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Regulation of secondary cell wall lignification by abiotic and biotic constraints

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Abstract

The appearance of lignin, 400 million-years ago, has been crucial for the successful land colonization by plants. This complex phenolic polymer which confers hydrophobicity and resistance to degradation to secondary cell walls, is also neosynthesized in response to both biotic and abiotic stresses. Lignin biosynthesis during development is regulated by a complex hierarchical transcriptional gene regulatory network. Notably, distinct

abiotic stresses can co-opt different genes of this network, leading to different lignification patterns. Co-optation of this developmental network is a mean to facilitate adaptation to stress by promoting functional adaptation. Supporting this hypothesis, a growing number of transcription factors known to be involved in the regulation of secondary cell wall, were shown to integrate both developmental and environmental signals leading to an increased tolerance to abiotic stresses, such as drought or salinity. On the other hand, some transcription factors known to be involved in stress response were shown to impact lignin biosynthesis as part of the functional adaptation performed by plants to cope with natural constraints. In this review, we focus on the most recent findings on the cross-talk between stress-signaling pathways and regulation of lignin biosynthesis in response to abiotic stress and to a lesser extent to biotic stresses. We consider the transcriptional regulation level which has received much attention and the emerging roles of posttranscriptional and posttranslational regulations in the control of lignin biosynthesis in response to stresses. We end discussing future outlines and challenges to fill gaps in this emerging and exciting research field.



1. Introduction

Lignin, the second most abundant biopolymer after cellulose, represents as much as 30% of the total biomass produced on earth (Boerjan, Ralph, & Baucher, 2003; Boudet, Kajita, Grima-Pettenati, & Goffner, 2003). This complex phenolic heteropolymer massively deposited in the secondary cell walls (SCW) of xylem, confers mechanical support, imperviousness, water conductivity and resistance to biodegradation. Lignin is indeed one of the most difficult biopolymers to degrade due to the nature and heterogeneity of its linkages (Vanholme, Demedts, Morreel, Ralph, & Boerjan, 2010). Thanks to these unique properties, lignin has played a crucial role in the emergence of vascular plants and the colonization of terrestrial habitats (Weng & Chapple, 2010).

1.1 Lignification is increased in response to various biotic and abiotic stresses

Besides the crucial roles that lignin plays in plant development, it is also known for several decades that induced lignification is one plant defense response to environmental cues such as wounding, mechanical stress or pathogen attack (for reviews see Cabane, Afif, & Hawkins, 2012; LeGall et al., 2015; Vance, Kirk, & Sherwood, 1980 and reference therein). Increased lignification is indeed a common response to biotic and abiotic stress (Dong & Lin, 2021; Moura, Bonine, de Oliveira Fernandes, Dornelas, & Mazzafera, 2010 and references therein) that contribute to plant

adaptation and tolerance to stresses. For instance, reduced lignification increases xylem vulnerability to embolism, whereas higher lignification in xylem can improve drought resistance in plants (Pereira, Domingues-Junior, Jansen, Choat, & Mazzafera, 2018). Salt stress enhances lignin biosynthesis that solidifies the cell wall (Chun et al., 2019). Similarly, enhanced lignification could contribute to cell wall strengthening, preventing cell collapse caused by freezing temperatures (LeGall et al., 2015). Abnormal SCW lignification was associated with the collapse of sieve transport element in *Arabidopsis* C4H mutants (Polo et al., 2020) and in trees submitted to severe drought stress (Donaldson, 2002).

1.2 Lignin is a highly flexible phenolic polymer during development and response to stresses

Lignin is generated by oxidative radical coupling polymerization of mainly three *p*-hydroxycinnamyl alcohol precursors also called monolignols, which differ by the degree of methoxylation of the phenolic hydroxyl group in their aromatic ring (Boerjan et al., 2003). The three main hydroxycinnamyl alcohols (*p*-coumaryl, coniferyl and sinapyl alcohols) give rise to *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units, respectively (Ralph, 2010). These C6–C3 units are interconnected by a series of ether and carbon–carbon linkages in various bonding patterns (Boudet, Lapiere, & Grima-Pettenati, 1995). The most frequent interunit β -O-4 are the targets of depolymerization processes, while the carbon–carbon and 4-O-5 bonds are more resistant to degradation. The respective proportion of H, G, S lignin has a major impact on the chemical nature and the frequency of the different types of inter-monomeric linkages which in turn affect the resistance to degradation of the polymer. In the last two decades, other monomers deriving from the monolignol synthetic pathway were shown to be incorporated in lignin including hydroxycinnamic acids and aldehydes, coniferyl and sinapyl acetates (Ralph, 2010; Vanholme, De Meester, Ralph, & Boerjan, 2019) highlighting the chemical flexibility of lignin. This high flexibility was further extended to monomers not derived from the monolignol pathway. For instance, triclin which derives from the shikimate pathway was shown to be a true lignin monomer in monocots acting as a nucleation site initiating lignin polymer chains (Lan et al., 2015).

Lignin content and monomeric composition vary greatly within and between species, among tissues and organs of the same plant and even in individual cell layers (Lourenço et al., 2016; Plomion, Leprovost, & Stokes, 2001). For this reason, we think it is most appropriate to use the

plural “lignins” as suggested by Boudet et al. (1995). Most gymnosperms lignins do not contain S units, while angiosperms possess both G and S units (Boudet et al., 1995). H lignins are always poorly represented as compared to G and S units. Noteworthy, lignins synthesized in response to stresses have been shown to display distinct structural features from those of developmental lignins (Cesarino, 2019; Lange, Lapierre, & Sandermann, 1995 and references therein). This suggests that the neosynthesized lignins possess different physical properties that allow them to play a role in stress responses. For instance, in a number of cases, it was shown that defense-induced lignins appear to have increased levels of H units compared with lignins found in vascular cell walls (Doster & Bostock, 1988; Hammerschmidt, Bonnen, Bergstrom, & Baker, 1985; Lange et al., 1995; Ride, 1975; Robertsen & Svalheim, 1990). In response to cold (Hausman, Evers, Thiellement, & Jouve, 2000; Ployet et al., 2018), drought or salt (Byrt, Munns, Burton, Gilliam, & Wege, 2018), modifications of the structure and composition of lignin polymer were observed. The syringyl/guaiacyl (S/G) ratio is increased in response to salt stress in roots and stems in maize (Oliveira et al., 2020), to cold stress in *Eucalyptus* xylem cells (Ployet et al., 2018) but is decreased in response to drought in young shoots of *Populus trichocarpa* (Hori et al., 2020). In response to salinity and osmotic stress, the pattern of ferulic acid (FA) incorporation within the SCW of maize and wheat vascular tissues was linked to an increase in lignin content and S/G ratio (Oliveira et al., 2020). FA is esterified to arabinoxylans and participates with lignin monomers in oxidative coupling pathways to generate ferulate-polysaccharide-lignin complexes that cross-link the cell wall. The structure of the lignin produced *de novo* seems to largely depend on the type and intensity of the stress and on the plant species (Cesarino, 2019). Furthermore, the functional relevance for lignin polymer modifications in response to biotic and abiotic constraints is not fully understood. It should be pointed out that the tissue localization of “defense” lignins is also different from those of structural lignins (reviewed recently in Cesarino, 2019).

