Analysis of *Pyricularia oryzae* and *P. grisea* from Different Hosts Based on Multilocus Phylogeny and Pathogenicity Associated with Host Preference in China

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ABSTRACT

Pyricularia oryzae and *P. grisea* are important agents of major diseases on a wide range of gramineous hosts. Whereas *P. oryzae* is the most important pathogen causing outbreaks of rice blast, *P. grisea* is mainly a pathogen of crabgrass. In this study, 103 *P. oryzae* and 20 *P. grisea* isolates were collected from seven species of plants, and we analyzed their phylogeny, pathogenicity, and relationship with host preferences to investigate the differences among them from different hosts. Based on phylogenetic analysis of multilocus sequences, 16 isolates from crabgrass and four isolates from green bristlegrass were identified as *P. grisea* and another 103 isolates from crabgrass, green

bristlegrass, goose grass, foxtail millet, wild millet, rice, and sedge belonged to *P. oryzae*. Results of pathogenicity tests by artificial inoculation demonstrated that six of 10 *P. oryzae* isolates from rice and three of 44 *P. oryzae* isolates from green bristlegrass showed cross-infectivity on green bristlegrass and rice, respectively. Taken together, our results demonstrated that isolates from green bristlegrass and crabgrass consist of both *P. oryzae* and *P. grisea* and that *P. oryzae* isolates showed cross-infectivity between rice and green bristlegrass, suggesting that host shifts may occur for *P. oryzae* and *P. grisea*.

Rice (*Oryza sativa*) is a staple food crop in the world (Gnanamanickam 2009), and some fungi are currently considered a major threat to global rice security. The most destructive disease of rice is rice blast, which is caused by an important heterothallic ascomycetous pathogen, *Pyricularia oryzae*. This disease causes symptoms on leaves, stems, nodes, roots, and panicles of rice, leading to severe yield loss (Sesma and Osbourn 2004; Talbot 2003; Valent and Chumley 1991).

In the genus *Pyricularia*, *P. oryzae* and *P. grisea* are the most studied species, and *P. oryzae* is the most destructive member (Klaubauf et al. 2014; Okagaki et al. 2016; Shirke et al. 2016). The species of *Pyricularia* share some morphological traits, while *P. oryzae* and *P. grisea* can be differentiated from other species by morphological traits (Klaubauf et al. 2014). However, no detectable morphological characteristics that are distinct between *P. grisea* and *P. oryzae* have been reported until now. The name *P. grisea* has been widely used since one isolate from crabgrass was first identified in 1880 (Saccardo 1880), and another name, *P. oryzae*, has been used since an isolate from rice was identified in 1892 (Choi et al. 2013). Both names were regarded as synonymous because there is no significant morphological difference to distinguish them (Rossman et al. 1990; Sprague 1950).

Traditionally, fungal species have been identified by the morphological species concept (MSC) (Taylor et al. 2000). MSC is widely suitable for sexually reproducing organisms, including animals, plants, and fungi. However, MSC cannot be easily applied to fungi, which are cryptic species or rarely reproduce by a sexual stage (Harrington and

Rizzo 1999; Hirata et al. 2007; Taylor et al. 2000). The advancement of molecular biology techniques provides a method for distinguishing species called the phylogenetic species concept (PSC) based on the concordance of multilocus DNA sequences (Hirata et al. 2007). PSC has become popular for use in filamentous fungi and has been successfully used to identify cryptic species in animal and plant pathogenic fungi (Bidochka et al. 2005; Cruse et al. 2002; Hughes et al. 2018; Steenkamp et al. 2002; Taylor and Fisher 2003). The teleomorph of *P. oryzae* was found by mating in the laboratory, but sexual reproduction has not been found in nature (Choi et al. 2013; Hebert 1971; Rossman et al. 1990; Yaegashi and Udagawa 1978). In 2002, two cryptic species in *P. grisea* were recognized by multilocus phylogenetic analysis and given a new species name: the authors assigned the name *P. oryzae* to isolates from rice and other grasses, whereas *P. grisea* was assigned to the isolates only from crabgrass, following the original specimen (Couch and Kohn 2002).

Different host-limited forms of *P. oryzae* can infect a broad range of host species, including crops and weeds such as rice (*Oryza sativa*), foxtail millet (*Setaria italica*), wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), oat (*Avena sativa*), wild millet (*Eriochloa villosa*), green bristlegrass (*S. viridis*), crabgrass (*Digitaria sanguinalis*), and goose grass (*Eleusine indica*) (Chiapello et al. 2015; Choi et al. 1996, 2013; Dean et al. 2005; Inoue et al. 2017). To date, the pathogenicity of many *P. oryzae* isolates from different hosts has been tested. For example, foxtail millet, barley, crabgrass, and goose grass isolates are virulent to rice (Choi et al. 2013; Urashima et al. 1993). However, in other studies, crabgrass, goose grass, green bristlegrass, and foxtail millet isolates could not infect rice, because these isolates are host specific, and barley is highly susceptible to many isolates from different hosts (Klaubauf et al. 2014; Zhong et al. 2016).

The objectives of this study were as follows: (i) to analyze the phylogenetic diversity within *P. oryzae* and *P. grisea* isolated from seven species of plants in China, (ii) to determine the pathogenicity of all of the isolates to rice and the pathogenicity of rice isolates to green bristlegrass, and (iii) to understand the role of green bristlegrass in the rice blast cycle in paddy fields.

