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Study of pathogenicity and genetic diversity of *Magnaporthe oryzae* isolated from rice hybrid Wuyou 308 and detection of resistance genes

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Abstract: To understand the cause of loss of rice blast resistance, we studied the pathogenicity of *Magnaporthe oryzae* strains isolated from rice hybrid Wuyou 308 and evaluated its resistance genes. A total of 62 *M. oryzae* strains were isolated and tested in 7 Chinese rice varieties with varying degrees of resistance to rice blast and 30 blast-resistant monogenic lines. Fourteen physiological races of *M. oryzae* were identified: 8.55% belonging to the ZA group, 86.67% to the ZB group, and 5.00% to the ZC group. ZB15 was the most abundant race (45.00%). Five resistance genes, *Pi-3(1)*, *Pi-z5*, *Pi-k*, *Pi-kp(C)*, and *Pi-k(C)*, conferred good resistance to the 62 strains, with resistance frequencies of 95.56, 91.11, 88.89, 82.22, and 82.22%, respectively. In contrast, *Pi-a(2)* had a resistance frequency of 0%. The hybrid combination Wuyou 308 was found to carry *Pi-ta* and *Pi-b* genes. Because *Pi-ta* and *Pi-b* both showed low resistance frequencies to *M. oryzae* isolated from Jiangxi, the hybrid rice variety Wuyou 308 could be infected by most of the 62 *M. oryzae* strains. The emergence and spread of rice blast disease in Wuyou 308 may thus be difficult to avoid when climatic conditions are favourable.

Keywords: genetic diversity; pathogenic differentiation; rice blast; Wuyou 308

Rice blast, caused by the fungus *Magnaporthe oryzae*, is a devastating disease that can seriously affect rice yield. Because this disease is species-specific and exhibits pathogenic differentiation, the breeding of resistant rice varieties would seem to be the most effective and economical method of prevention and control (E et al. 2008). Over the long course of evolution, however, rice blast fungi have become genetically diverse and virulence variability. When the fungus interacts with resistance genes, these characteristics facilitate changes in population genetic structure, with new and old avirulence genes constantly exchanged and variety resistance ultimately lost (Lee et al. 2009; Moytri et al. 2012). Consequently, the resistance of most newly introduced varieties is lost within 3 to 5 years after release,

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with subsequent outbreaks and spread of rice blast (Lei et al. 2000).

Wuyou 308 (*Oryza sativa* L.) is a three-line *indica* hybrid rice bred from Wufeng A × Guanghui 308 by the Rice Research Institute of the Guangdong Academy of Agricultural Science in China. This variety has been grown in Jiangxi Province for many years and was selected because of its high quality and resistance as one of five major late rice varieties by the Provincial Department of Agriculture in 2015. In that same year, however, rice blast, mainly infecting Wuyou 308, broke out in Gandong and Jitai Basin. The disease affected 70 000 ha of land, 13 000–20 000 ha of which had a disease rate of 50–80%. This outbreak was thus one of the most important events related to annual rice production in Jiangxi Province. In this study, we identified physiological races, including dominant ones, of rice blast strains from Wuyou 308 specimens, analysed their genetic relationships, and performed comparative analyses to clarify the cause of serious occurrences of rice blast on Wuyou 308. The results of this study may help researchers accurately predict and develop rice varieties with prolonged resistance to rice blast. Our findings will also provide a theoretical basis for the identification of resistant varieties appropriate for cultivation in Jiangxi Province.

MATERIAL AND METHODS

Plant material. The following test materials were used in this study: the hybrid rice combination Wuyou 308, provided by the Seed Management Bureau of Jiangxi Province; 7 Chinese hosts with differential resistance to rice blast, namely, Tetep, Zhenlong 13, Sifeng 43, Dongnong 363, Guandong 53, Hejiang 18, and Lijiang Xintuan Heigu; 6 rice blast-resistant co-isogenic lines bred by the Institute of Crop Cultivation, Chinese Academy of Agricultural Sciences; and 24 rice blast-resistant monogenic lines bred by the International Rice Research Institute in cooperation with Japan.

PCR primers. A total of 13 primer pairs (KMS02, KMS20, A5, G5, D4, D5, and SMS17 (Li et al. 2007); FG01, FG02, and FG03 (Luo et al. 2013); and MS355, MS363, and MS677 (Zhang et al. 2010)) were synthesised by Shanghai Introgen and used to analyse the genetic diversity of *M. oryzae*. Detailed information is provided in Table 1.

