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Pathogenicity of Rice Blast Fungus *Magnaporthe oryzae* on *Brachypodium distachyon*

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Abstract: Inoculation methods for rice blast fungus *Magnaporthe oryzae* to *Brachypodium distachyon* were developed to investigate the infection process and symptom development in comparison with those on rice (*Oryza sativa*) and barley (*Hordeum vulgare*). *M. oryzae* could infect leaves, sheathes, stems and panicles of *B. distachyon* and cause blast disease. Spraying conidial suspension on either intact seedlings or leaf segments induced typical symptoms on *B. distachyon*. During the intact seedling inoculation, the symptom developed on *B. distachyon* leaves closely resembled that on rice; but the lesions on *B. distachyon* had better uniformity in shapes and sizes than those on rice or barley. In the leaf segments inoculation, only initial and low-developed lesions could be found on rice, while normal symptoms on *B. distachyon* and barley. Inoculated with low-virulent mutants of *M. oryzae*, *B. distachyon* produced low-level symptoms. The symptom level of each mutant on *B. distachyon* corresponded well to that on rice. In addition, typical infection processes presented on *B. distachyon* leaves: forming melanized appressoria, penetrating into host epidermis and then forming hyphae in epidermal cells. According to these results, *B. distachyon* can be used as a candidate for studying fungus-plant interactions and as a probable source of disease resistance.

Key words: *Brachypodium distachyon*; *Magnaporthe oryzae*; interaction; model plant; pathogenicity

Rice blast is the most destructive disease on rice (*Oryza sativa*). Plant-fungus interaction is a hot topic in plant pathology. In recent years, the rice blast fungus (*Magnaporthe oryzae*) is becoming a valuable model for such research. Besides rice, *M. oryzae* infects barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), millet (*Eleusine coracana*) and other species of the *Poaceae* family (Igarashi et al, 1986; Ekwamu, 1991). Investigating interactions of this fungus on different hosts (even non-host) to reveal differences among them are relevant to both plant pathology research and disease control. Du et al (1995, 1996) investigated *Pyricularia* spp. derived from different grasses in Zhejiang Province, China and used the cross-protection among them to control the disease. Maeda et al (2009, 2010) and Park et al (2009) analyzed the interaction of *M. oryzae* on *Arabidopsis* and isolated related genes. However, the knowledge is still limited so far on mechanism and application of the interaction of *M. oryzae* to non-rice hosts. Therefore, establishing efficient interaction systems of the fungus on non-rice plants, especially on the *Poaceae* species, and clarifying their susceptibility, infection features and symptom development, are important for both phyto-pathologic research and new resistant resources discovery.

Brachypodium distachyon is a wild annual temperate grass classified in the *Poaceae* family and *Pooideae* subfamily. It has sister relationship to the ancestor of the four 'core pooid' tribes, i. e., *Triticeae*, *Aveneae*, *Bromeae* and *Poeae* (Davis and Soreng, 1993; Kellogg, 2001), and closely relates to main cereal crops such as *T. aestivum*, *H. vulgare*, *Avena sativa*, *O. sativa*, *Zea mays*, *Sorghum bicolor* and the biofuel crop *Panicum virgatum* (Caetano-Anolles, 2005). *B. distachyon* has small physical stature, short lifecycle, simple growth requirement, self-fertility and strong reproduction ability (Chen et al, 2008). Various chromosome ploidies, such as diploid (2n = 10), tetraploid (4n = 20) and hexploid (6n = 30), were found in *B. distachyon* (Shi et al, 1993). The genome of diploid Bd21 of *B. distachyon* has been sequenced. It spans 271.9 Mb (The International *Brachypodium* Initiative, 2010) and has similar component and structure to those of other *Poaceae* members (Wang et al, 2007). The biolistic bombardment and *Agrobacterium*-mediated transformation (AtMT) systems of *B. distachyon* have already been established and presented high regeneration rates (Li et al, 2008). By AtMT, T_1 generation could be obtained within eight months (Alves et al, 2009). Therefore, *B. distachyon* was regarded as an ideal experimental

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model plant for functional genomics of *Poaceae* plants and biofuel crops (Li et al, 2008).