1.3 The complex monolignol pathway involves multigenic families

Lignin resistance to degradation which is crucial for plant development and defense against environmental cues, is on the other hand, a major obstacle for industrial processes such as pulp and paper and lignocellulosic biofuel production (Wang et al., 2018). It also decreases forage digestibility by cattle (Barrière, Courtial, Soler, & Grima-Pettenati, 2015). Large research efforts

have been devoted to engineering lignin amount and composition to reduce biomass recalcitrance (Chanoca, de Vries, & Boerjan, 2019). This has been a driving force to decipher the lignin biosynthetic pathway whose topology has been revisited several times in the last decades. Indeed, the analyses of transgenic plants or mutants downregulated for lignin biosynthetic genes revealed that the monolignol biosynthetic pathway was more complex and reticulated than initially thought (reviewed in Boerjan et al., 2003; Boudet et al., 2003; Grima-Pettenati & Goffner, 1999; Humphreys & Chapple, 2002; Ralph et al., 2004; Vanholme et al., 2010). More recently, co-expression network analyses have allowed the discovery of new alternative routes such as that involving the caffeoyl shikimate esterase (CSE; Vanholme et al., 2013). In brief, 11 enzymatic reactions are implicated in the synthesis of monolignols, which involves the general phenylpropanoid pathway starting with the deamination of phenylalanine and leading to the production of hydroxy-cinnamoyl CoA esters. The enzymes involved in this short sequence of reactions are phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H) and 4-coumarate CoA ligase (4CL). The hydroxycinnamoyl-CoA esters are not only the precursors of lignin but also of a wide range of end products including flavonoids, anthocyanins and condensed tannins, which vary according to species, cell type and environmental signals (Dixon & Paiva, 1995). In order to produce monolignols, hydroxycinnamoyl-CoA esters undergo successive hydroxylation and O-methylation of their aromatic rings (Boerjan et al., 2003) involving the following enzymatic activities: hydroxycinnamoyl-transferase (HCT); CSE; *p*-coumarate 3-hydroxylase (C3H); caffeoyl-CoA 3-O-methyltransferase (CCoAOMT); ferulate 5-hydroxylase (F5H) and caffeate/5-hydroxyferulate O-methyl-transferase (COMT). CSE catalyzes the conversion of caffeoyl shikimate into caffeate and its activity, combined with that of 4CL to produce caffeoyl-CoA, bypasses the second/reverse reaction of HCT (Vanholme et al., 2013). The conversion of the side-chain carboxyl to an alcohol group is catalyzed successively by cinnamoyl CoA reductase (CCR) and cinnamyl alcohol dehydrogenase (CAD), two enzymes considered to be the most specific of the monolignol biosynthesis pathway (Lacombe et al., 1997). The monolignols are synthesized in the cytoplasm and translocated to the apoplast, where they are dehydrogenated to monolignols radicals by the action of laccases and peroxidases (Berthet et al., 2011; Zhao et al., 2013). These monolignols radicals then couple with each other in a combinatorial fashion generating a variety of chemical bonds. It is worth noting that most of the lignin biosynthesis genes belong to gene families (Carocha et al., 2015) and that the role of individual gene family members

is not clearly established. Family members involved in vascular lignification have been identified thanks to their expression patterns, promoter analyses, analyses of transgenics and enzymatic properties (Carocha et al., 2015; Raes, Rohde, Christensen, Van de Peer, & Boerjan, 2003). Members of some gene families have been identified as stress responsive genes such as *AtCCR2* from *Arabidopsis* (Lauvergeat et al., 2001). In contrast to *AtCCR1* involved in constitutive lignification, *AtCCR2* which is poorly expressed during development, is strongly and transiently induced during the incompatible interaction with *Xanthomonas campestris* pv. *campestris* leading to a hypersensitive response. *AtCCR2* was proposed to be involved in the biosynthesis of phenolics whose accumulation may lead to resistance (Lauvergeat et al., 2001) (Table 1).



2. Objective

In this review, we focus on the most recent findings on the cross-talk between stress signaling and regulation of lignin biosynthesis in response to abiotic stress and to a lesser extent to biotic stresses. We first consider the transcriptional regulation level, describing briefly the transcriptional regulatory network regulating lignin biosynthesis during development and further showing that abiotic stresses can co-opt the SCW regulatory network. We provide a survey of (i) the stress-associated transcription factors that can modulate the lignin pathway to enhance stress resistance and (ii) the SCWs-transcription factors recruited by stress signaling pathways whose overexpression leads to better tolerance to environmental constraints. The emerging roles of posttranscriptional and posttranslational regulations in the control of lignin biosynthesis in response to stresses will also be presented. We end discussing future outlines and challenges to fill gaps in this emerging and exiting research field.



3. Transcriptional regulation of the lignin pathway

3.1 A complex transcriptional network regulates lignin biosynthesis

The biosynthesis of lignified secondary cell walls involves hundreds of genes whose expression needs to be tightly regulated in time and space. The coordination between transcriptional regulation of synthesis for each polymer (lignins, cellulose, hemicelluloses) is complex and vital to cell function. In the last two decades, tremendous progress had been made in our understanding of the transcriptional regulation of the deposition of lignified SCWs

Table 1 Transcriptional and posttranscriptional regulators involved at the crossroads between stress signaling pathways and lignin deposition.

