Synthetical Evaluation on the Qualitative and Quantitative Resistance of Rice Germplasms to *Magnaporthe oryzae*

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Abstract [**Objectives**] This study was conducted to screen out rice resources resistant to rice blast (*Magnaporthe oryzae*). [**Methods**] The qualitative and quantitative resistance of 1 659 rice resources from 45 countries and regions to rice blast were evaluated by disease nursery in upland condition and the test of the spectrum to rice blast isolates. [**Results**] There were 292 entries which accounted for 17.6% showed high blast resistance (0 and 1 disease scale), 68 entries (counted for 4.1%) showed resistance to blast (3 disease scale); and the number of the entries showed intermediate resistance, intermediate susceptible and susceptible were 208 (with the corresponding percentage of 12.5%), 471 (28.4%), 620 (37.4%) respectively. Among the tested entries, 27 entries including BG1222, BL122, BTX, IR37704-131-2-3-2, and LEBONNET had showed broad-spectrum blast resistance with the resistance frequency of higher than 90%, Quantitative resistance evaluation was conducted on some key resources, and 14 entries, of which are BR27, DRAGO, IR100, QINLIUAI、SERIBU GANTANG, YUEXIANGZHAN and so on, showed good quantitative resistances, and 8 entries had higher quantitative resistances than IR36. [**Conclusions**] This study provides important blast resistance resources for the local rice breeding program and has a significant value for the discovery of new blast resistance genes and its application in the blast resistance breeding.

Key words Rice germplasm; *Magnaporthe oryzae*; Resistance

Rice blast (*Magnaporthe oryzae*) is the most devastating and important disease in rice production, and the application of resistant varieties is an economic and effective measure to control this disease[1]. The diversity of pathogenicity differentiation of *Magnaporthe oryzae* and its variability often lead to resistance degradation or even loss in disease resistant varieties applied in production after 3 - 5 years of promotion. Therefore, continuously exploring and applying new resistance sources and breeding new types of resistant varieties are of great significance for the sustained and effective control of rice blast[2].

There are two main methods for identifying and evaluating rice blast in rice resources: natural induction identification in disease nurseries in diseased areas and indoor artificial inoculation identification. The former includes identification of seedling blast in upland nurseries and identification of leaf and panicle blast in irrigated nurseries, and the latter includes identification of mixed-strain inoculation and resistance spectrum determination. The identification of seedling blast in upland nurseries and mixed-strain inoculation are often used for primary screening, while the identification of leaf and panicle blast in irrigated nurseries and resistance spectrum determination are used for fine identification of resources[3-5]. The qualitative resistance to rice blast refers to the resistance (levels 0 - 3) and susceptibility (levels 4 - 9) that rice exhibits to pathogens, with a wide or narrow resistance spectrum; and the quantitative resistance refers to the level of resistance of rice to pathogens when it has been affected by the disease (phenotypically exhibiting 4 - 5 levels of seedling and leaf blast), usually measured by the proportion of diseased leaf area[3,5-6]. In this study, the qualitative and quantitative resistance of 1 659 rice resources from different countries and regions to rice blast were evaluated by disease nursery identification and indoor resistance spectrum determination methods, aiming to screen rice blast-resistant resources and provide important resource materials for disease resistance breeding and the exploration of new blast resistance genes.

Materials and Methods

Experimental materials

In this study, 1 659 rice resources from 45 countries and regions were mainly sourced from the International Rice Blast Nursery (IRBN) from 1994 to 2012, and some rice varieties were collected by The Plant Protection Research Institute, Guangdong Academy of Agricultural Sciences (Table 1).

