Doctoral thesis/dissertation Digest Form

Thesis/dissertation Title: Roles of protein ubiquitination regulation in xylem vessel cell differentiation in *Arabidopsis thaliana*

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Xylem vessels execute the function of long-distance conduction of water in land plants to survive on the dry condition. These specialized tissues undergo characteristic differentiation process including secondary wall deposition (SCW) and programmed cell death (PCD). These processes are initiated by a specific group of plant-specific NAC transcription factors, VASCULAR-RELATED NAC-DOMAIN (VND) protein family in Arabidopsis. For further understanding of VND family-based molecular mechanisms of xylem vessel cell differentiation, *seiv* (*suppressor of ectopic xylem vessel cell differentiation induced by VND7*) mutants were isolated as suppressor mutants of Arabidopsis VND7-inducible line (*VND7-VP16-GR*).

In this study, I analyzed novel dominant *seiv* mutants, *seiv2* to *seiv9*. In wild-type *VND7-VP16-GR*, VND7 is functionally activated by the treatment with the synthetic glucocorticoid dexamethasone (DEX), therefore ectopic xylem vessel cell differentiation was promoted, while the ectopic xylem vessel cell differentiation was inhibited in *seiv* mutants particularly in above-ground tissues. The induction level of VND7-downstream genes was remarkably decreased in the shoot regions of *seiv* mutants, in accordance with the observation above. To identify the responsible genes for *seiv* mutants, whole-genome resequencing analysis combined with genomic fragment introduction were performed. I found that *seiv5*, *seiv6*, and *seiv9* mutants possessed a single nucleotide substitution in the gene encoding PLANT U-BOX 36 (PUB36), an uncharacterized F-box protein (FBX), and UBIQUITIN-SPECIFIC PROTEASE 1 (UBP1), respectively. These genes are all expected to function in protein ubiquitination. Thus, the misregulation of protein ubiquitination should lead to the *seiv* phenotypes.

RNA-seq data suggested that the mutations of *FBX* and *UBP1* genes can disturb the transcriptional activation of xylem vessel cell differentiation induced by VND7, as well as transcriptional regulation for seedling development. Quantitative proteome analysis and genome-wide protein ubiquitination status of *seiv6* and *seiv9* seedlings, with the reference of the wild-type *VND7-VP16-GR* and vector control plants. Collectively, the regulation of protein ubiquitination was disturbed in *seiv6* and *seiv9* mutants, resulting in the incompetence of VND7-based induction of xylem vessel cell differentiation, neither at the transcriptional level or at the protein level.

Interestingly, I found that the Lys94 in the conserved NAC domain of VND7 was ubiquitinated in the DEX treatment-depending manner in the wild-type *VND7-VP16-GR*, but such ubiquitination was not occurred in *seiv6* and *seiv9*. Transient reporter expression assay demonstrated that the substitutions of Lys94 to Arg in VND7 significantly decreased the transactivation activity of VND7, indicating that the ubiquitination at Lys94 is important for the VND7 activity.

Together, this work will provide a novel perspective of protein ubiquitination, as one of active regulatory layer for xylem vessel cell differentiation coordinated with environments.