B. distachyon is susceptible to a variety of important plant fungal pathogens. For example, the leaf rust fungus *Puccinia triticina* and the stripe rust fungus *P. striiformis* could penetrate and colonize on *B. distachyon* leaves. The powdery mildew fungus *Blumeria graminis* induced papilla response on *B. distachyon*. Thus, *B. distachyon* can be used to research the plant-fungus interactions (Draper et al, 2001; Parker et al, 2008). However, such research is still very limited and efficient methods are required at present. In this study, we developed inoculation methods for rice blast fungus to *B. distachyon*, investigated the disease development and symptom characteristics, and compared them with those on rice and barley. Our work provided a tool for the research of plant-fungus interaction and discovery of resistance genes.

MATERIALS AND METHODS

Materials

Fungal strains, host cultivars and cultivation methods

The wild type of *M. oryzae* used was Guy11. The mutant strains *Δcpka* (Mitchel and Dean, 1995), *Δmac1* (Choi and Dean, 1997), *Δmpg1* (Talbot et al, 1993) and *Δpmk1* (Xu and Hamer, 1996) were kindly provided by Professor LIN Fucheng at the Institute of Biotechnology, Zhejiang University, China (Table 1). All the strains were cultured on complete media (CM) using routine procedures described by Talbot et al (1993). The *B. distachyon* variety used was Bd21, a kind gift from Professor AN Hailong at the Faculty of Life Sciences, Shandong Agricultural University, China. The barley variety was ZJ-8 and the rice variety was CO-39.

Instruments and reagents

Leica DM2500 (Germany) was used for fluorescence microscopy. Aniline blue was purchased from Sigma (Germany) and other reagents were from Huadong Medicine Ltd. Co., China.

Inoculation

Inoculum preparation

The spores of *M. oryzae* were harvested from 10 d colonies on complete media and suspended in sterilized water to 2×10^4 conidia/mL.

Smearing inoculation on seedlings

The spore suspension of *M. oryzae* was smeared on leaves, sheaths, stems and panicles of *B. distachyon* seedlings by using a painting brush. The inoculated seedlings were incubated at 28 °C, alternating 12 h light/12 h dark, with the inoculated seedlings kept moist in a sterilized plastic bag. The symptoms were checked at 9 d post-inoculation (dpi).

Spray inoculation on seedlings

The seedlings of 10-day-old barley, 21-day-old rice and 15- to 90-day-old *B. distachyon* were inoculated with the spore suspension of *M. oryzae* by using a mini sprayer. The inoculated plants were kept in dark for 24 h and then transferred to 28 °C, 12 h/12 h alternating light/dark for 9 d.

Spray inoculation on detached leaves

The young leaves (the second top leaves of tillers) of 10-day-old barley, 21-day-old rice and 15- to 90-day-old *B. distachyon* were removed and cut into 5 cm segments, and then spray inoculated. The inoculated leaves were kept in darkn for 24 h and then incubated at 28 °C, 24 h continuous light for 9 d.

Droplet inoculation on detached leaves

The young leaves of *B. distachyon* were collected, cut into 5 cm segments and inoculated with 20 μL droplets of the spore suspension of *M. oryzae* by using a pipette. The inoculated leaves were kept in dark for 24 h and then incubated at 28 °C, 24 h continuous light for 9 d.

Microscopy of fungal structure and plant surface

The droplet-inoculated *B. distachyon* leaf segments were treated with ethanol for 3−4 times to remove the chlorophyll, dissociated with 5% KOH for 0.5 h at 65 °C and then stained with aniline blue. The fungal structures

Table 1. *M. oryzae* mutants used in this study.

Mutant	Mutant phenotype	Related gene	Full name of gene	Possible gene function
Δcpka	Host surface sensing; appressorium formation defective; abolished pathogenicity	*CPKA*	cAMP-dependent protein kinases	Kinase; activating *PMK1* signaling of appressorium initiation
Δmac1	Appressorium formation defective; reduced pathogenicity	*MAC1*	*Magnaporthe* adenylate cyclase	Activating the cAMP signaling pathway
Δmpg1	Appressorium formation defective; reduced pathogenicity	*MPG1*	*Magnaporthe* pathogenicity gene 1	Host surface hydrophobin protein assembly; surface thigmotropic signaling
Δpmk1	Lack of melanization in appressorium; inability to cease nuclear division in appressorium; abolished pathogenicity	*PMK1*	Pathogenicity MAP-Kinase 1	Signaling in appressorium maturation; regulation of melanin biosynthesis; cession of nuclear division inside appressorium