Identification of disease resistance in nurseries

The experiment set up a rice blast nursery in Lvtian town, Conghua City, Guangdong Province. ① Upland nursery identification method: The planting of materials was carried out by the upland direct seeding method. Each material was seeded in a row, approximately 50 cm in length, with about 50 seeds, in three replicates. The inducing variety CO39 was seeded on both sides of each material. When the leaf blast level of the inducing variety

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CO39 reached level 9, the conditions of test materials were investigated. ② Irrigated nursery identification method for leaf and panicle blast: Thirty clumps was planted for each material (6 rows for 5 per row), in two replicates. One or two plants of the inducing variety (susceptible variety CO39) were transplanted on both sides of each row of each variety in each column. The leaf blast investigation was conducted in the late tillering stage of rice, while the panicle blast investigation was conducted in the early yellow ripe stage of rice.

The disease level investigation of the disease nursery experiment was conducted according to the international rice blast nursery method, and the rice blast resistance grading standard was referred to SES[7]. The investigation of diseased leaf area was conducted according to the method of Notteghem *et al*.[6].

Resistance spectrum determination

The upland nursery and cultivation method was adopted. The rice materials were germinated and then bunch-planted in enamel pots (30 cm × 20 cm × 5 cm), according to 24 materials per pot and 8 – 10 seeds per material. When the rice seedlings grew to the stage of one leaf and one heart, they were fertilized with ammonium sulfate, at a rate of 0.5 g per pot, for a total of 3 times before inoculation. When the rice seedlings grew to the age of 3.5 – 4.0 leaves, artificial spray inoculation was carried out, and the inoculation liquid volume was 20 ml/pot. After inoculation, the plants were placed in dark boxes and kept moist at 25 ℃ for 24 h. Then, they were cultured in a greenhouse and kept moist at 25 – 28 ℃ until the disease developed on rice seedlings. Each material was set with two replicates. An investigation was conducted 7 d after inoculation. There were 64 – 68 inoculated strains, all of which were single spore-isolated strains, belonging to ZA, ZB, ZC, ZF, and ZG, with ZB and ZC races being the main ones. The tested materials were inoculated with a single strain, respectively.

Resistance frequency (%) = (Number of strains not causing the disease/Total number of inoculated strains)

Results and Analysis

Qualitative resistance evaluation

Evaluation of seedling blast in upland nurseries According to the upland nursery identification method, 1 659 rice resources from 45 countries and regions from 1994 to 2012 were identified for resistance to rice blast. As shown in Fig. 1, there were 292 materials with high resistance (levels 0 – 1), accounting for 17.6%; there were 68 materials with resistance (level 2), accounting for 4.1%; and 208, 471, and 620 materials were, respectively, moderately resistant (level 3), moderately susceptible (levels 4 – 5), and susceptible (levels 6 – 9), accounting for 12.5%, 28.4%, and 37.4%, respectively. Among the resistant (levels 0 – 3) resources, 1 is from Oceania, 391 from Asia, 85 from Africa, 83 from America, and 6 from Europe. They accounted for 33.3%, 30.7%, 48.3%, 49.4%, and 15.4% of the tested materials in their respective regions, respectively (Table 1).

Resistance spectrum determination and field resistance evaluation Thirty one resistant resources (levels 0 – 3) identified in upland disease nurseries were tested for indoor resistance spectrum and field natural induction identification in the early crop of 2012 and the late crop of 2013, respectively, using thirty two isolates of *M. oryzae* with rich pathogenicity, to further clarify their resistance levels. The results (Table 2) showed that there were 9 materials showing 100% resistance frequency to the tested strains, namely BL122, BTX, CNA6870, IR37704-13-1-3-2, IR47686-13-1-1, IR5533-PP856-1, TOX1011-4-A2, TOX3107-56-1-2-2, and TOX955-208-2-102; and other resource materials that exhibited broad-spectrum resistance to tested pathogens included ALIANCA, 4732, 5173, B7291D-SM-12, BG1222, LEBONNET, MILAGROSA N. E., P30 DAWN, TSUYUAKE, and WHD-1S-75-1-127. These disease-resistant resources showed good resistance to leaf blast and panicle blast in affected areas.