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MATERIALS AND METHODS

Isolation of *P. oryzae* and *P. grisea* isolates. During 2010 to 2017, 113 nonrice blast isolates were collected from 12 different locations in China (Supplementary Fig. S1). One isolate was isolated from sedge (*Cyperus rotundus*), whereas all other isolates were isolated from five different species of Poaceae: green bristlegrass (*S. viridis*; $n = 48$), crabgrass (*D. sanguinalis*; $n = 36$), foxtail millet (*S. italica*; $n = 12$), goose grass (*Eleusine indica*; $n = 12$), and wide millet (*Eriochloa villosa*; $n = 4$). One rice blast isolate, P131, originated from Japan, and nine rice blast isolates collected from rice fields in China were maintained in our laboratory (Fang et al. 2018; Xue et al. 2012). All isolates were collected from blast-infected leaves and purified by single spore isolation. Fungal isolates were cultured on oatmeal tomato agar medium (OTA; 40 g of oatmeal, 20 g of agar, 0.6 g of CaCO_3 , and 150 ml of tomato juice per liter) at 26°C under continuous fluorescent light and were then maintained on filter paper and stored at -20°C for long-term conservation (Chen et al. 2011).

DNA extraction, PCR, and sequencing. Genomic DNA was extracted from vegetative mycelia with the cetyltrimethylammonium bromide protocol (Xu and Hamer 1996). DNA amplification reactions were performed for the actin, beta-tubulin, and calmodulin genes as previously described (Couch and Kohn 2002). The PCR reactions were performed in 25- μl mixtures with 1 μl of genomic DNA, 12.5 μl of 2 $\times$ Taq PCR MasterMix (Biomed, China), and 10 μM of each primer. The following PCR primers were used: ACT-512F (5'-ATGTGCAAGGCCGGTTTCGC-3') and ACT-783R (5'-TACGAGTCCTTCTGGCCCAT-3'), Bt1a (5'-TTCCCCCGTCTCCACTTCTTCATG-3') and Bt1b (5'-GACGAGATCGTTCATGTTGAATC-3'), and CAL-228F (5'-GAGTTCAAGGAGGCCTTCTCC-3') and CAL-737R (5'-CATCTTTCTGGCCATCATGG-3') (Carbone and Kohn 1999; Glass and Donaldson 1995; Klaubauf et al. 2014). DNA sequencing was performed at Tsingke Biological Technology Company (Beijing, China).

Phylogenetic analysis. DNA sequences were aligned with an online version of MAFFT (<https://mafft.cbrc.jp/alignment/software/>), and the alignments were manually adjusted with MEGA version 6.0.6 (Tokyo Metropolitan University, Tokyo, Japan). Phylogenetic analyses were performed with the individual and combined data sets to test the robustness of each included locus. Combined sequences of three genes (actin, beta-tubulin, and calmodulin) were concatenated with SequenceMatrix 1.7.8 (National University of Singapore, Singapore). The incongruence-length difference for these combined data (1,487 characters) was tested using the partition homogeneity test (PHT) (Farris et al. 1995), performed with 100 replicates and implemented in PAUP* version 4.0 alpha (Swofford 2003). Maximum parsimony trees were constructed with PAUP* version 4.0 alpha for all data sets as described previously (Crous et al. 2006) and they were generated by heuristic searches with a reference taxon of *Nakataea oryzae* (GenBank accession numbers AF395975, AF396004, and AF396030), and 1,000 bootstrap replicates. *N. oryzae* was originally allocated to the genus *Magnaporthe* and named as *M. salvinii* (Klaubauf et al. 2014; Zhang et al. 2016). The DNA sequences were deposited in GenBank (GenBank accession numbers MH589425 to MH589793). Trees and DNA sequence alignments were deposited in TreeBASE (accession number S24260).

Pathogenicity assays. All isolates were cultured on OTA plates at 26°C for 5 days, and conidiation was analyzed as described previously (Peng and Shishiyama 1988). The pathogenicity test was performed by spraying the conidial suspension with a concentration of 1×10^5 per ml in 0.025% Tween 20 onto 4-week-old seedlings of green bristlegrass, crabgrass, and susceptible rice cultivar LTH as described previously (Peng and Shishiyama 1988). Two inoculation methods, scratching and spraying, were used to test the pathogenicity on rice. Inoculated plants were incubated in a humid and dark chamber at 26°C for 24 h and moved to a greenhouse with 12-h light/

12-h dark conditions for 6 days. For each isolate, three replicate plastic pots of plants were used for the pathogenicity assay with three independent experiments. Infections were evaluated in two ways: (i) no reaction in three independent experiments or (ii) visible blast lesions on leaves observed in at least two independent experiments. To observe conidia generated from blast lesions, diseased leaves were incubated on water agar plates at 26°C with continuous light for at least 36 h and were observed under an Olympus IX71 microscope (Olympus, Tokyo, Japan).

Morphological examination. To characterize conidial morphology, conidial length and width were observed under an Olympus IX71 microscope, measured with Image-Pro Plus version 6.0.0 software (Media Cybernetics, Silver Spring, MD), and statistically analyzed by IBM SPSS version 17.0 software (IBM, Chicago, IL). At least 40 conidia were measured for each isolate. The length of a conidium from apex to base was defined as the conidial length, and the widest part of each conidium was defined as the conidial width. The mean $\pm$ standard deviation was determined, and significant differences of all data were calculated with the Tukey test.

