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Evaluation of Resistance of US Rice Breeding Lines to the Rice Blast Pathogen

Chunda Feng and James C. Correll

Abstract

Rice blast, caused by the fungus *Magnaporthe oryzae* (anamorph: *Pyricularia oryzae*), is a ubiquitous disease that threatens rice production in the USA and worldwide. Growing resistant cultivars is the most economical and effective way to manage this disease. Multiple races exist in the *M. oryzae* population in the USA. It is necessary to know the resistance spectrum of rice cultivars to the prevalent rice blast races in the areas where they are grown. Twelve isolates of *M. oryzae* collected from the southern US rice-growing region were used in this study. The genetic diversity of these isolates was evaluated with genetic and molecular methods, and the pathogenicity to different rice blast resistance genes was determined by the disease reaction of two sets of near-isogenic lines containing one blast *R* gene per line. From 2005 to 2016, about 200 Uniform Regional Rice Nursery (URRN) breeding lines have been tested with 9–12 reference isolates annually, and a total of 2377 breeding lines have been tested. The varieties with good resistance to rice blast disease have been identified. The results could be useful for the management of rice blast disease in the southern US rice production area.

Keywords: rice, blast disease, avirulence gene, resistance gene, breeding lines

1. Introduction

Rice is one of the most important staple food crops worldwide, feeding over half of the world's population [1]. The demand for rice continues to increase with the increase in the global population. The USA grows approximately 1.5 million hectares of rice annually and produces about 8–11 million metric tons of rice valued at 3.6 billion dollars (**Figure 1**) [2]. Although the USA is a relatively small rice producer accounting for less than 2% of the total rice production worldwide, it is a major rice exporter that occupies 6–13%, with an average of 10%, of the world rice export market (**Figure 1**), making the USA one of the top rice exporters in the world [3].

Rice blast disease, caused by the fungus *Magnaporthe oryzae* (anamorph: *Pyricularia oryzae*), is one of the most important diseases on rice worldwide and is responsible for approximately 30% of rice production losses globally [1, 4]. A wide range of management practices have been used to reduce losses from rice blast. For example, cultural practices such as crop rotation, controlling the timing and amount of nitrogen applied, and managing the flood depth in the field may reduce the impact of blast [5]. A number of fungicides also are effective in managing rice

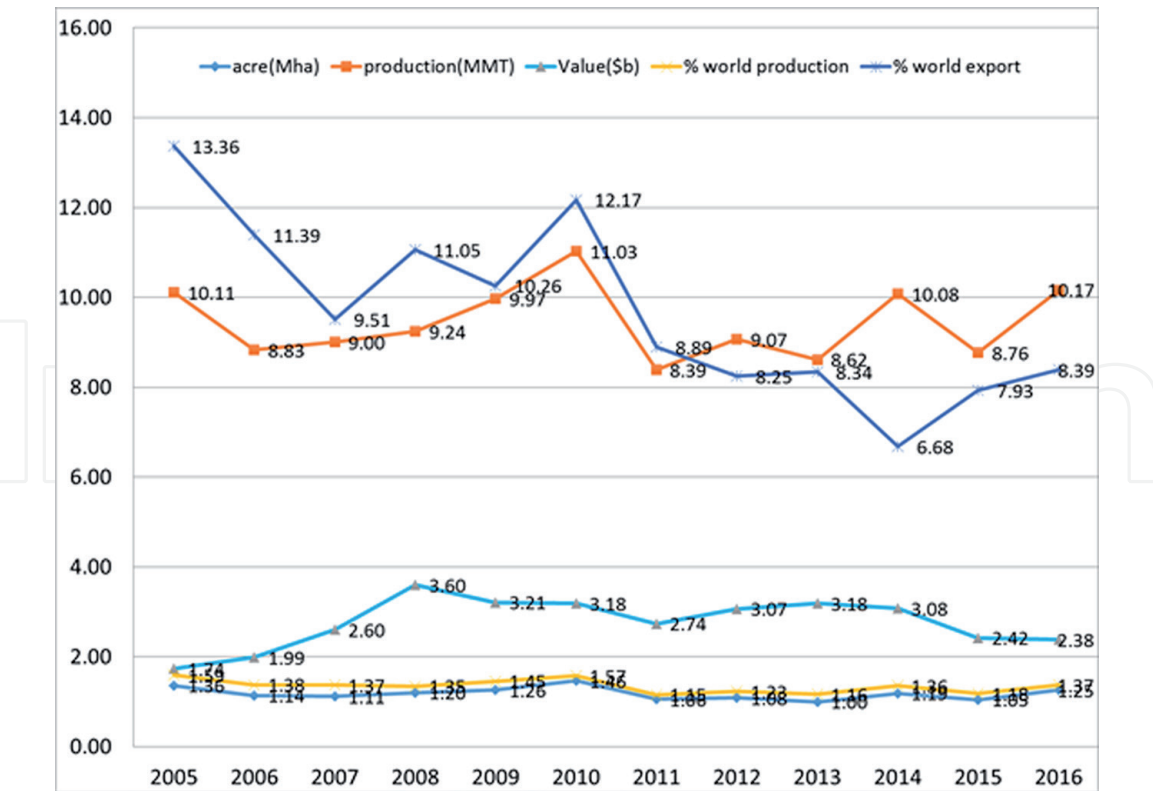


Figure 1. Rice production in the USA and its percentages of world total rice production and export. Mha, million hectare; MMT, million metric tons.

