Characterization of Field Isolates of *Magnaporthe oryzae* with Mating Type, DNA Fingerprinting, and Pathogenicity Assays

Jinbin Li, Agricultural Environment and Resources Research Institute, Yunnan Academy of Agricultural Sciences (YAAS), Kunming, Yunnan Province 650205, China; **Lin Lu**, Flower Research Institute, YAAS, Kunming, China; **Yulin Jia**, United States Department of Agriculture–Agriculture Research Service, Dale Bumpers National Rice Research Center, Stuttgart, AR; **Qun Wang**, Agricultural Environment and Resources Research Institute, YAAS, Kunming, China; **Yoshimichi Fukuta**, Tropical Agriculture Research Front, Japan International Research Center for Agricultural Sciences, Ishigaki, Okinawa 907-0002, Japan; and **Chengyun Li**, The Ministry of Education Key Laboratory for Agricultural Biodiversity and Pest Management, Yunnan Agricultural University, Kunming, Yunnan Province 650201, China

Abstract

Li, J., Lu, L., Jia, Y., Wang, Q., Fukuta, Y., and Li, C. 2016. Characterization of field isolates of *Magnaporthe oryzae* with mating type, DNA fingerprinting, and pathogenicity assays. *Plant Dis.* 100:298-303.

Due to the harmful nature of the rice blast fungus, *Magnaporthe oryzae*, it is beneficial to characterize field isolates to help aid in the deployment of resistance (*R*) genes in rice. In the present study, 252 field isolates of *M. oryzae*, collected from rice fields of Yunnan Province in China, were assessed for mating type, DNA fingerprinting, and disease reactions to differential rice lines. In total, 94 isolates (37.3%) were *MAT1-1* and 158 (62.7%) were *MAT1-2* based on polymerase chain reaction assays, and some of them were verified with the tester isolates. All *MAT1-1*

and *MAT1-2* isolates were virulent to some of the International Rice Research Institute–Japan International Research Center for Agricultural Sciences monogenic lines harboring 22 major resistance genes as differential varieties. Three simple-sequence repeat markers were used to examine genetic diversity in all isolates. The existence of regional patterns of genetic diversity, sexual reproduction potential, and pathogenicity suggests that *M. oryzae* populations have been independently asexually adapted in rice fields during crop cultivation.

Blast disease of rice caused by the filamentous ascomycetous fungus *Magnaporthe oryzae* is one of the most challenging diseases worldwide. Rice (*Oryza sativa*) is an important food that feeds half of the world population (Olofsson et al. 1995). However, yield losses due to blast disease have been observed annually, and can be from 10 to 100% under severe epidemic conditions (Ebbola 2007; Talbot 2003).

M. oryzae is known to amplify asexually, and a high degree of pathogenic variation was found in commercial rice fields (Zeigler et al. 1994). The sexuality of *Magnaporthe* spp. was first reported by Hebert (1971), and is controlled by a single locus, *MAT1* (Yoder et al. 1986). The fertility was only possible between individuals of opposite mating types, such as *MAT1-1* and *MAT1-2*. However, the ability of *M. oryzae* isolates to produce a fertile female (perithecia) is apparently controlled by multiple genes that segregate independently of mating type and pathogenicity on different hosts (Kolmer and Ellingboe 1988). High fertility is common in *M. grisea* strains from nonrice hosts (Yaegashi and Nishihara 1976). Thus far, the final sexual reproductive form (teleomorph) was found from isolates of rice, wheat, turfgrass, and finger millet (Consolo et al. 2005; Le et al. 2010; Maciel et al. 2014; Takan et al. 2012; Tredway et al. 2003). The hermaphroditic isolates were found in southern China (Li et al. 1992, 1996) and the Indian Himalayas (Kumar et al. 1999) and were used as tester strains for detecting the mating type of the field isolates (Chao and Ellingboe 1991). In

China, the fertile isolates of both mating types were documented (Hayashi et al. 1997; Li et al. 1996). The two mating types can be found in the same region but the sexual recombination might be a potential mechanism for existing genetic diversity of *M. grisea* (Chen et al. 2004). Moreover, computer simulations predicted that the observed genetic diversity in field isolates was likely due to sexual recombination (Mekwatanakarn et al. 1999). A contemporary pathogenic population of *M. oryzae* was predicted to reproduce sexually in Southeast Asia naturally (Saleh et al. 2012). However, some studies suggest that there were no sexually fertile rice pathogens in commercial rice fields (Devulapalle and Suryanarayanan 1995; Viji and Gnanamanickam 1998).