RESULTS

Phylogenetic analysis of *P. oryzae* and *P. grisea*. The single most parsimonious trees were generated from a combined sequence of three genes (actin, beta-tubulin, and calmodulin; Fig. 1), and individual genes (Supplementary Figs. S2, S3, and S4) from 123 isolates. In addition, sequence data of 10 isolates used by Couch and Kohn (2002) and nine isolates used by Choi et al. (2013) were included as controls (gray characters in Fig. 1). In the PHT, incongruence ($P < 0.05$) was found among phylogenies inferred from the actin, beta-tubulin, and calmodulin genes. On the whole, the tree in each phylogeny had the same topology, and two clades with high bootstrap values were resolved in phylogenies inferred for *P. oryzae* and *P. grisea*. Sixteen isolates from *D. sanguinalis*, four isolates from *S. viridis*, the isolate W95-12 (the control strain from *S. viridis* used in Choi et al. 2013), and the remaining eight isolates (the control strains from *D. sanguinalis* used in Couch and Kohn 2002 and Choi et al. 2013) were clustered within the clade of *P. grisea*, and they were distinguishable from other isolates from *O. sativa* and other grasses. However, 20 *D. sanguinalis* isolates and the isolates W06-20 and W95-06 (the control strains from *D. sanguinalis* used in Choi et al. 2013) belonged to the clade of *P. oryzae* from *O. sativa*, *S. viridis*, *Eleusine indica*, *C. rotundus*, *S. italica*, and *Eriochloa villosa*. In the maximum parsimony tree inferred from the actin sequence, four *Eriochloa villosa* isolates in the clade of *P. oryzae* were clustered within a sub-branch and distinguishable from other *P. oryzae* isolates. In the tree inferred from beta-tubulin, three goose grass isolates and four rice isolates were clustered within a sub-branch and distinguishable from other *P. oryzae* isolates. In the trees inferred from the combined sequences of three genes (Fig. 1) and inferred from the calmodulin gene, three green bristlegrass isolates (HuBG-1, HuBG-3, and YNG-1) were clustered into a clade with 10 rice isolates, control *D. sanguinalis* isolates W06-20 and W95-06, and control *O. sativa* isolates BK-6, A264, and YHL-873. Therefore, from the supplementary figures we found that three green bristlegrass isolates, HuBG-1, HuBG-3, and YNG-1, had closer phylogenetic relationships with rice isolates, *D. sanguinalis* isolates W06-20 and W95-06, when compared with other grass isolates.

Pathogenicity of crabgrass and green bristlegrass isolates on the host. Phylogenetic analysis suggested that 20 and 16 isolates from crabgrass were *P. oryzae* and *P. grisea*, respectively, and 44 and four isolates from green bristlegrass were *P. oryzae* and *P. grisea*, respectively. To confirm this result, we selected SZG-2 (*P. oryzae* isolate) and DGG-3 (*P. grisea* isolate) to inoculate their host, green bristlegrass, and BJM-1 (*P. oryzae* isolate) and BJM-8 (*P. grisea* isolate) to inoculate their host, crabgrass, by the spray inoculation approach. Typical blast disease lesions with

subfusiform or dotted shapes were observed on leaves of crabgrass and green bristlegrass at 7 days postinoculation (Fig. 2A). Moreover, abundant conidiophores and conidia formed on the lesions after incubation on water agar for at least 36 h (Fig. 2A). Four isolates were isolated from the lesions and named SZG-2-G, DGG-3-G, BJM-1-C, and BJM-8-C. The colonial morphology of these four isolates and the original isolates SZG-2, DGG-3, BJM-1, and BJM-8 were the same (Fig. 2B), respectively, after 6 days of inoculation on the OTA plates. The results showed that these four isolates maintained their pathogenicity to their own hosts.

Cross-infectivity of *P. oryzae* isolates from rice and green bristlegrass. To confirm the potential pathogenicity of the 123 isolates to rice, a pathogenicity test was performed for the 123 isolates. We found that three *P. oryzae* isolates (YNG-1, HuBG-1, and HuBG-3) from green bristlegrass produced blast lesions on rice susceptible cultivar LTH (Fig. 3A and B) in addition to 10 rice isolates by both scratching and spraying inoculation approaches. However, the other 110 nonrice isolates did not produce blast lesions under the same condition. To further confirm the

pathogenicity on rice, symptomatic leaves were incubated on water agar for 36 h. Gray mycelia then grew over the lesions on the leaves, and conidia were produced at the tips of conidiophores on the infected leaves (Fig. 3C to F).

We also tested the pathogenicity of 10 rice isolates to green bristlegrass for investigating the cross-infectivity potential between rice and green bristlegrass. Six rice isolates (P131, 11-856-35-1, 11-856-37-1, 14-1-1, DG-3, and PJ-61-1) formed blast lesions on leaves of green bristlegrass (Fig. 4A). However, the other four rice isolates failed to form blast lesions on green bristlegrass. Similarly, gray mycelia grew over the lesions on the leaves, and conidia were produced at the tips of conidiophores by incubating the symptomatic leaves on water agar for 36 h (Fig. 4B to G). Taken together, these findings suggest that isolates from rice and green bristlegrass have cross-infectivity capacity.

Conidial morphology of all of the isolates. Conidia of all 123 isolates were pyriform with two septa and three cells, and their mean lengths, widths, and length/width ratios are shown in Supplementary Table S1. Conidial morphology of representative

Fig. 1. The maximum parsimony tree of *Pyricularia oryzae* and *P. grisea* inferred from combined sequences. Labels on the phylogeny are as follows from left to right: isolate number, host, and phylogenetic group. Sequences used in a previous study (Choi et al. 2013; Couch and Kohn 2002) were integrated as controls (gray characters). The bootstrap support values from 1,000 replicates are indicated at the nodes and the scale bar represents the number of changes. The tree length is 1,635. The consistency index (CI), the retention index (RI), and the rescaled consistency index (RC) are 0.917, 0.993, and 0.900, respectively.

isolates from rice, green bristlegrass, foxtail millet, wild millet, goose grass, and crabgrass are shown in Figure 5A to F, respectively. Among the mean length/width ratios values of all of the isolates, the length/width ratio of PJ-61-1 from rice was 2.05 ± 0.06 as the minimal one (Fig. 5A), whereas the length/width ratio of GYM-6 from crabgrass was 3.69 ± 0.05 as the maximal one (Fig. 5F). The length/width ratios of 19 *P. grisea* isolates (ranging from 3.08 ± 0.03 to 3.69 ± 0.05) were obviously larger than those of other isolates and were >3.0 , but the length/width ratio of *P. grisea* isolate SZM-6 from crabgrass was 2.99 ± 0.05 . The length/width ratios of the two *P. oryzae* isolates SZN-9 (Fig. 5E) and SZN-4 from goose grass were 3.13 ± 0.06 and 3.04 ± 0.04 , respectively. However, the length/width ratios of other *P. oryzae* isolates were <3.0 . The standard

deviations of length, width, and length/width ratio of all 123 isolates were ≤ 0.39 , 0.28 , and 0.11 , respectively. None of the length value for each isolate was significantly different ($P > 0.05$) or the width or length/width ratio value for each isolate. However, the difference among the lengths of all the isolates was significant ($P < 0.05$), and the differences among the widths or the length/width ratios of all of the isolates were significant ($P < 0.05$).

