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MYB Transcription Repressors Regulate Plant Secondary Metabolism

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ABSTRACT

MYB transcription factors (TFs) belong to one of the largest and important gene families, which regulate development under changing environmental conditions, primary and secondary metabolism, and plant response to stresses (biotic and abiotic stresses). MYB repressors have a conserved N-terminal domain like other MYB TFs, but the C-terminal domain makes them structurally and functionally different from the rest. MYB repressors usually possess some repressive motifs, such as EAR (ethylene-responsive element binding factor-associated amphiphilic repression motif), SID (Sensitive to ABA and Drought 2 protein interact motif), and TLLLFR motifs, which contribute to their repression function through a variety of complex regulatory mechanisms. In this review, we summarize recent developments in research of MYB repressors and suggest directions to future research.

I. Introduction

MYB transcription factors (TFs) are identified in many eukarya (Jia et al., 2004), and are well-researched in plants. The first plant MYB transcription factor (TF) was isolated from maize (*Zea mays*) in the 1980s and showed 40% sequence homology to the animal MYB proto-oncogene (Paz-Ares et al., 1987). Subsequently, the structure and function of various MYB proteins have been extensively studied in many plant species. MYB proteins usually have the following two discrete regions: N-terminal and C-terminal. The N-terminal region has a conserved MYB domain that is crucial for DNA binding, whereas the C-terminal region is variable defines the functional multiplicity of this class of TFs. Generally, the MYB domain contains one to four imperfect repeats (R), and each repeat comprised of 50-53 amino acid residues has three α-helices. The second and third helices form a 3D helix-turn-helix (HTH) structure with a hydrophobic moiety, which contributes to the recognition of the specific target gene (Ogata et al., 1996). The third helix is responsible for direct contact with the major DNA groove (Jia et al., 2004). Depending on the location and number of these repeats, MYB proteins can be divided into the following four main classes: 1R (R1/2, R3-MYB), 2R (R2R3-MYB), 3R (R1R2R3-MYB), and 4R (R1/R2-like repeats) (Figure 1) (Dubos et al., 2010).

MYB proteins are essential for regulating pathways involved in primary and secondary metabolism, growth and development, and biotic and abiotic stress responses (Dubos et al., 2010; Liu et al., 2015; Ma and Constabel, 2019). The MYB proteins function by directly or indirectly binding the *cis*-acting element of DNA to activate or inhibit the downstream genes. In this review, we summarized the structure and function of MYB repressors and the regulatory mechanisms of these TFs in plant secondary metabolism.

II. Structure of MYB repressor proteins

A. Signature features of MYB repressors

The DNA-binding domain of MYB repressors is in the N-terminal region, highly conserved, and consists of different numbers of repeats. The repressive function of these proteins usually depends upon the
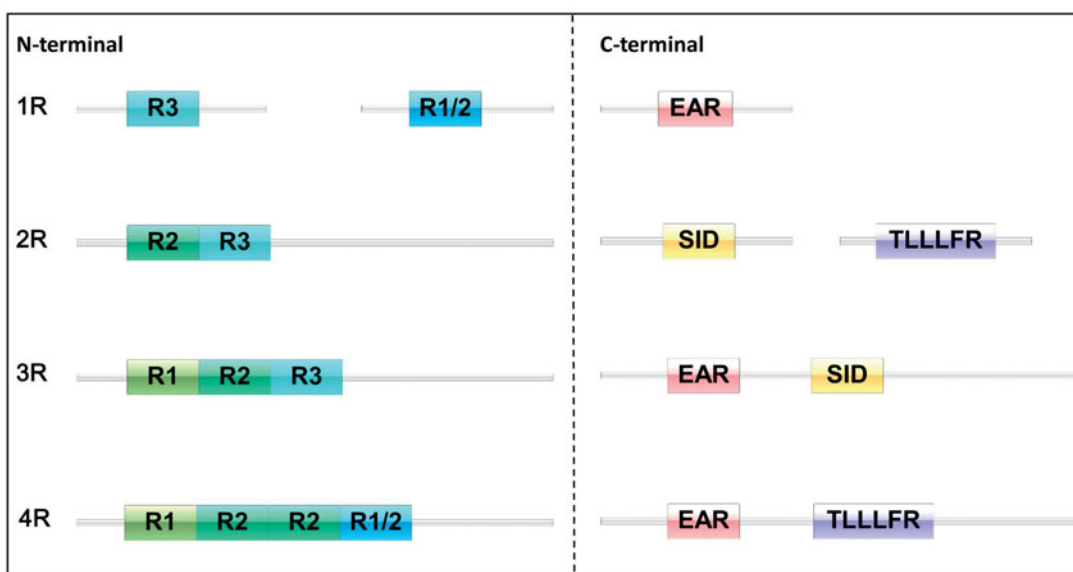
Figure 1. Four types of MYB transcription factors. The N-terminal of MYBs are divided into four groups. R1, R2, and R3 are three types of R repeat with similarity to R1, R2 or R3 of c-Myb. In the 1R group, MYB only contains 1R repeat R3 or R1/2. The 2R group contains R2 and R3 repeat, which are also known as R2R3-MYB subfamily. The 3R contains three R repeats: R1, R2, and R3. The 4R group contains four R repeats, one R1 and R1/2, and two R2 subgroups. The C-terminal of MYB repressors often contain one or two distinct motifs like EAR motif (ERF-associated amphiphilic repression), SID motif (Sensitive to ABA and Drought 2 protein interact motif), and TLLLFR motif.

