Combinatorial interactions of MYB and bHLH in flavonoid biosynthesis and their function in plants

Article · January 2014

CITATIONS
4

READS
1,019

4 authors, including:

Mohammed Nuruzzaman
Central South University
14 PUBLICATIONS **98** CITATIONS

SEE PROFILE

Journal of Plant Biology Research 2014, 3(2): 65-77

eISSN: 2233-0275
pISSN: 2233-1980
http://www.inast.org/jpbr.html

JPBR
Journal of Plant Biology Research

REGULAR ARTICLE

Combinatorial interactions of MYB and bHLH in flavonoid biosynthesis and their function in plants

Sadia Afrin[1]， Mohammed Nuruzzaman [1], Jie Zhu[1] and Zhi-Yong Luo[1, 2*]

[1]Molecular Biology Research Center, School of Life Sciences, Central South University, Changsha 410078, China.
[2]State Key Laboratory of Medical Genetics, Xiangya School of Medicine, Central South University, Changsha 410078, China.

ABSTRACT

MYB and basic helix-loop-helix (bHLH) are two important transcription factor (TF) families found in plants and animals. MYB-bHLH interactions control multiple enzymatic steps in flavonoid (anthocyanin and proanthocyanidin) biosynthesis pathway in plants. Moreover, they play roles in trichome and root hair formation, activation of vacuolar acidification, phytochrome A signaling, glucosinolate biosynthesis and abscisic acid (ABA) regulated gene expression. This review provides an insight into flavonoid biosynthesis by the interactions of MYB and bHLH TFs and their multitude biological functions that are important for various fundamental aspects of plant biology.

Keywords: flavonoid biosynthesis; MYB/bHLH transcription factors, MBW complex.

INTRODUCTION

MYB and bHLH transcription factors (TFs) are widely distributed in all eukaryotic organisms [1, 2]. MYB proteins are defined by a highly conserved MYB DNA binding domain at the N-terminus [1]. The first gene defined as encoding a MYB domain-containing protein was *v-myb* in the genome of avian myeloblastosis virus (AMV), therefore called 'MYB' [3]. Plant MYB proteins are classified into three major groups: R2R3-MYB, R1R2R3-MYB and a heterogeneous group together mention as the MYB-related proteins [4, 5]. Among these groups, R2R3-MYBs are the largest group of plant MYB TFs that includes hundreds of members of terrestrial plants where they are involved in diverse physiological and biochemical processes including the regulation of secondary metabolism, control of cell morphogenesis, regulation of meristem formation, floral and seed development and the control of the cell cycle. Some were also involved in various defense and stress responses and in light and hormone signaling pathways [6]. The first plant *MYB* gene *C1* was isolated from *Zea mays* in 1987 and encoded a c-myb-like TF concerned in anthocyanin biosynthesis [7], whereas, the bHLH motif was initially noticed by Murre and colleagues (in 1989) in two murine TFs known as E12 and E47 [8]. MYB and bHLH domain were highly conserved and comprise approximately 50-53 and 60 amino acids respectively. These two important TF families have been extended significantly in plants: approximately 339 and 162 genes in *Arabidopsis thaliana* and 230 and 111 genes in *Oryza sativa*. These two transcription activators can form homo and/or hetero-dimers within the members of the same family or between different TF family members and form strong transcriptional complexes [3]. Combinatorial interaction between MYB and bHLH TFs in flavinoid biosynthesis has been elucidated in various plant species. For example, the information seized on the combinatorial

* Corresponding author: Zhi-Yong Luo, Molecular Biology Research Center, School of Life Sciences, Central South University, Changsha 410078, China Tel: +86-731-84805025; E-mail: luo_zhiyong@hotmail.com

performance of these two TFs in *Z. mays* and *A. thaliana* provides an outstanding allusion to explore flavonoid biosynthesis in crops and other plants [3, 9]. However, members of these two TFs families can also interact with a number of other regulatory proteins such as WD-repeat (WDR) proteins that have been progressively identified as a key regulator in many eukaryotic cellular processes. The WD-repeat is a structural motif comprising approximately 44-60 amino acids usually ending with the amino acid sequence tryptophan (W)-aspartic acid (D) and hence the name WD [6, 10]. It has been shown that the cooperative interaction of MYB and bHLH proteins regulate diverse functions in plants such as trichome and root hair formation [11], activation of vacuolar acidification [12], phytochrome A signaling [13], glucosinolate biosynthesis [14] and ABA regulated gene expression [15]. Here, we review an update about flavonoid biosynthesis (anthocyanin and proanthocyanidin) by the interactions of MYB and bHLH TFs associated with other protein families and their diverse roles in plants.

1. MYB-bHLH interactions in flavonoid biosynthesis pathway

Flavonoids are ubiquitous low molecular weight polyphenolic secondary metabolites that are widespread throughout the plant kingdom ranging from mosses to angiosperms. They accumulate in most vascular plants and play an essential role in many fundamental aspects of plant biology including pigmentation, pollen viability, plant-microbe interactions and UV protection [16]. The majority of flavonoids are conserved among plant species. Flavonoid biosynthesis starts with the amino acid phenylalanine and the end products include anthocyanins, flavones/isoflavones and condensed tannins (also known as proanthocyanidins, PAs) [17]. In flavonoid biosynthesis pathway two classes of genes can be distinguished: (I) the biosynthetic genes encoding enzymes directly participate in the formation of flavonoids and (II) regulatory genes that control the expression of the biosynthetic genes [18]. In various plant species, it has been shown that tissue-specific regulation of the biosynthetic genes involved in flavonoid biosynthesis is directly controlled by the cooperative interactions between MYB and bHLH TFs [17]. Cooperation between MYB and bHLH proteins have been mainly studied in *Petunia hybrida*, *Antirrhinum majus* and *Z. mays* as regulators of anthocyanin biosynthesis and more recently in *A. thaliana* as regulators of anthocyanin and proanthocyanidin biosynthesis [19]. In addition, members of these two TFs families also interact with a number of other regulatory proteins such as WD-repeat (WDR) proteins that take part in chromatin remodeling through histone protein modification, thereby influence transcription processes [20, 21]. WDR proteins are not thought to have any catalytic activity rather they seem to be a docking platform in anthocyanin and proanthocyanidin biosynthesis pathways. A significant number of WDR proteins involved in the regulation of the flavonoid pathway have been identified so far, including *P. hybrida* Anthocyanin 11 (AN11), *A. thaliana* TTG1 [10], *Perilla frutescens* PFWD [21], *Z. mays* Pale Aleurone Color1 (PAC1) [22], *Medicago trunculata* WD40-1[23] and *Vitis vinifera* WDR1 and WDR2 [24]. These WDR proteins appear to be highly conserved among the plant species. In most of the plant species MYB, bHLH and WDR protein combine together and form MYB-bHLH-WDR (MBW) complex that either activate and/or repress the expression of sets of target genes thus regulate the flavonoid biosynthesis pathway. Formation of the MBW complex has so far been assumed to be unique to plants. Most of these regulatory protein complexes have been demonstrated to be functionally conserved among plant species [16, 17]. The coordinated expression of flavonoid pathway genes by MBW complexes exemplifies combinatorial gene regulation in plants. Based (partly) on the genes regulated by MBW complexes, the flavonoid biosynthesis pathway is subdivided into two steps, 'early' steps that are more or less independent of MBW complexes and 'late' steps that are dependent on this complexes (Figure 1) [25, 26].