DNA extraction. DNA was extracted from Wuyou 308 leaves using a MiniBEST Universal Genomic DNA Extraction kit Ver. 5.0 (Takara, Japan). After incubation in a 42 °C oven for 3 days to break dormancy, Wuyou 308 seeds were directly sown in a germination box containing a nursery matrix. After 2 weeks of cultivation, leaves were collected and used for DNA extraction with a CTAB method (Wang et al. 2015). Specific primers for amplification of resistance genes *Pi-ta, Pi-b, Pi-1, Pi-9, Pi-k*, and *Pi-z* are listed in Table 2.

Pathogenicity testing. To assess the pathogenicity of different strains of *M. oryzae*, Thirty-seven rice varieties were seeded onto plates (35 × 30 ×

Table 1. SSR primers specific for *Magnaporthe oryzae*

Primer	Sequence (5'-3')		Annealing temperature (°C)
	F	R	
KMS02	CGCAAAGAATTCAAACCCGC	GGAGACGACACTGGGGTGCT	55
KMS20	CGCCCTTCAAAAACCAAGGG	AATTGCGACAAGTCGCTCCC	57
SMS17	GTGACAGCGAGGATTTCGAT	ATCGTCGTCAGAGTCGGAGT	55
A5	CGTTTGGCAAAATTAAAGCA	CGTTTGGGCGTTCTTTTGTA	56
D4	TGCATGAAGCTGATTTGCTC	CAGGGGATGAACTCCGATGG	55
D5	CGTGCCAGAAAGACCTGAAGC	CAGGGGATGAACTCCGATGG	55
G5	GTAACCAGGCCGTTTCAAGA	GGAGGTTGCAGAAGGACAGA	55
FG01	AACGTGACAATGTGAGCACC	GCCATGTTCTAAGGTGCTGAG	55
FG02	TCAGTAGGCTTGGAATTGAAAAA	CTTGATTGGTGGTGTGTTG	55
FGO3	TCACATTTGCTTGCTGGACT	AGACAGGGTTGACGGCTAAA	55
MS355	AACCCTCCGTGCACCTTAG	GCTTCTTCTCGCTTGCTCTT	56
MS363	TCTCGGGAAGCTGATTGAGT	CTAACGGCCGGCTAACAAAC	56
M3677	TCGTGAGGGTTCCTATCTGC	GACCTTTATCGGATGCGTCT	56

F – forward; R – reverse

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5 cm^3). At the 3.5- to 4-leaf stage, inoculation was performed by spraying spores of *M. oryzae* onto the seedlings. A 40-mL volume of the spore suspension, which included 0.1% Tween 20, was sprayed per plate. After inoculation, the seedlings were cultured in a moist incubator in darkness at 25 °C for 24 h and then cultured in a moist room at 25–28 °C until disease symptoms were evident. Pathogenicity testing was performed 7 days after inoculation, with two repeats conducted per strain. The level of disease was determined according to rice blast classification criteria proposed by the International Rice Research Institute, where plants classified at levels 1–3 and 4–9 are considered to be resistant and susceptible, respectively (Zhu et al. 2004). In addition, physiological races of the fungus isolated in this study were identified using the method proposed by the National Joint Test Group of Physiological Races of *M. oryzae* (Sun et al. 1984).

Calculation of pathogenic frequency and virulence frequency. Pathogenicity was calculated as described by Yang et al. (2007). The pathogenic frequency (PF, %) of a particular *M. oryzae* strain to all tested rice varieties was calculated as follows: PF = (number of susceptible varieties/total number of tested varieties) × 100%. Strong, relatively strong, moderate, and weak pathogenicity was then defined as PF ≥ 70%, 50% ≤ PF ≤ 70%, 30% ≤ PF ≤ 50%, and PF < 30%, respectively. The virulence frequency (VF, %) of a particular rice blast fungal strain was calculated according to the following formula: VF = (number of rice varieties infected by the tested rice blast fungal strain/total number of rice varieties) × 100%.