distinct motifs in the C-terminal region (Figure 1). In the study of *Arabidopsis thaliana* MYB family, the majority of MYB repressors belonged to the subgroup 4 of R2R3-MYB proteins, whose C-terminal has a conserved motif, C1 or C2. C1 LlsrGIDPx^{T}/$_{s}$HRx^{I}/$_{L}$ is referred to as the GIDP domain and C2 pdLNL^{D}/$_{E}$Lxi^{G}/$_{S}$ is referred to as an EAR motif (ethylene-responsive element binding factor-associated amphiphilic repression motif). Additionally, some members have Zinc-finger domain $CX_{1-2}CX_{7-12}CX_{2}C$ (Kranz et al., 1998). These conserved domains are considered responsible for the repression activity of these proteins. EAR motif was first identified in AtERF4 as pdLNL^{D}/$_{E}$Lxi^{G}/$_{S}$ (Ohta et al., 2001). Members of other TF families, such ERF (ethylene-responsive element binding factor), ZCT (zinc finger *Catharanthus* protein), and AUX/IAA (auxin/indole-3-acetic acid proteins), also contain EAR domains and act as repressors in the regulation of signaling pathways (Kazan, 2006). The EAR-containing TF AtMYB4 is a negative regulator of the sinapate ester biosynthesis induced by UV-B in Arabidopsis (Jin et al., 2000). In rice, the negative regulator of disease resistance NRR (Chern et al., 2005) and its homologs in Arabidopsis NIMIN2 (Weigel et al., 2001) and tobacco G8-1 (Horvath et al., 1998) have an LDLNxxP in the C-terminal, which resembles the EAR motif. Tobacco ZFT1 lost its repressive activity when the EAR domain was deleted (Uehara et al.,

2005). The repressing activity of ZCT1, ZCT2, and ZCT3 in *Catharanthus roseus* is also dependent on the LxLxL type EAR motif (Pauw et al., 2004). Although the EAR motif is a ubiquitous core suppression domain among a large number of plant TFs, the TLLLFR and R/KLFGV motifs exhibit repressive functions (Matsui et al., 2008; Ikeda and Ohme-Takagi, 2009). The TLLLFR was first identified in Arabidopsis AtMYBL2 (R3-MYB) repressor (Matsui et al., 2008), and subsequently in VvMYBC2L-1, VvMYBC2L-2, VvMYBC2L-3, PtMYB165, and PtMYB194 (Cavallini et al., 2015; Yoshida et al., 2015). R/KLFGV was identified as a repressive domain in various TFs, including MYB, HSF, and AP2by transient expression analysis, however, the regulatory mechanism remains unclear and requires further investigation (Ikeda and Ohme-Takagi, 2009).

B. Classification of MYB repressors

The characteristics of MYB repressors were summarized by using the online tool MEME (http://meme-suite.org/) to search specific motifs of 37 MYB repressors (Table 1). The majority contained an EAR motif with the conserved sequence LxLxL (Figure 2A). Moreover, 14 of those 37 proteins contained conserved GY/FDFLGL sequences, also known as SAD2 interacting motif (SID motif) (Figure 2B). The protein sequence alignment of those 14 SID-containing MYB

Table 1. MYB transcriptional repressors in plants.

