Thesis/dissertation Title Studies on TED6 and TED7 during secondary cell wall deposition in xylem vessels

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## Approved Digest

Tracheary elements of xylem vessels are characterized by the formation of secondary cell wall (SCW) between the plasma membrane and primary cell wall. TRACHEARY ELEMENT DIFFERENTIATION-RELATED6 (TED6) and TED7, encoding closely related plasma membrane proteins, were identified to be important for SCW formation based on aberrant SCW observed in vessels of Arabidopsis roots upon suppression of TED6 and TED7 as well as the ability of TED6 to interact with the SCW-related cellulose synthase (CesA7). However, it is still unclear how these proteins function during SCW formation.

Homolog search in several plant species revealed that TED6 and TED7 are conserved only in angiosperms but not in other basal taxa analyzed in this study. Subsequent analysis using  $\beta$ -glucuronidase (GUS) reporter lines showed that the expression of TED6 and TED7 was restricted only to differentiating vessel elements of all organs examined, but not in other cell types that also have SCWs. Since TED6 and TED7 are found only in angiosperms, which develop vessels distinct from those in other plant lineages, it is plausible that the restricted expression of TED6 and TED7 reflects a specialized function in vessel-specific morphogenesis during SCW formation. This may lead to better water conducting capacity, giving rise to successful colonization of the land by angiosperm plants.

Observation of YFP-tagged TED6 and TED7 driven by its own promoters during ectopic SCW deposition (using VND7-induction system) revealed dynamic in protein localization, which they localized broadly on cell surface at the early stages of SCW formation and later became confined underneath SCW domains. Plasmolysis experiment confirmed that both TED6 and TED7 localized to the plasma membrane and cell walls. In addition, disruption of microtubules and actin organization resulted in rapid changes in TED6 and TED7 localizations suggesting possible contribution of microtubules and actin to the protein function. Moreover, BiFC assays suggest the possible interactions of TED6 and TED7 with cytoskeletal components; ACTIN7 and TUBULIN7, which were identified in previous research, as well as between TED6 and TED7 themselves. Together, these findings suggest a possible contribution of TED6 and TED7 during the evolution of water-conducting cells from tracheids to vessels and these two proteins may function in guiding or marking the sites for SCW deposition by interacting with cellulose synthase complexes and the cytoskeleton.