Identification of the Rice Blast Resistance Gene *Pib* in the National Small Grains Collection

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ABSTRACT

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The *Pib* gene in rice confers resistance to a wide range of races of the rice blast pathogen, *Magnaporthe oryzae*, including race IE1k that overcomes *Pita*, another broad-spectrum resistance gene. In this study, the presence of *Pib* was determined in 164 rice germplasm accessions from a core subset of the National Small Grains Collection utilizing DNA markers and pathogenicity assays. The presence of *Pib* was evaluated with two simple sequence repeat (SSR) markers and a dominant marker (Pib-dom) derived from the *Pib* gene sequence. Pathogenicity assays using two avirulent races (IE1k and IB1) and a virulent race (IB54) were performed to verify the resistance responses of accessions. Of the 164 accessions evaluated, 109 contained the *Pib* gene as determined using both SSR markers and pathogenicity assays, albeit different haplotypes were detected. The remaining 52 germplasm accessions were different in their responses to the blast races IB54, IE1k, and IB1, thus indicating the presence of *R* gene(s) other than *Pib*. The accessions characterized in this study could be used for marker-assisted breeding to improve blast resistance in *indica* and *japonica* cultivars worldwide.

Rice blast caused by *Magnaporthe oryzae* B. Couch is the most destructive disease affecting rice production worldwide. The use of resistant cultivars has been the most economical and efficient method for controlling this disease. However, the lifespan of many resistant cultivars is only a few years, due to the loss of resistance in the face of hyper-variability of the pathogen (20,28, 31). The inheritance of major-gene-mediated resistance to the blast pathogen has been studied extensively worldwide. Major resistance (*R*) genes are effective in preventing infection by races of *M. oryzae* containing the corresponding avirulence (*AVR*) genes (11,32). Presently, more than 70 blast *R* genes have been identified, and 13 of them have also been characterized using molecular markers and subsequently used to develop resistant cultivars (2,6, 7,9,12–16,21–23,29,30,35,38,39).

Molecular markers tightly linked to major *R* genes are useful for marker assisted selection (MAS). Although a large number of blast *R* genes have been fine mapped based on closely linked markers and/or some of them cloned based on marker information, there are only a few published examples where the markers had a direct impact on plant breeding. DNA markers derived from the cloned *Pib* and *Pita* blast *R* genes are currently used in several rice breeding programs (18). Allele-specific DNA markers were also developed to distinguish *Pikm* and *Pik* (8). In addition, polymerase chain reaction (PCR)-based single nucleotide polymorphism markers for genes at the *Piz* locus are also known to be used in breeding programs (14) and linked markers have been successfully used to identify germplasm that carry *Piz* (30). The *R* gene in rice lines, BL8, BL9, BL10, and BL11, was named by Kiyosawa (19) as *Pib* and has been used extensively in rice breeding programs in Japan, China, and Indonesia (19,26,27,38). The gene encoding a cytoplasmic protein with a nucleotide binding site and leucine rich repeats (NBS-LRR) was the first cloned blast *R* gene (35). The availability of a high-density linkage map (13) and DNA markers in the *Pib* region (27) has facilitated the identification of additional molecular markers more closely linked to *Pib* (10). *Pib* was introduced into the U.S. rice cultivar 'Saber' (25) from the *indica* 'Teqing', a cultivar from China and the gene has been identified in rice cultivars resistant to blast races IA45, IB1, IH1, IB45, IG1, IE1k, IC17, and IE1k (24,25). Resistance to race IE1k can be attributed to the presence of only *Pib* or *Piz* in U.S. rice cultivars. Race IB1 can be used to distinguish the presence of either of these genes because *Piz* provides resistance to IE1k, but is ineffective against IB1, whereas *Pib* is effective against both.

The objectives of this study were to (i) identify the *Pib* gene in a core collection of 1,790 rice germplasm accessions, collected from 113 countries, representing an estimated 70% of the genetic diversity of the entire U.S. Department of Agriculture (USDA) National Small Grains Collection of rice germplasm (37), using previously identified simple sequence repeat (SSR) markers closely linked to the *Pib* gene and a dominant marker derived from *Pib*; and (ii) determine disease reactions of the accessions containing *Pib* to differential U.S. blast races. The results indicated that the molecular markers served as an effective diagnostic tool for the presence of *Pib*.

