Regulation of secondary cell wall biosynthesis by a NAC transcription factor from *Miscanthus*

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Abstract
Cell wall recalcitrance is a major limitation for the sustainable exploitation of lignocellulosic biomass as a renewable resource. Species and hybrids of the genus *Miscanthus* have emerged as candidate crops for the production of lignocellulosic feedstock in temperate climates, and dedicated efforts are underway to improve biomass yield. However, nothing is known about the molecular players involved in *Miscanthus* cell wall biosynthesis to facilitate breeding efforts towards tailored biomass. Here, we identify a *Miscanthus sinensis* transcription factor related to SECONDARY WALL-ASSOCIATED NAC DOMAIN1 (SND1), which acts as a master switch for the regulation of secondary cell wall formation and lignin biosynthesis. MsSND1 is expressed in growth stages associated with secondary cell wall formation, together with its potential targets. Consistent with this observation, MsSND1 was able to complement the secondary cell wall defects of the *Arabidopsis snd1 nst1* double mutant, and ectopic expression of MsSND1 in tobacco leaves was sufficient to trigger patterned deposition of cellulose, hemicellulose, and lignin reminiscent of xylem elements. Transgenic studies in *Arabidopsis thaliana* plants revealed that MsSND1 regulates, directly and indirectly, the expression of a broad range of genes involved in secondary cell wall formation, including MYB transcription factors which regulate only a subset of the SCW differentiation program. Together, our findings suggest that MsSND1 is a transcriptional master regulator orchestrating secondary cell wall biosynthesis in *Miscanthus*.

Keywords
biomass, cell wall, lignin, *Miscanthus*, transcription factor, transcriptional networks

1 | INTRODUCTION

The bulk of the globally available renewable biomass is formed by plant secondary cell walls (SCWs). A major limitation for tapping into the potential of this sustainable source of energy is cell wall recalcitrance, or the resistance to deconstruction, conferred by the properties of and interactions between SCW components. SCWs are deposited by specialized cells after cessation of growth and differ from the growth-controlling primary cell wall mainly by the incorporation of the aromatic polyphenol lignin, which confers mechanical support and impregnation to water conducting tissues (Lucas et al., 2013). As lignin is extremely resistant to degradation and coats the...
SCW polysaccharide network of cellulose and hemicellulose, it has become the main target for efforts aiming at decreasing cell wall recalitrance (Marriott et al., 2014; Sibout et al., 2016; Van Acker et al., 2014; Wilkerson et al., 2014). Attempts to decrease lignin content or alter lignin composition frequently caused undesirable phenotypes such as collapsed xylem vessels, dwarfing, or increased susceptibility to pathogens (Bonawitz & Chapple, 2013). However, dwarfism in response to interference with lignin biosynthesis was in some cases caused by signaling, rather than by insufficient cell wall integrity, and plants with altered lignin content, but without growth penalty could be obtained by circumventing the signaling feedback (Bonawitz et al., 2014; Gallego-Giraldo, Escamilla-Trevino, Jackson, & Dixon, 2011a; Gallego-Giraldo, Jikumaru, Kamiya, Tang, & Dixon, 2011). This demonstrates that plant growth, in principle, tolerates alternative cell wall structures if secondary responses can be controlled. Thus, a thorough understanding of cell wall biosynthesis, maintenance, and perception can greatly facilitate tailoring biomass for broad application.

The herbaceous monocot genus Miscanthus harbors perennial C4 grasses that originate from subtropical and tropical regions in East Asia. Due to low water and modest nutrient requirements, high photosynthetic efficiency, cold and drought tolerance, and high biomass yield, Miscanthus has emerged as leading second-generation bioenergy crop for the production of lignocellulosic biomass in temperate climates (Lewandowski et al., 2016; van der Weijde et al., 2013). To improve biomass composition and yield, Miscanthus has sought genetic improvement at several global breeding programs have been initiated, aiming to capitalize on existing genetic and phenotypic variation within and between Miscanthus species (Robson et al., 2013). In addition, these efforts are complemented by in-depth cell wall profiling of various genotypes (da Costa et al., 2014, 2017). However, understanding of the molecular regulation involved in SCW formation in Miscanthus has received thus far little attention.

The biosynthesis of SCW cellulose, hemicellulose, and lignin in Arabidopsis is controlled by a sophisticated network of transcription factors (TFs) that coordinately integrate developmental and environmental cues into SCW formation (Taylor-Teeples et al., 2015). The network is organized in a multitiered hierarchical manner by TFs of the NAM, ATAF1,2, and CUC2 (NAC) and MYB classes (Nakano, Yamaguchi, Endo, Rejab, & Ohtani, 2015). A group of closely related NAC TFs including NAC SECONDARY WALL THICKENING PROMOTING FACTOR1 (NST1), SECONDARY WALL-ASSOCIATED NAC DOMAIN1 (SND1)/NST3, VASCULAR-RELATED NAC-DOMAIN6 (VND6), and VND7 is situated in the first tier and can be viewed as master switches regulating SCW formation (Nakano et al., 2015), which includes transcriptional upregulation of enzymes involved in SCW biosynthesis, but also controlling the machinery for its patterned deposition (Kubo et al., 2005; Oda & Fukuda, 2013). NST1 and SND1/NST3 have been shown to redundantly regulate the SCW thickening in fibers, whereas VND6 and VND7 are implicated in the SCW program of xylem elements (Kubo et al., 2005; Mitsuda, Seki, Shinozaki, & Ohme-Takagi, 2005; Mitsuda et al., 2007; Zhong, Demura, & Ye, 2006; Zhong, Richardson, & Ye, 2007b). Downstream of NAC TFs, MYB46 and MYB83 were identified in Arabidopsis as second-tier regulators of SCW formation (Ko, Kim, & Han, 2009; McCarthy, Zhong, & Ye, 2009; Zhong, Richardson, & Ye, 2007a). Targets of MYB46 and MYB83 in the third tier comprise a number of MYB TFs, such as MYB58, MYB63, and MYB85, as well as other types of TFs, which are proposed to fine-tune transcriptional regulation of the SCW formation (Ohman et al., 2013; Zhong, Lee, Zhou, McCarthy, & Ye, 2008; Zhou, Lee, Zhong, & Ye, 2009). Finally, the activators of the SCW differentiation program comprise biosynthetic enzymes for the main components cellulose, xylan, and monolignols, as well as enzymes involved in lignin polymerization and programmed cell death. Some of these metabolic enzymes are regulated by both the lower-tier MYBs and the master switches, constituting feed-forward loops (Nakano et al., 2015; Taylor-Teeples et al., 2015). Master switches of the NAC family capable of activating the SCW differentiation program have been identified in species such as Brachypodium, Eucalyptus, maize, Medicago, pine, poplar, rice, and switchgrass, indicating a high degree of conservation of the regulatory network of SCW biosynthesis in vascular plants (Valdivia et al., 2013; Yoshida et al., 2013; Zhao et al., 2010; Zhong, Lee, & Ye, 2010a; Zhong et al., 2011, 2015).