Gene alias	Species	Gene accession	Closest ortholog(s) in At	Effect on lignification	Effect on stress resistance	Reference
Abiotic stress-signaling TFs regulating CW lignification						
<i>At</i> SHN2	<i>Arabidopsis thaliana</i>	AT5G11190	—	+ Lignin	+ Drought	Ambavaram, Krishnan, Trijatmiko, & Pereira, 2011
<i>Os</i> ERF71	<i>Oryza sativa</i>	LOC4340383	RAP2.12 (AT1G53910)	+ Lignin	+ Drought	Lee et al., 2016
<i>Os</i> ERF83	<i>Oryza sativa</i>	LOC4334864	ERF1 (AT3G23240)	+ Lignin	+ Drought	Jung et al., 2021
<i>Dc</i> DREB1A	<i>Daucus carota</i>	—	CBF3 (AT4G25480)	+ Lignin	+ Drought	Li et al., 2020
<i>Ba</i> DBL1	<i>Bryum argenteum</i>	QDB64566	—	+ Lignin	+ Salt/osmotic	Liang et al., 2021
<i>Egu</i> CBF14	<i>Eucalyptus gunnii</i>	Eucgr. A02834†	AT4G25490	+ Lignin	+ Cold	Cao et al., 2020
<i>Md</i> MYB88	<i>Malus domestica</i>	KY569647	MYB88 (AT2G02820)	+ Lignin	+ Drought	Geng et al., 2018
<i>Md</i> MYB124	<i>Malus domestica</i>	KY569648	MYB124 (AT1G14350)	+ Lignin	+ Drought	Geng et al., 2018
<i>Os</i> TF1L	<i>Oryza sativa</i>	LOC4345188	AT4G00730	+ Lignin	+ Drought	Bang et al., 2019
<i>Vl</i> bZIP30	<i>Vitis vinifera</i>	LOC100266389	AREB3/DPBF3/bZIP66 (AT3G56850) DBF4/EEL/bZIP12 (AT2G41070)	+ Lignin	+ Drought	Tu et al., 2020
<i>Pu</i> C3H35	<i>Populus ussuriensis</i>	MW792496	OXS2/C3H30 (AT2G41900)	+ Lignin	+ Drought	Li et al., 2022

Continued

Table 1 Transcriptional and posttranscriptional regulators involved at the crossroads between stress signaling pathways and lignin deposition.—cont'd

Gene alias	Species	Gene accession	Closest ortholog(s) in At	Effect on lignification	Effect on stress resistance	Reference
TFs involved in CW lignification co-opted by abiotic stresses						
NAC family						
SND1	<i>Arabidopsis thaliana</i>	AT1G32770	—	+ Lignin	+ Salt	Jeong et al., 2018
Bp NAC012	<i>Betula platyphylla</i>	KT344119	SND1/NST3/ ANAC012 (AT1G32770)	+ Lignin	+ Salt/osmotic	Hu, Zhang, & Yang, 2019
XND1	<i>Arabidopsis thaliana</i>	AT5G64530	—	– SCW	– Drought	Tang et al., 2018
Ag NAC1	<i>Apium graveolens</i> L.	MT847625	ANAC0002/ATAF1 (AT1G01720) ANAC102 (AT5G63790) ATAF2 (AT5G08790) ANAC032 (AT1G77450)	+ Lignin	+ Salt	Duan et al., 2020
MYB family						
Ej MYB1	<i>Eriobotrya japonica</i>	KF767453	MYB63 (AT1G79180) MYB58 (AT1G16490)	+ Lignin	Induced by cold	Xu et al., 2014
Bpl MYB46	<i>Betula platyphylla</i>	KP711284	MYB46 (AT5G12870) MYB83 (AT3G08500)	+ Lignin	+ Salt/osmotic	Guo et al., 2017

<i>Md</i> MYB46	<i>Malus domestica</i>	MD03G1176000†	MYB83 (AT3G08500) MYB46 (AT5G12870)	+ Lignin	+ Salt/osmotic	Chen et al., 2019
<i>Pto</i> MYB170	<i>Populus tomentosa</i>	KY114929	MYB61 (AT1G09540)	+ Lignin	+ Drought	Xu et al., 2017
<i>Eg</i> MYB1	<i>Eucalyptus gunnii</i>	Eucgr.G01774†	MYB4 (AT4G38620)	– Lignin	+ Drought	Legay et al., 2010, Soler et al., unpublished
<i>Bp</i> MYB4	<i>Betula platyphylla</i>	KA257119	MYB4 (AT4G38620)	– Lignin	+ Salt/osmotic	Yu et al., 2021
Biotic stress–signaling TFs regulating CW lignification						
<i>At</i> MYB46	<i>Arabidopsis thaliana</i>	AT5G12870	—	+ SCW	+ <i>B. cinerea</i>	Ramirez et al., 2011
<i>At</i> MYB15	<i>Arabidopsis thaliana</i>	AT3G23250	—	+ Lignin	+ Biotic stress	Chezem, Memon, Li, Weng, & Clay, 2017; Kim et al., 2020
<i>Os</i> MYB30	<i>Oryza sativa</i>	LOC4330027	MYB15 (AT3G23250)	+ Lignin	+ Biotic stress	Kishi-Kaboshi, Seo, Takahashi, & Hirochika, 2018
<i>Os</i> MYB55	<i>Oryza sativa</i>	BAF15232	MYB15 (AT3G23250)	+ Lignin	+ Biotic stress	Kishi-Kaboshi et al., 2018
Posttranscriptional regulation (sRNAs) of CW lignification						
<i>Ptr</i> -miR473	<i>Populus trichocarpa</i>			+ Lignin	Induced by UV-B	Lu et al., 2005
miRNA828	<i>Ipomoea batatas</i>			+ Lignin	Induced by wounding	Lin, Lin, Lin, Chen, & Jeng, 2012

Continued

Table 1 Transcriptional and posttranscriptional regulators involved at the crossroads between stress signaling pathways and lignin deposition.—cont'd

