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Rice blast resistance gene profiling of Thai, Japanese and international rice varieties using gene-specific markers

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Research Article

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Abstract

Rice blast disease, caused by *Magnaporthe oryzae*, is one of the most damaging diseases of rice worldwide. Cultivation of rice varieties carrying resistance genes is the most economic and successful strategy to control the disease. In this study, 451 rice varieties from around the world including 363 Thai landrace rice varieties, 21 Thai improved rice varieties, 43 Japanese rice varieties and 24 worldwide rice varieties were screened by PCR technique using gene-specific markers for 10 rice blast resistance genes: *Pi9*, *Piz-t*, *Pi50*, *Pigm(t)*, *Pid2*, *Pid3*, *Pia*, *Pik*, *Pi54* and *Pita*. The results showed that 382 (99.48%) Thai rice varieties have at least one resistance gene and two rice varieties, 'Hom' and 'Bak muay', contained eight out of ten screened rice blast resistance genes. 320 rice varieties (83.33%) contained three or more rice blast resistance genes. The frequency of the rice blast resistance gene ranges from 87.76–9.64 per cent, of which the *Pid3* gene has the highest frequency and the *Pi54* gene has the lowest frequency. Two major resistance genes, found in Japanese rice varieties, are the *Pik* gene (76.74%) and the *Pi9* gene (72.09%). While two major resistance genes, found in the international rice varieties are the *Pi9* gene (66.67%) and the *Pi54* gene (62.50%). The disease resistance gene profile of each rice variety obtained from this study will benefit the rice blast resistant breeding programme in the future.

Introduction

Rice is the most important staple food source for more than half of the world's population, where more than 90% of rice is cultivated in Asia (Yadav and Kumar, 2018). Rice blast disease caused by the fungus *Magnaporthe oryzae* is one of the most frequent and destructive diseases of rice causing a heavy loss of yield in all rice-growing regions (Wang *et al.*, 2014). One of the most effective methods to control this disease is using resistant cultivars. The interaction between rice and rice blast fungus follows the gene-for-gene concept. A resistance gene (*R*) in a plant corresponds to an avirulence gene (*AVR*) in a blast pathogen. The *AVR* gene synthesizes an effector protein, which is recognized by a resistance protein, a product of the rice *R* gene. This interaction can activate hypersensitive response (HR) and finally leads to active defence responses (Flor, 1971; Gururani *et al.*, 2012). At present, more than 100 rice blast resistance genes are mapped on different rice chromosomes, which 25 resistance genes have been successfully cloned including *Pib*, *Pita*, *Pid2*, *Pi9*, *Pi2*, *Piz-t*, *Pi36*, *Pi37*, *Pikm*, *Pi5*, *Pit*, *Pid3*, *Pi21*, *Pish*, *Pb1*, *Pik*, *Pikp*, *Pikh*, *Pia*, *Pi1*, *Pi64* and *Pi50* (Kalia and Rathour, 2019).

Thailand has diverse rice cultivation areas. The north and the south of Thailand feature mountainous geography. Rice varieties growing in these regions are mostly landraces, which are well adapted to the colder climates. The northeast region is well known for the cultivation of long-grain and glutinous rice varieties. In the central region, long-grain and non-glutinous rice varieties are cultivated during the rainy season (The Global Rice Science Partnership, 2013). The diverse geography and climatic features provide a high level of rice genetic diversity in Thailand, which also has been demonstrated by Moonsap *et al.* (2019) using Indel markers. There are more than 100,000 landraces, improved and elite rice varieties, which many of them exhibit resistant reactions to rice blast disease (Srikeaw, 2010). It is very important to identify these rice varieties that have the potential to be used as a source for the blast-resistant in the

rice breeding programme and to develop a Thai rice core germplasm so that everyone can benefit from using this core set of germplasm for the blast-resistant purposes.

Molecular markers have been widely used to find variation in organisms both at individuals and population levels (Tharachand *et al.*, 2012). In this study, we aimed to identify rice blast resistance genes in Thai, Japanese and worldwide rice varieties and to develop a catalogue of the rice blast resistance genes using gene-specific DNA markers. Ten rice blast resistance genes were selected for the cataloguing based on the importance of their potential for the rice blast-resistance to the rice blast fungus population in Thailand and around the world.