blast disease [4]; however, it is not a preferred management option due to environmental concerns and cost. Growing resistant cultivars is the most economical and effective way to manage this disease [4, 6]. Many rice blast *R* genes have been characterized, some of which have been widely used in rice breeding programs worldwide [6–8]. The *R* genes recognize the corresponding specific avirulence genes from the pathogen and initiate defense mechanism [9]. For example, the *R* gene *Pita* can interact with the counterpart *AVR-Pita* from the pathogen and confer resistance [10]. However, the changes in avirulence genes can result in the loss of function of the corresponding *R* genes. For example, the *R* gene *Pita* has deployed in rice cultivars in the southern USA and provided durable resistance for a long period of time [11], but the resistance of the *Pita* gene was overcome by race IE1k in 2004 [12].

The population of *M. oryzae* in the southern USA has been intensively studied [13–18]. Multiple races exist in the *M. oryzae* population in the USA. For example, race IB49 and IC17 were the most prevalent races in Arkansas [13–15], with occasional epidemics due to race IE1k or “race K” type isolates [12]. Near-isogenic lines, each containing a targeted blast resistant gene, in either a Japonica-type variety Lijiangxingtuanheigu (LTH) background [19] or Indica-type CO39 background [20], have been used for race identification in Asia [21]. In the USA, the *M. oryzae* population has been intensively studied [13–18, 22], but the relationship between races to individual rice blast *R* genes in the USA is largely unknown [22]. In addition, it is necessary to evaluate the resistance spectrum of newly developed rice breeding lines to the prevalent rice blast races in the southern US rice-growing region before they are released.

The objective of this study was to summarize the disease reactions of a wide range of rice germplasm from the Uniform Regional Rice Nursery (URRN) lines to 12 reference isolates of the rice blast pathogen from 2005 to 2016.

2. Diversities of the 12 US reference isolates of *M. oryzae*

2.1 Twelve US reference isolates of *M. oryzae*

Twelve isolates of *M. oryzae*, collected from the southern USA, were used as reference isolates to test the URRN lines during 2005–2016 (**Table 1**). Among them, six isolates (49D, #24, A119, A264, A598, IB33) were collected from AR; four isolates (TM2, ID13, ZN7, and ZN15) were collected from TX; one isolate, IB54, from LA; and one isolate, ZN46, from FL (**Table 1**). These isolates represented 10 races, including IB49 (49D, A119, and A598), IB33, IB54, IC1 (ZN46), IC17 (A264), ID13, IE1 (ZN7), IE1k (TM2), and IG1 (#24). Most isolates were used each year on a different set of URRN lines. Isolate IB33 has been tested in 11 years but not in 2007. Isolate IB54 has not been tested until 2009. Isolate ID13 has been tested in 7 years, but not in 2005, 2007, 2009, 2013, and 2014.

2.2 Genetic diversity of the 12 reference isolates

The genetic diversity of the 12 reference isolates was evaluated by vegetative compatibility analysis [13] and molecular methods. Vegetative compatibility analysis indicated that three isolates A598, ZN15, and ZN46 belonged to vegetative compatibility group (VCG) US-01; isolates TM2, #24, and A264 belonged to VCG US-02; two isolates 49D and A119 belonged to VCG US-03; and other two isolates IB33 and IB54 belonged to VCG US-04 (**Table 1**). The VCG of isolate ID13 was not determined.

Using Pot 2 primers [23], the repetitive element-based polymerase chain reaction (Rep-PCR) was used to DNA fingerprint the 12 reference isolates. The amplicon patterns of 49D, IB33, and IB54 based on Pot 2 primers were identical; TM2 and ZN7 were identical to each other; isolate 24 and A264 were identical to each other, but they had one extra band compared to that of TM2 and ZN7; ZN15 and ZN46 had similar patterns (**Figure 2**). The mating types of these isolates were determined by using mating-type-specific primers [24]. The results suggested that six isolates, 49D,

Isolate	Vegetative compatibility group (VCG)	Mating type	RACE	Year	Origin
49D	US-03	I	IB49	1985	AR
TM2	US-02	II	IE1K	2004	TX
#24	US-02	II	IG1	1992	AR
A119	US-03	II	IB49	1992	AR
A264	US-02	II	IC17	1993	AR
A598	US-01	I	IB49	1992	AR
IB33	US-04	I	IB33		AR
IB54	US-04	I	IB54	1959	LA
ID13		II	ID13	1982	TX
ZN7	US-02	II	IE1	1995	TX
ZN15	US-01	I	IB1	1996	TX
ZN46	US-01	I	IC1	1996	FL

Table 1.
Background information on the 12 US reference isolates of *M. oryzae* used in this study.