In addition to mating type, molecular markers such as restriction fragment length polymorphism (Viji et al. 2000), amplified fragment length polymorphism (Takan et al. 2012), random amplified polymorphic DNA (Singh and Kumar 2010), and simple-sequence repeats (SSR) (Brondani et al. 2000; Kaye et al. 2003; Suzuki et al. 2009; Zheng et al. 2008) have been effectively deployed to study genetic identity of *M. oryzae* population structure in a particular ecosystem.

The international differential varieties Raminad Str. 3, Zenith, NP-125, Usen, Dular, Kanto 51, Sha-tiao-tsao, and Caloro have been used to characterize pathotypes for guiding resistant breeding (Atkins et al. 1967; Xing et al. 2013). In China, a set of near isogenic lines (NIL) with five resistance (*R*) genes (*Pib*, *Pik*, *Pik-m*, *Pik-p*, and *Pita-2*) in the susceptible *japonica* landrace variety Lijiangxintuanheigu (LTH) was developed (Ling et al. 2000). In Japan, two sets of differential varieties were developed (Kiyosawa 1981, 1984). A set of NIL with *indica* variety CO39 carrying *Pita*, *Piz-5*, *Pil*, and *Pi31* was developed in the International Rice Research Institute (IRRI), the Philippines (Mackill and Bonman 1992). Under the IRRI–Japan International Research Center for Agricultural Sciences (JIRCAS) collaboration, a set of differential varieties—monogenic lines carrying 24 *R* genes: *Pia*, *Pib*, *Pii*, *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, *Pik-s*, *Pish*, *Pit*, *Pita*, *Pita-2*, *Piz*, *Piz-5*, *Piz-t*, *Pil*, *Pi3*, *Pi5(t)*, *Pi7(t)*, *Pi9(t)*, *Pi11(t)*, *Pi12(t)*, *Pi19(t)*, and *Pi20* in LTH—was released (Kobayashi et al. 2007; Tsunematsu et al. 2000).

The objectives of the present study were to (i) determine the mating type of 252 *M. oryzae* isolates from rice-growing regions in Yunnan, (ii) determine genetic identity of these isolates using SSR

Corresponding authors: C. Li; E-mail: licheng_yun@163.com; and Y. Jia; E-mail: Yulin.Jia@ars.usda.gov

J. Li and L. Lu contributed equally to this report.

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*The e-Xtra logo stands for “electronic extra” and indicates that one supplementary figure and one supplementary table are published online.

Accepted for publication 25 September 2015.

<http://dx.doi.org/10.1094/PDIS-06-15-0660-RE>
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markers, and (iii) evaluate pathogenicity and fitness of these isolates using IRRI monogenic lines. Ultimately, our studies will help to understand the role of sexual recombination in rice blast fungus in Yunnan Province.

Materials and Methods

Fungal isolates. Rice blast isolates were collected from rice-growing regions of Baoshan (BS), Dehong (DH), Honghe (HH), Lijiang (LJ), Kunming (KM), Lincang (LC), Qujing (QJ), Puer (PE), Wenshan (WS), Yuxi (YX), Xishuangbanna (XSBN), and Zhaotong (ZT) prefectures of Yunnan Province, China. Five samples were collected in each block and placed in a sealed envelope. Each sample was placed on moistened sterile filter paper in a 9-cm petri dish, creating a high-humidity environment, and incubated at 25°C for 24 h. Single spores were isolated by the tip of a glass pin under a stereomicroscope, and cultured on potato dextrose agar (PDA; potato at 200 g liter⁻¹, dextrose at 20 g liter⁻¹, and agar at 15 g liter⁻¹; Beijing Solarbio Science and Technology Ltd., Beijing). Single-spored isolates were grown on filter papers on oatmeal agar (OMA; oatmeal at 40 g liter⁻¹, dextrose at 20 g liter⁻¹, and agar at 15 g liter⁻¹; Wako Pure Chemical Industries Ltd., Osaka, Japan), and processed as described by Valent et al. (1986) for long-term storage at -20°C.