Here, we identify and functionally characterize a NAC TF of the SND1 class from Miscanthus sinensis, MsSND1 is expressed in tissues undergoing SCW formation together with its potential targets. Expression of MsSND1 was sufficient to complement the SCW defects of the Arabidopsis snd1 nst1 double mutant. In addition, transient expression of MsSND1 in tobacco leaves triggered patterned deposition of cellulose, hemicellulose, and lignin reminiscent of SCW differentiation in mesophyll cells, in sharp contrast to what was observed after expression of a lower-tier MYB TF. Transgenic Arabidopsis thaliana lines carrying an inducible version of MsSND1 revealed that MsSND1 lines carrying an inducible version of MsSND1 regulate directly and indirectly the expression of a broad range of genes involved in SCW formation. Together, our findings suggest that MsSND1 is a transcriptional regulator capable of orchestrating SCW biosynthesis in Miscanthus sinensis.

2 MATERIALS AND METHODS

2.1 Phylogeny

Amino acid sequences were aligned with ClustalW and the neighboring phylogenetic tree with 1000 bootstraps was conducted using MEGA6 software (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). References for Arabidopsis transcription factors used in the alignment are mentioned in the introduction. Populus sequences were taken from Ref. (Ohtani et al., 2011), Brachypodium sequences were taken from Ref. (Valdivia et al., 2013), maize and rice sequences were from Ref. (Zhong et al., 2011), and Panicum sequences were from Ref. (Zhong et al., 2015). Miscanthus sequences are listed in Table S3. Alignment of MYB transcription factors included sequences from Arabidopsis (McCarthy et al., 2009; Stracke, Werber, & Weisshaar, 2001; Zhong et al., 2006, 2007a, 2008), Antirrhinum (Tamagnone et al., 1998), Eucalyptus (Goicoechea et al., 2007, 2010).
Arabidopsis mutant seeds (Mitsuda et al., 2007) were obtained from the Nottingham Arabidopsis Stock Center (NASC). Alignment of MsSND1 and AtSND1 was performed with Clustal Omega (Sievers et al., 2011).

2.2 Molecular cloning

Plasmid constructs were assembled via GreenGate cloning (Lampropoulos et al., 2013). The protein-coding region of the MsSND1 gene was amplified by PCR using cDNA from Miscanthus sinensis and appropriate primers with BsaI restriction site overhang (see Table S1). More details about cloning such as primers, modules, and assembled constructs for plant transformation can be found in Table S1.

2.3 Plant materials, growth conditions, and treatments

Miscanthus sinensis (identification number: Sin-13) collected in Honshu, Japan (Clifton-Brown & Lewandowski, 2002), were grown from seeds in glasshouse under 8/16-hr (light/dark) photoperiod until rosette stage (identification number: Sin-13) collected in Honshu, Japan (Clifton-Brown & Lewandowski, 2002), were grown from seeds in glasshouse under 8/16-hr (light/dark) photoperiod until rosette stage. Arabidopsis thaliana ecotype Columbia-0 (Col-0) was grown in soil at 21°C under 8/16-hr (light/dark) photoperiod until rosette stage and then transferred to 16/8-hr photoperiod. Arabidopsis plants were stably transformed by the floral dip method as described (Clough & Bent, 1998). For selection, plants were grown on half-strength MS plates supplemented with 25 mg/L hygromycin or 7.5 mg/L glufosinate ammonium (Sigma-Aldrich); 10-day-old transgenic seedlings of mCherry-GR-Ms Triple Gateway (Clontech) were incubated in liquid LB medium containing respective pSOUP+ was transformed with the respective construct. Transgenic clones were incubated in liquid LB medium containing respective respective construct. Transgenic clones were incubated in liquid LB medium containing respective antibiotics for 2 days. The bacteria were transferred to infiltration medium (10 mM MgCl2, 10 mM MES, 0.15 mM acetosyringone at pH 5.7). The suspension was set to OD600 0.4 and infiltrated with a needless syringe into 4- to 6-week-old Nicotiana benthamiana leaves. After 5 days, the leaves were embedded in 6% agarose, hand-sectioned, and stained.

2.4 Transient transformation of Nicotiana benthamiana leaves

For overexpression experiments, Agrobacterium tumefaciens ASE (pSOUP+) was transformed with the respective construct. Transgenic clones were incubated in liquid LB medium containing respective antibiotics for 2 days. The bacteria were transferred to infiltration medium (10 mM MgCl2, 10 mM MES, 0.15 mM acetosyringone at pH 5.7). The suspension was set to OD600 0.4 and infiltrated with a needless syringe into 4- to 6-week-old Nicotiana benthamiana leaves. After 5 days, the leaves were embedded in 6% agarose, hand-sectioned, and stained.

2.5 Tissue staining and microscopy

Lignin was stained with either HCl-phloroglucinol or basic fuchsin as described in Ref. (Valdivia et al., 2013). Cellulose was stained with the fluorochrome calcofluor white M2R (fluorescent brightener 28). Hemicelluloses were detected with xylan and arabinoxylan-specific LM11 as primary (McCartney, Marcus, & Knox, 2005) and Alexa 488-conjugated donkey anti-rat IgG (H+L; Thermo Fisher) as secondary antibody according to the procedure described elsewhere (McCartney, Steele-King, Jordan, & Knox, 2003). Cell walls were stained with SCRI Renaissance 2200 staining (Musielak, Schenkel, Kolb, Henschen, & Bayer, 2015). HCl-phloroglucinol staining was imaged under brightfield with a 20.0 × 0.40 NA objective on a Leica DM IRB inverted microscope. Fluorescent images were captured with a confocal Leica TCS SP5i microscope equipped with a 40.0 × 1.25 NA objective. Basic fuchsin- and SCRI Renaissance-stained tissues were excited at 561 nm and 405 nm to detect emission at 593/40 nm and with DAPI filter, respectively. Calcofluor white-stained tissues were irradiated with UV light and detected with DAPI filter. Immunolabeled tissues with LM11 and anti-rat Alexa 488 conjugate were excited using the 488-nm Argon laser line to detect emission with Alexa 488 filter settings. The fluorescent protein mCherry was imaged at 543-nm excitation and 600/80-nm detection. Orthogonal sections and scale bars were produced with ImageJ software.