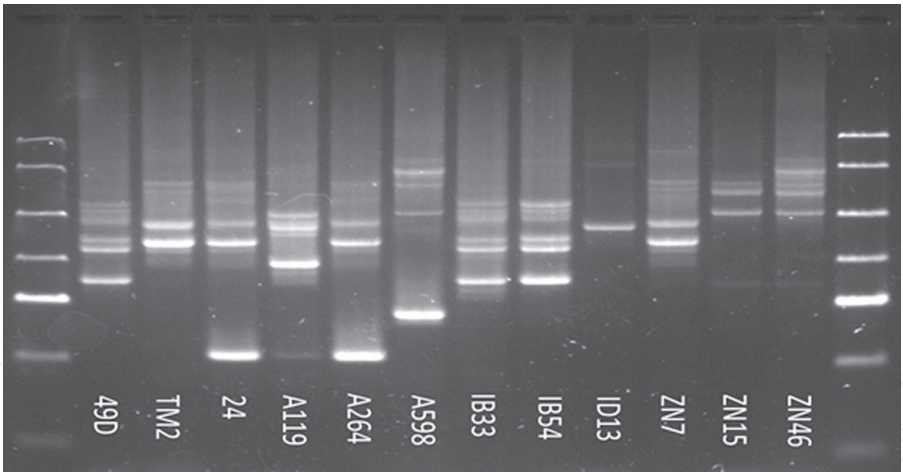


Figure 2.
Rep-PCR band patterns of 12 reference isolates amplified with Pot 2 primers.

A598, IB33, IB54, ZN15, and ZN46, belonged to mating type I, while other six isolates TM2, #24, A119, A264, ID13, and ZN7 belonged to mating type II (**Figure 3**).

Seven avirulence genes were assessed using specific primers to each gene (**Table 2**) [25–28]. The entire *AVR-Pita* fragment could be amplified from nine isolates with primers YL149/YL169, but not from isolates TM2, IB33, and ID1. The coding regions of the avirulence gene *AVR-Pib* was found in all 12 reference isolates (amplified with the *AVR-Pib* F3/R3 primers); however, the promoter region of the *AVR-Pib* gene (amplified with the *AVR-Pib* F2/R2 primers) was not found in isolates 49D, IB33, and IB54. The avirulence gene *AVR-Pikm* was only found in four isolates, 49D, IB33, IB54, and ID13. The other four avirulence genes, *AVR-CO39*, *AVR-Pi9*, *AVR-Pikz*, and *AVR-Piz-t*, were present in all 12 reference isolates (**Figure 4**).

2.3 Testing the US reference isolates on IRRI near-isogenic rice lines

2.3.1 IRRI rice blast near-isogenic lines

The 12 US reference isolates were tested on 31 LTH NILs (containing 24 blast *R* genes) and 20 CO39 NILs (containing 14 *R* genes) in three independent tests, with two replications in each test. Two cultivars, M204 and Francis, were included as susceptible controls.

2.3.2 Inoculation of blast pathogen and disease screening

Rice seed was planted in plastic trays filled with river sand mixed with potting soil in the greenhouse at the University of Arkansas, Fayetteville, AR, USA. Iron

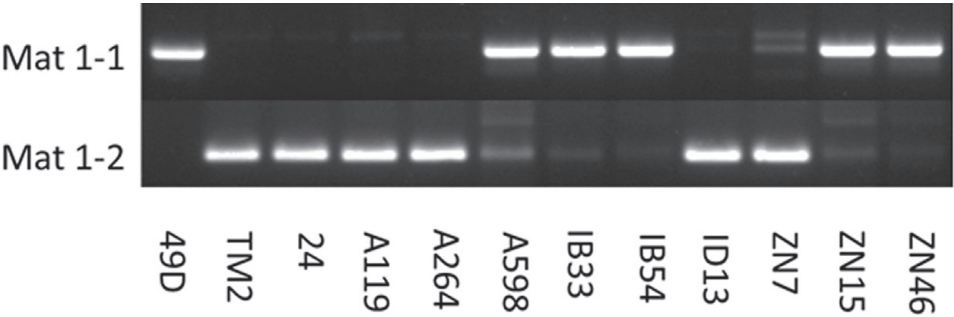


Figure 3.
Mating type analysis of the 12 reference blast isolates.

