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Genome-scale analysis of ABC transporter genes and characterization of the ABCC type transporter genes in *Magnaporthe oryzae*

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ABSTRACT

Rapid adaptation to various environmental stresses is a prerequisite for successful infection in fungal pathogens. ABC transporters are responsible for regulating intracellular levels of cytotoxic or xenobiotic compounds, suggesting a crucial role in pathogenesis. Here, we report genome-scale identification of putative ABC transporter genes in *Magnaporthe oryzae*. A total of 50 ABC transporter genes were predicted and phylogenetic analysis divided them into 11 subfamily groups: ABCA, ABCB, ABCC-1, ABCC-2, ABCD, ABCE, ABCF, ABCG-1, ABCG-2, ABCI, and YDR061W-like. In the 11 ABCC subfamily genes, the transcript levels were elevated during infection stages and after exposure to various abiotic stresses. Based on expression pattern, three representative genes, *MoABC5*, *MoABC6* and *MoABC7*, were selected. Functional analysis of *MoABC5*, *MoABC6* and *MoABC7* revealed that the genes may be responsible for virulence, abiotic stress tolerance, and conidiation, respectively. Our data will be providing valuable information to examine the role of ABC transporter genes in *M. oryzae*.

1. Introduction

For successful infection of the host plant, phytopathogenic fungi need to adapt to a specific host environment and subsequently overcome the cytotoxic and antifungal compounds such as phytoalexins produced by the host plant [1]. The ATP-binding cassette (ABC) transporter protein family is one of the largest gene families in most organisms. They are key players in tolerance and resistance of toxic substances, either sequestering the toxic hydrophobic compounds into specialized designated organelles, or by directing them for secretion [2]. In humans, it is important for maintaining the blood–brain barrier or for mediating cellular resistance to chemotherapeutic drugs [3,4]. In plants, ABC proteins are involved in stomatal movement in response to various stresses and are required for normal seed germination and lateral root development [5,6]. In phytopathogenic fungi, the transporter proteins are involved in resistance mechanisms against cytotoxic compounds or fungicides for successful disease development [7].

In eukaryotes, the ABC transporters are integral membrane proteins transporting a wide range of substrates such as lipids, drugs, and heavy metals. Nine different subfamilies have been defined by their structure and the location of the nucleotide-binding domain (NBD), N-terminal extension (NTE), and the transmembrane segment (TMD) [8]. The general structure of ABC transporters includes four core domains, two NBDs and two TMDs. Most recently, fungal ABC transporters were analyzed at the genome scale using a set of 27 fungal species [9]. They classified highly conserved subfamilies of ABC proteins and group-specific, diversified ABC protein subfamilies. However, ABC transporter genes in *Magnaporthe oryzae* have not been confirmed in detail. Furthermore, the genes have not been systematically explored at the genome-level.

Unlike other subfamilies, most of the ABCC subfamily proteins were found to be full-length transporters with an N-terminal hydrophobic region present in most eukaryotes. In *Arabidopsis*, AtABCC1 and AtABCC2 are responsible for detoxification of toxic compounds by expulsion from the cell or by sequestration in the vacuole [10]. In animals, some of ABCC transporters act either as ATP-gated channels or as potassium channel regulators although they are not primarily active. However, a few studies have been carried out on this subfamily in phytopathogenic fungi.

Only a few ABC transporter genes have been functionally analyzed in fungal species other than human pathogens such as *Candida albicans*

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[11], *Aspergillus fumigatus* [12], and *Cryptococcus neoformans* [13–15]. In *M. oryzae*, four ABC transporter genes, *ABC1* to *ABC4*, have been studied [16–19]. The *M. oryzae* ABC1 [16] and ABC4 [19] are required for pathogenicity [16], helping the fungus to cope with the cytotoxic environment during infection. In addition, ABC2 [17] and ABC3 are required for multidrug resistance. ABC3 specifically helps to overcome cytotoxicity and oxidative stress within the appressoria during early stages of infection-related morphogenesis and likely imparts defense against certain antagonistic and xenobiotic conditions encountered during pathogenic development [18].

In this study, we report genome-scale identification of *M. oryzae* ABC transporter genes in detail. We found that 50 ABC transporter genes are present in this fungus. Gene expression analysis revealed that they are induced during early and late infection stages and under various abiotic stresses. Functional analysis of three genes, *MoABC5*, *MoABC6* and *MoABC7*, from ABCC subfamily revealed that *MoABC5* and *MoABC6* are involved in pathogenicity and hyphal growth, respectively. Drawing together the results from the expression and the functional analyses, evidence supports the role of the ABCC subfamily proteins in tolerance of stress conditions encountered during colonization of the host, directly linked to pathogen fitness.

2. Results

2.1. ABC transporter genes in M. oryzae

In a previous study, 50 putative ABC transporter genes were identified in *M. oryzae* genome by Kovalchuk and Driessen [9]. The 50 genes were divided into nine different subfamilies, ABCA, ABCB, ABCC, ABCD, ABCE, ABCF, ABCG, ABCI, and YDR061w-like. However, no comparisons were made between the 50 genes. Based on this previous work, we collected the 50 genes from *M. oryzae* genome database (Table S1). Subfamilies of ABCB (19 genes, 38%), ABCC (11 genes, 22%), and ABCG (8 genes, 16%) occupied higher proportions than the other subfamilies in *M. oryzae*. We mapped the loci of the 50 genes to putative *M. oryzae* chromosomes by chromosome location analysis (http://cfgp.riceblast.snu.ac.kr). The genes were mapped onto all seven chromosomes and a dispensable chromosome (Fig. 1A). We found that the members of a single subfamily can be scattered around different chromosomes instead of linkage and the protein sizes are variable, ranging from 206 (MGG_13339) to 1683 (MGG_04855) residues (Table S1). All the ABC transporter genes were conserved in other 32 organisms including chromista, fungi, metazoa, and vidiriplantae, except MGG_13339 (Table S2).

