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ORIGINAL ARTICLE

The NAC Transcription Factor OsSWN1 Regulates Secondary Cell Wall Development in *Oryza sativa*

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Abstract Rice, as a major crop in the world, produces huge agronomic biomass residues besides food, which consist of cellulose, hemicelluloses and lignin. Many master regulators of secondary wall synthesis were identified in the model plant *Arabidopsis*. In this study, we investigated the function of a NAC (NAM, ATAF, and CUC2) transcription factor related to secondary cell wall biosynthesis, which is highly expressed in rice sclerenchyma tissue and is named *OsSWN1*. Our results showed that engineering of *OsSWN1* could exhibit multiple features regulated to agronomic traits and bioenergy research. Over-expression of *OsSWN1* caused an erect-leaf and enclosed-flower phenotype. Secondary cell wall-related genes were actively expressed in transgenic plants with obvious ectopic lignin deposition in the leaf collar, while increased lignin content and decreased the sugar yield correspondingly. In addition, down-regulation of *OsSWN1* expression levels decreased lignin content and increased the sugar yield in transgenic plants. Bioinformatics analysis revealed that *OsSWN1*-like genes are highly conserved in switchgrass and sorghum, suggesting a possibility of manipulating the expression level of the *OsSWN1* orthologs in the bioenergy crops for biofuel production.

Key words: Enzymatic digestibility, Erect leaf, NAC transcription factor, *Oryza sativa*, Secondary cell wall biosynthesis

Introduction

As a stable food crop for about 50% of the world's population, rice produces half of the agronomic biomass residues, which represent a potential source of bioethanol (Kim 2003). More than 90% of this dry plant biomass consists of secondary cell walls (Somerville 2007), which are mainly composed of cellulose, hemicellulose, and lignin, also called lignocellulosic biomass. As a key component of the secondary cell wall, cellulose is the major source for bioethanol production. However, cellulose is bound with lignin to form a tight crystal complex in vascular plants. Although this complex is essential for mechanical support and water transport, the presence of lignin in the biomass residue results in recalcitrance to saccharification, which is a major limiting factor for biofuel production. Lignin modification can decrease the cost of biomass pretreatment and improve fermentable sugar yields (Chen and Dixon 2007).

Plant cell wall biogenesis involves many distinct but interactive biochemical pathways. Annotations of the Arabidopsis and rice genomes revealed over 1,000 cell wall-related genes encompassing precursor-generating enzymes, polysaccharide synthases, glycosyl transferases, structural proteins, and a host of enzymes involved in polysaccharide modifications and depolymerizations (Somerville et al. 2004; Yokoyama et al. 2004). Many genes in Arabidopsis that are involved in the cellulose, lignin, pectin, and hemicellulose synthesis pathways have been cloned and characterized (Saxena and Brown 2005; Somerville 2006). Recently, different sets of transcription factors have been identified as key regulators of secondary cell wall biosynthesis in Arabidopsis. In the NAC transcription factor family, AtNST1, AtNST2, and AtNST3 are master switches in regulating secondary cell wall biosynthesis. Specifically, AtNST1 and AtNST2 are necessary for anther dehiscence (Mitsuda et al. 2005), while AtNST1 and AtNST3 control secondary cell wall deposition in stems (Mitsuda et al. 2007; Mitsuda et al. 2005; Zhong et al. 2006; Zhong et al. 2007b). Other research

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has indicated that AtNSTs turn on the entire secondary cell wall biosynthetic pathway through initiating a regulatory network, including some MYB domain transcription factors (McCarthy et al. 2009; Zhong et al. 2007a) and C3H zinc finger transcription factors (Ko et al. 2009). As secondary-level master regulators, MYBs can be regulated directly or indirectly by AtNSTs (Zhong et al. 2008; Zhong et al. 2007a). All of these key regulators involved in secondary cell wall biosynthesis have a common trait: they are highly expressed in lignin-rich tissues such as the interfascicular fibers in Arabidopsis. Several orthologous genes in poplar and *Medicago truncatula* also found in secondary wall formation (Zhao et al. 2010; Zhong et al. 2011b).

In monocots, some rice and corn NST orthologs were renamed Secondary Wall NAC domain proteins (SWNs) and could restore the drooping phenotype of *nst1nst3* double mutants in Arabidopsis (Yoshida et al. 2013; Zhong et al. 2011a). The *OsSWN2* chimeric repressor alters secondary wall formation in rice (Yoshida et al. 2013). In this study, we characterized the rice sclerenchyma-specific transcription factor *OsSWN1*, which is highly expressed in rice sclerenchyma and vascular tissues and encodes a NAC transcription factor. Overexpression (OX) of *OsSWN1* resulted in altered morphology and increased lignin content in transgenic rice, while silencing of *OsSWN1* gene caused reduced lignin content. We further demonstrated that *OsSWN1* can regulate the expression of many secondary cell wall-related genes in rice, which include many brittle culm genes. As a consequence, *OsSWN1* can modify the enzymatic digestibility of raw transgenic rice straw through changing cell wall genes expression. Because the *OsSWN1* gene is highly conserved in maize, sorghum, and switchgrass, manipulation of the orthologous genes in these species may generate new feedstocks for efficient biofuel production in the future.

