

Rice plants response to the disruption of *OsCSLD4* gene

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The plant cell wall is a complex polysaccharide network and performs important developmental and physiological functions far beyond supplying the physical constrains. Plant cells have the ability to react to cell wall defects as exhibited by changes in gene expression, accumulation of ectopic lignin, stress responses and growth arrest. It is a major challenge to understand how plants sense and respond to wall integrity since very little is known about the signaling involved in the responses. Cellulose synthase-like D (CSLD) proteins mediating the biosynthesis of a wall polysaccharide polymer make up a common subfamily to all plants. Recently, we have reported the functional characterization of *CSLD4* in rice. Mutations in *OsCSLD4* show morphological alterations and pleiotropic effects on wall compositions and structure. Our study demonstrates that *OsCSLD4* play a critical role in cell wall formation and plant growth. Here we show the subtle wall alterations by separating the culm residues into five fractions. Quantitative RT-PCR analysis further revealed that the expression of various genes involved in xylan synthesis and cell cycle regulation was altered in mutant plants, as the responses to *OsCSLD4* disruption. Therefore, plants may have fine sensory machinery to react to wall defects and modulate growth for adapting to the changes.

Introduction

The plant cell wall is a complex network of polysaccharides mainly containing cellulose, hemicelluloses and pectins.¹ Hemicellulosic polysaccharides, synthesized and modulated by numerous

putative glycosyltransferases (GT) or glycosyl hydrolases (GH), are macro molecules responsible for the crosslinking of cellulose microfibrils for providing a load-bearing matrix.^{2,3} Cellulose synthase like (CSL) genes belonging to an important GT superfamily encode proteins presumed to catalyze the biosynthesis of various β -linked glycan backbones.³

Traditional biochemical techniques and identification of relative mutants are general ways for understanding the specific functions of CSLs. Using these approaches, the polysaccharides synthesized by CSLA, CSLC, CSLF and CSLH have been discovered.⁴⁻⁷ However, the enzymatic activities of the other CSLs, such as CSLD, are less understood, even though several *csld* mutants have been reported in Arabidopsis and rice.⁸⁻¹³

Although the biochemical function of CSLD is still unknown, through identification of mutants in CSLD subfamily, all the CSLD members in Arabidopsis exhibit diverse developmental roles in root hair and pollen tube elongation and plant growth,⁸⁻¹³ indicating that the polysaccharide synthesized by CSLDs is essential for plant growth. Increasing evidence has shown that plants have the ability to sense the wall perturbations and trigger a set of cellular changes, including growth inhibition, lignin ectopic accumulation and wall compositional alterations.^{1,14} In contrast to yeast, in which a cell-wall-integrity-signaling pathway has been reasonably well characterized,^{15,16} very little is known about the signaling involved in plants response to wall defects. We recently reported mutation in *OsCSLD4* causes pleiotropic effects on polysaccharides synthesis and plant growth.¹³ Here, we describe the alterations in xylan synthesis and cell growth

Key words: *OsCSLD4*, cell wall biosynthesis, plant development, wall integrity, rice

Submitted: 10/19/09

Accepted: 10/19/09

Previously published online:
www.landesbioscience.com/journals/psb/
article/10369

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Addendum to: Li M, Xiong G, Li R, Cui J, Tang D, Zhang B, et al. Rice cellulose synthase-like D4 is essential for normal cell-wall biosynthesis and plant growth. *Plant J* 2009; 60:1055-69; PMID: 19765235; DOI: 10.1111/j.1365-313X.2009.04022.x.

Table 1. Monosaccharide compositional analysis of the wall fractions derived from culms of mutant and wild-type plants

Fractions		RHA	FUC	ARA	XYL	MAN	GLC	GAL	Total
EPG	WT	0.2	0.1	0.3	0.9	1.0	0.6	0.2	3.3
	<i>oscsld4</i>	0.3	0.3	0.6	1.2	0.9	0.6	0.5	4.5
Na ₂ CO ₃	WT	0.1	0.4	0.2	0.4	0.1	0.3	0.2	1.6
	<i>oscsld4</i>	0.1	0.2	0.4	0.6	0.1	0.7	0.3	2.4
1 N KOH	WT	0.2	0.1	6.1	34.7	0.1	2.8	1.4	45.4
	<i>oscsld4</i>	0.2	0.1	5.8	23.4	0.1	5.4	2.0	37.0
4 N KOH	WT	0.1	0.1	1.1	8.5	0.1	3.0	0.4	13.2
	<i>oscsld4</i>	0.1	0.1	1.3	5.7	0.1	6.4	0.8	14.6
4 N insol	WT				294.7				
	<i>oscsld4</i>				252.6				
Total	WT	0.5	0.7	7.8	44.5	1.2	301.4	2.2	358.2
	<i>oscsld4</i>	0.7	0.8	8.2	30.9	1.2	265.7	3.7	311.2

