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Analysis of rice blast resistance gene *Pi-z* in rice germplasm using pathogenicity assays and DNA markers

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Abstract The *Pi-z* gene in rice confers resistance to a wide range of races of the rice blast fungus, *Magnaporthe oryzae*. The objective of this study was to characterize *Pi-z* in 111 rice germplasm accessions using DNA markers and pathogenicity assays. The existence of *Pi-z* in rice germplasm was detected by using four simple sequence repeat (SSR) markers (RM527, AP4791, AP5659-1, AP5659-5) closely linked to *Pi-z*, and was verified using pathogenicity assays with an avirulent strain (IE1k) and two virulent races (IB33 and IB49). Among 111 germplasm accessions evaluated, 73 were found to contain the *Pi-z* gene using both SSR markers and pathogenicity assays. The remaining 38 germplasm accessions were found to be inconsistent in their responses to the blast races IB33, IEIk and IB49 with expected SSR marker alleles, suggesting the presence of unexpected SSR alleles and additional *R* gene(s). These characterized germplasm can be used for genetic studies and marker-assisted breeding for improving blast resistance in rice.

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Abbreviations

AVIR	Avirulent
D	Day
MAS	Marker assisted selection
R	Resistance
SSR	Simple sequence repeat
VIR	Virulent
PIC	Polymorphism information content

Introduction

Rice blast disease, caused by the filamentous ascomycete fungus *Magnaporthe oryzae* (formerly *Magnaporthe grisea*), is a major fungal disease threatening rice production worldwide. Genetic resistance in rice to *M. oryzae* belongs to a classic gene-for-gene system where a resistance (*R*) gene is effective in preventing infection by the races of *M. oryzae* containing the corresponding avirulence (*AVR*) gene (Silue et al. 1992). Resistant cultivars, fungicides, suitable planting dates, optimum fertilizer applications and adequate flood depth are useful tools to manage the disease (Bonman 1992; Lee 1994). Among them, the utilization of *R* genes is the most economical and environmentally-benign method for control of this disease. Thus far, more than 80 race-specific *R* genes

to *M. oryzae* have been identified and some of them have been molecularly cloned and tagged for marker-assisted selection (MAS) (Yu et al. 1991; McCouch et al. 1994; Godwa et al. 2003; Ballini et al. 2008; Jia et al. 2009).

Among the tagged blast *R* genes, the *Pi-z* gene, first identified by Kiyosawa (1967) in the medium grain cultivar 'Zenith', has been effectively introgressed into numerous rice cultivars around the globe to prevent infection by a wide range of races of the rice blast pathogen. In the U.S., *Pi-z* is usually found in tropical *japonica* medium grain rice cultivars (Fjellstrom et al. 2006). This gene has been shown to confer resistance to five U.S. races of blast (IH-1, IG-1, IC-17, IE-1 and IE1k), and susceptibility to two races (IB49 and IB33) Historically, IE1k as an avirulent race and IB49 and IB33 as virulent races have been used for predicting the presence of *Pi-z* in a rice germplasm (Marchetti et al. 1987; Conaway-Bormans et al. 2003). The *Pi9/2/zt* complex mapped at the same chromosomal location of *Pi-z(t)* has been recently characterized (Liu et al. 2002; Zhou et al. 2006). Therefore, *t* is removed from *Pi-z(t)* throughout this manuscript. Four simple sequence repeat (SSR) markers associated with the *Pi-z* gene have been identified and recommended for use in germplasm characterization and MAS in the U.S. (Fjellstrom et al. 2006).

Marker-assisted selection (MAS) is a recent tool in breeding for improved resistance to rice blast (Jia 2003). For MAS, selection is made based on DNA markers closely linked to a blast *R* gene that confers resistance to a particular race of the pathogen. MAS can be used to screen seeds or seedlings under laboratory conditions, which is much faster than traditional pathogenicity assays where accurate selection can only be made during the later stages of plant growth. In addition, MAS can avoid the overlapping effects of other matched pairs of *R* and *AVR* genes. However, disagreements between markers and disease reactions can occur in some breeding lines due to different genetic backgrounds and/or potential recombination events between markers and trait. Accurate identification of a particular *R* gene in diverse elite germplasm using DNA markers and differential blast races is an essential step for ensuring the accuracy of *R* gene utilization in using MAS for different rice breeding programs.

The objectives of this study were to identify the *Pi-z* gene in 111 rice germplasm using SSR markers closely linked to the *Pi-z* gene, to determine disease reactions of these germplasm to differential U.S. blast races.

