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Thesis/dissertation Title

Studies on roles for nonsense-mediated mRNA decay in auxin response during seedling development and de novo shoot organogenesis

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Nonsense-mediated mRNA Decay (NMD) is one of mRNA surveillance mechanisms to shape transcriptome among eukaryotes. In plants, the downregulation of NMD activity was first discovered to trigger upregulation of pathogen-related and wounding response. Later, phytohormones such as salicylic acid and jasmonic acid were found to be associated with the NMD-regulated defense-related genes. Notably, auxin-related genes were screened in addition to salicylic acid-related and jasmonic acid-related genes in the same works. However, the relationship between NMD and auxin had not been further analyzed in detail. In my thesis work, I aimed to reveal the roles for NMD in modulating auxin response in plants by studying two NMD-deficient mutants: *upf1-1* (point mutation mutant) and *upf3-1* (T-DNA insertion mutant) for early seedling development and *de novo* shoot organogenesis.

To make clear the impact of NMD on auxin signaling during seedling development, RNA short-read sequencing (RNA-seq) and Nanopore long-read sequencing were performed on the RNA samples extracted from 2-day-old seedlings. Interestingly, from the RNA-seq data, auxin-related genes were found enriched in the sets of genes up-regulated and down-regulated in *upf1-1* and *upf3-1*, respectively. Majority of these enriched auxin-related genes were comprised of early auxin-responsive genes i.e., *SAUR, GH3* and *IAA* genes. These findings were further supported by Nanopore long-read sequencing analysis, which successfully revealed significant different in the transcript architecture of *SAUR, GH3* and *IAA* genes between the wild-type and *upf* mutants. To check auxin response by phenotypic observations, various auxin-related developmental processes including apical hook formation, hypocotyl length under dark condition, and gravitropism response were examined in the seedlings of wild-type and *upf* mutants. In comparison to

the wild-type, roots of *upf* mutants were significantly wavier and aberrantly skewed. Subsequent gravitropism analysis also revealed that *upf* mutants were defective gravitropism response. Collectively, the transcriptomic and phenotypic data indicated that the *upf* mutation would affect auxin response in seedlings through the mRNA amount and/or architecture of auxin response genes.

Intriguingly, the phenotypic data obtained from *de novo* shoot organogenesis demonstrated more severe phenotypes than those in the seedling experiment. Although the hypocotyl explants of *upf* mutants showed relatively normal callus development, the subsequent shoot induction was aberrant; under the optimum shoot inducing condition, massive root-like structures, instead of adventitious shoots, were formed in the *upf* mutants. During early stages of shoot induction, the protuberances of the *upf* mutants were noticeably grown and elongated much quicker than those of wild-type. To monitor auxin distribution in the *upf* mutants, I performed the DR5rev::GFP reporter analysis. The results showed that strong GFP signals were observed in the tip of the protuberances of *upf* mutants at early stages of shoot inducing culture, although the GFP signals in the wild-type were still weak at the same timing. Besides, the distribution pattern of DR5-GFP signals in the mutants were virtually similar to that in lateral roots, supporting the idea that the *upf1-1* and *upf3-1* callus would generate adventitious roots, not adventitious shoots, under the shoot inducing condition. Microarray analysis of calli indicated that auxin-related genes were also significantly affected in *upf* mutants. The RNA-seq data on seedlings has suggested that splice variants of *IAA* genes were likely to be differently regulated by UPF1 and UPF3. Thus, two splice mRNA isoforms of *IAA7* gene, *IAA7.1* and *IAA7.2*, were selected for further RT-qPCR analyses. The RT-qPCR results demonstrated that the expression ratio of *IAA7.1* and *IAA7.2* in *upf* mutants was indeed altered in the *upf* mutants in comparison to the wild-type.

In conclusion, my findings have successfully demonstrated that NMD is one of the important layers for modulating auxin response at different developmental/growth stages, and/or environmental conditions, via the NMD eliciting features embedded in transcripts