Materials and methods

Plant materials

Three hundred and eighty-four Thai rice varieties including 363 landrace rice and 21 improved rice varieties, 43 Japanese rice and 24 International rice varieties were used in this study (online Supplementary Table S1). IRRI-inbred blast resistant lines (IRBLs) provided by the International Rice Research Institute (IRRI) were used as a positive control. Lijiangxintuanheigu (LTH) was used as IRBL background and susceptible control.

DNA extraction

Rice seeds were soaked in water for five days and transferred to a mini tray with sterile soil in greenhouse condition. Genomic DNA was extracted from 21-day-old leaf samples by cetyltrimethylammonium bromide (CTAB) method (20 mM EDTA, 0.1 M Tris-HCl pH 8.0, 1.4 M NaCl, 2% CTAB, plus 0.4% β-mercaptoethanol) (Doyle and Doyle, 1987). DNA quality was checked by a 1% of agarose gel electrophoresis, stained with ViSafe Green Gel Stain (Vivantis Technologies Sdn. Bhd., Malaysia), and photographed by The Gel Doc XR + System (Bio-Rad Laboratories, Inc., USA). DNA quantity was checked by Nano Drop 2000 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

DNA marker analysis

The primer sequences of ten DNA markers for rice blast resistance genes are shown in Table 1. The PCR amplification was performed in a 20 µl reaction containing, 12.6 µl of sterile distilled water, 2 µl of 10 × PCR buffer (100 mM Tris-HCl, 500 mM KCl), 0.25 µl of 50 mM $MgCl_2$, 0.5 µL of 10 mM dNTPs solution mix, 1 µl of each 5 µM primer, 0.3 µL of Taq DNA polymerase (5 units/µl) (Vivantis, Shah Alam, Malaysia), and 2 µl of rice genomic DNA (50 ng). The PCR cycling programme consists of an initial denaturation for 2 min at 94 °C, 35 cycles of 30 s of denaturation at 94 °C, 30 s of annealing temperature depending on the primer pair (Table 1), 30 s of extension at 72 °C and a final extension at 72 °C for 5 min. The PCR products were stained with 1 µl of 10X-orange-G loading dye ViSafe Green Gel Stain (Vivantis, Shah Alam, Malaysia) and run using 1.5% agarose gel electrophoresis at 100 V for 40 min. The gels were photographed under ultraviolet light by The Gel Doc XR + System (Bio-Rad Laboratories, Inc., Singapore). For CAPs marker, 5 µl of PCR product was digested with 3 units of fast digest restriction enzymes (Table 1), 1 µl of 10 × digesting buffer, and 3.7 µl of sterile distilled water at 37 °C for overnight. The product was determined using 2% agarose gel electrophoresis and photographed under ultraviolet light. The DNA bands were scored R and S as the blast-resistant and -susceptible rice varieties respectively. All DNA markers were repeated at least twice, which showed the same results.

R gene sequence confirmation

To confirm the sequences of rice blast resistance genes from gene-specific primers, three positive samples from each gene were randomly selected. PCR products were purified with a Qiaquick gel extraction kit (QAIGEN, Germany) and sequenced by a commercial sequencing service provider (U2Bio, Bangkok, Thailand) using gene-specific primers. The DNA sequences were confirmed by the alignment with the reference gene sequences obtained from the GenBank database.

Results

Distribution frequency of rice blast resistance genes in Thai, Japanese and International rice varieties