Results

Identification of the Sclerenchyma-specific NAC Transcription Factor *OsSWN1* in Rice

To identify functional genes involved in lignin biosynthesis in sclerenchyma tissue, we searched the Yale Virtual Center for Cellular Expression Profiling of Rice database (http://bioinformatics.med.yale.edu/riceatlas) for highly expressed genes in the sclerenchyma-related region (Jiao et al. 2009). We found that a gene encoding a NAC transcription factor, named *OsSWN1*, was highly expressed in the mature epidermis region and in the root tip vascular bundle (Fig. S1). Moreover, co-expression analysis showed that *OsSWN1*

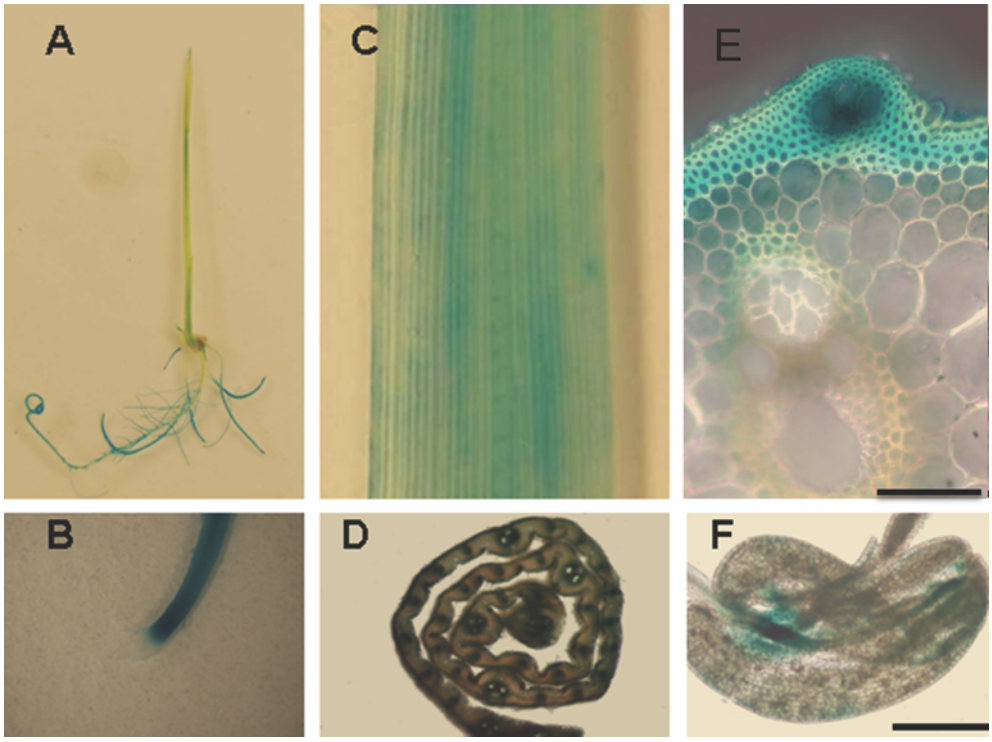

Fig. 1. OsSWN1 promoter activity revealed by GUS staining in transgenic rice. (**A**) GUS activity in 1-week-old seedlings, (**B**) GUS activity in roots of 1-week-old seedlings, (**C**) GUS activity in leaves of 1-month-old plants, (**D**) GUS activity in vascular tissue of young leaves (cross-section) of 1-month-old plants, (**E**) GUS activity in sclerenchyma and vascular tissues of culms (cross-sections) of 1-month-old plants. Bar = 200 μm, (**F**) GUS activity in anthers of flowering plants. Bar = 0.5 mm.

was co-expressed with several key secondary cell wall biosynthesis genes, such as *OsCESA4* and *OsCESA7* (Tanaka et al. 2003) (Table S2). Therefore, we selected *OsSWN1* as our candidate gene to study its function in the lignin biosynthesis pathway in rice.