Materials and methods

Plant materials and growth

The USDA core collection consisting of 1,790 accessions from 113 countries representing 70% of genetic diversity of the entire USDA collection (Yan et al. 2007) was screened with AP5659-1, the marker most closely linked to the *Pi-z* gene prior to the purification of the collection by single seed decent (Agrama et al. 2010). One gram of seed from each accession from the purified core was provided by the Genetic Stock Collections of *Oryza* at Dale Bumpers National Rice Research Center (DB NRRC) for pathogenicity assays. Four rice cultivars from the Molecular Plant Pathology Program at DB NRRC, Bengal (PI 561735), Jefferson (PI 593892) [+*Pi-z*], Wells (PI 612439), and Zhe733 (PI 629016) [−*Pi-z*], were used as the controls. Eight seeds of each accession were germinated in 96 well inserts (10 × 20 × 2 cm) (Hummert International, Missouri USA). Prior to seeding the inserts were placed in trays [26.67 × 53.34 × 6.35 (in cm), Model # INT0804, Hummert International, Missouri, USA] and filled with silt loam soil (pH 5.5–5.8) fertilized with Osmocote Pro 15-9-12 (Scotts-Sierra Horticultural Products Company, OH), autoclaved and stored at −20°C for three days. The trays were completely filled with water. Rice plants were grown for 3 weeks in the greenhouse maintained at 23–29°C during the day in winter (November–April) and 29–32°C in summer (May–October) and 22–25°C during the night all year long until the 3–4 leaf stage, for pathogenicity assay and subsequent DNA extraction.

Pathogenicity assay

Pathogenicity assays were performed on 131 experimental germplasm and four control germplasm. *M. oryzae* isolates, an avirulent (AVR) race IB49 (isolate ZN61), virulent (VIR) races, race IE1k (isolate TM2), and race IB33 (isolate FL9) were

selected for pathogenicity tests. There were four replicates for each germplasm. The *Pi-z* gene could be verified by the pattern of resistance or susceptibility to a pair of *AVR* and *VIR* races. Pathogen inoculation was performed using a modified procedure based on Valent et al. (1991). Briefly, plants were inoculated with 40 ml of a spore suspension (5×10^5 spores/ml, 0.25% gelatin) using a hand atomizer (100 kPa) connected to an air compressor. Inoculated plants were maintained at approximately 95% relative humidity in a clear polyethylene autoclave bag 24 × 36 (in cm) and 1.5 mm thick at room temperature (Product code 018143 Fisher Scientific, USA). Approximately 24 h after inoculation, plants were moved to the greenhouse for an additional 6 days. Disease reactions were assessed 7 days after inoculation using a rating scale (Fig. 1). For each accession, 7–8 seedlings were evaluated and each pathogenicity assay was conducted three times.

DNA extraction

DNA was extracted using a rapid DNA extraction procedure (Xin et al. 2003). DNA was extracted from bulked leaves from each of four replicates for further analysis. After extraction, sample DNAs were prepared for PCR through a Biomek 2000 Lab Automation Work Station (Beckman and Coulter, Brea, CA) using manufacturer protocols.

SSR marker selection

Five SSR markers from which data are already available from the USDA purified core collection (Agrama et al. 2009) were used to screen current germplasm to preclude any seed mixtures or experimental error. The markers selected were RM224, RM208, RM231, RM447, and RM171 because all five markers are robust and have high PIC (polymorphism information content) value. Four SSR markers, RM527, AP4791, AP5659-1 and AP5659-5, from the five markers identified by Fjellstrom et al. (2006) as associated with the presence of the Piz gene were selected for the present study because: (1) AP5659-1 was used for the initial screening of the core prior to purification and Fjellstrom et al. (2006) found this marker displays a unique 220 nt allele in germplasm carrying the *Pi-z* gene; (2) Fjellstrom et al. (2006) indicated AP5659-5 has a 279 nt allele for all germplasm with *Pi-z*, although this also was found in an accession carrying *Pi-9*, another *R* allele at the *Pi-z* locus (Liu et al. 2002). Fjellstrom et al. (2006) found that RM 527 had a 217 nt allele in all *Pi-z* germplasm but this allele was also found in germplasm not carrying *Pi-z*; (3) AP4791 is another marker that can be used to detect association with *Pi-z*; and (4) AP5659-3 was reported to co-segregate with the *Pi-z* resistance, and considered the most closely linked to *Pi-z* of those identified to date.

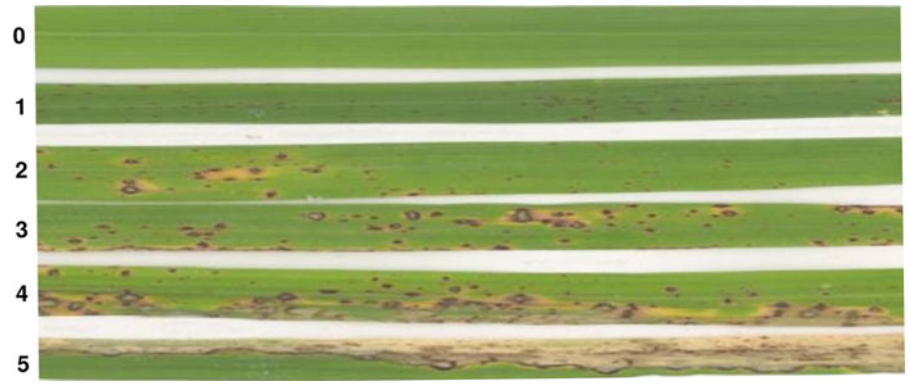

Fig. 1 Improved evaluation standard for determining disease reactions of rice germplasm. Resistant (0–2): No lesion formation *0*; Lesions covering less than 5% of total leaf area, lesions restricted at the site of infection *1*; Lesions covering between 5 to 10% of the total leaf area; restricted spindle lesions at diameter less than 2 mm *2*; Susceptible (3–5): Lesions in several locations on the leaf to form a large eye-shaped brown area (diameter greater than 2 mm) *3*; Lesions covering greater than 50% of the leaf area, diseased area with lesion greater than 30% of the total leaf area *4*; Lesions covering greater than 70% of the total leaf area *5*. *Note*: Improvement was based on disease reactions of both indica and japonica cultivars to blast. Plants at the three to four leaf stages were inoculated and the second youngest leaf was evaluated one week after inoculation

