Influence of osmotic condition on secondary cell wall formation of xylem vessel cells induced by the master transcription factor VND7

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Abstract  Xylem vessels, which conduct water from roots to aboveground tissues in vascular plants, are stiffened by secondary cell walls (SCWs). Protoxylem vessel cells deposit cellulose, hemicellulose, and lignin as SCW components in helical and/or annular patterns. The mechanisms underlying SCW patterning in the protoxylem vessel cells are not fully understood, although VASCULAR-RERATED NAC-DOMAIN 7 (VND7) has been identified as a master transcription factor in protoxylem vessel cell differentiation in Arabidopsis thaliana. Here, we investigated deposition patterns of SCWs throughout the tissues of Arabidopsis seedlings using an inducible transdifferentiation system that utilizes a chimeric protein in which VND7 is fused with the activation domain of VP16 and the glucocorticoid receptor (GR) (VND7-VP16-GR). In slender- and cylinder-shaped cells, such as petiole and hypocotyl cells, SCWs that were ectopically induced by the VND7-VP16-GR system were deposited linearly, resulting in helical and annular patterns similar to the endogenous patterns in protoxylem vessel cells. By contrast, concentrated linear SCW deposition was associated with unevenness on the surface of pavement cells in cotyledon leaf blades, suggesting the involvement of cell morphology in SCW patterning. When we exposed the seedlings to hypertonic conditions that induced plasmolysis, we observed aberrant deposition patterns in SCW formation. Because the turgor pressure becomes zero at the point when cells reach limiting plasmolysis, this result implies that proper turgor pressure is required for normal SCW patterning. Taken together, our results suggest that the deposition pattern of SCWs is affected by mechanical stimuli that are related to cell morphogenesis and turgor pressure.

Key words: Arabidopsis thaliana, plasmolysis, secondary cell wall, turgor pressure, xylem vessel.

Plant cell walls are multifunctional structures that play crucial roles in cell morphogenesis, cell–cell adherence, and pathogen defense. To achieve these functions, plant cells have specialized cell walls that vary depending on the tissue and cell type. In vascular plants, xylem vessels are essential to conduct water and transport minerals from the roots to other organs and tissues. Xylem vessel cells require mechanical strength to resist water pressure and pressure from their surrounding cells. They contain rigid secondary cell walls (SCWs) with tissue-dependent deposition patterns: helical or annular patterns in protoxylem vessels and reticular or pitted patterns in metaxylem vessels (reviewed in Oda and Fukuda 2012; Schuetz et al. 2013). These patterns suggest that SCWs in xylem vessel cells are mechanically optimized structures and that their formation is strictly regulated during plant development.

Previously, we demonstrated that VASCULAR-RELATED NAC-DOMAIN (VND) transcription factors regulate the differentiation of xylem vessel cells in Arabidopsis thaliana (Kubo et al. 2005). Of the seven VND genes in Arabidopsis, VND6 and VND7 are master regulators in the differentiation of metaxylem and protoxylem vessel cells, respectively (Kubo et al. 2005). Since overexpression of VND6 and VND7 can ectopically induce metaxylem- or protoxylem vessel-like cells that deposit SCWs in their specific patterns (Kubo et al. 2005), the SCW patterning should be regulated transcriptionally by these VND genes. Furthermore, we developed a VND-dependent transdifferentiation system for vessel elements that utilizes a chimeric protein of VND6 or VND7 with the activation domain of VP16 from herpes virus and the rat glucocorticoid receptor (GR), VND6/7-VP16-GR (Yamaguchi et al. 2010). VND6/7-VP16-GR is post-translationally activated by the synthetic steroid hormone dexamethasone (DEX),

Abbreviations: CFW, calcofluor white; CMT, cortical microtubule; DEX, dexamethasone; DIC, differential interference contrast; GFP, green fluorescent protein; GR, GLUCOCORTICOIDE RECEPTOR; HG, homogalacturonan; PCW, primary cell wall; SCW, secondary cell wall; SYP, SYNTAXIN OF PLANTS; VND, VASCULAR-RELATED NAC-DOMAIN.
allowing the ectopic deposition of SCWs in the chimeric-protein-expressing cells. The transdifferentiated cells expressing VND7-VP16-GR form SCWs in helical- and/or annular-shaped patterns similar to those of protoxylem vessel cells (Schuetz et al. 2014; Watanabe et al. 2015; Yamaguchi et al. 2010). In previous studies, the protoxylem vessel-like cells induced by VND7-VP16-GR could be transdifferentiated from a broad range of cell types such as leaf pavement and hypocotyl epidermis in Arabidopsis, and Arabidopsis and tobacco (Nicotiana tabacum) suspension culture cells, although they possess a variety of cellular characteristics based on their original cell identities before transdifferentiation. While the VND7-VP16-GR system is a powerful tool for analyzing the molecular mechanism underlying SCW formation of xylem vessel cells, it remains to be investigated whether the deposition patterns of SCWs are affected by the cellular characteristics. Here, we demonstrated tissue- and cell-type-dependent deposition patterns of SCWs using the VND7-VP16-GR system.

