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Functional Genomics Approaches in Rice-*Magnaporthe grisea* Interactions

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Rice blast, caused by *Magnaporthe grisea*, is considered as a model system for studying fungal pathogen-plant interactions not only due to the great economic importance involved, but also due to the genetic and molecular genetic tractability of both the fungus and the host. Although much has been known about pre-penetration stages, little is known about molecular mechanisms and genes responsible for infectious growth and reproduction of the fungus in host tissues to complete the disease cycle. To elucidate the mechanisms involved in the late stages of pathogenesis at genomics level, expressed sequence tag (EST) analysis was conducted to identify rice genes involved in defense responses against infection by the blast fungus *Magnaporthe grisea* and fungal genes involved in infectious growth within the host in a compatible interaction. Two different cDNA libraries were constructed: a cDNA library from rice leaves (*Oryza sativa* cv. Hwacheong) infected with *M. grisea* strain KJ201 and a suppression subtractive cDNA library to enrich the fungal genes expressed *in planta*. Sequencing of 4,000 clones generated 2,315 non-redundant ESTs. Based on the similarity searches against genome drafts of *M. grisea*, Indica-type rice, and dbEST entries with BlastN algorithm, 708 and 1,542 ESTs could be identified to encode fungal and rice genes, respectively. Of the 708 fungal genes, 319 ESTs did not show significant homology to about 20,000 ESTs of *M. grisea* suggesting that these ESTs might be novel genes uniquely or preferentially expressed during infectious growth. Twenty most abundant ESTs showed different expression profiles when compared with the proportions in other libraries constructed from mycelia, conidia, or appressorial stages of *M. grisea*. Transcriptional profiling and high-throughput functional analysis of fungal genes expressed *in planta* is in progress.

In the second approach to understand pathogenicity factors of this fungus at whole genome level, we developed large-scale insertional mutagenesis technique using *Agrobacterium tumefaciens*-mediated transformation (ATMT) and high-throughput phenotype assay system. Seventeen thousands of transformants were generated thus far, and their phenotypes including fungal growth rate, pigmentation, ability of conidiation, conidial germination, appressorium formation and pathogenicity are being evaluated. Among the first screened 8,040 transformants, 800 pathogenicity-defective mutants that contain one or more developmental defects were obtained. The tagged sequences from pathogenicity-defective mutants are being identified by TAIL-PCR technique. This approach would offer highly efficient means for characterizing fungal genes that are important for pathogenicity of *M. grisea*.

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