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MYB-mediated regulation of lignin biosynthesis in grasses☆

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ABSTRACT

Recent analyses of cell wall components of various grass mutant and transgenic lines have provided information on characteristic transcriptional regulation of cell wall formation in grasses, although its knowledge yet remains limited compared with that for eudicot cell wall formation. MYB transcription factors, which are regarded as downstream regulators operating under NAM, ATAF1/2, and CUC2 (NAC) domain transcription factors, have been suggested to be involved in direct regulation of cell wall biosynthesis. In this review, we discuss MYB-mediated transcriptional regulation of the biosynthesis of grass lignins, including grass-specific lignin components such as γ-acyl groups and flavone tricin units. Grass mutant and transgenic lines harboring modified cell-wall-associated *MYB* genes display altered composition of the γ-acylated and tricin-incorporated lignin units and/or modified expression of enzyme genes involved in the formation of these grass-specific lignin components along with conserved monolignol biosynthetic genes. These findings implicate important roles for MYB transcription factors in coordinated regulation of grass lignin biosynthesis including γ-acylated and tricin-incorporated lignin biosynthesis.

1. Introduction

Lignocellulose, which is derived mainly from secondary cell walls of vascular plants, is the most abundant renewable raw materials for fuels, woody materials, and paper feedstocks [1]. A large proportion of the wood biomass used as fuels, which comprises almost half of the world's annual wood consumption, is derived from natural forests, which has accelerated deforestation predominantly in developing countries [1]. Non-wood lignocelluloses derived from agricultural wastes (e.g., grass straws and herbaceous fibers) and grass biomass plants [e.g., *Miscanthus* spp. and switchgrass (*Panicum virgatum*)] are an additional promising source of lignocellulosic biomass [2]. Other large grass species, such as sugarcane (*Saccharum* spp.), *Erianthus* spp., sorghum (*Sorghum bicolor*), and bamboos are also potent biomass feedstocks [1–7]. In general, delignification efficiency of grass biomass is higher than that of tree biomass [1], which might be beneficial for fractionation of lignins and cell-wall polysaccharides toward biorefinery processes. Utilization of grass lignocellulose is therefore important to reduce current deforestation and to meet future demands of lignocellulosic biomass for biorefinery applications.

Lignin, a major component (15 %–30 %) of lignocellulosic biomass, is a phenylpropanoid polymer filling the interspace of cell-wall polysaccharides, i.e., cellulose and hemicelluloses in the secondary cell walls of vascular plants [8–11]. Lignin plays essential roles in plant growth, such as provision of mechanical support, water transport, and abiotic/biotic stress management [8–10]. However, lignin acts as a major obstacle for the efficient use of lignocellulosic polysaccharides (e.g., pulping and fermentable sugar production), hence plant biomass with reduced and/or easily removable lignins is advantageous [12–17]. On the other hand, lignin is also regarded as a valuable source of aromatic chemicals [18–21] and contributes primarily to the increased heating value of lignocellulosic biomass [1], which has in turn promoted generation of lignin-enriched plants [22–27].

Because of the importance of grass lignocelluloses, structure and biosynthesis of grass lignins have been intensively studied. Grass lignins incorporate several unique units [17,28], although they share the three monolignol-derived aromatic units, i.e., *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) aromatic units [8–11] with eudicot lignins. One of the typical grass lignin units is tricin (T) units derived from incorporation of a flavone tricin into lignin polymers as a nucleation site [29,30], although T lignins are also cell wall constituents of certain non-grass monocots and the eudicots such as *Medicago truncatula* and alfalfa (*Medicago sativa*) albeit at much lower abundances [31,32]. Genes that encode enzymes associated with T lignin biosynthesis of maize (*Zea*

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mays) [33,34], sorghum [35], and rice (*Oryza sativa*) [36–38] have been identified. Another unique component of grass lignins is ester-linked *p*-coumarate (*p*CA) at the γ-position of the lignin backbones, arising from the incorporation of γ-*p*-coumaroylated monolignols which serve as grass-specific lignin monomers [39–41]. In addition, ferulate (FA)-bound lignin units derived from analogous γ-feruloylated monolignols are present in grass lignins [42], although the majority of FAs is linked to arabinoxylans, which are hemicellulosic polysaccharides typical of grasses [43,44]. Such γ-*p*-coumaroylated and γ-feruloylated monolignols are synthesized by the actions of BAHD acyltransferase enzymes, i.e., *p*-COUMAROYL-COA:MONOLIGNOL ACYLTRANSFERASE (PMT) [39–41] and FERULOYL-CoA:MONOLIGNOL ACYLTRANSFERASE (FMT) [42], respectively. These grass lignin components – T, *p*CA, and FA – are a potent source of beneficial physiologically active substances [30,31,45]. In addition, T-truncated rice cell walls are saccharified more efficiently by enzymatic hydrolysis than the wild-type lignocellulose [36,37] and *p*CA introduction to eudicot *Arabidopsis thaliana* lignins improves their solubility in a mild alkaline solution [46]. Thus, grass lignin structures have attracted substantial attention with regard to their contribution to enhanced applications of grass biomass as well as their physiological functions.

