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Although transposons occupying large portions of the genome in various plants were once thought to be junk DNA, they play an important role in genome reorganization and evolution. Active DNA transposons are important tools for gene functional analysis. The endogenous non-autonomous transposon, *nDart1-0*, in rice (*Oryza sativa* L.) is expected to generate various transposon-insertion mutants because *nDart1-0* elements tend to insert into genic regions under natural growth conditions. The transpositions of *nDart1-0* were promoted by an active autonomous element, *aDart1-27*, on chromosome 6. By using the endogenous *nDart1*/*aDart1-27* system in rice, a large-scale *nDart*-inserted mutant population was easily generated under normal field conditions, and the resulting tagged lines were free of somaclonal variation.

I. A Gain of Function Mutant

The *nDart1*-promoted gene tagging line was developed using the endogenous *nDart1*/*aDart1* system to generate various rice mutants effectively. While the dominant mutants were occasionally isolated from the tagging line, it was unclear what causes dominant mutations. Efficient selection and analysis of dominant mutants to analyze the gene functions in rice is very useful. A semidominant mutant, *Bushy dwarf tiller1* (*Bdt1*), which has the valuable agronomic traits of multiple tillering and dwarfism, was obtained from the tagging line. The *Bdt1* mutant carried a newly inserted *nDart1* at 38-bp upstream of the transcription initiation site of a non-protein-coding gene, *miR156d*. This insertion caused an upstream shift of the *miR156d* transcription initiation site and, consequently, increased the functional transcripts producing mature microRNAs. These results indicate

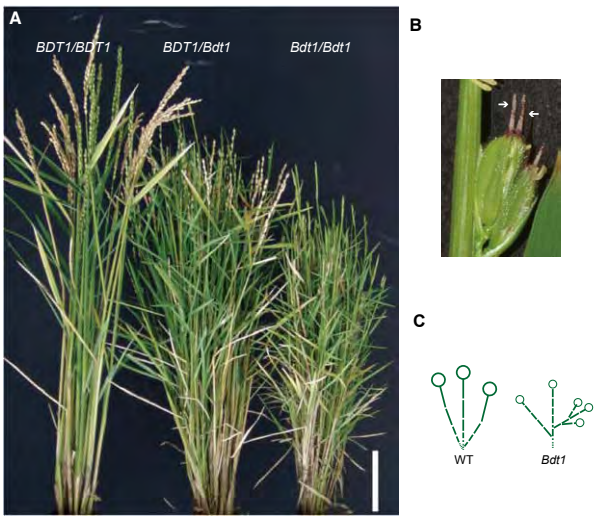

Figure 1. Phenotype of *Bushy dwarf tillers1* (*Bdt1*). (A) Three month old plants, (B) Abnormal panicles of *Bdt1*/*Bdt1* plants. White arrowheads indicate overgrown bracts and leaf-like structures, respectively. (**C**) Morphological phenotypes of WT and *Bdt1* plants. Each broken line and each circle represents an internode and a panicle, respectively.

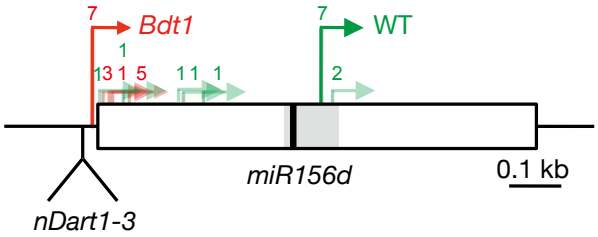

Figure 2. Transcription initiation sites of *BDT1* gene. Major transcription initiation sites of *miR156d* in WT and *Bdt1*plants. Red and green arrows indicate transcription initiation sites of miR156d in WT and *Bdt1* plants, respectively. Numbers above the arrows represent the numbers of clones that correspond to the transcription initiation site. The left end of the white box indicates the reported 5' terminal of the full-length cDNA (AK073452) in Nipponbare (http://rapdb.dna.affrc.go.jp/). The gray and black boxes show the corresponding positions of the pre-miR156d and miR156d sequences.

that the total amount of miR156d is controlled not only by transcript quantity but also by transcript quality. Furthermore, transgenic lines introduced an *miR156d* fragment that flanked the *nDart1* sequence at the 5' region, suggesting that insertion of *nDart1* in the gene promoter region enhances gene expression as a cis-element. This study demonstrates the ability of *nDart1* to produce gain-of-function mutants as well as further insights into the function of transposable elements in genome evolution.

Publication List:

〔Original paper〕

● Hayashi-Tsugane, M., Maekawa M., and Tsugane, K. (2015). A gain-of-function *Bushy dwarf tiller* 1 mutation in rice microRNA gene *miR156d* caused by insertion of the DNA transposon *nDart1*. Sci. Rep. *5*, 14357.