| Name	Species	Motif	Function	Accession	Reference
AtMYB4	*Arabidopsis thaliana*	EAR, SID	phenylpropanoid	AT4G38620	Jin et al., 2000
AtMYB7	*A. thaliana*	EAR, SID	flavonol	AT2G16720	Fornale et al., 2014
AtMYB23	*A. thaliana*	EAR	trichomes	AT5G40330	Hiratsu et al., 2003
AtMYB60	*A. thaliana*	EAR	anthocyanin	AT1G08810	Park et al., 2008
ATMYBD	*A. thaliana*	TLLLFR	anthocyanin	AT1G70000	Nguyen et al., 2015
AtMYBL2	*A. thaliana*	TLLLFR	flavonoid, anthocyanin	AT1G71030	Matsui et al., 2008
EjMYB2	*Eriobotrya japonica*	EAR, SID	lignin	KF767454	Xu et al., 2014
EgMYB1	*E. gunnii*	EAR, SID	lignin	AJ576024	Legay et al., 2010
FaMYB1	*Fragaria × ananassa*	EAR	anthocyanin, flavonol	AF401220	Aharoni et al., 2001
FcMYB1	*F. chiloensis*	EAR	anthocyanin	GQ867222	Salvatierra et al., 2013
FtMYB11	*F. tataricum*	EAR, SID	phenylpropanoid	KU498041	Zhou et al., 2017
FtMYB13	*F. tataricum*	SID	rutin	KY290579	Zhang et al., 2018
FtMYB14	*F. tataricum*	EAR, SID	rutin	KY290580	Zhang et al., 2018
FtMYB15	*F. tataricum*	EAR, SID	rutin	KY290581	Zhang et al., 2018
GmMYB100	*Glycine max*	EAR	isoflavonoid, flavonol	Glyma07g33960.1	Yan et al., 2015
LlMYB1	*Leucaena leucocephala*	EAR, SID	lignin, phenylpropanoid	GU901209	Omer et al., 2013
LOC_Os08g43550.1	*Oryza. sativa*	EAR, SID			
MtMYB2	*Medicago truncatula*	EAR	anthocyanin	XM_003616340	Jun et al., 2015
MusaMYB31	*Musa cultivar Rasthali*	EAR	lignin, polyphenols	KU507534	
NtMYB2	*Narcissus. tazetta*	EAR	anthocyanin	KY860527	Anwar et al., 2018
PhPH4	*Petunia × hybrida*	EAR	anthocyanin	AB982129	
PhMYB27	*Petunia × hybrida*	EAR	anthocyanin	KF985023	Spelt et al., 2000
PhMYBx	*Petunia × hybrida*		anthocyanin	KF985022	Albert et al., 2014
PpMYB18	*Prunus persica*	EAR	PAs, anthocyanin	KT159234	Zhou et al., 2017
PtrMYB165	*Populus trichocarpa*	EAR, TLLLFR	flavonoid, anthocyanin	Potri.010G114000	Ma et al., 2018
PtrMYB57	*P. trichocarpa*	EAR	PAs, anthocyanin	Potri.T011400.1	Wan et al., 2017
PtrMYB194	*P. trichocarpa*	EAR, TLLLFR	flavonoid, anthocyanin	Potri.008G128500	Ma et al., 2018
PtrMYB221	*P. trichocarpa*	EAR, SID	secondary wall biosynthesis	KX273518	
PtrRML1	*P. trichocarpa*		anthocyanin	Potri.012G031200	Hu et al., 2016
TaMyb1D	*Triticum. aestivum*	EAR	lignin, flavonoids	KY679302	Wei et al., 2017
SlTHM27	*Solanum lycopersicum*	EAR, SID		NM_001247046	Lin et al., 1996
Traes_7AS_2EE7C2628.11	*Triticum aestivum*	EAR, SID			
VvMYB4a	*Vitis vinifera*	EAR, SID	anthocyanin	EF113078	Cavallini et al., 2015
VvMYB4b	*V. vinifera*	EAR, SID	anthocyanin, phenolic	FJ792820	Cavallini et al., 2015
VvMYB4-like	*V. vinifera*	EAR	anthocyanin	XP_0022733328.1	Pérez-Díaz et al., 2016
VvMYBC2-L1	*V. vinifera*	EAR, TLLLFR	proanthocyanidins (PAs)	JX050227	Huang et al., 2014
VvMYBC2-L3	*V. vinifera*	EAR, TLLLFR	anthocyanin	KM046932	Cavallini et al., 2015
ZmMYB11	*Zea mays*	EAR, SID-like	lignin	GRMZM2G000818	Vélez-Bermúdez et al., 2015
ZmMYB31	*Z. mays*	EAR, SID-like	lignin, phenylpropanoid	NP_001105949	Fornale et al., 2014
ZmMYB42	*Z. mays*	EAR, SID-like	phenylpropanoid	HQ858694	Yilmaz et al., 2009
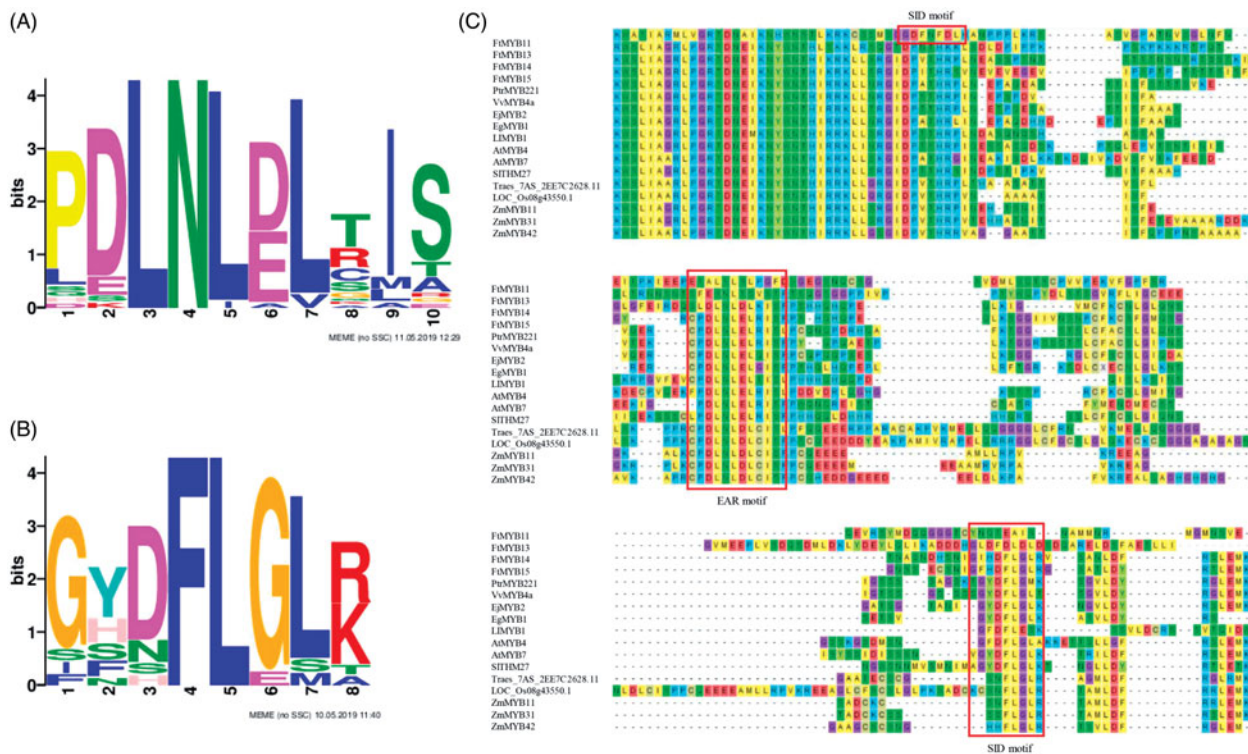
Figure 2. EAR motif and SID motif in MYB transcription factors. (A) The positional enrichment of EAR motif in 37 MYB repressors. The most conservative sequence of EAR motif is LxLxL. (B) The positional enrichment of SID motif in 16 MYB repressors. The SID motif contains a conservative sequence G^Y/$_F$DFLGL motif, which is the SAD2 binding site. (C) The alignment of 16 MYB repressors and distribution of EAR motif and SID motif. EAR motif located at C terminal behind MYB R repeat. Only FtMYB13 does not contain EAR motif. The SID motif of FtMYB11 locates at N terminal, near the downstream of R repeat upstream of EAR motif. Other MYB repressors' SID motifs are downstream of EAR motif.