Transcriptional regulation of secondary cell wall formation has been extensively studied using eudicot and grass species from a biomass breeding perspective, although fewer studies have been conducted on grass species than on eudicots. Secondary cell-wall-associated NAM, ATAF1/2, and CUC2 (NAC) domain transcription factors such as VASCULAR-RELATED NAC-DOMAIN (VND7) and NAC SECONDARY WALL THICKENING PROMOTING FACTOR (NST3)/SECONDARY WALL-ASSOCIATED NAC DOMAIN PROTEIN (SND1), which act as triggers for differentiation of xylem and fiber cells, are accepted as master switches for secondary cell wall formation [47–57]. The master switches directly regulate expressions of downstream transcription factor genes, which are associated with regulation of lignin and cell-wall polysaccharide biosynthesis. On the other hand, the master switch *NAC* genes are directly or indirectly regulated by various other transcription factors including WRKYs [58–62], basic helix-loop-helix (bHLH) MYC2 and MYC4 [63] and a E2 factor (E2F) E2Fc [64] as well as downstream MYB transcription factors [52]. Many recent works have manipulated the upstream cell-wall regulators for modification [27,59,61,65–67] and reconstruction [68,69] of lignocelluloses.

Cell-wall-associated MYB transcription factors, which are major downstream regulators operating under NAC transcription factors, have been intensively studied using eudicot species such as *Arabidopsis* [52,54,70–72], *Populus* spp. [73–77], *Eucalyptus gunnii* [78,79] and *Antirrhinum majus* [80]. On the other hand, although our understanding of the functions of cell-wall-associated MYB transcription factors in grasses is still considerably limited, recent studies have highlighted distinct functions of grass MYBs compared with those of eudicot MYBs [56]. In this review, we focus on characterization of grass cell walls with modified expression and/or knockout of cell-wall-associated *MYB* genes, and discuss the association of a MYB-mediated regulatory network with grass cell wall biosynthesis, which may provide information for guiding the future direction of grass biomass breeding toward biorefinery applications.

2. Functions of MYB activators are partially shared in *Arabidopsis* and grasses

In plants, R2R3-type MYB transcription factors play important roles in the biosynthesis of phenylpropanoids, such as monolignols (major lignin precursors), and flavonoids [81–83]. In the eudicot *Arabidopsis*, AtMYB46 and AtMYB83 (MYB46/83 clade, Fig. 1a) are directly upregulated by the master switches (NACs), and redundantly activate secondary cell wall formation in xylem vessels and fibers [84–86] (Table 1). AtMYB85 (MYB42/85 clade, Fig. 1a) [72], and AtMYB58 and AtMYB63 (MYB58/63 clade, Fig. 1a) [87] have been regarded as further downstream regulators. AtMYB85, AtMYB58, and AtMYB63 can target and/or upregulate lignin biosynthetic genes in *Arabidopsis* (Table 1), but do not upregulate genes involved in cell-wall polysaccharide biosynthesis and flavonoid biosynthesis [72,87], which indicates that AtMYB85, AtMYB58, and AtMYB63 act as lignin-specific activators in *Arabidopsis*.

The functions of these MYB transcription factors as a lignin activator may be conserved in *Arabidopsis* and grasses. Rice OsMYB46, maize ZmMYB46, and switchgrass PvMYB46 (PvMYB46A) (MYB46/83 clade, Fig. 1a) can act as a functional ortholog of AtMYB46 and AtMYB83 in *Arabidopsis* [88,89] (Table 1). Rice *OsMYB42/85* and *OsMYB58/63* (Fig. 1a) overexpression augments the intensity of phloroglucinol−HCl staining, which detects cinnamaldehyde end-groups in lignin polymers, of vascular bundles and sclerenchyma fibers, and elevates the expression level of at least one lignin biosynthetic gene encoding CINNAMYL ALCOHOL DEHYDROGENASE (CAD) [90] (Table 1). Switchgrass *PvMYB42/85* (*PvMYB42/85A*) and *PvMYB58/63* (*PvMYB58/63A*) overexpression increases lignin content, lignin S/G ratio, and expression levels of lignin biosynthetic genes, such as those encoding FERULATE 5-HYDROXYLASE (F5H) or CONIFERALDEHYDE 5-HYDROXYLASE (CAld5H) [61].

Maize ZmMYB167, which belongs to the MYB42/85 clade (Fig. 1a), upregulates lignin biosynthesis in *Brachypodium* and maize [91] (Table 1). However, *ZmMYB167* expression distinctly impacts on plant growth, tissue-specific lignification, and lignin S/G ratio of the two species [91]. In addition, *ZmMYB167* overexpression decreases glucose yield from enzymatic hydrolysis of *Brachypodium*, whereas the glucose yield from maize is unchanged [91], which may reflect structural differences of lignocelluloses from the two transformants.

