functional role of this MYB was observed in *Arabidopsis*, resulting in anthocyanin accumulation in the flower stalks.

It has been well demonstrated that anthocyanin production in the lily is controlled via activation of the transcript levels of structural genes by several MYBs whose sequences are phylogenetically related to each other (Nakatsuka et al. 2009; Yamagishi et al. 2010, 2012, 2014; Yamagishi 2011, 2016; Suzuki et al. 2016); however, their functional roles are slightly different. For example, in the Asiatic lily, LhMYB6 controlled the formation of red spots in the tepals and leaves, whereas LhMYB12 did so in the tepals, filaments, and styles (Nakatsuka et al. 2009; Yamagishi et al. 2010; Suzuki et al. 2016). LhMYB12-Lat (an allele of LhMYB12 from the Asiatic lily) and LhMYB12 (from the Oriental lily) that are grouped in the same clade as LhMYB12 from the Asiatic lily exhibited the same pigmentation in the tepals as that of LhMYB12 in the Asiatic lily by enhancing the transcript levels of the structural genes (Yamagishi 2011; Yamagishi et al. 2012, 2014). These MYBs interacted with LhbHLH2, which was confirmed by co-expression of LhbHLH2 and MYB6 or MYB12 in the lily bulb scales. However, LrMYB15 from *Lilium regale*, which is a homolog of LhMYB6, was thought to be responsible for anthocyanin production (Yamagishi 2016) because anthocyanin accumulation was observed only in the organs where LrMYB15 was expressed, whereas LhbHLH2 was expressed in all the organs. However, this appeared to be a light-induced MYB because under shade conditions the MYB could not be detected, and anthocyanin production was lower than that under light conditions. A similar function of LrMYB15 has been confirmed in tobacco. RhMYB10 from the rose, which is a divergent of the above MYBs from the lily, also regulated anthocyanin production; however, its functional roles have not yet been clearly revealed (Lin-Wang et al. 2010).

Like the abovementioned MYBs in lilies, sequences of the anthocyanin regulatory MYBs from orchids are also phylogenetically related to each other. In *Phalaenopsis* spp., MYBs (PeMYB2, PeMYB11, and PeMYB12) pigmented the flower by activating the structural genes *PeF3H5, PeDFR1*, and *PeANS3* (Hsu et al. 2015), and their inactivation resulted in the loss of color pigmentation. Moreover, they determined the induction of distinct color patterns in floral organs such as the sepals, petals, and lip; however, the formation of red spots on the lip was determined by PeMYB11 and full pigmentation in the central lobe of the lip was influenced by PeMYB12. In most species, the co-expression of MYB and bHLH, rather than an expression of a single factor, strongly induces anthocyanin production; however, in this species, co-expression of PeMYBs and PebHLHs did not affect anthocyanin production, which was probably controlled by the MYBs. OgMYB1 from *Oncidium* spp., which is a homolog of PeMYB11, also enhanced red pigments in lip tissues via activation of the structural genes *OgCHI* and *OgDFR* (Chiou and Yeh 2008). DwMYB2 from *Dendrobium* spp. enhanced pigmentation in flowers via regulation of *DFR* and *ANS* genes (Li et al. 2017) and was phylogenetically related to the anthocyanin regulatory MYB MgLAR1, but not related to the above MYBs. Recently, PmMYBa1 from *Prunus mume* that was previously identified as a R2R3-MYB domain increased anthocyanin in petals by regulation of late biosynthesis genes (LBGs) (Zhang et al. 2017).

In summary, two or more MYBs have been shown to control anthocyanin production in several ornamental plants via transcriptional regulation of the structural genes, however, only one anthocyanin regulatory MYB has been shown to date in some important ornamental plants. Based on phylogenetic results, sequences of most MYBs are related by divergent evolution from a common ancestor. Generally, most MYBs within the same species are more homologous than MYBs of different species; however, some MYBs within the same species that act as suppressors are not related to anthocyanin regulatory MYBs but are grouped in a clade with MYB suppressors from other species.

Fruits

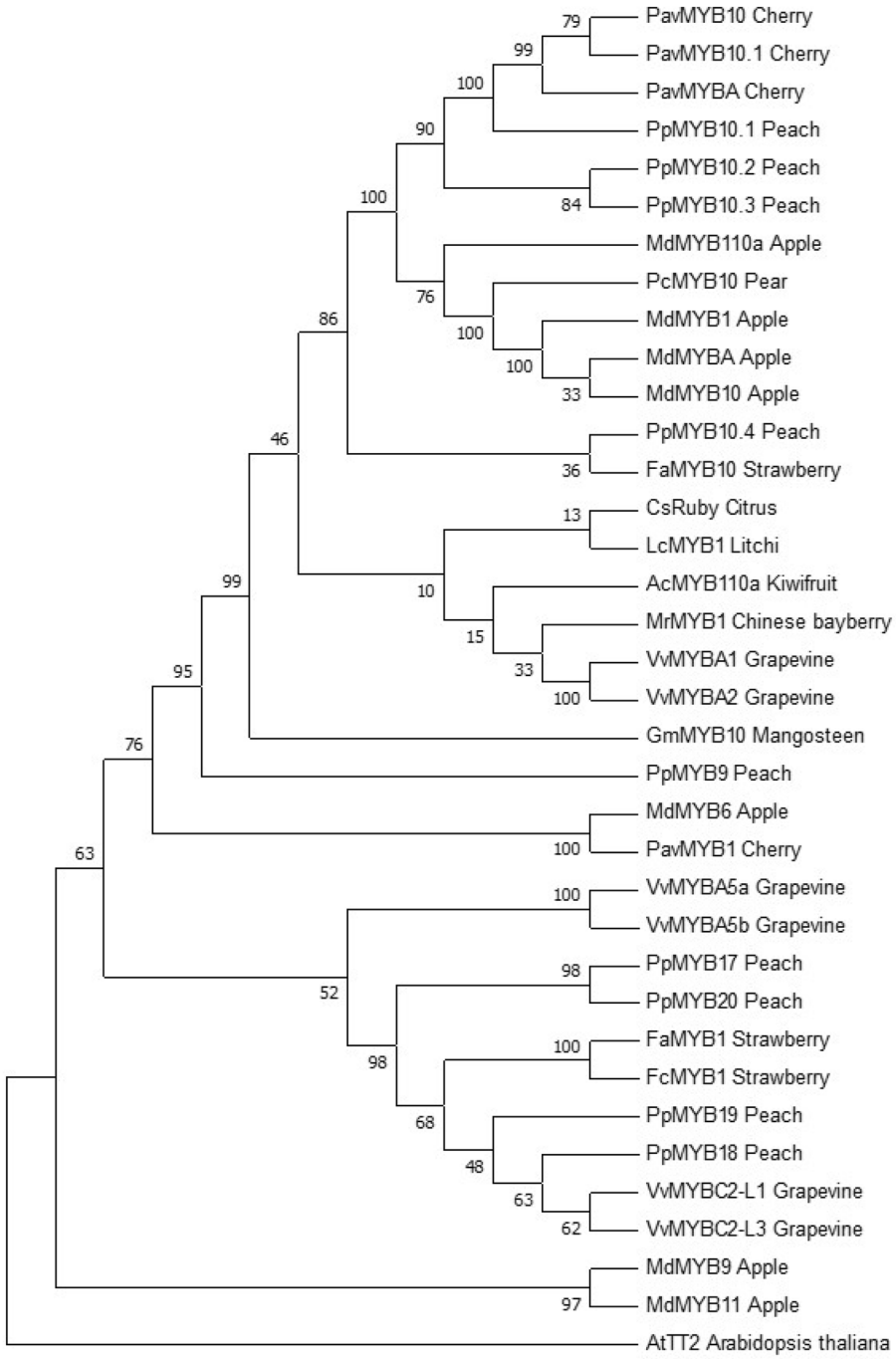
In fruit crops, fruit color that is determined by anthocyanins not only indicates maturity and quality but also increases consumer preference, because the marketability of fruit highly depends on the red color. The quality of wine that is made from fruits such as grapes and apples is determined by the anthocyanin concentration, which affects the bitterness, astringency, and color. Klatsky (2002) suggested that cardiovascular disease and cancer might be ameliorated by moderate consumption of red wine. In addition, anthocyanins provide nutrients and health benefits for humans by increasing the antioxidant properties of cells and tissues (Bagchi et al. 2003; Pervaiz 2003). Thus, regulation of the color development of fruits is of particular interest. Recently,

MYBs that are involved in the promotion or suppression of anthocyanin production in fruit crops have been identified. Here, we describe which MYBs are associated with anthocyanin production or suppression in many fruit crops (Table 2) as well as showing their phylogenetic relationships (Fig. 4).