Thai, Japanese and International rice varieties were screened for ten major rice blast resistance genes: *Pi9*, *Piz-t*, *Pi50*, *Pigm(t)*, *Pid2*, *Pid3*, *Pia*, *Pik*, *Pi54* and *Pita* using gene-specific DNA markers. The DNA markers for the *Pi9*, *Piz-t*, *Pi50*, *Pik*, *Pia* and *Pita* genes are dominant allele-specific markers. A resistant allele showed a positive DNA band with expected size, while a susceptible allele showed an absent band (Fig. 1). The gene-specific marker for the *Pi9* gene successfully detected the resistant allele in 199 Thai rice varieties (51.82%), 31 Japanese rice varieties (72.09%) and 16 International rice varieties (66.67%) (Table 2). A 257 bp DNA band from the *Piz-t* gene-specific marker was detected in 70 (18.23%), 2 (4.65%) and 2 (8.33%) rice varieties from Thailand, Japan and worldwide respectively (Table 2). The *Pi50* gene was found in 99 Thai rice varieties (25.78%) and was not detected in Japanese and International rice varieties (Table 2). Forty-eight Thai rice varieties (12.50%), 33 Japanese rice varieties (76.74%) and 7 international rice varieties (29.17%) showed 1144 bp positive amplicon of the *Pik* gene-specific marker (Table 2). The presence of the blast resistance gene *Pia* was determined by the visualization of 906 bp amplicon in 133 (34.63%), 7 (16.28%) and 2 (8.33%) Thai, Japanese and International rice varieties respectively (Table 2). The *Pita* gene was detected by the 1024 bp amplicon in 127 (33.07%), 7 (16.28%) and 2 (8.33%) Thai, Japanese and International rice varieties (Table 2). The PCR products of three positive samples from each gene containing the rice blast resistance allele were sequenced. The sequences were deposited in GenBank and accession numbers of all sequences were shown in online Supplementary Table S2. Sequences were then compared with the reference blast resistance gene sequences from NCBI GenBank (DQ285630.1, CCD28558.1, AKS24975.1, KY225903.1, AB604626 and AF207842.1 for *Pi9*, *Piz-t*, *Pi50*, *Pik*, *Pia* and *Pita* respectively). The alignment results confirmed the presence of the rice blast resistance genes.

The *Pigm(t)* and *Pi54* genes were screened by the gene-specific Indel markers, which gave two different amplicon sizes. The resistant allele of the *Pigm(t)* showed the 555 bp amplicon and the susceptible allele showed the 461 bp amplicon (Fig. 1). The resistant allele was observed in 205 Thai rice varieties (53.39%), 15 Japanese rice varieties (34.88%) and 8 International rice varieties (33.33%) (Table 2). Two Thai rice varieties contained

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Table 1. Gene-specific markers for rice blast resistance genes

Target gene	Primer sequence (5′→3′)	AT (°C)	Target size (bp)	Reference
Pi9	F: CCCAATCTCCAATGACCCATAAC R: CCGGACTAAGTACTGGCTTCGATA	56	500	Liu *et al.* (2002)
Pi54[a]	F: CAATCTCCAAAGTTTTCAGG R: GCTTCAATCACTGCTAGACC	55	R: 261 S: 359	Ramkumar *et al.* (2011)
Pia	F: GAGCAATGCCCAATCTCCAG R: TTTACCGTTCACTGACGCAG	60	906	IRRI, Unpublished
Pi50	F: CTTGACATCCAAACCGCACC R: TAGGCCTAGCCAATTTTTGCC	60	1172	Xiao *et al.* (2017)
Pigm(t)[a]	F: CAGTGAAACGAACGCTATG R: AATAGGAAGGGTTGATGTTG	56	R: 555 S: 461	Deng *et al.* (2006)
Pi-ta	F: AGCAGGTTATAAGCTAGGCC R: CTACCAACAAGTTCATCAAA	58	1024	Jia *et al.* (2002)
Pik	F: GGAAAGCTGATATGTTGTCG R: ACTCGGAGTCGGAGAGTCAG	58	1144	Ariya-anandech *et al.* (2018)
Pid2[b]	F: TTGGCTATCATAGGCGTCC R: ATTTGAAGGCGTTTGCGTAGA	58	1057	Chen *et al.* (2004)
Pid3[b]	F: TACTACTCATGGAAGCTAGTTCTC R: AGCACTTCTTGACTACTGTCTGCCT	52	178	Shang *et al.* (2009)
Piz-t	F: TTGCTGAGCCATTGTTAAACA R: ATCTCTTCATATATATGAAGGCCAC	60	257	Hayashi *et al.* (2006)

[a]Indel marker, resistant allele and susceptible allele were detected by different sizes.
[b]CAPs marker, PCR products were digested with a restriction enzyme to identify resistant or susceptible allele, the *Pid2* gene was digested with *Mlu*I enzyme and the *Pid3* gene was digested with *Bam*HI enzyme.