Genetic analysis of 62 *M. oryzae* strains from Wuyou 308. After isolation and purification of *M. oryzae*, fungal DNA was extracted using a MiniBEST Universal Genomic DNA Extraction kit Ver. 5.0 (Takara, Japan). PCR amplifications were then performed as described by Liu et al. (2015) in 25-μL volumes comprising 1.25 μL of upstream and downstream primers, 11.5 μL of ddH_2O, 10 μL of 2× Easy *Taq* SuperMix, and 1 μL of DNA template. The product (5 μL) was loaded with 2 μL of 1000× SYBR Green I and 3 μL of loading buffer onto a 1.0% agarose gel and electrophoresed at 120 V for 30 min. After electrophoresis, the gel was observed and imaged using a gel imaging system. A statistical analysis was performed using Quantity One software (Bio-Rad, USA). An SSR database based on the primer amplification results was established, and the electrophoresed DNA fragments were converted into binary data: the presence or absence of a DNA fragment was assigned a value of 1 or 0, respectively. Genetic distances were calculated according to Nei (1987) in DPS 7.05 (Data Processing System). A cluster analysis was performed using the unweighted pair-group method with arithmetic averages.

Detection of resistance genes in Wuyou 308. To scan for resistance genes *Pi-1*, *Pi-ta*, *Pi-9*, *Pi-k*, *Pi-z*, and *Pi-b*, DNA of Wuyou 308 was PCR-amplified with gene-specific primers. The amplified regions were separated by agarose gel electrophoresis and visualised under ultraviolet light. Gene detection was performed according to Dai et al. (2012), Xie et al. (2015), Liu et al. (2016), and Zhang et al. (2016).

RESULTS

Physiological races of *M. oryzae* isolated from Wuyou 308. As shown in Table 3, 62 fungal strains

Table 2. Name, sequences and expected fragment size of primers specific for resistance genes

Pi-gene	Prime	Sequence (5'-3')	Fragment size (bp)	Reference
Pi-ta	YL155, YL87	AGCAGGTTATAAGCTAGGCC CTACCAACAAGTTCATCAAA	1040	Wang et al. (2010)
Pi-b	Lys145	TCGGTGCCTCGGTAGTCAGT GGGAAGCGGATCCTAGGTCT	803	Liu et al. (2008)
Pi-z	Z565962	AAGAAATAATATTTTGAAACATGGC AAAGCCATGGTGGTAACTGGTATGTG	267	Xie et al. (2015)
Pi-k	Pik-SNP	TTCGAGGCCCTACCAAGACA CATGGAAGGCTATCCTTGGTA	100	Xie et al. (2015)
Pi-9	PB-8	CCGGACTAAGTACTGGCTTCGATA	500	Dai et al. (2012)
Pi-1	M-Pi1	GTGCTGCTGTGGCTAGTTTG AGTCCCCGCTCAATTTTTCT	460	Liu et al. (2016)

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were classified according to physiological race and tested for pathogenicity. A total of 14 physiological races belonging to three groups were detected. Among the 62 strains, 8.33% belonged to the ZA group, 86.67% to ZB, and 5.00% to ZC. ZB15 was the dominant race (45%), followed by ZB13 (21.67%) and ZA31 (6.67%). Of the 62 strains, FC-10 and WZ-1 had the strongest pathogenicity, with a PF of 76.67%. CR-7 had the weakest pathogenicity, with a PF of only 26.67%. The average PF of the 62 strains was 52.06%.

Pathogenicity of Wuyou 308-derived *M. oryzae* strains towards resistant monogenic rice lines. As mentioned above, the VF of the 62 strains ranged from 4.44% to 100%. The 30 monogenic lines had varying degrees of resistance to the different strains: *Pi-3(1)* had the highest resistance, with a VR of 4.44%, while *Pi-a(2)*, with a VR of 100%, was the least resistant. As shown in Table 4, five monogenic lines, namely, *Pi-3(1)*, *Pi-z5*, *Pi-k*, *Pi-kp(C)*, and *Pi-k(C)*, exhibited good resistance to the 62 Wuyou 308-derived *M. oryzae* strains, with VRs of 4.44, 8.89, 11.11, 17.78, and 17.78%, respectively.

