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Global analysis of RNA-dependent RNA polymerase-dependent small RNAs reveals new substrates and functions for these proteins and SGS3 in Arabidopsis

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RNA silencing pathways control eukaryotic gene expression transcriptionally or post transcriptionally in a sequence-specific manner. In RNA silencing of Arabidopsis thaliana, small RNAs (smRNAs) are often derived from long double-stranded RNA (dsRNA) molecules synthesized by one of the six genomically encoded RNA-dependent RNA Polymerase (RDR) proteins. However, the full complement of the RDR-dependent smRNAs and functions that these proteins and their RNA-binding cofactors play in plant RNA silencing has not been fully uncovered.

To address this gap, we performed a global genomic analysis of all six RDRs and two of their cofactors to find new substrates for RDRs and targets of the resulting RDR-derived siRNAs to uncover new functions for these proteins in plants. Based on these analyses, we identified new substrates for six RDRs as well as the RDR2 cofactor RNA-directed DNA methylation 12 (RDM12) and the RDR6 cofactor suppressor of gene silencing 3 (SGS3). These findings revealed that the target substrates of SGS3 are not limited to those solely utilized by RDR6, but that this protein seems to be a more general cofactor for the RDR family of proteins. Additionally, we found that RDR6 and SGS3 are involved in the production of smRNAs that target transcripts related to abiotic stresses, including water deprivation, salt stress, and ABA response, and as expected the levels of these mRNAs are increased in rdr6 and sgs3 mutant plants. Correspondingly, plants that lack these proteins (rdr6 and sgs3 mutants) are hypersensitive to ABA treatment, tolerant to high levels of PEG8000, and have a higher survival rate under salt treatment in comparison to wild-type plants. In total, our analyses have provided an extremely data-rich resource for uncovering new functions of RDR-dependent RNA silencing in plants, while also revealing a previously unexplored link between the RDR6/SGS3-dependent pathway and plant abiotic stress responses.

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