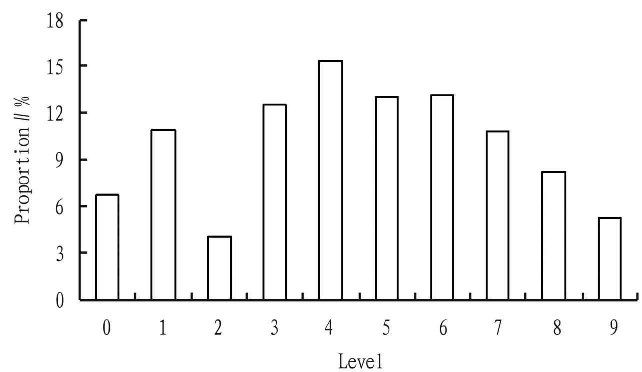

Fig. 1 Resistance performance and proportions with different resistance levels of 1 659 rice resources in upland nurseries

Quantitative resistance evaluation

In the identification experiment of rice blast in upland disease nurseries, 28.4% of the materials showed susceptibility to rice blast, but their disease levels were all at 4 or 5. In order to screen out resources with good quantitative resistance from these resources, in the crop of 2014, a multiple-cycle nursery infection experiment of rice blast and quantitative testing of diseased leaf area expansion were conducted on some resources in the historical disease areas of rice blast in Lvtian town, Conghua City, with B40 and IR36 as susceptible and resistant controls of quantitative resistance, respectively. According to the method of Notteam *et al*.[6], the testing of diseased leaf area expansion after the onset of disease was conducted on resource materials to evaluate the quantitative resistance levels of the resources. A total of 25 resources were tested, and among them, the final proportion of diseased leaf area (DLA) of susceptible control B40 was 82%, while the DLAs of other materials were smaller than that of the susceptible control; and the DLA of the resistant control IR36 was 7.2%, and the materials with superior quantitative resistance to IR36 included B3016-TB-260-3-2-1-1-3, TAINUNG 70, SEIBU GANTANG, GIGANGTE VERCELLI, IR100, DRAGO, and BR27, and materials with similar resistance to IR36 included QILIUAI, SUWEON 349, ORYZICA LIANOS 4, and YUEXIANGZHAN (Table 3).

Table 1 Identification of resistance of international rice germplasms to rice blast in upland nursery