2.6 Gene expression analysis

Developing Miscanthus leaves were cut at their node, dissected, and immediately frozen in liquid nitrogen. Total RNA was isolated from ground plant material from 100 mg Arabidopsis seedlings or 30 mg Miscanthus leaves using GeneMatrix Universal RNA Purification Kit (EURx/Roboklon) including an on-column DNase I digestion. cDNA was synthesized according to the manufacturer’s instructions of Superscript III Reverse Transcriptase (Invitrogen) for Arabidopsis samples and RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher) for Miscanthus samples with oligo dT primer and 1 μg RNA. qPCRs were prepared in 15 μl volume containing 2 μl of 1:8 diluted cDNA, 1 μl 5 μM of each forward and reverse primer, 0.3 μl 10 mM dNTPs, 0.3 μl of 1:400 diluted SYBR® Green I (Sigma-Aldrich), 0.3 μl of JumpStart™ Taq DNA polymerase with 1.5 μl of the corresponding buffer (Sigma-Aldrich), and 8.6 μl H2O. Ct values, primer efficiencies, and melting curves were measured in a Rotor-Gene Q thermocycler and evaluated by Q Series Software Q (Qiagen). Primer sequences can be found in Table S2. Stably expressed housekeeping genes in Miscanthus sinensis leaf gradient were identified in a Miscanthus transcriptome (Barling et al., 2013) by blasting common housekeeping genes from Arabidopsis (Czechowski, Stitt, Altman, Udvardi, & Scheible, 2005). Primers were designed for protein phosphatase 2A subunit 3A (PP2A), clathrin adaptor AP2M (Clath), peroxin 4 (UBC21), and TIP41-like protein (TIP) based on sequences in the Miscanthus transcriptome (Table S2). During the course of the leaf gradient, cytoskeleton reference genes like tubulin or actin were differentially expressed in leaf base and tip. Hence, the most stable reference genes PP2A and UBC21 were selected for normalization. Geometrical mean of three biological replicates is shown. Gene expression of Arabidopsis was normalized against clathrin adaptor subunit (At5g46630) mRNA.
described in (Czechowski et al., 2005). The qPCR products were either sequenced or checked on acrylamide gel in cases of small amplicons.

2.7 | Accession numbers

Sequence data from this article can be found in the GenBank databases under the following accession numbers: KY930620 for MsSND1; KY930621 for MsSCM1; MF996502 for MsSCM2; KY930622 for MsSCM3; MF996501 for MsSCM4; and KY930623 for MsLAC2. Arabidopsis accession numbers are as follows: At1g32770 for AtSND1; At4g35350 for XCP1; At5g12870 for MYB46; At3g08500 for MYB83; At5g44030 for CesA4; At5g17420 for CesA7; At4g18780 for CesA8; At2g38080 for LAC4; At2g28110 for IRX7; At5g54690 for IRX8; At1g51680 for 4CL1; At4g34050 for CCoAOMT1; At5g46630 for Clathrin; At5g25760 for UBC21; and At1g13320 for PP2A.

3 | RESULTS AND DISCUSSION

3.1 | Identification of Miscanthus sinensis SND1 through phylogenetic analysis of the NAC transcription factor family

In Miscanthus, the molecular players involved in cell wall biosynthesis are largely unknown. To facilitate the identification of potential targets for future crop improvement, we queried the previously published Miscanthus transcriptome (Barling et al., 2013) for TFs involved in SCW biosynthesis using known factors from Arabidopsis thaliana and other plants. A phylogenetic analysis of NAC TFs from different angiosperm lineages showed that the subfamily Ic may be further divided into three classes (Figure 1). Members of classes II and III are implicated in xylem differentiation (Kubo et al., 2005; Zhou, Zhong, & Ye, 2014), whereas members of class I are described to be involved in fiber differentiation (Zhong et al., 2006). In the Miscanthus transcriptome, we found various transcripts putatively encoding SCW TFs and concentrated on the Miscanthus sinensis SND1 candidate. The predicted protein shares 41.5% identity and 51.5% similarity on amino acid level with AtSND1 (Fig. S1). The structurally and functionally essential N-terminal DNA-binding motif, known as the NAC domain, shows the highest similarity with 93% within the amino acid sequence, suggesting that MsSND1 may possess similar DNA-binding characteristics as AtSND1. The more divergent C-terminus that contains the transcriptional activation domain (Duval, Hsieh, Kim, & Thomas, 2002; Zhong et al., 2006) is 63 amino acids longer in MsSND1 compared to AtSND1 that may reflect different activation properties. In summary, our analysis indicates a close relationship between MsSND1 and AtSND1.

3.2 | High MsSND1 expression coincides with vascular development and SCW formation in Miscanthus leaves

In monocots, leaf differentiation follows a linear pattern from the leaf sheath toward the leaf blade, resulting in a continuous developmental gradient along the leaf. To explore the role of MsSND1 in