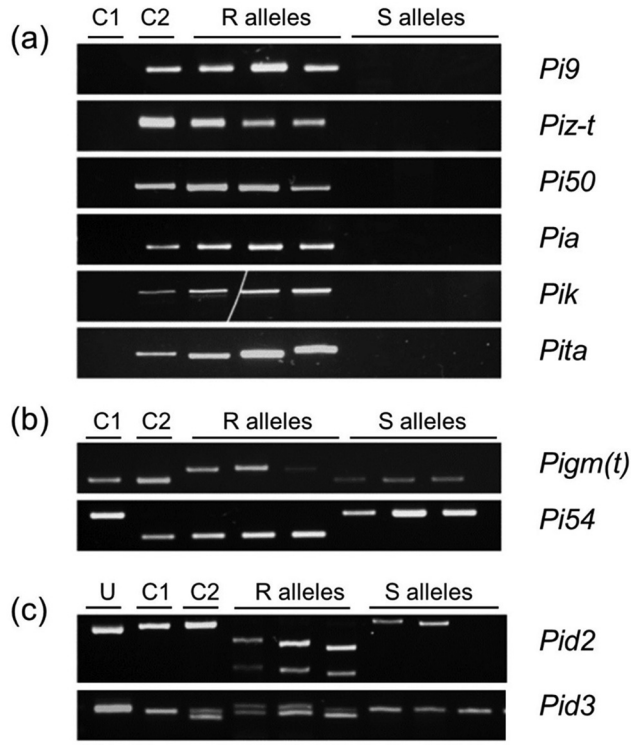

Fig. 1. Gel electrophoresis detection of ten rice blast resistance genes using gene-specific primers, (a) the results of the dominant allele-specific marker (b) the results of the co-dominant allele-specific marker, InDel (c) the results of the co-dominant allele-specific marker, CAPs. Rice varieties used as a negative control (C1) include Nipponbare, KDML105 and Lijiangxintuanheigu (LTH). Rice varieties used as a positive control (C2) include IRBL9-w (*Pi9*), IRBLzt-T(*Piz-t*), IRBLz5-CA(*Pi50*), IRBLa-A (*Pia*), Jao Hom Nin (*Pik*, *Pi54*), IRBLta-K1(*Pita*), Nipponbare (*Pigm(t)*, *Pid2*, *Pid3*). U indicates an uncut amplicon. Note no IRBL positive control (C2) for *Pigm(t)* and *Pid2* genes.

Pigm(t) resistant allele, 'Nhew dam' and 'Hom', and one rice variety, 'Nhew ubon1' which contained a susceptible allele were sequenced and the sequences were deposited in GenBank, accession number OM236475 to OM236477 respectively (online Supplementary Table S2). The sequence alignment showed 100% sequence identity with the reference *Pigm(t)* sequence (GenBank KU904633.2). There is a 94 bp insertion or deletion difference between the resistant and susceptible allele of the *Pigm(t)* rice blast resistance gene. The resistant allele of the *Pi54* gene showed a 261 bp amplicon and the susceptible allele showed a 359 bp amplicon (Fig. 1). Thirty-seven Thai rice varieties (9.64%), 14 Japanese rice varieties (32.56%) and 15 International rice varieties (62.50%) showed the resistant allele (Table 2). To confirm the resistant and susceptible allele of the *Pi54* allele, one rice variety 'Homdong', that contained a resistant allele, and two rice varieties 'Dorhom' and 'Khaw tud ngon' that contained a susceptible allele were sequenced and deposited in GenBank, accession number OM236490 to OM236492 respectively (online Supplementary Table S2). These sequences were aligned with the *Pi54* reference sequences (GenBank AP014967.1, AY914077.1) and the result confirmed that there is a 143 bp insertion or deletion region between resistant and susceptible allele.

