Recent Research Topics in the Laboratory of Plant Breeding and Genetics

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Summary

In the Laboratory of Plant Breeding and Genetics, we have studied genetics and molecular biology of *Brassica* and rice for 60 years. Based on the great achievements of the past, we are studying molecular genetics of plant reproduction systems, genetics of tolerances to environmental stresses in rice, and the *Brassica* and *Raphanus* genomes. These studies are apparently independent, but advance in one contributes to the other studies and leads to further development of plant breeding techniques.

Molecular Genetics of Self-incompatibility and Male-sterility

The molecular mechanism of self-incompatibility has been most intensively studied using *Brassica*. Self-pollen rejection by the stigma to inhibit self-fertilization is controlled by a single locus complex, *S*, having multiple alleles. One set of alleles of the genes in the *S* locus complex is termed "*S* haplotype." We have developed many lines homozygous for *S* haplotypes, i.e., *S*-tester lines, in *Brassica rapa*, *Brassica oleracea*, and *Raphanus sativus*, which are useful for genetic study of self-incompatibility and F_1 hybrid breeding using a self-incompatibility system (Nishio *et al.*, 2006). Each *S* haplotype has a unique *S*-locus structure (Fukai *et al.*, 2003), and interhaplotype recombination seldom occurs (Takuno *et al.*, 2007). Although the number of *S* haplotypes in a species has been estimated to be more than 50, such haplotypes tend to be lost in cultivated plants, even in gene banks (Takuno *et al.*, 2009). In determination of the nucleotide sequences of a large number of alleles of the pollen ligand gene (*SP11/SCR*) and those of the stigma receptor gene (*SRK*), we found very similar *S* haplotypes between different species (Sato *et al.*, 2002 ; Fujimoto *et al.*, 2006a). Pollination tests using interspecific hybrids or transgenic plants revealed that these *S* haplotypes have the same recognition specificity between different species

(Kimura *et al.*, 2002 ; Sato *et al.*, 2003). These results suggest that these similar *S* haplotypes were derived from the same ancestral *S* haplotypes and have maintained the same ancestral recognition specificity since divergence of *Brassica* species. Studying pollen-stigma recognition specificities of similar *S* haplotypes using recombinant proteins and transgenic plants, we proposed an evolutionary model of the generation of new *S* haplotypes (Sato *et al.*, 2004). We also revealed the cause of self-compatibility of self-compatible cultivars in *B. rapa* and *B. oleracea* and a self-compatible amphidiploid species, *Brassica napus* (Fujimoto *et al.*, 2006b ; Okamoto *et al.*, 2007).

Since there are many *S* haplotypes in a species, confusion and contamination of *S* haplotypes sometimes occur. For identification of *S* haplotypes, PCR-RFLP analysis of *SLG*, an *S*-locus gene homologous to *SRK*, has been used (Nishio *et al.*, 1996). However, there are several *S* haplotypes not identifiable by this method. We found dot-blot analysis of *SP11/SCR* alleles to be highly useful for *S* haplotype identification (Fujimoto and Nishio, 2003). This method was improved using allele-specific oligonucleotides and applied to *S* haplotype identification of Rosaceae fruit trees (Kitashiba *et al.*, 2008).

Male-sterility is also an important trait used for F_1 hybrid seed production. A novel cytoplasmic male-sterile line was developed by intergeneric hybridization between *Diplotaxis muralis* and *B. rapa* followed by repeated back-crossings of Brassica in our laboratory (Hinata and Konno, 1979). A mitochondrial gene responsible for cytoplasmic male-sterility was identified using a newly developed restorer line of this male-sterility (Shinada *et al.*, 2006). Recently a candidate male-sterility gene of a male-sterile mutant in rice was identified and the function of this gene is investigated (Shiokai *et al.*, unpublished).

Development of SNP Techniques and Their Application to Genetic Analysis in Rice

Based on the technique of dot-blot analysis of *S* haplotypes, we developed a novel technique for single nucleotide polymorphism (SNP) analysis, i.e., dot-blot-SNP (Shirasawa *et al.*, 2006). With this method, all the SNPs and short indels which had been tested were successfully analyzed. Since SNPs are the most common polymorphisms of genomes, they are good DNA markers for QTL analysis and fine mapping of genes. Although the original method of dot-blot-SNP was costly in terms of preparation of labeled oligonucleotide probes, a recently developed bridge hybridization method enables production of new SNP markers at low cost (Shiokai *et al.*, 2009a). The dot-blot-SNP technique is suitable for genotyping of a large number of individuals, more than several thousand, and therefore applicable to selection of plants in conventional cross-breeding programs by SNP genotyping of important genes with known functions, the number of

which will increase dramatically by genome studies of various crop species. In such studies, preparation of a large number of plant DNA samples is labor-intensive. A recently developed DNA preparation method, the leaf-punch method (Shiokai *et al.*, 2009b), is considered to be an ideal DNA preparation method which is both simple and cost-efficient.