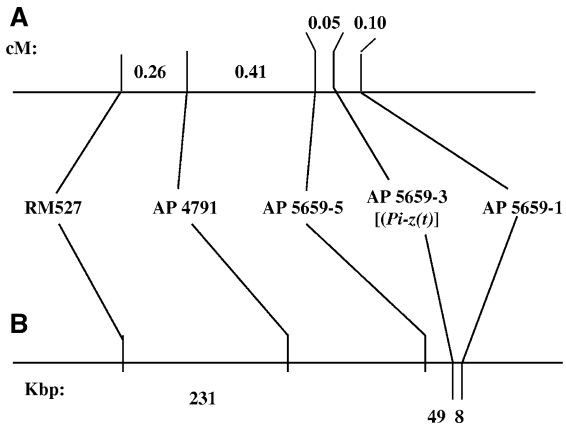

Fig. 2 Genetic and physical maps of the *Pi-z* gene as defined by SSR markers. Genetic map showing indicated SSR markers spanning the *Pi-z* locus (**a**) and physical map of the *Pi-z* locus as delimited by indicated SSR markers (**b**). Modified from Fjellstrom et al. (2006)

However, this marker has a null allele in some medium and long grain cultivars and therefore the marker was not selected (Fjellstrom et al. 2006, Fig. 2).

SSR marker analysis

Fluorescently labeled SSR markers were analyzed by capillary electrophoresis. For each marker, forward primers were labeled with fluorescent dyes (6FAM, NED, and Hex) from Applied Biosystems (Foster City, CA, USA) or Integrated DNA Technologies (Coralville, IA, USA). Reverse primers were not labeled. DNA was amplified with MJ Research Tetrad thermocyclers (Waltham, MA, USA) under the following PCR conditions: (1) initial denaturation at 94°C for 5 min; (2) 35 cycles of 94°C for 1 min, 55–67°C (marker dependent) for 1 min, and 72°C for 2 min; (3) 5 min final extension at 72°C. Two–three PCR products were pooled based on color and size range of the amplified PCR products using a Mini Prep75 (Tecan Group Ltd., Männedorf, Switzerland) instrument based on the manufacturer protocols. PCR products were diluted between 500 and 1,000×, and 2 µl of the diluted product were added to 9 µl of formamide-containing ROX-labeled size standards (Applied Biosystems, Foster City, CA). DNA was denatured by heating at 94°C for 5 min. The reaction was run on an ABI Prism 3730 DNA Analyzer (Applied Biosystems) following the manufacturer instructions. Fragment size and SSR marker genotype analysis were performed with Gene Mapper® software version 3.7 (Applied Biosystems). Analyzed alleles were exported into a Microsoft Excel spreadsheet.

Statistical analysis

To verify accuracy of Fjellstrom et al. (2006) previous observed association of AP5659-1 220 nt allele with *Piz* resistance, χ^2 analysis was conducted assuming Hardy–Weinberg equilibrium of IE1k resistance in general rice lines using software GraphPad χ^2 calculator at http://www.graphpad.com.

Results and discussion

Identification and verification of germplasm with potential *Pi-z* resistance

An initial screen of the core collection with marker AP5659-1 identified 143 germplasm with the 220 nt allele that Fjellstrom et al. (2006) indicated was associated with the presence of the *Pi-z* gene. When seed from the purified core was obtained 12 germplasm failed to germinate in time to perform pathogenicity assays. In the remaining 131 germplasm, 114 retained the 220 nt allele, while the remaining 17 germplasm either contained a different allele or failed to amplify although PCR was attempted three times, thus indicating a possible null allele. The 17 germplasm with different AP5659-1 results between the purified and unpurified core are likely due to changes in the germplasm during purification. To preclude seed mixtures and/or erroneous germplasm we screened the 131 germplasm with six SSR's previously run on the purified core collection (five high PIC value SSR's and RM527 from the *Pi-z* region). Fourteen germplasm were either mixtures or failed to match previous marker data on the purified core.