Pro35S:VND7-VP16-GR, which expresses VND7-VP16-GR under the control of the cauliflower mosaic virus 35S promoter in the Arabidopsis ecotype Columbia (Col), was used for induction of the transdifferentiation of xylem-vessel-like cells (Yamaguchi et al. 2010). Surface-sterilized seeds were sown on 0.8% (w/v) agar in Murashige and Skoog medium and grown at 22°C under continuous light. Four-day-old seedlings were transferred into 10 µM DEX solution with/without mannitol and incubated for three days, and then fixed with 4% (w/v) paraformaldehyde in phosphate-buffered saline (pH 7.4). The fixed samples were stained with 0.1% (w/v) calcofluor white (CFW, a fluorescent dye for β-glucans such as cellulose polysaccharides). CFW-stained SCWs patterns and fluorescent signals of green fluorescent protein (GFP) were inspected using the Carl Zeiss LSM800 confocal laser scanning microscope system. Image data were processed and analyzed using the Fiji software (Schindelin et al. 2012). SCW images were visualized as a z-stack projection with the maximum intensity projection method.

Cotyledon, hypocotyl, and root tissues of Pro35S:VND7-VP16-GR plants were observed after transdifferentiation (Figure 1). As reported previously (Yamaguchi et al. 2010), almost all of the cells in these tissues deposited SCW components linearly on a surface plane of those cells, resulting in helical- and/or annular-shaped patterns surrounding the cell. The thickness of SCWs to the cell surface plane varied cell by cell throughout all tissues that were observed in this study, even if they were located in the same tissue; however, their deposition patterns could be characterized by each cell type. In the cotyledon leaf blade, several cell types were present in the epidermis. A large part of the epidermis is occupied by pavement cells that have a jigsaw-puzzle-like shape. The stomatal guard cells and marginal cells located at the leaf edge have kidney- and slender-shaped morphology, respectively. Due to their cell morphology, the pavement cells and the guard cells generate concave and convex sides in the surface plane of view. There seemed to be a tendency for ectopically deposited SCWs to be concentrated on the concave side in these cells rather than on the convex side (Figure 1B, C). The linear SCWs in the pavement cells also frequently had a curved shape and were partially crossed or bundled between each other, showing more complex deposition patterns than in the other tissues. The marginal cells regularly deposited ectopic SCWs helically and/or annularly (Figure 1D). This deposition pattern was also observed in cylinder-shaped cells in the epidermis of the cotyledon petiole and the hypocotyl and cortex of the root (Figure 1E–I), although a scalariform-patterned SCW was occasionally formed in hypocotyl cells located at the adjacent region with a root in addition to helical- and annular-patterned SCWs. Compared to the jigsaw-puzzle-like- and kidney-shaped cells, slender- and cylinder-shaped cells have smoother surfaces. These results suggest that deposition patterns of ectopic SCWs are affected by cell morphology in the VND7-VP16-GR system.