For mapping of genes or quantitative trait locus (QTL) analysis using *japonica* rice cultivars, DNA markers showing polymorphism between different cultivars are necessary. Since *japonica* rice cultivars are closely related, they have few DNA polymorphisms in common, even in SNPs. Screening of SNPs was carried out using PCR-RF-SSCP analysis and mismatch-cleavage analysis (Shirasawa *et al.*, 2004, 2007). Mutations of the *wx* gene in rice mutants and glutinous cultivars were efficiently identified by PCR-RF-SSCP analysis (Sato and Nishio, 2003; Hori *et al.*, 2007). Mismatch-cleavage analysis using *Brassica* petiole extract was applied to reverse-genetic selection of mutants from a rice population mutagenized with gamma-ray irradiation (Sato *et al.*, 2006).

Tolerances to environmental stresses are important breeding objectives. We detected QTLs for tolerances to low temperature at the booting stage and high temperature at the seed filling stage on chromosome 3 and 6, respectively, in rice using SNP markers. Near-isogenic lines having the QTLs showed significantly higher tolerances. Producing SNP markers in these QTL regions, we are narrowing down the chromosomal regions possibly containing stress-tolerance genes.

Genome Study of *Brassica* and *Raphanus*

SNP techniques developed for the study of rice genetics are useful for genome study of *Brassica*. Producing 300 DNA markers including 120 EST-based SNP markers, we constructed a linkage map of 241 DNA markers in *B. rapa* and used these markers for QTL analysis of flowering time and leaf morphological traits (Li *et al.*, 2009). Synteny analysis of *B. rapa* and *Arabidopsis thaliana* using sequences of the EST markers enabled identification of candidate genes controlling flowering time and leaf morphological traits. We intend to use this linkage map in genetic analysis of reproductive traits such as interspecific incompatibility, breakdown of hybrid embryos, and also self-incompatibility.

Receiving generous funding from the Bio-oriented Technology Research Advancement Institution (BRAIN), we started a *Raphanus* genome project in 2008. In this project, we are planning to construct a linkage map of 3,000 EST-based SNP markers and to isolate BAC clones harboring these SNP markers to cover about 95% of the *Raphanus* genome. These markers and BAC clones will be helpful tools for elucidation of genes controlling unique traits of *Raphanus*.

References

Fukai E, Fujimoto R, Nishio T (2003) Genomic organization of the *S* core region and the *S* flanking regions of a class-II *S* haplotype in *Brassica rapa*. *Mol Genomics Genet* **269**: 361-369.

Fujimoto R, Nishio T (2003) Identification of *S* haplotypes in *Brassica* by dot blot analysis of SP11 alleles. *Theor Appl Genet* **106**: 1433-1437.

Fujimoto R, Okazaki K, Fukai E, Kusaba M, Nishio T (2006a) Comparison of the genome structure of the self-incompatibility (*S*) locus in interspecific pairs of *S* haplotypes. *Genetics* **173**: 1157-1167.

Fujimoto R, Sugimura T, Fukai E, Nishio T (2006b) Suppression of gene expression of a recessive *SP11/SCR* allele by an untranscribed *SP11/SCR* allele in *Brassica* self-incompatibility. *Plant Mol Biol* **61**: 577-587.

Hinata K, Konno N (1979) Studies on a male sterile strain having the *Brassica campestris* nucleus and the *Diplotaxis muralis* cytoplasm. I. On the breeding procedure and some characteristics of the male sterile strain. *Japan J Breed* **29**: 305-311.

Hori Y, Fujimoto R, Sato Y, Nishio T (2007) A novel *wx* mutation caused by insertion of a retrotransposon-like sequence in a glutinous cultivar of rice (*Oryza sativa* L.). *Theor Appl Genet* **115**: 217-224.

Kimura R, Sato K, Fujimoto R, Nishio T (2002) Recognition specificity of self-incompatibility maintained after the divergence of *Brassica oleracea* and *Brassica rapa*. *Plant J* **29**: 215-223.