To further demonstrate the differences in conidial lengths, widths, and length/width ratios of the 123 isolates, isolates from the same host were treated as a group. *P. grisea* isolates from crabgrass and green bristlegrass are indicated as crabgrass (G) and green bristlegrass (G), respectively. *P. oryzae* isolates from crabgrass and green bristlegrass are indicated as crabgrass (O) and green

Fig. 2. Pathogenicity of crabgrass and green bristlegrass isolates on the host. **A**, Disease lesion on green bristlegrass infected by SZG-2 and DGG-3 and on crabgrass infected by BJM-1 and BJM-8; conidiation structure of SZG-2, DGG-3, BJM-1, and BJM-8 on disease lesions of their host leaves. Bar = 50 μ m. **B**, Colony morphology of *Pyricularia oryzae* and *P. grisea* isolates.

bristlegrass (O), respectively. Finally, eight different groups were constituted based on hosts and species, and the sedge isolate HuNS-1 was deleted from this analysis. Their conidial lengths, widths, and length/width ratios were then calculated. As shown in Table 1, we found that conidial lengths and length/width ratios for isolates from the crabgrass (G) and green bristlegrass (G) groups were larger than others but their conidial widths were smaller than others. However, there were no significant differences in conidial length, width, and length/width ratio of isolates from the rice, goose grass, foxtail millet, wild millet, crabgrass (O), and green bristlegrass (O) groups.

DISCUSSION

P. oryzae is the most important causal agent of blast disease on a broad range of hosts, including rice and other species of Gramineae. *P. oryzae* has been known to infect more than 50 host species of Gramineae to date (Choi et al. 2013; Gladioux et al. 2018). In this study, a unique set of 102 *P. oryzae* isolates and 20 *P. grisea* isolates from six different species of gramineous plants and one *P. oryzae* isolate from the sedge family was gathered. Their phylogeny and pathogenicity were analyzed to uncover the differences among them. To our knowledge, this is the first analysis report for *P. oryzae*

Fig. 3. Pathogenicity test of *Pyricularia oryzae* and *P. grisea* isolates on rice. Typical blast lesions were observed on the leaves after 7 days by **A**, scratching and **B**, spraying inoculation; SZG-2 was a negative control. Conidiophore and conidia on the lesions caused by isolates **C**, P131, **D**, HuBG-3, **E**, HuBG-1, and **F**, YNG-1. Bar = 50 μ m.

Fig. 4. Pathogenicity test of rice isolates on green bristlegrass. **A**, Typical blast lesions were observed on the leaves after 7 days by spraying inoculation; 22-1-2 was a negative control. Conidiophore and conidia on the lesions caused by isolates **B**, P131, **C**, 11-856-35-1, **D**, PJ-61-1, **E**, 11-856-37-1, **F**, DG-3, and **G**, 14-1-1. Bar = 50 μ m.

and *P. grisea* from different hosts in China, and the following key findings can improve our understanding of *P. oryzae* and *P. grisea*. Multilocus phylogenetic analysis has been widely used in many studies (Choi et al. 2013; Couch and Kohn 2002; Crous et al. 2006; Klaubauf et al. 2014). In this study, multilocus phylogenetic analysis was performed for the actin, beta-tubulin, and calmodulin genes. The PHT was described as the incongruence-length

difference test by Farris et al. (1995). Incongruence ($P < 0.05$) was found among phylogenies inferred from the actin, beta-tubulin, and calmodulin genes in this study. However, according to related reports, gene alignments should always be combined together, even though gene alignments are not congruent (Farris et al. 1995; Irestedt et al. 2004). The maximum parsimony trees, inferred from actin and beta-tubulin, clustered 123 isolates into two clades but did

Fig. 5. Conidial morphology of *Pyricularia oryzae* and *P. grisea* isolates from **A**, rice, **B**, green bristlegrass, **C**, foxtail millet, **D**, wild millet, **E**, goose grass, and **F**, crabgrass. Bar = 10 μ m. The values in the figure are the means $\pm$ standard deviations of length, width and length/width ratio of isolates; the unit of length and width is in micrometers.

TABLE 1. Conidial length, width, and length/width ratio of *Pyricularia oryzae* and *P. grisea* isolates from different hosts^a

Host (no. of isolates)	Conidial size (μ m)		Length/width ratio
	Length	Width	
Rice (10)	22.07 ± 1.79	9.30 ± 0.39	2.39 ± 0.19
Foxtail millet (12)	24.48 ± 2.10	9.26 ± 0.38	2.70 ± 0.14
Goosegrass (12)	25.69 ± 1.26	9.34 ± 0.34	2.78 ± 0.15
Crabgrass (O) (20)	23.57 ± 0.78	8.77 ± 0.27	2.70 ± 0.10
Crabgrass (G) (16)	29.06 ± 1.20	8.49 ± 0.31	3.40 ± 0.21
Green bristlegrass (O) (44)	22.98 ± 1.70	8.87 ± 0.47	2.62 ± 0.14
Green bristlegrass (G) (4)	29.25 ± 0.60	8.73 ± 0.15	3.37 ± 0.04
Wild millet (4)	25.48 ± 0.53	9.11 ± 0.13	2.81 ± 0.02