Material source		Number of materials for each disease level				Name of resistant material
		Levels 0 – 3	Levels 4 – 5	Levels 6 – 9	Total	
Oceania (3 materials)	Australia	1	0	2	3	PD450
Asia (1273 materials)	Bengal	17	20	30	67	BR27, GOAI, MOLLADIGA, *etc.*
	China	59	32	41	132	4732, BTX, Xianzi3150, *etc.*
	India	19	36	85	140	ARC7098, PTB33, RCPL3-2, *etc.*
	Indonesia	21	12	14	47	B3532F-TB-1, DANAU LAUT TAWAR, BAHAGIA, *etc.*
	Iran	2	0	0	2	TCHAMPA, IRAT271
	International Rice Research Institute (IRRI, Philippines)	175	142	211	528	IR37704-131-2-1-3-2, BL22, 5173, *etc.*
	Japan	24	30	50	104	KAGAHIKARI, SAKAKIMOCHI, TSUYUAKE
	The Republic of Korea	39	32	49	120	UNBONG9, CHEOLWEON 48, SUWEON 405, *etc.*
	Lebanon	0	1	0	1	No
	Malaysia	4	6	1	11	MR103, BAHAGIA, SERIBU GANTANG, *etc.*
	Myanmar	0	2	2	4	No
	Nepal	0	1	1	2	No
	Pakistan	1	3	2	6	DM25
	Philippines	12	11	12	35	R63, C1136-3, SINANDOM, *etc.*
	Sri Lanka	5	8	5	18	BG1222, BG1492, BW306-2, *etc.*
	Taiwan	1	1	2	4	TAIKENG 8
	Thailand	8	13	25	46	RD19, SENG, DWOT82-2-1, *etc.*
	Vietnam	4	1	1	6	TETEP, MTL113, OM269-65, *etc.*
Africa (176 materials)	The Republic of Côte d'Ivoire	4	4	1	9	BOND, WAB32-55, WAB99-10, *etc.*
	Egypt	5	7	6	18	GAZA176, GZ4120-205, GZ4196-36, *etc.*
	Haiti	1	0	0	1	HD1-4
	International Institute for Tropical Agriculture (IITA, Nigeria)	33	8	3	44	ITA120, ITA257, ITA301, *etc.*
	French Institute of Tropical Agriculture and Food Crops (IRAT, The Republic of Côte d'Ivoire)	16	23	7	46	IRAT 299, IDSA 3, IRAT 318, *etc.*
	Madagascar		1	1	2	No
	Malawi	0	0	2	2	No
	Nigeria	0	2	3	5	No
	Senegal	1	0	0	1	WB56-50
	Sierra Leone	10	4	5	19	WAR72-1-1-1-4, WAR89-3-A8-1-B-B-2, WAR90-1-6-2-B-B-3, *etc.*
	Togo	1	0	0	1	MK14-87
	Africa Rice Center (WARDA, the Republic of Côte d'Ivoire)	12	10	4	26	WAB96-30, WAB30-24, WAB56-57, *etc.*
	West Africa	1	0	0	1	MOROBEREKAN
	Zaire	1	0	0	1	RT1031-69
Americas (168 materials)	Brazil	15	14	6	35	TRES MARIAS, GUAR ANI, CAIAPO, *etc.*
	International Center for Tropical Agriculture (CIAT, Colombia)	21	17	11	49	BRIRGA 410, CT6516-1-2-2, P5166F2-25-2, *etc.*
	Columbia	21	2	3	26	5173, BAKKA BATJERE, CICA9
	Costa Rica	0	1	0	1	No
	Cuba	0	1	0	1	No
	The Dominican Republic	0	0	1	1	No
	Salvador	0	0	1	1	No
	Republiek Suriname	0	2	1	3	No
	U. S. A	26	11	14	51	LEMONT, LABELLE, NEW BONNET, *etc.*
Europe (39 materials)	France	0	0	1	1	No
	Hungary	0	2	7	9	No
	Italy	5	5	4	14	ALPE, GIGANGTE VERCELLI, PAND, *etc.*
	Peru	0	0	3	3	No
	The Republic of Türkiye	1	1	10	12	87020-TR968-1-1-1

The data in parentheses represent the number of identified rice samples from the continent; and resistant materials refer to materials with disease level ≤3. 'No' means that there are no disease-resistant materials with disease level ≤3 in the identified materials from the country.

Table 2 Determination of resistance spectrum and field resistance evaluation of some key resistant sources to *M. oryzae*