Diverse fruit colors determined by different levels of anthocyanin accumulation, such as white, red, and dark red–purple, are observed in different Chinese bayberry (*Myrica rubra*) cultivars and are strongly associated with the transcript level of MrMYB1, which suggests that it is the transcription level that determines the anthocyanin content in ripe fruit (Niu et al. 2009). According to phylogenetic analysis, the sequences of MYBs from kiwifruit (AcMYB110a), Chinese berry (MrMYB1), and grape (VvMYBA1 and VvMYBA2) are phylogenetically related and the MYB strongly influences the red pigmentation in the respective fruits as described for MrMYB1 (Fraser et al. 2013; This et al. 2007). Similarly, other genetically related MYBs (CsRuby and LcMYB1) have been shown to enhance anthocyanin in blood oranges and litchi fruits (Butelli et al. 2012; Lai et al. 2014). In fact, these MYBs are variations of GmMYB10, which induces red pigmentation in mangosteen (Palapol et al. 2009). Based on the phylogenetic results, VvMYBA1 and VvMYBA2 are homologous to each other, as are VvMYB5a and VvMYB5b; however, the two pairs are not grouped in the same clade and their roles in anthocyanin regulation are also slightly different. VvMYBA1 and VvMYBA2 regulate anthocyanin in grape

Table 2 Identified R2R3-MYBs that promote or suppress anthocyanin structural genes in fruit plants

Species	R2R3-MYBs	Proven target gene	References
Grapevine	VvMYBA1	*UFGT*	Kobayashi et al. (2002)
	VvMYBA2	*UFGT*	Walker et al. (2007)
	VvMYB5a	*CHI, F3'5'H, ANS, ANR, LAR1, UFGT*	Deluc et al. (2006)
	VvMYB5b	*CHI, F3'5'H, ANS, ANR, LAR1, UFGT*	
	VvMYBC2-L1	*Supress PA and flavonoid genes*	Huang et al. (2014)
	VvMYBC2-L3	*Supress PA and flavonoid genes*	Cavallini et al. (2015)
	VvMYB4-like	*Supress PA and flavonoid genes*	Pérez-Díaz et al. (2015)
Pear	PcMYB10	*F3H, DFR, ANS*	Pierantoni et al. (2010) Li et al. (2012) Feng et al. (2010)
Mangosteen	GmMYB10	*UFGT*	Palapol et al. (2009)
Apple	MdMYBA	*ANS*	Takos et al. (2006)
	MdMYB1	*DFR, GT*	Espley et al. (2007)
	MdMYB9	*CHI, CHS, F3H, DFR, ANS, ANR*	Telias et al. (2011)
	MdMYB10	*CHI, CHS, F3H, DFR, ANS, UFGT*	An et al. (2015)
	MdMYB110a	*CHS*	Chagne et al. (2013)
	MdMYB11	*CHI, CHS, F3H, DFR, ANS, ANR*	Ban et al. (2007) Vimolmangkang et al. (2013)
Cherry	PavMYBA	*DFR, ANS, UFGT*	Lin-Wang et al. (2010)
	PavMYB1	*CHS, LDOX, UFGT*	Sooriyapathirana et al. (2010)
	PavMYB10	*ANS, UFGT*	Shen et al. (2014)
	PavMYB10.1		Starkevič et al. (2015) Jin et al. (2016)
Chinese bayberry	MrMYB1	*F3H, F3'H, DFR, ANS, UFGT*	Niu et al. (2009)
Peach	PpMYB9	*DFR*	Ravaglia et al. (2013)
	PpMYB10.1	*CHS, F3H, UFGT*	Rahim et al. (2014)
	PpMYB10.2	*CHS, F3H, UFGT*	Zhou et al. (2014)
	PpMYB10.3	*CHS, F3H, UFGT*	Zhou et al. (2015, 2016)
	PpMYB10.4	*Early and Late flavnonoid genes*	
	PpMYB17	*Supress DFR*	
	PpMYB18	*Supress DFR*	
	PpMYB19	*Supress DFR*	
	PpMYB20	*Supress DFR*	
Kiwifruit	AcMYB110a	*F3GT1*	Fraser et al. (2013)
Strawberry	*FaMYB10*	*Early and Late flavonoid genes*	Aharoni et al. (2001)
	FaMYB1	*Supress ANS*	Medina-Puche et al. (2014)
	FcMYB1	*Supress ANS*	Kadomura-Ishikawa et al. (2015)
Litchi	*LcMYB1*	*UFGT*	Lai et al. (2014)



berries by activating the expression of the last LBG, *UFGT* (This et al. 2007), whereas VvMYB5a and VvMYB5b are involved in tannin production during the early stage of berry development, but potentially regulate anthocyanin in ripe berry tissues (Deluc et al. 2006). However, VvMYBC2-L1 and VvMYBC2-L3, which are not related to the above MYBs from grapes, suppressed proanthocyanidin accumulation in grapevines by downregulation of structural genes (Huang et al. 2014; Cavallini et al. 2015) and are grouped in the same clade as MYBs from the peach (PpMYB17, PpMYB18, PpMYB19, and PpMYB20) and the strawberry (FaMYB1 and FcMYB1) that have also been reported as anthocyanin suppressor MYBs in their respective species (Zhou et al. 2016). Silencing FaMYB1 in the fruits significantly increased anthocyanin content and, conversely, its overexpression lowered the anthocyanin content by reducing transcript levels of *ANS* (Kadomura-Ishikawa et al. 2015). Similarly, high expression of FcMYB1 imparted a white color to the fruit (Salvatierra et al. 2013) and its downregulation increased anthocyanin by upregulation of *ANS* and

◂**Fig. 4** Phylogenetic analysis of protein sequences of R2R3-MYB transcription factors associated with regulation or suppression of anthocyanin in fruit plants. The evolutionary history was inferred using the Neighbor-Joining method. The bootstrap consensus tree inferred from 1500 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% of the bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1500 replicates) are shown next to the branches (Felsenstein 1985). The evolutionary distances were computed using the p-distance method and are in units of the number of amino acid differences per site. The analysis included 36 amino acid sequences. All ambiguous positions were removed for each sequence pair. There were a total of 430 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al. 2016). All R2R3-MYB sequences were retrieved from the GenBank database and accession numbers are as follows: Grapevine VvMYBA1 (BAD18977), VvMYBA2 (BAD18978), VvMYBA5a (AAS68190), VvMYBA5b (AAX51291), VvMYBC2-L1 (AFX64995), VvMYBC2-L3 (AIP98385); Pear *Pyrus communis* PcMYB10 (ABX71487); Mangosteen *Garcinia mangostana* GmMYB10 (ACM62751); Apple *Malus domestica* MdMYB (BAF80582), MdMYB1 (ABK58136), MdMYB6 (AAZ20429), MdMYB9 (ABB84757), MdMYB10 (ACQ45201), MdMYB110a (AFC88038), MdMYB11 (AAZ20431); Cherry *Prunus avium* PavMYBA (AHL45015), PavMYB1 (XP_021829544), PavMYB10 (ALH21137), PavMYB10.1 (ALM31949); Chinese bayberry *Morella rubra* MrMYB1 (ADG21957); Peach *Prunus persica* PpMYB9 (ALO81019), PpMYB10.1 (XP_007216530); *Prunus cerasifera* PpMYB10.2 (AKV89248), PpMYB10.3 (AKV89249); *Prunus persica* PpMYB10.4 (AHL68405), PpMYB17 (ALO81020), PpMYB18 (ALO81021), PpMYB19 (ALO81022), PpMYB20 (ALO81023); *Citrus sinensis* CsRuby (AFB73913); Kiwifruit AcMYB110a (AHY00342); Strawberry *Fragaria ananassa* FaMYB10 (ABX79947), FaMYB1 (AAK84064); Litchi *Fragaria chiloensis* FcMYB1 (ADK56163); *Litchi chinensis* LcMYB1 (APP94121); *Arabidopsis thaliana* AtTT2 (NP_198405.1), which is involved in regulation of proanthocyanidin biosynthesis, is included as an outgroup

strong suppression of the transcript levels of anthocyanidin reductase (*ANR*) and leucoanthocyanidin reductase (*LAR*). The phylogenetic analysis results (Fig. 2) support the evolutionary relationships among the anthocyanin MYB suppressors (PpMYB17, PpMYB18, PpMYB19, PpMYB20, FaMYB1, FcMYB1, VvMYBC2-L1, and VvMYBC2-L3).

