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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 nDart-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 gain (*Lgg*). Harvested panicle and seeds.

II. Analysis of *Lgg* mutants

The identified *LGG* gene shows similarity to RNA binding proteins. Transgenic rice lines with knock-out (KO) and over-expressing (OE) in *LGG* gene showed large and small seed sizes, respectively. The section of lemma KO and OE plant were observed, and their cell numbers counted

(Figure 2). Increased cell numbers in *Lgg* mutant have induced the *Large Grain* phenotype. RNA seq analysis revealed that expression of cell-cycle and proliferation related genes was changed in transgenic plants.

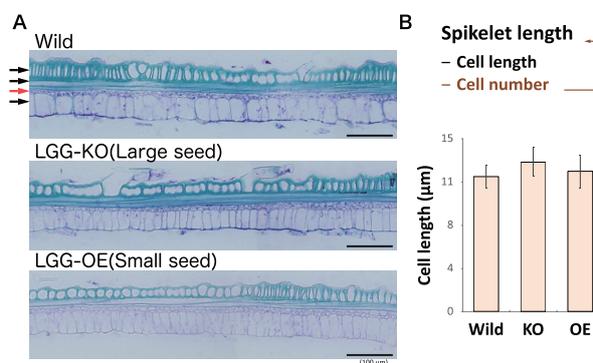


Figure 2. The section and cell number of lemma in transgenic plants. Bar = 100 μm. (A) the section of lemma in rice plants. The red arrow indicates counted cells. (B) Graph of cell number of lemma

Publication List:

[Original papers]

- Chiou, W.-Y., Kawamoto, T., Himi, E., Rikiishi, K., Sugimoto, M., Hayashi-Tsugane, M., Tsugane, K., and Maekawa, M. (2019). LARGE GRAIN Encodes a putative RNA-binding protein that regulates spikelet hull length in rice. *Plant Cell Physiol.* 60, 503-515. doi: 10.1093/pcp/pcz014
- Nishimura, H., Himi, E., Eun, C.-H., Takahashi, H., Qian, Q., Tsugane, K., and Maekawa, M. (2019). Transgenerational activation of an autonomous DNA transposon, Dart1-24, by 5-azaC treatment in rice. *Theor. Appl. Genet.* 132, 3347-3355. doi: 10.1007/s00122-019-03429-7
- Nishimura, H., Himi, E., Rikiishi, K., Tsugane, K., and Maekawa, M. (2019). Establishment of nDart1-tagged lines of Koshihikari, an elite variety of rice in Japan. *Breed. Sci.* 69, 696-701. doi: 10.1270/jsbbs.19049