FIGURE 1 Identification of MsSND1. Phylogeny of MsSND1 in relation to the NAC transcription factor subfamily Ic from angiosperm lineages Arabidopsis, Brachypodium, Panicum, Populus, Oryza, and Zea. Amino acid sequences were aligned with ClustalW, and the neighbor-joining phylogenetic tree with 1000 bootstraps was conducted using MEGA6 software (Tamura et al., 2013)
FIGURE 2  MsSND1 expression is correlated with vascular development and with the expression of its putative target genes Miscanthus sinensis leaves. (a,b) Gene expression profiles of the indicated genes over ten developmental zones as obtained by quantitative real-time PCR. The results of three biological replicates were combined, normalized against two reference genes (PP2A and UBC, [a]) and visualized as heat map in (b). Pictures of all three leaves are shown in Fig. S1. Cross-sections of the first three basal zones (d–f) from the Miscanthus leaf depicted in (c) were stained for lignin with HCl-phloroglucinol, indicating that the expression of MsSND1 and its putative targets is concomitant with the onset of vascular development. Scale bars: 100 μm. px, protoxylem; sf, sclerenchyma fibers (extraxylary fibers); te, tracheary elements; vb, vascular bundle; xf, xylary fibers; ys, young sclerenchyma
Miscanthus development, the expression profile of MsSND1 together with those of putative cell wall-related transcription factors and biosynthetic genes was determined along the developmental gradient in the leaf and visualized (Figure 2a,b, Fig. S2). The transcripts of R2R3-MYB TFs, lignin biosynthesis, and polymerization genes were identified in the Miscanthus transcriptome in the same manner as described for MsSND1. Along the leaf developmental gradient, the expression of MsSND1 was highest at the leaf sheath and decreased rapidly over the following two leaf segments (Figure 2a,b). We then analyzed, the expression pattern of putative downstream targets of SND1 related to AtMYBs involved in the SCW transcriptional network. In this case, relationship to previously described factors was more ambiguous; therefore, we named them Miscanthus sinensis SECONDARY CELL WALL MYBs 1-4 (MsSCM1-4). MsSCM1-3 fall into the clade of AtMYB20, AtMYB43, and AtMYB85, respectively, whereas MsSCM4 is most closely related to AtMYB63 and AtMYB58 (Zhong et al., 2008; Zhou et al., 2009). The expression of these MYB TFs, as well as that of lignin biosynthesis genes peaked in the second segment to decline toward the leaf blade at different rates depending on the gene. Putative orthologues from maize and rice revealed very similar expression patterns, supporting our results (Li et al., 2010; Wang et al., 2014). These observations suggest evolutionary conservation of the SCW transcriptional network in accordance with previous reports (Zhong et al., 2010a), and demonstrate feasibility of a homology-based approach for identifying SCW-related genes in Miscanthus.

To corroborate these results, the expression profiles of the putative SCW-related genes were correlated with SCW differentiation in leaf segments of Miscanthus (Figure 2d–f). At the leaf sheath, the fundamental organization of vasculature is initiated very early in development. Lignification of vascular tissue started in protoxylem cells of the first segment, shortly followed by lignification of tracheary elements and sclerenchyma fibers (extraxylary fibers) in the second and third leaf segment (Figure 2d–f). SCW deposition and lignification of cells within the vascular bundles appeared more prominent than in sclerenchyma fibers (Figure 2e), which may be required to secure integrity of the tissue during early stages of leaf maturation. Extraxylary fibers, the adaxial threads of sclerenchyma fibers that are in contact with the outer bundle sheath, and the thin abaxial subepidermal strip of sclerenchyma fibers seemed to lignify and deposit SCWs in a similar fashion, different from vascular cells. This was further confirmed by basic fuchsin staining of the leaf cross-sections. Basic fuchsin was able to stain cells in the vascular tissue but was unable to stain sclerenchyma fibers (Fig. S2b–e). Notably, sclerenchyma cells were found to possess a distinct lignin composition in alfalfa (Vallet, Chabbert, Czaninski, & Monties, 1996), which is probably the result of different lignification mechanisms employed by xylary and extraxylary fibers (Smith et al., 2013). The high expression of MsSND1, candidate MYB TFs, and lignin biosynthesis genes that occurs concurrently with differentiation and SCW formation of xylary and extraxylary elements suggests a possible involvement of the investigated genes in this process.

3.3 | MsSND1 complements the SCW deficiency of the Arabidopsis snd1 nst1 double mutant

To substantiate the hypothesis that MsSND1 is functionally equivalent to AtSND1, we tested whether expression under control of the AtSND1 promoter, MsSND1 restored vertical growth (a) and lignification of secondary cell walls in the interfascicular fibers similar to the positive control AtSND1 (b–e). Cross-sections of inflorescence stems were stained with HCl-phloroglucinol for lignin. Scale bars: 100 μm

FIGURE 3 MsSND1 can replace Arabidopsis SND1. Complementation of Arabidopsis snd1 nst1 double mutant by expression of MsSND1 under control of the AtSND1 promoter. MsSND1 restored vertical growth (a) and lignification of secondary cell walls in the interfascicular fibers similar to the positive control AtSND1 (b–e). Cross-sections of inflorescence stems were stained with HCl-phloroglucinol for lignin. Scale bars: 100 μm
was capable to rescue the vertical growth of snd1 nst1 similar to AtSND1 (Figure 3a). Cross-sections of inflorescence stems stained for lignin confirmed that both MsSND1 and AtSND1 are able to complement the snd1 nst1 double mutant and restore loss of lignin in stem fiber cells (Figure 3d). Even though this result supported our hypothesis that MsSND1 and AtSND1 are functionally similar, a previous study has revealed that only partial activation of the regulatory network is already sufficient to rescue the snd1 nst1 mutant, albeit with compositional changes to the SCW (Sakamoto & Mitsuda, 2015). Therefore, we continued with a more profound functional characterization of MsSND1 to shed light on its regulatory role in SCW formation.

**FIGURE 4** Ectopic expression of MsSND1 is sufficient to induce secondary cell wall deposition and patterning. Ectopic deposition of secondary cell wall components in *Nicotiana benthamiana* leaves induced by transient expression of AtSND1 and MsSND1. Cross-sections of tobacco leaves stained for lignin with basic fuchsin (a–c), for cellulose with Calcofluor White (d–f) and for xylan and arabinoxylan with LM11 as primary and Alexa 488 as secondary antibody (g–i). The control leaves were transformed with an empty vector control (c, f, i). Scale bar: 40 μm

**FIGURE 5** Ectopic secondary cell wall patterning differs between MsSND1 and downstream MYB factors. Ectopic deposition of lignin in tobacco leaves induced by ectopic, transient expression of MsSND1, MsSCM1, MsSCM3, MsSCM4, and MsSCM2 in *Nicotiana benthamiana* leaves. Cross-sections of leaves were stained for lignin with basic fuchsin. The control leaves were transformed with an empty vector control. Scale bar: 50 μm
3.4 | Expression of MsSND1 leads to strong ectopic SCW deposition in tobacco leaves