In apples (*Malus domestica*), three MYBs (MdMYB1, MdMYB10, and MdMYBA) that have strong sequence homology enhance anthocyanin production in fruit skin via upregulation of structural genes (Takos et al. 2006; Ban et al. 2007; Espley et al. 2007; Telias et al. 2011; Vimolmangkang et al. 2013). These MYBs are divergent from MYB110a, which produces red coloration in the apple flesh cortex (Chagné et al. 2013). The MYBs MdMYB9 and MdMYB11, which are not phylogenetically related to the above MYBs, also promote anthocyanin accumulation by activating early biosynthesis genes (EBGs) and LBGs (*MdCHS, MdCHI, MdF3H, MdANS, MdDFR*, and *MdANR*) (An et al. 2015). PcMYB10 from pears is grouped with the anthocyanin MYBs (MdMYB1, MdMYB10, and MdMYBA) and was thought to be participating in the anthocyanin pathway (Feng et al. 2010; Pierantoni et al. 2010; Li et al. 2012) because the expression level of this MYB was significantly higher in the red-skinned pear cultivars (Aoguan and Max Red Bartlett) than in the yellow Williams pear (Feng et al. 2010; Pierantoni et al. 2010). However, in the pear cultivar Wujiuxiang, this MYB was more likely to be induced by low temperatures and enhanced transcript levels of the structural genes *F3H, DFR*, and *ANS* during fruit development (Li et al. 2012). Sequences of the MYBs from cherries (PavMYBA, PavMYB10, and PavMYB10.1), peaches (PpMYB10.1, PpMYB10.2, and PpMYB10.3), and apples (MdMYB1, MdMYB10, MdMYB110a, and MdMYBA) are related by divergent evolution from a common ancestor and, as observed in apples, the MYBs from the cherry and peach also participated in anthocyanin accumulation in their fruits (Lin-Wang et al. 2010; Sooriyapathirana et al. 2010; Ravaglia et al. 2013; Rahim et al. 2014; Shen et al. 2014; Zhou et al. 2014, 2015, 2016; Starkevič et al. 2015; Jin et al. 2016). The MYBs are more closely related within species than among species and their regulatory roles are also similar. Generally, in the cherry, PavMYBA activated the promoters of the LBGs involved in the anthocyanin pathway by interacting with bHLH (Shen et al. 2014); similarly, PavMYB10 and PavMYB10.1 also interacted with bHLH and WD40 to bind with promoters of the LBGs (Jin et al. 2016). In peaches, although PpMYB10.4 and PpMYB9 are anthocyanin regulatory MYBs, they are not closely related to other peach MYBs (PpMYB10.1, PpMYB10.2, and PpMYB10.3), and the sequences of these two MYBs are not homologous. This is related to their tissue-specificity because the MYBs (PpMYB10.1, PpMYB10.2, and PpMYB10.3) are responsible for anthocyanin accumulation in fruit, whereas PpMYB10.4 and PpMYB9 are responsible for accumulation in leaves and flowers, respectively (Zhou et al. 2014, 2016). FaMYB10 was observed to be a potential MYB for anthocyanin biosynthesis because it is primarily expressed in ripe red strawberry fruit, and when its expression was transiently silenced in fruit receptacles, anthocyanin was not produced (Fig. 5) (Medina-Puche et al. 2014).

Like ornamental plants, the majority of MYBs that regulate anthocyanin in fruits are related by divergent evolution from a common ancestor and those within the same species are more homologous than those from different species (with a few exceptions). Generally, fruit anthocyanin-related MYBs from peaches, cherries, and apples are closely related, while the suppressor MYBs from different fruit species are also closely related to each other. The present review reveals the MYBs (more than 30) that enhance anthocyanin accumulation in different fruit crops via regulation of structural genes and provides information about their phylogenetic relationships.

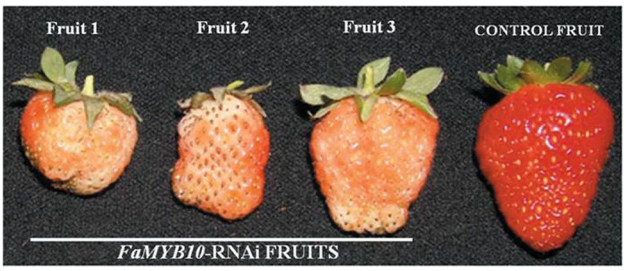


Fig. 5 Role of FaMYB10 in anthocyanin accumulation in strawberry. This image was taken from the study by Medina-Puche et al. (2014) and is copyrighted by Oxford University Press

Vegetables

Vegetables are not only one of the fundamental components of the human diet but are also important to human health because they are important sources of essential vitamins and minerals, as well as secondary metabolites such as anthocyanins that can protect against several human diseases (Hou 2003; Lila 2004). R2R3-MYBs have been considered closely related to the anthocyanin biosynthetic pathway in several plant species (Davies et al. 2012) and the regulatory roles of MYBs in anthocyanin biosynthesis have been reported for some vegetables. In the present review, we highlight the MYBs and their regulated target genes involved in anthocyanin production in vegetable crops (Table 3) and present their phylogenetic relationships (Fig. 6).

To date, several anthocyanin regulatory MYBs have been functionally characterized in various vegetable crops, mostly *Brassica* spp. including cabbage, cauliflower, purple kale, purple kohlrabi (*B. oleracea*), mustard (*B. juncea*), and heading Chinese cabbage (*B. rapa*). In cabbage, three MYBs, BoMYB1, BoMYB2, and BoMYB3, participate in anthocyanin biosynthesis; however, BoMYB2 is more likely to be related to anthocyanin production because its transcript level in red cultivars is higher than that of other MYBs (Yuan et al. 2009), together with an increase in the expression levels of most structural genes (*CHS, F3H, F3'H, DFR, LDOX*, and *GST*). In addition, upregulation of the co-TFs (bHLH) has been observed in the red cultivars. Thus, it is likely that BoMYB2 up-regulated the transcript levels of the structural genes in red cabbage by interacting with bHLH (Yuan et al. 2009). The MYB (MYB2) from cauliflower and heading Chinese cabbage, which is a homolog of BoMYB2 from cabbage, also enhanced anthocyanin production via upregulation of structural genes by interacting with bHLHs, as achieved by BoMYB2 from cabbage (Chiu et al. 2010; Chiu and Li 2012; He et al. 2016); however, BoMYB2 from cauliflower mainly induced the pigments in curds and seed endosperm (Chiu et al. 2010; Chiu and Li 2012). Moreover, BoPAP2 from purple kohlrabi (*B. oleracea*), which is grouped in a clade with the above three MYBs, also enhanced pigmentation via up-regulation of structural genes by interacting with bHLH (Zhang et al. 2015a). Specifically, in the leaves of the cultivar Kolibri (purple), transcript levels of BoPAP2 were 990-fold higher than those in the cultivar Winner (green), whereas in the skins of swollen stems, BoPAP2 transcript levels in Kolibri were 452-fold higher than those in Winner. RsMYB1 from radishes

Table 3 Identified R2R3-MYBs that regulate anthocyanin structural genes in vegetable plants

Species	R2R3-MYB TF	Proven target gene	References
Cabbage	BoMYB2	*CHS, F3H, F3'H, DFR, LDOX*, and *GST*	Yuan et al. (2009)
Kohlrabi	BoPAP2	Early and Late flavonoid genes	Zhang et al. (2015a, b)
Chinese cabbage	BrMYB2	*CHS, F3'H, DFR, ANS, UFGT*, and *GST*	He et al. (2016)
Tomato	SlANT1	*Early and Late flavonoid genes*	Kiferle et al. (2015)
	SlANT2	*DFR*	Mathews et al. (2003)
			Jia et al. (2015)
Pepper	CaMYB	*CHS,CHI, F3H, F3'5'H, DFR, ANS, UFGT*	Borovsky et al. (2004)
	CaMYB1	*DFR*	Li et al. (2011)
	CaMYB2	*DFR*	Zhang et al. (2015a, b)
Mustard	BjMYB1	*F3H, F3'H, DFR*, and *ANS*	Xie et al. (2014)
	BjMYB2	*F3H, F3'H, DFR*, and *ANS*	
	BjMYB3	*F3H, F3'H, DFR*, and *ANS*	
	BjMYB4	*F3H, F3'H, DFR*, and *ANS*	
Eggplant	SmMYB1	*CHS,CHI, F3H, F3'5'H, DFR, ANS, UFGT*	Zhang et al. (2014)
Potato	StAN1	*F3'5'H, DFR*	Jung et al. (2009)
	StMYBA1	*F3'5'H, DFR*	Liu et al. (2016)
	StMYB113	*F3'5'H, DFR*	
Onion	AcMYB1	*CHS, DFR*	Schwinn et al. (2016)
Radish	RsMYB1	*DFR, ANS*	Park et al. (2011)
Lablab pods	LpPAP1	*CHS, ANS, FLS*	Cui et al. (2016)