To confirm previously published AP5659-1 association with IE1k resistance presumably from the *Pi-z* locus we conducted χ^2 analyses on the purified germplasm that retained the 220 nt AP5659-1 allele assuming that IE1k resistance in the Core Collection is in Hardy–Weinberg Equilibrium. Under that assumption, if we select germplasm using a marker that lacks an association with IE1k resistance 50% of the germplasm should be resistant to IE1k and 50%

Table 1 χ^2 analysis of germplasm that contained the 220 nt AP5659-1 allele

Germplasm	Disease reaction to the race IE1k[a]	χ^2 (1:1)
101	R	33.96
13	S	33.96
Total 114		67.92*

* $P < 0.0001$

[a] Disease reaction was determined using 0–2 as resistant (R) and 3–5 as susceptible (S)

should be susceptible to IE1k. The assumption of Hardy–Weinberg equilibrium for IE1k resistance was verified by performing χ^2 analysis on data published by Wang et al. (2010) in which germplasm were screened for the presence of blast resistance gene *Pi-ta*, and pathogenicity assays were performed with both IE1k and IB49. *Pi-ta* confers resistance to IB49 but not IE1k. 50% of this germplasm were IE1k resistant, χ^2 0.53. However our current germplasm did not fit a model of Hardy–Weinberg equilibrium indicating marker AP5659-1 is selecting for IE1K resistance (Table 1). This analysis could not be conducted on the other markers because we do not have data from the entire core collection with marker's AP5659-5 and AP4791 and we did not analyze all core collection germplasm that contained the RM527 217 nt allele. Standard association mapping could not be performed on this germplasm because there is inadequate representation of both IE1k susceptible germplasm and the alleles Fjellstrom et al. (2006) identified as associated with *Piz* resistance are extremely over-represented in the current germplasm.

The *Pi-z* resistance

In the present study, we relied on previously identified markers spanning the *Pi-z* locus and differential blast races to identify germplasm accessions with *Pi-z* and with additional *R* genes from 1,790 rice germplasm (Yan et al. 2007). In addition we verified accuracy of our germplasm using SSRs for which data was available from the purified core collection. We excluded the fourteen germplasm that were either mixtures or failed to contain the same alleles observed in previous core collection data (Agrama et al. 2009) at any of the six marker loci used for germplasm verification from further analysis or presentation. Six germplasm gave inconsistent pathogenicity results and were also excluded from further analysis or presentation. The remaining 111 germplasm were used to identify the presence of *Pi-z* and additional *R* genes.

The gene-for-gene theory predicts that a germplasm is resistant due to *Pi-z* when this germplasm is (i) resistant to an *AVR* race IE1k (ii) and susceptible to a virulent (*VIR*) race, IB33 or IB49. As expected, the cultivar Bengal carrying *Pi-z* was found to be resistant to IE1k and susceptible to both IB33 and IB49. The cultivar Wells lacking *Pi-z* was found to be susceptible to all three races. The cultivar Zhe733 carrying *Pi42*(*t*) and *Pi43*(*t*) was found to be resistant to all three *M. oryzae* races (Lee et al. 2009). The cultivar Jefferson carrying *Pi-z* was found to be susceptible to IB33 and IB49 but resistant to IE1k (Table 2). Using these *M. oryzae* differential races the current study found that 77 germplasm accessions were resistant to IE1k and susceptible to IB33 and IB49, indicating the possible presence of *Pi-z* in these accessions (Fig. 3). Out of the 77 accessions with *Pi-z* as detected by pathogenicity assays, 40 had identical marker alleles for all four SSR markers (Table 2). The presence of the same marker alleles in these germplasm suggests that they contain a single *Pi-z* haplotype. This finding is important because these 40 germplasm were collected from several geographic regions of the world: the United States, South America, Europe, Asia and Africa (Table 2). The most likely reason for this haplotype similarity is that the original donor parent for the *Pi-z* gene may contain the same genomic fragment for all these cultivars. In contrast, 33 germplasm accessions showed 1–3 of the *Pi-z* allele (haplotype) markers, suggesting that these rice germplasm contain different *Pi-z* haplotypes, presumably either inherited from different donors or the result of recombination during the breeding process. Although pathogenicity data supported the presence of *Pi-z*, no expected maker (null) alleles were found in the remaining four germplasm accessions. The existence of the *Pi-z* gene in these four germplasm cannot be verified using our present markers and differential blast races.

If observed resistance in these germplasm is due to *Pi-z* only, susceptibility to IB49 and IB33 is expected. By pathogenicity assays, a total of 16 germplasm accessions were found to be resistant to all three races (Fig. 3; Table 2). These findings suggest the presence of additional *R* genes in these germplasm. There were nine germplasm that were susceptible to all isolates

tested, eight of which showed the presence of 2–4 expected marker alleles, however, Montakcl from Egypt was the only cultivar which was susceptible to all isolates evaluated and did not show any expected marker allele. There were five germplasm that were resistant to IB49 and three that were resistant to IB33 in addition to being resistant to IE1k indicating the presence of additional *R* genes. There was one germplasm that was resistant to both IB33 and IB 49 but susceptible to IE1k suggesting *Pi-z* was absent or non functional.