To confirm the expression pattern of *OsSWN1*, we fused the 2.3-kb DNA fragment upstream of the translation start site of *SWN1* with the β-glucuronidase (GUS) gene and generated the *OsSWN1* promoter-GUS transgenic rice. Histochemical analysis was performed with three independent transgenic lines. GUS activity assays revealed that *OsSWN1* was highly expressed in sclerenchyma tissue of the culm (Fig. 1E), suggesting that *OsSWN1* may be involved in rice secondary cell wall biosynthesis in sclerenchyma. In addition, we also observed GUS staining in leaf veins and vascular tissues, where lignin is deposited (Fig. 1C and 1D), and found GUS activity in mature anther tissue (Fig. 1F), where secondary cell wall thickening is important for anther dehiscence. Strong GUS staining was also detected in roots, but not in the root cap region where no vascular tissue or sclerenchyma is formed (Fig. 1A and 1B). Recently, OsSWN1 is found prominently in developing fiber cells around epidermis and bundle sheath tissues in rice (Yoshida et al. 2013; Zhong et al. 2011a), which is consistent with our results.

Overexpression (OX) and RNAi Silencing of *OsSWN1* in Transgenic Plants Showed Abnormal Developmental Phenotypes

To characterize the function of *OsSWN1*, we generated *OsSWN1* OX and RNAi transgenic lines through the *Agrobacterium*-mediated transformation. Over 20 independent *OsSWN1* OX and RNAi lines were obtained separately. Most of the *OsSWN1* OX lines showed a semi-dwarf and a nearly sterile phenotype (the latter characteristic was possibly due to abnormal flowers) (Fig. 2A and 2C). Only three independently transformed *OsSWN1* OX lines can get few seeds, namely *OsSWN1* OX-4, -5, and -6. The expression level of *OsSWN1* was much higher in *OsSWN1* OX plants than in wild-type (WT) plants (Fig. S1). We also found that *OsSWN1* OX plants had shorter internodes, which was consistent with the semi-dwarf phenotype. The relative length as a percentage of internodes, however, was similar for *OsSWN1* OX and WT plants (Fig. 2E). Besides the semi-dwarf and sterile phenotype, *OsSWN1* OX plants also displayed an erect-leaf phenotype with a decrease in the bending angle of the lamina joint (Fig. 2B), which is a classic BR-deficiency effect (Tanaka et al. 2009; Yamamuro et al. 2000). In flowering stage, *OsSWN1* OX plants also showed Enclosed Flower phenotype with 100% (Fig. 2C). In contrast to *OsSWN1* OX plants, RNAi lines grew relatively normally through the whole life cycle, except that they showed a sterile phenotype due to anther indehiscence (Fig. 2F).