Genetic analysis of 62 *M. oryzae* strains isolated from Wuyou 308. A total of 57, 48, 32, 35, 39, 20,

Table 3. Physiological race identification and pathogenicity analysis on *Magnaporthe oryzea* of Wuyou 308

Strain	Physiological race	Pathogenicity frequency (%)	Strain	Physiological race	Pathogenicity frequency (%)
WA-1	ZB25	66.67	FC-14	ZB15	43.33
WA-2	ZB15	36.67	FC-15	ZB15	43.33
WA-3	ZB3	60.00	FC-16	ZB13	43.33
WA-4	ZA31	50.00	FC-17	ZB23	60.00
WA-5	ZB7	70.00	FC-18	ZB15	63.33
WA-6	ZB1	56.67	CR-1	ZB13	60.00
WA-7	ZB15	53.33	CR-2	ZB15	33.33
WA-8	ZB15	36.67	CR-3	ZB31	43.33
WA-9	ZB15	40.00	CR-4	ZB13	53.33
WA-10	ZB11	56.67	CR-5	ZB13	30.00
WA-11	ZB15	46.67	CR-6	ZB13	63.33
WA-12	ZB15	36.67	CR-7	ZB15	26.67
WA-13	ZC11	46.67	CR-8	ZB15	33.33
WA-14	ZB13	43.33	CR-9	ZA15	63.33
WA-15	ZB15	50.00	CR-10	ZB13	40.00
WA-16	ZB31	60.00	CR-11	ZB15	55.55
WA-17	ZB15	66.67	WZ-1	ZB13	76.67
WA-18	ZB15	63.33	WZ-2	ZA15	70.00
FC-1	ZB13	70.00	WZ-3	ZA15	56.67
FC-2	ZB13	36.67	WZ-4	ZB15	70.00
FC-3	ZB15	56.67	WZ-5	ZB15	73.33
FC-4	ZB15	50.00	WZ-6	ZB13	63.33
FC-5	ZC13	66.67	WZ-7	ZA15	70.00
FC-6	ZB15	43.33	WZ-8	ZB15	55.56
FC-7	ZB31	53.33	LC-1	ZB15	50.00
FC-8	ZB15	66.67	LC-2	ZB15	53.33
FC-9	ZB15	36.67	LC-3	ZB15	60.00
FC-10	ZB25	76.67	LC-4	ZB15	43.33
FC-11	ZB7	60.00	LC-5	ZB13	50.00
FC-12	ZB7	60.00	LC-6	ZB13	53.33
FC-13	ZB13	43.33	LC-7	ZB15	50.00

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37, 21, 25, 22, 44, 52, and 38 DNA fragments were amplified by primers KMS02, KMS20, SMS17, A5, D4, D5, G5, FG01, FG02, FG03, MS355, MS363, and MS677, respectively, which indicated that the 62 strains had high genetic diversity. Representative amplification results are shown in Figures 1 and 2.

The electrophoretic data were analysed with Quantity One software (Bio-Rad). In the cluster dendrogram shown in Figure 3, the 62 strains were divided, at a genetic distance of 2.29, into four lineages. The largest lineage was L1 comprising 32 strains corresponding to 51.61% of the total (Table 5).

One monosporic strain each was randomly selected from the four lineages: FC-16 (L1), WZ-1 (L2), WA-3 (L3), and CR-10 (L4). The strains were sprayed onto Wuyou 308 at the three-leaf stage. After 7 days of moist cultivation, all infected plants exhibited disease symptoms (Figure 4).

Detection of resistance genes in Wuyou 308. Specific markers were used to search for resistance genes *Pi-ta*, *Pi-b*, *Pi-1*, *Pi-9*, *Pi-k*, and *Pi-z* in Wuyou 308. After PCR amplification, electrophoresis, staining, and UV imaging, two bands corresponding to 1,040 bp and 803 bp were detected (Figure 5A, B),

Table 4. Virulence frequency of monogenic lines by rice blast strains from Wuyou 308