Step 1: Early biosynthetic gene regulation

In the initial steps of the flavonoid pathway, Phenylalanine is metabolized to yield coumaroyl-CoA by a series of enzymatic reactions. Then chalcone synthase (*CHS*) catalyzes the production of naringenin chalcone by combining one coumaroyl CoA molecule with three malonyl CoA molecules [16, 27, 28]. *CHS* is the first committed enzyme in flavonoid biosynthesis. Studies have shown that these enzymes interact via protein-protein interactions. *CHS* is believed to act as a central role for the enzymes involved in the

flavonoid pathway. Chalcone is then isomerized to flavanone by chalcone isomerase (*CHI*) and from this step the pathway diverges to form different classes of flavonoids. In the next step, flavanones are converted to dihydroflavonols by flavanone 3-hydroxylase (*F3H*) (Figure 1) [16, 28]. These enzymes are known as early biosynthetic genes (EBGs) in *A. thaliana*. These genes leading to the production of flavonols are activated by co-activator independent and functionally redundant R2R3-MYB regulatory genes MYB11, MYB12 and MYB111 in *A. thaliana* [26, 29]. However, in monocot species, these EBGs are regulated by the combinatorial interactions of MYB and bHLH protein families. For example, in *Z. mays* MYB and bHLH proteins are encoded by two multi-gene families PL/C1 and B/R respectively are committed to regulate these EBGs gene successfully [22] (Figure 1). In other plants such as *P. hybrida* and *A. majus* distinct sets of MYB-bHLH interactions are also responsible for regulating the EBGs (*CHS* up to *F3H*) in flavonoid biosynthesis pathway [30].

Step 2: Late biosynthetic gene regulation

In this step, dihydroflavonol reductase (*DFR*) catalyzes the reduction of dihydroflavonols to flavan-3,4-diols (leucoanthocyanins) which are then converted to anthocyanins through a series of enzymatic steps [16, 27, 28]. *ANS/LDOX* and *UFGT* genes are required for anthocyanin biosynthesis known as late biosynthetic genes (LBGs) in *A. thaliana.* There is an interesting exception is presented by the *F3´H* biosynthetic gene that appears to be regulated by both EBGs and LBGs (Figure 1) consistent with its requirement in the synthesis of both quercetin type flavonols and cyanidin type anthocyanins [26, 29]. In *A. thaliana* LBGs (*DFR*, *ANS/LDOX*, *UFGT*) are regulated by various MBW complexes. For example, MBW complex comprised of R2R3 MYBs, MYB75 (production of anthocyanin pigments1, PAP1)/MYB90 (PAP2)/MYB113/114, the bHLH factors, glabrous3 (GL3)/enhancer of glabrous3 (EGL3)/transparent testa8 (TT8) and the WDR protein, transparent testa glabra1 (TTG1) can effectively regulate the LBGs of flavonoid biosynthesis pathway in *A. thaliana* (Figure 1). The bHLH TFs, GL3, EGL3 and TT8 play partially redundant roles in control of the anthocyanin pathway where EGL3 plays a major role in activation of LBGs [26]. Combinatorial interactions of MYB and bHLH TFs also play a vital role to regulate anthocyanin biosynthetic genes (both EBGs and LBGs) in flower and fruit plants. It has been shown that in dicots distinct sets of MYB-bHLH interactions are responsible for regulating the early part (*CHS* up to *F3H*) or/and the late part (*DFR* to *3GT*) of anthocyanin biosynthesis pathway [30]. For example, in *P. hybrida*, two different ternary MBW complexes composed by the WDR protein AN11 and bHLH anthocyanin1 (AN1) proteins interacting with the R2R3-MYB anthocyanin2 (AN2) proteins in petals or the R2R3-MYB anthocyanin4 (AN4) in anthers for anthocyanin biosynthesis [12, 31].

In flowers of *Ipomoea purpurea* anthocyanin biosynthesis is activated by an MBW complex composed of the MYB1, bHLH2 and WDR1 proteins respectively [32, 33], whereas R2R3-MYB TF, NtAN2 and bHLH TF, NtAN1 collectively regulate anthocyanin accumulation in the flowers of *Nicotiana tabacum*. The NtAN2-NtAN1 complex strongly activates the LBGs and moderately the EBGs [34, 35]. In *A. majus*, the R2R3-MYBs, rosea1, rosea2 and venosa control floral anthocyanin accumulation through interaction with bHLH factors, delila or mutabilis. The bHLH delila is required for the expression of LBGs (including *F3H, DFR, ANS/LDOX, UFGT*), whereas the R2R3-MYB proteins differentially regulate some EBGs and LBGs. In fact, rosea1 controls *F3H, FLS, F3´H, DFR, ANS/LDOX, UFGT* and then rosea2 activates only *CHI* and *F3´H* and finally venosa activates *CHI* and the same biosynthesis genes as rosea1 except *DFR* [36].

In fruits plant *M. domestica*, MYB1, MYB10 and MYBA act as activators of anthocyanin synthesis in skin and flesh when co-expressed with bHLH factors, bHLH3 and bHLH33 [37-39], whereas a set of R2R3-MYBs (MYBA1/2/5a/5b), bHLH (MYC1, MYCA1) and WDR (WDR1 and WDR2) proteins were responsible for anthocyanin biosynthesis in *Vitis vinifera* [24, 40]. Recently anthocyanin biosynthesis has also been studied in some vegetables. Orthologous genes of the *Arabidopsis* MBW complex have been identified in cauliflower and red cabbage [41, 42]. In leaves of red cabbage MYB TF, MYB2 combined with a bHLH gene, TT8 and regulates some EBGs together with LBGs (i.e. *CHS, F3H, F3´H, DFR, ANS/LDOX*) [42]. However, in monocot species, combinatorial interactions of MYB and bHLH TFs are also able to