In sorghum, *SbMYB60*, which encodes a protein in the MYB58/63 clade (Fig. 1a), has been manipulated for grass biomass valorization. Overexpression of *SbMYB60* upregulates monolignol biosynthetic genes and enhances accumulation of lignins, especially S lignins, and soluble phenolics, including caffeic, ferulic, and sinapic acids [22] (Table 1). *SbMYB60* overexpression substantially elevates the calorific value (100 cal g^{-1} on average among transgenic lines) of sorghum biomass, which is possibly due to increased contents of lignin and soluble phenolics [22]. *SbMYB60* overexpression also activates primary carbon metabolism, which suggests that SbMYB60 can shunt carbon sources toward phenylpropanoid biosynthesis [24]. However, it should be noted that sorghum overexpressing *SbMYB60* shows growth reduction [22], which is similar to rice overexpressing *OsMYB58/63* [90] and switchgrass overexpressing *PvMYB58/63* [61]. The growth reduction might be linked to excess lignification and/or metabolic changes [22,24]. In addition, *SbMYB60* overexpression improves resistance of sorghum against a major pathogen, *Fusarium thapsinum*, under greenhouse conditions, although it is ineffective under field conditions [25].

Genes that encode SECONDARY WALL ASSOCIATED MYBs (SWAMs) (Fig. 1a) have been found in grasses and eudicots but not in Brassicaceae, including *Arabidopsis* [92]. *Brachypodium distachyon* BdSWAM1 interacts with promoters of cellulose and lignin biosynthetic genes and activates secondary cell wall thickening [92] (Table 1). *BdSWAM1* overexpression enhances biomass accumulation, whereas *BdSWAM1* dominant repression improves bioethanol productivity [92]. SWAM transcription factors may strongly impact on secondary cell wall formation in grasses as well as eudicots, although they are not conserved in the Brassicaceae [92].

3. MYB58/63 is involved in cell-wall polysaccharide biosynthesis in grasses

In addition to the function as a lignin activator, MYB58/63 proteins (Fig. 1a) may be associated with cell-wall polysaccharide biosynthesis in grasses (Table 1). For example, rice OsMYB58/63, as well as AtMYB63, activates secondary cell-wall-specific cellulose synthase gene *OsCesA7* in rice protoplasts [93]. The *cis*-regulatory element AC-II, to which

