

Expression Patterns of Transposable Elements in *Magnaporthe oryzae* under Diverse Developmental and Environmental Conditions

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The genome of the rice blast fungus *Magnaporthe oryzae* contains several types of transposable elements (TEs), and some TEs cause genetic variation that allows *M. oryzae* to evade host detection. We studied how five abundant TEs in rice pathogens, Pot3, Pot2, MAGGY, Line-like element (MGL) and Mg-SINE, are expressed under diverse conditions related to growth, development, and stress. Expression of Pot3 and Pot2 was activated in germinated conidia and mycelia treated with tricyclazole. Retrotransposon MAGGY was highly expressed in appressoria and tricyclazole-treated mycelia. MAGGY and Pot2 were also activated during the early and late stages of perithecia development. MGL was up-regulated in conidia and during conidial germination but not during appressorium formation. No noticeable expression of Mg-SINE was observed under most conditions. Our results should help investigate if and how condition-specific expressions of some TEs contribute to the biology and evolution of *M. oryzae*.

Keywords: Genetic variation, *Magnaporthe oryzae*, Repeat sequences, Rice blast, Transposable elements

Transposable elements (TEs) are present in the genomes of bacteria, plants, animals, and fungi and appear to have played significant roles in their evolution (Seidl and Thomma, 2017). TEs are classified into two groups based on how they propagate in the host genome. Retrotransposons move via RNA intermediates (class I), while class II TEs encode an enzyme that catalyzes their transposition (Daboussi and Capy, 2003). Several studies showed that fungal TEs could be activated during specific developmental processes or under

stressful conditions, such as exposure to Cu²⁺ ions, oxidative stress, and heat shock (Ikeda et al., 2001; Mhiri et al., 1997). Condition-specific activation of TEs and subsequent movement might benefit fungi, especially those that asexually reproduce, by generating beneficial genetic changes (Kang et al., 2001; Schmidt et al., 1998; Teng et al., 1996).

The rice blast fungus *Magnaporthe oryzae* is one of the most damaging pathogens in rice cultivated areas around the world. The genomes of rice pathogenic isolates contain many different TEs, including class II TEs (e.g., Pot3 and Pot2) and class I retrotransposons (e.g., MAGGY, Mg-SINE and Line-like element [MGL]). DNA fingerprinting analyses of diverse field isolates using TEs as probes showed high degrees of

their variability, which led to the wide application of some TEs as makers for population genetic studies (Kang and Lee, 2000). The activity of some TEs caused race variation, resulting in the breakdown of host resistance. For example, insertion of Pot3 into the promoter or coding sequence of *AVR-Pita1*, an avirulence gene, caused gain-of-virulence against specific rice varieties (Kang et al., 2001; Seidl and Thomma, 2017). Insertion of MGL into *ACR1*, a gene involved in conidiation, generated a variant of *M. oryzae* that produces acropetal conidia (Nishimura et al., 2000).

Isolates from infected rice carry high copy numbers of some TEs, such as Pot3 (Hamer et al., 1989) and MAGGY (Farman et al., 1996; Tosa et al., 1995). Most of rice pathogenic isolates contain over 50 copies of Pot3 and 30–50 copies of MAGGY in their genome. On the other hand, grass-pathogenic isolates only contain 0–5 copies of Pot3 and 0–30 copies of MAGGY (Farman et al., 1996), suggesting a potential association between the copy number of some TEs and host adaption. Our previous study of a large group of *M. oryzae* isolates collected over multiple years in Korea using Pot3 and MAGGY as probes for DNA fingerprinting showed high degrees of genetic diversity associated with these TEs (Park et al., 2008). However, most of the isolates collected in the same year only displayed minor differences (e.g., the disappearance of a commonly present band, the appearance of a new band), suggesting that changes in Pot3 and MAGGY do not occur very frequently. Field populations of *M. oryzae* display high degrees of TE-associated polymorphisms due to accumulated changes over many years. Specific developmental and environmental conditions likely influence the activity of TEs and their transposition in *M. oryzae*. Accordingly, studies on which conditions affect TEs are needed to understand how *M. oryzae* changes and if we can take specific measures to slow down the rate of such TE-mediated genetic variation. The present study aimed to enhance this understanding by investigating how five *M. oryzae* TEs, including Pot3, Pot2, MAGGY, Mg-SINE and MGL, are expressed under diverse growth and developmental conditions (Table 1).