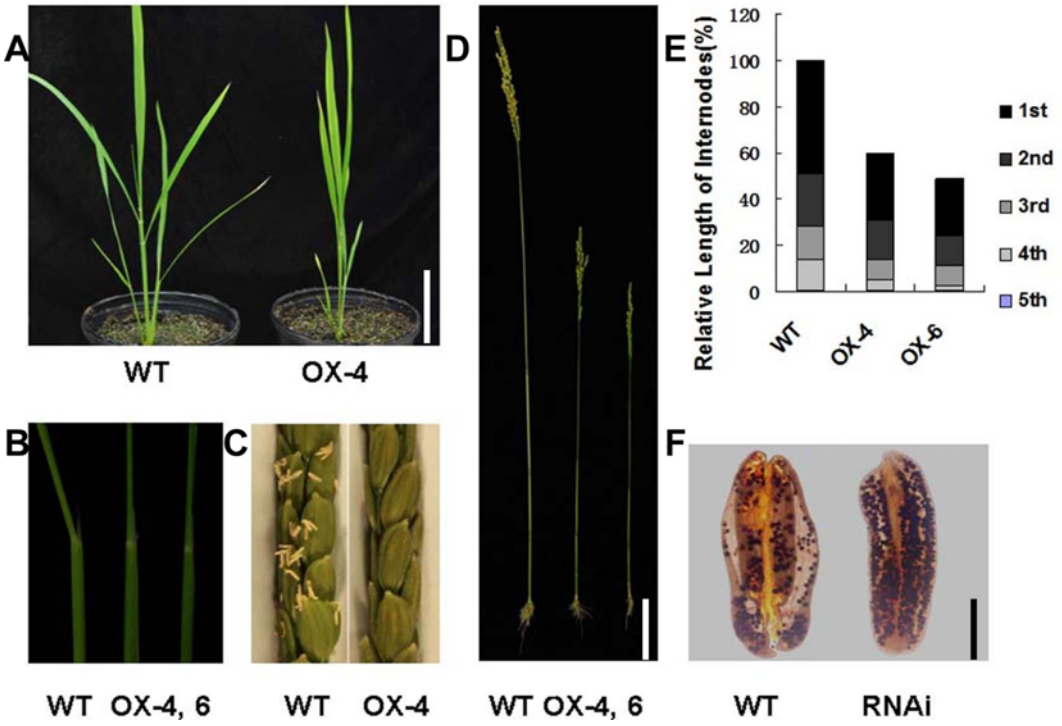

Fig. 2. Phenotypes of *OsSWN1* overexpression (OX) and RNAi transgenic plants. (**A**) Gross morphology of 1-month-old wild-type (WT) and OX plants. Bar = 10 cm, (**B**) Lamina joints of third leaf from flag leaf of 1-month-old WT and OX plants, (**C**) Flowers of WT and OX plants, (**D**) Internodes of mature WT and OX plants. Bar = 10 cm, (**E**) Relative length of internodes in WT and OX plants, (**F**) Anthers of WT and RNAi plants. Bar = 0.5 mm

OX and RNAi Silencing of *OsSWN1* in Transgenic Plants Led to Changes in Enzymatic Hydrolysis Efficiency Through Affecting Lignin Content

To determine lignin distribution in both *OsSWN1* OX and RNAi plants, we performed histochemical staining using a Wiesner reaction according to the standard protocol for lignin localization in rice (Li et al. 2003; Zhang et al. 2006). Overexpression of *OsSWN1* induced obvious ectopic lignification in leaf collar tissues (Fig. 3A-B); In addition, light microscopy revealed that the sclerenchyma and vascular regions in leaves had thicker cell walls in *OsSWN1* OX plants than in WT plants (Fig. 3C-D).

To accurately evaluate the cell wall content in the *OsSWN1* OX and RNAi plants, we extracted the total composition of biomass of the transgenic and WT raw straw according to the method previously described (Li et al. 2003; Zhang et al. 2006). This assay showed that lignin content was increased in *OsSWN1* OX-4 plants and *OsSWN1*OX-6 plants. Strikingly, lignin content was decreased in the RNAi plants (Table 1). Generally, less *OsSWN1* expression level is well linked with the lower lignin content in the straw corresponded to the higher cellulose content. These results demonstrated that OsSWN1 is a positive regulator of lignin biosynthesis in rice.

To evaluate the potential of our transgenic plants in biofuel production, we checked the sugar yield of raw rice straw by enzymatic hydrolysis. As shown in Fig. 5, lower glucose yield was obtained in the OX lines; conversely, the higher glucose yield was obtained with RNAi lines (Fig. 5). In addition, the RNAi plants with lower lignin content did not show any morphological changes. Therefore, the *OsSWN1* gene could be used to modify plant lignin content for efficient biofuel production.

OsSWN1 Regulates the Expression Level of Genes Involved in Secondary Cell Wall Biosynthesis

To reveal the regulatory mechanism of the OsSWN1-mediated cell wall pathway, we performed a whole-genome Affymetrix microarray analysis using the RNA isolated from the *OsSWN1* OX lines and WT plants. The microarray analysis showed that many classic secondary cell wall biosynthesis-related genes were upregulated in the *OsSWN1* OX lines compared with WT plants (Table S1). We selected some for quantitative real-time PCR (Q-PCR) analysis to validate the microarray results. For example, the important cellulose synthase genes *OsCESA4* and *OsCESA7*, which are necessary for secondary cell wall biosynthesis and linked with rice brittle culm trait (Tanaka et al. 2003; Yan et al. 2007), were upregulated up to about 20 times over WT plants. The *Laccase* gene (LOC_Os03g16610) that is involved in lignin polymerization was also induced more than 7-fold in the *OsSWN1* OX plants relative to WT plants. In addition, a MYB-

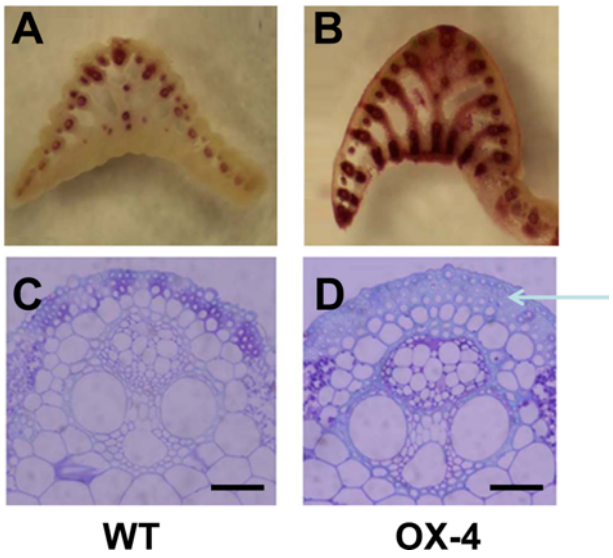

Fig. 3. Ectopic lignification and secondary wall thickening in WT and *OsSWN1* overexpression (OX) plants. (**A**, **B**) Phloroglucinol staining of leaf collar cross-sections in WT and OX plants, (**C**, **D**) Light micrographs of leaf collar cell walls in WT and OX plants. Arrow indicates thicker cell walls in sclerenchyma and vascular tissues in OX plants. Bar = 100 μm.

