

# Pathogenicity Analysis on *Magnaporthe oryzae* from Hybrid Combination Wuyou 308

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**Abstract** Eighteen blast isolates were obtained from hybrid combination Wuyou308 using the *Magnaporthe oryzae* pathogen isolation method. Race identification of these isolates was conducted based on seven Chinese blast differentials and 11 blast monogenic lines. The results indicated that the isolates were identified as the races of ZB13, ZB15 and ZC13, accounting for 66.67%, 27.78%, 5.56%, respectively, and the resistance genes including *Pi-ta2* and *Pi-sh*, *Pi-i* were highly susceptible to these isolates, while the resistance genes like *Pi-kh*, *Pi-l*, *Pi-2*, *Pi-9* and *Pi-50* showed good resistance to tested pathogens. All isolates were compatible to the original rice hybrid Wuyou308. Three isolates including GDHY-308-1401 were used for testing their pathogenicity to 45 local varieties. The results demonstrated that 13 varieties appeared highly susceptible to the tested isolates, accounting for 28.89%; two varieties appeared moderately susceptible to the tested isolates, accounting for 4.44%; 30 varieties showed moderately/highly resistance, accounting for 66.67%. Among them, some of new hybrid combinations such as Wufengyou 9802, Wuyou 613, Wuyou 1179 showed good resistance to the inoculated strains, and they were recommended to be candidates in the rice region where Wuyou308 showed susceptibility.

**Key words** Wuyou308; *Magnaporthe grisea*; Pathogenicity; Rice variety; Combination

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Rice blast is an important rice disease in South China, which often causes serious rice yield reduction. The application of disease-resistant varieties is an economical and effective measure to control the disease<sup>[1]</sup>. *Magnaporthe grisea* exhibits pathogenic differentiation to varieties with different backgrounds, and its pathogenic types also change with the planting of different varieties. It is of great significance to know the pathogenicity characteristics of *M. grisea* in time for regional variety layout and its application strategy<sup>[2]</sup>. Wuyou 308, a hybrid rice combination, is widely planted in rice areas in South China and the middle and lower reaches of the Yangtze River, and shows good blast resistance in production<sup>[3]</sup>. However, this variety suffers from severe rice blast in some rice areas of Guangdong Province since the early season in 2013. Finding out the pathogenicity of *M. grisea* that causes Wuyou 308 to lose its resistance is of great guiding significance for the application of this variety and the rotation of varieties in planted areas.

## Materials and Methods

### Experimental materials

The seven Chinese varieties from which *M. grisea* races were identified were Tetepu, Zhenlong 13, Sifeng 43, Dongnong 363,

Guandong 51, Hejiang 18, and Lijiang Xintuan Heigu.

Eleven monogenic lines of rice blast were GDBL1 (harboring resistance gene *Pikh*), GDBL2 (harboring *Pi1*), GDBL3 (harboring *Pi9*), GDBL4 (harboring *Pita2*), GDBL5 (harboring *Pi50*), GDBL6 (harboring *Pikp*), GDBL7 (harboring *Pish*), GDBL8 (harboring *Piz*), GDBL9 (harboring *Pii*), IRBLzt (harboring *Pizt*), and IRBLz5 (harboring *Pi2*). A total of 45 cultivated varieties (combinations) were tested.

### Strain isolation and culture

The standard samples of neck blast were disinfected with 0.5% strong chlorine for 2 min, washed with sterile water, and placed in Petri dishes for 3–5 d while preserving moisture, and single spore isolation was performed by a spore shaking-off method. The isolates were cultured in test tubes containing yeast starch culture medium for 7–10 d, and then transferred to a corn kernel culture medium and cultured in it for 10–13 d. When the mycelia grew full of corn kernels, the mycelia were washed off from the surface of corn kernels with sterile water. The corn kernels were added in disinfected enamel dishes (25 cm × 19 cm × 2 cm), each of which was covered with a layer of wet gauze, and culture was continued under sunlight for 3–4 d. The spores attached to corn kernels were washed off with sterile water, and the corn residues were separated with two layers of plastic gauze. The concentration of inoculation spore liquids was adjusted to  $1 \times 10^5$  spores/ml. The culture and spore production of above isolates were carried out at 25 °C.