Race IB49 versus IB33

In the rice blast system, a pair of blast races is adequate to identify the corresponding *R* gene (Silue et al. 1992). An additional blast race will increase the complexity for *R* gene identification because it may contain a different avirulence gene because each *AVR* gene is sufficient to trigger the corresponding *R* gene mediated resistance. IB33 was a laboratory-generated strain (F. Lee, unpublished data); and IB49 was a field isolate; both of which were highly similar in fingerprinting (Correll et al. 2000; Zhou et al. 2007). If a pair of *AVR/VIR* cannot detect an *R* gene, it may suggest that there are *R* genes in rice that interfere with expected disease reactions. In the present study, 16 accessions were found to be resistant to all three blast races. The presence of the *Pi-z* gene in these germplasm can not be verified although 12 of them contain one to four expected alleles using differential blast races. Under these situations, MAS is a better choice because it can detect the presence of a particular *R* gene in rice (Jia 2003).

R gene modifier

Historically, it has been commonly observed that an *R* gene has different phenotypical effects in different germplasm and/or under different environmental conditions. These differences are often conditioned by *R* gene modifiers. Some of these modifiers are critical for complete resistance. In the present study, we also found nine germplasm accessions that may contain 0–4 *Pi-z* marker alleles that were susceptible to all three races tested. Although it is possible that mutations in the coding region of *Pi-z* can result in the loss of resistant function, these findings suggest that some of these germplasm may have at least one nonfunctional critical modifier rendering susceptibility.

Identification of additional *R* genes

In our study, five germplasm accessions, Chao Puak Deng and Assaw from China, Biribra from Ghana, CA902/b/2/2 from Chad, and Agami Mont-1 from Egypt, were found to be resistant to both IE1k and IB49 and susceptible to IB33, suggesting these germplasm accessions contain additional *R* genes. In addition, three germplasm accessions, Perititovo 1417 from Madagascar R 100/2 from Zaire and Ku Mun Do No. 84 from Korea, were also determined to carry additional *R* genes to IB33. The cultivar Wanni Dahanala from Sri Lanka was known to be resistant to IB49 and IB33 but susceptible to IE1K, indicating the absence of *Pi-z* yet conferring the presence of additional *R* genes. Three accessions (PI184675-4 from Iran; Ken Yen from China, and GP No. 22232 from Germany) did not show any *Pi-z* haplotype alleles for the SSR markers, yet conferred resistance to all three races. Despite the nature of *R* genes it is unknown if these materials could be useful as resistant donors.

Geographic distribution of germplasm carrying *Pi-z*

Rice germplasm with *Pi-z* was found in 42 countries from six continents, Asia, Europe, North America, South America, Africa and Australia (Table 2). The most *Pi-z* containing germplasm was found in the U.S. and Puerto Rico, 5 + 4 of 77, respectively. Cote D' Ivoire of West Africa was the next to the US and Puerto Rico where seven of 77 germplasm with *Pi-z* in Cote D' Ivoire were verified in the present study. However, we have not found any germplasm with *Pi-z* from India and China where most of the rice is being grown in the world.

In conclusion, we not only verified the *Pi-z* gene in 73 of 77 rice germplasm using previously identified DNA markers but also demonstrated the usefulness of DNA markers and pathogenicity assays with differential blast races for germplasm characterization. We verified differential blast races; IE1k, IB33 and IB49 should be useful for predicting the existence of the *Pi-z* gene for conventional breeding for blast resistance. All of these new findings presented by this

Table 2 Summary of disease reaction, pathogenicity assays, SSR marker profile for the analysis of the *Pi-z* gene in rice germplasm