^a Values are presented as the mean $\pm$ standard deviation. *P. oryzae* isolates from crabgrass and green bristlegrass are indicated as crabgrass (O) and green bristlegrass (O). *P. grisea* isolates from crabgrass and green bristlegrass are indicated as crabgrass (G) and green bristlegrass (G).

not resolve the phylogeny of three green bristlegrass isolates (YNG-1, HuBG-1, and HuBG-3), which could infect rice. In 2002, *P. grisea* was nomenclaturally defined to isolates from crabgrass (Couch and Kohn 2002). However, in 2013, both *P. grisea* and *P. oryzae* isolates were isolated from crabgrass in Korea. Moreover, some *P. oryzae* isolates showed cross-infectivity between rice and crabgrass. The control crabgrass *P. oryzae* isolates W06-20 and W95-06 could infect rice, and Southern blot analysis with the MGR586 sequence also confirmed that W95-06 had originated from rice (Choi et al. 2013). In this study, *P. oryzae* and *P. grisea* isolates were also isolated from crabgrass. However, both *P. oryzae* and *P. grisea* isolates from crabgrass are nonpathogenic to rice, according to the results of pathogenicity tests. Moreover, according to the maximum parsimony trees inferred from the combined sequences of three genes (Fig. 1) and inferred from calmodulin gene, HuBG-1, HuBG-3, and YNG-1, had closer phylogenetic relationships with isolates from rice, control isolates W06-20 and W95-06 from crabgrass. In previous reports, some different species of Pyriculariaceae are pathogenic to sedge family plants. For example, *C. iria* and *C. rotundus* are susceptible to *Pseudopyricularia cyperi* isolates, and *C. brevifolius* can be invaded by *P. ctenantheicola* isolates (Klaubauf et al. 2014). In this study, one *P. oryzae* isolate, HuNS-1, was isolated from *C. rotundus*.

We tried to find the differences between *P. oryzae* and *P. grisea* on conidial morphology and found that the conidial length/width ratio was more than 3.0 in 19 *P. grisea* isolates and two *P. oryzae* isolates. However, the conidial length/width ratio was <3.0 in 101 *P. oryzae* isolates and one *P. grisea* isolate. However, this finding cannot be regarded as a morphological difference between *P. oryzae* and *P. grisea*, and more isolates should be further investigated.

In this study, the pathogenicity of all 123 isolates on rice was tested. One-hundred and thirteen nonrice isolates from six different hosts, collected from 12 different locations that were mostly distributed over rice-producing areas of Northeast and South China, but only three *P. oryzae* isolates (YNG-1, HuBG-1, and HuBG-3) from green bristlegrass were virulent to rice besides of 10 rice isolates. This finding may suggest a closer relationship of these three green bristlegrass isolates to rice isolates than to isolates from other grasses. Green bristlegrass is a model system for C4 photosynthesis, for analysis of bioenergy traits, and for the study of grass genomics and evolution. It is native to Eurasia but is now a ubiquitous weed in China (Hu et al. 2018; Huang et al. 2014). In this study, the largest number of isolates was from green bristlegrass, as 44 *P. oryzae* isolates and four *P. grisea* isolates were isolates from green bristlegrass, and the pathogenicity of 10 rice isolates on green bristlegrass was also tested. We found that six of 10 rice isolates (P131, 11-856-35-1, 11-856-37-1, 14-1-1, DG-3, and PJ-61-1) can cause blast lesions on leaves of green bristlegrass.

These results suggest that cross-infectivity exists between rice and green bristlegrass. Most *P. oryzae* isolates from rice were pathogenic to green bristlegrass. Given cross-infectivity on rice and green bristlegrass, we hypothesized that green bristlegrass could be a shelter for *P. oryzae* in rice fields. Some *P. oryzae* isolates from green bristlegrass may be the primary inoculum of the rice blast. Cross-infectivity between rice and green bristlegrass could result in variation and rapid adaptation to a new host of *P. oryzae*. Moreover, infection of a novel host is a major route for disease emergence by pathogenic fungi (Gladieux et al. 2018). Therefore, it is important to eradicate weeds around paddy fields.

P. oryzae is a model fungus for understanding the interactive mechanisms of plant pathogenic fungi and shows variability with respect to host range and cultivar specificity (Chen et al. 2013; Leung et al. 1988; Yoshida et al. 2016). Host-range expansion and host shifts are the main reasons for fungal populations or frequent species evolution (Chiapello et al. 2015). The emergence of wheat blast disease in Brazil was the result of host shifts and host expansion capabilities of *P. oryzae* (Inoue et al. 2017; Maciel et al. 2014). In the last decade, it has been reported that some *P. grisea*

isolates from Korea can infect rice and green bristlegrass (Choi et al. 2013), some *P. grisea* isolates from India can infect barnyard grass and perennial ryegrass (Klaubauf et al. 2014). In our study, four *P. grisea* isolates from China can also infect green bristlegrass. Moreover, *P. oryzae* can infect crabgrass, and this phenomenon was also observed in some isolates from Korea (Choi et al. 2013) and China (in this study). Asia is the geographic origin of cultivated rice (*O. sativa*), which is considered to have been domesticated from wild rice (*O. rufipogon*) thousands of years ago (Cheng et al. 2003; Huang et al. 2012; Khush 1997). There are more complex host conditions and more complicated ecological environments and genetic diversity within domesticated populations and wild relatives in Asia than in other geographic areas (Couch et al. 2005; Klaubauf et al. 2014; Wang et al. 2018). Therefore, in Asia, host shifts or host-range expansion in nature may occur in *P. oryzae* and *P. grisea*.