Table 1. Composition of raw rice straw.

Composition (%)*	WT	OX-090-4	OX-090-6	RNAi--22	RNAi--9
Holocellulose	55.32±2.10	54.70±0.19	56.20±0.53	57.17±1.67	61.20±0.12
Cellulose	36.79±1.57	33.52±0.22	34.05±0.05	41.78±1.49	40.33±0.00
Hemicellulose	18.53±0.53	21.18±0.03	22.15±0.58	15.39±0.17	20.86±0.12
-Xylan	13.61±0.36	16.47±0.07	17.10±0.15	10.93±0.45	14.76±0.11
-Galactan	1.91±0.08	1.82±0.02	1.87±0.12	1.82±0.03	2.42±0.01
-Arabinan	3.02±0.09	2.89±0.02	3.17±0.31	2.63±0.31	3.67±0.00
Lignin	18.94±0.28	20.13±0.38	19.32±0.71	15.18±0.24	17.55±0.04
- Acid-insoluble	16.65±0.31	17.83±0.40	17.06±0.68	13.00±0.20	15.34±0.05
- Acid-soluble	2.29±0.03	2.30±0.02	2.26±0.03	2.18±0.04	2.21±0.01
Others	25.73±2.37	25.17±0.57	24.48±0.18	27.65±1.43	21.25±0.16

*Composition was based on dry mass. Data was reported as mean ±S.D. of duplicates.

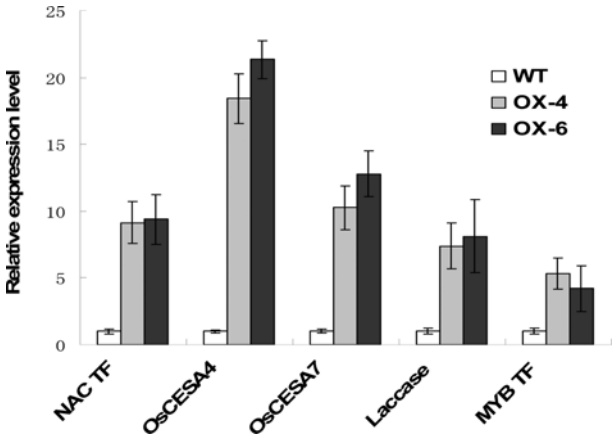

Fig. 4. Expression of genes related to secondary cell wall biosynthesis in *OsSWN1* overexpression (OX) plants determined by qRT-PCR. WT-Nipponbare, OX-4, and OX-6-OX transgenic lines, Error bars represent ± SD of three replicates, Gene ID in the assay. NAC TF: Os11g03370, OsCESA4: Os01g54620, OsCESA7: Os10g32980, Laccase: Os3G16610, MYB TF: Os02g49986.

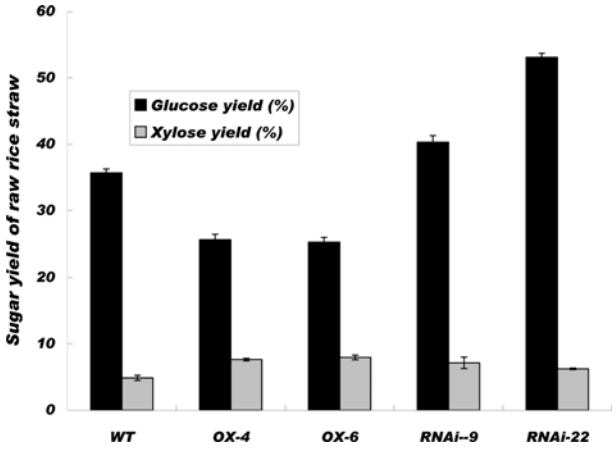

Fig. 5. Sugar yield of raw rice straw by enzymatic hydrolysis. Values are means (± SD) of three replicates

like transcription factor were also upregulated about 5-fold or greater in the *OsSWN1* OX plants (Fig. 4). This MYB gene (LOC_Os02g49986) is a homolog of the Arabidopsis gene *AtMYB20,* which is a positive regulator of secondary wall biosynthesis (Zhong et al. 2008). All together, these data suggested that *OsSWN1* is an important positive regulator in secondary cell wall biosynthesis of rice plants.