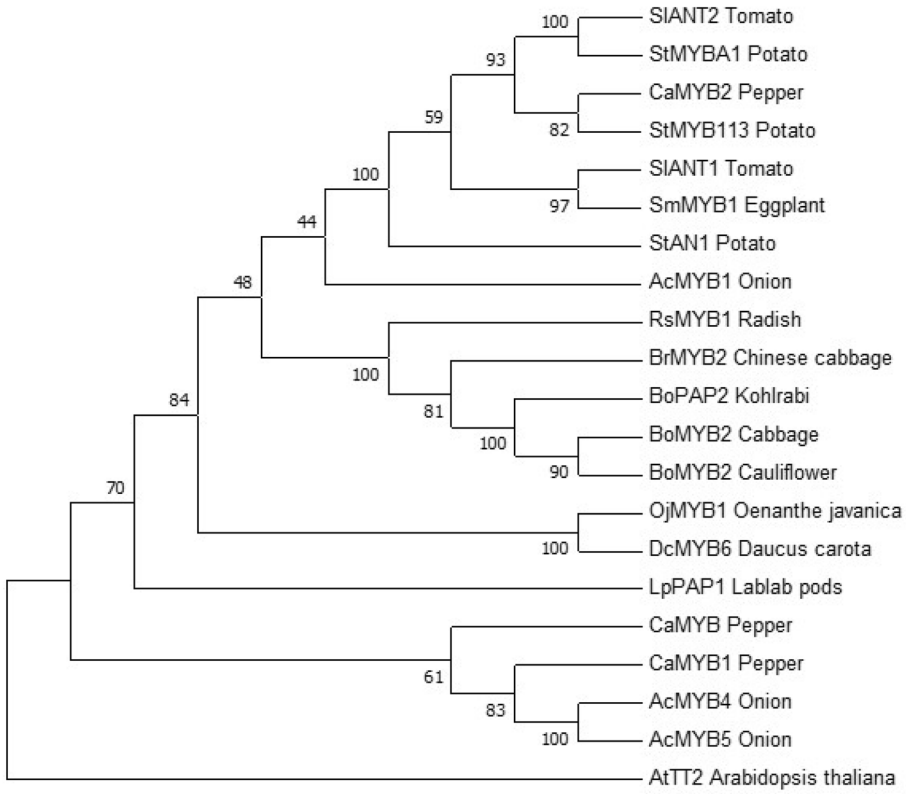


Fig. 6 Phylogenetic analysis of protein sequences of R2R3-MYB transcription factors associated with regulation of anthocyanin in vegetable plants. The evolutionary history was inferred using the Neighbor-Joining method. The bootstrap consensus tree inferred from 1500 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% of the bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1500 replicates) are shown next to the branches (Felsenstein 1985). The evolutionary distances were computed using the p-distance method and are in units of the number of amino acid differences per site. The analysis included 21 amino acid sequences. All ambiguous positions were removed for each sequence pair. There were a total of 423 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al. 2016). All R2R3-MYB sequences were retrieved from the GenBank database and accession numbers are as follows: Cabbage *Brassica oleracea* BoMYB1 (ADP76651), BoMYB2 (ADP76651), BoMYB3 (ADP76651); Cauliflower BoMYB2 (ADP76650); Kohlrabi BoPAP2 (ADP76650); Chinese cabbage BrMYB2 (AIP94023); Tomato *Solanum lycopersicum* SlANT1 (AAQ55181), SlANT2 (ACN22102); Pepper *Capsicum annuum* CaMYB (ABN11121), CaMYB1 (AAQ05796), CaMYB2 (CAE75745); Eggplant *Solanum melongena* SmMYB (AMK01804); Potato *Solanum tuberosum* StAN1 (AGC31676), StMYBA1 (AFD31816), StMYB113 (AND01219); Onion *Allium cepa* AcMYB1 (AQP25672), AcMYB4 (AQP25673), AcMYB5 (AQP25674); Radish *Raphanus sativus* RsMYB1 (AKM95888); *Lablab purpureus* LpPAP1 (AKP06425); *Daucus carota* DcMYB6 (ARD08872); *Oenanthe javanica* OjMYB1 (ASZ70608); Gerbera GmMYB12 (ACG69458); *Arabidopsis thaliana* AtTT2 (NP_198405.1), which is involved in regulation of proanthocyanidin biosynthesis, is included as an outgroup

(*Raphanus sativus*), in a clade with the above MYBs, also regulated anthocyanin biosynthesis in red radish cultivars via strong induction of structural genes by interacting with bHLH (Park et al. 2011), indicating that this MYB group induce anthocyanin via upregulation of structural genes by interacting with co-TFs (bHLHs). In the case of the mustard cultivars 'Zi Yang' and 'Lv Ying' (Fig. 7), induction of purple pigments in the leaves of the cultivar Zi Yang was due to strong expression of the MYBs (BjMYB1, BjMYB2, BjMYB3, and BjMYB41), which are homologs of the anthocyanin regulatory MYBs (AtPAP1, AtPAP2, AtMYB113, and AtMYB114, respectively) that activate transcription of the structural genes *F3H, F3'H, DFR*, and *ANS* (Xie et al. 2014); however, expression of the MYBs appeared to be light-induced, because the expression levels of BjMYB1, BjMYB2, BjMYB3, and BjMYB4 were significantly higher in seedlings grown in the light than in those grown in the dark.

The regulatory role of MYBs in anthocyanin production has also been documented in *Solanum* spp., including tomato, eggplant, and potato, in which the MYBs are phylogenetically related by divergent evolution from a common ancestor. Specifically, two MYBs [anthocyanin 1 (SlANT1) and anthocyanin 2 (SlANT2)] are involved in anthocyanin synthesis in tomatoes (*S. lycopersicum*) and are mainly responsible for the purple pigmentation in vegetative

Fig. 7 Phenotype of purple cultivar "Zi Ying" expressing high BjMYB (**a**) and green cultivar "Lv Ying" expressing low BjMYB (**b**). This image was taken from a publication by Xie et al. (2014) and is copyrighted by the American Chemical Society

tissues and fruits; however, the production of anthocyanin due to SlANT2 can only be seen under high-light or low-temperature conditions (Mathews et al. 2003; Kiferle et al. 2015). Recently, another two MYB encoding genes, *SlMYB7-like* and *SlMYB48-like*, were identified as possible positive regulators of anthocyanin synthesis in tomatoes (Jia et al. 2015). SmMYB1 from eggplant (*S. melongena*) is a homolog of SlANT1 from tomato and, like SlANT1, is also responsible for the purple pigmentation in fruits via upregulation of most structural genes (Fig. 8), while overexpression of SmMYB1 enhanced the same purple pigmentation in transgenic eggplants (Zhang et al. 2014). Three MYBs (StAN1, StMYBA1, and StMYB113) that are phylogenetically related to the above MYBs are also observed to have the same function in the potato (*S. tuberosum* L.) (Jung et al. 2009; Liu et al. 2016); however, they interacted with the co-TFs StbHLH1 and StJAF13. In addition, several MYBs have been reported to regulate anthocyanin in other important culinary crops such as pepper (*Capsicum annuum*) and onion (*Allium cepa*). Among the MYBs (CaMYB, CaMYB1, and CaMYB2) reported in peppers, CaMYB1 and CaMYB2 participate in anthocyanin accumulation in hot peppers (purple fruit) and during fruit developmental stages (Borovsky et al. 2004; Li et al. 2011) by enhancing the expression level of *CaDFR*, whereas CaMYB is responsible for regulation of anthocyanin structural genes in pepper leaves (Zhang et al. 2015b). These MYBs are not homologous to each other; thus, although they are derived from the same species

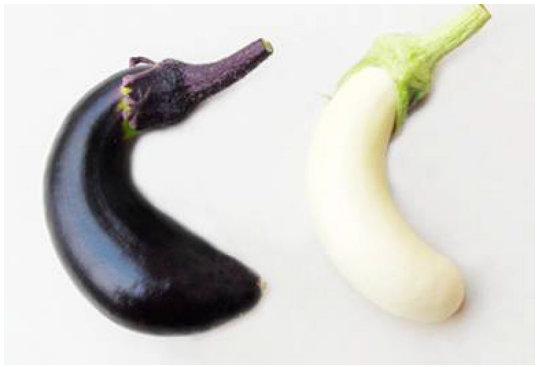


Fig. 8 Phenotypes of two eggplant cultivars Zi Chang (on the left) expressing high SmMYB1 and Bai Xue (on the right) expressing low SmMYB1. This image was taken from the study by Zhang et al. (2014) and is copyrighted by the American Chemical Society

and involved in anthocyanin production, their existence in different groups is probably related to variation in their specific roles. In white and red onions (*Allium cepa*) (Fig. 9), the MYBs are associated with pigmentation, i.e., AcMYB1 is observed to be the regulatory MYB in red onion, whereas AcMYB4 and AcMYB5, which are homologous to each other but not to AcMYB1, act as suppressors. AcMYB1 has been confirmed as an activator in onion by transient overexpression and RNAi methods (Schwinn et al. 2016). Interestingly, in purple lablab pods, LpPAP1 seemed to be a dark-induced anthocyanin activator, because it is highly expressed under dark conditions and regulates most of the structural genes (*LpPAL, LpF3H, LpF3'H, LpDFR*, and *LpANS*) (Cui et al. 2016); however, the genes and the MYB were markedly suppressed when removed from dark conditions, leading to suppression of anthocyanin accumulation in the purple pods. Recently, it has been shown that DcMYB6 from *Daucus carota* and OjMYB1 from *Oenanthe javanica*, which group in the same clade as LpPAP1, enhanced the accumulation of anthocyanin in the same way as LpPAP1 (Feng et al. 2018; Xu et al. 2017) via upregulation of structural

Fig. 9 Phenotypes of onions (white) expressing low AcMYB1 and (red) expressing high AcMYB1. This image was taken from a publication by Schwinn et al. (2016) and is copyrighted by Frontiers

genes; however, they interacted with its co-transcription factor bHLH because they contained the conserved bHLH-interaction motif.

In summary, several MYBs have been shown to play critical roles in anthocyanin accumulation in vegetable crops and they are related by divergent evolution from a common ancestor. Generally, the majority of MYBs grouping in the same clade exhibited identical regulatory modes; however, some MYBs were likely to be dependent on environmental stimulus, organs, and developmental stages to induce anthocyanin.

Metabolic engineering of R2R3-MYB TFs to improve anthocyanin content

Ornamental plants

Flower color, which is determined by anthocyanins, is one of the most important commercial traits that interest flower enthusiasts and breeders. The MYBs participating in floral pigment accumulation have been well described (see above), and flower color modification in ornamental plants has been achieved by genetic engineering of the MYBs. In the present review, we describe the recent progress in color modification in important flower species by metabolic engineering of the MYBs (Table 4).