Target gene	Primer name	Sequences
AVR-Pita	YL149	TGACCGCGATTCCCTCCATT
	YL169	CGACCGTTTCCGCC
AVR1-CO39	AVR1-CO39F1	GATCTGTAAATTACATA
	AVR1-CO39R1	GGATCCGCCGTCGCCTCC
AVR-Pi9	AVRPi9F	CTG CTC CAT CTT GTT TGG CC
	AVRPi9R	CAC TAG TAC AAG CAC TAA CC
AVR-Pib	AVR-PibF2	TGGAGAAGACTTTGATGC
	AVR-PibR2	TAGTTGCCATTATGCGTTC
	AVR-PibF3	ATGCGTTCCTCAACCACTTT
	AVR-PibR3	TTATTCCACGGTATATTTGCTGCC
AVR-Pikz	AVR-PikzF	TGACGCAGCTTGAGTTGT
	AVR-PikzR	TCCGAGCAATCAACTCTG
AVR-Pikm	AVR-PikmF	TTATCGCCCCCTATATTGC
	AVR-PikmR	TTATCGCCCCAACACGGA
AVR-Piz-t	AVR-Piz-tF	ATGCAGTTCTCAACCATC
	AVR-Piz-tR	CTATTGGCGCTGAGCCT

Table 2.
Primers used to amplify seven avirulence genes from the 12 US reference isolates of *M. oryzae*.

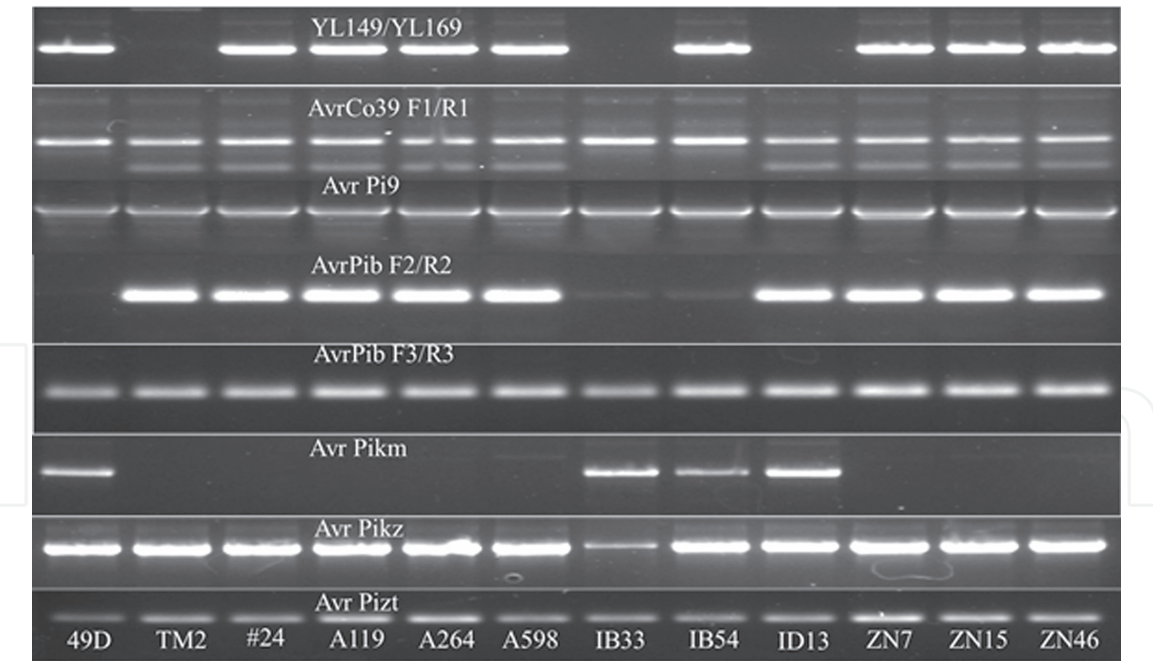


Figure 4.
Detection of seven avirulence genes in the 12 US reference isolates of *M. oryzae*.

sulfate was applied to the newly emerged seedlings. The plants were fertilized with Miracle-Gro All-Purpose Plant Food 20-20-20 once a week during each test. Plants were inoculated approximately 14–20 days after planting. Each isolate was grown on rice bran agar (RBA) [13] for approximately 7–10 days and then reinoculated on new RBA plates for 7–10 days. Spores were collected in cold water and adjusted to a concentration of 200,000 spores/ml per isolate. Each tray was inoculated with 50 ml of inoculum mixed with 0.02% Tween 20 with an air compressor sprayer. After

inoculation, the plants were incubated at 100% relative humidity in a mist chamber at approximately 22°C for 24 h, allowed to dry for 2–3 h before being moved to the greenhouse. The inoculated plants were incubated in the greenhouse for 6 days. On the 7th day after inoculation, 15–20 plants of each line were scored according to a standard 0–9 disease rating scale developed by IRRI [22]. Lines rated 0 to 3 were considered resistant, whereas those rated 4–9 were considered susceptible.

2.3.3 Virulence of US reference isolates of *M. oryzae* on IRRI rice NILs

Among the blast reference isolates, IB33, 49D, and TM2 were the most virulent isolates, whereby only 8–13 (15.7–21.6%) of the NILs were resistant. Isolates IB54, ID13, and #24 were the least virulent isolates with 33–38 (64.7–74.5%) of the NILs being resistant. Other isolates were intermediate in virulence (**Table 3**).