Accession number	Name	Origin	Disease reaction[a]			SSR markers				No. of expected *Pi-z* alleles	*Pi-z*[b]
			IB49 (ZN61)	IE1k (TM2)	IB33 (FL9)	AP5659-1	AP5659-5	AP4791	RM527		
GSOR310003	WC3398	Mexico	S 3	R 1	S 4	220	279	290	217	4	+
GSOR310010	Coray 4	Honduras	S 3	R 2	S 3	204	299	Null	238	0	–
GSOR310013	WC 4431	Panama	S 3	R 1	S 3	220	279	287	217	3	+
GSOR310021	PR325	Puerto Rico	S 3	R 1	S 3	220	279	287	217	3	+
GSOR310022	PR358	Puerto Rico	S 3	R 0	S 3	222	279	287	217	2	+
GSOR310035	Stg58-2158	United States, Arkansas	S 3	R 0	S 3	220	279	Null	217	3	+
GSOR310037	Stg625377	United States, Arkansas	S 3	R 0	S 3	220	279	290	217	4	+
GSOR310046	Clor11009	United States, Louisiana	S 3	R 0	S 5	220	279	290	217	4	+
GSOR310051	15	Iran	S 3	R 0	S 3	220	279	287	217	3	+
GSOR310064	PR433	Puerto Rico	S 3	R 0	S 3	220	279	287	217	3	+
GSOR310073	Fortuna Negro	Peru, Lima	S 4	R 2	S 3	Null	297	300	217	1	+
GSOR310074	Mejicano	Peru, Lima	S 3	R 1	S 3	220	279	290	217	4	+
GSOR310078	Saku	Mongolia	S 3	R 0	S 3	220	279	290	217	4	+
GSOR310107	1021	Guatemala, Jalapa	S 3	R 2	S 4	220	279	290	217	4	+
GSOR310146	WC3396	Jamaica	S 3	R 2	S 4	220	279	290	217	4	+
GSOR310177	AP439	Venezuela	R 2	R 0	R 1	220	279	290	217	4	–
GSOR310201	WC 1909	Japan	S 3	R 2	S 3	203	290	Null	233	0	–
GSOR310214	Campino	Portugal	S 3	R 0	S 3	220	279	290	217	4	+
GSOR310222	Baraggia	Italy, Piedmont	S 3	R 0	S 3	220	297	300	217	2	+
GSOR310265	Nilo 48A	El Salvador	S 3	S 4	S 3	220	279	290	217	4	–
GSOR310301	H57-3-1	Argentina, Buenos Aires	S 4	R 0	S 3	220	279	290	217	4	+
GSOR310302	H62-3-1	Argentina, Buenos Aires	S 5	S 3	S 5	220	276	290	217	3	–
GSOR310303	H71-11-1	Argentina, Buenos Aires	S 3	R 2	S 4	220	297	300	217	2	+
GSOR310321	Bankoram	Ghana, Ashanti	S 3	R 0	S 3	220	279	287	217	3	+
GSOR310322	PindeGogo Wiere	Suriname	S 3	R 0	S 4	220	279	287	217	3	+
GSOR310327	52/16-0-2	Papua New Guinea	S 4	R 0	S 5	220	279	290	217	4	+
GSOR310329	GPNO 15007	Senegal	S 4	S 3	S 4	220	279	290	217	4	–
GSOR310336	Chao Puak Deng	Laos	R 0	R 0	S 3	220	279	300	221	2	–
GSOR310391	Assaw	China, Sichuan	R 0	R 0	S 3	220	279	290	217	4	–
GSOR310404	Ken Yen	China	R 1	R 0	R 0	Null	295	296	221	0	–
GSOR310477	BIRIBRA	Ghana	R 0	R 1	S 4	220	279	300	221	2	–
GSOR310480	Djimoron	Guinea	S 3	R 2	S 4	220	279	300	221	2	+
GSOR310500	VARY LAVA 9	Madagascar	S 4	R 0	S 5	220	279	287	217	3	+

Table 2 continued

Accession number	Name	Origin	Disease reaction[a]			SSR markers				No. of expected *Pi-z* alleles	*Pi-z*[b]
			IB49 (ZN61)	IE1k (TM2)	IB33 (FL9)	AP5659-1	AP5659-5	AP4791	RM527		
GSOR310502	Perititovo 1417	Madagascar	S 3	R 0	R 1	220	279	287	238	2	–
GSOR310503	Manga Kely 694	Madagascar	S 3	R 0	S 3	220	279	287	217	3	+
GSOR310512	CA497/V/7	Chad	S 3	R 0	S 3	220	279	290	217	4	+
GSOR310518	Gaza	Mozambique	S 4	R 1	S 5	220	279	290	238	3	+
GSOR310525	India Pa Lil 92	Sierra Leone	S 3	R 2	S 5	220	279	287	238	2	+
GSOR310533	MAKALIOKA 752	Madagascar	S 3	R 1	S 4	220	279	290	238	3	+
GSOR310535	CA435/B/5/1	Chad	S 3	R 0	S 4	220	279	290	217	4	+
GSOR310538	PI 184675-4	Iran	R 0	R 0	R 0	202	290	304	235	0	–
GSOR310562	Higueyano	Dominican Republic, La Alt	S 3	R 0	S 5	220	279	290	217	4	+
GSOR310577	GPNO22232	Germany, Saarland	R 0	R 0	R 0	Null	294	296	221	0	–
GSOR310586	Baluola 11	Zaire	R 0	R 1	R 0	Null	297	293	217	1	–
GSOR310591	R 89	Zaire	R 0	R 0	R 0	220	279	290	217	4	–
GSOR310596	R 100/2	Zaire	S 3	R 1	R 0	220	279	290	217	4	–
GSOR310602	P 817	Russian Federation	S 4	R 0	S 5	220	279	290	238	3	+
GSOR310635	IM 16	Nigeria	S 3	R 0	S 3	220	279	290	217	4	+
GSOR310638	IRAT 104	Cote D'Ivoire	R 0	R 0	R 0	220	279	290	217	4	–
GSOR310642	IRAT132	Cote D'Ivoire	S 3	R 0	S 3	220	279	290	217	4	+
GSOR310643	IRAT 134	Cote D'Ivoire	S 3	R 0	S 3	220	279	290	217	4	+
GSOR310644	IRAT139	Cote D'Ivoire	S 3	R 0	S 3	220	279	290	217	4	+
GSOR310683	IITA130	Nigeria, Oyo	S 3	R 0	S 3	220	279	290	217	4	+
GSOR310692	Tox177-1-2-B	Nigeria, Oyo	S 3	R 0	S 3	220	279	290	217	4	+
GSOR310704	CT7378-2-1-3-1-4	Colombia, Valle	S 5	R 0	S 3	220	279	290	217	4	+
GSOR310707	Medusa	Italy, Lombardy	S 3	R 0	S 4	220	279	290	217	4	+
GSOR310712	IRAT13	Cote D'Ivoire	S 3	R 0	S 3	220	279	290	217	4	+
GSOR310734	CNAX 5072-2-1-2	Colombia	S 3	R 0	S 3	220	279	290	217	4	+
GSOR310762	IRAT44	Burkina Faso	S 3	R 0	S 3	220	279	290	217	4	+
GSOR310774	WAB502-13-4-1	Cote D'Ivoire	S 3	R 0	S 3	220	279	290	217	4	+
GSOR310775	WAB501-11-5-1	Cote D'Ivoire	S 3	R 0	S 3	220	279	290	217	4	+
GSOR310777	WC3532	Peru	S 3	R 0	S 3	220	279	290	217	4	+
GSOR310805	Chivacia-1	Venezuela, Aragua	S 3	R 0	S 3	220	279	290	217	4	+
GSOR310815	PR 147	Puerto Rico	R 1	R 0	R 0	220	279	287	217	3	–
GSOR310829	Stg 64M3390	United States, Arkansas	S 5	R 0	S 3	220	279	290	217	4	+

