学位論文内容の要旨

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学位論文題名

Studies on DNA rearrangements in rice blast fungus

（イネいもち病菌におけるDNA再編成に関する研究）

Rice blast caused by a pathogen *Pyricularia oryzae*, is one of the most destructive diseases in cultivated rice, which feeds one-half of the world's population. Resistance of rice plant to *P. oryzae* is known to follow Gene-for-Gene theory, where major resistance (*R*) gene is effective in preventing infection by a race of *P. oryzae* harboring the corresponding avirulence (*AVR*) gene. Although cultivation of resistant cultivars with R genes has been successfully applied for management of rice blast diseases, the strong selection pressure by R genes and rapid pathogen evolution have caused the repetitive breakdown of the resistance. A loss of function in an AVR gene allows the pathogen to avoid induction of resistance in the cultivar to gain pathogenicity to the cultivar, leading to an emergence of a new pathogenic race. On the other hand, AVR genes are responsible for production of effectors, and it is suggested that AVR genes should be recovered after the deletion, to regain fitness. However, there is only limited knowledge available on the mechanisms of AVR genes deletion and recovery. In this study, elucidation of deletion mechanism of AVR-Pik gene and investigation of parasexual recombination in the field condition were attempted.

1. Elucidation of the spontaneous deletion mechanism of *AVR-Pik* in the mutant Ina168m95-5 of *Pyricularia oryzae*

The mutations in *AVR-Pik* are thought to be due to a gradual selection pressure on the *Pik* allele. Ina168 strain had one copy of *AVR-Pik* and the spontaneous mutant strain Ina168m95-5 lacked *AVR-Pik*. For the characterization of *AVR-Pik* locus in Ina168 strain, the next-generation sequencing analysis of cosmid clones 20, 45, and 49 was performed, and sequence contig of 49 kb was determined. With additional inverse PCR analysis of upstream of *AVR-Pik* gene, finally sequence of approximately 55 kb in the *AVR-Pik* flanking region in strain Ina168 was determined. In the region, many copies of

transposable elements such as DNA transposon, retrotransposon and its solo-LTR(long terminal repeat) were identified. On the other hand, the sequence of Ina168m95-5 *AVR-Pik locus* was revealed by repetitive inverse PCR and sequencing, and finally identified as sequence contig of 56 kb. In the region, there was a conserved 10 kb region named u4 at its 3' end, but the rest of the sequence consisted of patially conserved fragment, inversely conserved fragment, and unique sequence. The origin of the unique sequence was retrieved in Ina168 cosmid library and two cosmid clones were identified. Finally the *AVR-Pik* deletion mechanism in Ina168m95-5 was proposed, which started by a double strand break in a transposon Pot2-like, followed by four homologous recombination events involving *AVR-Pik* locus and the two cosmid clones.

2. Identification of parasexual recombination in the field isolates of rice blast fungus

Parasexual recombination is a cellular event specific for fungi, which involves hyphal fusion of two individuals, followed by nuclear fusion and haploidization, resulting exchange of their DNA fragments. Parasexual recombination is believed to be one of mechanisms for genetic exchange in the *P. oryzae*, whose sexual stage had been only observed *in vitro*, but no evidence of parasexual recombination has been found in field condition. To identify the genetic recombination in the field isolates, the simple sequence repeat (SSR) markers, which are widely distributed throughout the genome were used. The population of *P. oryzae* from closely-located paddy field was screened for the candidate genotypes for the marker exchange by parasexual recombination. We have been identified 23 identified genotype sets from the population of rice blast fungus. These set of genotypes were further investigated for the evidence of partial DNA exchange using MGR586 repetitive DNA probe, and translocation of SSR marker using PFGE (pulsed-field gel electrophoresis)-separated chromosomal DNA. Hybridizing pattern changes may suggest that some exchange occurs between field isolates which can be proved as an evidence for parasexual recombination.

As a conclusion, this study revealed the mechanisms of DNA rearrangement in the rice blast pathogen. These informations are expected to be utilized in the establishment of new approaches to prevent mutations in the field.