LABORATORY OF BIOLOGICAL DIVERSITY

TSUGANE Group

Assistant Professor: TSUGANE, Kazuo

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. nDart1-0-Inserted Mutants

It is essential to develop an easy detection method of the nDart1-0-inserted gene for forward and reverse genetics. For high-copy-number elements, transposon display (TD) based on an amplified fragment-length polymorphism (AFLP) technique is a powerful tool to visualize multiple transposons. We successfully visualized 13 copies of nDart1 of Nipponbare through an nDart1-optimized TD method. In addition, an nDart1-0-specific iPCR procedure was also developed (Hayashi-Tsugane etl al. 2014). Among 8,984 lines, visible phenotypes were observed at the seedling, posttransplanting, reproductive, and mature stages. Fifty percent of the total tagged lines showed mutant phenotypes. Abnormal growth phenotypes, in particular, were easily detectable at the seedling stage, whereas both sterility and dwarf phenotypes were frequently observed at the mature stage. Some of these mutant phenotypes are shown in Figure 1. A variegated albino plant can survive under normal field conditions (a). Although chlorophyll mutants that revert to the wild phenotype are also observed at the seedling stage, these zebra mutants (b and c) retain a cross-banded phenotype even at the reproductive stage. A mutant (d) abnormally elongates the leaf and stem at the seedling stage, somewhat similar to the Gibberella fujikuroi-infected rice plant. Fuchsia leaf blades with revertant sectors (e) are observed under UV light. This is similar to the hcf (high chlorophyll fluorescence) mutant in maize. Under normal growth conditions, this mutant shows pale-green leaves and is lethal at the seedling stage. A mutant displaying an extremely short-statured phenotype (f) is named Thumbelina; this phenotype must be caused by the insertion of a DNA transposon because of its mutable phenotype (right in f), as a normally elongated shoot can often be observed in the thumbelina mutant (Tsugane et al., 2006). A semi-dominant mutant (g) shows a very short panicle with many tillers, and its heterozygous plant exhibits an intermediate phenotype between the wild type and the mutant. A mutant (right in h) has an abnormal ligule, resulting in an erect leaf. The short panicle phenotype is shown in (i) (right). The mutant shown in (j) expresses multiple glumes and high sterility, and a mutant (k) exhibiting the neck leaf-enveloped panicle phenotype, like the NECK LEAF1 mutant cloned by Wang et al. (2009), is observed. The mutant shown in (l) displays a phenotype similar to that of a PLASTCHRON mutant.

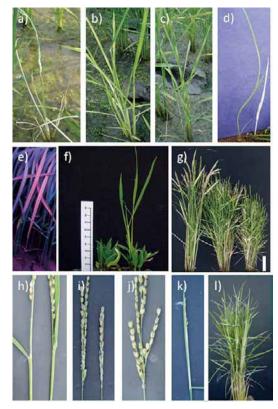


Figure 1. Phenotypes of *nDart*-tagged line mutants. Scale bar = 10 cm.

Publication List

[Original paper]

• Hayashi-Tsugane, M., Takahara, H., Ahmed, N., Himi, E., Takagi, K., Iida, S., Tsugane, K., and Maekawa, M. (2014). A mutable albino allele in rice reveals that formation of thylakoid membranes requires SNOW-WHITE LEAF1 gene. (2014). Plant Cell Physiol. 55, 3-15.