The *Pid2* and *Pid3* genes were screened by the gene-specific CAPs markers. The 1057 bp PCR product of the *Pid2* gene was digested with the *Mlu*I enzyme, the resistant allele showed two DNA bands with 657 and 400 bp fragments (Fig. 1). This resistant allele was detected in 190 Thai rice varieties (49.48%) but no Japanese rice and International rice varieties contained the *Pid2* resistant allele (Table 2). The 1057 bp PCR product of the *Pid2* gene-specific primer before the digestion with *Mlu*I enzyme from three Thai rice varieties 'RD12', 'KDML105' and 'Khaw jao Prachinb' were sequenced and the sequences were deposited

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Table 2. Distribution of rice blast resistance genes in Thai rice germplasm, Japanese rice and International rice varieties

Rice set	No.	*Pi9*	*Piz-t*	*Pi50*	*Pigm(t)*	*Pid2*	*Pid3*	*Pia*	*Pik*	*Pi54*	*Pita*
Thai germplasm	384	199 (51.82%)	70 (18.23%)	99 (25.78%)	205 (53.39%)	190 (49.48%)	337 (87.76%)	133 (34.63%)	48 (12.5%)	37 (9.64%)	127 (33.07%)
Japanese rice	43	31 (72.09%)	2 (4.65%)	0 (0%)	15 (34.88%)	0 (0%)	2 (4.65%)	7 (16.28%)	33 (76.74%)	14 (32.56%)	7 (16.28%)
International rice	24	16 (66.67%)	2 (8.33%)	0 (0%)	8 (33.33%)	0 (0%)	8 (33.33%)	2 (8.33%)	7 (29.17%)	15 (62.5%)	2 (8.33%)
Total	451	246 (54.55%)	74 (16.40%)	99 (21.95%)	228 (50.55%)	190 (42.13%)	347 (76.94%)	142 (31.48%)	88 (19.51%)	66 (14.63%)	136 (30.16%)

Table 3. The geographical distribution of the resistant alleles for the rice blast resistance genes in Thai landrace rice varieties

Source of Thai rice varieties	Total	*Pi9*	*Pizt*	*Pi50*	*Pigm(t)*	*Pid2*	*Pid3*	*Pia*	*Pik*	*Pi54*	*Pita*
Northern landrace	96	67 (69.79%)	11 (11.46%)	35 (36.46%)	61 (63.54%)	31 (32.29%)	81 (84.38%)	18 (18.75%)	15 (15.63%)	11 (11.46%)	26 (27.08%)
Northeast landrace	87	58 (66.67%)	28 (32.18%)	38 (43.68%)	38 (43.68%)	48 (55.17%)	82 (94.25%)	35 (40.23%)	6 (6.90%)	3 (3.45%)	34 (39.08%)
Central landrace	88	36 (40.91%)	7 (7.95%)	8 (9.09%)	46 (52.27%)	42 (47.73%)	80 (90.91%)	45 (51.14%)	12 (13.64%)	10 (11.36%)	32 (36.36%)
Southern landrace	92	22 (23.91%)	18 (19.57%)	16 (17.39%)	54 (58.70%)	58 (63.04%)	73 (79.35%)	24 (26.09%)	10 (10.87%)	13 (14.13%)	29 (31.52%)
Improved rice	21	16 (76.19%)	6 (28.57%)	2 (9.52%)	6 (28.57%)	11 (52.38%)	21 (100%)	11 (52.38%)	5 (23.81%)	0 (0%)	6 (28.57%)
Total	384	199 (51.82%)	70 (18.23%)	99 (25.78%)	205 (53.39%)	190 (49.48%)	337 (87.76%)	133 (34.63%)	48 (12.50%)	37 (9.64%)	127 (33.07%)