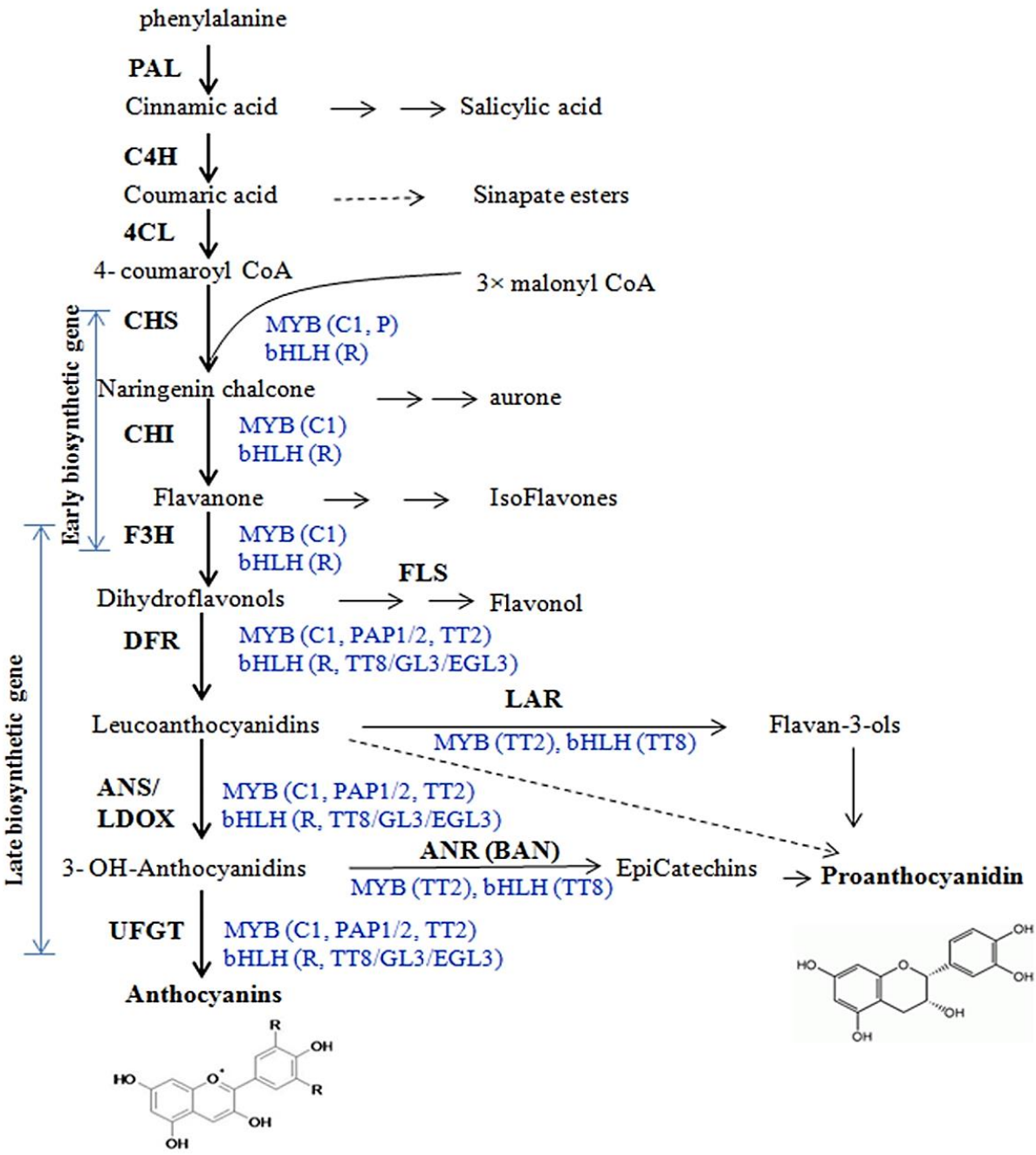

Figure 1. Simplified diagram of flavonoid (anthocyanin and proanthocyanidin) biosynthesis pathway. Enzymes that catalyze the reactions are shown in capital and bold letters on the left hand side and transcription factors on the right hand side of the arrows. Unbroken arrows indicate single enzymatic conversions and broken or double arrows indicate multiple enzymatic steps. Transcription factors C1, R and P are from maize, whereas PAP1/2, TT2, TT8, GL3 and EGL3 are from *A. thaliana*. Abbreviations: bHLH, basic Helix-Loop-Helix; BAN, BANYULS; CHS, chalcone synthase; C4H, cinnamate 4-hydroxylase; CHI, chalcone-flavanone isomerase; 4CL, 4-coumaroyl-coenzyme A ligase; DFR, dihydroflavonol 4-reductase; F3H, flavanone 3-hydroxylase; LAR, leucoanthocyanidin reductase; LDOX, leucoanthocyanidin dioxygenase; PAL, phenylalanine ammonia lyase; PAP1/2, production of anthocyanin pigment1/2; TT2/8, transparent testa2/8; UFGT, UDP glucose-flavonol glucosyl transferase.

regulate LBGs. For example, combinatorial action of MYB (PL/C1) and bHLH (B/R) proteins are responsible for LBGs regulation, while a WDR protein PAC1 is required for full activation of anthocyanin biosynthetic genes in seeds and roots of *Z. mays* [30]. By contrast, MYB P1 which promotes phlobaphene synthesis in kernels activates a subset of anthocyanin biosynthetic genes (i.e. *CHS, CHI, DFR and FLS*) without bHLH interactors [43, 44] indicating the existence of a partial overlap in the regulation of early biosynthetic genes.

In the side chain of flavonoid biosynthesis pathway leucoanthocyanidin reductase (*LAR*) catalyzes the synthesis of catechin (an initiating monomer of PA synthesis) from 3,4-*cis*-leucocyanidin [45]. Anthocyanidins are also converted to PAs by anthocyanidin reductase (*ANR*) that is encoded by the BANYULS (*BAN*) gene [46]. *BAN* expression is strictly restricted to PA accumulating cells during seed coat development [47]. This specific expression pattern appears to be mainly conferred by TT2, an R2R3-MYB TF [47, 48]. TT2 acts as a master regulator as its mutation affects the expression of LBGs *DFR*, *LDOX* and proanthocyanidin-specific genes *BAN* in developing region [48, 49]. Two additional regulatory genes *TT8* and *TTG1* were also shown to participate in the control of *BAN* expression that encoded by bHLH and WDR protein respectively [49, 50] (Figure 1). It appears that the above three TFs work in concert and positively regulate the expression of *BAN*, *DFR* and *LDOX*. Another category of TFs includes TT1 (zinc finger protein), TT16 (MADS domain protein) and TTG2 that coordinate cell and organ development for PA deposition [50, 51]. TT1 and TT16 are essential for PA biosynthesis, as both of these proteins modify the spatial pattern of *BAN* gene expression by regulating the LBGs of flavonoid biosynthesis pathway [47].

Recent studies reveal that there is an interesting difference in flavonoid biosynthesis between monocot and dicot species like *Z. mays* and *A. thaliana* respectively. Regardless the type of biosynthetic genes (early or late) in monocot *Z. mays*, MYB-related protein and a bHLH-containing protein interact and activate the flavonoid biosynthetic genes (*CHS, CHI, F3H, DFR, ANS/LDOX, UFGT*) as a single unit by a ternary MBW complex, where in dicot *A. thaliana*, MBW complex only regulate the LBGs in flavonoid biosynthesis pathway. Collectively, it appears that both in monocot and dicot species, combinatorial interactions of MYB and bHLH TFs can regulate flavonoid biosynthetic genes (EBGs and/or LBGs), thus regulate the pathway.

2. Function

Combinatorial interactions among TFs are central to gene regulation of any given cellular process [52, 53]. The physical interaction and regulatory cooperation between specific sub-classes of MYB and bHLH TFs are involved in controlling various development and physiological processes in a wide range of plant species (Table 1) [54, 55]. Though flavonoid biosynthesis is probably the best-studied example of cooperation between MYB and bHLH proteins [56], on the other hand their cooperative interactions are also able to perform various functions in different plant species.

2.1. Trichome and root hair formation

Hair is single or multicellular, absorptive (root hair) or secretory (glandular hair) and sometimes only a superficial outgrowth (covering hair) of the epidermal cells. Trichomes (leaf hairs) are large, branched, single cells and easily visible with the bare eye that initiated and developed on young leaves in a regular spacing pattern [72]. It is very difficult to differentiate between hairs and trichomes. Generally trichomes are connected to the vascular system, whereas hairs have no vascular connection. Like flavonoid biosynthesis, *A. thaliana* trichome and root hair formation are also directed by the interplay of specific sub-groups of R2R3-MYB and bHLH TFs, in cooperation with the WDR protein TTG1, forming MBW complex [11]. Various MBW complexes control trichome initiation [73, 74], trichome branching [63] and root hair formation [75]. Some of these functions are highly conserved in other plants, such as cotton fiber development [72]. For illustration, Glabra1 (GL1) and GL3/EGL3 form a complex in *Arabidopsis* trichome formation, homologs of GL1 and GL3/ EGL3 have been identified in cotton and shown to play a role in trichome development [76], GL3 and GL1 interact when over expressed together in plants. Yeast two hybrid analysis showing that TTG1 and GL1 physically interact with GL3/EGL3 but not with each other [25, 76]. Glabra2 (GL2), a

Table 1. MYB and bHLH TFs associated with WDR proteins and their functions in different plant species