Many whole-genome sequences of *P. oryzae* and *P. grisea* isolates have been reported. More information was obtained from whole-genome sequences and this information has made more detailed classification of isolates from different hosts (Gladieux et al. 2018). In this study, 113 nonrice isolates and nine rice isolates from 12 and four different locations in China, respectively, and one rice isolate from Japan were compared, and considerable morphology and sequence discrepancy existed in all of these isolates. *P. oryzae* showed various pathotypes based on several pathogenicity tests (Choi et al. 2013; Dayakar et al. 2000; Urashima et al. 1993; Zhong et al. 2016). Phylogenetic analyses indicated that abundant genetic diversity exists in *P. oryzae* from different hosts or locations (Chiapello et al. 2015; Gladieux et al. 2018; Hirata et al. 2007; Klaubauf et al. 2014). A driving force of fungi population and species evolution is rapidly adapting to a new host (Zhong et al. 2016). To this end, sequencing whole genomes of *P. oryzae* and *P. grisea* isolates from rice and grasses may tell us why *P. oryzae* and *P. grisea* can adapt to new hosts rapidly, and better classification results will be obtained from the whole-genome sequences of the same species from different hosts.

Taken together, our results showed that isolates from rice, goose grass, foxtail millet, wild millet, and sedge belong to *P. oryzae*, and isolates from green bristlegrass and crabgrass consist of *P. oryzae* and *P. grisea*. Moreover, *P. oryzae* isolates showed cross-infectivity between rice and green bristlegrass. Therefore, green bristlegrass may play a role in the rice blast cycle in paddy fields.

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LITERATURE CITED

- Bidochka, M. J., Small, C. L., and Spironello, M. 2005. Recombination within sympatric cryptic species of the insect pathogenic fungus *Metarhizium anisopliae*. *Environ. Microbiol.* 7:1361-1368.
- Carbone, I., and Kohn, L. M. 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* 91:553-556.
- Chen, C., Lian, B., Hu, J., Zhai, H., Wang, X., Venu, R. C., Liu, E., Wang, Z., Chen, M., Wang, B., Wang, G. L., Wang, Z., and Mitchell, T. K. 2013. Genome comparison of two *Magnaporthe oryzae* field isolates reveals genome variations and potential virulence effectors. *BMC Genomics* 14:887.
- Chen, X. L., Yang, J., and Peng, Y. L. 2011. Large-scale insertional mutagenesis in *Magnaporthe oryzae* by *Agrobacterium tumefaciens*-mediated transformation. *Methods Mol. Biol.* 722:213-224.
- Cheng, C., Motohashi, R., Tsuchimoto, S., Fukuta, Y., Ohtsubo, H., and Ohtsubo, E. 2003. Polyphyletic origin of cultivated rice: Based on the interspersed pattern of SINEs. *Mol. Biol. Evol.* 20:67-75.
- Chiapello, H., Mallet, L., Guérin, C., Aguilera, G., Amselem, J., Kroj, T., Ortega-Abboud, E., Lebrun, M. H., Henrissat, B., Gendral, A., Rodolphe, F., Tharreau, D., and Fournier, E. 2015. Deciphering genome content and evolutionary relationships of isolates from the fungus *Magnaporthe oryzae* attacking different host plants. *Genome Biol. Evol.* 7:2896-2912.