Discussion

Although several regulators of secondary cell wall biosynthesis have been reported in the dicotyledonous plant Arabidopsis, little is known about such regulators in monocotyledonous plants, which includes the major crops. Understanding regulators of secondary cell wall biosynthesis in monocots is important because those species that are considered to be the major bioenergy model plants, such as switchgrass and *Brachypodium*, are monocots. Considering the obvious structural differences between dicots and monocots, one cannot assume that the regulatory processes are the same. In this study, we identified the sclerenchyma-associated NAC transcription factor gene *OsSWN1* and demonstrated that OsSWN1 is a novel regulator of secondary wall biosynthesis in rice, a model monocot crop.

Different from Arabidopsis, rice and other biofuel grass species contain sclerenchyma, which is located at the outside layer of culm and root. Sclerenchyma provides mechanical strength and protection and is therefore vital for growth and development. The brittle-culm phenotype in rice is mainly caused by a deficiency in cell wall thickness within the sclerenchyma tissue. So far, several rice brittle-culm mutants have been reported, all of which share a similar thinner cell wall phenotype in sclerenchyma compared with WT (Tanaka et al. 2003; Yan et al. 2007). The brittle-culm phenotype may be related to CESA genes in rice, of which there are 11 CESA genes in the rice genome (Wang et al. 2010). OsCESA4, -7, and -9 have a similar expression pattern and specifically function in secondary cell wall biosynthesis. All OsCESA4, -7, and -9 mutants have the brittle-culm phenotype. Our microarray data showed that OX of *OsSWN1* upregulated the expression level of OsCESA4, -7, and -9. In addition, some putative MYB transcription factors were coordinately regulated in the *OsSWN1* OX plants. One MYB gene, LOC_Os02g49986, has a conserved MYB domain similar to AtMYB20, a reported functional gene regulated by the *AtNST* genes in Arabidopsis secondary wall biosynthesis (Zhong et al. 2008). Together, these data indicate that OsSWN1 is a novel transcription factor (activator) in rice and may act as an important regulator in rice secondary wall biosynthesis.

Gene function redundancy is common in cell wall biosynthesis. In Arabidopsis, three homologs of *OsSWN1* (*AtNST1–NST3*) have overlapping functions in controlling secondary cell wall biosynthesis in stem and anther development (Mitsuda et al. 2007; Mitsuda et al. 2005). Similarly, *MtNST1*, the only homolog in *Medicago truncatula*, can regulate secondary cell wall biosynthesis in both stems and anthers (Zhao et al.; Zhao et al. 2010). In rice, there is a putative paralog of the NAC transcription factor (LOC_Os08g02300), which has high sequence similarity in the N-terminal NAC domain with OsSWN1, but not in the C-terminal. Although *OsSWN1* RNAi lines contained less lignin, the plants grew normally and did not have an obvious morphological phenotype. However, they had an anther-indehiscent phenotype as the *nst1:nst2* double mutant in Arabidopsis (Mitsuda et al. 2005). Reduced lignin deposition was also observed in the sclerenchyma tissue of the RNAi lines. The enzymatic hydrolysis assay based on glucose yield was correlated with the lignin content in rice straw. This

result indicates that OsSWN1 can modify lignin content in rice biomass tissues. Because lignin content was reduced by about 50% in the *Mtnst1* mutant (Zhao et al. 2010), an *OsSWN1* homolog in *Medicago truncatula*, it is likely that the *OsSWN1* paralog LOC_*Os08g02300* in rice may have a redundant function similar to that of *OsSWN1* in culm and leaf tissue when *OsSWN1* is silenced. Unfortunately, we were unable to obtain transgenic plants in which both genes were silenced.

The *OsSWN1* OX plants displayed diverse developmental phenotypes, e.g., a dwarf habit and decreasing fertility. These are similar to findings in Arabidopsis, which indicated that overexpression of secondary wall regulatory genes often resulted in ectopic lignin deposition and a dwarf, curled-leaf phenotype (Mitsuda et al. 2005; Zhong et al. 2007a). Interestingly, *OsSWN1* overexpression plants also displayed an erect-leaf phenotype, similar to rice BR-deficient mutants. Because erect leaves may increase light capture for photosynthesis, researchers have proposed that some rice erect-leaf mutants may have increased biomass and grain yield at high planting densities (Morinaka et al. 2006; Sakamoto et al. 2006). The *OsSWN1* OX plants also showed enclosed flowering phenotype which may benefit transgenic pollen pollution issue and further investigation is required concerning the use of the gene in rice production.