Phylogenetic analysis was carried out to determine the evolutionary relationship of the 50 genes. The resulting tree showed that they are classified into 11 subgroups (Fig. 1B). ABCB and ABCC subfamilies were more closely related to each other than to the other subfamilies. Interestingly, one gene (MGG_06024) from ABCC subfamily was separated as a distinct branch on its own. Moreover, ABCG subfamily was also divided into two branches.

All the identified *M. oryzae* ABC transporter proteins have more than two AAA ATPase domains (IPR003593) and two to four ABC transporter-like domains (IPR003439). The locations of the ATPase and ABC transporter-like domains are variable. Unlike other subfamilies, ABCB and ABCC transporters contain integral membrane type 1 ABC transporters (IPR017940) and transmembrane domain type 1 ABC transporters (IPR011527), resulting in close position of the two groups in the topology of the phylogenetic tree (Fig. 1B). ABCG transporters in *M. oryzae* contain ABC2 type transporter domain (IPR013525) (Fig. 2).

2.2. ABCC subfamily genes are expressed during infection and various abiotic stresses

Previous reports on ABC1 to 4 transporter encoding genes in *M. oryzae* have shown that they regulate cytotoxicity and mediate tolerance against antifungal agents as well as oxidative stress, allowing the pathogen to perform successful infection. Therefore it was suggested that these genes are required for pathogenicity [16–19]. As these four genes belong to ABCA (*ABC4*), ABCB (*ABC3*), and ABCG (*ABC1* and *ABC2*) subfamilies in *M. oryzae*, we directed our attention to the ABCC subfamily, which was the largest subfamily whose members have not been studied previously.

To obtain insights into the physiological roles of ABCC transporters, we performed expression analysis of 11 ABCC transporters in various conditions including cell developmental stages, infection stages, and abiotic stress conditions (Table S3 and Fig. 3). The results showed that the 11 transporter genes are classified into three major groups based on their expression patterns (Fig. S1). All the 11 genes were up-regulated under LiCl, sorbitol, and Iprobenfos treatments, implying ABCC transporter genes are generally required for those abiotic stress conditions in *M. oryzae*, whereas we did not find conditions where all 11 genes are down-regulated.

Group I contains three transporter genes, MGG_04855, MGG_03736, and MGG_08309, which are activated mainly by hygromycin. Group II-1 contains five transporter genes, MGG_13880, MGG_06024, MGG_01674, MGG_11025, and MGG_05044, which are up-regulated mainly by carbon starvation, heat shock, Triflumizol, Isoprothiolane and nitrogen starvation. Group II-2 contains three transporter genes, MGG_07567, MGG_05746, and MGG_05009, which are activated when grown on minimal medium, or at 72 h post inoculation on rice, or under nitrogen starvation, heat shock stress, NaCl, KCl, Triflumizol, and Isoprothiolane-treated conditions.

2.3. Targeted gene disruption of three ABCC transporter genes

Building on the information from the expression analysis, we examined the function of the three genes, MGG_04855, MGG_05044, and MGG_05009, from each Group I, II-1, and II-2 (Table S4). We designated gene names; *MoABC5* for MGG_05009, *MoABC6* for MGG_05044, and *MoABC7* for MGG_04855. We performed targeted gene disruption for each of the three genes. Gene disruption constructs with hygromycin gene cassettes were created as in Fig. S2 and were introduced into the wild-type strain to generate KO mutants. The transformants were primarily screened for hygromycin resistance. Southern blot analysis with appropriate probes confirmed successful gene disruption with a single integration event (Fig. S2). To confirm that the phenotypes shown by Δ*Moabc5* or Δ*Moabc6* or Δ*Moabc7* mutants are the result of gene inactivation, we carried out transcription analysis by qRT-PCR. This clarified that all Δ*Moabc5* or Δ*Moabc6* or Δ*Moabc7* mutants were down-regulated compared with that of the wild-type. The transcript abundance from complementation strains was identical to that of wild-type (Fig. S2).

2.4. Phenotype of ΔMoabc5, ΔMoabc6, and ΔMoabc7

The effects of deletion of *MoABC5*, *MoABC6*, and *MoABC7* genes on *M. oryzae* development and pathogenicity are summarized in Table 1. Deletion mutants of *MoABC6* and *MoABC7* genes exhibited significant reduction of conidiation compared to the wild-type. However, we observed no significant difference in germination and appressorium formation between the mutants and the wild-type (Table 1).

In spray-inoculation tests, the Δ*Moabc5* mutant showed reduced virulence on a susceptible rice cultivar, Nakdongbyeo, whereas the wild-type KJ201, Δ*Moabc6*, Δ*Moabc7* and the complemented transformants of each deletion mutant caused typical susceptible-type spreading lesions (Fig. 4 and Table 1). Dramatic differences in disease severity were observed, suggesting that *MoABC5* gene may be involved in pathogenesis (Table 1).

There was also no apparent difference between the wild-type and the mutants for mycelial growth on CM, MM, nitrogen starvation, carbon starvation, 1 M sorbitol, 1 M KCl, and 1 M NaCl. However,