Table 2 continued

Accession number	Name	Origin	Disease reaction[a]			SSR markers				No. of expected *Pi-z* alleles	*Pi-z*[b]
			IB49 (ZN61)	IE1k (TM2)	IB33 (FL9)	AP5659-1	AP5659-5	AP4791	RM527		
GSOR310831	Zenith	Puerto Rico	S 5	R 0	S 4	220	279	290	217	4	+
GSOR310833	71Cr-308	United States, Louisiana	S 3	R 0	S 5	220	279	290	217	4	+
GSOR310886	WC2656	Zaire	S 3	R 0	S 3	220	279	290	217	4	+
GSOR310889	Brazilero Perla	El Salvador, La Libertad	R 1	R 0	R 0	220	279	287	217	3	–
GSOR310892	Mamoriaka	Madagascar	S 3	R 0	S 4	220	279	287	238	2	+
GSOR310915	Zale	Myanmar, Rangoon	S 3	R 0	S 3	Null	295	293	221	0	–
GSOR310965	Vary Tarva Osla	Portugal	S 3	R 0	S 4	220	297	300	217	2	+
GSOR310966	Perlita Jalapa	Guatemala	S 5	R 0	S 3	220	279	287	217	3	+
GSOR311012	WC 5015	Mexico, Federal District	S 4	R 0	S 3	220	279	290	217	4	+
GSOR311025	IR647-PDI-C1	Philippines, Luzon	S 3	R 0	S 3	220	295	296	221	1	+
GSOR311037	H75-23-1	Argentina, Buenos Aires	S 4	R 0	S 3	220	279	290	217	4	+
GSOR311049	Blakka Tere Thelma	Suriname	S 3	R 0	S 4	220	279	287	217	3	+
GSOR311060	Hal Suduwi	Sri Lanka	S 4	R 0	S 4	220	279	287	223	2	+
GSOR311069	Lay Sort	Laos	S 3	R 0	S 3	220	279	290	217	4	+
GSOR311080	YRL-1	Australia	S 3	R 0	S 3	220	279	290	217	4	+
GSOR311099	Ku Mun Do No. 84	Korea	S 4	R 0	R 0	220	279	290	217	4	–
GSOR311104	EEA 406	Brazil, Rio Grande do Sul	S 3	R 0	S 3	220	276	290	217	3	+
GSOR311118	Cadung Ket	Vietnam	S 3	R 0	S 3	220	279	296	221	2	+
GSOR311150	Five months	Guyana	S 3	R 0	S 4	220	279	290	217	4	+
GSOR311180	Sapundali Local	India	S 3	R 2	S 3	220	279	290	238	3	+
GSOR311186	CA 902/8/2/2	Chad	R 0	R 0	R 0	220	279	290	217	4	–
GSOR311198	WC6570	Spain	R 1	R 0	R 0	220	279	287	217	3	–
GSOR311200	Kalila 50	Madagascar	R 2	R 0	R 0	220	279	287	217	3	–
GSOR311204	CA 902/b/2/2	Chad	R 1	R 0	S 3	220	279	290	217	4	–
GSOR311222	Agami Mont-1	Egypt	R 0	R 0	S 3	220	276/279	287	217	3	–
GSOR311257	Basala BaatkaS-R	Zaire	R 0	R 0	R 0	220	279	290	217	4	–
GSOR311269	Shimla Early	Iraq	S 4	S 3	S 5	220	279	290	238	3	–
GSOR311272	Sadri Dum Sufaid	Iran	S 5	R 0	S 3	220	279	300	221	2	+
GSOR311277	Ghoal Champa	Iran	S 4	R 0	S 3	220	279	300	221	2	+
GSOR311278	Montakcl	Egypt	S 3	S 4	S 5	Null	300	270	223	0	–
GSOR311305	IB 94	Nigeria	S 3	R 0	S 3	220	279	290	217	4	+

