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Characterization of early flowering mutant caused by activation tagging

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Objectives

We have tried to functionally characterize a gene responsible for early flowering phenotype by utilizing activation tagging method.

Materials and Methods

1. Material

Plant *Arabidopsis thaliana.*

2. Methods

-Isolate an early flowering mutant by screening activation tagging mutant pool

-Clone the flanking region of the inserted T-DNA

-Genetically analyze the segregation pattern of mutant phenotype

-Study gene expression of genes near T-DNA inserted region using RT-PCR

-Do complementation test to confirm the responsible gene for the phenotype

Results and Discussion

A mutant showing early flowering phenotype was isolated from activating tagging mutant pool transformed with activation tagging vector, pSKI015 (Weigel et al., 2000). The mutant flowered a week earlier than wild type and the mutant phenotype was semi-dominant. One copy of T-DNA was inserted in the mutant and the mutant phenotype was co-segregated with the T-DNA indicating mutant phenotype is probably caused by T-DNA insertion. We cloned the flanking region of T-DA insertion by plasmid rescue. T-DNA was inserted between ORFs, At1g73260 and At1g73270. The 35S enhancer was on the promoter region of the At1g73260 and directed toward to the 5′ region of the At1g73260 ORF. When we surveyed gene expression of all ORFs along 10kb region from the inserted position, only At1g73260 (a putative trypsin inhibitor) gene expression was activated. We cloned the cDNA of At1g73260 and studied its expression in Arabidopsis. It showed strong expression in root than other tissue. Currently we isolated sense & anti sense transgenic Arabidopsis of this gene and conducting complementation test.

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