MATERIALS AND METHODS

Plant materials. Prior to the purification of the collection by single seed descent (1), the USDA core collection consisting of 1,790 accessions was screened with SSR marker RM208 tightly linked to the *Pib* gene and a dominant marker Pib-dom, derived from a portion of the *Pib* sequence (10). Four grams of seed from each accession of the purified core was provided by the Genetic

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Stocks *Oryza* Collection (GSOR, www.ars.usda.gov/spa/dbnrrc/gsor) at Dale Bumpers National Rice Research Center (DB NRRC). Twelve seeds of each accession were germinated in 96 well inserts (10 × 20 × 2 cm) (Hummert International, MO). Prior to seeding, the inserts were placed in trays (26.67 × 53.34 × 6.35 cm, Model INT0804, Hummert International) and filled with silt loam soil (pH 5.5 to 5.8) fertilized with Osmocote Pro 15-9-12 (Scotts-Sierra Horticultural Products Company, OH), autoclaved, and stored at –20°C for 3 days. The trays were completely filled with water. Seedlings were grown in winter (November to December 2010) for 4 weeks in the greenhouse maintained at 23 to 29°C during the day and 22 to 25°C during the night until the three to four leaf stage, in preparation for pathogenicity assays and subsequent DNA extraction.

Pathogenicity assays. Pathogenicity assays were performed on 164 germplasm accessions confirmed by SSR markers as having *Pib*, and a positive control 'Saber' (PI 633624). The race IE1k detects the presence of *Pib* and *Piz*; however, IB1 detects the presence of *Pib*, not *Piz* (Fig. 1). Single spores of virulent (VIR) isolate (unnamed isolate-race IB54), AVR isolates TM2 (race IE1k), and an unnamed isolate-race IB1 of *M. oryzae* were selected for pathogenicity tests in the present study. There were four replicates for each germplasm accession. The presence of the *Pib* gene in each accession was verified by the pattern of resistance or susceptibility to both AVR and VIR isolates. Pathogen inoculation was performed using a modified procedure based on Valent et al. (33). Briefly, plants were inoculated with 40 ml of a spore suspension (5×10^5 spores/ml, 0.25% gelatin) using a hand atomizer connected to an air compressor (100 kPa). Inoculated plants were maintained at approximately 95% relative humidity in a clear polyethylene autoclave bag (24 × 36 cm and 1.5 mm thick) at room temperature (Product code 018143, Fisher Scientific). Approximately 24 h after inoculation, removing the humidity chamber, plants were moved to the greenhouse for an additional 6 days. Disease reactions were assessed 7 days after inoculation using a visual rating scale from 0 to 5, as previously described (30). For each accession, seven to eight seedlings were evaluated and each pathogenicity assay was conducted three times.

DNA extraction. DNA was extracted from bulked leaves from each of four replicates used in the pathogenicity assay using a rapid DNA extraction procedure (36). After extraction, sample DNA was prepared for PCR through a Biomek 2000 Lab Automation Work Station (Beckman and Coulter, Brea, CA) using manufacturer protocols.

DNA markers and analysis. Three SSR markers from which data are already available from the purified core collection (1) were used to screen current germplasm to preclude any seed mixtures or experimental error. The markers selected were RM224, RM171, and RM231 because all three markers are robust and have high PIC (polymorphism information content) value. Three markers previously identified as associated with the presence of *Pib* (10), RM208, Pib-dom, and RM166 were used to screen purified accessions from the core collection as verification of previous marker screen on unpurified accessions. Fluorescently labeled markers were analyzed by capillary electrophoresis based on the methods previously described (10). For each marker, forward primers were labeled with fluorescent dyes (6FAM, NED, and Hex) from Applied Biosystems (Foster City, CA) or Integrated DNA Technologies (Coralville, IA). Reverse primers were not labeled. DNA was amplified using MJ Research Tetrad thermocyclers (Waltham, MA) under the following PCR conditions: (i) initial denaturation at 94°C for 5 min; (ii) 35 cycles of 94°C for 30 s, 55 to 61°C (marker dependent) for 30 s, and 72°C for 1 min; (iii) 5 min final extension at 72°C. PCR products were pooled based on color and size range of the amplified PCR products and the DNA was denatured by heating at 94°C for 5 min. PCR products were diluted 200, 500, and 2,000×, and 2 µl of the diluted product was added to 9 µl of formamide-containing ROX/LIZ (dependent on the size of the product) labeled size standards (Applied Biosystems). PCR products from different primer pairs having different size ranges and labels were combined for simultaneous analysis using a Mini Prep75 (Tecan Group Ltd., Männedorf, Switzerland) instrument based on the manufacturer protocols, and analyzed to determine the size of the SSR alleles. The reaction was run on an ABI Prism 3730 DNA Analyzer (Applied Biosystems) following manufacturer's instructions. Fragment size and SSR marker genotype analysis were performed with Gene Mapper software version 3.7 (Applied Biosystems). Allele sizes for all SSR markers used in the present study are displayed in Table 1.