The activity of selected TEs during and after the sexual cycle of *M. oryzae* was previously studied (Eto et al., 2001), which showed the activation of MAGGY, but not Pot3, Pot2, Mg-SINE and MGL, during the sexual development. Resulting progeny displayed polymorphisms associated with MAGGY, indicating its transposition. In addition, Ikeda et al. (Ikeda et al., 2001) also reported that the promoter of

MAGGY is activated by various stresses, including heat shock, CuSO₄ treatment, and oxidative stress. Many studies in other organisms also showed that various environmental and stressful conditions, such as heat shock, heavy metals, and ultraviolet irradiation, activate various TE elements (Bradshaw and McEntee, 1989; Rolfe et al., 1986; Walbot, 1999).

Twenty-four different conditions/materials evaluated in the current study can be classified into three groups (Table 1). The first group included conditions/materials that represent the following stages of growth or development. We harvested conidia from 1-day-old culture of 70-15, a genome-sequenced laboratory strain (Dean et al., 2005), on oatmeal agar using sterilized distilled water. They were subsequently collected using disposable bottle top filter (0.45 µm) unit (Millipore Corporation, Billerica, MA, USA). Germinated conidia were collected after incubating some of the collected conidia in diluted (1/4) complete medium (Talbot et al., 1997) at 70 rpm for 3 hr. The appressorial sample was prepared by placing a suspension of the collected conidia ($3-4 \times 10^4$ conidia/ml) on Gelbond, a synthetic material with a hydrophobic surface that induces appressorium formation, for 4 hr. Mycelial cultures of 70-15 and 70-6 on oatmeal agar were collected after 5 days of incubation. Samples that represent two different stages of sexual development were prepared after crossing 70-15 with 70-6 as follows: Sterilized 1×8 cm Whatman filter paper was placed on the center of each oatmeal agar plate inoculated with 70-15 and 70-6 (at opposite ends of the plate). Filter papers that had been colonized by these isolates, which undergo sexual crosses, were collected after 12 days (premature perithecia) and 21 days (mature perithecia).

Mycelia cultured under a nitrogen or carbon starvation condition were prepared as previously described (Talbot et al., 1997). The sample for infected rice was collected after 7 days post-inoculation of rice cv. Hwacheng with *M. oryzae* strain KJ201 (Jeon et al., 2007), a Korean field isolate that is highly virulent to this cultivar. For testing the effect of individual stresses on TE expression, 70-15 was cultured in 50 ml of complete medium (CM) broth in 250-ml Erlenmeyer flasks at 25°C for 4 days using an orbital shaker (70 rpm). Resulting mycelia were collected, washed with sterilized distilled water, and then resuspended in CM broth containing each of the following agents: 100 mM of thiamine hydrochloride (T3902, Sigma-Aldrich, St. Louis, MO, USA), 500 ppm tricyclazole, 10 mM methyl viologen (856177, Sigma-Aldrich), 0.1, 1, and

Table 1. Twenty-four conditions/materials evaluated

Samples ^a	Treatment time or materials employed
Asexual and sexual development	
Conidia	Conidia of 70-15 produced on oatmeal agar
Germinated conidia	Conidia inoculated in half CM for 8 hr
Appressorium formation	Conidia placed on Gelbond for 4 hr
Culture of strain 70-15	5-Day-old culture on oatmeal agar
Culture of strain 70-6	5-Day-old culture on oatmeal agar
Mycelia collected from the contact zone between two strains	10-Day-old culture of 70-15 and 70-6 on oatmeal agar
Premature perithecia	12 Days after crossing between 70-15 and 70-6 on oatmeal agar
Mature perithecia	21 Days after crossing between 70-15 and 70-6 on oatmeal agar
Nutrient availability and infection	
Nitrogen starvation	16 hr
Carbon starvation	16 hr
Complete medium	16 hr
Infected rice	7 Days after infecting cv. Hwacheong with KJ201
Stress	
Heat shock	Mycelia at 42°C for 45 min
Cold shock	Mycelia at 4°C for 45 min
Complete medium	6 hr
Thiamine 100 mM	6 hr
3-Tricyclazole 500 ppm	6 hr
Methyl viologen 100 mM	6 hr
CuSO ₄ 0.1 mM	6 hr
CuSO ₄ 1 mM	6 hr
CuSO ₄ 10 mM	6 hr
p-coumaric acid 10 µg	6 hr
p-coumaric acid 50 µg	6 hr
p-coumaric acid 100 µg	6 hr

CM, complete medium.