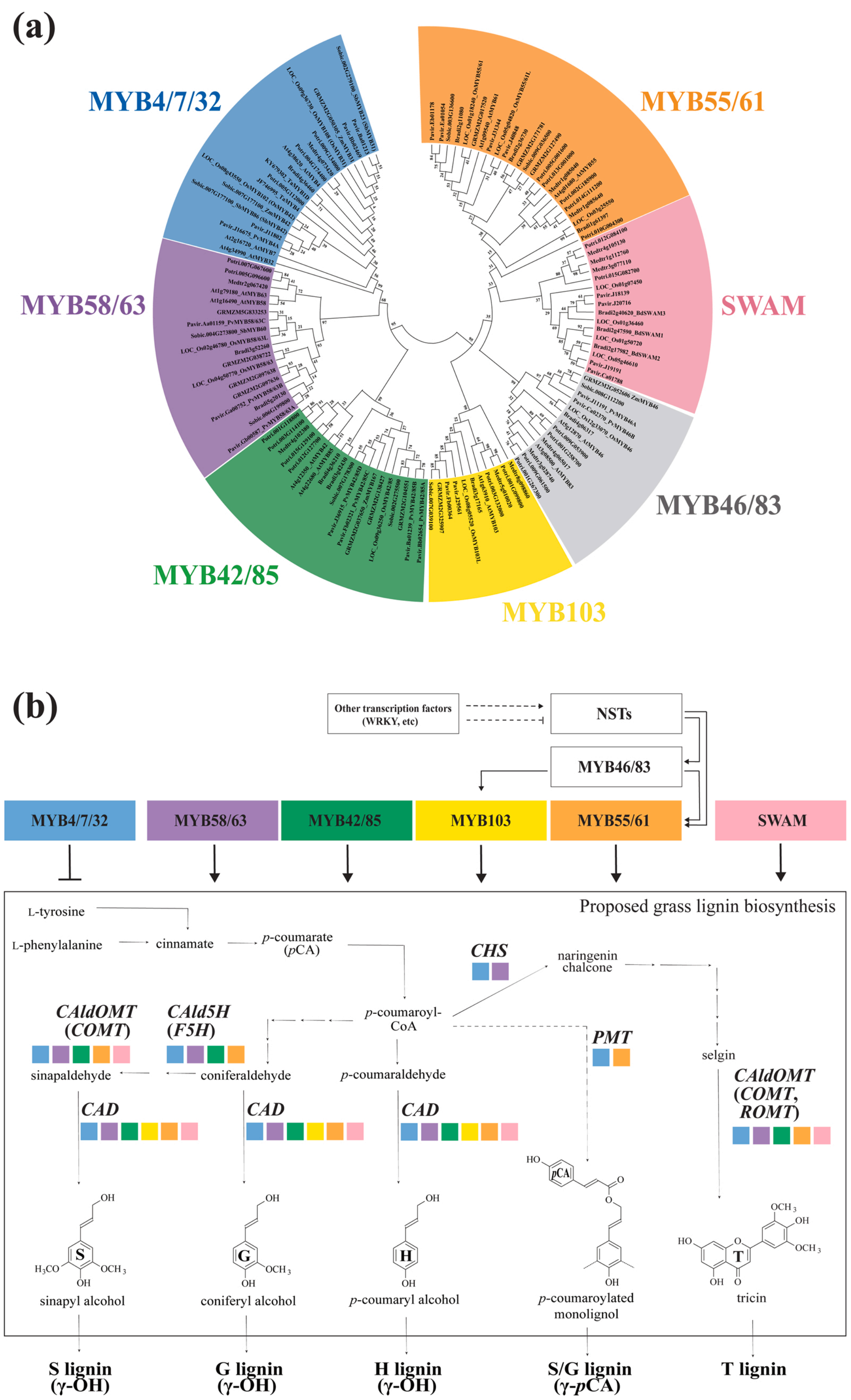

Fig. 1. Phylogenetic analysis of cell-wall-associated R2R3-type MYB proteins (a) and schematic representation of the regulation of grass lignin biosynthesis by the MYB transcription factors (b). (a) The phylogenetic tree of amino acid sequences that contain the R2R3 domains of MYB proteins from *Arabidopsis thaliana*, poplar (*Populus trichocarpa*), *Medicago truncatula*, rice (*Oryza sativa*), maize (*Zea mays*), wheat (*Triticum aestivum*), sorghum (*Sorghum bicolor*), *Brachypodium distachyon,* and switchgrass (*Panicum virgatum*) were used for the analysis. The MYB proteins were classified into seven clades such as those containing AtMYB46 and AtMYB83 (MYB46/83 clade), AtMYB42 and AtMYB85 (MYB42/85 clade), AtMYB58 and AtMYB63 (MYB58/63 clade), AtMYB55 and AtMYB61 (MYB55/61 clade), AtMYB103 (MYB103 clade), AtMYB4, AtMYB7, and AtMYB32 (MYB4/7/32 clade), and BdSWAM1 (SWAM clade). More detailed information on construction of the tree was described in the supplementary material. (b) Genes involved in monolignol biosynthesis (*CAld5H/F5H* and *CAD*), *p*-coumaroylated monolignol biosynthesis (*PMT*), flavonoid biosynthesis (*CHS*), and monolignol/tricin biosynthesis (*CAldOMT*) in grasses are upregulated or downregulated by manipulating *MYB* genes. Coloration of squares below enzyme genes matches that of MYB transcription factors that affect expressions of the genes.

Table 1

Modifications of plant cell walls and gene expressions by manipulating *MYB* genes.