RESULTS AND DISCUSSION

In the present study, a total of 178 rice accessions were initially identified by utilizing the SSR marker RM208 and the dominant marker Pib-dom, which were shown to have strong association with the *Pib* gene in a previous study (10). A total of 164 of these 178 accessions were verified to match data from the purified core

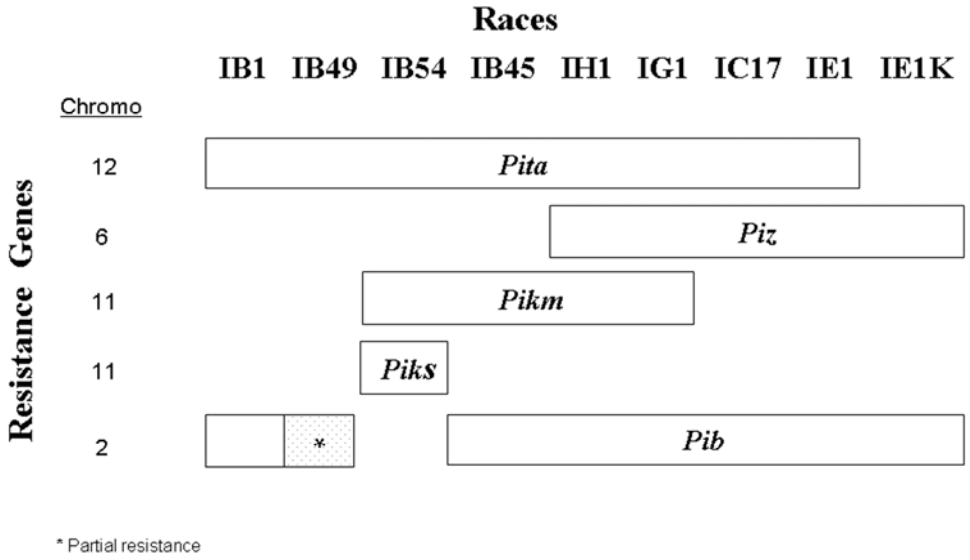

Fig. 1. Major blast resistance genes and their response to various U.S. blast races. Races are named according to their reactions on the international set of differential cultivars used in Atkins et al (3).

collection with three high PIC value SSRs and RM208. The remaining 14 accessions were removed due to suspected seed mix. Based on the gene-for-gene theory, *Pib* should be present only if the germplasm accession is (i) resistant to *AVR* races, such as IE1k and IB1, and (ii) susceptible to a virulent (*VIR*) race, such as IB54 (10). Utilizing these differential races, *Pib* was detected in 128 of 164 germplasm accessions since they were resistant to IE1k and IB1 but susceptible to IB54 (Fig. 1). Out of the 128 accessions with *Pib*, 89 had alleles typically associated with the presence of *Pib* for all three markers (RM208 [179], RM166 [318], and Pib-dom [360]) examined (Fig. 2; Table 2). The presence of all three *Pib* marker alleles in these germplasm accessions suggests that they all contain the same *Pib* haplotype. This was unexpected because these 89 germplasm accessions were collected from several geographic regions of the world, including Central and South America, Europe, Asia, and Africa (Table 2). Although these cultivars are not known to have direct parentage in common, it is still possible that they inherited *Pib* from the same donor. In contrast, 17 germplasm accessions contained just two of the *Pib* associated marker alleles, suggesting that these accessions contain different *Pib* haplotypes. Additionally, 16 germplasm accessions followed the gene-for-gene concept but had no *Pib* associated marker alleles. Thus, the presence of *Pib* utilizing our present markers could not be verified in these 16 accessions.

In contrast, 28 germplasm accessions having 0 to 3 *Pib* marker alleles were found to be resistant to all races, IE-1k, IB54, and IB1, suggesting these accessions contain other *R* genes that are responsible for resistance to IB54 (such as *Pita* or *Pi-ks*) (24) and perhaps IE-1k and IB1. Because of the incongruity between the markers and the race reactions, the presence or absence of *Pib* cannot be verified in these accessions (Fig. 2) (4). As examples of accessions resistant to all three races, Juma 61 had two *Pib* associated alleles, T442-57 had one *Pib* associated allele, and accessions C1-6-5-3 and Hansraj had no *Pib* associated alleles, indicating that resistant reactions observed were due to other *R* genes. Moreover, the cultivar Saber carries all three of the *Pib* resistant alleles and was resistant to IE1k and IB1 as predicted, but was also resistant to IB54 due to the presence of the *Pi-km* allele (Table 2, Fig. 1) (25).

Finally, a total of four germplasm accessions, R 647 from China, 17465-4 and Bilo from Fiji, and BR-IRGA-410 from Brazil, had all the expected marker alleles for having the *Pib* gene but were susceptible to all three tested races. One accession RP2199-16-2-2-1 from India having all expected marker alleles for *Pib* was resistant to IB1, but susceptible to both IE1k and IB54 (Table 2).