repressors and three homologs of AtMYB4 (SlTHM27, LOC_Os08g43550.1, and Traes_7AS_2EE7C2628.11) (Table 1) showed that SID motifs are relatively conservative in dicot and monocotyledon. Compared to the conserved ERF motif in most plants, SID motifs exhibit differences between dicot and monocotyledon (Figure 2C), and indicated that the SID motif is probably related to the relationship between plants and their evolutionary differences.

Three hundred MYB protein sequences (downloaded from: http://planttfdb.cbi.pku.edu.cn) were identified containing an EAR motif or R/KLFGV and TLLLFR motif among nine species, including seven crop plants (*Brassica napus*, *Oryza sativa* subsp. *indica*, *O. sativa* subsp. *japonica*, *Solanum lycopersicum*, *Triticum aestivum*, *Z. mays* and *Fagopyrum tataricum*), one well-researched model plant (*A. thaliana*), and *Aquilegia coerulea*, the intermediate plant between dicots and monocots. A neighbor end-joining phylogenetic tree was constructed, which classified these proteins into 27 subgroups based on the bootstrap values greater than 70 (Figure 3). Subgroup 4, comprised of R2R3 MYB proteins having an EAR motif, are widespread in plants. Members of subgroup

22 also have an EAR motif in the N-terminal, such as AtMYB77 (AT3G50060.1) that can enhance auxin signaling under IAA and ABA treatment (Zhao *et al.*, 2014). These types of MYB proteins might play a complex role in regulating plant signaling. There are many proteins that have an EAR motif in the subgroup 20, but their function is not known. Among these 300 sequences, only AtMYBL2 has a TLLLFR motif, At5g61620 has an R/KLFGV motif, and At3g16350, At5g47390, At5g56840 have both an EAR motif and R/KLFGV. At5g61620, At3g16350, At5g47390, At5g56840 are homologs of AtMYBD (a MYB-like protein), and their idiographic function has not been identified.

III. MYB repressors function

After the first MYB was identified in maize (Paz-Ares *et al.*, 1987), many MYB TFs in plants have been characterized (Jin *et al.*, 2000; Legay *et al.*, 2010; Xu *et al.*, 2014; Jun *et al.*, 2015; Zhou *et al.*, 2017). Through functional characterization of MYB repressors, a large proportion of these repressors are involved in the biosynthesis of phenylalanine and
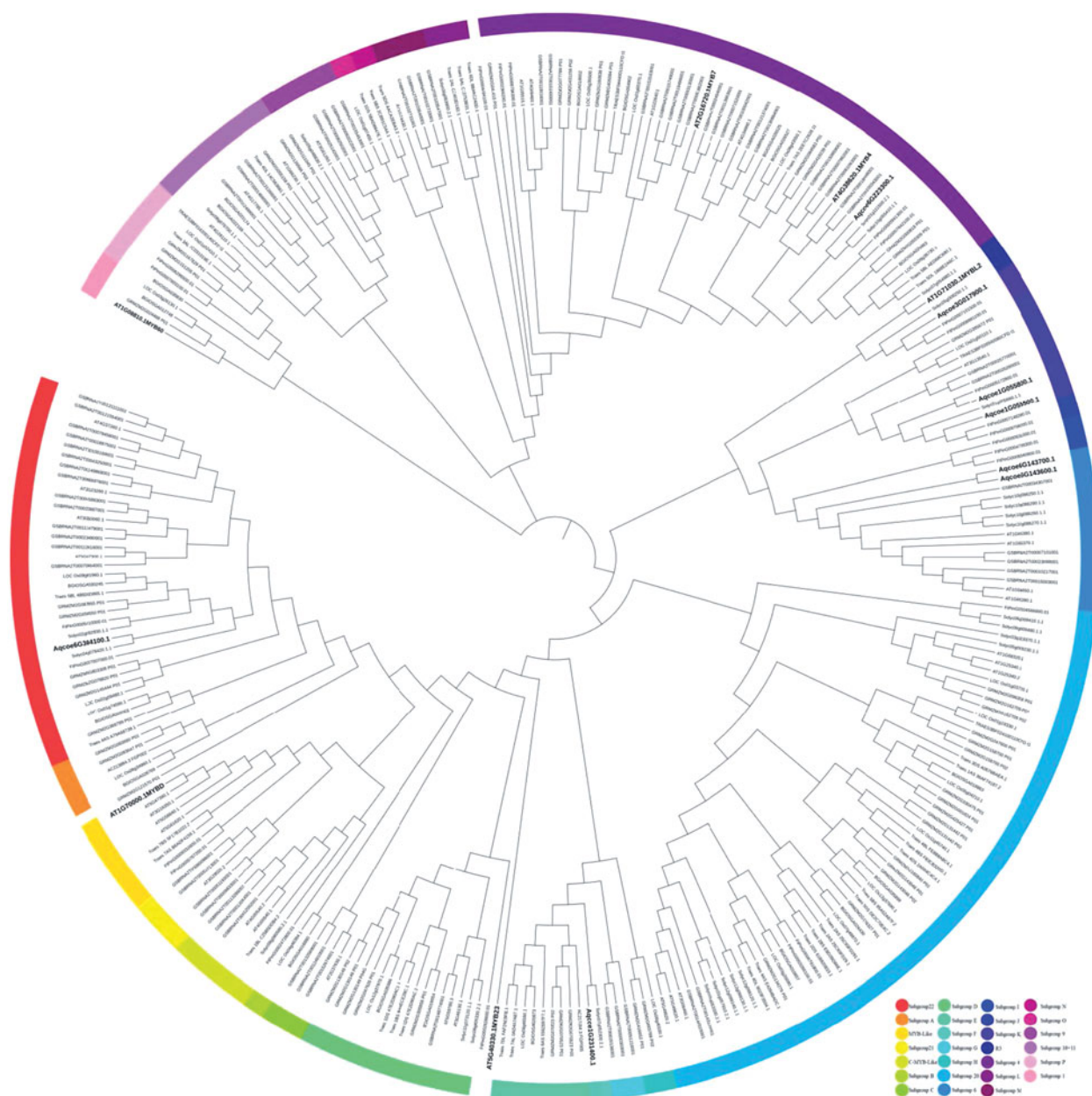
Figure 3. Neighbor-joining phylogenetic tree of 300 MYB transcription factors. Neighbor-joining phylogenetic tree constructed using 300 amino acid sequences including the MYB proteins, which contain an EAR motif from *A. thaliana*, *B. napus*, *O. sativa* subsp. *indica*, *O. sativa* subsp. *japonica*, *S. lycopersicum*, *T. aestivum*, *Z. mays*, *F. tataricum*, *A. coerulea*.