Monogenic lines	Gene	Donor	Type	Resistant frequency (%)
IRBLa-A	*Pi-a(1)*	Aichi-asahi	Japonica	17.78
IRBLa-C	*Pi-a(2)*	CO39	Indica	0
IRLBLi-F5	*Pi-i*	Fujisaka 5	Japonica	51.11
IRBLks-F5	*Pi-ks(1)*	Fujisaka 5	Japonica	28.89
IRBLks-S	*Pi-ks(2)*	Shin 2	Japonica	64.44
IRBLk-Ka	*Pi-k*	Kanto51	Japonica	88.89
IRBLkp-K60	*Pi-k^{p}*	K60	Indica	57.78
IRBLkh-K3	*Pi-k^{h}*	K3	Indica	37.78
IRBLz-Fu	*Pi-z*	Fukunishiki	Indica	60.00
IRBLz5-CA	*Pi-z^{5}*	C101A51	Indica	91.11
IRBLzt-T	*Pi-z^{t}*	Toride l	Indica	68.89
IRBLta-CT2	*Pi-ta(2)*	C105TTP2L9	Indica	55.56
IRBLta-CP1	*Pi-ta*	C101PKT	Indica	15.56
IRBLb-B	*Pi-b*	BL1	Indica	17.78
IRBLt-K59	*Pi-t*	K59	Indica	24.44
IRBLsh-S	*Pi-sh(1)*	Shin 2	Japonica	55.56
IRBLsh-B	*Pi-sh(2)*	BL1	Japonica	22.22
F80-1	*Pi-k(C)*	Lizhijiang	Japonica	82.22
F98-7	*Pi-k^{m}*	Huabeidami	Japonica	51.11
F124-1	*Pi-ta(C)*	Tadukan	Indica	40.00
F128-1	*Pi-ta^{2}*	Tadukan	Indica	73.33
F129-1	*Pi-k^{p}(C)*	Pusur	Indica	82.22
F145-2	*Pi-b(C)*	Tijahaja	Indica	13.34
IRBL1-CL	*Pi-1(1)*	C101LAC	Indica	64.64
IRBL3-CP4	*Pi-3(1)*	C104PKT	Indica	95.56
IRBL5-M	*Pi-5(t)*	Shin 2	Japonica	24.44
IRBL7-M	*Pi-7(t)*	RIL29	Japonica	28.89
IRBL9-W	*Pi-9(t)*	WHD-IS-75-1-127	Wild rice	68.89
IRBL12-M	*Pi-12(t)*	RIL10	Japonica	35.56
IRBL19-A	*Pi-19(t)*	Aichi-asahi	Japonica	48.89

(*C*), (*1*) and (*2*) were attached to the same genes, but derived from different donor parents, to distinguish from each other; (*C*) was attached to the genes from China

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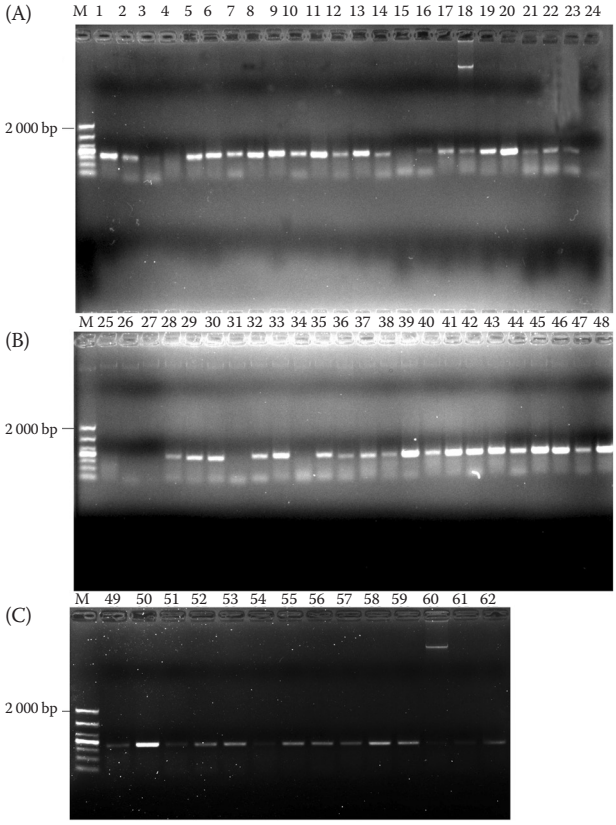

Figure 1. Results of PCR amplification of different *Magnaporthe oryzea* strains with primer KMS02: (A) M – size marker DL2000, lanes 1–24: strains WA1, WA2, WA3, WA4, WA5, WA6, WA7, WA8, WA9, WA10, WA11, WA12, WA13, WA14, WA15, WA16, WA17, WA18, FC1, FC2, FC3, FC4, FC5, FC6; (B): M – size marker DL2000, lanes 25–48: strains FC7, FC8, FC9, FC10, FC11, FC12, FC13, FC14, FC15, FC16, FC17, FC18, CR1, CR2, CR3, CR4, CR5, CR6, CR7, CR8, CR9, CR10, CR11, WZ1; (C): M – size marker DL2000, lanes 49–62: strains WZ2, WZ3, WZ4, WZ5, WZ6, WZ7, WZ8, LC1, LC2, LC3, LC4, LC5, LC6, LC7