Inconsistencies in the marker and phenotype analysis in this study can be attributed to genetic variability among the rice accessions and within the pathogen. Although we used single spore isolates, the rice blast fungus is known to be hyper-variable and thus it is impossible to have near-isogenic isolates (AVR/VIR strains of each race) (4,5) to test the gene for gene concept. MAS can overcome some disadvantages in pathogenicity assays for monitoring *R* genes; however, the power of MAS is dependent on how reliable the markers are, as the presence of marker alleles sometimes does not indicate the presence of functional *R* genes (17,18). In this study, five accessions which putatively possessed *Pib* based on markers were susceptible to all three races, with three of these accessions having all three *Pib* associated marker alleles. The associated marker alleles in these cases do not indicate the presence of a functional *Pib* gene, suggesting that marker analysis alone would not work using these five germplasm accessions. These incongruities and others demonstrate that the markers for *Pib* do not always predict gene function. One reason that the *Pib* gene may not be expressed is due to a promoter mutation or additional mutations in the *Pib* gene outside of the region used to design the dominant marker. However, it may take considerable effort to examine promoter and transcript levels for this gene because expression of *Pib* has been seen to be influenced by diverse environmental factors (34,35). It may be that sequence analysis of the *Pib* gene coding region in these accessions could infer possible mutations that result in a nonfunctional copy of *Pib* and allow the development of an improved or functional *Pib* marker in the future.

TABLE 1. Summary of *Pib* associated allele sizes, annealing temperatures, and sequences of dominant and simple sequence repeat markers at the *Pib* locus

Marker	Size (bases)	Recombination distance from *Pib*	Annealing temperature (°C)	Forward primer	Reverse primer
Pib-dom	360	0.0	55	GAACAATGCCCAAACTTGAGA	GGGTCCACATGTCAGTGAGC
RM208	179	0.0	55	TCTGCAAGCCTTGTCTGATG	TAAGTCGATCATTGTGTGGACC
RM166	316	2.3	61	GGTCCTGGGTCAATAATTGGGTTACC	TTGCTGCATGATCCTAAACCGG

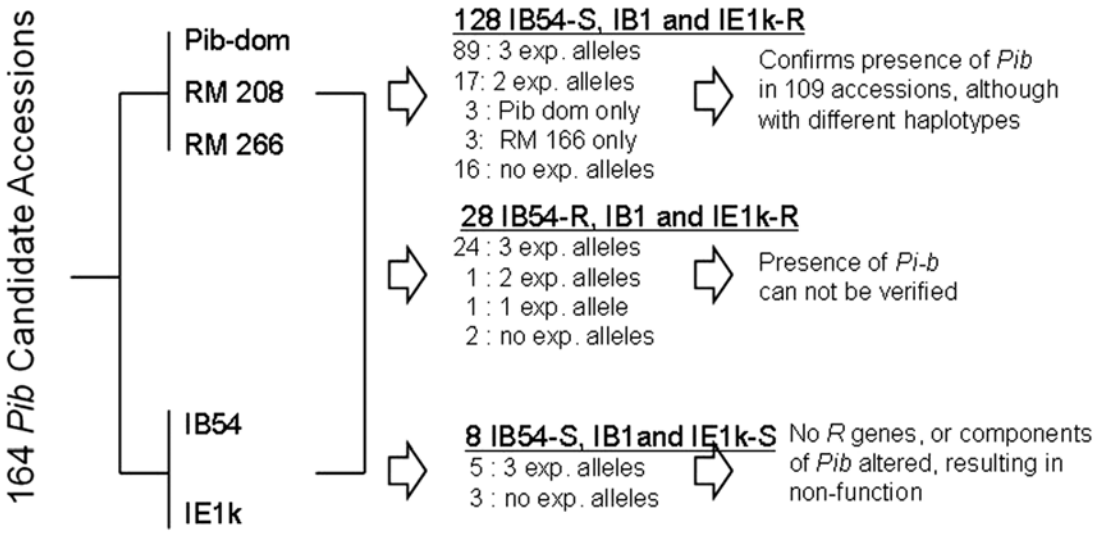

Fig. 2. Analysis of the *Pib* gene in rice germplasm using disease reaction and simple sequence repeat (SSR) markers. The diagram shows results of disease reactions and expected SSR marker alleles for germplasm in different categories.