Mating type assays. One set of standard isolates were used for testing mating type: isolates 94-64-1b (*MAT1-1*) and 95-23-4a (*MAT1-2*) (Li et al. 2002). Both isolates were collected from rice fields in Yunnan. These two isolates are heterothallic and can produce perithecia readily. Crosses were made with modifications to the method of Valent et al. (1991). Briefly, one field isolate and one pair of tester isolates were placed symmetrically on OMA in a 9-cm petri dish. The plates were incubated at 25°C for 5 to 7 days in the dark, then transferred to continuous white fluorescent light at 20°C. After 15 to 20 days, mature perithecia at the intersection of growth between the mated individuals were evaluated. Fertility was defined as the formation of perithecia producing viable ascospores. The mating type of the isolates was determined in reciprocal crosses, so that 94-64-1b (*MAT1-1*) and 95-23-4a (*MAT1-2*) were used to identify mating type of the isolates. The isolates that failed to mate with any of the two testers were assigned as “undetermined”.

Rice cultivars, fungal culture, and pathogenicity assays. The rice monogenic lines containing 22 major *R* genes (*Pi11(t)* and *Pi20(t)* were excluded due to no seed), developed by the IRRI-JIRCAS collaboration (Tsunematsu et al. 2000), and the susceptible parent LTH were used for pathogenicity assays. In total, 190 single-spored isolates were examined. All isolates were stored at -20°C on filter papers and grown at room temperature (25°C) under blue and white fluorescent light on petri dishes containing OMA for spore production. Five plants per line plus five LTH were examined for disease reactions under homogeneous conditions. Disease reactions were determined using a modified standard pathogenicity assay, as previously described by Jia et al. (2003). Specifically, rice seedlings at the three- to four-leaf stage were placed in plastic bags and spray inoculated with a spore suspension at 1 to 5 × 10⁵ spores ml⁻¹. After inoculation, the plastic bags were sealed for 24 h before removing the plants from the bags. Subsequently, plants were kept in a greenhouse for an additional 6 days. The disease reactions were rated based on lesions on the second youngest leaf using a 0-to-5 scale, with 0 to 2 as resistant and 3 to 5 as susceptible, based on a procedure described by RoyChowdhury et al. (2011).

DNA preparation and mating type polymerase chain reaction. Fungal isolates were grown in complete liquid media (LB; tryptone at 10 g liter⁻¹, yeast extract at 5 g liter⁻¹, and NaCl at 10 g liter⁻¹;

Beijing Solarbio Science and Technology Ltd.) at 25°C for 6 to 8 days to produce mycelia under dark conditions. DNA was then isolated from the mycelia using the cetyltrimethylammonium bromide method (Tai and Tanksley 1990). *MAT1-1*- (MAT1-1 F and R) and *MAT1-2*- (MAT1-2 F and R) specific primers designed based on known sequences (Kang et al. 1994) were used to identify the mating type (Takan et al. 2012). The gene encoding for the mating type was amplified by polymerase chain reaction (PCR) using the following primers: *MAT1-1* F (5'-TGCGAATGCCTACATCCTGTACCGC-3') and *MAT1-1* R (5'-CGCTTCTGAGGAACGCAGACGACC-3') amplifying at 960 bp, and *MAT1-2* F (5'-TCTGCTTGAAGCTGCAATACAACGG-3') and *MAT1-2* R (5'-CATGCGAGGGTGCCATGATAGGC-3') amplifying at 802 bp (Takan et al. 2012). Specifically, PCR assays were performed using 2× Taq PCR MasterMix (Tiangen Biotech Co. Ltd., Beijing). Each PCR consisted of the following components: 25 µl of Taq PCR Master Mix (contains 25 U of Taq DNA polymerase, 10× Tiangen PCR buffer, 15 mM MgCl₂, and 200 µM each dNTP), 1 µl of each 10 µM primer, 2 µl of fungal genomic DNA, and 21 µl of distilled water (provided by Tiangen Kit). Reactions were performed in a Bio-Rad Thermal Cycler (C1000; Bio-Rad Laboratories, Life Science Research, Hercules, CA) with the following PCR program: 1 cycle at 95°C for 3 min for initial denaturation; followed by 29 cycles at 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s; and a final extension of 72°C for 7 min. The PCR products were separated by gel electrophoresis in a 1.5% agarose gel, stained with cyanine dyes (Rye et al. 1992), and visualized by Bio-Rad GelDoc XR System. Each sample was repeated twice. The sizes of the amplified fragments were estimated by a DL2000 DNA Ladder (Tiangen Biotech Co. Ltd.).