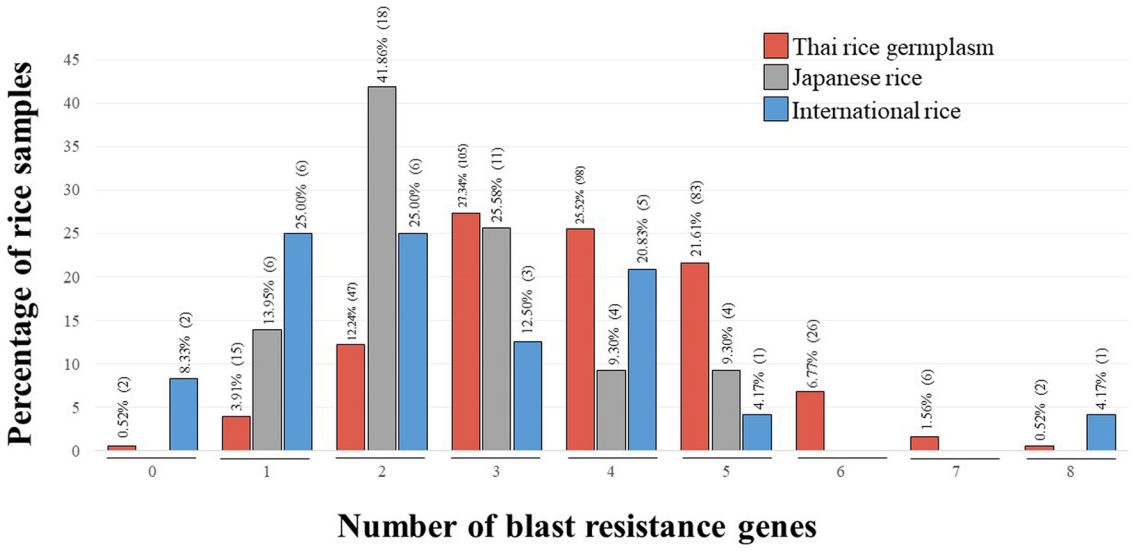

Fig. 2. Distribution of rice varieties bearing variable numbers of the rice blast resistance genes. Bar graphs show the different number of blast resistance genes of each group, ranging from 0 to 8 genes. Hight of the bar charts shows the percentage of rice samples and the number of samples shown in the bracket.

in GenBank, accession number OM236478 to OM236480 respectively (online Supplementary Table S2). The sequence alignment with the *Pid2* reference sequence (GenBank KP738455.1) confirmed the presence of the *Pid2* gene. For the *Pid3* gene, 178 bp PCR product of the *Pid3* gene-specific primer was digested with the *Bam*HI enzyme (Fig. 1). The resistant allele of the *Pid3* gene showed two DNA bands with 153 and 35 bp fragments in 337 Thai rice varieties (87.76%), 2 Japanese rice varieties (4.65%) and 8 International rice varieties (33.33%), respectively (Table 2). The 178 bp PCR product before digestion with *Bam*HI enzyme from three Thai rice varieties 'Je san', 'KDML105' and 'Nhew dang' were sequenced and the sequences were deposited in GenBank, accession number OM236481 to OM236483 respectively (online Supplementary Table S2). The sequence alignment with the *Pid3* reference sequence (GenBank FJ773286.1) confirmed the presence of the *Pid3* gene.

Geographical distribution of rice blast resistance genes in Thai landrace rice varieties

Rice blast resistance *Pid3* gene is the most frequently found in Thai germplasm with 337 from 384 (87.76%) rice varieties (Table 3). The *Pi9* gene was frequently found in northern, northeast landrace rice varieties and the improved rice varieties, 67 of 96 (67.79%), 58 of 87 (66.67%) and 16 of 21 (76.19%), respectively but only found 22 out of 92 (23.91%) in southern rice varieties (Table 3). The *Piz-t* and *Pi50* genes were mostly found in northeast rice varieties. The *Pigm(t)* gene was equally found in about 50% of all regions but found only 6 out of 21 (28.57%) in improved rice varieties. The *Pid2* gene was the most found in southern landrace rice varieties. The *Pia* gene showed the highest frequency in central landrace rice varieties (51.14%) and improved rice varieties (52.38%), while the *Pik* and *Pi54* genes were rarely found in Thai landrace and improved rice varieties (12.50 and 9.64% respectively) (Table 3). The presence of different rice blast resistance genes was geographically distinctly distributed. The *Pid3* and *Pi9* genes were dominant in northern and northeast regions. While the *Pid3*, *Pigm(t)* and *Pia* genes were dominant in the central region, and the *Pid2*, *Pid3* and *Pigm(t)* genes were dominant in the southern region. All improved rice varieties contain the *Pid3* resistant allele, while none contain the *Pi54* gene (Table 3).