TABLE 2. Summary of disease reaction pathogenicity assays (scored on a range from 0 to 5) and genetic marker profiles for the analysis of the *Pib* gene in rice germplasm accessions

Sample	Country	Name	SSR markers[a]			Disease reactions			Presence of *Pib*[b]
			Pib-dom	RM208	RM166	IB54	IE1k	IB1	
GSOR310285	Philippines	IR 532-1-47	360	179	316	S 3	R 0	R 0	+
GSOR310298	Guyana	51779	360	179	316	S 3	R 2	R 0	+
GSOR310326	Philippines	IR 1103-15-8-5-3-3-3	360	179	316	S 3	R 2	R 0	+
GSOR310340	Laos	Chao Hay b	360	179	316	S 4	R 0	R 0	+
GSOR310363	Colombia	P773-44-3-1	360	179	316	S 3	R 2	R 0	+
GSOR310367	Colombia	P 738-97-3-1	360	179	316	S 3	R 0	R 0	+
GSOR310368	Colombia	P 761-40-2-1	360	179	316	S 3	R 0	R 0	+
GSOR310481	India	Anandi	360	179	316	S 3	R 0	R 0	+
GSOR310485	Sri Lanka	Perum Karuppan	360	164	318	S 4	R 2	R 0	+
GSOR310487	Indonesia	Sigadis	360	179	316	S 4	R 0	R 0	+
GSOR310517	Hong Kong	Fa Loh Pak	×	164	316	S 3	R 2	R 0	+/?
GSOR310528	Brazil	J 312	360	179	316	S 3	R 0	R 0	+
GSOR310539	Mali	Segadis	360	179	316	S 4	R 0	R 0	+
GSOR310542	Bangladesh	BR51-319-9	360	179	316	S 3	R 0	R 0	+
GSOR310545	Indonesia	B462B-PN-31-2	360	179	316	S 4	R 0	R 0	+
GSOR310547	Peru	Huallaga	360	179	316	S 3	R 2	R 0	+
GSOR310548	Thailand	BKN 6820-6-3-2	360	179	316	S 3	R 2	R 0	+
GSOR310549	Sri Lanka	BG 90-2	360	179	316	S 3	R 0	R 0	+
GSOR310567	Guatemala	Tikal2	360	179	316	S 3	R 2	R 0	+
GSOR310574	Malaysia	SM II	360	179	316	S 3	R 2	R 0	+
GSOR310575	Haiti	Gros Riz	360	176	316	S 3	R 2	R 0	+
GSOR310576	India	Pusa 33	360	179	316	S 3	R 1	R 0	+
GSOR310612	Uzbekistan	Uz Begohef 2	360	179	316	S 3	R 2	R 0	+
GSOR310630	Thailand	BKN 6987-68-14	360	179	316	S 4	R 2	R 0	+
GSOR310631	Guinea	GPNO 22236	360	179	316	S 4	R 2	R 0	+
GSOR310632	Philippines	IR 4482-5-3-9-5	360	179	316	S 3	R 2	R 0	+
GSOR310636	Cote D'Ivoire	IRAT 8	360	179	316	S 3	R 0	R 0	+
GSOR310650	India	PR 106	360	179	316	S 3	R 0	R 0	+
GSOR310655	Chile	CH 272-132	360	179	316	S 3	R 0	R 0	+
GSOR310657	Egypt	CR418-3-12	360	179	316	S 3	R 0	R 0	+
GSOR310658	Egypt	CR 561-4-2-1	360	179	318	S 4	R 2	R 0	+
GSOR310663	Kazakhstan	Kasakstanica	360	164	318	S 3	R 0	R 0	+
GSOR310690	S. Korea	Milyang23/IR1545-339-2-2	360	179	316	S 3	R 0	R 0	+
GSOR310709	Bangladesh	BR19	360	179	316	S 3	R 0	R 0	+
GSOR310732	Colombia	C 3CU77-1CU-2CU-2CU-2CU-SMCU2	360	179	316	S 3	R 0	R 0	+
GSOR310735	Panama	Anayansi	360	179	316	S 3	R 0	R 0	+
GSOR310741	Cuba	Perla	360	179	316	S 3	R 0	R 0	+
GSOR310746	Cambodia	376	360	179	316	S 4	R 2	R 0	+
GSOR310748	Nepal	IR-44595	360	176	316	S 3	R 0	R 0	+
GSOR310757	India	RP2151-173-1-8	360	179	316	S 3	R 2	R 0	+
GSOR310770	China	Miyang	360	179	316	S 4	R 0	R 0	+
GSOR310773	Cuba	ECIA76-S89-1	360	179	316	S 3	R 0	R 0	+
GSOR311005	Philippines	IR 8-296-2-1	360	179	316	S 3	R 0	R 0	+
GSOR311024	India	RP1 332	360	179	316	S 3	R 2	R 0	+
GSOR311032	Guyana	50638	360	179	316	S 3	R 0	R 0	+
GSOR311033	Argentina	Fortuna Corrientes Sel Inta	360	179	421	S 3	R 0	R 0	+
GSOR311042	Philippines	IR 1314-28-1-2	360	179	316	S 3	R 0	R 0	+
GSOR311061	Philippines	Siryan	360	179	316	S 4	R 0	R 0	+
GSOR311066	Laos	Kh. Mack Fay	360	179	316	S 3	R 0	R 0	+
GSOR311073	Indonesia	Tukan Tuna	360	179	×	S 3	R 0	R 0	+
GSOR311076	Bulgaria	Sesilla	360	179	316	S 3	R 0	R 0	+
GSOR311113	Hong Kong, China	Shui Ya Jien	360	179	316	S 3	R 0	R 0	+
GSOR311152	Fiji	Rani	360	179	316	S 3	R 0	R 0	+
GSOR311153	Philippines	IR 2061-214-2-3	360	176	316	S 3	R 0	R 0	+
GSOR311154	Philippines	IR2151-598-3-5	360	179	316	S 3	R 0	R 0	+
GSOR311162	Guyana	60-283	360	179	318	S 3	R 2	R 0	+
GSOR311168	Philippines	IR9-60	360	179	316	S 3	R 0	R 0	+
GSOR311184	Thailand	Bang Tuey	360	176	316	S 4	R 0	R 0	+
GSOR311207	India	NP 97	×	164	316	S 3	R 2	R 0	+/?
GSOR311210	Philippines	IR 2151-745-3-1	360	179	316	S 3	R 2	R 0	+
GSOR311213	Bangladesh	Biplab	360	179	316	S 3	R 2	R 0	+
GSOR311214	Philippines	IR 1514A-E597	360	176	318	S 3	R 1	R 0	+
GSOR311217	Pakistan	Sella Manzkhora	360	179	316	S 3	R 0	R 0	+
GSOR311223	Indonesia	KN-1 B-361-BLK-2	360	172	316	S 3	R 0	R 0	+
GSOR311238	Sierra Leone	Chen Chu Ai	360	179	×	S 3	R 1	R 0	+
GSOR311248	Dominican Republic	Mingolo	360	179	316	S 4	R 0	R 0	+
GSOR311249	Dominican Republic	Tono Brea 439	360	179	316	S 5	R 0	R 0	+