phenylpropanoid-derived compounds (Table 1). These secondary metabolites were required for plant growth and development, the resistance against biotic and abiotic stresses, and the nutritional value. For instance, *MYB308* and *MYB330* were selected from six highly expressed genes in the flowers of the *Antirrhinum* spp. (Jackson *et al.*, 1991), and the heterologous expression of these, in tobacco, resulted in the regulation of monolignol, phenolic acid, and lignin (Tamagnone *et al.*, 1998).

The genome sequence of *A. thaliana* released in the year 2000 greatly promoted the research on the

functions of various genes, including MYB repressors (Arabidopsis Genome Initiative, 2000). The EAR-containing AtMYB4 protein negatively regulated the sinapate ester accumulation (Jin *et al.*, 2000). AtMYB7, a homolog of AtMYB4, repressed the expression of *DFR* and UDP sugar glycosyltransferase (*UGT)* genes that encode key enzymes in the flavonoid pathway (Fornale *et al.*, 2014). Ectopic expression of the UV-B irradiation responsive gene *AtMYB60* in lettuce (*Lactuca sativa*) inhibited the anthocyanin biosynthesis by suppressing the expression of *DFR* (Park *et al.*, 2008). A leaf specific one-repeat MYB, MYBL2

(Kirik and Baumlein, 1996), was identified as a negative regulator of the flavonoid biosynthesis in *A. thaliana* (Matsui *et al.*, 2008; Dubos *et al.*, 2010). Subsequently, overexpression of MYBL2 reduced the anthocyanin biosynthesis in Arabidopsis (Matsui *et al.*, 2008). AtMYB23 repressed the expression of *Glabra2* (*GL2*), which affected the development of epidermal cells of leaf, stem, and roots (Matsui *et al.*, 2005).

MYB repressors were also identified and characterized in other dicots. Ectopic expression of eucalypt (*Eucalyptus gunnii*) gene *EgMYB1* in poplar (*Populus trichocarpa*) and Arabidopsis results in the reduction of lignin biosynthesis, thus, repressing secondary wall formation (Legay *et al.*, 2010). EjMYB2 identified from loquat (*Eriobotrya japonica*) down-regulated the lignin biosynthesis genes by countering the inductive effects of the activator EjMYB1 (Xu *et al.*, 2014). The transcription repressor PtrMYB165 of poplar suppressed the flavonoid biosynthesis by repressing the flavonoid promoters, like PtrbHLH131 (Ma *et al.*, 2018). FtMYB11, FtMYB13, FtMYB14, FtMYB15, and FtMYB16 are repressors of rutin biosynthesis in *F. tataricum*, through spatially repressing the key enzyme genes (Zhou *et al.*, 2017; Zhang *et al.*, 2018). Moreover, FtMYB16 interfered with the transport of rutin precursors by directly repressing the expression of multidrug and toxic compound extrusion (MATE) transporter gene *FtMATE1* (Li *et al.*, 2019).

MYB repressors are involved in the regulation of proanthocyanidin (PA) and anthocyanin biosynthesis in various plants. In grape, VvMYBC2-L3, VvMYB4b, VvMYB4a, and VvMYB4-like reduced PA and anthocyanin accumulation and down-regulated structural and regulatory genes of the flavonoid biosynthesis pathway (Cavallini *et al.*, 2015; Pérez-Díaz *et al.*, 2016). Overexpression of *NtMYB2* from Chinese narcissus (*Narcissus tazetta*) in tobacco repressed anthocyanin biosynthesis genes, particularly the *UFGT* gene (Anwar *et al.*, 2018). Expression of *FaMYB1* from strawberry in tobacco resulted in decreased flavonoids accumulation (Aharoni *et al.*, 2001). RNAi-based gene silencing of *FcMYB1*, the homolog of *FaMYB1*, in *Fragaria chiloensis* resulted in the overexpression of anthocyanidin synthase (ANS) and down-regulation of anthocyanidin reductase and leucoanthocyanidin reductase (Salvatierra *et al.*, 2013). MtMYB2 functions as part of a multidimensional regulatory network to regulate anthocyanin and PA accumulation during various developmental stages (Jun *et al.*, 2015). Although the first plant MYB was found in maize (*Z. mays*), MYB repressors are not well studied in monocotyledonous plants. In maize, three MYB