Next, we examined SCW patterning under low turgor-pressure conditions. Hypertonic conditions cause a
decrease of turgor pressure because of the movement of water out of the cell, resulting in plasmolysis (Lang et al. 2014). Because cell wall components are deposited at just outside of the plasma membrane, plasmolysis should affect cell morphogenesis. When cells reach the point of limiting plasmolysis, the turgor pressure becomes zero. At pressure potentials below that of limiting plasmolysis, mechanical stress on the cell wall is abolished. We induced hypertonic conditions by treatment with mannitol. In hypocotyl cells, the plasma membrane was highly associated with the primary cell wall (PCW) at concentrations below 0.4 M mannitol, whereas contact between the PCW and the plasma membrane was abolished at 0.5 M mannitol. This result showed that the concentration of mannitol required for limiting plasmolysis to occur in hypocotyl cells is between 0.4 and 0.5 M in our experimental conditions (Figure 2). Pro35S:VND7-VP16-GR seedlings were treated with a 0 and 0.5 M mannitol solution containing 10 μM DEX for three days. After the treatment, the cells were fixed and observed as described above. Aberrant deposition patterns of SCWs were observed in the hypocotyl and the cotyledon at the 0.5 M mannitol concentration of mannitol. Although the SCWs tended to be deposited more on the concave side of the cotyledon, the density and thickness of the SCWs on the cell surface were decreased by the mannitol treatment (Figure 3A, B). The mannitol treatment also caused a decrease in the density of SCWs in the hypocotyl (Figure 3C, D). The interval between linear SCWs was larger in the 0.5 M treatment than that of the control treatment. Furthermore, confocal microscopy in high magnification revealed that the linearity of the deposited SCWs was disordered by the mannitol treatment, resulting in SCW patterning with large curvatures (Figure 3E, F). Interestingly, we could also observe SCW deposition at plasma membrane regions where do not contact with PCWs at the 0.5 M treatment (Figure 3G–I). These results indicated that turgor pressure is required for SCW patterning in xylem vessel cell differentiation rather than for SCW production around the point of limiting plasmolysis.

Our findings demonstrated that deposition patterns of SCWs in VND7-mediated SCW formation were not uniform among all of the Arabidopsis tissues. The
SCW deposition patterns appeared to be associated with cell morphology. In cell morphogenesis, the cell wall plays a crucial role in elongation and expansion of growing cells. For instance, pollen tube cells that elongate by tip growth actively synthesize and secrete pectin polysaccharides (PCW components) into the tip region of the cells (Chebli et al. 2012; Lund et al. 2020). The homogalacturonan (HG) domain of pectin polysaccharides is highly methylesterified at the tip region, whereas a lower degree of methylesterified HG is preferentially distributed along the remainder of the pollen tube PCW in Arabidopsis and tobacco pollen tubes (Bosch et al. 2005; Mravec et al. 2017). Demethylesterification of galacturonic acid residues of HG is involved in cross-linking between pectin polysaccharide chains (described in Hocq et al. 2017; Kunieda et al. 2020; Voiniciuc et al. 2013), which impacts the mechanical properties of cell walls. In fact, the knockout mutant of Arabidopsis POLLEN-SPECIFIC PECTIN METHYLESTERASE 1, AtPPME1, which catalyzes the demethylesterification of HG, shows irregular morphology of pollen tubes (Tian et al. 2006). Furthermore, differences in the mechanical stiffness of PCWs between the lobe and neck sides in Arabidopsis leaf pavement cells have been demonstrated by atomic force microscopy. Between the pavement cells facing each other, PCWs are stiffer at the lobe side compared to the neck side, contributing to the formation of the jigsaw-puzzle-like shape (Majda et al. 2017). Therefore, the cell mechanical properties that mediate cell morphogenesis would be involved in the deposition patterns of SCWs in the VND7-VP16-GR system, as they are in PCW formation.

We also showed that plasmolysis altered SCW patterning in the VND7-VP16-GR system, suggesting an involvement of turgor pressure in SCW patterning. Since SCWs were able to be deposited at the plasma membrane region where is away from PCWs, contact closely between plasma membrane and PCW may not be absolutely required for regulation of biosynthesis of SCW components. Further analyses are required to elucidate a relationship between SCW production and turgor pressure. SCWs mainly consist of cellulose, hemicelluloses, and lignin in xylem vessel cells (Turner et al. 2007). Cortical microtubules (CMTs) are key molecules in SCW patterning because they organize the migration of cellulose synthases that produce cellulose microfibrils on the plasma membrane. CMT orientation is affected by osmotic stresses caused by hypertonic reagents such as mannitol (Lang et al. 2014). Additionally, it has been reported that orientation of the CMTs is affected by the mechanical stimuli generated by physical interactions between neighboring cells (Sampathkumar et al. 2014; Verger et al. 2018) and that CMTs function in sensing mechanical stimuli (Hamant et al. 2019). It is likely that CMTs similarly function in the VND7-VP16-GR system. Taken together, our findings indicate that mechanical stimuli from the extracellular environment, such as contact pressure between adjacent cells, or generated from within cells, such as turgor pressure, likely function in SCW patterning in the VND7-VP16-GR system.

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