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Although transposons occupying a large portion 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*, in rice (*Oryza sativa* L.) is said to generate various transposon-insertion mutants because *nDart1* elements tend to insert into genic regions under natural growth conditions. The transpositions of *nDart1* 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 *nDart1*-inserted mutant population was easily generated under normal field conditions, and the resulting tagged lines were free of somaclonal variation. The *nDart1/aDart1-27* system was introduced into a rice variety, Koshihikari, named MK-1. 3000 MK-1 plants were grown in field conditions (IPSR, Okayama Univ.). All plants' genomes were isolated for identifying the insertion sites of *nDart1*.

I. Large grain (*Lgg*) mutation in rice

Seed size and number were controlled by various genes in the plants. It was reported that expression changes in high contribution genes for seed size, number and panicle shape resulted in a decrease of the total yield. A strategy for boosting rice yield based on molecular biology is to stack the finely tuned gene expressions. The *Lgg* mutant which was isolated from MK-1 plants bore slightly larger grains (Figure 1) as a dominant inheritance. Transposon-display identified the insertion site of *nDart1* in the *Lgg* mutant.

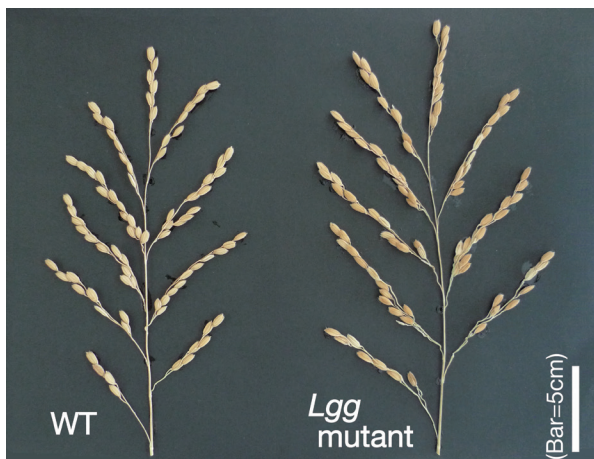


Figure 1. Phenotype of *Large grain (Lgg)*. Harvested panicle and seeds.

II. Analysis of *Lgg* mutants

The identified *LGG* gene shows similarity to RNA binding proteins. In the *Lgg* mutants, insertion of *nDart1* and genomic deletion was confirmed in a 5' untranslated region of the *LGG* gene. It was estimated that genomic deletion in *Lgg* mutants derived from microhomology in both the

nDart1 and *LGG*-genomic region. The expression of the *LGG* gene in *Lgg* mutants was lower than WT. *Lgg* mutation was essentially caused by the insertion of *nDart1*, knock-out mutant using genome editing (GE) and over-expressing (OE) lines were generated (Figure 2). GE lines showed large grain phenotypes that were the same as *Lgg* mutants. On the contrary, harvested seeds from the OE line were smaller than the WT and GE lines. To confirm that *Large grain* in *Lgg* mutants was caused by increasing the cell size or cell number or both, we developed a cross section method of rice seeds by using cryomicrotome (Chiou *et al.* 2018).

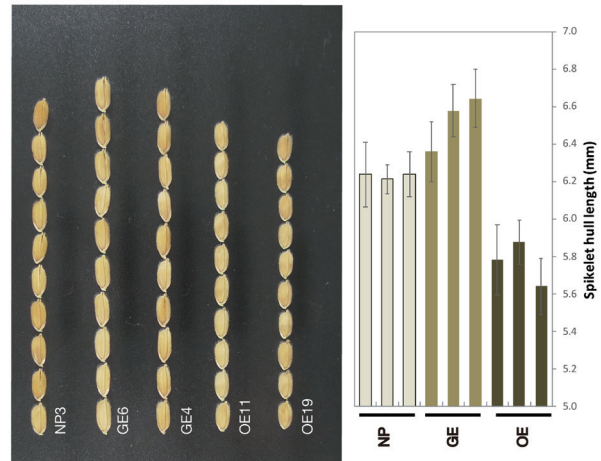


Figure 2. Phenotypes of wild type (NP), the genome-editing line (GE), and the overexpression line (OE). (Left) The spikelets. Bar = 5 mm. (Right) Spikelet hull length of NP, GE and OE plants. Different letters indicate $P < 0.05$ by Fisher's Least Significant Difference test. $n = 10$, mean \pm SD.

Publication List:

[Original paper]

- Chiou, W.Y., Tsugane, K., Kawamoto, T., and Maekawa, M. (2018). Easy sectioning of whole grain of rice using cryomicrotome. *Breeding Science* 68, 381-384.