NILs containing gene *Pi3(t)* were susceptible to all reference isolates tested. NILs containing *R* genes *Pia*, *Pi19(t)*, *Pii*, *Piks*, and *Pizt*, were only resistant to one isolate. Those lines containing *Pikh*, *Pikp*, and *Pita* were resistant to two isolates. NILs containing the *R* genes, *Pi1*, *Pi7(t)*, *Pik*, *Pikp*, *Pikm*, *Pit*, and *Piz*, were resistant to three isolates. NILs containing *Pi9(t)* or *Pi12(t)* were resistant to all isolates (**Table 4**). Lines containing genes *Pib*, *Pi11(t)*, and *Pita-2/Ptr* [29] were resistant to 9 or 11 isolates.

Line	A598	ZN15	ZN46	24	A264	ZN7	TM2	49D	A119	IB33	IB54	ID13
R	16	21	17	33	31	15	13	11	27	8	38	34
%	31.4	41.2	33.3	64.7	60.8	29.4	25.5	21.6	52.9	15.7	74.5	66.7
S	35	30	34	18	20	36	38	40	24	43	13	17
%	68.6	58.8	66.7	35.3	39.2	70.6	74.5	78.4	47.1	84.3	25.5	33.3

Table 3.
Number and percentage of rice NILs resistant or susceptible to blast isolates.

ID	Gene	49D	TM2	24	A119	A264	A598	IB33	IB54	ID13	ZN7	ZN15	ZN46
		IB49	IE1K	IG1	IB49	IC17	IB49	IB33	IB54	ID13	IE1	IB1	IC1
IRBLA-A	<i>Pia</i>	S	S	S	S	S	S	S	R	S	S	S	S
IRBLA-C	<i>Pia</i>	S	S	S	S	S	S	S	R	S	S	S	S
IRBLI-F5	<i>Pii</i>	S	S	S	S	S	S	R	R	S	S	S	S
IRBLKS-F5	<i>Piks</i>	S	S	S	S	S	S	S	R	S	S	S	S
IRBLKS-S	<i>Piks</i>	S	S	S	S	S	S	R	R	S	S	S	S
IRBLK KA	<i>Pik</i>	S	S	R	S	S	S	S	R	R	S	S	S
IRBLKP-K60	<i>Pikp</i>	S	S	R	S	S	S	S	S	R	S	S	S
IRBLKH-K3	<i>Pikh</i>	S	S	R	S	S	S	S	S	R	S	S	S
IRBLZ FU	<i>Piz</i>	S	R	S	S	R	S	S	S	R	S	S	S
IRBLZ 5-CA	<i>Piz-5</i>	R	S	S	R	R	R	R	R	R	R	R	S
IRBLZT-T	<i>Piz-t</i>	S	S	S	S	S	S	S	R	S	S	S	S
IRBLTA CT 2	<i>Pita</i>	S	S	R	S	R	S	S	S	S	S	S	S
IRBLB-B	<i>Pib</i>	S	R	R	R	R	R	S	S	R	R	R	R
IRBLT-K59	<i>Pit</i>	S	S	S	R	R	S	S	R	S	S	S	S
IRBLSH-S	<i>Pish</i>	R	R	R	R	R	R	R	R	R	R	R	R
IRBLSH-B	<i>Pish</i>	S	S	R	R	R	R	S	R	S	S	R	S