Variety name	Source	Resistance frequency//%			Field resistance	
		B group	C group	Total resistance frequency	Leaf blast	Panicle blast
CNA6870	Brazil	100	100	100	3	NH
JAVAE		93.8	100	93.8	2	3
CNA687		93.8	100	93.8	3	NH
CNA3891		93.8	100	93.5	3	1
WAB30-24	Africa Rice Center	87.5	88.9	90.6	3	1
MILAGROSA N. E.	Philippines	93.8	100	96.6	1	1
CARREON		62.5	88.9	78.1	5	NH
5173	Columbia	96.6	100	93.8	1	1
TOX3107-56-1-2-2	International Institute for Tropical Agriculture	100	100	100	1	NH
TOX1011-4-A2		100	100	100	1	3
TOX955-208-2-102		100	100	100	3	1
ALIANCA	International Center for Tropical Agriculture	100	100	96.9	1	1
BL122	International Rice Research Institute	100	100	100	1	1
IR37704-131-2-1-3-2		100	100	100	1	1
IR47686-13-1-1		100	100	100	1	1
IR5533-PP856-1		100	100	100	1	1
5173		93.75	100	96.9	1	3
P30 DAWN		93.8	100	96.6	1	1
WHD-1S-75-1-127		93.8	100	96.6	1	NH
IR60		87.5	100	93.5	2	3
LEBONNET	U. S. A	93.8	100	96.9	3	1
BR14	Bengal	87.5	66.7	75.0	3	NH
TSUYUAKE	Japan	93.75	100	96.9	2	3
BG1222						
Sri Lanka	93.75	100	96.9	1	3	
BW311-1		75	88.9	78.1	4	1
CNTBR82074-210-1-2-1	Thailand	87.5	100	90.7	5	3
NP125	India	81.2	66.7	75.0	5	5
B6149F-MR-7	Indonesia	87.5	100	93.5	1	1
B7291D-SM-12		93.8	100	96.6	3	1
BTX	China	100	100	100	1	1
4732		93.8	100	96.6	1	1

The number of strains used for spectrum determination is 32; and NH indicates no heading and no experimental data.

Table 3 Evaluation of quantitative resistance to rice blast in 25 rice resources

Resource name	Source	Proportion of diseased leaf area//%				
		I	II	III	IV	V
B40(ck, S)	Indonesia	3.8 ±1.9	17.7 ±1.6	32.5 ±2.6	62.3 ±4.2	82.0 ±0.0 a
SWARNA	India	1.1 ±0.2	10.3 ±1.3	12.5 ±0.7	26.7 ±3.0	39.3 ±1.9 b
ARBORIO	Italy	1.1 ±0.2	8.5 ±1.2	10.4 ±0.8	28.2 ±2.6	39.0 ±2.1 b
METICA 1	Columbia	2.2 ±0.3	4.2 ±0.3	16.6 ±2.5	32.6 ±2.7	32.6 ±2.7 c
MTU 1003	India	2.3 ±0.5	4.3 ±0.9	8.5 ±0.5	17.2 ±2.1	31.5 ±1.8 c
BW295-4	Sri Lanka	1.9 ±0.6	12.2 ±1.1	28.4 ±3.3	31.0 ±1.4	31.3 ±1.6 c
HPU741	India	2.1 ±0.2	10.6 ±1.1	23.5 ±1.5	24.4 ±0.9	28.1 ±1.2 d
Chianung Si-Pi661020	China	2.1 ±0.2	10.5 ±1.0	22.4 ±1.7	23.9 ±1.3	27.0 ±1.2 de
IR3880-29	International Rice Research Institute	2.3 ±0.2	9.6 ±0.3	21.6 ±0.7	22.2 ±1.3	25.2 ±1.0 e
KMP34	India	2.2 ±0.3	9.1 ±0.2	21.5 ±1.6	22.4 ±3.6	22.3 ±0.8 f
CAIAPO	India	1.9 ±0.2	2.7 ±0.4	4.0 ±0.3	8.3 ±0.5	16.5 ±0.7 g
MAHSURI	Malaysia	2.2 ±0.4	2.4 ±0.2	4.4 ±1.1	8.3 ±0.6	16.3 ±0.4 g
MTU 1001	India	2.2 ±0.2	2.2 ±0.2	3.1 ±0.2	15.2 ±1.9	15.4 ±1.8 g

(Continued)

(Table 3)

