A novel active DNA transposon, *nDart*, and its application for gene tagging in rice

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Rice (*Oryza sativa*L.) is an important staple food for more than half of the world population and a model plant for other cereal species. Draft sequences of the genomes of two rice subspecies, *japonica* and *indica*, together with high-quality sequences of three out of 12 chromosomes of the *japonica* cultivar Nippponbare have been published. The genomic sequencing predicted about 50,000 genes, a large proportion of which have no recognizable homologues in *Arabidopsis*. Under theses circumstances, a new reverse genetic approach such as a gene tagging with a transposon would be important to study function of unknown genes. Although gene tagging using endogenous transposons is regarded to be a powerful reverse genetic approach, only the retrotransposon *Tos17*has been used systematically for transposon tagging in rice and its tagging efficiency is very low (5-10%) because of the high occurrence of somaclonal variations: genetic and epigenetic changes induced by tissue culture, which is a prerequisite process for activating dormant *Tos17* element.

Recently, although an active DNA transposon, named *mPing*, has been identified in rice, it is dormant in natural condition and activated by gamma-ray irradiation or cell and anther culture that can induce additional mutations or somaclonal variations. We identified a novel active DNA transposon, called *nDart*, which is a non-autonomous endogenous element in rice that is a causative element in spontaneous leaf variegation mutant that was discovered among progeny of a hybrid between a *japonica* and an *indica* rice. The leaf variegation was regarded to be a recurrent somatic mutation from the recessive *pyl-v* (pale yellow leaf-variegated) allele to the dark-green *Pyl*-revertant (*pyl-r*) allele. We also obtained stable mutant (*pyl-stb*) exhibiting only pale-yellow leaves. In the F2 populations from a cross between the *pyl-stb* mutant and a parental line, the occurrence of the variegated *pyl-v* mutants to the stable pyl-stb plants showed a ratio of 3 to 1. The results indicate that a non-autonomous DTE is inserted at the *pyl*locus and that its excision depends on the autonomous element present in the parental line used.

The availability of the genomic sequences of both *japonica* and *indica* subspecies could facilitate map-based cloning on the *pyl-v*allele and we were able to pinpoint the *pyl-v* locus, in which an insertion of approximately 0.6 kb was found within a putative gene, termed *OsClpP5*. The *OsClpP5* gene encodes a protein showing 92% homology to the *AtClpP5* of the ATP-dependent caseinolytic protease from *Arabidopsis*. The insertion of 0.6 kb, named *nDart*, carries 19-bp perfect terminal inverted repeats (TIRs) with 8-bp target duplication and belongs to the Ac/Ds superfamily.

No somaclonal variation could occur in *nDart*-induced mutant lines because no tissue culture was involved in *nDart*activation. This novel active DNA transposon would facilitate the development of a new rice transposon tagging system, thereby contributing to the functional genomics of rice.

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RESEARCH INTERESTS

1) Toshihiro Kobayashi, **Changho Eun**, Hidetoshi Hanai, Yoshikatsu Matsubayashi, Youji Sakagami and Hiroshi Kamada (1999) Phytosulphokine-a, a peptidyl plant growth factor, stimulates somatic embryogenesis in carrot. *J. Exp. Bot.* 50(336): 1123-1128.
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6) M. Umehara, T. Kobayashi, **C. Eun**, K. Higashi, H. Hanai, Y. Matsubayashi, Y. Sakagami and H. Kamada (2003) Stimulatory and inhibitory conditioning factors regulating somatic embryogenesis in carrot: Recent advances. On "Agrobiotechnology and Plant Tissue Culture", pp. 25-36.