^aThe evaluated samples were grouped into three.

10 mM CuSO₄, and 10, 50, 100 µg p-coumaric acid (C9008, Sigma-Aldrich). These treatments lasted 6 hr. Heat shock and cold shock treatments were performed by placing cultures at 45°C for 45 min and 4°C for 45 min, respectively.

All fungal and plant samples collected were immediately put into liquid nitrogen and then kept at -70°C until RNA extraction. RNA extraction and Northern hybridization were performed as follows. After grinding the samples finely in the presence of liquid nitrogen, they were mixed vigorously with a 1:1 mixture of RNA extraction buffer (200 mM Tris-HCl pH 8.0; 400 mM LiCl; 25 mM EDTA; 1.0% sodium dodecyl sulfate

[SDS]) and equilibrated acidic phenol. After one phenol extraction and two chloroform extractions, RNAs were precipitated using 8 M LiCl (1/3 volume of extracted RNA solution). Ten micrograms of RNAs were fractionated on a 1% denaturing agarose gel, and RNA gel blotting was performed following the standard procedures (Sambrook et al., 1989).

RNA hybridization was conducted using labeled Pot3, Pot2, MAGGY, Mg-SINE and MGL. Probes for Pot3 and MAGGY were prepared as previously described (Park et al., 2003). Pot2 probe was prepared by isolating the 1.3 kb *Hpa*I/*Sal*I restriction fragment from plasmid p33. Probes for Mg-SINE