SN.	Plant Species	MYB proteins	bHLH proteins	WDR proteins	Function in plants	Ref
1.	*Arabidopsis thaliana*	PAP1/2, MYB113/114	GL3/EGL3/ TT8	TTG1	Anthocyanin biosynthesis	[57]
2.	*A. thaliana*	TT2	TT8	TTG1	Proanthocyanidin accumulation in developing seeds	[48]
3.	*A. thaliana*	MYBL2	TT8/GL3/ EGL3/MYC1	TTG1 (experimentally not established)	Anthocyanin biosynthesis	[58]
4.	*A. thaliana*	MYB75/ GL1	TT8/GL3/ EGL3	-	Anthocyanin accumulation and trichome initiation	[59]
5.	*A. thaliana*	GL1	EGL3/GL3	TTG1	Enhance trichome initiation	[60]
6.	*A. thaliana*	WER	EGL3/GL3	TTG1	Root hair formation	[25, 61]
7.	*A. thaliana*	TRY/CPC/ ETC	EGL3/GL3	TTG1	Trichome and root hair formation	[61-63]
8.	*A. thaliana*	MYB5/23	EGL3	TTG1	Mucilage synthesis, seed coat development and trichome morphogenesis	[64]
9.	*A. thaliana*	MYB5	EGL3/TT8	TTG1	Control outer seed coat differentiation	[65]
10.	*A. thaliana*	LAF1	HFR1	COPI	Phytochrome A signaling	[13]
11.	*A. thaliana*	MYB34	MYC2	-	Glucosinolate Biosynthesis	[14]
12.	*Antirrhinum majus*	Rosea1, rosea2 and venosa	Delila or mutabilis	-	Anthocyanin biosynthesis	[3]
13.	*Brassica oleracea*	Purple	bHLH1	WD40	Anthocyanin biosynthesis	[42]
14.	*Gentian triflora*	MYB3	bHLH1	-	Anthocyanin biosynthesis	[66]
15.	*Gerbera hybrida*	MYB10	MYC1	-	Anthocyanin biosynthesis	[67]
16.	*Petunia hybrida*	AN2/4	AN1	AN11	Anthocyanin/ proanthocyanidin biosynthesis	[68]
17.	*P. hybrid*	AN2/4	AN1	AN11 (experimentally not established)	Activates vacuolar acidification	[12]
18.	*Ipomoea purpurea*	MYB1	bHLH2	WDR1	Anthocyanin biosynthesis	[32, 33]
19.	*Malus domestica*	MYB1/10	bHLH3/33	-	Anthocyanin biosynthesis	[38]
20.	*M. domestica*	TT2	TT8	TTG1 (experimentally not established)	Anthocyanins regulation	[69]
21.	*Nicotiana tabaccum*	AN2	AN1	-	Anthocyanin biosynthesis	[34, 35]
22.	*Oryza sativa*	C1	Ra/RC	-	Proanthocyanidin biosynthesis	[70]
23.	*Vitis vinifera*	MYBA1/2/5a/5b	MYC1, MYCA1	WDR1 and WDR2	Anthocyanin biosynthesis	[24, 40]
24.	*Zea mays*	C1/PL	R/B	PAC1	Anthocyanin/ proanthocyanidin biosynthesis	[71]

homeodomain (HD-Zip) is a direct target of GL3 and EGL3 [74] and TTG2 is directly regulated by GL1. This activation is believed to be instigated through the formation of GL1-GL3-TTG1and GL1-EGL3- TTG1 (MBW) regulatory complexes, thereby regulating trichome cell fate [74] (Figure 2a). To date, at least four homologous single MYB proteins such as triptychon (TRY) [61], caprice (CPC) and enhancer of triptychon and caprice 1 and 2 (ETC1 and 2) [62, 63] have been identified as negative regulators of trichome initiation and patterning. Protein interaction analysis in yeast suggested that TRY or CPC would interrupt the functionality of the activating MBW complex by competitive interaction with the bHLH [25, 77] (Figure 2a). Nevertheless, plenty of the data that have explained the molecular mechanism of these regulators is either indirect or acquired from another similar pathway such as root hair patterning. For instance, evidence for the existence of the MYB-bHLH-TTG1 complex is exclusively based on protein interaction studies in yeast [25, 76]. The expression of CPC in the root epidermis is GL3/EGL3 dependent and directly regulated by the MYB werewolf (WER), a GL1 equivalent protein in root hair patterning [78, 79] (Figure 2b). GL2, CPC and ETC1 are straightly activated by GL3 and this targeting is GL1 dependent [74], but loss of TTG1 or GL1 disorganize the distribution of GL3 [73]. From these discussions, it could be postulated that GL1-GL3/ EGL3-TTG1 and WER-GL3/EGL3-TTG1complexes regulate GL2 expression, which promotes differentiation into trichomes and root hairless cells, respectively. MYB proteins such as TRY and CPC inhibit the formation of these complexes, resulting in inhibition of GL2 expression. Based on above data, a hypothetical regulatory network for trichome and root hair formation is delineated in Figure 2 (a, b).

2.2. Activation of vacuolar acidification

In most plant species, the pigmentation of flowers and fruits are derived from the accretion of anthocyanin pigments (flavonoids) in the vacuoles of epidermal or sub-epidermal cells. The absorption spectrum of anthocyanins depends on the pH of environment and the color of a tissue depends upon the pH of the vacuolar lumen, therefore, making flower color suitable and consistent [80]. So, it is clear that pH of vacuole is important for pigmentation and also important for a variety of

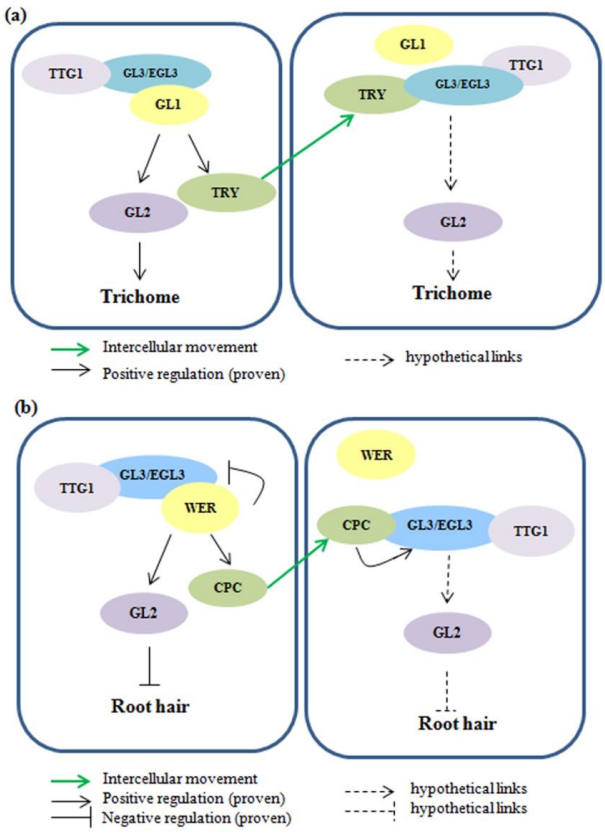

Figure 2 (a, b). MYB-bHLH interactions in root trichome and hair differentiation

(a) In trichome fate determination TTG1/GL1/GL3/EGL3 act as regulators, GL2 act as activators and TRY act as inhibitors. The GL1-GL3/EGL3-TTG1complex promotes *GL2* and *TRY* expression. The TRY protein moves into neighboring cells where it competes with GL1 for binding to GL3/EGL3. Neither the TRY-GL3/EGL3-TTG1 complex nor dissociated GL1 can promote *GL2* or *TRY* expression, thus differentiate into trichome cells. Abbreviations: EGL3, enhancer of Glabra3; GL3, glabra3; GL1, glabrous1; TTG1, transparent testa glabra1; TRY, triptychon.

