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A novel ABC transporter gene *ABC2* involved in multidrug susceptibility but not pathogenicity in rice blast fungus, *Magnaporthe grisea*☆

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Abstract

We cloned a novel ATP-binding cassette (ABC) transporter gene *ABC2* from the rice blast fungus, *Magnaporthe grisea*. ABC2 protein had nucleotide-binding folds (NBF) and predicted transmembrane domains (TMD_6) arranged in a duplicate $[NBF–TMD_6]_2$ configuration and showed the highest amino acid homology with BMR1 of *Botrytis cinerea*. Transcription of the gene was up-regulated by treatment with many toxicants, including several blasticides, demethylation inhibitors (DMI), and antibiotics. *ABC2* disruptants displayed an increased sensitivity to bitertanol, myclobutanil, and tebuconazole (DMIs), camptothecin (alkaloid) and cycloheximide (antibiotic). This result demonstrated that ABC2 is a member of multidrug transporters. 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.

Keywords: ATP-binding cassette transporter; *Magnaporthe grisea*; Multidrug susceptibility

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1. Introduction

Fungicide treatment is the most important method for the control of plant diseases caused by phytopathogenic fungi. Fungicide resistant strains have appeared in many phytopathogenic fungi, such as *Botrytis cinerea*, *Penicillium digitatum*, and *Venturia inaequalis*, which have led to difficulties in the control of these diseases [1].

Rice blast fungus, *Magnaporthe grisea* (Hebert) Barr [anamorph, *Pyricularia grisea* Sacc.], is the most devastating rice pathogen. Strains of this fungus which are resistant to phosphorothiolates, kasugamycin, and blasticidin S first appeared in Japan in the 1970s [1], and carpropamid resistant strains also appeared in 2001 in Japan [2]. To develop the efficient control strategy towards such strains, it is important to clarify the fungicide resistance mechanism.

Until now, molecular mechanisms of fungicide resistance such as mutation of target protein [1,3], overproduction of target enzyme [4], and detoxification of fungicide [1,3] 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* [5–7], *P. digitatum* [8,9], and *B. cinerea* [10–13].

ABC transporters form a large protein superfamily found in almost all living organisms [14]. In the genome of *Saccharomyces cerevisiae*, for example, a total 30 such proteins are found [15]. ABC transporters actively transport chemically or functionally diverse compounds from the inside of the cell to the outside using the energy from ATP hydrolysis [14]. PDR5 of *S. cerevisiae* extrudes more than 100 compounds [16]. For this reason, ABC proteins are often called 'multidrug transporter'. All ABC proteins have a similar molecular architecture with a hallmark domain organization that includes the presence of evolutionarily conserved nucleotide-binding folds (NBF), containing the Walker A and Walker B motifs and the ABC signature [17,18], as well as several transmembrane domains (TMD). In most ABC transporters, the TMDs and NBFs are arranged in a duplicate configuration—$[NBF–TMD_6]_2$ or $[TMD_6–NBF]_2$ [15].

In addition, the natural functions of some ABC transporters concerning pathogenicity and microorganism competition have been demonstrated. For example, ABC1 of *M. grisea* [19], BcatrB of *B. cinerea* [12], and Gpabc1 of *Gibberella pulicaris* [20] act as pathogenicity factors. It is thought that these ABC proteins are involved in efflux of phytoalexins or phytoanticipins. BcatrB is involved in competition with phenazine-producing *Pseudomonas* spp. by extruding this antibiotic [21].

We previously cloned a novel ABC transporter gene (*ABC2*) fragment from *M. grisea* by degenerate polymerase chain reaction (PCR) [22]. In this paper, we present the corresponding full-length gene structure of *ABC2*. The contribution of *ABC2* to fungicide susceptibility and pathogenicity towards rice was examined by creating *ABC2* disruptants. Furthermore, comparison of *ABC2* with *ABC1* was carried out.

2. Materials and methods

2.1. Fungal strains and culture conditions

Magnaporthe grisea strain P-2b (MAFF235001, race: 303) and all the transformants were maintained on potato dextrose agar (PDA, Difco laboratories, Sparks, MD) at 25 °C in the dark. For nucleic acid extraction, *M. grisea* strains used were cultured in potato dextrose broth (PDB, Difco) for 3–4 days at 25 °C with agitation at 120 strokes/min. Conidia were produced on oatmeal plates during incubation at 25 °C. After 2 weeks of culture, the aerial mycelia were brushed off, and the plates were incubated for a further 3 days under BLB lamps (FL20S BL-B, Matsushita Electric Industrial, Osaka, Japan). Conidial germination and appressorium formation on the cellophane membrane were observed 4 and 12 h after collection of conidia, respectively.

2.2. Toxicants

Iprobenfos (IBP), edifenphos (EDDP), fthalide, tricyclazole, pyroquilon, carpropamid, benomyl, thiophanate-methyl, iprodione, bitertanol, myclobutanil, tebuconazole, triflumizole, fenarimol, mico-