MYB gene	Impacts on cell wall and gene expression	
	Knockout/Knockdown/Dominant repression	Overexpression/Heterologous expression
AtMYB46 (At5g12870)	***Arabidopsis***: cell wall thickness (↓) [84]	***Arabidopsis***: ectopic lignification, lignin biosynthetic genes (↑), cell-wall polysaccharide biosynthetic genes (↑) [84]
AtMYB83 (At3g08500)	***Arabidopsis***: cell wall thickness (↓) [85]	***Arabidopsis***: ectopic lignification, lignin biosynthetic genes (↑), cell-wall polysaccharide biosynthetic genes (↑) [85]
OsMYB46 (LOC_Os12g33070)		***Arabidopsis***: ectopic lignification, lignin biosynthetic gene *4CL* (↑), cell-wall polysaccharide biosynthetic genes (↑) [88]
ZmMYB46 (JN634085)		***Arabidopsis***: ectopic lignification, lignin biosynthetic gene *4CL* (↑), cell-wall polysaccharide biosynthetic genes (↑) [88]
PvMYB46 (*PvMYB46A*; Pavir.J11191)		***Arabidopsis***: ectopic lignification, lignin biosynthetic genes (↑), cell-wall polysaccharide biosynthetic genes (↑) [89]
AtMYB85 (At4g22680)	***Arabidopsis***: cell wall thickness (↓), cell-wall polysaccharide content (↓) [72]	***Arabidopsis***: ectopic lignification [72]
OsMYB42/85 (LOC_Os09g36250)	**Rice:** lignin staining coloration (↓), lignin biosynthetic gene *CAD* (↓) [90]	**Rice:** lignin staining coloration (↑), lignin biosynthetic gene *CAD* (↑) [90]
PvMYB42/85 (*PvMYB42/85A*; Pavir. Bb02654)	**Switchgrass:** lignin biosynthetic genes (↓) [61]	**Switchgrass:** lignin content (↑), lignin S/G (↑), lignin biosynthetic genes (↑), cell-wall polysaccharide biosynthetic genes (↓) [61]
ZmMYB167 (GRMZM2G037650)		***Brachypodium***: ectopic lignification, lignin content (↑), lignin S/G (↑), *p*CA content (↑) [91] **Maize:** lignin content (↑), *p*CA content (↑), FA content (↑) [91]
AtMYB58 (At1g16490)	***Arabidopsis***: cell wall thickness (↓), lignin monomer yield (↓) [87]	***Arabidopsis***: ectopic lignification, lignin monomer yield (↑), lignin biosynthetic genes (↑) [87]
AtMYB63 (At1g79180)	***Arabidopsis***: cell wall thickness (↓), lignin monomer yield (↓) [87]	***Arabidopsis***: ectopic lignification, lignin monomer yield (↑), lignin biosynthetic genes (↑) [87] **Rice:** lignin content (↑) [23]
OsMYB58/63 (LOC_Os04g50770)		**Rice:** lignin staining coloration (↑), lignin biosynthetic gene *CAD* (↑) [90]
PvMYB58/63 (*PvMYB58/65A*; Pavir. Gb00587)	**Switchgrass:** lignin biosynthetic genes (↓) [61]	**Switchgrass:** lignin content (↑), lignin S/G (↑), lignin biosynthetic genes (↑), cell-wall polysaccharide biosynthetic genes (↑), flavonoid biosynthetic genes (↑) [61]
SbMYB60 (Sobic.004G273800)		**Sorghum:** ectopic lignification, lignin content (↑), lignin S/G (↑), soluble phenolic content (↑), cellulose staining coloration (↑), cellulose content (↓), xylan content (↓), lignin biosynthetic genes (↑), cell-wall polysaccharide biosynthetic genes (↑) [22,24]
BdSWAM1 (Bradi2g47590)	***Brachypodium***: cell wall thickness (↓), lignin content (↓), lignin biosynthetic genes (↓), cell-wall polysaccharide biosynthetic genes (↓) [92]	***Brachypodium***: cell wall thickness (↑), lignin biosynthetic gene *CAld5H/COMT* (↑), cell-wall polysaccharide biosynthetic genes (↑) [92]
AtMYB55 (At4g01680)		**Rice:** lignin content (↑) [23]
AtMYB61 (At1g09540)	***Arabidopsis*** cell wall thickness (↓), lignin staining coloration (↓) [94,95]	**Rice:** lignin content (↑), lignin S/G (↑), *p*CA level (↑), tricin level (↑) [23] ***Arabidopsis***: ectopic lignification [94,95]
OsMYB55/61 (*OsMYB61a*; LOC_Os01g18240)	**Rice:** lignin staining coloration (↓), lignin content (↓), cellulose content (↓), mixed-linkage glucan content (↓), lignin biosynthetic genes (↓), cell-wall polysaccharide biosynthetic genes (↓) [90,97]	**Rice:** lignin staining coloration (↑), lignin biosynthetic gene *CAD* (↑) [90]
AtMYB103 (At1g63910)	***Arabidopsis***: cell wall thickness (↓), lignin S/G (↓), lignin biosynthetic gene *CAld5H/F5H* (↓) [72,98]	***Arabidopsis***: cell wall thickness (↑) [72]
OsMYB103 (*OsMYB103L*; LOC_Os08g05520)	**Rice:** lignin staining coloration (↓), cell-wall polysaccharide content (↓/↑), lignin biosynthetic gene *CAD* (↓), cellulose biosynthetic genes (↓) [90,99,100]	**Rice:** lignin staining coloration (↑), cellulose staining coloration (↑), cellulose content (↑), lignin biosynthetic gene *CAD* (↑), cellulose biosynthetic genes (↑) [90,99,100]
AtMYB4 (At4g38620)	***Arabidopsis***: sinapoyl malate content (↑), flavonol content (↓), anthocyanin content (↑), lignin biosynthetic gene *C4H* (↑) [103,105]	***Arabidopsis***: sinapoyl malate content (↓), lignin biosynthetic genes (↓/↑), flavonoid biosynthetic genes (↓) [103,106]
AtMYB7 (At2g16720)	***Arabidopsis***: flavonol content (↑), anthocyanin content (↓), lignin biosynthetic genes (↑), flavonoid biosynthetic genes (↑) [105]	***Arabidopsis***: flavonol content (↓), lignin biosynthetic genes (↓), flavonoid biosynthetic genes (↓) [105]
AtMYB32 (At4g34990)	***Arabidopsis***: lignin biosynthetic gene *CAldOMT/COMT* (↑), flavonoid biosynthetic genes (↓) [104]	
OsMYB108 (*OsMYB31*; LOC_Os09g36730)	**Rice:** lignin content (↑), lignin S/G (↑), *p*CA content (↑), FA content (↑), lignin biosynthetic genes (↑), flavonoid (tricin) biosynthetic genes (↑) [26]	
ZmMYB31 (GRMZM2G050305)		***Arabidopsis***: lignin staining coloration (↓), lignin content (↓), sinapoyl malate content (↓), anthocyanin content (↑), lignin biosynthetic genes (↓), flavonoid biosynthetic genes (↑) [110] **Sugarcane:** lignin content (↓), lignin S/G (↓), lignin biosynthetic genes (↑/↓) [113]

(*continued on next page*)

Table 1 (*continued*)

MYB gene	Impacts on cell wall and gene expression	
	Knockout/Knockdown/Dominant repression	Overexpression/Heterologous expression
ZmMYB42 (GRMZM2G419239)		***Arabidopsis***: lignin staining coloration (↓), lignin content (↓), lignin S/G (↓), sinapoyl malate content (↓), flavanol content (↓), cell-wall polysaccharide contents (↑/↓), lignin biosynthetic genes (↓), flavonoid biosynthetic genes (↓) [109] **Sugarcane**: lignin content (↓), Lignin S/G (↓), lignin biosynthetic genes (↑/↓) [113]
TaMYB1D (KY679302)		**Tobacco**: lignin biosynthetic genes (↓), flavonoid biosynthetic genes (↓) [117]
TaMYB4 (JF746995)		**Tobacco**: lignin content (↓), lignin S/G (↑), flavonoid content (↑), lignin biosynthetic genes (↓) [118]
PvMYB4 (Pavir.J16675)		**Tobacco**: lignin content (↓), lignin S/G (↑), lignin biosynthetic genes (↓) [119] **Switchgrass**: lignin content (↓), *p*CA content (↓), lignin biosynthetic genes (↓), cell-wall polysaccharide biosynthetic genes (↓), flavonoid biosynthetic genes (↓) [61,119]

Arrows indicate changes of cell wall thickness, cell wall staining coloration, contents of cell wall components, lignin S/G ratio, or gene expression levels: ↑, overall upregulated; ↓, overall downregulated; ↑/↓, partially upregulated and partially downregulated. References are described in the parentheses.