Determination of allele frequency and diversity analysis by SSR. Seventeen pairs of SSR markers (Jinbin Li and Chengyun Li unpublished data) were developed based on the genome sequence (www.broadinstitute.org/annotation/fungi/magnaporthe), and three highly polymorphic SSR markers were identified for diversity analysis (Table 1). The alleles for each SSR locus across the samples were scored based on fragment length of the PCR product with genetic analysis system of GenomeLab GeXP (Beckman Coulter, Inc., Life Science Division, Indianapolis, IN), and these sizes were used for statistical analysis.

Data analysis. The genetic diversity of SSR marker loci was analyzed using methods described in previous publications (Nei 1973; Zhan and McDonald 2004; Zhan et al. 2003, 2005). Total genetic diversity was partitioned into within and among mating types to determine genetic differentiation (*G_{ST}*) for selectively neutral loci between the two mating type groups (Nei 1973). Standard deviations for *G_{ST}* were generated using a bootstrap analysis of the original SSR data of the genotypes with 1,000 replicates, with the software POPGENE v 1.32 (Yeh et al. 1999). The standard deviations were used to determine whether the estimates of *G_{ST}* between the opposite mating types were significantly larger than zero.

Results

Mating types in Yunnan Province. Using the tester strains, we only identified 26 mating types (data not shown). These mating types were verified with PCR-based assays. The mating type of the remaining isolates was determined by PCR (Fig. 1). In total, 94 (37.3%) isolates were *MAT1-1* and 158 (62.7%) isolates were *MAT1-2*. Both *MAT1-1* and *MAT1-2* were detected in all of the prefectures (Fig. 1). Most of the *MAT1-2* isolates (more than 60%) were found in DH, BS, WS, KM, ZT, LC, and LJ populations, while most of the *MAT1-1* isolates (more than 60%) were in HH, XSBN, YX, and

Table 1. Simple-sequence repeat primers of *Magnaporthe oryzae* used in this study

Primer	Forward (5' to 3')	Reversed (5' to 3')	Chr. ^a	Size (bp)	Motif
D4	TGCATGAAGCTGATTTGCTC	TTTGACTCTCGCTCCCTCTC	II	230	TA
G5	GTAACCAGGCCGTTTCAAGA	GGAGGTTGCAGAAGGACAGA	II	270	TATG
H4	CGCGGTAATGGAAGCTACA	AGCGAGCTGTGTTGACCTTT	VI	155	TG

^a Chromosome.

PE populations. *MAT1-1* and *MAT1-2* were found in near equal numbers in QJ (Fig. 1). It is worth noting that the frequency of *MAT1-2* isolates in the *indica* rice-growing region was higher than that of the *MAT1-1* isolates in the *japonica* rice-growing region (Table 2). These findings demonstrated that *MAT1-1* and *MAT1-2* isolates coexist in a small ecosystem (Table 2; Fig. 1). Thus far, we have not found any single isolate of the perfect state (the stage where sexually produced spores are formed) of *M. oryzae*, suggesting that sexual recombination has not occurred.

G_{ST} between mating types. G_{ST} for three SSR markers between the *MAT1-1* and *MAT1-2* mating type groups ranged from 0.0314 to 0.4400 when the analysis was based on individual regional populations and was 0.3137 when the isolates were combined (Table 3). The estimated G_{ST} values were significantly larger than zero, indicating that G_{ST} exists in select neutral loci between the two mating type

groups. The isolates from PE, YX, and XSBN were excluded from G_{ST} analysis due to sample size.

To examine genetic variation of the *M. oryzae* mating type population, the observed number of alleles (na), effective number of alleles (ne), Nei's gene diversity (h) (Nei 1973), Shannon's information index (I) (Lewontin 1972), number of polymorphic loci (NPL), and the percentage of polymorphic loci (PPL) were calculated and summarized in Table 4. The values of na, ne, h, I, NPL, and PPL of the *MAT1-2* population were higher than those of *MAT1-1* (Table 4), suggesting that the *MAT1-2* population was more diverse than that of *MAT1-1*. The phylogram was constructed by POPGEN software. *MAT1-1* and *MAT1-2* populations in the same region were clustered in different branches and different regions were clustered in the same branch, suggesting that certain G_{ST} exists between the *MAT1-1* and *MAT1-2* populations from different collection regions (Fig. 2).