The overexpression of MYBs from gerberas, *Arabidopsis*, radishes, snapdragons, petunias, and tomatoes resulted in anthocyanin production in the flowers and other tissues (Mol et al. 1998; Borevitz et al. 2000; Mathews et al. 2003;

Table 4 Metabolic engineering of R2R3-MYBs to modify flower color or improve anthocyanin contents in fruit and vegetable crops

R2R3-MYBs	Overexpression in plant species	References
GMYB10	Gerbera	Elomaa et al. (2003), Laitinen et al. (2008)
C1	*Phalaenopsis* spp.	Ma et al. (2008)
C1	*Doritis* spp.	Griesbach and Klein (1993)
C1	*Cymbidium*	Albert et al. (2010)
AaMYB2	*Cymbidium*	
ROSEA1	Lisianthus	Schwinn et al. (2014)
RsMYB1	Chrysanthemum	Naing et al. (2015), Kee et al. (2016)
RsMYB1	Petunia	Ai et al. (2017)
PhDPL	Petunia	Quattrocchio et al. (1998)
PhPHZ	Petunia	Albert et al. (2011)
PhAN2	Petunia	Cavallini et al. (2014)
VaMYBA1	Petunia	Schwinn et al. (2014)
VaMYB5b	Petunia	Ben et al. (2008)
ROSEA1	Petunia	
AtPAP1	Petunia	
AtPAP1	Rose	Zvi et al. (2012)
VvMybPA1	Grape	Terrier et al. (2009)
VvMybPA2	Grape	
MdMYB9	Apple	An et al. (2015)
MdMYB11	Apple	
MdMYB10	Strawberry	Kortstee et al. (2011)
McMYB10	Crabapple and apple	Tian et al. (2015)
BoMYB2	Cauliflower	Chiu et al. (2010)
AtPAP1	*Brassica napus*	Ben et al. (2008)
SlANT1	Tomato	Mathews et al. (2003)
SlANT2	Tomato	Kiferle et al. (2015)
Ros1 + Del	Tomato	Butelli et al. (2008)
AtPAP1	Tomato	Zuluaga et al. (2008)
LeAN2	Tomato	Meng et al. (2015)
SmMYB1	Eggplant	Zhang et al. (2016)
Pr-D	Cauliflower	Chiu and Li (2012)
AcMYB1	Garlic	Schwinn et al. (2016)

Allan et al. 2008; Laitinen et al. 2008; Ai et al. 2016; Li et al. 2016). Of these MYBs, GmMYB10 was found to mainly induce cyanidin- and pelargonidin-based anthocyanins in transgenic gerbera flowers (Elomaa et al. 2003; Laitinen et al. 2008). In addition, the single expression of C1 (MYB), or its homolog AaMYB1 from *A. andraeanum*, could enhance pigmentation in petals in *Cymbidium* orchid cultivars (Albert et al. 2010). In fact, production of pigments in the white flowers of *Phalaenopsis* and *Doritis* by co-expression of C1 (MYB) and leaf color (Lc) from maize (bHLH) was reported more than two decades ago (Griesbach and Klein 1993; Ma et al. 2008). Similarly, the expression of AmROSEA1 alone or in combination with Lc in lisianthus and petunia also resulted in higher pigmentation in the flower petals and sepals than that in the control lines, by regulating the transcript levels of *CHS* and *ANS* (Schwinn et al. 2014); however, induction of a novel color was seen in petunias, particularly in the petal throat region and anthers. Recently, overexpression of RsMYB1 from radishes in chrysanthemum, petunia, and ornamental tobacco resulted in upregulated anthocyanin structural genes (Naing et al. 2015; Kee et al. 2016; Lim et al. 2016; Ai et al. 2017), and higher pigmentation was also observed in petunias and tobacco.

Homologous expression of PhAN2, PhDPL, and PhPHZ in petunias could increase pigmentation in flowers (Quattrocchio et al. 1998; Albert et al. 2011). Likewise, heterologous expression of the grapevine MYBs VvMYBA1 and VvMYB5b in petunias also increased color pigmentation in the petals (Cavallini et al. 2014). A similar pigmentation in petunia flowers could be obtained by overexpression of the MYB AtPAP1 (production of anthocyanin pigment 1), where it increased not only the levels of anthocyanins but also the emissions of floral volatiles (benzenoids) (Ben et al. 2008). Additionally, the role of the PAP1 was confirmed in roses, in which it significantly activated transcript levels of some structural genes and induced approximately six- to nine-fold higher anthocyanin content than that in the control (Zvi et al. 2012).

Fruit and vegetable crops

The health benefits of anthocyanins that have high antioxidant activity have been highlighted earlier in the present review; therefore, it is important to develop fruits and vegetables enriched with anthocyanins to obtain the desired quantity of health-promoting components in our daily diets. Anthocyanin production by metabolic engineering is an alternative method to obtain anthocyanin-rich fruits and vegetables. Metabolic engineering of MYBs to improve anthocyanin levels has been reported in some fruit and vegetable crops (Table 4). A common approach is to overexpress heterologous or homologous anthocyanin regulatory MYBs, when available, in the species of interest.

In grapes, the overexpression of VvMybPA1 or VvMybPA2 dramatically increased proanthocyanidin accumulation (Terrier et al. 2009), whereas, in apples, transgenic lines overexpressing MdMYB9 or MdMYB11 promoted anthocyanin accumulation (An et al. 2015). Increased anthocyanin content in the strawberry due to heterologous expression of apple MYB10 has been reported (Kortstee et al. 2011) and it has also been demonstrated that homologous expression of McMYB10 in crabapples and apples could promote anthocyanin accumulation (Tian et al. 2015).

Overexpression of BoMYB2 in the purple cauliflower enhanced anthocyanin production in the seed endosperm (Chiu et al. 2010), and overexpression of AtPAP1 in transgenic *B. napus* (canola) also enhanced the intense purple coloration and antioxidant capacity in the leaves up to fourfold (Akagi et al. 2010). In tomato, overexpression by activation tagging of the MYB SlANT1 increased anthocyanins in the fruit (Mathews et al. 2003). In addition, the combined expression of *ROSEA1* and *DELILA* genes or individual expression of MYBs (SlANT1 and SlANT2) produced purple- or red-colored tomatoes by strong promotion of anthocyanin structural genes (Butelli et al. 2008; Kiferle et al. 2015). Transgenic tomato plants expressing AtPAP1 and LeAN2 significantly enhanced anthocyanin levels in the leaves, stems, roots, flowers, and fruits under normal growth conditions (Zuluaga et al. 2008; Meng et al. 2015). Similarly, compared to wild-type eggplants, transgenic eggplants overexpressing SmMYB1 under normal growth conditions exhibited higher levels of anthocyanin in the leaves, petals, stamens, fruit peels, and especially in the fruit flesh, by transcriptional activation of most of the anthocyanin structural genes (Zhang et al. 2016). Moreover, overexpression of Pr-D/BoMYB2 in cauliflowers, AcMYB1 in onions, and Lc from maize in garlic also regulated anthocyanin accumulation (Fig. 10) via activation of structural genes (Chiu and Li 2012; Schwinn et al. 2016).

Fig. 10 Overexpression of AcMYB1 from onion in garlic enhanced anthocyanin accumulation. This image was taken from a publication by Schwinn et al. (2016) and is copyrighted by Frontiers

Conclusions and future perspectives

There is increasing interest in the modification of flower colors and the development of anthocyanin-rich fruits and vegetables. The identification of MYBs and the characterization of their roles in controlling anthocyanin biosynthesis in crop plants are crucial prerequisites for such modifications. The present review describes MYBs that have been reported thus far as anthocyanin promoters or suppressors in horticultural crops (flowers, fruits, and vegetables) and highlights their specific roles and their targeted genes involved in anthocyanin biosynthesis. In addition, phylogenetic analysis highlighted the different relationships of MYBs that are linked to production or suppression of anthocyanins in horticultural crops. Thus, the present review facilitates a better understanding of the regulation of anthocyanin accumulation in plants by MYBs and of the phylogenetic relationships between anthocyanin promoters and suppressors. Further, our review shows that in horticultural crops, the anthocyanin pathway can be manipulated by overexpressing R2R3-MYBs, which will pave the way for the development of new varieties and other commercially important plants, as well as the production of anthocyanin-rich foods by metabolic engineering approaches.

Acknowledgements This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ01368505)" Rural Development Administration, Republic of Korea. We sincerely thank to Dr. Khaing Thandar Soe (Seoul National University, South Korea) for her kind assistance in collection of difficult-to-access articles.