OsMYB58/63, AtMYB58, and AtMYB63 can bind [87,93], is present in the promoter regions of lignin, cellulose, and hemicellulose biosynthetic genes in rice, whereas the element AC-II was detected only upstream of lignin biosynthetic genes in *Arabidopsis* [93]. These results collectively suggest that diversity of *cis*-regulatory element composition induces distinct transcriptional regulation by MYB58/63 proteins in *Arabidopsis* and rice [93]. *PvMYB58/63* overexpression upregulates cellulose and xylan biosynthetic genes in switchgrass [61], which supports the notion that MYB58/63 proteins can activate both lignin and cell-wall polysaccharide biosynthesis in grasses. Sorghum *SbMYB60* overexpression positively affects polysaccharide biosynthetic genes, although *SbMYB60* shows low co-expression values with cellulose biosynthetic genes [24]. Taken together, these findings indicate that MYB58/63 proteins may play a unique role in grasses, such as activation of cell-wall polysaccharide biosynthesis, although the function may differ among grass species.

4. MYB55/61 and MYB103 act as a secondary cell wall activator under gibberellin-mediated signaling

AtMYB61 (MYB55/61 clade, Fig. 1a) activates secondary cell wall formation, and enhances allocation of carbon sources to developing vasculature, root, and seed tissues in *Arabidopsis* [94,95]. *AtMYB61* overexpression induces ectopic lignification of *Arabidopsis* [94,95] (Table 1).

Heterogenous expression of *AtMYB61* and *AtMYB55* (MYB55/61 clade, Fig. 1a), as well as *AtMYB63*, has been tested in rice to generate and characterize lignin-enriched grass biomass for lignin-oriented biorefinery applications [23]. *AtMYB61* expression increases lignin content of rice culm tissues by up to 53 % [23] (Table 1). Rice OsMYB55/61 (OsMYB61a) (MYB55/61 clade, Fig. 1a) also acts as a secondary cell wall activator [90,96,97]. OsMYB55/61 plays an essential role in activating cellulose synthase genes through gibberellin-mediated signaling in rice [96]. *OsMYB55/61* knockout reduces the contents of cell wall components such as lignin, cellulose, and mixed-linkage glucans, and downregulates secondary cell wall biosynthetic genes in rice [97]. In addition, transient overexpression of *OsMYB55/61* and its paralog *OsMYB55/61-L* (*OsMYB61b*) (Fig. 1a) activates secondary cell wall biosynthetic genes in rice protoplasts [97].

AtMYB103 (MYB103 clade, Fig. 1a) is directly upregulated by the master switches AtVND6/7, AtNST1/2, and AtSND1, as well as AtMYB46/83, and can upregulate the secondary-cell-wall-related cellulose synthase gene *CesA8* [72] (Table 1). *Arabidopsis atmyb103* mutants show downregulation of *CAld5H/F5H*, which is responsible for S lignin biosynthesis, and reduction of the lignin aromatic S/G ratio compared with those of the wild-type controls, although AtMYB103 does not directly activate *F5H* in a promoter assay [98]. Microarray data suggested that AtMYB103 is involved in a complex regulatory network [98], which may affect F5H-mediated S lignin biosynthesis in *Arabidopsis*.

In contrast, however, no evidence suggests that MYB103 proteins are associated with S lignin biosynthesis in grasses. Rice *OsMYB103* (*OsMYB103L*) (Fig. 1a), which has been isolated as a gene responsible for the culm easily fragile phenotype, is involved in gibberellin-SLENDER RICE1 (SLR1) signaling for upregulation of cellulose biosynthesis [99] (Table 1). *OsMYB103* overexpression increases the cellulose content of rice [100]. Enhanced expression of *OsMYB103* strengthens the intensity of phloroglucinol-HCl staining of lignin in rice culm tissues and upregulates the lignin biosynthetic gene *CAD2* [90]. Further investigation of function(s) of MYB103 proteins in grass cell walls will be beneficial for a deeper understanding of grass cell wall formation.