(b) In *Arabidopsis*, there is a relatively high level of WER relative to CPC, which enables to form a WER-GL3/EGL3 complex and promote GL2 and CPC transcription. The CPC protein moves into neighboring cells where it competes with WER for binding to GL3/EGL3. Neither the CPC-GL3/EGL3-TTG1 complex nor dissociated WER can promote *GL2* or *CPC* expression. GL3/EGL3 expression is positively regulated by *CPC* and negatively regulated by *WER*, *GL3*, *EGL3* and *TTG1*. Cells expressing *GL2* differentiate into hairless cells. Abbreviations: CPC, caprice; EGL3, enhancer of glabra3; GL3, glabra3; TTG, transparent testa glabra; WER, werewolf.

physiological processes, such as osmoregulation, ion transport and storage of metabolites. Moreover, it plays an imperative role in cell growth, because the enlargement of the vacuole volume is more responsible for cellular growth rather than of the cytoplasm [81, 82]. Combinatorial interactions of MYB and bHLH TFs could activate vacuolar acidification in different plant species. For explanation, *P. hybrida* genes *AN2* and *AN1*encoded by MYB and bHLH TFs respectively that is required for anthocyanin synthesis and acidification of the vacuole in petal cells. *Petunia* AN11, a WDR protein forms a complex with AN1 and AN2 (petal limbs) or AN4 (anthers) that control vacuolar acidification thus raise flower color suitable in *P. hybrida* [12, 31].

2.3. Phytochrome A signaling

Another example of MYB-bHLH association is the interaction of LAF1 (long after far-red light1) and HFR1 (long hypocotyl in far-red1) during regulation of phytochrome A (phyA) signaling. LAF1, a MYB TF and HFR1, a bHLH TF regulate the part of phyA signaling pathway mostly independently and also cooperate with each other, that proved by mutant analyses [13, 83]. Phytochrome A plays a vital role in seedling depilation in *A. thaliana* [84]. Analysis of hypocotyl lengths suggests that HFR1 and LAF1 are likely to operate close to the bottom of the phyA signaling cascade consistent with their biochemical functions [83]. However, it is not yet well known whether LAF1 and HFR1factor mediate the phyA signals by a direct transcriptional interaction.

2.4. Dehydration and abscisic acid (ABA) inducible gene expression

MYB-bHLH complex can also activate the dehydration and abscisic acid (ABA) inducible gene expression. In *Arabidopsis*, *rd22* (dehydration-responsive gene) induction is mediated by ABA and requires protein biosynthesis for ABA-dependent gene expression. Promoter analysis of rd22 reveals that two closely located putative recognition sites for the bHLH protein MYC and one putative recognition site for MYB is required in support of dehydration and ABA induced gene expression. Abe H. et al reported ABA-inducible genes *ATMYBS* and *rd22BP1* that encodes by the MYB and bHLH related proteins respectively. Transient trans-activation experiment indicates that both the ATMYB2 (MYB) and rd22BP1 (bHLH) proteins function as transcriptional activators in the dehydration and ABA-inducible expression of the *rd22* gene [15].

2.5. Glucosinolate biosynthesis

The specific interaction of bHLH-MYB plays a crucial role in the regulation of defense related secondary metabolites production, such as glucosinolate biosynthesis. These natural chemicals most likely contribute to plant defense against pests and diseases, but are also enjoyed in small amounts by humans and are believed to contribute to the health promoting properties. Yeast two-hybrid and pull-down experiments indicated that bHLH TF interacts directly with GS-related MYBs. Glucosinolate biosynthetic genes are regulated by six R2R3-MYB TFs. MYB28, MYB29 and MYB76 act in concert as a complex interaction to control aliphatic glucosinolate biosynthetic genes [85-87], whereas MYB34, MYB51 and MYB122 control indole glucosinolate biosynthetic genes. Furthermore, bHLH TFs (including MYC2, MYC3 and MYC4) is required to regulate the expression of glucosinolate pathway genes. Among these MYB and bHLH TFs, MYB34 and MYC2 contribute to enhance the formation of MYB-bHLH complex and might potentiate glucosinolate biosynthesis [88].

Based on above evidence, it is intriguing to recommend that R2R3-MYB consistently functions in a combinatorial manner with bHLH TF families. It is also clear that TFs often interact with other regulatory proteins. Combination of such transcriptional network might be an attractive and biotechnical challenging goal for manipulation of natural plant product biosynthesis and their possible biotechnological application. The cooperative interactions between MYB and bHLH TFs organizes regulatory networks that control anthocyanin and proanthocyanidin biosynthesis and their other functions in various plant species are summarized diagrammatically in Figure 3.

3. Conclusions

Combinatorial interactions between MYB and bHLH TF families play important role in regulating biosynthetic genes in flavonoid biosynthesis pathway and also involve in the regulation of many plant cellular processes. In the last decades, an increasing number of flavonoid biosynthesis pathways have been elucidated in a variety of plant

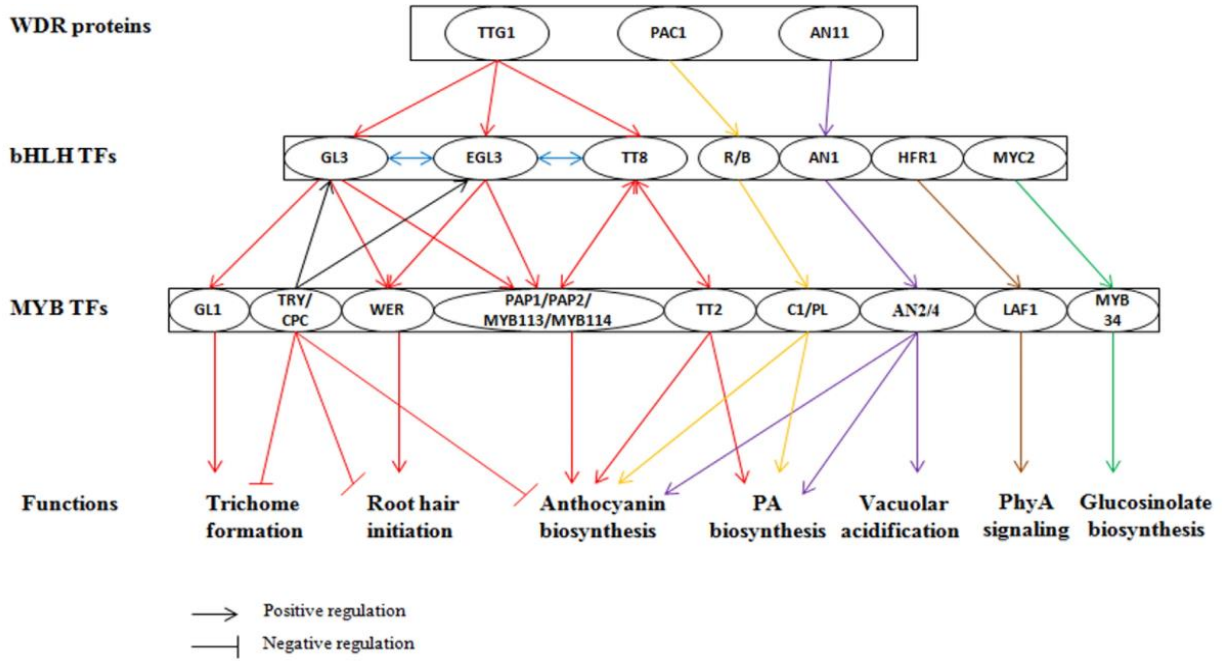

Figure 3. Functions of MYB-bHLH interactions in plants
This model shows that bHLH and MYB TFs interact with WDR proteins and regulate diverse functions in different plant species. Here TTG1, GL3, EGL3, TT8, GL1, TRY/CPC, WER, PAP1/PAP2/MYB113/MYB114, TT2, HFR1, LAF1, MYC2, MYB34 proteins were from *A. thaliana*; PAC1, R/B, C1/PL proteins were from *Z. mays* and AN11, AN1, AN2/4 proteins were from *P. hybrida*.

species. Recent evidences show that anthocyanin biosynthesis pathway is controlled by a common basic regulatory system consisting of a ternary complex of MYB-bHLH-WDR proteins. More evidences indicate that pH of vacuole is also important for flavonoid biosynthesis pathway, because anthocyanins change their color depending on the pH of the vacuole. Combinatorial interactions of these two important TFs not only control the biosynthetic genes of flavonoid biosynthesis pathway but also control pH of the vacuolar lumen, therefore, making flower color suitable and consistent [80]. Biosynthetic gene (both early and late) regulation and control vacuolar pH by the interaction of these two important TFs in flavonoid biosynthesis pathway have a great importance in metabolic engineering. Further efforts towards metabolic engineering by the combinatorial interactions of MYB and bHLH TFs in flavonoid biosynthesis pathway will certainly provide improvement of floriculture industries in the near future.