Author contributions AHN collected the literature and wrote the manuscript; CKK advised and assisted with the writing of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

Aharoni A, Ric De CH, Wein M, Sun ZK, Greco R, Kroon A, Mol JNM, O'Connell AP (2001) The strawberry FaMYB1 transcription factor suppresses anthocyanin and Xavonol accumulation in transgenic tobacco. Plant J 28:319–332

Ai TN, Naing AH, Arun M, Lim SH, Kim CK (2016) Sucrose-induced anthocyanin accumulation in vegetative tissue of petunia plants requires anthocyanin regulatory transcription factors. Plant Sci 252:144–150

Ai TN, Naing AH, Arun M, Jeon SM, Kim CK (2017) Expression of RsMYB1 in petunia enhances anthocyanin production in vegetative and floral tissues. Sci Hortic 214:58–65

Akagi T, Ikegami A, Yonemori K (2010) DkMyb2 wound-induced transcription factor of persimmon (*Diospyros kaki* Thunb.) contributes to proanthocyanidin regulation. Planta 232:1045–1059

Albert NW, Lewis DH, Zhang H, Irving LJ, Jameson PE, Davies KM (2009) Light-induced vegetative anthocyanin pigmentation in petunia. J Exp Bot 60:2191–2202

Albert NW, Arathoon S, Collette VE, Schwinn KE, Jameson PE, Lewis DH (2010) Activation of anthocyanin synthesis in *Cymbidium* orchids: variability between known regulators. Plant Cell Tiss Organ Cult 100:355–360

Albert NW, Lewis DH, Zhang H, Schwinn KE, Jameson PE, Davies KM (2011) Members of an R2R3-MYB transcription factor family in petunia are developmentally and environmentally regulated to control complex floral and vegetative pigmentation patterning. Plant J 65:771–784

Albert NW, Davies KM, Lewis DH, Zhang H, Montefiori M, Brendolise C (2014) A conserved network of transcriptional activators and repressors regulates anthocyanin pigmentation in eudicots. Plant Cell 26:962–980

Allan AC, Hellens RP, Laing WA (2008) MYB transcription factors that colour our fruit. Trends Plant Sci 13:99–102

An XH, Tian Y, Chen KQ, Liu XJ, Liu DD, Xie XB (2015) *MdMYB9* and *MdMYB11* are involved in the regulation of the JA-induced biosynthesis of anthocyanin and proanthocyanidin in apples. Plant Cell Physiol 56:650–662

Bagchi D, Sen CK, Ray SD, Das DK, Bagchi M, Preuss HG (2003) Molecular mechanisms of cardioprotection by a novel grape seed proanthocyanidin extract. Mutat Res 523–524:87–97

Ban Y, Honda C, Hatsuyama Y, Igarashi M, Bessho H, 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 48:958–970

Ben ZMM, Negre-Zakharov F, Masci T, Ovadis M, Shklarman E (2008) Interlinking showy traits: co-engineering of scent and colour biosynthesis in flowers. Plant Biotechnol 6:403–415

Borevitz JO, Xia YJ, Blount J, Dixon RA, Lamb C (2000) Activation tagging identifies a conserved MYB regulator of phenylpropanoid biosynthesis. Plant Cell 12:2383–2393

Borovsky Y, Oren-Shamir M, Ovadia R, Jong WD, Paran I (2004) The A locus that controls anthocyanin accumulation in pepper encodes a MYB transcription factor homologous to anthocyanin2 of petunia. Theor Appl Genet 109:23–29

Butelli E, Titta L, Giorgio M, Mock HP, Matros A, Peterek S (2008) Enrichment of tomato fruit with health promoting anthocyanins by expression of select transcription factors. Nat Biotech 26:1301–1308

Butelli E, Licciardello C, Zhang Y, Liu J, Mackay S, Bailey P (2012) Retrotransposons control fruit-specific, cold-dependent accumulation of anthocyanins in blood oranges. Plant Cell 24:1242–1255

Cavallini E, Zenoni S, Finezzo L, Guzzo F, Zamboni A, Avesani L (2014) Functional diversification of grapevine MYB5a and MYB5b in the control of flavonoid biosynthesis in a petunia anthocyanin regulatory mutant. Plant Cell Physiol 55:517–534

Cavallini E, Matus JT, Finezzo L, Zenoni S, Loyola R, Guzzo F (2015) The phenylpropanoid pathway is controlled at different branches by a set of R2R3-MYB C2 repressors in grapevine. Plant Physiol 167:1448–1470

Chagné D, Lin-Wang K, Espley RV, Volz RK, How NM, Rouse S (2013) An ancient duplication of apple MYB transcription factors is responsible for novel red fruit-flesh phenotypes. Plant Physiol 161:225–239

Chiou CY, Yeh KW (2008) Differential expression of MYB gene (OgMYB1) determines color patterning in floral tissue of Oncidium Gower Ramsey. Plant Mol Biol 66:379–388

Chiu LW, Li L (2012) Characterization of the regulatory network of BoMYB2 in controlling anthocyanin biosynthesis in purple cauliflower. Planta 236:1153–1164

Chiu LW, Zhou X, Burke S, Wu X, Prior RL, Li L (2010) The purple cauliflower arises from activation of a MYB transcription factor. Plant Physiol 154:1470–1480

Cui B, Hu Z, Zhang Y, Hu J, Yin W, Feng Y (2016) Anthocyanins and flavonols are responsible for purple color of *Lablab purpureus* (L.) sweet pods. Plant Physio Biochem 103:183–190

Davies KM, Albert NW, Schwinn KE (2012) From landing lights to mimicry: the molecular regulation of flower colouration and mechanisms for pigmentation patterning. Funct Plant Biol 39:619–638

Deluc L, Barrieu F, Marchive C, Lauvergeat V, Decendit A, Richard T (2006) Characterization of a grapevine R2R3-MYB transcription factor that regulates the phenylpropanoid pathway. Plant Physiol 140:499–511

Elomaa P, Uimari A, Mehto M, Albert VA, Laitinen RAE, Teeri TH (2003) Activation of anthocyanin biosynthesis in *Gerbera hybrida* (Asteraceae) suggests conserved protein-protein and protein-promoter interactions between the anciently diverged monocots and eudicots. Plant Physiol 133:1831–1842

Espley RV, Hellens RP, Putterill J, Stevenson DE, 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

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Feng S, Wang Y, Yang S, Xu Y, Chen X (2010) Anthocyanin biosynthesis in pears is regulated by a R2R3-MYB transcription factor PyMYB10. Planta 232:245–255

Feng K, Xu ZS, Que F, Liu JX, Wang F, Xiong AS (2018) An R2R3MYB transcription factor, OjMYB1, functions in anthocyanin biosynthesis in *Oenanthe javanica*. Planta 247:301–315

Fraser LG, Seal AG, Montefiori M, McGhie TK, Tsang GK, Datson PM (2013) An R2R3 MYB transcription factor determines red petal colour in an *Actinidia* (kiwifruit) hybrid population. BMC Genom 14:28

Griesbach RJ, Klein TM (1993) In situ genetic complementation of a flower color mutant in *Doritis pulherrima* (Orchidaceae). Lindleyana 8:223–226

He Q, Zhang ZF, Zhang LG (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 Food Chem 64:132–145

Hichri I, Barrieu F, Bogs J, Kappel C, Delrot S, Lauvergeat V (2011) Recent advances in the transcriptional regulation of the flavonoid biosynthetic pathway. J Exp Bot 62:2465–2483

Hou DX (2003) Potential mechanisms of cancer chemoprevention by anthocyanins. Curr Mol Med 3:149–159

Hsu CC, Chen YY, Tsai WC, Chen WH, Chen HH (2015) Three R2R3-MYB transcription factors regulate distinct floral pigmentation patterning in *Phalaenopsis* orchids. Plant Physiol 133:1831–1842

Huang W, Sun W, Lv H, Luo M, Zeng S, Pattanaik S (2013) A R2R3-MYB transcription factor from *Epimedium sagittatum* regulates the flavonoid biosynthetic pathway. PLoS ONE 8:e70778

Huang YF, Vialet S, Guiraud JL, Torregrosa L, Bertrand Y, Cheynier V (2014) A negative MYB regulator of proanthocyanidin accumulation, identified through expression quantitative locus mapping in the grape berry. New Phytol 201:795–809

Huang W, Khaldun ABM, Lv H, Du L, Zhang C, Wang Y (2016) Isolation and functional characterization of a R2R3-MYB regulator of the anthocyanin biosynthetic pathway from *Epimedium sagittatum*. Plant Cell Rep 35:883–894

Jia X, Shen J, Liu H, Li F, Ding N, Gao C (2015) Small tandem target mimic-mediated blockage of microRNA858 induces anthocyanin accumulation in tomato. Planta 242:283–293

Jin W, Wang H, Li M, Wang J, Yang Y, Zhang X (2016) The R2R3 MYB transcription factor PavMYB10. 1 involves in anthocyanin biosynthesis and determines fruit skin colour in sweet cherry (*Prunus avium* L.). Plant Biotechnol J 14:2120–2133

Jung CS, Griffiths HM, De Jong DM, Cheng S, Bodis M (2009) The potato developer (D) locus encodes an R2R3 MYB transcription factor that regulates expression of multiple anthocyanin structural genes in tuber skin. Theor Appl Genet 120:45–57

Kadomura-Ishikawa Y, Miyawaki K, Takahashi A (2015) RNAi-mediated silencing and overexpression of the *FaMYB1* gene and its effect on anthocyanin accumulation in strawberry fruit. Biol Plantarum 59:677–685

Kee ES, Naing AH, Lim SH, Han JS, Kim CK (2016) MYB transcription factor isolated from *Raphanus sativus* enhances anthocyanin accumulation in chrysanthemum cultivars. 3 Biotech 6:79

Kiferle C, Fantini E, Bassolino L, Povero G, Spelt C, Buti S et al (2015) Tomato R2R3-MYB proteins SlANT1 and SlAN2: same protein activity, different roles. PLoS ONE 10:e0136365

Klatsky AL (2002) Alcohol and cardiovascular diseases: a historical overview. Ann N Y Acad Sci 957:7–15

Kobayashi S, Ishimaru M, Hiraoka K, Honda C (2002) Mybrelated genes of the Kyoho (Vitis labruscana) regulate anthocyanin biosynthesis. Planta 215:924–933

Kortstee AJ, Khan SA, Helderman C, Trindade LM, Wu Y, Visser RG (2011) Anthocyanin production as a potential visual selection marker during plant transformation. Transgenic Res 20:1253–1264