In order to examine the ability of MsSND1 to stimulate SCW formation, we transiently overexpressed MsSND1 in Nicotiana benthamiana leaves. After 6 days of incubation, leaf cross-sections were stained for lignin, cellulose, and hemicellulose. Overexpression of MsSND1 and AtSND1 led to patterned ectopic deposition of lignified SCW by epidermis, palisade cells, and spongy mesophyll cells (Figure 4). Interestingly, the patterns of ectopic SCW deposition are reminiscent of tracheary elements which has been previously observed after induced overexpression of NAC and MYB SCW master switches from several species such as Arabidopsis, poplar, Eucalyptus, switchgrass, rice, maize, and Brachypodium (Kubo et al., 2005; Zhong et al., 2007a,b; Zhong et al., 2010a; Zhong, Lee, & Ye, 2010b; Zhong et al., 2011; McCarthy et al., 2009; Valdivia et al., 2013; Yoshida et al., 2013). Cortical microtubules have long been known to play a crucial role in patterned deposition of SCW by guiding exocyst complex and secretory vesicles at the sites of SCW deposition (Baskin, 2001; Oda & Fukuda, 2013; Vukasinovic et al., 2017; Watanabe et al., 2015; Wightman & Turner, 2008). Indeed, several genes involved in cytoskeleton organization and vesicle transport like MIDD1, FRA1, kinesins, or tubulin were found to be regulated by NAC TFs (Ohashi-Ito, Oda, & Fukuda, 2010; Yamaguchi et al., 2011; Zhong et al., 2010b). Therefore, it is tempting to speculate that overexpression of MsSND1 does not only activate the biosynthetic cell wall machinery but also impacts cytoskeleton organization and vesicle transport resulting in the specific patterns of SCW deposition. The high similarity of the observed SCW patterns and deposition after overexpression of AtSND1 and MsSND1 supports the assumption that they are functionally similar. In sharp contrast, expression of MsSCM1-3 (related to AtMYB20, AtMYB43, and AtMYB85) and MsSCM4 (related to AtMYB63 and AtMYB58) (Fig. S3) resulted in uniform lignin deposition around N. benthamiana mesophyll cells without apparent patterning (Figure 5), demonstrating that individual SCW TFs differ in their functionality and target spectrum. Taken together, transient expression of MsSND1 in tobacco leaves demonstrates that MsSND1 is capable of initiating a SCW differentiation program resulting in ectopic deposition of patterned cellulose, hemicellulose, and lignin.

3.5 | Induction of mCherry-GR-MsSND1 leads to transdifferentiation of xylem-like elements and allows localization and tracking of the fusion protein

To further evaluate the function of MsSND1, we established a post-translational MsSND1 induction system in Arabidopsis consisting of MsSND1 fused to the fluorophore mCherry and the ligand-binding domain of the rat glucocorticoid receptor (GR; Figure 6a). Briefly, in the absence of the steroid ligand, the heat-shock protein 90 (HSP90) builds a cytosolic complex with GR, preventing the fusion protein, in this case mCherry-GR-MsSND1 from entering the nucleus (Dalman,
Scherrer, Taylor, Akil, & Pratt, 1991). Upon application of the synthetic steroid dexamethasone (DEX), mCherry-GR-MsSND1 is released from cytosolic retention and able to act as transcriptional regulator in the nucleus. Under normal growth conditions, transgenic Arabidopsis carrying a constitutively expressed mCherry-GR-MsSND1 construct exhibited no obvious differences to wild-type plants concerning germination, growth, or development (Figure 6b). In contrast, activation of mCherry-GR-MsSND1 with DEX for 7 days led to an arrest of growth in seedlings of three independent transgenic Arabidopsis lines (Figure 6b). Furthermore, induction of mCherry-GR-MsSND1 resulted in cell death, as indicated by loss of chlorophyll. The transgenic line 16-2 seemed to show the strongest activation of mCherry-GR-MsSND1, as growth was immediately arrested and loss of chlorophyll was the fastest, and thus was chosen for subsequent experiments. Nonetheless, cell death after mCherry-GR-MsSND1 activation occurred much slower than observed in mCherry-GR-MsVND7 lines (Fig. S4) and previously described (Yamaguchi et al., 2010), suggesting functional similarity to class I NAC TFs like AtSND1 or BdSWN8, as they share a weaker induction of cell death (Valdivia et al., 2013).

In untreated mCherry-GR-MsSND1 seedlings, detection of the mCherry signal in root tips revealed a cytosolic localization of the fusion protein mCherry-GR-MsSND1 (Figure 6c). After 24 h of DEX treatment, the mCherry signal was exclusively detected in nuclei of root cells (Figure 6d). The sharp boundaries of the mCherry signal, excluding nuclei, in Figure 6c indicate that the mCherry-GR-MsSND1 fusion protein only marginally leaked into the nucleus, consistent with normal growth and development of noninduced seedlings. This was further validated by lignin staining using basic fuchsin in the absence and presence of DEX. In noninduced seedlings, lignification was only detected in vascular elements (Figure 6e, g). In contrast, DEX application for 4 days led to ubiquitous SCW deposition in the seedling including leaf mesophyll cells and root ground tissue (Figure 6f, h). In line with what was observed after transient expression of MsSND1 in Nicotiana benthamiana leaves, induction of mCherry-GR-MsSND1 in Arabidopsis gave rise to patterns of SCW deposition reminiscent of tracheary elements. This result again supports a high degree of conservation of the molecular mechanism involved in SCW patterning and biosynthesis throughout vascular plants (Bomal et al., 2008; Li et al., 2012; Zhong et al., 2010a).

**FIGURE 7** Induction of mCherry-GR-MsSND1 activates expression of genes involved in SCW formation. Expression analysis of candidate genes directly and indirectly targeted by MsSND1. Ten-day-old heterozygous Arabidopsis mCherry-GR-MsSND1 seedlings (line 16) were treated with cycloheximide (CHX) and/or dexamethasone (DEX). Gene expression is normalized against clathrin adaptor subunit. Bars depict means ± SE from three technical replicates. The same experiment was performed with an independent transgenic line with similar results (Fig. S5).
establishment of a precisely inducible mCherry-GR-MsSND1 Arabidopsis line allows localization and tracking of the fusion protein and corroborates the finding that MsSND1 is capable of activating the SCW program resulting in patterned SCW deposition reminiscent of xylem elements.