Gene alias	Species	Gene accession	Closest ortholog(s) in At	Effect on lignification	Effect on stress resistance	Reference
sRNA8105	<i>Ipomoea batatas</i>			+ Lignin	Induced by wounding	Lin et al., 2013
<i>Zm</i> miR528	<i>Zea mays</i>			– LIGNIN	– Lodging	Sun et al., 2018
miR397	<i>Setaria viridis</i>			– Lignin	– Salt	Nguyen, Brown, Pegler, Eamens, & Grof, 2020
<i>Vun</i> - miR408-3p	<i>Vigna unguiculata</i>			+ Lignin	+ Drought/salt	Mishra, Sahu, & Shaw, 2022
<i>Bm</i> - miR172c-5p	<i>Bacopa monnieri</i>			– Lignin	– Drought	Jeena, Joshi, & Shukla, 2021
Posttranslational regulation of CW lignification						
ZML2	<i>Zea mays</i>	LOC100277669	GATA28/TIFY2A (AT1G51600)	– Lignin	Degraded by wounding	Velez-Bermudez et al., 2015
<i>Zm</i> MYB11	<i>Zea mays</i>	LOC541744	MYB4 (AT4G38620)	– Lignin	Degraded by wounding	Velez-Bermudez et al., 2015
<i>Egr</i> H1.3	<i>Eucalyptus grandis</i>	Eucgr.I02364†	H1.3 (AT2G18050)	– Lignin		Soler et al., 2017
<i>Eg</i> MYB1	<i>Eucalyptus gunnii</i>	Eucgr.G01774†	MYB4 (AT4G38620)	– Lignin	+ Drought	Legay et al., 2010; Soler et al., unpublished

<i>Pd</i> MPK6 (Map kinase)	<i>Populus</i>	Potri.007G139800†	MPK6 (AT2G43790)			Gui et al., 2019
LTF1 (MYB)	<i>Populus</i>	KP770010	MYB4 (AT4G38620)	– Lignin	Degraded by wounding	Gui et al., 2019
<i>Pto</i> UBC34	<i>Populus tomentosa</i>	MH708242	UBC34 (AT1G17280)	+ Lignin	Induced by salt and heat	Zheng et al., 2019
<i>Pto</i> MYB221	<i>Populus tomentosa</i>	—	—	– Lignin		Zheng et al., 2019
<i>Pto</i> MYB156	<i>Populus tomentosa</i>	KT990214	MYB4 (AT4G38620)	– Lignin		Zheng et al., 2019
SnRK2	<i>Arabidopsis thaliana</i>	AT3G50500 AT5G66880 AT4G33950	—		Induced by ABA	Liu, Yu, Rao, Li, & Dixon, 2021
NST1	<i>Arabidopsis thaliana</i>	AT2G46770	—	+ Lignin		Liu et al., 2021

When available, accessions from GenBank or Phytozome V13 (genes marked with †) are listed. For each gene, the fourth column provides the closest *Arabidopsis thaliana* ortholog(s), mentioned in the references cited, or obtained by using BLASTP of NCBI database (best hits >80% of query protein identity).

(for reviews see Camargo, Ployet, Cassan-Wang, Mounet, & Grima-Pettenati, 2019; Grima-Pettenati, Soler, Camargo, & Wang, 2012; Hussey, Mizrachi, Creux, & Myburg, 2013; Schuetz, Smith, & Ellis, 2013; Wang & Dixon, 2012 and reference therein). In *Arabidopsis*, a complex hierarchical regulatory network has been proposed to control SW deposition in which the Transcription Factors (TF) NAM/ATAF/CUC (NAC) such as NST1, SND1, NST2, VND6 and VND7 act as first-level master switches. MYeloBlastosis (MYB) transcription factors (MYB46, MYB83 and MYB55) and NAC TF (SND3 and XND1) (Yamaguchi et al., 2011; Zhong, Lee, & Ye, 2010) act as second-level master switches to regulate a battery of downstream transcription factors and secondary cell wall biosynthesis genes. Noteworthy, among the TF involved in the regulation of the biosynthesis of the polymers of the secondary cell wall, only three MYB were reported to regulate specifically the lignin biosynthesis pathway in *Arabidopsis*: *AtMYB58*, *AtMYB63* and *AtMYB85* (Zhong & Ye, 2009). MYB58 and MYB63 have been shown to directly activate nearly all of the genes in the monolignol pathway (Zhou, Lee, Zhong, & Ye, 2009). Overexpression of *MYB58* or its co-ortholog *MYB63* specifically activated the monolignol pathway and lignin accumulation at the expense of biomass production (Zhou et al., 2009). Most of the transcriptional regulatory network underlying the lignified SCW is conserved in other plants (Ohtani & Demura, 2019) but differences have been highlighted between *Arabidopsis* and woody angiosperms for instance (Camargo et al., 2019; Nakano, Yamaguchi, Endo, Rejab, & Ohtani, 2015 and references therein). In addition, the role of lignin-specific SG3-type R2R3-MYBs (MYB58/63) in vascular cell wall lignification seems to be conserved in dicot plants (Zhong & Ye, 2009), but it is less clear in monocots. The orthologs of *MYB58/63* in sorghum (*Sorghum bicolor*) and maize (*Zea mays*), *SbMYB60* and *ZmMYB19/Zm1* only induced marginal increases in lignin content, respectively (Scully et al., 2018). Moreover, recent discoveries of new transcription factors in *Arabidopsis*, and other plant species, suggest that the regulatory network of lignin biosynthesis in plants extends beyond the established NAC-MYB network (Behr, Guerriero, Grima-Pettenati, & Baucher, 2019; Rao & Dixon, 2018).