Kitashiba H, Zhang S-L, Wu J, Kenta Shirasawa K, Nishio T (2008) *S* genotyping and S screening utilizing *SFB* gene polymorphism in Japanese plum and sweet cherry by dot-blot analysis. *Mol Breed* **21**: 339-349.

Li F, Kitashiba H, Inaba K, Nishio T (2009) A *Brassica rapa* linkage map of EST-based SNP markers and its application to QTL analysis for flowering time and leaf morphological traits. *DNA Research* in press.

Nishio T, Kusaba M, Watanabe M, Hinata K (1996) Registration of *S* alleles in *Brassica campestris* L by the restriction fragment sizes of *SLG*s. *Theor Appl Genet* **92**: 388-394.

Nishio T, Izumida A, Hanzawa H, Sakamoto K (2006) Development of *S* tester lines of *Brassica oleracea*, *Brassica rapa*, and *Raphanus sativus* as genetic resources. *Acta Hort* **706**: 141-144.

Okamoto S, Odashima M, Fujimoto R, Sato Y, Kitashiba H, Nishio T (2007) Self-compatibility in *Brassica napus* is caused by independent mutations in *S*-locus genes. *Plant J* **50**: 391-400.

Sato K, Nishio T, Kimura R, Kusaba M, Suzuki T, Hatakeyama K, Ockendon DJ, Satta Y (2002) Coevolution of the *S*-locus genes *SRK*, *SLG*, and *SP11/SCR* in *Brassica oleracea* and *B. rapa*. *Genetics* **162**: 931-940.

Sato Y, Fujimoto R, Toriyama K, Nishio T (2003) Commonality of self-recognition specificity of *S* haplotypes between *Brassica oleracea* and *Brassica rapa*. *Plant Mol Biol* **52**: 617-626.

Sato Y, Nishio T (2003) Mutation detection in rice waxy mutants by PCR-RF-SSCP. *Theor Appl Genet* **107**: 560-567.

Sato Y, Okamoto S, Nishio T (2004) Diversification and alteration of recogni-

tion specificity of the pollen ligand SP11/SCR in self-incompatibility of *Brassica* and *Raphanus*. *Plant Cell* **16**: 3230–3241.

Sato Y, Shirasawa K, Takahashi Y, Nishimura M, Nishio T (2006) Mutant selection of progeny of gamma-ray-irradiated rice by DNA heteroduplex cleavage using *Brassica* petiole extract. *Breed Science* **56**: 179–183.

Shinada T, Kikuchi Y, Fujimoto R, Kishitani S (2006) An alloplasmic male-sterile line of *Brassica oleracea* harboring the mitochondria from *Diplotaxis muralis* expresses a novel chimeric open reading frame, orf72. *Plant Cell Physiol* **47**: 549–553.

Shiokai S, Shirasawa K, Sato Y, Nishio T (2009a) Improvement of the dot-blot-SNP technique for efficient and cost-effective genotyping. *Mol Breed* in press

Shiokai S, Kitashiba H, Shirasawa K, Nagano K, Nishio T (2009b) Leaf-punch method to prepare a large number of PCR templates from plants for SNP analysis. *Mol Breeding* **23**: 329–336.

Shirasawa K, Monna L, Kishitani S, Nishio T (2004) Single nucleotide polymorphisms in randomly selected genes among *japonica* rice (*Oryza sativa* L.) varieties identified by PCR-RF-SSCP. *DNA Research* **11**: 275–283.

Shirasawa K, Shiokai S, Yamaguchi M, Kishitani S, Nishio T (2006) Dot-blot-SNP analysis for practical plant breeding and cultivar identification in rice. *Theor Appl Genet* **113**: 147–155.

Shirasawa K, Maeda H, Monna L, Kishitani S, Nishio T (2007) The number of genes having different alleles between rice cultivars estimated by SNP analysis. *Theor Appl Genet* **115**: 1067–1074.

Takuno S, Fujimoto R, Sugimura T, Sato K, Okamoto S, Zhang S-L, Nishio T (2007) Effects of recombination on the hitchhiking diversity in *Brassica* self-incompatibility locus complex. *Genetics* **177**: 949–958.

Takuno S, Oikawa E, Kitashiba H, Nishio T (2009) Assessment of genetic diversity of accessions in Brassicaceae genetic resources by frequency distribution analysis of *S* haplotypes. Submitted.