Many novel functions by the interactions of these two TF families have been identified mostly in model plants and some other plant species. These functions are important to physiological and developmental processes in plants. More recently, the evidences show that combination of these two TF families control trichome initiation, trichome branching and root hair formation that are highly conserved in other plants. The results of such studies combined with numerous high throughput protein-protein interactions, are likely to provide a much intelligible picture of the regulatory complexes that function in the control of most genes. Another example of MYB-bHLH association is phytochrome A (phyA) signaling and the activation of dehydration and abscisic acid (ABA) inducible gene expression. In plants, the specific interaction of these two TFs plays a crucial role in the regulation of defense related secondary metabolites production, such as glucosinolate biosynthesis. It implies that combinatorial interactions of MYB and bHLH TF families are able to biosynthesis various important secondary metabolites. Applying such knowledge in other plant species will increase their importance in plant

field and bring a great achievement in secondary metabolite biosynthesis.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (30470189, 81071821, 81101655), the Fundamental Research for the Central University of China (721500011), the key Project of Hunan Provincial Natural Science Foundation of China (13JJ2016).

REFERENCES

[1] Du, H., Yang, S. S., Liang, Z., Feng, B. R., Liu, L. and Huang, Y. B. 2012. Genome-wide analysis of the MYB transcription factor superfamily in soybean. BMC plant biology 12: 106.

[2] Jones, S. 2004. An overview of the basic helix-loop-helix proteins. GENOME BIOLOGY 5: 226.

[3] Feller, A., Machemer, K., Braun, E. L. and Grotewold, E. 2011. Evolutionary and comparative analysis of MYB and bHLH plant transcription factors. The Plant Journal 66: 94-116.

[4] Yanhui, C., Xiaoyuan, Y., Kun, H., Meihua, L., Jigang, L. and Zhaofeng, G. 2006. The MYB transcription factor superfamily of Arabidopsis: expression analysis and phylogenetic comparison with the rice MYB family. Plant molecular biology 60: 107-124.

[5] Stracke, R., Werber, M. and Weisshaar, B. 2001. The R2R3-MYB gene family in Arabidopsis thaliana. Current opinion in plant biology 4: 447-456.

[6] Du, H., Zhang, L., Liu, L., Tang, X. F., Yang, W. J. and Wu, Y. M. 2009. Biochemical and molecular characterization of plant MYB transcription factor family. Biochemistry (Moscow) 74: 1-11.

[7] Paz-Ares, J., Ghosal, D., Wienand, U., Peterson, P. and Saedler, H. 1987. The regulatory c1 locus of Zea mays encodes a protein with homology to myb proto-oncogene products and with structural similarities to transcriptional activators. The EMBO Journal 6: 3553.

[8] Murre, C., McCaw, P. S. and Baltimore, D. 1989. A new DNA binding and dimerization motif in immunoglobulin enhancer binding, daughterless, MyoD, and myc proteins. Cell 56: 777-783.

[9] Mol, J., Grotewold, E. and Koes, R. 1998. How genes paint flowers and seeds. Trends in Plant Science 3: 212-217.

[10] Van Nocker, S. and Ludwig, P. 2003. The WD-repeat protein superfamily in Arabidopsis: conservation and divergence in structure and function. BMC genomics 4: 50.

[11] Pesch, M. and Hülskamp, M. 2004. Creating a two-dimensional pattern de novo during Arabidopsis trichome and root hair initiation. Current opinion in genetics & development 14: 422-427.

[12] Quattrocchio, F., Verweij, W., Kroon, A., Spelt, C., Mol, J. and Koes, R. 2006. PH4 of petunia is an R2R3 MYB protein that activates vacuolar acidification through interactions with basic-helix-loop-helix transcription factors of the anthocyanin pathway. The Plant Cell Online 18: 1274-1291.

[13] Jang, I. C., Yang, S. W., Yang, J. Y. and Chua, N. H. 2007. Independent and interdependent functions of LAF1 and HFR1 in phytochrome A signaling. Genes & development 21: 2100-2111.

[14] Schweizer, F., Fernández Calvo, P., Zander, M., Diez Diaz, M., Fonseca, S. and Glauser, G. 2013. arabidopsis basic helix-loop-helix transcription factors myc2, myc3, and myc4 regulate glucosinolate biosynthesis, insect performance, and feeding behavior. The Plant Cell Online 25: 3117-132.

[15] Abe, H., Yamaguchi Shinozaki, K., Urao, T., Iwasaki, T., Hosokawa, D and Shinozaki, K. 1997. Role of Arabidopsis MYC and MYB homologs in drought-and abscisic acid-regulated gene expression. The Plant Cell Online 9: 1859-1868.

[16] ML, F. F., Rius, S. P. and Casati, P. 2011. Flavonoids: biosynthesis, biological functions, and biotechnological applications. Frontiers in plant science 3: 222.1-15.

[17] Hichri, I., Barrieu, F., Bogs, J., Kappel, C., Delrot, S., and Lauvergeat, V. 2011. Recent advances in the transcriptional regulation of the flavonoid biosynthetic pathway. Journal of Experimental Botany 62: 2465-2483.

[18] Barton D and Meth-Cohn O. 1999. Comprehensive natural products chemistry: Newnes.

[19] Vom Endt, D., Kijne, J. W. and Memelink, J. 2002.Transcription factors controlling plant secondary metabolism: what regulates the regulators? Phytochemistry 61: 107-114.

[20] Suganuma, T. and Workman, J. L. 2010. WD40 repeats arrange histone tails for spreading of silencing. Journal of molecular cell biology 2: 81-83.

[21] Sompornpailin, K., Makita, Y., Yamazaki, M. and Saito, K. 2002. A WD-repeat-containing putative regulatory protein in anthocyanin biosynthesis in Perilla frutescens. Plant molecular biology 50: 485-495.

[22] Carey, C. C., Strahle, J. T., Selinger, D. A. and Chandler, V. L. 2004. Mutations in the pale aleurone color1 regulatory gene of the Zea mays anthocyanin pathway have distinct phenotypes relative to the functionally similar TRANSPARENT TESTA GLABRA1 gene in

Arabidopsis thaliana. The Plant Cell Online 16: 450-464.

[23] Pang, Y., Wenger, J. P., Saathoff, K., Peel, G. J., Wen, J. and Huhman, D. 2009. A WD40 repeat protein from Medicago truncatula is necessary for tissue-specific anthocyanin and proanthocyanidin biosynthesis but not for trichome development. Plant physiology 151: 1114-1129.

[24] Matus, J., Poupin, M., Cañón, P., Bordeu, E., Alcalde, J. and Arce Johnson, P. 2010. Isolation of WDR and bHLH genes related to flavonoid synthesis in grapevine (Vitis vinifera L.). Plant molecular biology 72: 607-620.

[25] Zhang, F., Gonzalez, A., Zhao, M., Payne, C. T. and Lloyd, A. 2003. A network of redundant bHLH proteins functions in all TTG1-dependent pathways of Arabidopsis. Development 130: 4859-4869.