3.2 Abiotic stresses can co-opt the SCW transcriptional regulatory network

The landmark interactome study of Taylor-Teeple et al. (2015) has identified hundreds of novel regulators and provided considerable insight into

the developmental regulation of xylem cell differentiation. The approach consisting in a large-scale yeast one-hybrid analysis of secondary cell wall-related transcription factors expressed in *Arabidopsis* roots, revealed that the complexity of the transcriptional regulation of the SCW was underestimated. This study highlighted a complex protein-DNA network between *Arabidopsis* transcription factors and metabolic genes with gene expression regulated by a series of feed-forward loops. It also showed that E2Fc can regulate the secondary wall biosynthesis network acting either as a transcriptional repressor or as an activator. E2Fc has the ability to affect the expression levels of many other transcription factors, including VND7, VND6, MYB46 as well as cellulose, hemicelluloses and lignin-associated genes in a dose-dependent manner. As E2Fc is also involved in regulating cell division through its interaction with the RETINOBLASTOMA-RELATED protein (RBR), it may function to coordinate cell division and cell differentiation during plant development (Ohtani & Demura, 2019). Importantly, the authors filtered the gene network to include only genes that were differentially expressed in response to salt stress or iron deprivation in the root vasculature of *Arabidopsis*. They showed that REVOLUTA (REV), the most upstream regulator in response to iron deprivation, regulates lignin biosynthesis. The expression of VND7 and MYB46, the most upstream regulators in response to salt stress, was greatly increased but the expression of the lignin genes was not altered. Notably, VND7 was ectopically expressed leading to an additional strand of metaxylem in roots exposed to high salinity. This demonstrated that distinct abiotic stresses can co-opt different genes of the xylem regulatory network. Co-optation of a developmental network is a mean to facilitate adaptation to stress by promoting functional adaptation (Taylor-Teeples et al., 2015).

In the last decade, transcription factors known to be involved in stress response were shown to impact lignin biosynthesis as part of the functional adaptation performed by plants to cope with natural constraints. Moreover, a growing number of transcription factors known to be involved in the regulation of SCW formation were shown to integrate both developmental and environmental signals leading to an increased tolerance to abiotic stresses, such as drought and salinity.

3.3 Abiotic stress-signaling transcription factors can modulate lignin deposition

Abiotic stress signaling in plants involves first the perception of the stress through sensors, which triggers signaling cascades within the cell involving

protein kinases (Chen et al., 2021). These signaling pathways result finally in the activation of stress responsive genes by transcription factors that will set up a wide range of cell protection mechanisms. Stress responsive genes include many different genes coding for chaperones, detoxification enzymes, ionic transporters, osmolytes biosynthesis enzymes, aquaporins (Zhu, 2016) that will enable the plant to withstand the cellular consequences of environmental constraints. Besides these cellular protection mechanisms, several reviews have pointed out cell wall modifications occurring in response to abiotic stresses that contribute to plant tolerance toward these stresses (Coleman, Brunner, & Tsai, 2021; Houston, Tucker, Chowdhury, Shirley, & Little, 2016; LeGall et al., 2015; Rui & Dinneny, 2020; Tenhaken, 2015). We focus here on recent studies showing that stress response signaling pathways can directly regulate SCW lignification.

APETALA2/ETHYLENE RESPONSIVE FACTOR (AP2/ERF) TFs, which are pivotal regulators of several stress responses, among which drought response (Xie et al., 2019), have been demonstrated to target genes belonging to the lignin biosynthesis pathway leading to further increased plant tolerance to drought. In *Arabidopsis thaliana*, the AP2/ERF *AtSHN2*, involved in wax/cutin deposition and drought tolerance, can modify lignin deposition by the direct regulation of NAC and MYB TFs (Ambavaram et al., 2011). In rice (*Oryza sativa*), overexpression of *OsERF71* confers higher drought tolerance associated with an upregulation of genes involved in lignin biosynthesis, as well as an increase of lignin content in the roots. The authors have shown that *OsERF71* binds directly to the promoter of *OsCCR1*, a key enzyme of the lignin biosynthesis pathway (Lee et al., 2016). Similarly, overexpression of *OsERF83* leads to increased drought tolerance and is associated with upregulation of lignin biosynthesis genes in rice (Jung et al., 2021). A DEHYDRATION-RESPONSIVE ELEMENT BINDING (DREB) TF from carrot (*DdDREB1A*) has been shown to increase lignification through the induction of lignin biosynthesis genes while being a positive regulator of drought tolerance (Li et al., 2020). During osmotic stress, a DREB transcription factor from the moss *Bryum argenteum* (*BaDBL1*) upregulates genes related to lignification pathway, enhancing lignin content and plant tolerance (Liang et al., 2021). Overexpression of *Eucalyptus grandis* *DREB/CBF14* transcription factor led to an increased lignification of xylem cells accompanied by an increase of the S/G ratio (Cao et al., 2020), mimicking the phenotype obtained with trees submitted to long-term chilling temperatures (Ployet et al., 2018). Besides, in the *CBF*-overexpressing lines, lignin biosynthesis genes were upregulated (Cao et al., 2020), suggesting an interplay between

cold-response CBF-signaling pathway and lignin biosynthesis pathway. However, it remains unclear whether CBF transcription factors directly regulate the expression of SCW related genes by direct binding of their promoters.

Other families of transcription factors involved in drought response have also been pointed out for their crosstalk with the lignification pathway. In apple (*Malus domestica*), *MdMYB88* and *MdMYB124*, previously described for their role in freezing tolerance have been shown to bind directly the promoters of *MdVND6* (key regulator of xylem vessel differentiation and SCW deposition) and *MbMYB46* (SCW master regulator) under drought conditions, leading to an increased lignification of root cell walls (Geng et al., 2018). In rice, overexpression of *OsTF1L*, a TF belonging to the HD-Zip transcription factor family, known for their role in plant growth as well as stress response, leads to increased drought tolerance along with accumulation of lignin in shoots. *OsTF1L* has been shown to bind directly to the promoters of lignin biosynthesis genes, thus enhancing lignin deposition and contributing to water deficit tolerance (Bang et al., 2019). In grapevine, a b-ZIP transcription factor, *VbZIP30*, regulates drought tolerance and lignin accumulation by direct binding of the promoters of stress-responsive and SCW-related genes, *VvNAC17* and *VvPRXN1* (Tu et al., 2020). In *Populus ussuriensis*, *PuC3H35*, a CCCH-type zinc finger protein known for its role in plant growth and stress response, is responsible for lignin deposition in roots under drought stress by direct binding in the promoter of *EARLI1* (Li et al., 2022), which has been described in *Arabidopsis* as a positive regulator of *CCR1*, a key enzyme in lignin biosynthesis (Shi et al., 2011). Altogether, these examples constitute further proof of the capacity of stress-signaling-pathways to co-opt the lignification pathway in order to contribute to better abiotic stress tolerance.