(*continued on following page*)

[a] × indicates lack of amplification product detected for simple sequence repeat (SSR) marker.

[b] + indicates accessions containing *Pib* with different haplotypes; ? indicates accessions containing additional *R* genes; – indicates accessions which do not contain *Pib*; and * indicates accessions with no marker alleles with pathogenicity response similar to accessions containing *Pib.*

TABLE 2. (*Continued from previous page*)

Sample	Country	Name	SSR markers[a]			Disease reactions			Presence of *Pib*[b]
			Pib-dom	RM208	RM166	IB54	IE1k	IB1	
GSOR311294	Senegal	CAS 209	360	179	316	S 3	R 2	R 0	+
GSOR311298	Thailand	Jek Chuey 159	360	179	316	S 3	R 2	R 0	+
GSOR311302	Sierra Leone	SL 22-613	360	179	421	S 4	R 2	R 0	+
GSOR311306	Nigeria	Mange2	360	179	316	S 3	R 0	R 0	+
GSOR311310	India	Archana	360	179	316	S 4	R 0	R 0	+
GSOR311317	Philippines	IR 1615-246	360	179	318	S 3	R 0	R 0	+
GSOR311325	Italy	Bajang Allorio	360	179	316	S 3	R 0	R 0	+
GSOR311344	Philippines	IR 9209-26-2	360	179	316	S 4	R 2	R 0	+
GSOR311348	S. Korea	Seogwangbyeo	360	179	316	S 3	R 0	R 0	+
GSOR311359	Colombia	17632	360	179	316	S 3	R 0	R 0	+
GSOR311360	Colombia	19965	360	179	316	S 3	R 0	R 0	+
GSOR311366	China	Te Qing	360	179	316	S 3	R 0	R 0	+
GSOR311380	Bangladesh	BR24	360	179	316	S 3	R 0	R 0	+
GSOR311399	Colombia	Amistad 82	360	179	316	S 3	R 0	R 0	+
GSOR311402	Ecuador	INIAP 11	360	179	316	S 4	R 0	R 0	+
GSOR311403	Colombia	Panama 1048	360	179	316	S 3	R 0	R 0	+
GSOR311405	Colombia	Huri 282	360	179	316	S 3	R 0	R 0	+
GSOR311423	Philippines	IR 58614-B-B-8-2	360	176	316	S 3	R 0	R 0	+
GSOR311424	Japan	BL 1	360	179	418	S 3	R 0	R 0	+
GSOR311430	Cuba	ECIA 66	360	179	316	S 3	R 2	R 0	+
GSOR311433	Philippines	IR 54055-142-2-1-2-3	360	179	316	S 4	R 0	R 0	+
GSOR311435	Vietnam	CM1, Haipong	360	179	316	S 3	R 0	R 0	+
GSOR311438	Liberia	2071-621-2	360	179	316	S 3	R 2	R 0	+
GSOR311439	China	4582	360	172	316	S 3	R 0	R 0	+
GSOR311441	China	GP-2	360	179	316	S 3	R 0	R 0	+
GSOR311442	Philippines	IR58025 B	360	179	316	S 3	R 0	R 0	+
GSOR311443	China	Gui 99	360	179	316	S 3	R 0	R 0	+
GSOR311445	China	Z 535	360	179	316	S 4	R 0	R 0	+
GSOR311447	China	Xiangzhaoxian NO. 15	360	179	316	S 4	R 0	R 0	+
GSOR311448	China	Hunanruanmi	360	179	316	S 3	R 0	R 0	+
GSOR311449	China	Zhongyu No. 6	360	179	316	S 3	R 0	R 0	+