3.6 | Induction of mCherry-GR-MsSND1 activates expression of genes involved in SCW formation

Next, we assessed the ability of MsSND1 to act as transcriptional regulator of SCW formation. Therefore, we induced mCherry-GR-MsSND1 Arabidopsis lines with or without pretreatment with the protein translation inhibitor cycloheximide (CHX), to determine direct and indirect targets. Expression levels of selected genes involved in SCW formation were measured via qRT-PCR (Figure 7). Activation of mCherry-GR-MsSND1 with DEX elevated the transcript levels of CesA4, CesA7, and CesA8, xylan biosynthesis (IRX7 and IRX8), lignin biosynthesis (4CL1 and CCoAOMT1), cell death (XCP1), lignin polymerization (LAC4), and MYB TFs (MYB46/83). Besides for XCP1, 4CL1, and CCoAOMT1, the background expression levels in the controls were very low. Upon DEX application, gene expression was strongly upregulated up to 70-fold in case of CesA7 (Figure 7). However, induction of mCherry-GR-MsSND1 with DEX in the presence of CHX promoted the expression of XCP, MYB46, and MYB83, while genes associated with cellulose, hemicellulose, lignin biosynthesis, and polymerization seem to be indirect targets. The independent mCherry-GR-MsSND1 line 18 exhibited the same activation pattern as mCherry-GR-MsSND1 line 16 but with generally lower relative values (Fig. S5). Thus, MsSND1 can activate many of the same downstream target genes as previously identified for secondary wall NACs (SWNs) like AtSND1, AtVND6, and AtVND7 (Ohashi-Ito et al., 2010; Zhong et al., 2010b).

4 | CONCLUSION

Cell wall recalcitrance, which is mainly conferred by incorporation of lignin into SCWs, remains a major limitation to explore lignocellulosic biomass as renewable resource. Concomitantly, considerable efforts are directed at increasing production of lignocellulosic biomass that can be valorized in a sustainable manner. However, the molecular mechanisms involved in lignification and SCW formation in Miscanthus have received only little attention. In this study, we identified and functionally characterized Miscanthus sinensis SND1 as a transcriptional master regulator orchestrating SCW formation, most likely in extraxylary fibers. In addition, we could identify other TF from the SCW transcriptional network, which appears to only regulate a subset of the differentiation program. Despite the fact that research on Miscanthus is still hampered by challenging transformation procedures, our approach shows that utilization of the increasingly available genomic resources and transfer of knowledge from model plants greatly facilitate the investigation of molecular mechanisms. The functional characterization of MsSND1 contributes to a deeper understanding of the molecular network of TFs involved in SCW formation in Miscanthus. It is tempting to speculate that lower-tier TFs such as MsSCM4 (related to AtMYB63/AtMYB58) could affect lignin qualities more specifically, suggesting that may serve as potential targets for breeding programs. Moreover, the possible involvement of MsSND1 in extraxylary fibers differentiation may represent a target for cell wall engineering (Yang et al., 2013) as it offers the opportunity to manipulate lignin specifically in extraxylary fibers without affecting xylem elements and thereby bypassing severe growth defects.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

P.G., T.R., and S.W. designed research; P.G., C.V., and F.H. performed research and analyzed data; P.G., T.R., and S.W. wrote the manuscript.

REFERENCES

Barling, A., Swaminathan, K., Mitros, T., James, B. T., Morris, J., Nagaboina, O., ... Moose, S. P. (2013). A detailed gene expression study of the Miscanthus genus reveals changes in the transcriptome associated with the rejuvenation of spring rhizomes. BMC Genomics, 14, 864. https://doi.org/10.1186/1471-2164-14-864

Baskin, T. I. (2001). On the alignment of cellulose microfibrils by cortical microtubules: A review and a model. Protoplasma, 215, 150–171. https://doi.org/10.1007/BF01280311

Bonal, C., Bedon, F., Caron, S., Mansfield, S. D., Levasseur, C., Cooke, J. E., ... Mackay, J. (2008). Involvement of Pinus taeda MYB1 and MYB8 in phenylpropanoid metabolism and secondary cell wall biosynthesis: A comparative in planta analysis. Journal of Experimental Botany, 59, 3925–3939. https://doi.org/10.1093/jxb/erm234

Bonawitz, N. D., & Chapple, C. (2013). Can genetic engineering of lignin deposition be accomplished without an unacceptable yield penalty? Current Opinion in Biotechnology, 24, 336–343. https://doi.org/10.1016/j.copbio.2012.11.004

Bonawitz, N. D., Kim, J. I., Tobimatsu, Y., Ciesielski, P. N., Anderson, N. A., Ximenes, E., ... Chapple, C. (2014). Disruption of Mediator rescues the stunted growth of a lignin-deficient Arabidopsis mutant. Nature, 509, 376–380. https://doi.org/10.1038/nature13084