3.4 SCW-TFs recruited by abiotic stress-signaling pathways

Members of the NAC and the MYB TF family involved in the regulation of SCW were shown to be co-opted by stress signaling pathways:

SND1, a member of the NAC TF family well-known to be a level-one master positive regulator of SCW was shown recently to have a dual function (Jeong et al., 2018). Compared to wild type, the *snd1* knockout mutant seedlings accumulated less anthocyanin and exhibited low tolerance to salt stress. The authors demonstrated that *SND1* binds not only to the promoter of *Myb46* (a second-level master regulator of SCW), but also to the

promoter of the *ABI4* gene, thereby reducing ABA levels under normal growth conditions. Similar findings were found for its ortholog, *BpNAC012* in birch (Hu et al., 2019). Chromatin immunoprecipitation (ChIP) assays revealed that *BpNAC012* could bind both to the *cis*-elements CGT[G/A] and SNBE site in the promoters of abiotic stress-responsive genes and SCW-associated genes, respectively. Similarly, the overexpression of *AgNAC1* from celery (*Apium graveolens* L.), a positive regulator of lignin biosynthesis was recently shown to enhance plants' resistance to salt stress (Duan et al., 2020). Through a genome-wide association analysis, XYLEM NAC DOMAIN 1 (*XND1*), a transcriptional repressor regulating the expression of genes involved in both PCD and secondary wall formation (Zhao, Avci, Grant, Haigler, & Beers, 2008) was identified as a negative regulator of *Arabidopsis* root hydraulic conductivity (Tang et al., 2018). Phenotyping of *xnd1* mutants and natural *XND1* variants show that *XND1* modulates root hydraulic conductivity through action on xylem formation and potential indirect effects on aquaporin function and that it diminishes drought stress tolerance.

The expression of *EjMYB1* from Loquat (*Eriobotrya japonica*), a co-ortholog of the lignin-specific activators *AtMYB58/63* in *Arabidopsis*, is induced at low-temperature, concomitantly to an increase of the lignin content in the fruit (Xu et al., 2014). *EjMYB1* directly activates lignin genes by binding to AC elements found in their promoters, leading to flesh lignification (Xu et al., 2014). *BplMYB46* from *Betula platyphylla* (ortholog of *AtMYB46*) was one of the first examples highlighting the crosstalk between the abiotic stress and secondary cell wall biosynthesis pathways in trees (Guo et al., 2017). Overexpression of *BplMYB46* not only increases lignin deposition, secondary cell wall thickness and the SW biosynthesis genes (including phenylalanine ammonia lyase, *PAL*), but also improves salt and osmotic tolerance in transgenic birch plants (Guo et al., 2017). *BplMYB46* enhances abiotic stress tolerance in birch, *via* increasing scavenging of reactive oxygen species (ROS) and regulating expression of key ROS-scavenging enzymes: superoxide dismutase (SOD) and peroxidase (POD). Guo et al. (2017) further demonstrated that *BplMYB46* binds to the TC-box, GT-box and E-box motifs in addition to the MYBCORE and AC-box, present in lignin biosynthesis genes. The TC-box and GT-box motifs located in the promoters of SOD and POD, respectively are associated with stress tolerance. Similarly, *MdMYB46* could enhance the salt and osmotic stress tolerance in transgenic *Arabidopsis* and apple (*Malus × domestica* Borkh.). *MdMYB46* not only promotes the biosynthesis of secondary cell wall and deposition of

lignin by directly binding to the promoter of lignin biosynthesis genes (*CAD*, *COMT*, *CCR*) but also enhances stress tolerance, by activating stress-responsive genes belonging to the AP2/ERF family and dehydration-responsive genes through direct binding to their promoters. Therefore, *MdMYB46* could enhance the salt and osmotic stress tolerance in apple not only by activating lignin biosynthesis pathway, but also by directly activating genes involved in stress-signal transduction (Chen et al., 2019). The respective roles of the two paralogs *PtoMYB170* and *PtoMYB216* from *Populus tomentosa* (co-orthologs of *AtMYB61*) were examined by Tian et al. (2013) and Xu et al. (2017). Using overexpression in poplar, they first showed that *PtoMYB216* and *PtoMYB170* have conserved functions in regulating lignin biosynthesis. Both TFs specifically activate the expression of the upstream genes in the lignin biosynthetic pathway which results in ectopic deposition of lignin in cells that are normally nonlignified (Tian et al., 2013). However, the CRISPR/Cas9 mutant of *PtoMYB170* displays pendent stem phenotype resulting from reduced lignin deposition and more flexible xylem fibers. This strong phenotype suggests that the functional redundancy between *PtoMYB216* and *PtoMYB170* is weak because *PtoMYB216* cannot replace the function of *PtoMYB170* in lignin deposition (Xu et al., 2017). Interestingly, heterologous expression of *PtoMYB170* in transgenic *Arabidopsis* enhanced stomatal closure in the dark and resulted in drought tolerance of the transgenic plants through reduced water loss. *PtoMYB170* was specifically expressed in guard cells of transgenic *Arabidopsis* while *PtoMYB216* was not. These findings suggest a neofunctionalization of one of the two paralogs which acquired a new function in enhancing drought tolerance. After *BpMYB46* (Guo et al., 2017), *PtoMYB170* was the second example of tree SCW-TF able to integrate both developmental and stress signals. We have found that poplar plants overexpressing the *Eucalyptus* transcription factor *EgMYB1*, known to repress lignin biosynthesis (Legay et al., 2010), were far more resistant to drought than wild-type plants (unpublished). *BpMYB4* from *B. platyphylla*, a close ortholog of *EgrMYB1* was shown to have the same repressive activity on lignin biosynthesis. It is also induced by several abiotic stresses and promotes ROS scavenging through the positive regulation of resistance-related genes like POD and SOD (Yu et al., 2021).

3.5 Transcription factors involved in the cross talk between biotic stress and lignification

The crosstalk between lignin biosynthesis and defense is extremely complex (Xie et al., 2018). It has been widely observed that lignin deposition and

lignin biosynthetic genes are induced during the attack of various pathogens. However, contra-intuitively, “weakening” the cell wall by knocking out essential genes involved in lignin biosynthesis sometimes leads to enhanced resistance toward specific pathogens. The consequences of SCW modifications on pathogenic infection and their role in plant resistance to pathogens have been extensively reviewed in [Miedes, Vanholme, Boerjan, and Molina \(2014\)](#). We consider here only examples of modulation of SCW-transcription factors.