GSOR311456	China	Erxi No. 149	360	179	316	S 3	R 0	R 0	+
GSOR311459	China	71198	360	179	316	S 3	R 0	R 0	+
GSOR311467	China	Jinnuo No. 6	360	176	316	S 3	R 2	R 0	+
GSOR311468	China	Dian No. 01	×	172	316	S 3	R 0	R 0	+/?
GSOR311471	China	You No. 51	360	179	316	S 3	R 0	R 0	+
GSOR311477	China	H 323	360	179	316	S 3	R 0	R 0	+
GSOR311478	China	CDR 22	360	179	×	S 3	R 0	R 0	+
GSOR311481	China	Shufeng 121	360	179	316	S 3	R 0	R 0	+
GSOR311511	China	MPH 501	360	179	316	S 3	R 0	R 0	+
GSOR311518	Bangladesh	Bhujon Kolpo	360	179	316	S 3	R 0	R 0	+
GSOR311519	Bangladesh	Khoia	360	179	316	S 3	R 0	R 0	+
GSOR311520	Bangladesh	Bogra	360	179	316	S 3	R 0	R 0	+
GSOR311521	Philippines	IR 56450-28-2-2	360	179	316	S 3	R 0	R 0	+
GSOR311525	Indonesia	S972B-22-1-3-1-1	360	179	316	S 4	R 0	R 0	+
	United States	Saber	360	179	316	R 0	R0	R 0	+
GSOR310164	Mexico	C1-6-5-3	×	164	×	R 0	R 0	R 0	?
GSOR310350	Papua New Guinea	C 8435	360	179	316	R 1	R 0	R 0	?
GSOR310540	Thailand	T442-57	×	176	316	R 1	R 0	R 0	?
GSOR310543	Costa Rica	CR 1113	360	179	316	R 1	R 2	R 0	?
GSOR310566	Ecuador	INIAP 7	360	179	316	R 1	R 2	R 0	?
GSOR310648	Zimbabwe	IR 400	360	179	316	R 0	R 0	R 0	?
GSOR310687	Philippines	IR 9660-48-1-1-2	360	179	316	R 1	R 2	R 0	?
GSOR310688	S. Korea	Milyang 56	360	179	316	R 1	R 0	R 0	?
GSOR310689	S. Korea	Raegyeong	360	179	316	R 1	R 0	R 0	?
GSOR310730	Dominican Republic	Juma 61	360	×	316	R 0	R 0	R 0	?
GSOR310750	Nigeria	Faro 37	360	179	316	R 0	R 0	R 0	?
GSOR310751	India	RP1821-5-17-2	360	179	316	R 0	R 0	R 0	?
GSOR310752	Cuba	ECIA 128	360	179	316	R 0	R 0	R 0	?
GSOR310753	Egypt	GZ1368-5-4	360	179	316	R 0	R 0	R 0	?
GSOR310756	Dominican Republic	J355-6-2-1-1	360	179	316	R 0	R 0	R 0	?
GSOR310772	Brazil	CL Seleccion 56	360	179	316	R 0	R 1	R 0	?
GSOR311039	Philippines	IR 1321-19	360	179	316	R 0	R 0	R 0	?
GSOR311044	Philippines	IR 773A1-36-2-1-3	360	179	316	R 0	R 0	R 0	?
GSOR311082	Pakistan	Hansraj	×	164	×	R 0	R 0	R 0	?
GSOR311097	Portugal	Indo Yiaia Lonica	360	179	316	R 0	R 0	R 0	?
GSOR311219	S. Korea	Suweon 258	360	179	316	R 1	R 0	R 0	?
GSOR311244	Peru	INTI	360	179	316	R 0	R 0	R 0	?
GSOR311304	Nigeria	Adny 11	360	179	316	R 0	R 0	R 0	?
GSOR311409	Mexico	Campeche A 80	360	179	316	R 1	R 0	R 0	?
GSOR311411	Peru	San Martin 86	360	179	316	R 1	R 0	R 0	?
GSOR311421	Philippines	C2764-10-2	360	179	316	R 1	R 0	R 0	?
GSOR311503	China	Zhong 413	360	179	316	R 0	R 0	R 0	?