The majority of Thai landrace rice varieties carry several rice blast resistance genes

Almost all Thai landrace rice varieties in this study (382 from 384, 99.48%) contained at least one rice blast resistance gene (Fig. 2, online Supplementary Table S1). The highest number of eight rice blast resistance genes were found in two rice varieties 'Hom' (*Pi9*, *Piz-t*, *Pi50*, *Pid2*, *Pid3*, *Pigm(t)*, *Pia* and *Pik* genes) and 'Bak mouy' (*Pi9*, *Piz-t*, *Pi50*, *Pid2*, *Pid3*, *Pigm(t)*, *Pia* and *Pita* genes) (online Supplementary Table S1). Six rice varieties 'Kaen-Jan', 'Leung-Aon', 'Hom phae pha lo', 'Phitsanulok 60-1', 'Se sa' and 'Sang Yod Phattalung' have seven rice blast resistance genes (online Supplementary Table S1) and 117 (30.47%) Thai rice varieties have at least 5 rice blast resistance genes (Fig. 2).

Rice blast resistance genes in Japanese and International rice varieties

All Japanese rice varieties carried at least one rice blast resistance gene, but none has more than 5 resistance genes. Four Japanese rice varieties namely, 'Nankai-Mochi 50', 'Tosanishiki', 'Kantou-Mochi 201' and 'Etsunan 230' carry the highest number of five rice blast resistance genes. Noted that these four Japanese rice varieties show three different combinations of the resistance genes ('Nankai-Mochi 50' and 'Tosanishiki' have the same combination of resistance genes) (online Supplementary Table S1). Of 24 International rice varieties included in this study, only one rice variety 'PLA3' from India has eight rice blast resistant genes and only one rice variety 'Irri338' from Korea has five rice blast resistance genes. The rest of screened International rice varieties contain only one or two resistance genes. Two rice varieties namely, 'Nucleoryza' from Austria and 'L.K.V.R.' from Hungary have no resistance gene. Our results indicate that rice germplasm from Thailand, Japan and worldwide have different rice blast resistance gene profiles and that Thai landrace rice

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varieties serve as good sources of the rice blast resistance genes for the breeding programme.

Discussion

We demonstrated that the gene-specific DNA markers can be successfully used to identify the resistant allele of the rice blast resistance gene. Our study showed that 382 of 384 Thai rice varieties (~99%) contained at least one rice blast resistance gene with two rice varieties carrying the highest number of eight rice blast resistance genes. While all 43 Japanese rice varieties found at least one rice blast resistance gene and 91.67% of the screened International rice varieties in this study contained the rice blast resistance genes. Only two of 384 Thai rice varieties showed no positive result for the rice blast resistance gene. The genetic frequency of 10 major rice blast resistance genes in Thai rice germplasm ranged from 9.64 to 87.76%. This result was consistent with a previous report by Teerasan *et al.* (2019) that Thai landrace rice varieties and recommended rice varieties contain several rice blast resistance genes, *Pid3*, *Pi54* and *Pigm(t)*. Kim *et al.* (2010) reported that the aromatic rice germplasm contains many major rice blast resistance genes, *Piz*, *Piz-t*, *Pik*, *Pik-m*, *Pik-p* and *Pit*. Imam *et al.* (2014) reported the genetic frequency of nine rice blast resistance genes, *Pi-z*, *Piz-t*, *Pi-k*, *Pik-p*, *Pik-h*, *Pi-ta/Pi-ta2*, *Pi-ta*, *Pi-9* and *Pi-b*, ranged from 6 to 97% in the selected set of Indian rice germplasm. Singh *et al.* (2015) screened 10 rice blast resistance genes in 192 rice accessions using SSR markers and found that the genetic frequencies of *Piz-5*, *Pi9*, *Pitp(t)*, *Pi1*, *Pi5(t)*, *Pi33*, *Pib*, *Pi27(t)*, *Pik-h* and *Pita* ranging from 19.79 to 54.69%, and 17 accessions harboured 7–8 blast resistance genes and Liang *et al.* (2017) reported the frequency of 11 major rice blast resistance genes, *Pi-d2*, *Pi-z*, *Piz-t*, *Pi-9*, *Pi-36*, *Pi-37*, *Pi5*, *Pi-b*, *Pik-p*, *Pik-h* and *Pi-ta2*, ranged from 9.4 to 100.0% in 32 Chinese rice varieties. All the studies indicated that landrace rice varieties serve as a great source of rice blast resistance genes.