[26] Gonzalez, A., Zhao, M., Leavitt, J. M. and Lloyd, A. M. 2008. Regulation of the anthocyanin biosynthetic pathway by the TTG1/bHLH/Myb transcriptional complex in Arabidopsis seedlings. The Plant Journal 53: 814-827.

[27] Petroni, K. and Tonelli, C. 2011. Recent advances on the regulation of anthocyanin synthesis in reproductive organs. Plant science 181: 219-229.

[28] Grotewold E. 2006.The genetics and biochemistry of floral pigments. Annu Rev Plant Biol 57: 761-780.

[29] Stracke, R., Ishihara, H., Huep, G., Barsch, A., Mehrtens, F. and Niehaus, K. 2007. Differential regulation of closely related R2R3-MYB transcription factors controls flavonol accumulation in different parts of the Arabidopsis thaliana seedling. The Plant Journal 50: 660-677.

[30] Schijlen, E. G., Ric de Vos, C., van Tunen, A. J. and Bovy, A. G. 2004. Modification of flavonoid biosynthesis in crop plants. Phytochemistry;65: 2631-2648.

[31] Gerats, T. and Strommer, J. 2008. Petunia: evolutionary, developmental and physiological genetics: Springer.

[32] Mori, A., Nitasaka, E., ta, Y., Saitoh, M. and Hoshino 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 and Cell Physiology 47: 457-470.

[33] Park, K. I., Ishikawa, N., Morita, Y., Choi, J. D., Hoshino, A. and Iida, S. 2007. A bHLH regulatory gene in the common morning glory, Ipomoea purpurea, controls anthocyanin biosynthesis in flowers, proanthocyanidin and phytomelanin pigmentation in seeds, and seed trichome formation. The Plant Journal 49: 641-654.

[34] Bai, Y., Pattanaik, S., Patra, B., Werkman, J. R., Xie, C. H. and Yuan, L. 2011. Flavonoid-related basic helix-loop-helix regulators, NtAn1a and NtAn1b, of tobacco have originated from two ancestors and are functionally active. Planta 234: 363-375.

[35] Pattanaik, S., Kong, Q., Zaitlin, D., Werkman, J. R., Xie, C. H. and Patra, B. 2010. Isolation and functional characterization of a floral tissue-specific R2R3 MYB regulator from tobacco. Planta 231: 1061-1076.

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

[37] Takos, A. M., Jaffé, F. W., Jacob, S. R., Bogs, J., Robinson, S. P. and Walker, A. R. 2006. Light-induced expression of a MYB gene regulates anthocyanin biosynthesis in red apples. Plant Physiology 142: 1216-1232.

[38] Espley, R. V., Hellens, R. P., Putterill, J., Stevenson, D. E., Kutty-Amma, S. and Allan, A.C. 2007. Red colouration in apple fruit is due to the activity of the MYB transcription factor, MdMYB10. The Plant Journal 49: 414-427.

[39] Ban, Y., Honda, C., Hatsuyama, Y., Igarashi, M., Bessho, H., and 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 and Cell Physiology 48: 958-970.

[40] Hichri, I., Heppel, S. C., Pillet, J., Léon, C., Czemmel, S. and Delrot, S. 2010. The basic helix-loop-helix transcription factor MYC1 is involved in the regulation of the flavonoid biosynthesis pathway in grapevine. Molecular plant 3: 509-523.

[41] Chiu, L. W., Zhou, X., Burke, S., Wu, X., Prior, R. L. and Li, L. 2010. The purple cauliflower arises from activation of a MYB transcription factor. Plant physiology 154: 1470-1480.

[42] Yuan, Y., Chium, L.W. and Li, L. 2009. Transcriptional regulation of anthocyanin biosynthesis in red cabbage. Planta 230: 1141-1153.

[43] Grotewold, E., Drummond, B. J., Bowen, B. and Peterson, T. 1994. The myb homologous P gene controls phlobaphene pigmentation in maize floral organs by directly activating a flavonoid biosynthetic gene subset. Cell 76: 543-553.

[44] Falcone Ferreyra, M. L., Rius, S., Emiliani, J., Pourcel, L., Feller, A. and Morohashi, K. 2010. Cloning and characterization of a UV-B-inducible maize flavonol synthase. The Plant Journal 62: 77-91.

[45] Tanner, G. J., Francki, K. T., Abrahams, S., Watson, J. M., Larkin, P. J. and Ashton, A. R. 2003 Proanthocyanidin biosynthesis in plants purification of legume leucoanthocyanidin reductase and molecular cloning of its cDNA. Journal of Biological Chemistry 278: 31647-1656.

[46] Xie, D. Y., Sharma, S. B., Paiva, N. L., Ferreira, D. and Dixon, R. A. 2003. Role of anthocyanidin reductase, encoded by BANYULS in plant flavonoid biosynthesis. Science 299: 396-399.

[47] Debeaujon, I., Nesi, N., Perez, P., Devic, M., Grandjean, O. and Caboche, M. 2003. Proanthocyanidin-accumulating cells in Arabidopsis testa: regulation of differentiation and role in seed development. The Plant Cell Online 15: 2514-2531.

[48] Nesi, N., Jond, C., Debeaujon, I., Caboche, M. and Lepiniec, L. 2001. The Arabidopsis TT2 gene encodes an R2R3 MYB domain protein that acts as a key determinant for proanthocyanidin accumulation in developing seed. The Plant Cell Online13: 2099-2114.

[49] Nesi, N., Debeaujon, I., Jond, C., Pelletier, G., Caboche, M. and Lepiniec, L. 2000. The TT8 gene encodes a basic helix-loop-helix domain protein required for expression of DFR and BAN genes in Arabidopsis siliques. The Plant Cell Online 12: 1863-1878.

[50] Baudry, A., Heim, M.A., Dubreucq, B., Caboche, M., Weisshaar, B. and Lepiniec, L. 2004. TT2, TT8, and TTG1 synergistically specify the expression of BANYULS and proanthocyanidin biosynthesis in Arabidopsis thaliana. The Plant Journal 39: 366-80.

[51] Sagasser, M., Lu, G.H., Hahlbrock, K. and Weisshaar, B. 2002. A. thaliana TRANSPARENT TESTA 1 is involved in seed coat development and defines the WIP subfamily of plant zinc finger proteins. Genes & development 16: 138-149.

[52] Istrail, S. and Davidson, E.H. 2005. Logic functions of the genomic cis-regulatory code. Proceedings of the National Academy of Sciences of the United States of America 102: 4954-4959.

[53] Martinez E. 2002. Multi-protein complexes in eukaryotic gene transcription. Plant molecular biology 50: 925-47.

[54] Tai, H.H., Goyer, C. and Murphy, A. M. 2013. Potato MYB and bHLH transcription factors associated with anthocyanin intensity and common scab resistance. Botany 91: 722-30.

[55] Ramsay, N.A. and Glover, B.J. 2005. MYB-bHLH-WD40 protein complex and the evolution of cellular diversity. Trends in plant science 10: 63-70.

[56] Koes, R., Verweij, W. and Quattrocchio, F. 2005. Flavonoids: a colorful model for the regulation and evolution of biochemical pathways. Trends in plant science 10: 236-242.

[57] Borevitz, J.O., Xia, Y., Blount, J., Dixon, R. A. and Lamb, C. 2000. Activation tagging identifies a conserved MYB regulator of phenylpropanoid biosynthesis. The Plant Cell Online 12: 2383-2393.

[58] Dubos, C., Le Gourrierec, J., Baudry, A., Huep, G., Lanet, E. and Debeaujon, I. 2008. MYBL2 is a new regulator of flavonoid biosynthesis in Arabidopsis thaliana. The Plant Journal 55: 940-953.