TABLE 2. (*Continued from previous page*)

Sample	Country	Name	SSR markers[a]			Disease reactions			Presence of *Pib*[b]
			Pib-dom	RM208	RM166	IB54	IE1k	IB1	
GSOR311513	China	ZAO 402	360	179	316	R 0	R 0	R 0	?
GSOR310278	Iraq	Amber 33	×	168	318	S 3	R 0	R 2	*
GSOR310319	India	BC5-55	×	164	318	S 3	R 2	R 0	*
GSOR310352	Malaysia	Padi Bangka	×	164	418	S 3	R 0	R 0	*
GSOR310436	Cuba	Zayas Bazan	×	176	318	S 3	R 0	R 0	*
GSOR310553	Iran	205	×	170	318	S 3	R 2	R 0	*
GSOR310555	Colombia	Colombia 1	×	176	318	S 5	R 1	R 0	*
GSOR310659	Egypt	YNA 223	×	168	418	S 3	R 0	R 0	*
GSOR310683	Nigeria	IITA 130	×	164	421	S 3	R 0	R 0	*
GSOR310686	Brazil	Pratao	×	164	418	S 3	R 0	R 0	*
GSOR310754	Argentina	H232-44-1-1	×	164	418	S 3	R 2	R 0	*
GSOR310856	China	WC 521	×	164	418	S 3	R 1	R 0	*
GSOR311059	Philippines	IR 1103-49-4-1-3-3-2	×	164	418	S 3	R 0	R 0	*
GSOR311239	Brazil	Pratao Tipo Guedes	×	164	421	S 4	R 0	R 0	*
GSOR311262	Zaire	R 46/3	×	164	421	S 3	R 0	R 0	*
GSOR311264	Zaire	Sechele	×	164	421	S 3	R 2	R 0	*
GSOR311436	China	Zhongyu No.1	×	172	318	S 3	R 0	R 0	*
GSOR310583	Fiji	17465-4	360	179	316	S 3	S 4	S 3	–
GSOR310668	Azerbaijan	Bak Saly Mestnyj	×	164	418	S 3	S 3	S 4	–
GSOR310685	Brazil	BR-IRGA-410	360	179	316	S 3	S 3	S 3	–
GSOR311253	Fiji	Bilo	360	179	316	S 3	S 3	S 3	–
GSOR311494	China	R 647	360	179	316	S 5	S 3	S 3	–
GSOR311524	India	RP2199-16-2-2-1	360	179	316	S 3	S 3	R 0	–
GSOR311640	India	ARC 10378	×	176	418	S 5	S 3	S 4	–
GSOR311668	Pakistan	Daudzai Field Mix	×	164	318	S 5	S 3	S 4	–

The use of multiple markers linked with various *R* genes can be used to determine the basis of resistance in diverse germplasm collections. The Pib-dom marker was derived from a portion of the *Pib* gene; however, it was not a perfect functional marker (10). In the present study, the Pib-dom marker appeared to be the most accurate, having the least number of disagreements (=7) between its presence and the race reactions expected with the presence of the *Pib* gene. RM208 and RM166 had 18 and 13 disagreements, respectively. Three of the disagreements between both the Pib-dom and RM208 markers and the disease reactions may be the result of other *R* genes being present besides *Pib* (indicated in Table 2 with "+/?" designations) that confer resistance to races IE1k and IB1. In conclusion, no single SSR marker allele was completely (100%) associated with the Pib-dom marker or with the presence of *Pib* as indicated by race reactions.

In the present study, rice germplasm with *Pib* was found in 40 countries, but predominantly from China, the Philippines, Colombia, and India (Table 2). In the United States, *Pib* is useful because it confers resistance to IE1k, a virulent race that has overcome resistance mediated by *Pita*. Field isolates that belong to IE1k have been found in commercial rice fields in the southern United States for decades (18). Stacking *Pib* or *Piz* with *Pita* in advanced breeding lines would achieve more durable resistance to blast (5).

In summary, we not only verified the *Pib* gene in 109 rice germplasm accessions but also demonstrated the usefulness of combining DNA markers and pathogenicity assays to confirm the presence of *R* genes (10). The pathogenicity assays helped verify the accuracy of DNA markers and also identified germplasm accessions that may possibly have additional blast *R* genes.

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