- Choi, J., Park, S. Y., Kim, B. R., Roh, J. H., Oh, I. S., Han, S. S., and Lee, Y. H. 2013. Comparative analysis of pathogenicity and phylogenetic relationship in *Magnaporthe grisea* species complex. *PLoS One* 8:2775-2783.
- Choi, W. B., Chun, S. J., and Lee, Y. H. 1996. Host range of Korean isolates of *Magnaporthe grisea*. *J. Plant Pathol.* 12:453-454.
- Couch, B. C., Fudal, I., Lebrun, M. H., Tharreau, D., Valent, B., van Kim, P., Nottéghem, J. L., and Kohn, L. M. 2005. Origins of host-specific populations of the blast pathogen *Magnaporthe oryzae* in crop domestication with subsequent expansion of pandemic clones on rice and weeds of rice. *Genetics* 170:613-630.
- Couch, B. C., and Kohn, L. M. 2002. A multilocus gene genealogy concordant with host preference indicates segregation of a new species, *Magnaporthe oryzae*, from *M. grisea*. *Mycologia* 94:683-693.
- Crous, P. W., Wingfield, M. J., Mansilla, J. P., Alfenas, A. C., and Groenewald, J. Z. 2006. Phylogenetic reassessment of *Mycosphaerella* spp. and their anamorphs occurring on *Eucalyptus*. II. *Stud. Mycol.* 55:99-131.
- Cruse, M., Telerant, R., Gallagher, T., Lee, T., and Taylor, J. W. 2002. Cryptic species in *Stachybotrys chartarum*. *Mycologia* 94:814-822.
- Dayakar, B., Narayanan, N., and Gnanamanickam, S. 2000. Cross-compatibility and distribution of mating type alleles of the rice blast fungus *Magnaporthe grisea* in India. *Plant Dis.* 84:700-704.
- Dean, R. A., Talbot, N. J., Ebbole, D. J., Farman, M. L., Mitchell, T. K., Orbach, M. J., Thon, M., Kulkarni, R., Xu, J. R., Pan, H., Read, N. D., Lee, Y. H., Carbone, I., Brown, D., Oh, Y. Y., Donofrio, N., Jeong, J. S., Soanes, D. M., Djonovic, S., Kolomiets, E., Rehmeier, C., Li, W., Harding, M., Kim, S., Lebrun, M. H., Bohnert, H., Coughlan, S., Butler, J., Calvo, S., Ma, L. J., Nicol, R., Purcell, S., Nussbaum, C., Galagan, J. E., and Birren, B. W. 2005. The genome sequence of the rice blast fungus *Magnaporthe grisea*. *Nature* 434:980-986.
- Fang, W. W., Liu, C. C., Zhang, H. W., Xu, H., Zhou, S., Fang, K. X., Peng, Y. L., and Zhao, W. S. 2018. Selection of differential isolates of *Magnaporthe oryzae* for postulation of blast resistance genes. *Phytopathology* 108: 878-884.
- Farris, J. S., Kallersjö, M., Kluge, A. G., and Bult, C. 1995. Constructing a significance test for incongruence. *Syst. Biol.* 44:570-572.
- Gladieux, P., Condon, B., Ravel, S., Soanes, D., Maciel, J. L. N., Nhani, A., Jr., Chen, L., Terauchi, R., Lebrun, M. H., Tharreau, D., Mitchell, T., Pedley, K. F., Valent, B., Talbot, N. J., Farman, M., and Fournier, E. 2018. Gene flow between divergent cereal- and grass-specific lineages of the rice blast fungus *Magnaporthe oryzae*. *MBio* 9:e01219-17.
- Glass, N. L., and Donaldson, G. C. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Appl. Environ. Microbiol.* 61:1323-1330.
- Gnanamanickam, S. S. 2009. Rice and its importance to human life. Pages 1-11 in: *Biological Control of Rice Diseases: Progress in Biological Control*. Springer, Dordrecht, The Netherlands.
- Harrington, T. C., and Rizzo, D. M. 1999. Defining species in the fungi. Pages 43-71 in: *Structure and Dynamics of Fungal Populations*. J. J. Worrall, ed. Kluwer Academic, Dordrecht, The Netherlands.
- Hebert, T. T. 1971. The perfect stage of *Pyricularia grisea*. *Phytopathology* 61:83-87.
- Hirata, K., Kusaba, M., Chuma, I., Osue, J., Nakayashiki, H., Mayama, S., and Tosa, Y. 2007. Speciation in *Pyricularia grisea* inferred from multilocus phylogenetic analysis. *Mycol. Res.* 111:799-808.
- Hu, H., Mauro-Herrera, M., and Doust, A. N. 2018. Domestication and improvement in the model C4 grass, *Setaria*. *Front. Plant Sci.* 9:719.
- Huang, P., Feldman, M., Schroder, S., Bahri, B. A., Diao, X., Zhi, H., Estep, M., Baxter, I., Devos, K. M., and Kellogg, E. A. 2014. Population genetics of *Setaria viridis*, a new model system. *Mol. Ecol.* 23:4912-4925.
- Huang, X., Kurata, N., Wei, X., Wang, Z. X., Wang, A., Zhao, Q., Zhao, Y., Liu, K., Lu, H., Li, W., Guo, Y., Lu, Y., Zhou, C., Fan, D., Weng, Q., Zhu, C., Huang, T., Zhang, L., Wang, Y., Feng, L., Furuumi, H., Kubo, T., Miyabayashi, T., Yuan, X., Xu, Q., Dong, G., Zhan, Q., Li, C., Fujiyama, A., Toyoda, A., Lu, T., Feng, Q., Qian, Q., Li, J., and Han, B. 2012. A map of rice genome variation reveals the origin of cultivated rice. *Nature* 490:497-501.
- Hughes, D. F., Tolley, K. A., Behangana, M., Lukwago, W., Menegon, M., Dehling, J. M., Stipala, J., Tilbury, C. R., Khan, A. M., Kusamba, C., and Greenbaum, E. 2018. Cryptic diversity in *Rhampholeon boulengeri* (Sauria Chamaeleonidae), a pygmy chameleon from the Albertine Rift biodiversity hotspot. *Mol. Phylogenet. Evol.* 122:125-141.
- Inoue, Y., Vy, T. T. P., Yoshida, K., Asano, H., Mitsuoka, C., Asuke, S., Anh, V. L., Cumagun, C. J. R., Chuma, I., Terauchi, R., Kato, K., Mitchell, T., Valent, B., Farman, M., and Tosa, Y. 2017. Evolution of the wheat blast fungus through functional losses in a host specificity determinant. *Science* 357:80-83.
- Irestedt, M., Fjeldså, J., Nylander, J. A., and Ericson, P. G. 2004. Phylogenetic relationships of typical antbirds (Thamnophilidae) and test of incongruence based on Bayes factors. *BMC Evol. Biol.* 4:23.
- Khush, G. S. 1997. Origin, dispersal, cultivation and variation of rice. *Plant Mol. Biol.* 35:25-34.