Resource name	Source	Proportion of diseased leaf area//%				
		I	II	III	IV	V
YUEXIANGZHAN	China	3.0±0.6	6.7±0.5	9.1±0.4	10.1±1.2	11.5±0.6 h
ORYZICA LIANOS 4	Columbia	2.0±0.3	2.5±0.9	4.6±0.5	8.5±0.6	8.6±0.6 i
SUWEON 349	The Republic of Korea	2.8±0.5	4.0±0.7	8.1±0.5	8.3±0.5	8.5±0.5 i
IR36(ck, R)	International Rice Research Institute	2.8±0.4	4.3±0.5	5.1±0.4	5.1±0.4	7.2±0.4 ij
QILIUAI	China	2.5±0.5	5.7±0.6	5.9±0.4	6.1±0.7	6.6±0.1 ijk
BR27	Bengal	1.3±0.1	2.4±0.3	4.3±0.5	4.4±0.6	5.2±0.8 jkl
DRAGO	Italy	1.1±0.0	1.2±0.1	4.3±0.5	4.4±0.4	4.8±0.5 klm
GDIR100	International Rice Research Institute	1.1±0.2	1.9±0.5	2.0±0.6	2.2±0.4	4.4±0.8 lmn
GIGANGTE VERCELLI	Italy	1.1±0.3	3.4±0.6	3.8±0.6	3.9±0.6	4.1±0.7 lmn
SERIBU GANTANG	Malaysia	2.1±0.2	2.4±0.6	2.5±0.2	4.0±0.8	4.1±0.8 lmn
TAINUNG 70	Taiwan	0.9±0.1	1.1±0.2	1.2±0.3	1.8±0.8	2.7±0.5 mn
B3016-TB-260-3-2-1-1-3	Indonesia	0.7±0.1	2.1±0.3	2.1±0.3	2.2±0.4	2.3±0.4 n

I is the initial stage of the disease, and the intervals between II and I, III and II, IV and III, and V and IV are all 5 d; the proportion of diseased leaf area refers to the proportion of diseased spot area to the entire leaf; and different lowercase letters following data within the same column indicate a significant difference in the final proportion of diseased leaf area between test resources.

Conclusions and Discussion

The diversity of pathogenicity differentiation of *M. oryzae* and its variability often lead to resistance degradation or even loss in disease resistant varieties after 3–4 years of promotion, and it is of great significance to continuously explore new resistance sources, especially durable resistant sources, for breeding durable resistant varieties[1]. Studies have shown that the persistence of rice blast is often composed of higher qualitative and quantitative resistance. Qualitative resistance is specifically manifested as broad-spectrum resistance, which exhibits non-affinity reactions to different pathogenic races, namely resistance reactions. Quantitative resistance refers to the fact that although a variety exhibits an affinity reaction to pathogens, its incidence is relatively mild, specifically manifested in a lower proportion of diseased leaf area[3-4]. This study screened a group of resource materials with good qualitative and quantitative resistance from rice resources from 45 different countries, including *Indica*, *Japonica*, and early middle rice populations (aus). The resistant resources with rich genetic backgrounds lay a solid foundation for further exploring new resistance genes and selecting rice varieties with persistent blast resistance.

Research has shown that the resistance sources of blast-resistant varieties in Guangdong Province mainly come from Sanhuangzhan 2, Qingliu'ai 1, Jingxian 89, IR36, and 28 Zhan[5]. Sanhuangzhan 2 has high qualitative and quantitative resistance, and its resistance is composed of multiple major resistance genes and multiple QTLs[4,8]; and the broad-spectrum resistance exhibited by 28 Zhan is mainly composed of the broad-spectrum resistance gene *Pi50* and two complementary resistance genes *Pita3* and *Pish*[9-10]. Fengsizhan, Yuejing Simiao 2, Wushan Simiao, Yuenong Simiao and other resistant varieties bred using resistance source 28 Zhan have been widely promoted and applied in Guangdong rice regions. Currently, new races of *M. oryzae* that can infect these varieties have emerged in some rice regions, and these new races also have strong infectivity against other resistant varieties. Breeding for blast resistance in Guangdong Provinces faces new challenges[11]. A deep understanding of the genetic background of applied resistance sources and targeted exploration of new resistance sources and genes are of great significance for future resistance breeding.

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