All plant cells have a primary cell wall but only cells that cease to expand have secondary cell wall lignification (Turner et al. 2001). It is reasonable to hypothesize that secondary cell wall biosynthesis and cell expansion have an antagonistic relationship. Expansin proteins contribute to plant cell expansion by loosening cell walls (Sampedro and Cosgrove 2005). Interestingly, the expression levels of seven expansin genes were down-regulated in the *OsSWN1* OX plants (Fig. S3B). The relationship between these genes and *OsSWN1* remains to be elucidated.

NAC proteins have a conserved N-terminal sequence as the DNA-binding domains and a highly diverse C-terminal sequence as the transcriptional regulation domains. The 151 NAC genes in rice and 117 in Arabidopsis are involved in diverse biological functions such as root formation, auxin signaling, biotic and abiotic stress response, and secondary wall biosynthesis (Nuruzzaman et al. 2010). Recently, a total of 1232 NAC proteins from 11 different plants were analyzed and grouped into eight subfamilies according to their phylogenetic relationships. OsSWN1 falls into the NAC-c family, together with the Arabidopsis NST proteins, and is further classified in the c-sc9 (monocots) and c-sc10 (dicots) subgroup separately based on motif patterns in the C-terminal regions (Shen et al. 2009). A BLAST search identified the putative orthologous genes of *OsSWN1* in Arabidopsis (AtNST1-3), Poplar *SWN1*, sorghum *SWN1* (Sb10g002120), maize *SWN1* (Q5NKQ3) and a partial swithgrass *SWN1* (CCGC2900). These protein has a high sequence similarity at the conserved N-terminal NAC domain. However, their C-terminal domains are distinctly diverged. In comparison with AtNSTs and Poplar SWN1 in dicots, the protein sequences of OsSWN1 and its orthologs

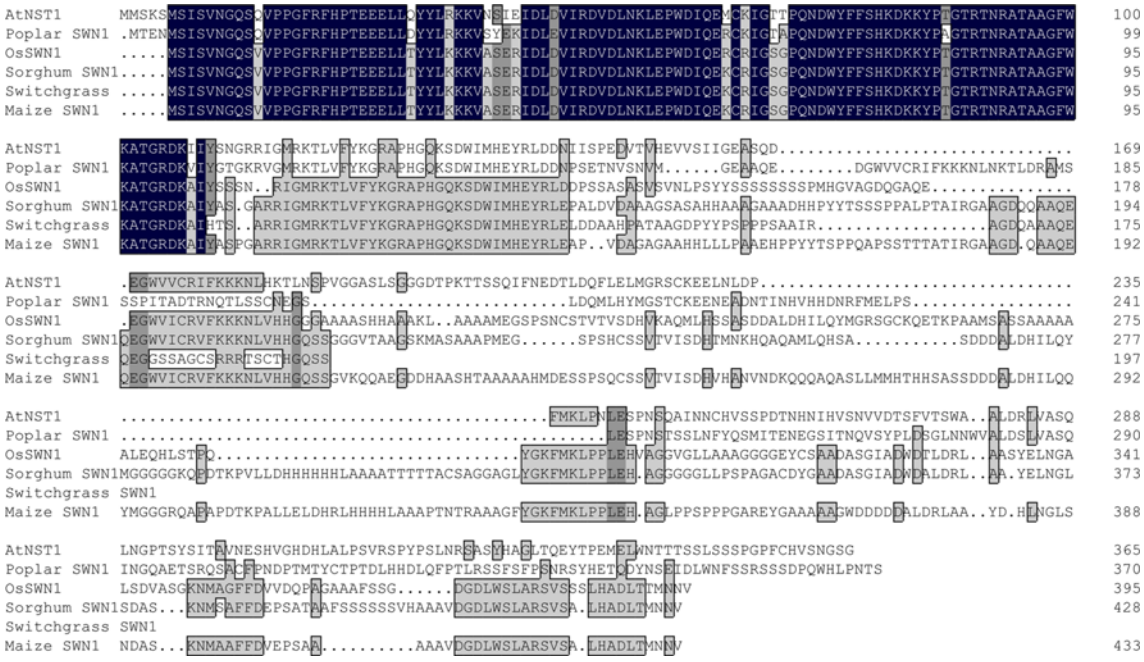

Fig. 6. Amino acid sequence alignments of OsSWN1 with its orthologues in Arabidopsis, Poplar, maize, sorghum and switchgrass. OsSWN1(LOC_Os06g04090), Sorghum SWN1 (Sb10g002120), Maize SWN1(Q5NKQ3) and Switchgrass SWN1 (CCGC2900), Black shadings indicate identical amino acids.

in other grass species are more conserved, even in the C-terminal region (Fig. 6), suggesting the highly conservative function of the *OsSWN1*-like genes in the grass species. Therefore, OsSWN1 may be a conserved NAC transcription factor among grass species family

We speculate that the *OsSWN1* orthologous genes in other monocotyledonous plants may have conserved function in secondary cell wall biosynthesis. As shown in Fig. 5, OX plants have less sugar yield and RNAi plants have more sugar yield than in the WT plants. Therefore, it may be possible to obtain new transgenic bioenergy crop plants with reduced content by silencing the *OsSWN1* orthologous genes in these plants. The novel biomass feedstock could have improved conversion efficiency for biofuel production and provide a resolution for huge agronomic biomass residues.