Pathogenicity of the mating type. Pathogenicity of each genotype was determined among *M. oryzae* isolates within each mating type group. The 22 *R* genes were overcome by *MAT1-1* and *MAT1-2*, showing that both of mating types were highly virulent, with 4.2 to 81.3 and 3.9 to 92.2%, respectively (Fig. 3). Virulence frequencies of the *MAT1-1* and *MAT1-2* populations on most *R* genes were similar; this suggests that they exhibit similar pathogenicity (Fig. 3).

Discussion

Yunnan Province is one of the origins of *M. oryzae* and is one of the oldest rice production regions in the world (Saleh et al. 2014; Tharreau et al. 2009). *M. oryzae* field isolates from this ecosystem are an ideal resource for studying genetic diversity and pathogen population structure. In the present study, we characterized 252 field isolates from two geographically representative ecosystems in Yunnan with mating type, DNA fingerprinting, and pathogenicity assays. We found that the *MAT1-2* population was more genetically diverse than that of *MAT1-1* (Table 4), which suggests that *MAT1-2* population may be more adaptive in Yunnan Province. We also found that the divergence between the *MAT1-1* and *MAT1-2* groups in *M. oryzae* populations based on the G_{ST} was greater than zero (Table 3), suggesting that the neutral loci between the two mating type groups has been selected. However, a correlation between mating type and the SSR profile has not been established as expected.

One study showed that the fertilities of 1,311 field strains in 43 counties were assayed by standard tester isolates, and *MAT1-2* isolates were more numerous than those of *MAT1-1*. Both mating types were found in 11 counties, while only *MAT1-1* was present in 3 counties and *MAT1-2* was present in 13 counties (Li et al. 1996). We showed that *MAT1-2* was predominant in Yunnan Province, which is consistent with a previous study (Li et al. 1996). In the present study, we found more isolates with *MAT1-2* in western Yunnan (BS, DH, and LC; Fig. 1) and more isolates with *MAT1-1* in southeastern Yunnan (PE, HH, YX, and XSBN; Fig. 1), which suggests that there exist two centers for mating type (Fig. 1). Although, the

Fig. 1. Distribution of mating types in *Magnaporthe oryzae* populations in Yunnan Province of China. BS, DH, HH, LJ, KM, LC, QJ, PE, WS, YX, XSBN, and ZT indicate isolates collected from Baoshan (58 isolates), Dehong (37 isolates), Honghe (12 isolates), Lijiang (9 isolates), Kunming (11 isolates), Lincang (9 isolates), Qujing (14 isolates), Puer (21 isolates), Wenshan (17 isolates), Yuxi (21 isolates), Xishuangbanna (12 isolates), and Zhaotong (31 isolates) prefectures, respectively. In total, 252 isolates were used in this study. The mating type of 26 isolates (21, 2, 2, and 1 isolates in Baoshan, Puer, Wenshan, and Xishuangbanna, respectively) was confirmed with both genetic crossing and molecular markers.

Table 2. Mating type of *Magnaporthe oryzae* detected by polymerase chain reaction-based assay

Rice production	Number of isolates and frequency (%)		Total isolates
	<i>MAT1-1</i>	<i>MAT1-2</i>	
<i>indica</i> region	49 (35.0)	91 (65.0)	140
<i>japonica</i> region	45 (40.2)	67 (59.8)	112
Total	94 (37.3)	158 (62.7)	252

Table 3. Genetic differentiation (G_{ST}) in neutral simple-sequence repeat markers between *MAT1-1* and *MAT1-2* groups in *Magnaporthe oryzae* populations

G _{ST} for locations ^a									
BS	DH	HH	KM	LC	LJ	QJ	WS	ZT	Total
0.0314	0.1024	0.2770	0.1170	0.2632	0.1099	0.4400	0.2564	0.0492	0.3137

^a BS, DH, HH, KM, LC, LJ, QJ, WS, and ZT indicate isolates collected from Baoshan, Dehong, Honghe, Kunming, Lincang, Lijiang, Qujing, Wenshan, and Zhaotong prefectures of Yunnan Province, respectively.