### Race identification and pathogenicity test

After the rice seeds were pre-germinated, they were hole-sown in enamel pots (30 cm × 20 cm × 5 cm), each of which was sown with 24 materials, and the seeding rate of each material was

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8–10 seeds. Rice seedlings were cultivated in dry land, and applied with ammonium sulfate after entering the stage of one leaf and one heart at a rate of 0.5 g per pot, for three times in total before inoculation. When the rice seedlings grew to 3.5–4 leaves, artificial spray inoculation was carried out according to an inoculum volume of 20 ml/pot. After inoculation, they were cultured in light-proof and sealed incubators while preserving moisture at 25 °C for 24 h. Next, the materials were cultured in a greenhouse at 25–28 °C while preserving moisture until the rice seedlings were diseased. At 7 d after inoculation, investigation was carried out. Race identification and pathogenicity determination of the isolates were set with two replicates. Race naming and disease level investigation were carried out according to the national identification method of *M. grisea* races and disease level investigation method<sup>[4]</sup>. In resistance type identification, 0–3 indicated resistance (R), and 4–9 indicated susceptible (S).

Table 1 Resistance response of *M. grisea* from Wuyou 308 to monogenic lines

Strain No.	Collection site	Race No.	Resistance response of monogenic lines in South China to identified races											
			GDBL 1	GDBL 2	GDBL 3	GDBL 4	GDBL 5	GDBL 6	GDBL 7	GDBL 8	GDBL 9	IRBL z1	IRBL z5	Wuyou 308
GDHY-308-1301	Heyuan	B15	R	R	R	S	S	R	S	S	S	R	S	S
GDHY-308-1302	Heyuan	B15	R	R	R	S	S	R	S	S	S	S	R	S
GDHY-308-1401	Heyuan	B15	R	R	R	S	R	S	S	S	S	R	R	S
GDHY-308-1402	Heyuan	B13	R	R	R	S	R	R	S	R	S	S	R	S
GDMZ-308-1301	Meizhou	B13	R	R	R	S	R	R	S	R	S	R	R	S
GDMZ-308-1302	Meizhou	B15	R	R	R	S	R	R	S	S	S	R	R	S
GDMZ-308-1303	Meizhou	B13	R	R	R	R	R	R	S	S	S	S	R	S
GDMZ-308-1401	Meizhou	B13	R	R	S	S	S	R	S	S	S	S	R	S
GDMZ-308-1402	Meizhou	B13	R	R	R	S	R	R	S	R	S	R	R	S
GDMZ-308-1403	Meizhou	B13	R	R	R	S	R	S	S	S	S	S	R	S
GDMZ-308-1404	Meizhou	B13	R	R	R	S	R	R	S	S	S	R	R	S
GDSG-308-1301	Shaoguan	B15	R	R	R	S	R	R	S	R	S	S	R	S
GDSG-308-1302	Shaoguan	C13	R	R	R	S	R	R	S	R	S	S	R	S
GDSG-308-1303	Shaoguan	B13	R	R	R	S	R	R	S	R	S	R	R	S
GDSG-308-1304	Shaoguan	B13	R	R	R	S	R	R	S	S	R	S	S	S
GDSG-308-1305	Shaoguan	B13	R	R	R	S	R	R	S	S	S	S	R	S
GDSG-308-1306	Shaoguan	B13	R	R	S	S	R	R	S	S	S	S	R	S
GDSG-308-1307	Shaoguan	B13	R	R	R	S	R	R	S	S	S	R	R	S

R stands for resistant, and S stands for susceptible. The genotype of GDBL1 is *Pikh*; the genotype of GDBL2 is *Pi1*; the genotype of GDBL3 is *Pi9*; the genotype of GDBL4 is *Pita2*; the genotype of GDBL5 is *Pi50*; the genotype of GDBL6 is *Pikp*; the genotype of GDBL7 is *Pish*; the genotype of GDBL8 is *Piz*; the genotype of GDBL9 is *Pii*; the genotype of IRBLz1 is *Pizt*; and the genotype of IRBLz5 is *Pi2*.

