A Novel ABC Transporter Gene *ABC2* Involved in Multidrug Susceptibility but not Pathogenicity in Rice Blast Fungus, *Magnaporthe grisea*

Young-Jin Lee[1], Kyosuke Yamamoto[1], Hiroshi Hamamoto[1], Ryoji Nakaune[2] and Tadaaki Hibi[1*].

Fungicide treatment is the most important method for the control of plant diseases caused by phytopathogenic fungi. But fungicide resistant strains have appeared in many phytopathogenic fungi. Until now, molecular mechanisms of fungicide resistance such as mutation of target protein, overproduction of target enzyme and detoxification of fungicide have been designated. Recently, it was demonstrated that active efflux of fungicides mediated by ATP-binding cassette (ABC) transporters also contributes to fungicide resistance in several filamentous fungi, such as *Aspergillus nidulans*, *Penicillium digitatum* and *Botrytis cinerea*.

First, we investigated the distribution of the consensus sequences of ABC transporter genes among several taxonomically distinct phytopathogenic fungi through southern hybridization, TA cloning and sequence analysis of their genomic DNA fragments. The result showed that almost of the taxonomically different fungi have the multigene of ABC transporters.

Among the investigated phytopathogenic fungi, we focused on the *Magnaporthe grisea* because it causes the serious problem of decreasing amount of rice crop. There are several reports that ABC transporter gene families of several fungi are involved in fungicide resistances. By degenerate PCR, we cloned five gene fragments encoding consensus amino acid sequences of ABC(ATP binding cassette) regions of their ABC transporters from genomic DNA. Northern hybridization probed by the five gene fragment was performed about total RNA isolated from *M. grisea* treated by 10 kinds of fungicides. Among the five probes, only when probed by the gene fragment of *ABC2*, transcriptional up-regulation was detected by treatment with many toxicants, including several blasticides, demethylation inhibitors (DMI) and antibiotics. So we named it as *ABC2* after we cloned the full length of the gene fragment probe by inverse PCR(IPCR). From above result, we suggested the possibility that *ABC2* is a candidative gene that might involved in resistance about the treated drugs.

Sequence analysis indicated that *ABC2* is an ORF of 4455 nucleotides encoding a deduced protein of 1484 amino acids that shared the highest amino acid homology with BMR1, previously reported ABC transporter protein of *Botrytis cinerea*. ABC2 had nucleotide-binding folds (NBF) and predicted transmembrane domains (TMD6) arranged in a duplicate [NBF-TMD6]2 configuration. The gene disruption experiment was performed to verify the exact role of the ABC2 in the fungicide resistance. To disrupt *ABC2*, protoplast of *M. grisea* was transformed by disruption vector(containing Hygromycin cassette) to target the intact *ABC2* on the genome by the homologous recombination and the distruptants were selected on the medium containing hygromycin.

These disruptants displayed an increased sensitivity to bitertanol, myclobutanil and tebuconazole (DMIs), camptothecin (alkaloid) and cycloheximide (antibiotic) compared to parent *M. grisea*. But, these five compounds are not blasticides that are used for *M. grisea*; however, it will be the first report that *ABC2* is a novel ABC transporter of multidrug resistance gene of *M. grisea*.

And since some phytopathogenic fungal ABC transporters including *ABC1*, another ABC transporter of *M. grisea*, are necessary for pathogenicity, we performed infection assay of *ABC2* disruptants towards rice. However, no obvious difference of disease severities between *ABC2* disruptants and wild-type strain was observed, demonstrating that *ABC2* is not involved in pathogenicity.