Shortly after *AtMYB46* was identified as a second-level master regulator of secondary cell wall biosynthesis in vascular tissues, it was also shown to be pivotal for mediating disease susceptibility to the necrotrophic fungal pathogen *Botrytis cinerea* ([Ramirez et al., 2011](#)). *Arabidopsis myb46* mutants (with impaired lignin biosynthesis) displayed enhanced disease resistance, which was thought to be caused by cell wall integrity damage-triggered high immunity level ([Ramirez et al., 2011](#)). Following infection, *AtMYB46* is able to modulate the induction of the pathogen-induced *Ep5C* gene, a type III cell wall-bound peroxidase, through binding to a new *cis*-element located in its promoter region. Different *myb46* mutants exhibit increased disease resistance to *B. cinerea*, a phenotype accompanied by selective transcriptional reprogramming of a set of genes encoding cell wall proteins and enzymes, of which extracellular type III peroxidases ([Ramirez et al., 2011](#)). Another MYB transcription factor, *AtMYB15*, was shown to be a part of the genetic pathway controlling defense-induced synthesis of G lignin units and basal immunity ([Chezem et al., 2017](#)). Treatment with bacterial flagellin FLG22 induced *MYB15* expression and resulted in ectopic lignification in WT plants but not in *myb15* mutants. In addition, *myb15* mutants showed increased susceptibility to *Pseudomonas syringae*, whereas an induced expression of *MYB15* restored basal resistance of *myb15* mutants to wild-type levels. *MYB15* directly binds to the secondary wall MYB-responsive element consensus sequence, which encompasses the AC elements, to drive synthesis of G lignin. This TF is also necessary for the basal synthesis of the preformed defense metabolite scopoletin ([Chezem et al., 2017](#)). Interestingly, the co-orthologs of *AtMYB15* in rice, *OsMYB30* and *OsMYB55*, were also shown to contribute to plant immunity by directly activating monolignol genes and inducing higher accumulation of FA, which is an important constituent of grass cell walls ([Kishi-Kaboshi et al., 2018](#)).



4. Posttranscriptional regulation of lignin biosynthesis under stress

The posttranscriptional control of lignin deposition has been demonstrated for developmental lignin. It involves alternative splicing, microRNAs, long coding RNAs (reviewed in Zhang, Tuskan, Tschaplinski, Muchero, & Chen, 2020 for poplar).

Increasing evidence suggests that stress lignins are also submitted to post-transcriptional regulation by small RNAs (Cesarino, 2019). A growing number of studies support a role for small RNAs in the regulation of the phenylpropanoid metabolism during stress responses. Small RNAs (sRNAs) are divided into two major classes, microRNAs (miRNAs) and small interfering RNAs (siRNAs), according to their biogenesis (Mallory & Vaucheret, 2006). Small RNAs participate in many molecular mechanisms and signaling transduction pathways that are associated with plant stress tolerance by repressing expression of their target genes.

In *P. trichocarpa*, the mechanical stress responsive Ptr-miR473 was predicted to target a UV-B-resistant gene (*UVR8*) believed to positively regulate phenylpropanoid metabolism associated with C4H for the production of cell wall phenolic compounds, such as lignin and flavonoids (Lu et al., 2005). In sweet potato (*Ipomoea batatas*) overexpression of a wounding-responsive miRNA828 enhanced lignin deposition and H₂O₂ accumulation through repressed expression of *IbMYB* and *IbTLD* (Lin et al., 2012). These TFs function in the repression of the phenylpropanoid pathway and in the activation of antioxidant enzymes, respectively. Therefore, miR828 induction results in derepression of phenylpropanoid genes and in lower activity of antioxidant enzymes, leading to the accumulation of lignin and H₂O₂ as a part of defense mechanisms (Lin et al., 2012). The posttranscriptional regulation of wounding-triggered lignin deposition in sweet potato also involves wounding-related small RNA-8105 (Lin et al., 2013). The 22 and 24 nt sRNA8105s bind to the first intron of *IbMYB1* before splicing to mediate RNA cleavage and DNA methylation of *IbMYB1*, respectively. The interaction of sRNA8105 with *IbMYB1* RNA also triggers production of secondary siRNAs to decrease expression of *IbMYB1* family genes. sRNA8105-mediated repression of the *IbMYB1* family genes upon wounding induces the expression of monolignol biosynthetic genes and leads to increased lignin

deposition (Lin et al., 2013). In maize, the monocot-specific miR528 was shown to regulate lignin biosynthesis under luxurious nitrogen fertilization, affecting lodging resistance (Sun et al., 2018). Nitrogen luxury induces the expression of ZmmiR528 that, in turn, negatively regulates the expression of the lignin-related laccase genes *ZmLAC3* and *ZmLAC5*, leading to lower lignin levels, decreased S/G ratio and reduced lodging resistance (Sun et al., 2018). Overexpression of microRNA397 (miR397) from the C4 monocot *Setaria viridis* in *Arabidopsis* led to repression of LACCASE (LAC) target gene expression, reduced lignin content and increased sensitivity to salt stress (Nguyen et al., 2020). Transgenic cowpea lines overexpressing artificial *vun-miR408-3p* displayed enhanced drought and salinity tolerance. Since *LACCASE 12* and *3* were identified as the potential target of *Vun-miR408-3p*, the authors hypothesized that the regulation of lignin content is an essential trait related to the drought tolerance in cowpea (Mishra et al., 2022). Recently, Jeena et al. (2021) showed that *Bm-miR172c-5p* is induced under drought stress and regulates lignin biosynthesis in *Bacopa monnieri*, by targeting the *F5H* gene. Overexpression of *Bm-miR172c-5p* precursor in *B. monnieri* represses *F5H* gene, leading to reduced lignification and secondary xylem thickness. By contrast, the mutation in *Bm-miR172c-5p* showed increased lignification and increased secondary xylem thickness, leading to better physiological response in drought stress conditions (Jeena et al., 2021).



5. Posttranslational regulation of lignin biosynthesis under stress

Several recent examples demonstrate that posttranslational mechanisms involving phosphorylation, translocation, degradation of transcription factors mediated through protein-protein interactions, are involved in the regulation of lignin biosynthesis under stress.