Table 4. Genetic variation between *MAT1-1* and *MAT1-2* populations^a

MT	N	na	ne	h	I	NPL	PPL
<i>MAT1-1</i>	44	1.5682 ± 0.50	1.0799 ± 0.12	0.0643 ± 0.09	0.1222 ± 0.15	25	56.8
<i>MAT1-2</i>	44	1.9318 ± 0.26	1.0957 ± 0.15	0.0748 ± 0.10	0.1433 ± 0.15	41	93.2
Total	44	2.0000 ± 0.00	1.0904 ± 0.13	0.0729 ± 0.09	0.1428 ± 0.14	...	...

^a MT = mating type; N = number of loci; na = observed number of alleles; ne = effective number of alleles; h = Nei's gene diversity (Nei 1973); I = Shannon's information index (Lewontin 1972); NPL = number of polymorphic loci; and PPL = percentage of all loci that are polymorphic regardless of allele frequencies, calculated by the default of the software POPGENE v 1.32 (Yeh et al. 1999).

MAT1-2-predominant center (BS, DH, and LC) and the *MAT1-1*-predominant center (PE, HH, YX, and XSBN) are separated by the Laobie and Wuliang mountain chains, the *MAT1-1*-predominant center was separated by the Ailao mountain chain (Supplementary Fig. S1). These findings suggest that isolates with two mating types in Yunnan may be contained because of the physical barrier of the mountain chains.

Mating types of *M. oryzae* are useful criteria for predicting genetic diversity and degree of virulence because they may have sexual reproduction potential (Valent et al. 1991). The distribution of mating types of *M. oryzae* is distinctly diverse worldwide. Both mating types were found in almost equal distribution in western Africa (Ivory Coast, Mali, and Burkina Faso); only *MAT1-1* of *M. oryzae* was found in Europe (France, Italy, and Spain). In Asia, two centers of diversity (*MAT1-1* and *MAT1-2*) were found in the Himalayan foothills: South China–Laos–North Thailand and western Nepal. Sexual reproduction was found only in the South China–Laos–North Thailand region that was identified as the putative center of origin of all *M. oryzae* populations on rice (Saleh et al. 2014). *MAT1-2* predominated over *MAT1-1* in a mating type bioassay in Thailand (Mekwatanakarn et al. 1999). Although the *MAT1-1* mating type was predominate in *M. oryzae* population of the Mekong Delta in Vietnam, in some provinces *MAT1-1* coexisted with *MAT1-2* at a lower frequency (Le et al. 2010). Similar to our findings, *MAT1-2* isolates predominated in southern China; only *MAT1-1* isolates were found in the southeastern China region and both mating types were present in southwestern China (Chen et al. 2004; Zeng et al. 2009).

A significant difference in pathogenicity was found between the *MAT1-1* and *MAT1-2* isolates on wheat; *MAT1-1* isolates had a greater pathogenicity than *MAT1-2* isolates in the wheat pathogen *Mycosphaerella graminicola* (Zhan et al. 2007). There are no G_{ST} in neutral loci between mating types; this is consistent with the hypothesis that differences in pathogenicity were not due to the effects of specific pathogenicity genes or nonrandom genetic backgrounds (Zhan et al. 2007). In the present study, the pathogenicity of the *MAT1-1* (54 isolates) and *MAT1-2* (136 isolates) populations was highly similar on the 22 monogenic lines (Fig. 2). These findings suggest that the mating type is independent of virulence and geographic distribution of the population.

Magnaporthe oryzae is one of the most destructive fungal pathogens on crop plants. Rice cultivars often are susceptible after growing for a short period of time due to the occurrence of more virulent isolates of *M. oryzae*. Many pathogenic eukaryotes use sexual recombination to create genetic diversity for pathogen adaptation and virulence in nature. However, the perfect stage of *M. oryzae*, with perithecia producing viable ascospore, was rarely reported. We found that *M. oryzae* isolates with *MAT1-1* and *MAT1-2* had similar pathogenicity on differential monogenic lines with 22 commonly deployed major blast *R* genes despite the G_{ST} that was noticed based on neutral markers. Taken together, we suggest that sexual recombination may not be needed for virulence, adaptation, and fitness of the rice blast fungus.