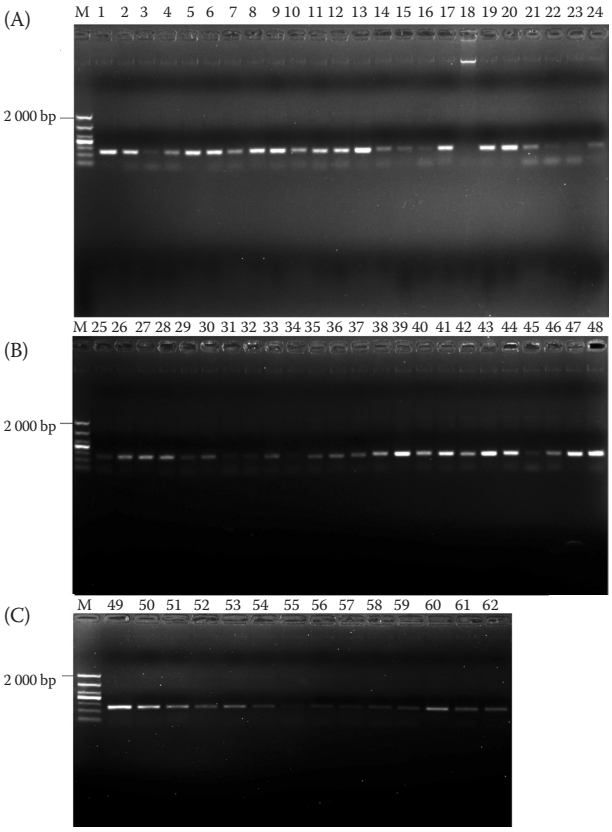

Figure 2. Results of PCR amplification of different *Magnaporthe oryzea* strains with primer MS363: (A): M – size marker DL2000, lanes 1–24: strains WA1, WA2, WA3, WA4, WA5, WA6, WA7, WA8, WA9, WA10, WA11, WA12, WA13, WA14, WA15, WA16, WA17, WA18, FC1, FC2, FC3, FC4, FC5, FC6; (B): M – size marker DL2000, lanes 25–48: strains FC7, FC8, FC9, FC10, FC11, FC12, FC13, FC14, FC15, FC16, FC17, FC18, CR1, CR2, CR3, CR4, CR5, CR6, CR7, CR8, CR9, CR10, CR11, WZ1; (C): M – size marker DL2000, lanes 49–62: strains WZ2, WZ3, WZ4, WZ5, WZ6, WZ7, WZ8, LC1, LC2, LC3, LC4, LC5, LC6, LC7

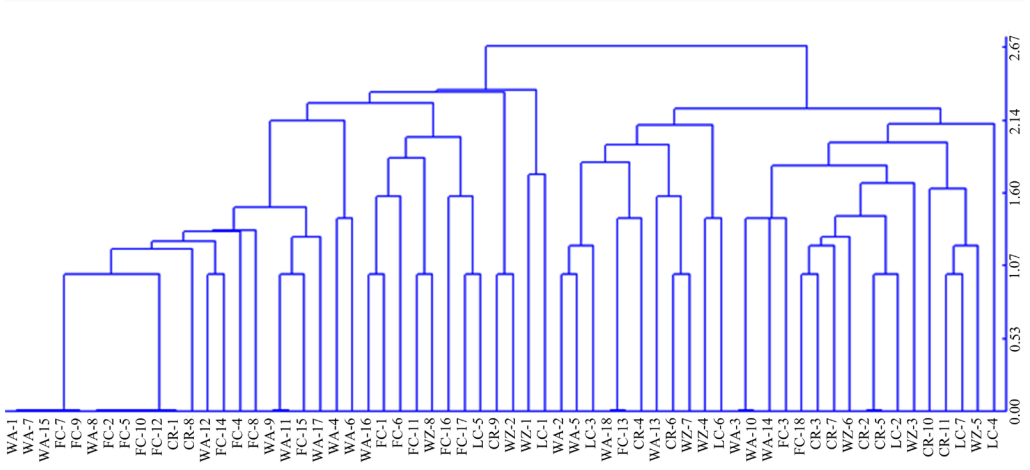

Figure 3. Cluster dendrogram based on SSRs in *Magnaporthe oryzea* isolated from Wuyou 308