ID	Gene	49D	TM2	24	A119	A264	A598	IB33	IB54	ID13	ZN7	ZN15	ZN46
		IB49	IE1K	IG1	IB49	IC17	IB49	IB33	IB54	ID13	IE1	IB1	IC1
IRBL 1-CL	<i>Pi1</i>	S	S	R	S	S	S	S	R	R	S	S	S
IRBL 3-CP 4	<i>Pi3</i>	S	S	S	S	S	S	S	S	S	S	S	S
IRBL 5-M	<i>Pi5(t)</i>	S	S	R	R	R	S	S	R	R	S	R	S
IRBL 7-M	<i>Pi7(t)</i>	S	S	R	R	R	S	S	S	R	S	S	S
IRBL 9-W	<i>Pi9</i>	R	R	R	R	R	R	R	R	R	R	R	R
IRBL 12-M	<i>Pi12(t)</i>	R	R	R	R	R	R	R	R	R	R	R	R
IRBL 19-A	<i>Pi19(t)</i>	S	S	S	S	R	S	S	S	S	S	S	S
IRBLKM TS	<i>Pikm</i>	S	S	R	S	S	S	S	R	R	S	S	S
IRBL 20-IR 24	<i>Pi20</i>	S	S	S	R	R	R	S	R	R	S	R	R
IRBLTA 2-PI	<i>Pita-2</i>	R	S	R	R	R	R	S	R	R	R	R	R
IRBLTA 2-RE	<i>Pita-2</i>	R	S	R	R	R	R	S	R	R	R	R	R
IRBLTA CP 1	<i>Pita</i>	S	S	R	S	S	S	S	R	S	S	S	S
IRBL 11-ZH	<i>Pi11(t)</i>	S	R	R	R	R	R	S	R	R	R	R	R
IRBLZ 5-CA (R)	<i>Piz-5</i>	R	R	S	R	R	S	R	R	R	S	R	S
LJXTHG	<i>LTH</i>	S	S	S	R	R	S	S	R	S	S	S	S
IR 85430	<i>Pish</i>	S	R	R	R	R	S	S	R	R	S	R	R
IR 85424	<i>Pish</i>	S	R	R	R	R	S	S	R	R	S	R	R
IR 93322	<i>Pish</i>	S	R	R	R	R	S	S	R	R	S	R	R
IR 85417	<i>Pib</i>	S	R	R	R	R	R	S	R	R	R	R	R
IR 85427	<i>Piz-5</i>	R	S	S	R	R	R	R	R	R	S	R	R
IR 85429	<i>Piz-t</i>	S	R	R	R	R	R	S	R	R	R	R	R
IR 85413	<i>Pi5(t)</i>	S	R	S	R	R	S	S	R	R	S	S	S
IR 85423	<i>Piks</i>	S	S	S	S	S	S	S	R	S	S	S	S
IR 85420	<i>Pik</i>	S	S	R	S	S	S	S	R	R	S	S	S
IR 85419	<i>Pik</i>	S	S	R	S	S	S	S	R	R	S	S	S
IR 85421	<i>Pikm</i>	S	S	R	S	S	S	S	R	R	S	S	S
IR 85422	<i>Pikp</i>	S	S	R	S	S	S	S	R	R	S	S	S
IR 85411	<i>Pi1</i>	S	S	R	S	S	S	S	R	R	S	S	S
IR 85414	<i>Pi7(t)</i>	S	S	R	S	R	S	S	R	R	S	S	S
IR 85426	<i>Pita</i>	S	S	R	R	R	S	S	R	S	R	S	S
IR 93324	<i>Pita</i>	S	S	R	R	R	S	S	R	S	R	S	S
IR 93323	<i>Pita-2</i>	R	S	R	R	R	R	R	R	R	R	R	R
IR 85425	<i>Pita-2</i>	R	S	R	R	R	R	S	R	R	R	R	R
IR 93325	<i>Pita-2</i>	R	S	R	R	R	R	S	R	R	R	R	R
CO39	<i>Pia</i>	S	S	S	S	S	S	S	S	S	S	S	S

Table 4.
Disease responses of rice NILs to US Magnaporthe oryzae reference isolates.

Four loci provided resistance to reference isolate 49D (race IB49) or IB33 (race IB33), 7 loci were resistant to isolate TM2 (race k), and 14, 16, and 17 loci were resistant to isolate IB54, isolate #24 (race IG1), and isolate ID13, respectively.

Discrepancy in disease reactions was observed among lines putatively containing the same target *R* genes. One NIL (IRBLSH-S), containing *Pish*, was resistant to all blast reference isolates, while four *Pish* containing lines were only resistant to six to eight isolates. Both NILs IRBLZT-T (in LTH background) and IR 85429 (in CO39 background) contain *R* gene *Pizt*. NIL IRBLZT-T was resistant to one isolate, while IR 85429 was resistant to 10 isolates. These discrepancies may have resulted due to a number of reasons including linkage drag from different donor parents. The *R* genes in the NILs also need to be confirmed with specific molecular markers.

Thus, NILs containing *Pia*, *Pi3*, *Pi19(t)*, and *Pi12(t)* were not useful for differentiating races of the US reference isolates tested. Resistance loci *Pi9(t)*, *Pi12(t)*, *Pib*, *Pi11(t)*, and *Pita-2* were the most effective *R* genes to the panel of US reference isolates evaluated and could be exploited to improve resistance to rice blast disease in the USA.

3. Evaluation of resistance of the US rice breeding lines to reference isolates of *M. oryzae*

3.1 Uniform Regional Rice Nursery (URRN) breeding lines

About 200 rice breeding lines, developed by the rice breeders from Arkansas, Louisiana, Mississippi, and Texas, were subjected to annual disease evaluations to the reference blast isolates at the University of Arkansas, Fayetteville, AR, in addition to the evaluation of yield and agronomic traits at various locations. A total of over 2000 breeding lines were tested during 2005–2016. The rice cultivars M204 and Francis were included in each test as the susceptible controls. The inoculation and disease scoring procedures were as described previously.

3.2 Pathogenicity of the reference isolates on the URRN lines

The susceptible control Francis was susceptible to all 12 isolates, while M204 was susceptible to 11 isolates but resistant to isolate IB54. Each year, in each test, the two susceptible controls consistently showed susceptible disease reactions with the disease rating scores ranging from 4 to 9, respectively.