Four rice blast resistance genes, *Pi9*, *Piz-t*, *Pi50* and *Pigm(t)* included in this study conferred broad-spectrum resistance to rice blast fungus. The *Pi9* rice blast resistance gene originated from wild rice species, *Oryza minuta* and it was introgressed to an Indica rice cultivar 75-1-127. It was resistant to 43 fungal isolates from 13 different countries (Liu *et al.*, 2002). About half of the screened Thai rice varieties showed the positive DNA band for the *Pi9* resistant allele. This result is consistent with the findings of Phaitreejit *et al.* (2011). The *Pigm(t)* rice blast resistance gene was found in the Chinese rice cultivar Gumei4 (GM4) (Deng *et al.*, 2006). Fifty-three per cent of Thai rice varieties in this study contained the resistant allele of the *Pigm(t)* gene. On the other hand, less than twenty per cent of Thai rice varieties in this study contained the *Piz-t* gene.

The *Pid2* rice blast resistance gene is a major resistance gene located on rice chromosome 6. It was reported in Chinese rice varieties 'Digu' (Chen *et al.*, 2004). In this study, approximately 50% of Thai rice varieties contained the *Pid2* gene. Ali *et al.* (2016) screened the rice blast resistance gene in 2509 accessions of rice germplasm from different geographic regions of Asia and Europe. They found that the *Pid2* gene was presented in 462 accessions from all regions except North Asia. In contrast, the *Pid3* rice blast resistance gene was first reported in the rice variety 'Gumei 2'. It was located on rice chromosome 6 (Shang *et al.*, 2009). Promchuay and Nilthong (2017) reported that 44 of 86 accessions from upland rice in the north of Thailand contained the *Pid3* gene.

The *Pik*, *Pi54* and *Pia* rice blast resistance genes were found at low frequency in Thai landraces rice varieties, which is consistent with the report by Ariya-anandech *et al.* (2018). 'Jao Hom Nin' (JHN) rice variety, one of the rice blast resistance gene donors for the rice breeding programme in Thailand, harboured the *Pik* gene (Chaipanya *et al.*, 2017). The *Pik* gene is shown to be an effective blast resistance gene for the rice blast population in Thailand (Chaipanya *et al.*, 2017; Longya *et al.*, 2019). It was used as a donor to produce resistant rice varieties including 'Ban Tang' and 'San Par Tong1' (Wongsaprom *et al.*, 2010; Nalampangnoenplab, 2011). The *Pi54* gene was highly resistant to the rice blast fungus isolates from north-western Himalayan (Sharma *et al.*, 2012) and the rice blast isolates from India (Ramkumar *et al.*, 2011). Since these *Pik*, *Pi54* and *Pia* genes were present at a low frequency in Thai landrace rice varieties and the improved rice varieties, they can be introgressed into Thai rice varieties to enhance the rice blast disease resistance.

The *Pita* and *Pita2* rice blast resistance genes were allelic and mapped near the centromere of chromosome 12 (Bryan *et al.*, 2000; Koide *et al.*, 2009). These genes were commonly used in rice breeding programmes worldwide. The *Pita* gene was found in many indica rice varieties. For example, the landrace cultivar 'Tadukan' in the Philippines and 'Tetep' in Vietnam, as well as in japonica rice in Japan and US (Bryan *et al.*, 2000; Jia *et al.*, 2002).

In summary, ten gene-specific DNA markers were used to screen for the rice blast resistance genes in Thai landrace rice varieties, Japanese rice varieties and the International rice varieties. The results indicated that Thai landrace rice varieties were a great source of the rice blast resistance gene pool, which can be used in the rice blast-resistant breeding programme in the future.

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