The alcohol insoluble wall residues (AIR) were extracted from the second internodes of *oscsld4* and wild-type culms; The destarched AIR was fractioned sequentially with endopolygalacturonase (EPG) plus pectin methylesterase (PME), 50 mM Na₂CO₃, 1 N KOH, and 4 N KOH; The fractions were hydrolyzed with sulfuric acid and the alditol acetates were analyzed by GC-MS; The insoluble residue after 4 N KOH extraction was used to determine cellulose content by colorimetric assay; The results are given as means (mg g⁻¹ of AIR) of four independent assays; The variance was not shown but is <15%.

in *oscsld4* being translated into changes in gene expression, to understand how plants monitor the integrity of cell walls and possess roles in plant development.

Mutation in *OsCSLD4* Causes Decreased Xylan and Cellulose Content in Mutant Culms

OsCSLD4 is an ortholog of *AtCSLD5*. The Arabidopsis *atcsld5* knock out and T-DNA insertion mutants exhibited reduced growth and altered xylan levels.¹⁰ Without chemical analysis of the wall, the direct or indirect alterations in wall composition resulted from the disruption of *AtCSLD5* have not been evaluated in detail. To further characterize the board changes of polysaccharides in *oscsld4*, we sequentially extracted the wall residues from the wild-type and mutant culms and performed sugar compositional analysis in the five fractions. As shown in Table 1, a number of wall compositional changes were observed. Some sugars in *oscsld4*, such as arabinose, xylose and glucose, were slightly increased in the fractions of pectin digested with endopolygalacturonase (EPG) plus pectin methylesterase (PME) or extracted with EGTA-Na₂CO₃. Contrastingly, xylose was decreased by ~32.5% in hemicellulose fractions extracted with 1 N and 4 N KOH, resulting in the significantly decreased total

sugars in these fractions. Because most arabinoxytan can be extracted by 1 N KOH, the decreased xylose in *oscsld4* may also be a consequence of the reduced arabinoxytan solubility. Another major wall defect is a significant reduction in cellulose content. Our report on its Golgi apparatus localization suggests that *OsCSLD4* is unlikely to participate in the synthesis of crystalline cellulose occurring at the plasma membrane.¹³ Deficiency in the synthesis of polysaccharide matrix might result in aberrant assembly of nascent cellulose microfibrils at the plasma membrane, which could, in turn, impede the further cellulose synthesis.¹⁷ The reduced cellulose is thus one of the possible responses to *OsCSLD4* disruption.

The pleiotropic polysaccharide modifications in *oscsld4* are possible results of that plants adapt to the loss of one polysaccharide component by altering the quantity of an alternative, or by modifying the structure of the wall.

The Expression of Genes Involved in Xylan Synthesis and Cell Cycle Regulation Is Altered in *oscsld4*

Xylan is a major component of hemicellulose in dicot and monocot walls, which is believed to be synthesized by protein complexes that include CSL proteins along with several GT and GH.¹⁸ Although

CSL involved in xylan synthesis remains elusive, previous studies have identified five GTs required for its formation.¹⁹⁻²¹ IRX7, IRX8 and PARVUS are probably responsible for the synthesis of a xylan primer, whereas IRX9 and IRX14 may be involved in xylan backbone production.²¹ The reduced xylan content promoted us to explore the expression of *IRX7*, *IRX14* and *PARVUS* homologs in *oscsld4* mutant and wild-type plants. Their expression levels were unexpectedly upregulated (Fig. 1A), indicating that the rice plants may perceive the defect in xylan and increase the expression of some genes involved in xylan synthesis as the compensatory reaction.