Materials and Methods

Plant Material and Growth Conditions

Rice seeds were surface sterilized with 75% ethanol for 1 min followed by 2% sodium hypochlorite for 20 min, and were washed with sterile-distilled water five times. For germination, seeds were placed on 1/2 Murashige and Skoog medium for about 7 d. The seedlings were transferred to soil and grown in a growth chamber at 26°C with 12 h light and at 20°C with 12 h dark or in a greenhouse ranging from 20-28°C with natural light.

Histochemical GUS Activity Assay

To analyze the *OsSWN1* expression pattern, we amplified a 2,342-bp promoter region by PCR with two primers: 5'-GAGGGGA-TGCTTGGAAGTTGG-3' and 5'-GCACCGACTGCCCGTTCACCG-3'. The amplified fragment was cloned into the pCXGUS-P vector at the BamHI site in front of the GUS gene (Chen et al. 2009). The Gus staining assay was performed as previously described (Kosugi et al. 1991). All samples were vacuum-infiltrated and incubated in the GUS solution for 10 min and were kept at 37°C for 3 h.

Construction of Plasmids and Rice Transformation

The japonica rice cultivar Nipponbare was used to generate all of the transgenic lines. To generate OX transgenic lines, we amplified the full-length CDS region of *OsSWN1* from Nipponbare cDNA using RT-PCR. The amplified fragment was completely sequenced and cloned into the PCXUN vector between the maize ubiquitin promoter and the nopaline synthase terminator (Chen et al. 2009). For the generation of RNAi lines, PCR product (348 bp) was amplified with two primers (5'-CACCGGAGGGCAGCCCGAGCAAC-3' and 5'-GCGTGCGCCGGGTGCGGTCCAGCGTGTCCCAGTC3') and subcloned into the pENTR entry vector (Invitrogen). With the Gateway cloning system, the target fragment was cloned into the pANDA vector (Miki et al. 2005). The Agrobacterium-mediated method was used for the rice transformation (Qu et al. 2006; Toki et al. 2006). Regenerated transgenic rice plants were planted in a greenhouse and observed for phenotype and seed production.

Lignin Staining and Observation

A Weiesner reaction (phloroglucinol- HCl staining) was used for the histochemical localization of lignin (Li et al. 2003). Fresh cross-sections of rice collars and culms were placed in a phloroglucin solution [2% in ethanol:water (95:5, v/v); Sigma] for 2 min, mounted in 50% HCl, and examined and photographed with a Nikon Eclipse 80i fluorescence microscope (Nikon) (Li et al. 2003).

Composition and Enzymatic Digestibility of Raw Rice Straw

Mature rice plants (10 plants each of WT, OX and RNAi lines) were air dried, cut into pieces, passed through a 20-mesh sieve (x-mm aperture), and stored at room temperature. The ground samples were subjected to compositional analysis and enzymatic hydrolysis according to NREL protocols.

Gene Expression Analyses

Total RNA was extracted with Trizol (Invitrogen) and treated with DNase for the removal of genomic DNA. cDNA was synthesized for RT-PCR using Promega's reverse transcription system. For real-time quantitative PCR, the SYBR GreenER Reagent System (Invitrogen) was used.

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Supporting Information

Fig. S1. *OsSWN1* expression level in different cell types.
Fig. S2. Relative expression levels of *OsSWN1* in OsSWN1 overexpression (OX) and RNAi transgenic plants determined by qRT-PCR.
Fig. S3. Representative of Semi-dwarf and erect leaf phenotype of OX transgenic plants in adult stage (A) and relative expression levels of three *Expansin* genes in control and two OX transgenic plants (B).
Table S1. Putative cell wall-related genes upregulated by more than twofold in OX rice plants.
Table S2. Positively co-expressed genes with *OsSWN1*.

Author's Contributions

MC, MB, CW and ZC performed experiments, data mining and gene discovery, bioinformatics analysis. MC and G-LW wrote the manuscript; MC, YL and G-LW revised the manuscript; MC, YL and G-LW designed the experimental plan and revised the manuscript. All the authors agreed on the contents of the paper and post no conflicting interest.

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