# Development of a Novel Breeding Method Using SNP-based Selection of Rice Genotypes(Frontiers in Rice Science -from Gene to Field-,The 100^<th> Anniversary of Tohoku University, International Symposium)

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# Development of a Novel Breeding Method Using SNP-based Selection of Rice Genotypes

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Although several agronomically important genes have been identified by the advance of plant genome studies, most plant breeding programs are still carried out by the conventional cross breeding method without using the genome information. Marker-assisted selection (MAS) has been used in the plant breeding of various crops, but it has a risk causing selection mistake due to recombination between the DNA marker and the selection target gene. DNA polymorphism analysis of the selection target gene itself is required for avoiding such problems. Since most of the DNA polymorphisms in genes are single nucleotide polymorphisms (SNPs) by spontaneous mutations, simple and cost-effective techniques for SNP analysis are required for the selection of the genotypes in the plant breeding.

We developed a technique for the SNP genotyping using dot-blot hybridization (dot-blot-SNP). High background signals of this method were removed by hybridization using a labeled oligonucleotide with an unlabeled competitive oligonucleotide. Not only single nucleotide substitutions but also indels can be analyzed with this technique. A large number, e.g., 864, of PCR products amplified from different plants can be blotted on one membrane, and more than ten membranes can be handled at once. Dot-blot-SNP was applied to genotyping of *Wx* and *Sd1* of plants in a segregating population of a rice breeding program. In dot-blot-SNP, unsuccessful DNA amplification by PCR can be identified by a mixed probe of a wild type and a mutant, and, therefore, wild-type-allele homozygotes, mutant-allele homozygotes, heterozygotes, and no DNA amplification can be clearly distinguished.

Since many SNPs between *japonica* rice cultivars have been identified, dot-blot-SNP is also useful for identification of cultivars. In reverse-genetic approach for selection of mutants, known as TILLING, most of the selected mutants are silent mutations. Such silent mutations are expected to be useful as markers for distinguishing different production areas growing the same cultivar.