repressors, MYB11, MYB31, MYB42 were identified in the down-regulation of a set of lignin genes by the interaction with a TIFY family protein ZML2 in the wound-induced lignification pathway (Vélez-Bermúdez *et al.*, 2015). The wheat (*T. aestivum*) MYB repressor TaMYB1D, which belongs to the subgroup 4 of the R2R3-MYB subfamily, has a dual function. Overexpression of *TaMYB1D* in tobacco (*Nicotiana tabacum*) resulted in the accumulation of lignin in stems and the enhanced expression of ROS- and stress-related genes in response to drought stress, however, the flavonoid content in leaves decreased (Wei *et al.*, 2017). Ectopic expression of banana (*Musa acuminata*) MYB repressor MaMYB3 in tomato decreased degradation of starch and delayed fruit ripening by inhibiting starch metabolism genes and a positive regulator of starch degradation MabHLH6 (Fan *et al.*, 2018).

IV. Regulation models of MYB repressors

A. The JAZ-MYB complex and the JAZ-MYB-SAD complex

In the 1990s, the consensus sequence (C/T)G(A/G)(A/C/G)GTT(A/G) was found to be an MYB-recognition element (MRE), by studying the c-MYB protein in mice (Howe and Watson, 1991). Later, MRE and ACE (ACGT-containing element) were identified as MYB-recognition elements in *A. thaliana* (Hartmann *et al.*, 2005). In loquat, EjMYB1 interfered with the function of EjMYB2 through competitive interaction with ACE in the promoter region of lignin biosynthesis genes, such as *Ej4CL1* (Ikeda and Ohme-Takagi, 2009; Xu *et al.*, 2014). These consensus sequences are widely distributed throughout plant genomes, and their function as *cis*-element needs to be studied by specific experiments. With the advancement in the field and improvement in our understanding, TFs do not always function independently but require interaction with other TFs for proper action. MYB repressors were involved in different plant life processes through complex regulatory mechanisms (Deng and Lu, 2017; Allan and Espley, 2018; Ma and Constabel, 2019).

In Arabidopsis, JAZ (jasmonate ZIM-domain) proteins interacted with the R2R3-MYBs through ZIM-domain and N-terminal (approximately pre-130 aa) of JAZ proteins and R2R3 MYB, respectively (Song *et al.*, 2011). In buckwheat, JAZ bound the SID-like motif of MYB TFs, and the Asp126 of MYB contributed a key role in the interaction (Zhou *et al.*, 2017). However, the specific motif responsible for the interaction between JAZ and MYB in the MYB protein

sequence remains to be studied. Two 1R MYB proteins, TRY (the small MYB proteins TRIPTYCHON) and At4g01060, and five R2R3 MYB proteins including the MYB repressor AtMYB4 were tested for interaction with R/B-like bHLH factors AtbHLH012 and TT8 in a yeast two-hybrid assay, and showed the amino acid motif [DE]Lx2[RK]x3Lx6Lx3R in MYBs was the core sequence for protein interaction (Zimmermann, et al., 2004; Kui et al., 2010). SAD2 (Sensitive to ABA and Drought 2 protein), an important β-domain protein in Arabidopsis, was involved in ABA signal transduction, salt stress and UV-B protection (Verslues et al., 2006; Zhao et al., 2007; Gao et al., 2008). SAD2 interacted with AtMYB4, which is required for the nuclear localization of AtMYB4 (Zhao et al., 2007). Soon afterward, a putative zinc-finger domain and a SID motif were found in the C-terminal of sub-group 4 R2R3-type MYB TFs MYB4, MYB7, and MYB32. Unlike the EAR motif that is widely distributed in the MYB protein family, the SID motif is relatively rare. Through multiple sequence alignment, the SID motif was determined to be relatively conserved in higher dicotyledons and diverse in Aquilegia, the earliest diverging branch of the eudicots and monocots (Figure 2), which may concern with different evolution history and physiological structure between dicots and monocots (Uncu et al., 2015). Furthermore, these putative sequences in monocots have not been studied yet. Protein interaction studies showed that the conserved $G^Y/_FDFLGL$ motif in MYB is the SAD2 binding site and the core amino acid is Asp (Zhou et al., 2015).

From the recent studies (Zhou et al., 2017; Zhang et al., 2018), it is not difficult to find that MYB repressors may have some connection with JA (Jasmonic acid) pathways. In F. tataricum, FtMYB11, a homolog of AtMYB4, containing an EAR motif and a SID-like motif repressed the expression of key enzyme genes, cinnamate 4-hydroxylase (C4H), 4- coumarate coenzyme ligase (4CL), chalcone synthase (CHS), and flavonol synthase (FLS) of the phenylpropanoid pathway. FtMYB11 interacted with FtJAZ1 and FtSAD2 in R2R3 region and SID-like motif respectively. Similar to AtMYB4, the Asp in SID-like motif was necessary for the interaction between FtMYB11 and FtSAD2. FtMYB11 together with FtJAZ1 and FtSAD2 bind with the MYB core element (MCE) in the promoter of FtMYB11 responsive genes of the phenylpropanoid pathway (Zhou et al., 2017). Later, JA-responsive repressors FtMYB13, FtMYB14, and FtMYB15 interacted with FtSAD2, whereas FtJAZ1 specifically interacted with FtMYB13, and these three proteins

repressed the phenylalanine ammonia lyase (FtPAL) gene expression (Zhang et al., 2018). The regulation of phenylpropanoid pathway by JAZ-MYB-SAD complex (Figure 4) both in Arabidopsis and F. tataricum gives a clue that this complex may have a ubiquitous regulation mechanism (Zhao et al., 2007; Zhou et al., 2015, 2017; Zhang et al., 2018).