[59] Qi, T., Song, S., Ren, Q., Wu ,D., Huang, H. and Chen, Y. 2011. The jasmonate-ZIM-domain proteins interact with the WD-repeat/bHLH/MYB complexes to regulate jasmonate-mediated anthocyanin accumulation and trichome initiation in Arabidopsis thaliana. The Plant Cell Online 23: 1795-1814.

[60] Payne, T., Clement, J., Arnold, D. and Lloyd, A. 1999. Heterologous myb genes distinct from GL1 enhance trichome production when overexpressed in Nicotiana tabacum. Development 126: 671-682.

[61] Schellmann, S., Schnittger, A., Kirik, V., Wada, T., Okada, K., Beermann, A. 2002. TRIPTYCHON and CAPRICE mediate lateral inhibition during trichome and root hair patterning in Arabidopsis. The EMBO Journal 21: 5036-5046.

[62] Hulskamp, M., Kirik, V., Simon, M., Wester, K., Schiefelbein, J. W. 2004. ENHANCER of TRY and CPC 2 (ETC2) reveals redundancy in the region-specific control of trichome development of Arabidopsis.

[63] Kirik, V., Simon, M., Huelskamp, M., Schiefelbein, J. 2004. The ENHANCER OF TRY AND CPC1 gene acts redundantly with TRIPTYCHON and CAPRICE in trichome and root hair cell patterning in Arabidopsis. Developmental biology 268: 506-513.

[64] Li, S. F., Milliken, O. N., Pham, H., Seyit, R., Napoli, R., Preston, J. 2009. The Arabidopsis MYB5 transcription factor regulates mucilage synthesis, seed coat development, and trichome morphogenesis. The Plant Cell Online 21: 72-89.

[65] Gonzalez, A., Mendenhall, J., Huo, Y. and Lloyd, A. 2009. TTG1 complex MYBs, MYB5 and TT2, control outer seed coat differentiation. Developmental biology 325: 412-421.

[66] Nakatsuka, T., Haruta, K. S., Pitaksutheepong, C., Abe, Y., Kakizaki, Y. and Yamamoto, K. 2008. Identification and characterization of R2R3-MYB and bHLH transcription factors regulating anthocyanin biosynthesis in gentian flowers. Plant and Cell Physiology 49: 1818-1829.

[67] Elomaa, P., Uimari, A., Mehto, M., Albert, V. A., Laitinen, R. A. and Teeri, T. H. 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 Physiology 133: 1831-1842.

[68] Gerats, T. and Strommer, J. 2009. Evolutionary, Developmental and Physiological Genetics, DOI 10.1007/978-0-387-84796-2.

[69] Brueggemann, J., Weisshaar, B. and Sagasser, M. 2010. A WD40-repeat gene from Malus× domestica is a functional homologue of Arabidopsis thaliana TRANSPARENT TESTA GLABRA1. Plant cell reports 29: 285-294.

[70] Furukawa, T., Maekawa, M., Oki, T., Suda, I., Iida, S. and Shimada, H. 2007. The Rc and Rd genes are involved in proanthocyanidin synthesis in rice pericarp. The Plant Journal 49: 91-102.

[71] Grotewold, E., Sainz, M. B., Tagliani, L., Hernandez, J. M., Bowen, B. and Chandler, V. L. 2000. Identification of the residues in the Myb domain of maize C1 that specify the interaction with the bHLH cofactor R. Proceedings of the National Academy of Sciences 97: 13579-13584.

[72] Zhao, M., Morohashi, K., Hatlestad, G., Grotewold, E. and Lloyd, A. 2008. The TTG1-bHLH-MYB complex controls trichome cell fate and patterning through direct targeting of regulatory loci. Development 135: 1991-1999.

[73] Ishida, T., Hattori, S., Sano, R., Inoue, K., Shirano, Y., Hayashi, H. 2007. Arabidopsis TRANSPARENT TESTA GLABRA2 is directly regulated by R2R3 MYB transcription factors and is involved in regulation of GLABRA2 transcription in epidermal differentiation. The Plant Cell Online 19: 2531-43.

[74] Morohashi, K., Zhao, M., Yang, M., Read, B., Lloyd, A. and Lamb, R. 2007. Participation of the Arabidopsis bHLH factor GL3 in trichome initiation regulatory events. Plant physiology 145: 736-746.

[75] Tominaga Wada, R., Nukumizu, Y., Sato, S. and Wada, T. 2013. Control of Plant Trichome and Root-Hair Development by a Tomato (Solanum lycopersicum) R3 MYB Transcription Factor. PloS one 8: e54019.

[76] Payne, C. T., Zhang, F. and Lloyd, A. M. 2000. GL3 encodes a bHLH protein that regulates trichome development in Arabidopsis through interaction with GL1 and TTG1. Genetics 156: 1349-62.

[77] Esch, J. J., Chen, M., Sanders, M., Hillestad, M., Ndkium, S., Idelkope, B. 2003. A contradictory GLABRA3 allele helps define gene interactions controlling trichome development in Arabidopsis. Development 130: 5885-5894.

[78] Ryu, K. H., Kang, Y. H., Park, Y. h., Hwang, I., Schiefelbein, J. and Lee, M. M. 2005. The WEREWOLF MYB protein directly regulates CAPRICE transcription during cell fate specification in the Arabidopsis root epidermis. Development 132: 4765-4775.

[79] Lee, M. M. and Schiefelbein, J. 2001. Developmentally distinct MYB genes encode functionally equivalent proteins in Arabidopsis. Development 128: 1539-1546.

[80] Yoshida, K., Toyama Kato, Y., Kameda, K. and Kondo, T. 2003. Sepal color variation of Hydrangea macrophylla and vacuolar pH measured with a proton-selective microelectrode. Plant and cell physiology 44: 262-268.

[81] Maeshima, M. 2001. Tonoplast transporters: organization and function. Annual review of plant biology 52: 469-497.

[82] Gaxiola, R. A., Fink G. R. and Hirschi, K. D. 2002. Genetic manipulation of vacuolar proton pumps and transporters. Plant Physiology 129: 967-973.

[83] Yang, S.W., Jang, I. C., Henriques, R. and Chua, N. H. 2009. FAR-RED ELONGATED HYPOCOTYL1 and FHY1-LIKE associate with the Arabidopsis transcription factors LAF1 and HFR1 to transmit phytochrome A signals for inhibition of hypocotyl elongation. The Plant Cell Online 21: 1341-59.

[84] Neff, M. M. and Chory, J. 1998. Genetic interactions between phytochrome A, phytochrome B, and cryptochrome 1 during Arabidopsis development. Plant Physiology 118: 27-35.

[85] Hirai, M.,Y., Sugiyama, K., Sawada, Y., Tohge, T., Obayashi, T., Suzuki, A. 2007. Omics-based identification of Arabidopsis Myb transcription factors regulating aliphatic glucosinolate biosynthesis. Proceedings of the National Academy of Sciences 104: 6478-6483.

[86] Gigolashvili, T., Engqvist, M., Yatusevich, R., Müller, C. and Flügge, U. I. 2008. HAG2/MYB76 and HAG3/MYB29 exert a specific and coordinated control on the regulation of aliphatic glucosinolate biosynthesis in Arabidopsis thaliana. New Phytologist 177: 627-642.

[87] Sønderby, I. E, Burow, M., Rowe, H. C., Kliebenstein, D. J, Halkier, B. A. 2010. A complex interplay of three R2R3 MYB transcription factors determines the profile of aliphatic glucosinolates in Arabidopsis. Plant physiology 153: 348-363.

[88] Fernández Calvo, P., Chini, A., Fernández Barbero, G., Chico, J. M., Gimenez Ibanez, S., Geerinck, J. 2011. The Arabidopsis bHLH transcription factors MYC3 and MYC4 are targets of JAZ repressors and act additively with MYC2 in the activation of jasmonate responses. The Plant Cell Online 23: 701-715.