- Klaubauf, S., Tharreau, D., Fournier, E., Groenewald, J. Z., Crous, P. W., de Vries, R. P., and Lebrun, M. H. 2014. Resolving the polyphyletic nature of *Pyricularia* (Pyrulariaceae). *Stud. Mycol.* 79:85-120.
- Leung, H., Borromeo, E. S., Bernardo, M. A., and Nottéghem, J. L. 1988. Genetic analysis of virulence in the rice blast fungus *Magnaporthe grisea*. *Phytopathology* 78:1227-1233.
- Maciel, J. L., Ceresini, P. C., Castroagudín, V. L., Zala, M., Kema, G. H., and McDonald, B. A. 2014. Population structure and pathotype diversity of the wheat blast pathogen *Magnaporthe oryzae* 25 years after its emergence in Brazil. *Phytopathology* 104:95-107.
- Okagaki, L. H., Sailsbery, J. K., Eyre, A. W., and Dean, R. A. 2016. Comparative genome analysis and genome evolution of members of the Magnaporthaceae family of fungi. *BMC Genomics* 17:135.
- Peng, Y. L., and Shishiyama, J. 1988. Temporal sequence of cytological events in rice leaves infected with *Pyricularia oryzae*. *Can. J. Bot.* 66:730-735.
- Rossman, A. Y., Howard, R. J., and Valent, B. 1990. *Pyricularia grisea*, the correct name for the rice blast disease fungus. *Mycologia* 82:509-512.
- Saccardo, P. A. 1880. *Conspectus generum fungorum italiae inferiorum*. *Michelia* 2:1-135.
- Sesma, A., and Osbourn, A. E. 2004. The rice leaf blast pathogen undergoes developmental processes typical of root-infecting fungi. *Nature* 431:582-586.
- Shirke, M. D., Mahesh, H. B., and Gowda, M. 2016. Genome-wide comparison of *Magnaporthe* species reveals a host-specific pattern of secretory proteins and transposable elements. *PLoS One* 11:e0162458.
- Sprague, R. 1950. *Moniliales*. Pages 414-417 in: *Diseases of Cereals and Grasses in North America*. Ronald Press Company Limited, New York, NY.
- Steenkamp, E. T., Wingfield, B. D., Desjardins, A. E., Marasas, W. F. O., and Wingfield, M. J. 2002. Cryptic speciation in *Fusarium subglutinans*. *Mycologia* 94:1032-1043.
- Swofford, D. L. 2003. PAUP* [Phylogenetic Analysis Using Parsimony (*and Other Methods)], Version 4. Sinauer Associates, Sunderland, MA.
- Talbot, N. J. 2003. On the trail of a cereal killer: Exploring the biology of *Magnaporthe grisea*. *Annu. Rev. Microbiol.* 57:177-202.
- Taylor, J. W., and Fisher, M. C. 2003. Fungal multilocus sequence typing – it's not just for bacteria. *Curr. Opin. Microbiol.* 6:351-356.
- Taylor, J. W., Jacobson, D. J., Kroken, S., Kasuga, T., Geiser, D. M., Hibbett, D. S., and Fisher, M. C. 2000. Phylogenetic species recognition and species concepts in fungi. *Fungal Genet. Biol.* 31:21-32.
- Urashima, A. S., Igarashi, S., and Kato, H. 1993. Host range, mating type, and fertility of *Pyricularia grisea* from wheat in Brazil. *Plant Dis.* 77:1211-1216.
- Valent, B., and Chumley, F. G. 1991. Molecular genetic analysis of the rice blast fungus, *Magnaporthe grisea*. *Annu. Rev. Phytopathol.* 29:443-467.
- Wang, W., Mauleon, R., Hu, Z., Chebotarov, D., Tai, S., Wu, Z., Li, M., Zheng, T., Fuentes, R. R., Zhang, F., Mansueto, L., Copetti, D., Sanciango, M., Palis, K. C., Xu, J., Sun, C., Fu, B., Zhang, H., Gao, Y., Zhao, X., Shen, F., Cui, X., Yu, H., Li, Z., Chen, M., Detras, J., Zhou, Y., Zhang, X., Zhao, Y., Kudma, D., Wang, C., Li, R., Jia, B., Lu, J., He, X., Dong, Z., Xu, J., Li, Y., Wang, M., Shi, J., Li, J., Zhang, D., Lee, S., Hu, W., Poliakov, A., Dubchak, I., Ulat, V. J., Borja, F. N., Mendoza, J. R., Ali, J., Li, J., Gao, Q., Niu, Y., Yue, Z., Naredo, M. E. B., Talag, J., Wang, X., Li, J., Fang, X., Yin, Y., Glaszmann, J. C., Zhang, J., Li, J., Hamilton, R. S., Wing, R. A., Ruan, J., Zhang, G., Wei, C., Alexandrov, N., McNally, K. L., Li, Z., and Leung, H. 2018. Genomic variation in 3,010 diverse accessions of Asian cultivated rice. *Nature* 557:43-49.
- Xu, J. R., and Hamer, J. E. 1996. MAP kinase and cAMP signaling regulate infection structure formation and pathogenic growth in the rice blast fungus *Magnaporthe grisea*. *Genes Dev.* 10:2696-2706.
- Xue, M., Yang, J., Li, Z., Hu, S., Yao, N., Dean, R. A., Zhao, W., Shen, M., Zhang, H., Li, C., Liu, L., Cao, L., Xu, X., Xing, Y., Hsiang, T., Zhang, Z., Xu, J. R., and Peng, Y. L. 2012. Comparative analysis of the genomes of two field isolates of the rice blast fungus *Magnaporthe oryzae*. *PLoS Genet.* 8:e1002869.
- Yaegashi, H., and Udagawa, S. 1978. The taxonomical identity of the perfect state of *Pyricularia grisea* and its allies. *Can. J. Bot.* 56:180-183.
- Yoshida, K., Saunders, D. G., Mitsuoka, C., Natsume, S., Kosugi, S., Saitoh, H., Inoue, Y., Chuma, I., Tosa, Y., Cano, L. M., Kamoun, S., and Terauchi, R. 2016. Host specialization of the blast fungus *Magnaporthe oryzae* is associated with dynamic gain and loss of genes linked to transposable elements. *BMC Genomics* 17:370.
- Zhang, N., Luo, J., Rossman, A. Y., Aoki, T., Chuma, I., Crous, P. W., Dean, R., de Vries, R. P., Donofrio, N., Hyde, K. D., Lebrun, M. H., Talbot, N. J., Tharreau, D., Tosa, Y., Valent, B., Wang, Z., and Xu, J. R. 2016. Generic names in Magnaporthales. *IMA Fungus* 7:155-159.
- Zhong, Z., Norvienyeku, J., Chen, M., Bao, J., Lin, L., Chen, L., Lin, Y., Wu, X., Cai, Z., Zhang, Q., Lin, X., Hong, Y., Huang, J., Xu, L., Zhang, H., Chen, L., Tang, W., Zheng, H., Chen, X., Wang, Y., Lian, B., Zhang, L., Tang, H., Lu, G., Ebbole, D. J., Wang, B., and Wang, Z. 2016. Directional selection from host plants is a major force driving host specificity in *Magnaporthe* species. *Sci. Rep.* 6:25591.