B. The MBW complex

The MBW complex is a widespread regulatory model of the phenylpropanoid biosynthesis pathway and epidermal cell fate. The MBW complex is comprised of three kinds of TFs: MYB, bHLH, and WDR (Figure 5) (Albert et al., 2011, 2014). Through the study of relative expression of TFs and structural genes of anthocyanin biosynthesis pathway in petunia (Petunia hybrida), the AN1 (bHLH) was regulated by AN11 (WDR) and AN2 (MYB), and these three TFs function in a complex regulatory hierarchy (Spelt et al., 2000). In A. thaliana, the interactions among TTG1 (WDR), TT2 (MYB), and TT8 (bHLH) were identified through the yeast two-hybrid system and yeast three-hybrid system. Mutant studies showed that this interaction was required for the expression of downstream genes, confirmed through histochemical studies and A. thaliana protoplasts transactivation assay (Baudry et al., 2004). WD40 repeat protein (WDR) made up of 40 residues contains a β-propeller protein group, and the core region has a glycine–histidine (GH) dipeptide and a tryptophan–aspartate (WD) dipeptide (Smith et al., 1999). WDR proteins do not execute an enzymatic function and can only interact with other protein or promote protein-protein interactions (Ramsay and Glover, 2005). bHLH proteins recognize and bind to specific DNA sequence through the bHLH domain (Voronova and Baltimore, 1990). MBW complex can facilitate or inhibit the expression of genes. Activation or inhibition of downstream genes was often related to MYB protein (Xu et al., 2015). For instance, in petunia, two light stress induced MYB repressors PhMYBx and PhMYB27 negatively regulated anthocyanin pathway gene expression by competing with another MYB activator (AN1) to form an inhibitory MYB complex (Albert et al., 2011; 2014). The regulation mechanism of strawberry MYB repressor FaMYB1 resembled the mechanism of petunia MBW (PHZ, AN1, and AN11) complex (Dubos et al., 2010; Paolocci et al., 2011). In the forage legume, Trifolium repens, Tr-MYB133, and Tr-MYB134, the biosynthesis of anthocyanin and PA by suppressing the activator MBW complex (Albert, 2015). PtrMYB57 formed an
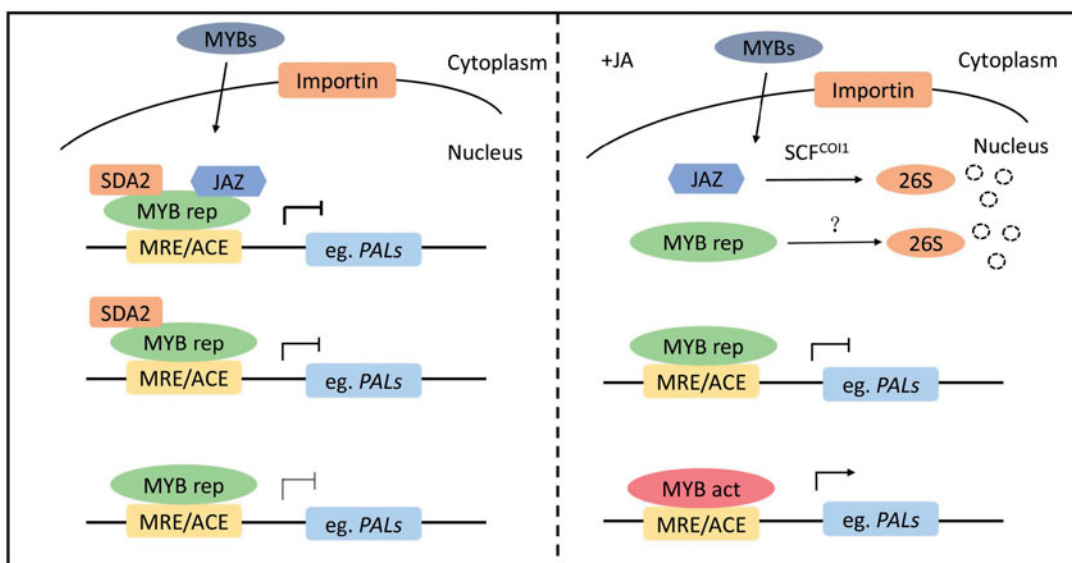
Figure 4. The JAZ-MYB-SAD complex. The importin such as sensitive to ABA and Drought 2 (SAD2) protein and Ftimportin-α1 recognizes the nuclear pore complex. MYB repressors function as transcription repressors by regulating the expression of other genes through binding their *cis*-acting element like MRE or ACE. When MYBs interact with them, the nuclear pore complex can transport the complex into the nucleus. In the nucleus, JAZ protein interacts with MYB repressors (MYB rep in the figure) and SAD acts as a co-repressor strongly repressing (bold line) the expression of the downstream genes like phenylalanine ammonia lyase (PALs) by binding to MYB binding sites like MRE or ACE. Some MYB repressors from a JAZ-MYB complex repress (plain line) the downstream genes. MYB repressors also can directly bind MYB binding sites like MRE or ACE and repress (thin line) downstream genes. However, in the presence of jasmonic acids (JAs), the inhibitory effect becomes weaker by the ubiquitin 26S proteasome degrading JAZ through JAZ proteins, binding JAs and from SCF^COI1 complex. Simultaneously, some MYB repressors are degraded through the ubiquitin 26S proteasome when JAs are present. Moreover, MYB activators (MYB act in the figure) compete with MYB repressors and bind to MRE or ACE activating the expression of downstream genes.
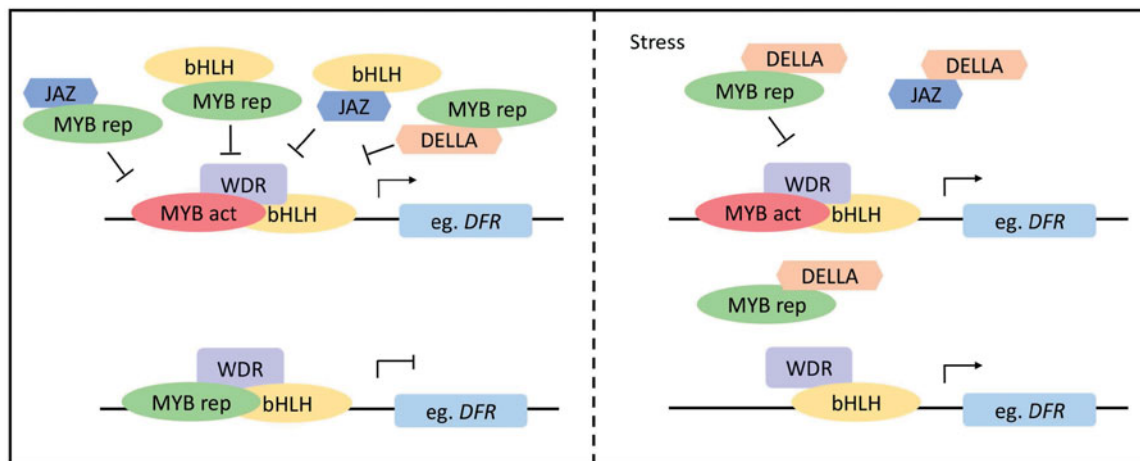