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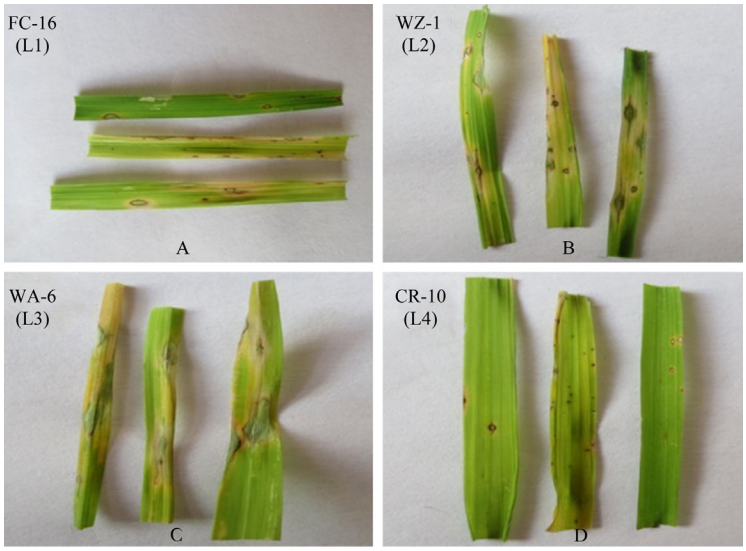

Figure 4. Results of inoculation of Wuyou 308 with different lineages of *Magnaporthe oryzea* strains. (A–D) Wuyou 308 infected with *M. oryzae* strains FC16 belonging to lineage L1 (A), WZ1 belonging to lineage L2 (B), WA6 belonging to lineage L3 (C), and CR10 belonging to lineage L4 (D)

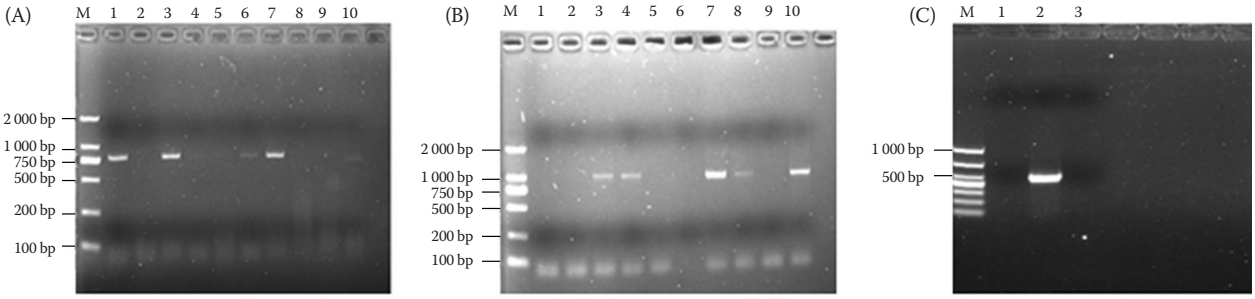

Figure 5. Molecular identification of rice blast resistance genes in Wuyou 308 and other rice varieties: (A): PCR screening for *Pib* in Wuyou 308, M – size marker DL2000, 1 – BL1 (positive control), 2 – Lijiangxintuanheigu (negative control), 3 – Wuyou 308, 4 – 03You 66, 5 – Ganxin 203, 6 – Chunguagnyihao, 7 – Jinyou 458, 8 – Zhuliangyou 819, 9 – Zhongzao 35, 10 – Zhunliangyou 608; (B): PCR screening for *Pita* in Wuyou 308, M – size marker DL2000, 1 – ddH_2O, 2 – LTH (negative control), 3 – Wuyou 308, 4 – C101PKT (positive control), 5 – Ganxin 203, 6 – Chunguagnyihao, 7 – Jinyou 458, 8 – Zhuliangyou 819, 9 – Zhongzao 35, 10 – Zhunliangyou 608; (C): PCR screening for *Pi9* in Wuyou 308, M – size marker DL1000, 1 – Lijiangxintuanheigu (negative control), 2 – WHD-IS-75-1-127 (positive control), 3 – Wuyou 308

which indicates that Wuyou 308 carries *Pi-ta* and *Pi-b* resistance genes. In contrast, no bands specific for *Pi-1* (460 bp), *Pi-9* (500 bp), *Pi-k* (100 bp), or *Pi-z* (267 bp) were observed (Figure 5C).