Clifton-Brown, J. C., & Lewandowski, I. (2002). Screening Miscanthus genotypes in field trials to optimise biomass yield and quality in...
Lampropoulos, A., Sutikovic, Z., Wenzl, C., Maegele, I., Lohmann, J. U., & Clough, S. J., & Bent, A. F. (1998). Floral dip: A simplified method for
Legay, S., Lacombe, E., Goicoechea, M., Brieg, E., Gallego-Giraldo, L., Jikumaru, Y., Kamiya, Y., Tang, Y., & Dixon, R. A.
Dalman, F. C., Scherrer, L. C., Taylor, L. P., Akil, H., & Pratt, W. B. (1991).
Czechowski, T., Stitt, M., Altmann, T., Udvardi, M. K., & Scheible, W. R.
da Costa, R. M., Pattathil, S., Avci, U., Lee, S. J., Hazen, S. P., Winters, A., & da Costa, R. M., Lee, S. J., Allison, G. G., Hazen, S. P., Winters, A., & Schwartz, K. U., Muller-Samann, K., … Kalinina, O. (2016). Pro
da Costa, R. M., Pattathil, S., Avci, U., Lee, S. J., Hazen, S. P., Winters, A., & … Bosch. (2017). A cell wall reference profile for Miscanthus
Czechowski, T., Stitt, M., Altmann, T., Udvardi, M. K., & Scheible, W. R. (2005). Genome-wide identification and testing of superior reference
Mitsuda, N., Iwase, A., Yamamoto, H., Yoshida, M., Seki, M., Shinozaki, K., & Ohme-Takagi, M. (2005). The NAC
Mitsuda, N., Iwase, A., Yamamoto, H., Yoshida, M., Seki, M., Shinozaki, K., & Ohme-Takagi, M. (2005). The NAC
McCartney, L., Marcus, S. E., & Knox, J. P. (2005). Monoclonal antibodies to plant cell wall xylans and arabinoxylans. The Journal of Histochemistry and Cytochemistry, 53, 543–546. https://doi.org/10.1369/jhc.4B6578.2005
McCartney, L., Steele-King, C. G., Jordan, E., & Knox, J. P. (2003). Cell wall pectic (1→4)-beta-d-galactan marks the acceleration of cell elongation in the Arabidopsis seedling root meristem. Plant Journal, 33, 447–454. https://doi.org/10.1046/j.1365-313x.2003.01640.x
Mitsuda, N., Iwase, A., Yamamoto, H., Yoshida, M., Seki, M., Shinozaki, K., & Ohme-Takagi, M. (2007). NAC transcription factors, NST1 and NST3, are key regulators of the formation of secondary walls in woody tissues of Arabidopsis. Plant Cell, 19, 270–280. https://doi.org/10.1105/tpc.106.047043
Mitsuda, N., Seki, M., Shinozaki, K., & Ohme-Takagi, M. (2005). The NAC transcription factors NST1 and NST2 of Arabidopsis regulate secondary wall thickenings and are required for anther dehiscence. Plant Cell, 17, 2993–3006. https://doi.org/10.1105/tpc.105.036004
Musielak, T. J., Schenkel, L., Kolb, M., Henschen, A., & Bayer, M. (2015). A simple and versatile cell wall staining protocol to study plant reproduction. Plant Reproduction, 28, 161–169. https://doi.org/10.1007/s00497-015-0267-1
Nakano, Y., Yamaguchi, M., Endo, H., Rejab, N. A., & Ohtani, M. (2015). NAC-MYB-based transcriptional regulation of secondary wall biosynthesis in land plants. Frontiers in Plant Science, 6, 288. https://doi.org/10.3389/fpls.2015.00288
Oda, Y., & Fukuda, H. (2013). Rho of plant GTPase signaling regulates the behavior of Arabidopsis kinesin-13A to establish secondary cell wall patterns. Plant Cell, 25, 4439–4450. https://doi.org/10.1105/tpc.113.117853
Ohashi-Ito, K., Oda, Y., & Fukuda, H. (2010). Arabidopsis VASCULAR-RELATED NAC-DOMAIN6 directly regulates the genes that govern programmed cell death and secondary wall formation during xylem differentiation. Plant Cell, 22, 3461–3473. https://doi.org/10.1105/tpc.110.075036
Ohman, D., Demedts, B., Kumar, M., Gerber, L., Gorzsas, A., Goeminne, G., … Sundberg, B. (2013). MYB103 is required for FERULATE-5-HYDROXYLASE expression and syringyl lignin biosynthesis in
Robson, P., Jensen, E., Hawkins, S., White, S. R., Kenobi, K., Clifton-Patzlaff, A., McInnis, S., Courtenay, A., Surman, C., Newman, L. J., Smith, R. A., Schuetz, M., Roach, M., Mansfield, S. D., Ellis, B., & Samuels, Sievers, F., Wilm, A., Dineen, D., Gibson, T. J., Karplus, K., Li, W., Vallet, C., Chabbert, B., Czaninski, Y., & Monties, B. (1996). Histochemistry and Cell Physiology, 36, 743–754. https://doi.org/10.1046/j.1365-313X.2003.01916.x

Sakamoto, S., & Mitsuda, N. (2015). Reconstitution of a secondary cell wall in a secondary cell wall-deficient Arabidopsis mutant. Plant and Cell Physiology, 56, 299–310. https://doi.org/10.1093/pcp/puc208

Siout, R., Le Bris, P., Legeez, F., Cezard, L., Renault, H., & Lapierre, C. (2016). Structural redesigning arabidopsis lignins into alkali-soluble lignins through the expression of p-Coumaryl-CoA: Monolignol transferase PMT. Plant Physiology, 170, 1358–1366. https://doi.org/10.1104/pp.15.01877

Sievers, F., Wilm, A., Dineen, D., Gibson, T. J., Karplus, K., Li, W., Higgins, D. G. (2011). Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Molecular Systems Biology, 7, 539. https://doi.org/10.1038/msb.2011.75

Smith, R. A., Schuetz, M., Roach, M., Mansfield, S. D., Ellis, B., & Samuels, L. (2013). Neighboring parenchyma cells contribute to Arabidopsis xylem lignification, while lignification of interfascicular fibers is cell autonomous. Plant Cell, 25, 3988–3999. https://doi.org/10.1105/tpc.113.117176

Stracke, R., Werber, M., & Weisshaar, B. (2001). The R2R3-MYB gene family in Arabidopsis thaliana. Current Opinion in Plant Biology, 4, 447–456. https://doi.org/10.1016/S1369-5266(00)00199-0

Tamagnone, L., Merida, A., Parr, A., Mackay, S., Culfianez-Macia, F. A., Roberts, K., & Martin, C. (1998). The AmMYB308 and AmMYB330 transcription factors from antirrhinum regulate phenylpropanoid and lignin biosynthesis in transgenic tobacco. Plant Cell, 10, 135–154. https://doi.org/10.1105/tpc.10.2.135

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution, 30, 2725–2729. https://doi.org/10.1093/molbev/ms3197

Taylor-Tipples, M., Lin, L., de Lucas, M., Turco, G., Toal, T. W., Gaudinier, A., ... Brady, S. M. (2015). An Arabidopsis gene regulatory network for secondary cell wall synthesis. Nature, 517, 571–575. https://doi.org/10.1038/nature14099

Valdivia, E. R., Herrera, M. T., Gianzo, C., Fidalgo, J., Revilla, G., Zarra, I., & Sampredo, J. (2013). Regulation of secondary wall synthesis and cell death by NAC transcription factors in the monocot Brachypodium distachyon. Journal of Experimental Botany, 64, 1333–1343. https://doi.org/10.1093/jxb/ers394

Vallet, C., Chabbert, B., Czaninski, Y., & Monties, B. (1996). Histochemistry of lignin deposition during sclerenchyma differentiation in alfalfa stems. Annals of Botany, 78, 625–632. https://doi.org/10.1006/anbo.1996.0170

Van Acker, R., Leple, J. C., Aerts, D., Storme, V., Goeminne, G., Ivens, B., ... Boerjan, W. (2014). Improved saccharification and ethanol yield from field-grown transgenic poplar deficient in cinnamoyl-CoA reductase. Proceedings of the National Academy of Sciences of the United States of America, 111, 845–850. https://doi.org/10.1073/pnas.1321673111

Vukasinovic, N., Oda, Y., Pejchar, P., Synek, L., Pecenková, T., Rawat, A., ... Zársky, V. (2017). Microtubule-dependent targeting of the exocytosis complex is necessary for xylem development in Arabidopsis. New Phytologist, 213, 1052–1067. https://doi.org/10.1111/nph.14267