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874

Lai B, Li XJ, Hu B, Qin YH, Huang XM, Wang HC (2014) LcMYB1 is a key determinant of differential anthocyanin accumulation among genotypes, tissues, developmental phases and ABA and light stimuli in *Litchi chinensis*. PLoS ONE 9:e86293

Laitinen RAE, Ainasoja M, Broholm SK, Teeri TH, Elomaa P (2008) Identification of target genes for a MYB-type anthocyanin regulator in *Gerbera hybrida*. J Exp Bot 59:3691–3703

Li JG, Li HL, Peng SQ (2011) Three r2r3-myb transcription factor genes from capsicum annuum showing differential expression during fruit ripening. Afr J Biotechnol 10:8267–8274

Li L, Ban Z, Li X, Wu M, Wang A, Jiang Y (2012) Differential expression of anthocyanin biosynthetic genes and transcription factor *PcMYB10* in pears (*Pyruscommunis* L.). PLoS ONE 7:e46070

Li C, Qui J, Yang G, Haung S, Yin J (2016) Isolation and characterization of a R2R3-MYB transcription factor gene related to anthocyanin biosynthesis in the spathes of *Anthurium andraeanum* (Hort.). Plant Cell Rep 35:2151–2165

Li C, Qui J, Ding L, Huang M, Huang S, Yang G (2017) Anthocyanin biosynthesis regulation of DhMYB2 and DhbHLH1 in *Dendrobium* hybrids petals. Plant Physiol Biochem 112:335–345

Lila MA (2004) Anthocyanins and human health: an *in vitro* investigative approach. J Biomed Biotechnol 5:306–313

Lim SH, Song JH, Kim DH, Kim JK, Lee JY, Kim YM (2016) Activation of anthocyanin biosynthesis by expression of the radish R2R3-MYB transcription factor gene RsMYB1. Plant Cell Rep 35:641–653

Lin-Wang K, Bolitho K, Grafton K, Kortstee A, Karunairetnam S, McGhie TK (2010) An R2R3 MYB transcription factor associated with regulation of the anthocyanin biosynthetic pathway in Rosaceae. BMC Plant Biol 10:50

Liu X, Xiang L, Yin X, Grierson D, Li F, Chen K (2015) The identification of a MYB transcription factor controlling anthocyanin biosynthesis regulation in chrysanthemum flowers. Sci Hortic 194:278–285

Liu Y, Lin-Wang K, Espley RV, Wang L, Yang H, Yu B (2016) Functional diversification of the potato R2R3 MYB anthocyanin activators AN1, MYBA1, and MYB113 and their interaction with basic helix-loop-helix cofactors. J Exp Bot 67:2159–2176

Lowry DB, Sheng CC, Lasky JR, Willis JH (2012) Five anthocyanin polymorphisms are associated with an R2R3-MYB cluster in *Mimulus guttatus* (Phrymaceae). Am J Bot 99:82–91

Ma H, Pooler M, Griesbach R (2008) Ratio of *Myc* and *Myb* transcription factors regulates anthocaynin production in orchid flowers. J Am Soc Hortic Sci 133:133–138

Martin C, Prescott A, Mackay S, Bartlett J, Vrljlandt E (1991) Control of anthocyanin biosynthesis in flowers of *Antirrbinum majus*. Plant J 1:37–49

Mathews H, Clendennen SK, Caldwell CG, Liu XL, Connors K, Matheis N (2003) Activation tagging in tomato identifies a transcriptional regulator of anthocyanin biosynthesis, modification, and transport. Plant Cell 15:1689–1703

Medina-Puche L, Cumplido-Laso G, Amil-Ruiz F, Hoffmann T, Ring L, Rodríguez-Franco (2014) *MYB10* plays a major role in the regulation of flavonoid/phenylpropanoid metabolism during ripening of *Fragaria* × *ananassa* fruits. J Exp Bot 65:401–417

Meng X, Wang JR, Wang GD, Liang XQ, Li XD, Meng QW (2015) An R2R3-MYB gene, *LeAN2*, positively regulated the thermotolerance in transgenic tomato. J Plant Physiol 175:1–8

Mol J, Grotewold E, Koes R (1998) How genes paint flowers and seeds. Trends Plant Sci 3:212–217

Morita Y, Saitoh M, Hoshino A, Nitasaka E, Iida S (2006) Isolation of cDNAs for R2R3-MYB, bHLH and WDR transcriptional regulators and Identification of c and ca mutations conferring white flowers in the Japanese morning glory. Plant Cell Physiol 47:457–470

Moyano E, Martinez-Garcia JF, Martin C (1996) Apparent redundancy in myb gene function provides gearing for the control of flavonoid biosynthesis in *Antirrhinum* flowers. Plant Cell 8:1519–1532

Naing AH, Ai TN, Jeon SM, Park KI, Lim KM, Kim CK (2015) Expression of RsMYB1 in chrysanthemum regulates key anthocyanin biosynthetic genes. Elect J of Biotechnol 18:359–364

Nakatsuka T, Haruta KS, Pitaksutheepong C, Abe Y, Kakizaki Y, Yamamoto K (2008) Identification and characterization of R2R3-MYB and bHLH transcription factors regulating anthocyanin biosynthesis in gentian flowers. Plant Cell Physiol 49:1818–1829

Nakatsuka A, Yamagishi M, Nakano M, Tasaki K, Kobayashi N (2009) Light-induced expression of basic helix–loop–helix genes involved in anthocyanin biosynthesis in flowers and leaves of Asiatic hybrid lily. Sci Hortic 121:84–91

Nishijima T, Morita Y, Sasaki K, Nakayama M, Yamaguchi H, Ohtsubo N (2013) A torenia (*Torenia fournieri* Lind. ex Fourn.) novel mutant 'flecked' produces variegated flowers by insertion of a DNA transposon into an R2R3-MYB gene. J Japanese Soc Horticult Sci 82:39–50

Niu SS, Xu CJ, Zhang WS, Zhang B, Li X, Lin-Wang K (2009) Coordinated regulation of anthocyanin biosynthesis in Chinese bayberry (*Myrica rubra*) fruit by a R2R3 MYB transcription factor. Planta 6:101–112

Palapol Y, Ketsa S, Lin-Wang K, Ferguson IB, Allan AC (2009) A MYB transcription factor regulates anthocyanin biosynthesis in mangosteen (*Garcinia mangostana* L.) fruit during ripening. Planta 229:1323–1334

Park NI, Xu H, Li X, Jang IH, Park S, Ahn GH (2011) Anthocyanin accumulation and expression of anthocyanin biosynthetic genes in radish (*Raphanus sativus*). J Agri Food Chem 59:6034–6039

Pérez-Díaz JR, Pérez-Díaz J, Madrid-Espinoza J, González-Villanueva E, Moreno Y, Ruiz-Lara S (2015) New member of the R2R3-MYB transcription factors family in grapevine suppresses the anthocyanin accumulation in the flowers of transgenic tobacco. Plant Mol Biol 90:63–76

Pervaiz S (2003) Resveratrol: from grapevines to mammalian biology. FASEB J 17:1975–1985

Pierantoni L, Dondini L, Franceschi PD, Musacchi S, Winkel BSJ, Sansavini S (2010) Mapping of an anthocyanin-regulating MYB transcription factor and its expression in red and green pear, *Pyrus communis*. Plant Physiol Bioch 48:1020–1026

Quattrocchio F, Wing JF, Leppen HTC, Mol JNM, Koes RE (1993) Regulatory genes controlling anthocyanin pigmentation are functionally conserved among plant species and have distinct sets of target genes. Plant Cell 5:1497–1512

Quattrocchio F, Wing JF, van der Woude K, Mol JNM, Koes R (1998) Analysis of bHLH and MYB–domain proteins: Species–specific regulatory differences are caused by divergent evolution of target anthocyanin genes. Plant J 13:475–488

Rahim MA, Busatto N, Trainotti L (2014) Regulation of anthocyanin biosynthesis in peach fruits. Planta 240:913–929

Ramsay NA, Glover BJ (2005) MYB-bHLH-WD40 protein complex and the evolution of cellular diversity. Trends Plant Sci 10:63–70

Ravaglia D, Espley RV, Henry-Kirk RA, Andreotti C, Ziosi V, Hellens RP (2013) Transcriptional regulation of flavonoid biosynthesis in nectarine (*Prunus persica*) by a set of R2R3 MYB transcription factors. BMC Plant Biol 13:68

Salvatierra A, Pimentel P, Moya-León MA, Herrera R (2013) Increased accumulation of anthocyanins in *Fragaria chiloensis* fruits by transient suppression of *FcMYB1* gene. Phytochemistry 90:25–36

Schwinn K, Venail J, Shang Y, Mackay S, Alm V, Butelli E (2006) A small family of MYB-regulatory genes controls floral pigmentation intensity and patterning in the genus *Antirrhinum*. Plant Cell 18:831–851

Schwinn KE, Boase MR, Bradley JM, Lewis DH, Deroles SC, Martin CR (2014) MYB and bHLH transcription factor transgenes increase anthocyanin pigmentation in petunia and lisianthus plants, and the petunia phenotypes are strongly enhanced under field conditions. Front Plant Sci 5:603

Schwinn KE, Ngo H, Kenel F, Brummell DA, Albert NW, McCallum JA (2016) The onion (*Allium cepa* L.) R2R3-MYB gene MYB1 regulates anthocyanin biosynthesis. Front Plant Sci 7:1865