Pathogenicity test of strains

Three strains, GDHY-308-1401, GDMZ-308-1401 and GDSG-308-1303, were selected to determine the pathogenicity of some varieties (combinations) in Guangdong Province. The results (Table 2) showed that among the 45 varieties (combinations) tested, 13 varieties (combinations) showed high susceptibility. They were Wuyou 308, Maba Yinzhan, Meiyou 998, Jinnong Simiao, Meixiangzhan, Wufengyou 316, Boyou 998, Yuxiang Youzhan, Hemeizhan, Hefengzhan, Fengmeizhan, Wufengyou 587 and Wuyou615, accounting for 28.9% of the total rice varieties (combinations) tested. Two rice varieties, Wufengyou 2186 and Huanghuazhan, were moderately susceptible, accounting for 4.4%

Results and Analysis

Race identification

Eighteen *M. grisea* strains were isolated from the neck blast standard samples of Wuyou 308 collected from Heyuan, Meizhou and Shaoguan, respectively. The results of race identification showed that three races were identified from the isolates, namely ZB13, ZB15 and ZC13, accounting for 66.67%, 27.78% and 5.56% of the isolates, respectively. The identification results from the 11 monogenic lines of *M. grisea* showed that monogenic lines with high susceptibility to the isolates were GDBL4, GDBL7 and GDBL9, which contain resistance genes PI-TA2, PI-SH and PI-I respectively; monogenic lines with good resistance to the isolates were GDBL1, GDBL2, IRBLz5, GDBL3 and GDBL5, which harbor resistance genes *Pi-kh*, *Pi-1*, *Pi2*, *Pi-9* and *Pi-50* respectively; and all isolates could be inoculated back to hybrid rice Wuyou 308, making the inoculated variety diseased (Table 1).

of the total rice varieties (combinations) tested. Thirty varieties (combinations) showed moderate resistance to high resistance. They were Meiyou 9822, Yinfengyou 9802, Hengfengyou Huazhan, Tianyou 122, Tianyou Huazhan, Meiyou 9802, T78 You 2155, Wuyou 1179, Fengyou 9802, Hengfengyou 9802, Yuyou 9822, Tianyou 998, Y Liangyou 1179, Huangguang Youzhan, Anfengyou 3301, Rongyou Huazhan, Wuyou 613, Huahang Simiao, Wufengyou 9802, Ningyou 1179, Yongfengyou 9802, Fengtianyou 9802, Wushan Simiao, Zhenfengyou 9822, Yuejing Simiao 2, Xinyinzhan, Qisiyinzhan, Guangyan 1, Shengba Simiao and Baixiangzhan, accounting for 66.7% of the total rice varieties (combinations) tested. Among them, hybrid rice combinations

Wufengyou 9802, Wuyou 613 and Wuyou 1179, of the same type as Wuyou 308, showed good resistance to inoculated strains.

Table 2 Resistance of rice varieties ( combinations) to *M. grisea* from Wuyou 308

Disease resistant type	Variety ( combination)	Level of disease resistance against various strains			
		GDHY-308-1401	GDMZ-308-1401	GDSG-308-1303	Mean
Susceptible to highly susceptible	Wuyou 308	8	8	8	8.0
	Maba Yinzhan	8	8	8	8.0
	Meiyou 998	8	8	8	8.0
	Jinnong Simiao	7	8	8	7.7
	Meixiangzhan	8	8	7	7.7
	Wufengyou 316	7	8	8	7.7
	Boyoun 998	7	7	8	7.3
	Yuxiang Youzhan	8	6	6	6.7
	Hemeizhan	6	7	7	6.7
	Hefengzhan	5	8	7	6.7
	Fengmeizhan	7	7	6	6.7
	Wufengyou 587	6	7	7	6.7
	Wuyou 615	6	5	7	5.7
	Wufengyou 2186	6	6	5	4.3
	Huanghuazhan	5	4	4	3.0
Moderately susceptible to moderately resistant	Meiyou 9822	3	3	3	3.0
	Yinfengyou 9802	3	3	3	2.3
	Hengfeng Youhuazhan	1	3	3	2.3
	Tianyou 122	1	3	3	2.3
	Tianyou Huazhan	1	3	3	2.3
	Meiyou 9802	1	3	3	2.3
	T78 you 2155	3	3	1	2.3
	Wuyou 1179	1	3	3	2.3
	Fengyou 9802	3	2	1	2.0
	Hengfengyou 9802	1	3	1	1.7
	Yuyou 9822	1	1	3	1.7
	Tianyou 998	1	1	3	1.7
	Y Liangyou 1179	1	3	1	1.7
	Huangguang Youzhan	1	3	1	1.7
	Anfengyou 3301	1	3	1	1.7
	Rongyou Huazhan	1	1	3	1.7
	Wuyou 613	3	1	1	1.7
	Huahan Simiao	1	1	2	1.3
	Wufengyou 9802	1	1	1	1.0
	Ningyou 1179	1	1	1	1.0
	Yongfengyou 9802	1	1	1	1.0
	Fengtianyou 9802	1	1	1	1.0
	Wushan Simiao	1	1	1	1.0
	Zhenfengyou 9822	1	1	1	1.0
	Yuejing Simiao 2	1	1	1	1.0
	Xinyinzhan	1	1	1	1.0
	Qisi Yinzhan	1	1	1	1.0
	Guangyan 1	1	1	1	1.0
	Shengba Simiao	1	1	1	1.0
	Baixiangzhan	1	1	1	1.0