oscsld4 mutant also shows significant reduction in plant growth due to retarded cell cycle progression.¹³ Because *OsCSLD4* is mainly expressed in tissues undergoing rapid growth, we therefore detected the expression of several genes involved in cell cycle regulation. As shown in Figure 1B, two *cyclins*, three *CDKs*, and *KRPI* (Kip-Related Protein 1), which govern the key G₁-to-S or G₂-to-M transition points,²² were upregulated in mutant plants. However, *E2FI*, a transcription factor that is in the down-stream of CDK/Cyclin complexes regulation pathway and activates the expression of S phase genes,²² was downregulated in *oscsld4*. This result, as well as diverse functions of CSLDs let us reason that the polysaccharide

produced by CSLDs may have a signaling role in development. Cell wall is an extremely complex reservoir of chemical and mechanical information,¹ which provides a wealth of potential signaling molecules that are capable of regulating plant growth.^{23,24} For example, galactoglucomannan can inhibit auxin-induced stem elongation in peas and pine stems.^{25,26} CSLA7 that catalyzes glucomannan backbone formation was then found playing a role in embryogenesis.^{27,28} Screening suppressors of *atcesa6* null mutant that attenuate growth effects led to the identification of a novel plasma-membrane receptor kinase (THE1) as a sensor of wall integrity, making a prominent progress in plant cell-wall-integrity-signaling pathway.¹⁴ Besides the growth responses, defective cell walls also trigger local and systemic stress responses. Mutations in the *AtCESA3* gene constitutively activate ethylene and jasmonate mediated signaling, thus increase powdery mildew resistance.²⁹ And the disruption of *AtCESA8* causes drought resistance.³⁰

Conclusion

Our study has revealed that rice plants have complex responses to the disruption of *OsCSLD4*, including the effects on various wall components and the expression of genes related to xylan synthesis and cell cycle regulation. All those probably reflect the ability of plants to perceive and react to the changes in cell wall. Further analysis is required to understand the systemic signaling mechanism that transduces the wall integrity information in specific cells to the machinery that regulates the wall remodeling and plant growth throughout the plant. Arabidopsis and rice each has five CSLD members. All those proteins could make the same glycan product, but contribute to different developmental roles. The wall-integrity-signaling system may be a conceivable way to decipher their diverse biological functions. Our findings here provide essential clues in understanding the co-regulated mechanism in response to the disruption of *OsCSLD4*.

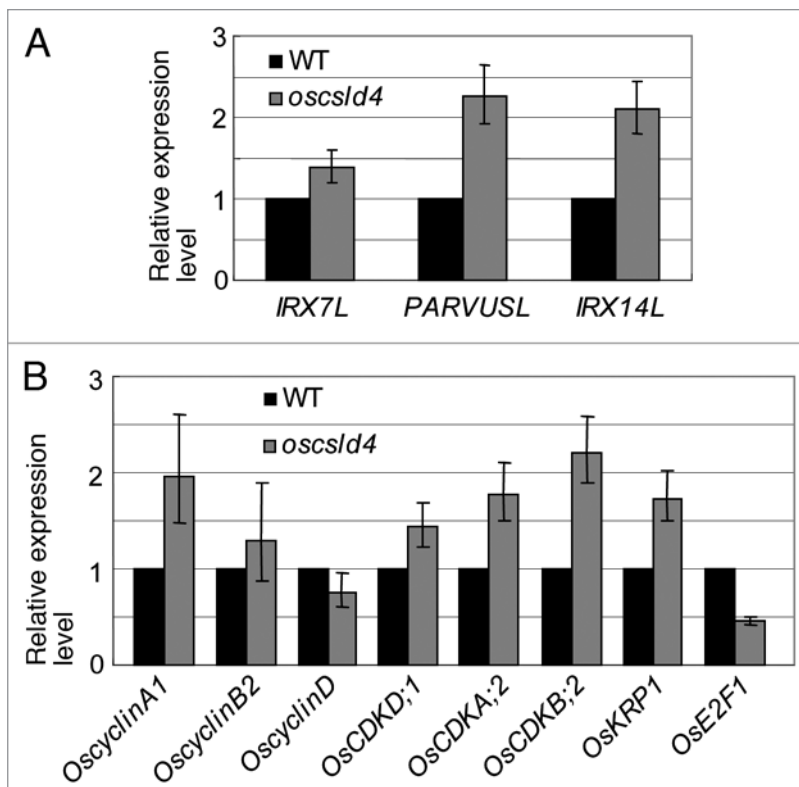


Figure 1. Real-time PCR analysis of the gene expression levels in *oscsld4* and wild-type plants. (A) The expression level of genes involved in xylan synthesis. (B) The expression level of genes of CDK/Cyclin complexes. The expression levels are given by counting that of wild type as 1.

Acknowledgements

This work was supported by the grant from the National Natural Science Foundation of China (90717117) and the Knowledge Innovation Program of the Chinese Academy of Sciences (KSCX2-YW-G-033).

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