Three maize (*Z. mays*) lignin repressors, MYB11, MYB31, and MYB42 were shown to participate in wound-induced lignification by interacting with ZML2, a protein belonging to the TIFY family (Velez-Bermudez et al., 2015). The three R2R3-MYB factors and ZML2 act as repressors of a set of lignin genes. In particular, MYB11 and ZML2 bind simultaneously to the AC-rich and GAT(A/C) *cis*-elements present in the promoter of the *COMT* gene. Upon wounding and methyl jasmonate treatments, MYB11 and ZML2 proteins are degraded and *COMT* transcription is induced. The authors proposed a molecular regulatory mechanism in which

the jasmonate-signaling cascade triggers the degradation of the MYB/ZML complex, resulting in the derepression of a set of lignin genes and, consequently, in the activation of lignin deposition in response to wounding (Velez-Bermudez et al., 2015). Soler et al. (2017) have shown that the *Eucalyptus* transcription factor *EgMYB1*, known to repress lignin biosynthesis (Legay et al., 2010), interacts specifically with a linker histone variant, *EgH1.3*. Linker histones can function as “hubs” to recruit many different proteins that affect the posttranslational modifications of core histones to change chromatin conformation and modify gene expression at specific *loci* (Over & Michaels, 2014). The interaction between *EgMYB1* and *EgH1.3* enhances the repression of *EgMYB1*'s target genes, strongly limiting the amount of lignin deposited in xylem cell walls. The authors suggested that a complex between *EgMYB1* and *EgH1.3* integrates developmental signals to prevent premature or inappropriate lignification of SCW, providing a mechanism to fine-tune the differentiation of xylem cells in time and space. Several indirect evidences suggest that the protein complex *EgMYB1* and *EgH1.3* could also integrate stress signals: (i) *H1.3* was originally discovered as a drought responsive gene in *Arabidopsis* (Ascenzi & Gantt, 1999), (ii) *EgMYB1*'s closer ortholog in *Arabidopsis* is *AtMYB4* whose expression is regulated by different environmental conditions such as wounding and UVB light (Jin et al., 2000), (iii) many stress-responsive genes are induced in *Arabidopsis* plants overexpressing both *EgMYB1* and *EgH1.3* (Soler et al., unpublished) and (iv) poplar plants overexpressing *EgMYB1* are drought resistant (unpublished results). Interestingly, LIGNIN BIOSYNTHESIS-ASSOCIATED TF 1 (LTF1) from *Populus*, a putative ortholog of *AtMYB4* and *EgMYB1*, was shown to be phosphorylated by the MAP kinase MPK6. Activation of *PdMPK6* in response to wounding promoted LTF1 phosphorylation in wood cells. In its unphosphorylated state, LTF1 functions as a repressor of lignin biosynthesis. When LTF1 becomes phosphorylated by *PdMPK6* in response to external stimuli such as wounding, it undergoes degradation through a proteasome pathway, resulting in activation of lignification. Because the MPK6 signaling module can be activated by various biotic and abiotic environmental stimuli such as cold, light, and drought, the authors proposed that LTF1 phosphorylation could act as a sensory switch to regulate lignin in response to stresses (Gui et al., 2019). Zheng et al. (2019) showed that a *P. tomentosa* E2 UBIQUITIN-CONJUGATING ENZYME 34 (*PtoUBC34*), is involved in the posttranslational regulation of lignin-associated transcriptional repressors *PtoMYB221* and *PtoMYB156*. *PtoUBC34* is localized at the endoplasmic reticulum (ER) membrane where

it interacts with *PtoMYB221* and *PtoMYB156*. This specific interaction allows for the translocation of TFs *PtoMYB221* and *PtoMYB156* to the ER and reduces their repression activity in a *PtoUBC34* abundance-dependent manner. Noteworthy, *PtoUBC34* is induced by treatment with sodium chloride and heat shock suggesting that this mechanism can operate in response to stresses. Very recently, Liu et al. (2021) have shown in *Arabidopsis* that ABA, a stress-signaling hormone, can regulate lignin biosynthesis through direct interaction between SnrK2, a central kinase in the ABA signaling pathway, and NST1, a master regulator of SCW formation. Once NST1 is phosphorylated by SnrK2 at Ser316 residue, it is activated and able to bind the promoters of SCW-related downstream target genes (Liu et al., 2021). Loss of function of NST1 in the *snd1* mutant background results in lack of lignified SCWs in the interfascicular fiber region of the stem, and the Ser316Ala mutant of NST1 fails to complement this phenotype and ABA-induced lignin pathway gene expression.



6. Concluding remarks

Recent studies highlight the crosstalk between the regulation of lignin biosynthesis and responses to stresses which occurs not only at the transcriptional level but also at the posttranscriptional and posttranslational levels. A growing number of SCW-related transcription factors are able to integrate both developmental and environmental signals and their overexpression led to an increased tolerance to abiotic stresses. On the other hand, some stress-signaling transcription factors were shown to impact lignin biosynthesis as part of the functional adaptation performed by plants to cope with environmental cues. The cell-tissue localization and the biochemical and molecular features of stress lignins need however to be further explored to better understand their roles in adaptation to stresses. Emerging-wide “omics” technologies revealed that the complexity of the transcriptional regulation of lignification during development goes far beyond the established NAC-MYB transcriptional regulatory network and that the network structure exhibits flexible changes induced by stress conditions (reviewed in Ohtani & Demura, 2019). Several additional regulatory factors have recently been found to overlay onto the NAC-MYB regulatory module, acting as molecular hubs that integrate developmental and environmental information into lignin biosynthesis. The concept of transcriptional hubs coordinating carbon and energy flux into growth, defense, and lignin biosynthesis is just emerging based on studies in *Arabidopsis* (Xie et al., 2018).

These molecular hubs are attractive targets for manipulation when aiming to control lignification in harmony with plant development and environmental adaptation (Ohtani & Demura, 2019). Moreover, as outlined by Chanoca et al. (2019), most lignin-engineering studies have been performed in controlled conditions and therefore do not provide sufficient insight into the interaction of the engineered plant with environmental factors. Indeed, the performance of lignin-engineered plants appears to be highly influenced by the environmental conditions.

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