Variation in a fungal pathogen's virulence to the host via parasexual and sexual recombination may improve the pathogen's adaptation to environmental conditions (Noguchi et al. 2006). Noguchi (2011) speculate that mutations and parasexual recombination are the primary causes of variations in *M. oryzae* pathogenicity in Japan because there have not been any reports on fertile crosses between Japanese field isolates. This suggests that sexual recombination and fungal virulence should benefit the effectiveness of *M. oryzae*.

Ebert (1994) state that there are two theories surrounding parasite evolution in regards to adaption and virulence. One theory is that virulent parasites become less virulent while the poorly suited parasites become virulent. The other theory is that virulence persists if there is proximity to an acceptable host (Ebert 1994). Ultimately, virulence is a balancing act between the positive and negative effects of parasitism. Whereas parasites evolve to retain virulence at an optimal level, hosts evolve to reduce virulence, infection, and multiplication of the

parasite without detrimental effects (Ebert and Hamilton 1996). Mutual selection is the underlying force for arms races between hosts and parasites.

M. grisea, which has the potential to reproduce sexually and asexually, can infect rice along with several other grasses. Although phenotypal and genotypical data suggest that *M. grisea* is asexual, Zeigler (1998) stresses that parasexual recombination should still be considered due to the deterring of meiosis by chromosome-length polymorphisms and translocations. Sexual fertility of *M. grisea* has been documented in South and East Asia, including documentation of sexual fertility losses due to progressive agricultural circumstances (Zeigler 1998). The mechanism of sexual recombination and pathogen virulence should be investigated further to see how it will benefit the development of effective management options for rice blast disease. In the present study, the finding that more *MAT1-2* than *MAT1-1* of

Fig. 2. Phylogram of *MAT1-1* and *MAT1-2* populations from different collection regions by simple-sequence repeat markers. BS, DH, HH, LJ, KM, LC, QJ, WS, and ZT indicate Baoshan, Dehong, Honghe, Lijiang, Kunming, Lincang, Qujing, Wenshan, and Zhaotong prefectures of Yunnan, respectively. *MAT1-1* and *MAT1-2* isolates were clustered in different branches, suggesting that genetic differentiation exists between populations from different collection regions. Red and black indicate *MAT1-1* and *MAT1-2*, respectively.

Fig. 3. Disease reactions of *Magnaporthe oryzae* isolates to monogenic lines carrying resistance (*R*) genes. The x-axis indicates the monogenic line carrying each *R* gene: numbers 1 to 22 are IRBLA-A for *Pia*, IRBL19-A for *Pi19(t)*, IRBL1-K59 for *Pit*, IRBL3-CP4 for *Pi3*, IRBLsh-S for *Pish*, IRBLta-CP1 for *Pita*, IRBLi-F5 for *Pii*, IRBLks-S for *Pik-s*, IRBLk-K for *Pik*, IRBL7-M for *Pi7(t)*, IRBLkp-K60 for *Pik-p*, IRBLta2-Pi for *Pita-2*, IRBLz5-CA-1 for *Piz-5*, IRBLkh-K3 for *Pik-h*, IRBLkm-Ts for *Pik-m*, IRBL1-CL for *Pi1*, IRBL12-M for *Pi12(t)*, IRBLb-B for *Pib*, IRBL5-M for *Pi5(t)*, IRBLz-Fu for *Piz*, IRBL9-W for *Pi9(t)*, and IRBLzt-T for *Piz-t*, respectively. The y-axis indicates the percentage of susceptibility.

M. oryzae were found in small rice-growing regions, both are virulent to some *R*-gene-carrying monogenic rice lines, and no sexual spores were found in 252 field isolates demonstrated that *M. oryzae* is highly adaptive, enabling it to cause disease on all known *R* genes in rice even without sexual recombination.

Acknowledgments

We thank Y. Zhu and Y. Guo of Yunnan Agricultural University for useful discussions and T. Bianco of the United States Department of Agriculture–Agricultural Research Service Dale Bumpers National Rice Research Center for proofreading and useful discussion. Financial support was provided by the National Natural Science Foundation of China (31460454 and 31160355), National Basic Research Program 973 (2012CB722901), and the Department of Sciences and Technology of Yunnan Province (2015HB076).

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