Wang, L., Czedik-Eysenberg, A., Mertz, R. A., Si, Y., Tohge, T., Nunes-Nesi, A., ... Brutnell, T. P. (2014). Comparative analyses of C4 and C3 photosynthesis in developing leaves of maize and rice. Nature Biotechnology, 32, 1158–1165. https://doi.org/10.1038/nbt.3019

Watanabe, Y., Meents, M. J., McDonnell, L. M., Barkwill, S., Sampathkumar, A., Cartwright, H. N., ... Mansfield, S. D. (2015). Visualization of cellulose synthases in Arabidopsis secondary cell walls. Science, 350, 198–203. https://doi.org/10.1126/science.aac7446

van der Weijde, T., Alvim Kamei, C. L., Torres, A. F., Vemmeris, W., Dolstra, O., Visser, R. G., & Trindade, L. M. (2013). The potential of C4 grasses for cellulosic biofuel production. Frontiers in Plant Science, 4, 107. https://doi.org/10.3389/fpls.2013.00107

Wightman, R., & Turner, S. R. (2008). The roles of the cytokinetic network during cellulosic deposition at the secondary cell wall. Plant Journal, 54, 794–805. https://doi.org/10.1111/j.1365-313X.2008.03444.x

Wilkinson, C. G., Mansfield, S. D., Lu, F., Withers, S., Park, J. Y., Karlen, S. D., ... Ralph, J. (2014). Monolignol furate transferase introduces chemically labile linkages into the lignin backbone. Science, 344, 90–93. https://doi.org/10.1126/science.1250161

Yamaguchi, M., Goue, N., Igarashi, H., Ohtani, M., Nakano, Y., Mortimer, J. C., ... Demura, T. (2010). VASCULAR-RELATED NAC-DOMAIN6 and VASCULAR-RELATED NAC-DOMAIN7 effectively induce transdifferentiation into xylem vessel elements under control of an induction system. Plant Physiology, 153, 906–914. https://doi.org/10.1104/pp.110.154013

Yamaguchi, M., Mitsuda, N., Ohtani, M., Ohme-Takagi, M., Kato, K., & Demura, T. (2011). VASCULAR-RELATED NAC-DOMAIN7 directly regulates the expression of a broad range of genes for xylem vessel formation. Plant Journal, 66, 579–590. https://doi.org/10.1111/j.1365-313X.2010.04514.x

Yang, F., Mitra, P., Zhang, L., Prak, L., Verhertbruggen, Y., Kim, J. S., ... Loque, D. (2013). Engineering secondary cell wall deposition in plants. Plant Biotechnology Journal, 11, 325–335. https://doi.org/10.1111/pbi.12016

Yoshida, K., Sakamoto, S., Kawai, T., Kobayashi, Y., Sato, K., Ichinose, Y., ...Mitsuda, N. (2013). Engineering the Osryz sativa cell wall with rice NAC transcription factors regulating secondary wall formation. Frontiers in Plant Science, 4, 383. https://doi.org/10.3389/fpls.2013.00383

Zhao, Q., Wang, H., Yin, Y., Xu, Y., Chen, F., & Dixon, R. A. (2010). Syringyl lignin biosynthesis is directly regulated by a secondary cell wall master switch. Proceedings of the National Academy of Sciences of the United States of America, 107, 14496–14501. https://doi.org/10.1073/pnas.1001970107

Zhong, R., Demura, T., & Ye, Z. H. (2006). SND1, a NAC domain transcription factor, is a key regulator of secondary wall synthesis in fibers of Arabidopsis. Plant Cell, 18, 3158–3170. https://doi.org/10.1105/tpc.106.047399

Zhong, R., Lee, C., McCarthy, R. L., Reeves, C. K., Jones, E. G., & Ye, Z. H. (2011). Transcriptional activation of secondary wall biosynthesis by rice and maize NAC and MYB transcription factors. Plant and Cell Physiology, 52, 1856–1871. https://doi.org/10.1093/pcp/pcr123

Zhong, R., Lee, C., & Ye, Z. H. (2010a). Evolutionary conservation of the transcriptional network regulating secondary cell wall biosynthesis. Trends in Plant Science, 15, 625–632. https://doi.org/10.1016/j.tplants.2010.08.007

Zhong, R., Lee, C., & Ye, Z. H. (2010b). Global analysis of direct targets of secondary wall NAC master switches in Arabidopsis. Molecular Plant, 3, 1087–1103. https://doi.org/10.1093/mp/ssq062
Zhong, R., Lee, C., Zhou, J., McCarthy, R. L., & Ye, Z. H. (2008). A battery of transcription factors involved in the regulation of secondary cell wall biosynthesis in Arabidopsis. Plant Cell, 20, 2763–2782. https://doi.org/10.1105/tpc.108.061325

Zhong, R., McCarthy, R. L., Haghighat, M., & Ye, Z. H. (2013). The poplar MYB master switches bind to the SMRE site and activate the secondary wall biosynthetic program during wood formation. PLoS ONE, 8, e69219. https://doi.org/10.1371/journal.pone.0069219

Zhong, R., Richardson, E. A., & Ye, Z. H. (2007a). The MYB46 transcription factor is a direct target of SND1 and regulates secondary wall biosynthesis in Arabidopsis. Plant Cell, 19, 2776–2792. https://doi.org/10.1105/tpc.107.053678

Zhong, R., Richardson, E. A., & Ye, Z. H. (2007b). Two NAC domain transcription factors, SND1 and NST1, function redundantly in regulation of secondary wall synthesis in fibers of Arabidopsis. Planta, 225, 1603–1611. https://doi.org/10.1007/s00425-007-0498-y

Zhong, R., Yuan, Y., Spiekerman, J. J., Guley, J. T., Egbsiuba, J. C., & Ye, Z. H. (2015). Functional characterization of NAC and MYB transcription factors involved in regulation of biomass production in switchgrass (Panicum virgatum). PLoS ONE, 10, e0134611. https://doi.org/10.1371/journal.pone.0134611

Zhou, J., Lee, C., Zhong, R., & Ye, Z. H. (2009). MYB58 and MYB63 are transcriptional activators of the lignin biosynthetic pathway during secondary cell wall formation in Arabidopsis. Plant Cell, 21, 248–266. https://doi.org/10.1105/tpc.108.063321

Zhou, J., Zhong, R., & Ye, Z. H. (2014). Arabidopsis NAC domain proteins, VND1 to VND5, are transcriptional regulators of secondary wall biosynthesis in vessels. PLoS ONE, 9, e105726. https://doi.org/10.1371/journal.pone.0105726

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