5. MYB4 acts as a repressor for phenylpropanoid biosynthesis

R2R3-MYB transcriptional repressors are crucial regulators of phenylpropanoid biosynthesis, which includes monolignol biosynthesis, and flavonoid biosynthesis in plants [101,102]. *Arabidopsis* AtMYB4 (MYB4/7/32 clade, Fig. 1a) has been characterized as a regulator of phenylpropanoid biosynthesis in response to ultraviolet irradiation [103]. Knockout mutation of *AtMYB4* upregulates the phenylpropanoid biosynthetic gene encoding CINNAMATE 4-HYDROXYLASE (C4H), whereas *AtMYB4* overexpression suppresses *C4H* in addition to other phenylpropanoid and flavonoid biosynthetic genes [103] (Table 1). *AtMYB7* and *AtMYB32* (Fig. 1a), which encodes close homologs of AtMYB4, also regulate flavonoid biosynthetic genes in vegetative and reproductive (anther) tissues, respectively (Table 1) [104,105]. Recent work has demonstrated that triple knockout of *AtMYB4*, *AtMYB7*, and *AtMYB32* partially restores growth defects and anthocyanin reduction, and upregulates flavonoid biosynthetic genes of *Arabidopsis ref4-3* mutants deficient in *MED5a* that encodes a subunit of transcriptional co-regulatory complex mediator [106]. On the other hand, *AtMYB4* knockout mutation alleviates dwarfism of *Arabidopsis ref8* mutants deficient in the function of *p*-COUMAROYL ESTER 3-HYDROXYLASE (C3′H), while not affecting the reduction in lignin content, augmentation of H lignin content, and modifications of contents of hydroxycinnamate esters and flavonoids [107]. This result suggests that AtMYB4 has unknown function(s) additional to regulation of phenylpropanoid metabolism.

Our deepest understanding of grass MYB4 homologs has been gleaned from studies on maize ZmMYB31 and ZmMYB42 (Fig. 1a)

[108–113] (Table 1). Heterologous expression of *ZmMYB31* and *ZmMYB42* suppresses lignification of *Arabidopsis* [109,110]. Chromatin immunoprecipitation (ChIP) has shown that ZmMYB31 and/or ZmMYB42 repressors directly target several lignin biosynthetic genes, such as those encoding 4-COUMARATE:CoA LIGASE (4CL), CAFFEOYL SHIKIMATE ESTERASE (CSE) and 5-HYDROXYCONIFERALDEHYDE *O*-METHYLTRASFERASE (CAldOMT, or CAFFEIC ACID *O*-METHYLTRASFERASE, COMT), in maize [97]. In addition to these genes, *CAld5H/F5H* genes, which are responsible for S lignin biosynthesis in sorghum [114] and rice [115,116], are direct targets of MYB4 homolog (s) in these two species [112]. However, ZmMYB31 and ZmMYB42 does not directly target *CAld5H/F5H* in maize [112], although *Arabidopsis* expressing *ZmMYB42* [109] and sugarcane expressing *ZmMYB31* and *ZmMYB42* [113] display reductions in *CAld5H/F5H* expression and lignin S/G ratio. Abundance of grass MYB4 homologs bound to the promoter of lignin biosynthetic genes is not always correlated with expression level of the *MYB4* genes [112], which might be explained by protein-protein interactions such as post-translational phosphorylation [76,77] and formation of protein complexes with other transcription factors [106].

Wheat (*Triticum aestivum*) TaMYB1D [117] and TaMYB4 [118] (MYB4/7/32 clade, Fig. 1a) are close homologs of ZmMYB31 and ZmMYB42, respectively. Heterologous expression of *TaMYB1D* and *TaMYB4* represses lignin biosynthetic genes in tobacco (*Nicotiana tabacum*) [117,118]. *TaMYB1D* expression increases a drought tolerance of tobacco plants [117] and *TaMYB4* expression reduces lignin content, especially G monomer content, of tobacco cell walls [118].

Switchgrass overexpressing *PvMYB4* (*PvMYB4A*) that encodes MYB4 homologs (Fig. 1a) has been characterized for polysaccharide-oriented biorefinery applications. *PvMYB4* expression represses lignin biosynthetic genes and reduces the lignin content of the eudicot tobacco and switchgrass [61,119] (Table 1). However, *PvMYB4* expression increases lignin aromatic S/G ratio in tobacco but not in switchgrass [119]. *PvMYB4* overexpression reduces the concentration of soluble phenolics, which are potential sugar fermentation inhibitors, in methanol extractives from switchgrass biomass, whereas soluble sugars were enriched, which is possibly due to suppression of the carbon flux into the phenylpropanoid biosynthetic pathway [120]. The altered biomass of switchgrass overexpressing *PvMYB4* displays improved enzymatic saccharification efficiency [119] and increased ethanol productivity via sugar fermentation [120]. In addition, a multiyear field cultivation trial revealed that the optimal expression level of *PvMYB4* is important for avoidance of growth defects, e.g., a diminished root system, while producing biomass that is relatively easy to convert to biofuels [121].

Recently, loss-of-function of a rice MYB4 transcription factor, OsMYB108 (OsMYB31) (Fig. 1) was shown to increase lignin content and upregulate monolignol biosynthetic genes [26] (Table 1), which further supports the notion that MYB4 transcription factors act as a lignin repressor. In addition, interestingly, *OsMYB108*-knockout rice cell walls display augmentation of grass-specific lignin units, such as *p*CA and T units [26]. This result provides an important insight into transcriptional regulation of grass cell wall formation, which is further discussed below.