Table 2 continued

Accession number	Name	Origin	Disease reaction[a]			SSR markers				No. of expected *Pi-z* alleles	*Pi-z*[b]
			IB49 (ZN61)	IE1k (TM2)	IB33 (FL9)	AP5659-1	AP5659-5	AP4791	RM527		
GSOR311306	Mange 2	Nigeria	R 0	R 0	R 0	Null	295	296	221	0	–
GSOR311309	IRAT 142	Cote D'Ivoire	S 3	R 0	S 3	220	279	290	217	4	+
GSOR311341	63-83	Cote D'Ivoire	S 3	R 0	S 3	220	279	290	217	4	+
GSOR311349	Tox 782-20-1	Nigeria, Oyo	R 0	R 1	R 0	220	279	290	217	4	–
GSOR311371	Estrela	Colombia, Valle	S 4	R 0	S 3	220	279	287	217	3	+
GSOR311403	Panama 1048	Colombia, Valle	R 0	R 0	R 0	220	279	287	238	2	–
GSOR311565	517	Uruguay,Treinta y Tres	S 3	R 0	S 3	Null	295	296	221	0	–
GSOR311583	Bakula	Sierra Leone, Southern	S 3	R 0	S 3	220	279	287	217	3	+
GSOR311590	Sadri Siah Dum	Iran	S 3	S 5	S 3	220	279	287	240	2	–
GSOR311624	Suduwi 305	Sri Lanka	S 3	R 0	S 3	220	279	287	238	2	+
GSOR311630	Hatadawee	Sri Lanka	S 3	S 5	S 3	220	279	306	221	2	–
GSOR311632	Wanni Dahanala	Sri Lanka	R 2	S 5	R 1	220	279	287	238	2	–
GSOR311634	Patchaiperumal	Sri Lanka, Kurunegala	S 3	S 4	S 3	220	279	306	221	2	–
GSOR311635	AMANE	Sri Lanka, Matale	S 3	S 5	S 3	220	279	306	221	2	–
GSOR311673	Sadri Ter Misri	Iran	S 3	R 0	S 3	220	279	287	240	2	+
PI561735	Bengal (control)		S 3	R 0	S 3	220	279	290	217	4	+
PI612439	Wells (control)		S 5	S 3	S 4	203	290	290	233	1	–
PI629016	Zhe 733 (control)		R 0	R 0	R 2	205	290	301	223	2	–
PI593892	Jefferson (control)		S 3	R 0	S 3	220	279	293	217	3	+

Germplasm that were mixed, did not match previous core collection data, or gave inconsistent pathogenicity results are excluded from this table

[a] A 0–5 scale was used to score for resistance and susceptibility. Plants were scored as resistant if for scores 0–2 and susceptible for scores of 3–5

[b] +indicates the presence of *Pi-z*, −indicates the presence of *Pi-z* can not be determined and verified

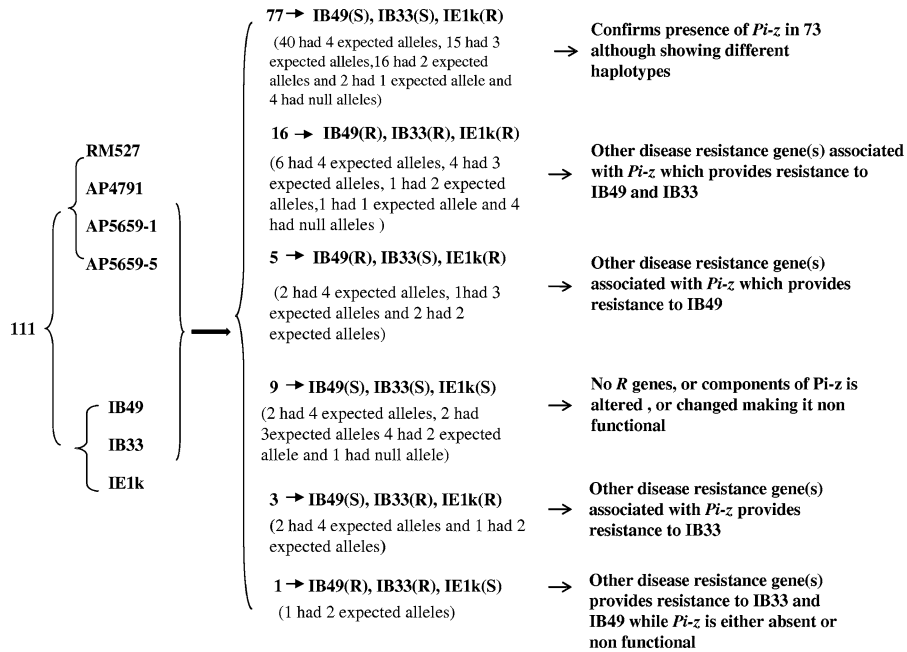

Fig. 3 Analysis of the *Pi-z* gene in rice germplasm using disease reaction and SSR marker. The diagram shows results of disease reactions and expected SSR marker alleles for germplasm in different categories

work were summarized in Table 2. For germplasm requests, please visit (http://www.ars.usda.gov/spa/dbnrrc/gsor) at GSOR of DB NRRC.

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