Average disease levels 6 – 9 stands for susceptible to highly susceptible; average disease levels 4 – 5 stands for moderately susceptible; and average disease levels 1 – 3 stands for moderately resistant to resistant.

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## Conclusions and Discussion

The distribution of physiological races of *M. grisea* in southern China's rice growing areas is mainly dominated by ZB and ZC groups with strong pathogenicity, and the monitoring results for many years show that ZB13, ZC13, ZC15 and ZB15 are stable dominant races in Guangdong Province<sup>[5–6]</sup>. The possible reason for this situation is that a single rice variety planted in a large area has led to the continuous rise of races with strong pathogenicity, so it is of great significance to maintain the diversity of *M. grisea* populations in a certain range for the prevention and control of rice blast. The results of this study indicate that in recent years, the severe susceptibility of Wuyou 308 to rice blast in multiple rice-growing areas in our province is mainly caused by dominant strains ZB13 and ZB15 of *M. grisea*. Resistant varieties are prone to loss of resistance after planting for several years, and highly-pathogenic populations ZB and ZC have become the absolute dominant populations in Guangdong Province, posing a great threat to rice production in our province, which requires high attention from relevant departments.

Monogenic line identification varieties of rice blast can well distinguish the resistance and susceptibility types of different physiological races to different disease-resistant genes<sup>[7–8]</sup>. In this study, 11 monogenic lines of rice blast were used to analyze the pathogenicity of *M. grisea* isolated from Wuyou 308, a rice variety resistant to blast, and the resistance and susceptibility of the isolates to different major resistance genes of rice blast was clarified. Eighteen isolates of *M. grisea* could infect *Pish* gene, and most of them could infect *Pi-ta2* and *Pi-i* genes. These three resistance genes are the main resistance genes in Guangdong Province, and have been widely used in disease resistance breeding of high quality rice in recent years.

Among the varieties tested in this study, conventional rice and hybrid rice varieties (combinations) containing disease resistance genes *Pi-ta2*, *Pi-sh* and *Pi-i*, such as Maha Yinzhan, Huan-guazhan, Jinnong Simiao, Meixiangzhan, Meiyu 998 and Boyou

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998, were susceptible to highly susceptible to tested strains. These varieties have certain risks when planted in areas where Wuyou 308 is infected, and special attention should be paid to the chemical control of rice blast.

*M. grisea* isolated from Wuyou 308 couldn't infect varieties (combinations) containing disease-resistant genes *Pi-kh*, *Pi-1*, *Pi-2*, *Pi-9* and *Pi-50*, such as Meiyu 9822, Meiyu 9802, Tianyou 998, Yuejing Simiao 2 and Xinyinzhan. Hybrid rice combinations Wufengyou 9802, Wuyou 613 and Wuyou 1179, of the same type as Wuyou 308, showed good resistance to inoculated strains, and they can be selected according to local conditions in the rice areas where Wuyou 308 is infected.

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