6. MYB transcription factors are associated with regulation of grass-specific lignin biosynthesis

In addition to the roles as an activator for the monolignol biosynthesis conserved in eudicots and grasses, MYB42/85, MYB58/63, and MYB55/61 transcription factors (Fig. 1a) may be involved in grass-specific lignin biosynthesis [122] in grasses. For example, maize ZmMYB167 (MYB42/85 clade, Fig. 1a) augments cell-wall-bound *p*CA contents along with total lignin contents in *Brachypodium* and maize [91] (Table 1). Likewise, heterologous expression of *Arabidopsis* AtMYB61 (MYB55/61 clade, Fig. 1 a) preferentially enriches *p*CA in rice cell walls [23] (Table 1). In line with this finding, loss-of-function of rice OsMYB55/61 (MYB55/61 clade, Fig. 1a) downregulates grass-specific BAHD acyltransferase genes [97], such as *OsAT4* (*OsPMT1*) [39–41] encoding an enzyme responsible for *p*-coumaroylation of monolignols. SbMYB60 (MYB58/63 clade, Fig. 1a) also upregulates cell-wall-associated BAHD acyltransferase genes in sorghum [24]. On the other hand, OsMYB108 (MYB4/7/32 clade, Fig. 1) has been suggested to downregulate *p*CA lignin biosynthesis (Table 1) through repression of *OsAT4/OsPMT1* in rice [26]. PvMYB4 (MYB4/7/32 clade, Fig. 1a) also represses cell-wall-bound *p*CA biosynthesis in switchgrass [119]. Taken together, these results suggest the MYB-mediated transcriptional network might regulate incorporation of *p*CA into grass lignins (Fig. 1b). Such grass-specific MYB functions might be partially explained by the distinct composition of MYB-bound *cis*-regulatory elements in grasses [93], although further investigation is required.

MYB transcription factors such as C1 [123] and P [124] in maize and Y1 in sorghum [125] are essential for flavonoid biosynthesis. In addition, it has been shown that MYB4 transcription factors serve as a regulator of flavonoid biosynthesis in both eudicots [103–107] and grasses [26,58]. Loss-of-function of OsMYB108, a rice homolog of AtMYB4 (Fig. 1 a), upregulates tricin biosynthetic genes such as those encoding APIGENIN 3′-HYDROXYLASE/CHRYSOERIOL 5′-HYDROXYLASE (A3′H/C5′H) [37] and FLAVONE SYNTHASE II (FNSII) [36] along with other genes involved in earlier steps of flavonoid biosynthesis, and proportionately augments lignin-incorporated T units with lignin aromatic units [26]. This result suggests that a flavonoid regulator MYB4 may play a crucial role in the regulation of T lignin biosynthesis in grasses (Fig. 1 b). In agreement with this notion, *OsCAldOMT1* (also known as *OsCOMT1* or *OsROMT9*), which encodes a bifunctional *O*-methyltransferase responsible both for S and T lignin biosynthesis [38,126], is upregulated in *OsMYB108*-knockout rice [26]. *CAldOMT/COMT* genes are direct targets of MYB4 homologs [112] and downregulated by overexpressing *MYB4* homologs in grasses [61,113,119] (Table 1 and Fig. 1 b).

Overexpression of other cell-wall-associated *MYB* genes also affect flavonoid biosynthesis in grasses (Fig. 1 b), although the effect might be an indirect consequence of the upregulation of *MYB* genes. For example, switchgrass PvMYB58/63, a homolog of lignin-specific activators AtMYB58 and AtMYB63 (Fig. 1 a), upregulates flavonoid biosynthetic genes [61]. In sorghum, several genes involved in flavonoid biosynthesis appeared to be highly co-expressed with *SbMYB60* which encodes a homolog of AtMYB58 and AtMYB63 (Fig. 1 a) [24]. In addition, it was reported that heterologous expression of *Arabidopsis AtMYB61* preferentially augments T lignins in rice cell walls [23]. Collectively, the MYB-mediated transcriptional network may be closely associated with the coordinal regulation of S/G/H lignin biosynthesis and T lignin biosynthesis in grasses.

7. Conclusions

Recent studies using grass mutant and transgenic lines have demonstrated distinct regulation of grass cell wall biosynthesis compared with that of eudicot cell wall biosynthesis. MYB58/63 transcription factors, which act as an activator for S/G/H monolignol biosynthesis in the eudicot *Arabidopsis* and grasses, are likely associated with cell-wall polysaccharide biosynthesis in grasses. In addition, MYB transcription factors in the MYB42/85, MYB58/63, MYB55/61 and MYB4/7/32 clades affect the expression levels of genes involved in acylation of monolignols and flavonoid biosynthesis in grasses. The association of MYB transcription factors, such as AtMYB61 (MYB55/61 clade) and OsMYB108 (MYB4/7/32 clade), with lignin-bound *p*CA and T biosynthesis in rice cell walls have been suggested. The MYB-mediated regulation of cell wall formation may be linked also to various primary metabolic processes, and can affect plant growth and resistance against biotic/abiotic stresses. A more comprehensive understanding of transcriptional regulation associated with grass cell wall formation is important for an enhanced capacity to breed grass biomass for biorefinery applications.

Author statement

We are enclosing herewith a manuscript entitled "**MYB-mediated regulation of lignin biosynthesis in grasses**" for consideration for publication in the Current Plant Biology journal. With the submission of this manuscript we would like to undertake that the above-mentioned manuscript has not been published elsewhere, accepted for publication elsewhere or under editorial review for publication elsewhere, and that our institute (Research Institute for Sustainable Humanosphere, Kyoto University) is fully aware of this submission.

Declaration of Competing Interest

Authors have no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.cpb.2020.100174.

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