The percentage of breeding lines resistant to each isolate in each year was quantified (**Figure 5**). The isolate IB33 was the most virulent isolate out of the 12 reference isolates tested, with 71.2–98% of the lines evaluated as susceptible for the 11 years tested. Overall 1963 out of 2177 lines tested (90.2%) were susceptible to IB33. Isolate 49D (race IB49) was highly virulent, with 70–90% of the lines tested susceptible for 10 of the 12 years examined. In 2007, however, 21.5% in 2007 and 48.7% in 2016 were evaluated as susceptible. Out of the 2377 lines tested in 12 years, 1673 lines (70.4%) were susceptible to 49D. Isolate TM2 (IE1k) was also considered highly virulent. In 2014 and 2015, about 75% of the lines were susceptible to TM2. In other years, over than 50% of the lines were susceptible. The lines tested in 2007 had the lowest percentage (33.5%) of susceptibility to this isolate. Overall, 1361 out of 2377 breeding lines (57.3%) were susceptible to TM2. Three isolates ID13, IB54, and #24 (IG1) were the least virulent; the percentages of susceptible breeding lines ranged between 7.5–26.7, 13.5–27.5, and 10.5–28.3%, respectively. Overall, the percentages of susceptible breeding lines to these three isolates were 18.7, 21.0, and

19.2%. The other six isolates were intermediately virulence on the lines tested with 40 to 50% of breeding lines were susceptible. In 2006, over 80% of the breeding lines were susceptible to isolate A119 (race IB49), but in the following years, only 25 to 50% of lines were susceptible to this isolate. In 12 years, 970 out of 2377 breeding lines (40.8%) were susceptible to A119.

3.3 Disease reaction of US rice breeding lines to the 12 reference isolates

All 12 reference isolates have been tested in 2010–2013 and 2016. In these 5 years, there were 45 lines that were rated as completely resistant to all isolates, and 101 lines only susceptible to one isolate. In 2010, there were 10 lines resistant to all isolates, 11 lines only susceptible to IB33, and 1 each only susceptible to TM2 and IB54. A total of 20 lines had no resistance to the 12 isolates. There were 14 lines from the 2011 set of germplasm that were resistant to all 12 isolates, 11 lines only susceptible to IB33, 1 only susceptible to TM2, and 2 only susceptible to 49D, and 8 lines susceptible to all 12 isolates. Five lines tested in 2012 were resistant to all 12 isolates, 12 lines were only infected by IB33, 1 and 3 lines were only susceptible to ID13 or 49D, respectively, while 7 lines were susceptible to all 12 lines. In 2013, 14 lines were evaluated as resistant to all isolates, 13 lines were only susceptible to IB33, 1 each only susceptible to ID13 or ZN7, 4 each only susceptible to 49D or TM2, while 5 lines were susceptible to all 12 isolates. Out of the 200 URRN lines tested in 2016, only 2 lines were resistant to all 12 isolates, 1 line only susceptible to TM2, 2 lines only susceptible to 49D, 32 lines were only susceptible to IB33, and 4 lines were susceptible to all 12 lines. In 2006 and 2008, 11 isolates were tested, but not IB54, 2 and 6 lines were resistant, and 28 and 13 lines were susceptible to all 11 isolates, respectively. In 2009, 2014, and 2015, isolate ID13 was not tested, but other 11 isolates were. There were 3, 1, and 4 lines resistant to and 11, 14 and 9 lines susceptible to all 11 isolates. In 2005, both IB54 and ID13 were not tested. No variety was found to be resistant to all 10 isolates tested. There were nine lines only susceptible to one isolate, six of them were susceptible to isolate IB33, and one each susceptible to 49D, TM2, and A598. There were 19 lines susceptible to all 10 isolates. Nine isolates were tested in 2007, but not IB33, IB54, and ID13. There were 60 lines resistant and 4 lines susceptible to all 9 isolates tested in 2007.