Shang Y, Venail J, Mackay S, Bailey PC, Schwinn KE, Jameson PE (2011) The molecular basis for venation patterning of pigmentation and its effect on pollinator attraction in flowers of *Antirrhinum*. New Phytol 189:602–615

Shen X, Zhao K, Liu L, Zhang K, Yuan H, Liao X (2014) A role for PacMYBA in ABA-regulated anthocyanin biosynthesis in red-colored sweet cherry cv. Hong Deng (*Prunus avium* L.). Plant Cell Physio 55:862–880

Sooriyapathirana S, Khan A, Sebolt A, Wang D, Bushakra J, Lin-Wang K (2010) QTL analysis and candidate gene mapping for skin and flesh colour in sweet cherry fruit (*Prunus avium* L.). Tree Genet Genomes 6:821–832

Spelt C, Quattrocchio F, Mol JNM, Koes R (2000) *Anthocyanin1* of petunia encodes a basic helix-loop-helix protein that directly activates transcription of structural anthocyanin genes. Plant Cell 12:1619–1631

Starkevič P, Paukštytė J, Kazanavičiūtė V, Denkovskienė E, Stanys V, Bendokas V (2015) Expression and anthocyanin biosynthesis-modulating potential of sweet cherry (*Prunus avium* L.) MYB10 and bHLH genes. PLoS ONE 10:e0126991

Sun SS, Gugger PF, Wang QF, Chen JM (2016) Identification of a R2R3-MYB gene regulating anthocyanin biosynthesis and relationships between its variation and flower color difference in lotus (Nelumbo Adans.). PeerJ 4:e2369

Suzuki K, Suzuki T, Nakatsuka T, Dohra H, Yamagishi M, Matsuyama K (2016) RNA-seq-based evaluation of bicolor tepal pigmentation in Asiatic hybrid lilies (*Lilium* spp.). BMC Genom 17:611

Takos AM, Jaffé FW, Jacob SR, Bogs J, Robinson SP, Walker AR (2006) Light-induced expression of a MYB gene regulates anthocyanin biosynthesis in red apples. Plant Physiol 142:1216–1232

Telias A, Lin-Wang K, Stevenson DE, Cooney JM, Hellens RP, Allan AC (2011) Apple skin patterning is associated with differential expression of *MYB10*. BMC Plant Biol 11:93

Terrier N, Torregrosa L, Ageorges A, Vialet S, Verriès C, Cheynier V (2009) Ectopic expression of VvMybPA2 promotes proanthocyanidin biosynthesis in grapevine and suggests additional targets in the pathway. Plant Physiol 149:1028–1041

This P, Lacombe T, Cadle-Davidson M, Owens CL (2007) Wine grape (*Vitis vinifera* L.) color associates with allelic variation in the domestication gene *VvmybA1*. Theor Appl Genet 114:723–730

Tian J, Peng Z, Zhang J, Song TT, Wan HH, Zhang ML (2015) McMYB10 regulates coloration via activating *McF3′H* and later structural genes in ever-red leaf crabapple. Plant Biotechnol J 13:948–961

Vimolmangkang S, Han Y, Wei G, Korban SS (2013) An apple MYB transcription factor, MdMYB3, is involved in regulation of anthocyanin biosynthesis and flower development. BMC Plant Biol 13:176

Walker AR, Elizabeth L, Jochen B, Debra AJ, Mc D, Mark RT (2007) White grapes arose through the mutation of two similar and adjacent regulatory genes. Plant J 49:772–785

Wu XM, Lim SH, Yang WC (2003) Characterization, expression and phylogenetic study of R2R3-MYB genes in orchid. Plant Mol Biol 51:959–972

Xie QL, Hu ZL, Zhang YJ, Tian SB, Wang ZJ, Zhao ZP (2014) Accumulation and molecular regulation of anthocyanin in purple tumorous stem mustard (*Brassica juncea* var. *tumida* Tsen et Lee). J Agric Food Chem 62:7813–7821

Xu ZS, Feng K, Que F, Wang F, Xiong AS (2017) A MYB transcription factor, DcMYB6, is involved in regulating anthocyanin biosynthesis in purple carrot taproots. Sci Rep 7:4532

Yamagishi M (2011) Oriental hybrid lily Sorbonne homologue of LhMYB12 regulates anthocyanin biosyntheses in flower tepals and tepal spots. Mol Breed 28:381–389

Yamagishi M (2016) A novel R2R3-MYB transcription factor regulates light-mediated floral and vegetative anthocyanin pigmentation patterns in *Lilium regale*. Mol Breed 36:1–14

Yamagishi M, Shimoyamada Y, Nakatsuka T, Masuda K (2010) Two R2R3-MYB genes, homologs of Petunia AN2, regulate anthocyanin biosyntheses in flower tepals, tepal spots and leaves of Asiatic hybrid lily. Plant Cell Physiol 51:463–474

Yamagishi M, Yoshida Y, Nakayama M (2012) The transcription factor LhMYB12 determines anthocyanin pigmentation in the tepals of Asiatic hybrid lilies (*Lilium* spp.) and regulates pigment quantity. Mol Breed 30:913–925

Yamagishi M, Toda S, Tasaki K (2014) The novel allele of the *LhMYB12* gene is involved in splatter-type spot formation on the flower tepals of Asiatic hybrid lilies (*Lilium* spp.). New Phytol 201:1009–1020

Yoshida K, Ma D, Constabel CP (2015) The MYB182 protein downregulates proanthocyanidin and anthocyanin biosynthesis in poplar by repressing both structural and regulatory flavonoid genes. Plant Physiol 167:693–710

Yuan Y, Chiu LW, Li L (2009) Transcriptional regulation of anthocyanin biosynthesis in red cabbage. Planta 230:1141–1153

Yuan YW, Sagawa JM, Frost L, Vela JP, Bradshaw HD (2014) Transcriptional control of floral anthocyanin pigmentation in monkeyflowers (*Mimulus*). New Phytol 204:1013–1027

Yuan YW, Rebocho AB, Sagawa JM, Stanley LE, Bradshaw HD (2016) Competition between anthocyanin and flavonol biosynthesis produces spatial pattern variation of floral pigments between *Mimulus* species. Proc Natl Acad Sci USA 113:2448–2453

Zhang YJ, Hu ZL, Chu GH, Huang C, Tian SB, Zhao ZP (2014) Anthocyanin accumulation and molecular analysis of anthocyanin biosynthesis-associated genes in eggplant (*Solanum melongena* L.). J Agric Food Chem 62:2906–2912

Zhang Y, Hu Z, Zhu M, Zhu Z, Wang Z, Tian S (2015a) Anthocyanin accumulation and molecular analysis of correlated genes in Purple Kohlrabi (*Brassica oleracea* var. *gongylodes* L.). J Agric Food Chem 63:4160–4169

Zhang Z, Li DW, Jin JH, Yin YX, Zhang HX, Chai WG (2015b) VIGS approach reveals the modulation of anthocyanin biosynthetic genes by CaMYB in chili pepper leaves. Front Plant Sci 6:500

Zhang Y, Chu G, Hu Z, Gao Q, Cui B, Tian S (2016) Genetically engineered anthocyanin pathway for high health-promoting pigment production in eggplant. Mol Breed 36:54

Zhang Q, Hao R, Xu Z, Yang W, Wang J, Cheng T (2017) Isolation and functional characterization of a R2R3-MYB regulator of *Prunus mume* anthocyanin biosynthetic pathway. Plant Cell Tiss Organ Cult 131:417–429

Zhou Y, Zhou H, Lin-Wang K, Vimolmangkang S, Espley RV, Wang L (2014) Transcriptome analysis and transient transformation suggest an ancient duplicated MYB transcription factor as a candidate gene for leaf red coloration in peach. BMC Plant Biol 14:388

Zhou H, Lin-Wang K, Wang H, Gu C, Dare AP, Espley RV (2015) Molecular genetics of blood-fleshed peach reveals activation of anthocyanin biosynthesis by NAC transcription factors. Plant J 82:105–121

Zhou H, Peng Q, Zhao J, Owiti A, Ren F, Liao L (2016) Multiple R2R3-MYB transcription tactors involved in the regulation of anthocyanin accumulation in peach flower. Front Plant Sci 7:1557

Zhu L, Shan H, Chen S, Jiang J, Gu C, Zhou G (2013) The heterologous expression of the chrysanthemum R2R3-MYB transcription factor *CmMYB1* alters lignin composition and represses flavonoid synthesis in *Arabidopsis thaliana*. PLoS ONE 8:e65680

Zimmermann IM, Heim MA, Weisshaar B, Uhrig JF (2004) Comprehensive identification of *Arabidopsis thaliana* MYB transcription factors interacting with R/B-like BHLH proteins. Plant J 40:22–34

Zuluaga DL, Gonzali S, Loreti E, Pucciariello C, Degl'Innocenti ED, Guidi L (2008) *Arabidopsis thaliana* MYB75/PAP1 transcription factor induces anthocyanin production in transgenic tomato plants. Funct Plant Biol 35:606–618

Zvi MMB, Shklarman E, Masci T, Kalev H, Debener T, Shafir S (2012) PAP1 transcription factor enhances production of phenylpropanoid and terpenoid scent compounds in rose flowers. New Phytol 195:334–335