Figure 5. The MBW complex. The MBW complex consists of three kinds of transcription factors, MYB, bHLH, and WDR. The downstream genes are inhibited when the MYB repressors bind with bHLH and WDR to form the MBW complex and get activated when the activator binds the complex. Otherwise, some MYB repressors interact with other TFs to repress activator MBW complex. MYB repressor (MtMYB2) binds with bHLH (MtTT9) to inhibit the activity of MBW complex (MtMYB4/MtMYB5-MtTT8-MtTTG1), thus, reducing the anthocyanin and PAs accumulation. MYB repressor (AtMYBL2) and JAZ interact with bHLH and MYB/bHLH, respectively, to suppress the MBW complex and reduce the anthocyanin accumulation. During stress, DELLA proteins sequester with MYB repressor (AtMYBL2) and JAZ to activate the MBW complex and subsequently increase the anthocyanin accumulation.

inhibitor MBW complex with bHLH131 and PtrTTG1 (WDR), decreased the biosynthesis of anthocyanins and PAs (Wan *et al.*, 2017). However, PtrRML1 (R3 MYB-LIKE1) reduced the anthocyanin accumulation by repressing MBW component genes (Hu *et al.*, 2016). Some MYB repressors interacted with other TFs to repress the activator MBW complex. MtMYB2 in *Medicago truncatula* bound with MtTT9 to inhibit

the activity of MYB activators (MtMYB4 and MtMYB5) in MtTT8-MtTTG1 MBW complex, and thus reduced the anthocyanin and PAs accumulation (Jun *et al.*, 2015). AtMYBL2 and JAZ interacted with bHLH and MYB/bHLH respectively to suppress the MBW complex and reduce anthocyanin accumulation. During stress, DELLA protein (RGA) interacted with AtMYBL2 and JAZ to activate the MBW complex and subsequently increase anthocyanin accumulation (Xie *et al.*, 2016). Additionally, DELLA and JAZ proteins modulated gibberellin (GA) and jasmonate (JA) signaling synergy by interacting with the bHLH TFs GL3/EGL3 and the MYB TF GL1. GA and JA induced degradation of DELLA and JAZ and resulted in the activation of the MBW complex (Qi *et al.*, 2014).

V. Conclusion and prospects

Plants have developed intricate regulatory mechanisms to respond to various environmental changes. TFs mediate genes expression via direct interaction with promoter sequences and/or with other TFs. The studies on MYB TFs indicate that a large proportion of MYB repressors belong to R2R3 MYB family and R3 MYB family, and their repression activity mainly depends on the repression motifs, such as EAR and SID in the C-terminus of protein. These motifs are important for binding with bHLH proteins or recruiting repressor and corepressors. However, the precise function and repression mechanism of TLLLFR motif needs to be studied. MYB TFs can regulate the gene expression by competitive binding with the *cis*-acting element in the promoter region. The whole genome ChIP-seq data will contribute to a better understanding of the MYB regulated genes and the identification of other variants of MYB binding sites in the promoters region of MYB regulated genes. A better understanding of the regulation mechanisms of MYB repressors requires some questions to be answered: how MYB repressors perceive environmental signals, what is the role of micro RNA in the regulation of MYB repressors, does post-translational modifications play any role in the regulation?

Disclosure statement

No conflict of interest was declared.

Author contributions

M.Z. and X.Z designed the paper. C.C., K.Z, and M.K. analyzed the data. C.C., K.Z., M.K., M.H., M.I.G., X.Z. and M.Z. wrote the paper.

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