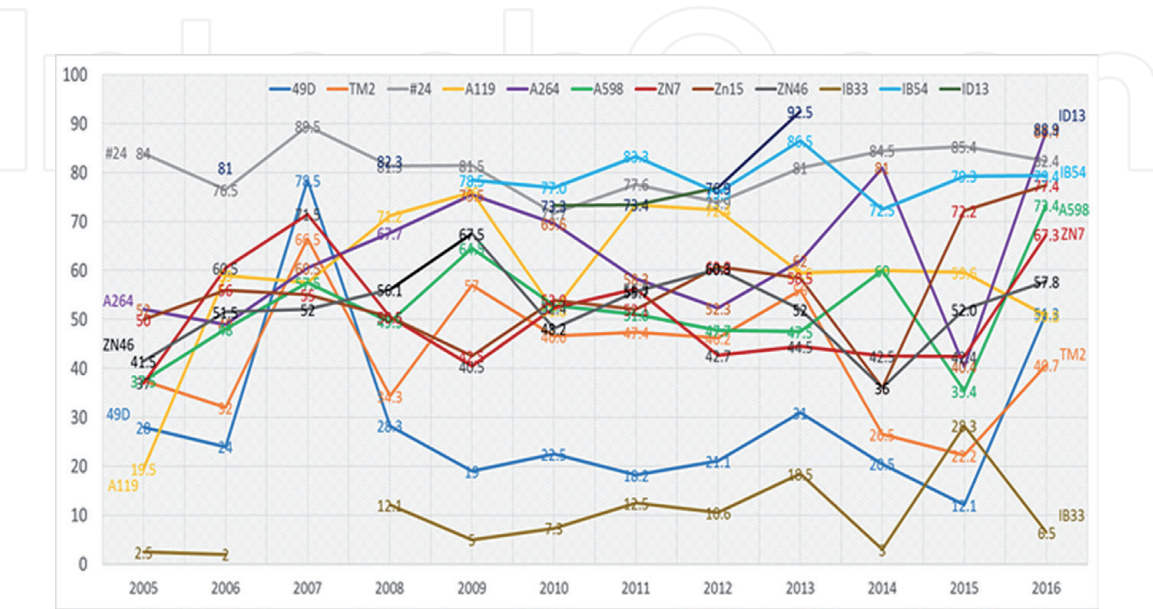


Figure 5.
Percentage of breeding lines resistant to each of the 12 reference US isolates of *M. oryzae* in each year from 2005 to 2016.

4. Discussion

Growing resistant cultivars has been demonstrated to be the most economical and effective way to manage rice blast disease. During 2005 to 2016, 2377 breeding lines were evaluated for disease resistance to the 12 reference isolates. Breeding lines resistant to all isolates have been found in each year of the period except 2005. Some lines were only susceptible to the most virulent isolate IB33. The use of the lines that have the broadest level of resistance to the spectrum of reference isolates would reduce the loss due to rice blast disease.

Based on the international differential cultivars and nomenclature, isolates A119, A598, and 49D are classified as race IB49 [18]. The disease reactions of many NILs tested to these three isolates were identical. However, these three isolates can be differentiated by some NILs (*R* genes), as *Pib*, *Pi11(t)*, and *Pi20* containing lines were resistant to A119 and A598 but susceptible to 49D; *Pi5(t)* and *Pit* containing lines were resistant to A119 but susceptible to 49D and A598. These results indicated that a set of differential cultivars should be chosen to more clearly demarcate races within the US rice blast pathogen population.

Any mutation, insertion, or deletion of the avirulence genes in the pathogen could cause the changes in its pathogenicity, thus resulting in the loss of function of the corresponding *R* gene and disease development. The coding region of *AVR-Pib* was found in all 12 reference isolates, but the promoter region was not amplified from isolates 49D, IB33, and IB54, and this may explain why the *Pib* gene containing line IRBLB-B cannot provide resistance to these three isolates. Some of the avirulence genes in the US population of *M. oryzae* have been studied [17, 18, 25]. However, the variation of other avirulence genes in the US population of *M. oryzae* needs to be evaluated.

Specific primers were used to detect the presence/absence of seven avirulence genes. Three avirulence genes, *AVR1-CO39*, *AVR-Pi9*, *AVR-Pikz*, *AVR-Pizt*, were present in all 12 reference isolates. According to the gene-for-gene concept [9], the corresponding *R* genes *Pi-CO39* line, *Pi9*, *Pikz*, and *Piz-t* would interact with these avirulence genes and initiate the defense response. It is unknown how many avirulence gene/*R* gene pairs could be involved in the resistance recognition process. When *AVR-Pita1* was introduced into strains that were virulent on *Pita* containing cultivars, those transformed strains lost their pathogenicity on *Pita* containing cultivars [30], suggesting that one *R* gene recognized one corresponding avirulence gene to initiate the resistance response. If this is the case, then cultivar CO39 and lines carrying *Pi9*, *Pikz*, and *Piz-t*, would have broad-spectrum resistance to the US isolates. It has been shown that *Pi9* containing line IRBL 9-W had resistance to all 12 reference isolates, but this is in contrast to the results of the NILs carrying *Pi-CO39*, *Pikz*, and *Piz-t* based on the reference isolates. If these avirulence genes in the reference isolates are functional, then the critical avirulence gene or combination of avirulence genes needs to be further evaluated for managing the disease.

A number of *R* genes to the blast pathogen disease have been identified from rice [4, 6–8]. Although more than 20 *R* genes were incorporated into the NILs, only *Pi9*, *Pi11(t)*, *Pi12(t)*, *Pib*, and *Pita-2* showed broad spectrum of resistance to the reference isolates of *M. oryzae* found in the southern USA. The *R* gene *Pita-2* has been widely used in US rice breeding programs, and has been effective, but incorporation of other *R* genes to develop more durable resistant cultivars will help to reduce the impact of rice blast disease.

5. Conclusions

The population of *M. oryzae* in the southern USA is very diverse. Breeding lines with broad spectrum of resistance to the reference isolates have been developed,

and incorporation of other *R* genes to develop more durable resistant cultivars will help to reduce the impact of rice blast disease.

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Conflict of interest

No conflict of interest.

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