DISCUSSION

In this study, the dominant physiological race isolated from the susceptible cultivar Wuyou 308 was

Table 5. The genetic lineage of 62 blast strains from Wuyou 308

Lineage	Strains	Proportion (%)
L1	WA-1, WA-7, WA-15, FC-7, FC-9, WA-8, FC-2, FC-5, FC10, FC-12, CR-1, CR-8, WA-12, FC14, FC-4, FC-8, WA-9, WA-11, FC-15, WA-17, WA-4, WA-6, WA-16, FC-1, FC-6, FC-11, WZ-8, FC-16, FC-17, LC-5, CR-9, WZ-2	51.61
L2	WZ-1, LC-1	3.23
L3	WA-2, WA-5, LC-3, WA-18, FC-13, CR-4, WA-13, CR-6, WZ-7, WZ-4, LC-6	17.74
L4	WA-3, WA-10, WA-14, FC-3, FC-18, CR-3, CR-7, WZ-6, CR-2, CR-5, LC-2, WZ-3, CR-10, CR-11, LC-7, WZ-5, LC-4	27.42

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ZB15, which represented 45% of the 62 isolates. In addition, the average PF of the 62 isolates towards the 30 resistant monogenic lines was 52.06%. According to these results, Wuyou 308 differs significantly from another susceptible cultivar, Lijiangxintuanheigu planted in Jianggangshan City (Lan et al. 2015), in terms of average PF as well as the types and frequencies of *M. oryzae* races present. These apparent differences are probably due to two factors. First, the two rice varieties have different types of resistance. As shown by our study, Wuyou 308 harbours *Pi-b*, *Pi-ta*, and presumably other unknown resistance genes, whereas Lijiangxintuanheigu has none (Ling et al. 2000). Second, their growing regions are different. Jinggangshan is mountainous and rainier than other places in Jiangxi Province: over the past 30 years, Jinggangshan City has had an annual average of 207.4 rainy days and 1916.1 mm of rainfall, which compares with 157.9 days and 1576.7 mm in all of Jiangxi Province (source: Jiangxi Meteorological Bureau). Of the five counties in which Wuyou 308 is grown, however, only Wan'an is mountainous, and it experiences less annual rainfall than Jinggangshan. In our investigation, the 62 strains of *M. oryzae* isolated from Wuyou 308 were classified into four genetic lineages. In a study by Ma et al. (2011), in contrast, 99 *M. oryzae* stains from different rice varieties in Jiangxi Province were divided into 14 genetic lineages. Strains of *M. oryzae* isolated from Wuyou 308 thus appear to be genetically more closely related to one another than to strains from other varieties.

Studies have also been carried out on the occurrence of rice blast on Wuyou 308 in other parts of China. For example, Wang et al. (2015) reported differences in the pathogenicity of *M. oryzae* isolated from significantly infected Wuyou 308 grown in Heyuan, Shaoguan, and Meizhou, Guangdong Province, in 2013. ZB13, ZB15, and ZC13 were identified as the dominant physiological races of the isolated strains, where they appeared at frequencies of 66.67, 27.78, and 5.56%, respectively. The genes responsible for high susceptibility to these strains were *Pi-ta2*, *Pi- sh*, and *Pi-i*, whereas high-resistance genes included *Pi-k*^{h}, *Pi-1*, *Pi-2*, *Pi-9*, and *Pi-50*. These results differ from our study findings, thus indicating that the pathogenicity of *M. oryzae* varies because of differences in environmental conditions, farming systems, and varieties.

Given our results, we believe rice blast occurs on Wuyou 308 in Jiangxi Province for two main reasons. First, the most abundant races among the 62 monosporic strains isolated from Wuyou 308 were ZB15 (45.00%), ZB13 (21.67%), and ZA31 (6.67%), and ZB15 and ZB13 are the dominant races in Jiangxi Province (Li et al. 2009; Lan et al. 2014). Consequently, no rare races have accumulated on Wuyou 308, i.e., no directional selection of strains by Wuyou 308 has taken place. Second, our screening for resistance genes in Wuyou 308 identified only *Pi-ta* and *Pi-b*, which have been found to confer only low resistance to *M. oryzae* in Jiangxi Province (resistance frequencies of 15.33% and 1.55%, respectively) in past research (Li et al. 2009). As long as environmental conditions are appropriate, the outbreak and spread of rice blast on Wuyou 308 may thus not be easily avoided.

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