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SWI3D, Chromatin Remodeling Factor, Regulates Efficient T-DNA Integration into Plant Genome

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Agrobacterium tumefaciens-mediated transformation is the most widely used genetic transformation system in plants. Recent research in plant transformation is concentrating now more on the problems associated with stable integration and reliable expression of the DNA once it has been integrated. As a first step to identify such a plant factors and determine their functions in the Agrobacterium-mediated plant transformation, We found Arabidopsis mutant, *uta* (untransformed by Agrobacterium), resistant to *Agrobacterium* infection using an in vitro root inoculation assay. *uta2* mutant showed as efficient transient GUS activity as that of wild-type, suggesting that *uta2* mutant has deficiency in T-DNA integration into the Arabidopsis genome. We also investigated whether SWI3 gene family involving in chromatin remodeling directly regulate T-DNA integration process during Agrobacteria-mediated plant transformation. Among them, only *swi3d* mutant showed deficient ability of inducing stable transformants such as tumor and selection marker resistance calli, but normal transient GUS activity in the in vitro root inoculation assay. SWI3 gene is specifically expressed at the pollen grains and meristem regions. More interestingly, its expression in root segments highly induced by microbial infection, but not by wounding. Taken together, SWI3D may induce structural change of chromatin in response to Agrobacterium infection, which facilitate T-DNA into the plant genome.

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Designing Artificial miRNA for the Efficient Biogenesis of Matured miRNA in Plants

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MicroRNAs (miRNA) have been shown to regulate not only diverse developmental processes but also the responses against various biotic and abiotic stresses. Their biogenesis results from processing the imperfectly paired hairpin precursors by DCL1 ribonuclease, which is generally produced from transcriptional units that are distinct from those of protein-coding genes. In addition to DCL1, HYL1 and HEN1 are required for successful miRMA biogenesis and accumulation. We investigated the effect of structures of miRMA precursor for efficient miRNA processing by using two homologous miRNAs. They have exactly same 21 nucleotides sequence in the miRNA region, but have a little different sequence in their complementary strands. The Arabidopsis transgenic plants overexpressing these two different miRNA precursors generated same miRNA, but showed different phenotypes. Using site-directed mutagenesis, we found a strong correlation between severity of phenotypes and the secondary structure of miRNA precursors, suggesting that particular structure of miRNA precursor is an important determinant for efficient miRNA processing in plant. Their further characterization might help develop the efficient artificial miRNA vector that is specifically silencing gene function, even multiple gene family functions simultaneously, on the basis of the high complementarity between miRNAs and their target mRNA.

Key words: miRNA, siRNA, DCL1, processing