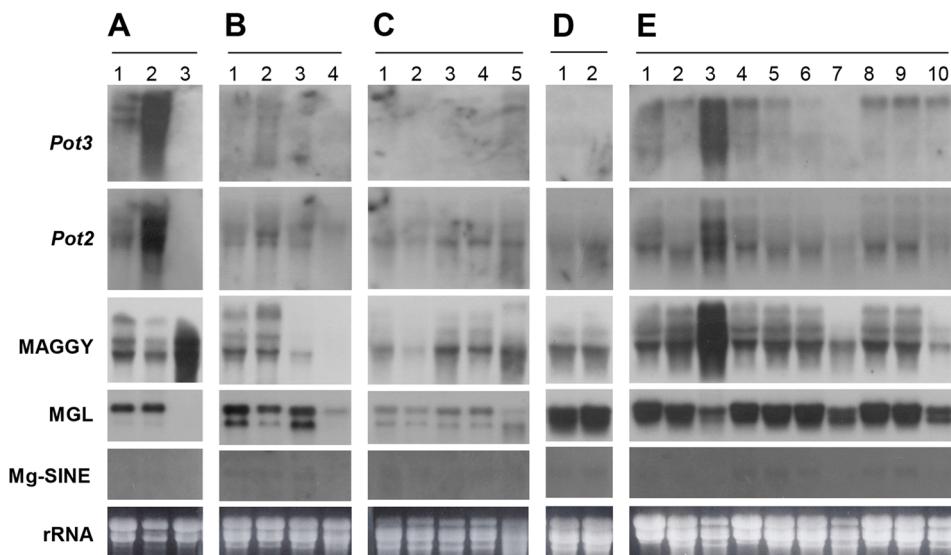


Fig. 1. Northern analysis of transposable element (TE) expression under diverse conditions. RNAs extracted from the specimens as described in Table 1 were fractionated via 1.0% formaldehyde-agarose gel, transferred to nylon membrane, and probed with five transposons. Fractionated RNAs stained with ethidium bromide are shown at the bottom panel. (A) 1, conidia of 70-15; 2, germinated conidia of 70-15; 3, appressoria of 70-15. (B) 1, mycelia cultured in complete medium (CM) with low nitrogen for 6 hr; 2, mycelia cultured in CM with low carbon for 6 hr; 3, mycelia cultured in CM for 6 hr; 4, rice cultivar Hwacheong infected with KJ201 (7 days post-inoculation). (C) 1, 70-15 (*Mat1-1*) mycelia; 2, 70-6 (*Mat1-2*) mycelia; 3, mycelia from the contacted area between 70-15 and 70-6; 4, premature perithecia; 5, mature perithecia. (D) 1, heat shock (42 °C, 45 min); 2, cold shock (4 °C, 45 min). (E) 1, CM; 2, thiamine 100 mM; 3, tricyclazole 500 ppm; 4, methyl viologen 10 mM; 5, CuSO₄ 0.1 mM; 6, CuSO₄ 1 mM; 7, CuSO₄ 10 mM; 8, *p*-coumaric acid 1 µg; 9, *p*-coumaric acid 5 µg; 10, *p*-coumaric acid 10 µg.

and MGL were prepared by isolating the 0.5 kb *Eco*RI fragment from plasmid SP076 and 0.7 kb *Xba*I fragment from plasmid SP077, respectively. These probes were labeled with ³²P using Rediprime TM II Random Prime Labeling System kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA) according to the manufacturer's instructions. RNA blots were hybridized with these probes at 42°C overnight in 5× SSPE (1× SSPE=0.18 M NaCl, 1 mM EDTA [pH 8.0], and 10 mM sodium phosphate [pH 7.4]) containing 0.2% SDS, 0.5× Denhard's solution (Sambrook et al., 1989), and 100 µg denatured salmon sperm DNA per ml. After hybridization, blots were washed twice in 2× SSPE containing 0.1× SDS for 10 min at 42°C. Signals were detected by autoradiography using an X-ray film.

Overall, expression patterns of Pot3 and Pot2 were highly similar but are distinct from that of MAGGY. No detectable transcriptional activation was found on Mg-SINE under all the conditions that we used. Activation of MGL element showed looks like constitutive expression under stress conditions, whereas Mg-SINE was up-regulated under some conditions, although degrees of its activation were very lower than those observed in other TEs.

Pot3 and Pot2 were highly up-regulated during conidial

germination but not in conidia or during appressorium formation. MAGGY was highly activated during appressorium formation. MGL was up-regulated in conidia and during conidial germination but not during appressorium formation (Fig. 1A).

Under nitrogen (-N) and carbon starvation (-C) conditions, in infected rice, and during the sexual cycle, we could not find any detectable activation of all TEs except MAGGY. MAGGY was activated under both -N and -C conditions, during mycelial growth on oatmeal agar, in premature and mature perithecia and during the sexual cycle (Fig. 1B, C). However, we could not detect any up-regulation under heat shock (Fig. 1D) although Ikeda et al. (2001) showed its up-regulation by heat shock. This difference might be because a different strain was used. We have also observed that the expression of Pot3, Pot2 and MAGGY was activated by tricyclazole. The degree of induction for MAGGY was higher than that for Pot3 and Pot2. However, we could not detect any noticeable activation of MG-SINE under stress conditions (Fig. 1E).

Each TE is likely to be regulated by different transcriptional factor(s). Although sequences of the putative promoter and

coding regions of Pot3 and Pot2 are different, their activation patterns were highly similar, suggesting their co-regulation by same transcription factors or signaling pathways. The regulation of MAGGY expression likely involves different transcription factors or transcription factors pathways.

Although most of the TEs analyzed here have been reported to transpose (Kachroo et al., 1995; Kang, 2001; Nishimura et al., 2000; Zhou et al., 2007), we could not find any noticeable transcriptional up-regulation of MGL and Mg-SINE under all the conditions tested, indicating Pot3, Pot2, and MAGGY is more sensitive and active under the more broad range of conditions. Of course, it is possible that MGL and Mg-SINE may be up-regulated by condition that we did not test. Some TEs are activated by different stress conditions (Mansour, 2007).

In summary, Pot3 and Pot2 are activated during conidial germination, whereas the expression of MAGGY is up-regulated during appressorium formation. In addition, Pot3, Pot2 and MAGGY are highly activated in response to tricyclazole treatment, but other agents that can cause stress did not significantly affect its expression. No noticeable transcriptional activation was observed for MGL and Mg-SINE under most of the conditions tested. These results suggest that the expression of TEs is affected by several growth- or development-related conditions, but which conditions activate each TE varies widely.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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