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## Investigation of rice blast resistant genes in Thai elite rice varieties (*Oryza sativa* L.) for improvement of broad-spectrum blast disease resistance variety

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**Abstract** The broad-spectrum blast disease resistance rice Huai variety (GS19567) has been reported to contain more than one blast resistance genes which one of them was mapped on chromosome 11. The blast resistance genes was investigated using gene specific primers in Huai and other 40 Thai elite rice varieties. The results obtained from polymerase chain reaction revealed that all tested varieties contained at least one blast resistant gene. The most frequent finding resistant gene was *Pib*, while *Pib* was missing in Huai variety. Therefore, in order to enhance broad spectrum blast disease resistance in a sustaining blast resistance variety, the resistance gene *Pib* is recommended to be used as candidate gene in breeding program.

**Keywords:** rice blast disease, *Pyricularia oryzae*, resistance genes

### Introduction

Rice (*Oryza sativa* L.), as one of the most important food crops of the world more than one half populations consume rice as staple food, but some rice varieties are susceptible to blast disease. Rice blast disease is one of the most devastating diseases of rice growing areas worldwide, caused by the fungus *Pyricularia oryzae* (anamorph *Magnaporthe oryzae*). It has been reported this disease caused about 10 to 20% yield loss in regular seasons and as high as 100% yield loss in years with blast epidemics. (Dean *et al.*, 2005) The most effective and economical way to control this disease was by using resistant cultivars, but resistance often lost in a few years after the cultivars were released because of the high variability of the rice blast fungus. Therefore, an urgent need was to develop the cultivars that carry durable resistance (*R*) genes against the fungus for rice blast. In the past few years, more than 85 major resistance genes were reported (Huang *et al.*, 2011). Most of them were

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located on chromosomes 6, 11 and 12. (Kinoshita, 1998; Sallaud *et al.*, 2003). Often, *R* genes for *P. oryzae* are identified in landraces cultivars or wild rice. (Tanksley and McCouch, 1997).

Thai indigenous varieties were selected by farmers and passed on for generations. It contained high level of genetic diversity and could serve as potential genetic resources for improving yield, resistance to pests and pathogens, and agronomic performance (Choudhury *et al.*, 2013). Thus, these rice materials provided the basis for genetic improvement of crops for specific traits and represent rich sources of novel allelic variation. It has been reported that the Huai variety (GS19769) showed a broad-spectrum resistance phenotype after spraying inoculated with a mix of blast isolates and one of the blast resistance genes was mapped on chromosome 11 (Parinthawong *et al.*, 2015). The objective of this study was to investigate the rice blast resistance genes in Huai variety and other 40 Thai elite rice varieties using PCR technique and gene specific primers. The results will provide a blast resistance gene list of each Thai elite rice variety. In addition, list of blast resistance gene containing or missing in Huai variety will help to enhance the broad-spectrum resistance of rice variety to blast disease.

## **Materials and methods**

### ***Plant materials***

A total of 40 varieties elite germplasms of rice in Thailand included Huai variety were analyzed in this study. Most of the varieties are categorized as good grain quality lines in terms of palatability. The details of the source of 40 varieties are listed in Table 1. The rice lines harboring particular single blast resistance gene (Near Isogenic Lines; NILs) including IRBL9-W, IRBLkh-K3, IRBLa-A, IRBLta-K1, IRBLk-Ka, IRBLb-B, as well as two well known varieties for blast resistance genetic resources, JHN and IR64, were considered as a positive control, and the susceptible NILs background variety Lijiangxintuanheigu (LTH) was used as negative control for gene profiling.

### ***DNA extraction and amplification***

Genomic DNAs were extracted from fresh leaves and grind into powder with liquid nitrogen and applied to Plant DNA Extraction Kit (VIVANTIS, Malaysia). The DNA was kept at 4 °C until use. Gene specific primers used for amplification of blast resistance genes were listed in Table 2. The 20 µl PCR reactions contained 10 ng of genomic DNA, 5 µM each of gene specific

forward and reverse primers, 10 µl OnePCR (GeneDireX Bio-Helix, Taiwan). The PCR amplification program consisted of 5 min denaturation at 94 °C, followed by 35 cycles of 30 sec denaturation at 94 °C, 1 min annealing at 55 – 60 °C and 2 min extension at 72 °C, and a final extension step of 5 min at 72 °C. The PCR products were resolved in 2.0% agarose gel by electrophoresis at 100V for 40 min in 0.5X TBE buffer. The gels were stained with Novel Juice (GeneDireX Bio-Helix, Taiwan) and visualized by UV light.

**Table1.** Forty Thai elite rice varieties used in blast resistance gene investigation

No.	Varieties	Region *	Blast** Resistance	No.	Varieties	Region *	Blast** Resistance
1	RD27	C	R	21	Chai Nat 1	All	R
2	RD41	N	R	22	Pathum Thani 1	C	R
3	RD6	N and NE	R	23	Leb Nok Pattani	S	S
4	RD43	All	R	24	Plai Ngahm Prachin Buri	C	R
5	RD59	N and C	R	25	Prachin Buri 2	C	R
6	RD7	All	S	26	Sang Yod Phattalung	S	S
7	RD61	N and C	R	27	Hahng Yi 71	NE	R
8	Jow Khao Chiangmai	N	R	28	Red Hawn Rice	NE	R
9	Phitsanulok 1	N and C	R	29	Yipun DOA1	N and NE	S
10	Khlong Luang 1	C	R	30	RD8	N	-
11	RD31	C	S	31	Homcholasit	C	-
12	Phitsanulok 60_1	All	S	32	RDP3	C	R
13	Suphan Buri 1	NE	R	33	Yipun DOA2	N	S
14	Niaw Ubon 1	All	S	34	RD29	N and C	R
15	Suphan Buri 2	C	R	35	Sunpatong 1	N	R
16	Suphan Buri 3	All	R	36	Phrae 1	N	R
17	Phitsanulok 2	C	R	37	RD10	N	S
18	Suphan Buri 60	C	R	38	RD14	N	R
19	Suphan Buri 90	C	R	39	KDML105	NE	S
20	Sakon Nakhon	NE	S	40	Jao Hom Nin	NE	R

\*C (Central), N (North), NE (Northeast) and S (South)

\*\*R (Resistances), S (Susceptible) and – (No data)

**Table 2.** Gene-specific PCR primers used in the identification of the rice blast resistance genes

<i>R genes</i>	Chrs	DNA marker	Sequence (5'-3')	Tm (C)	Exp. Size (bp)	Ref.
<i>Pi9</i>	6	pB8	F-CCCAATCTCCAATGACCCATAAC R- CCGGACTAAGTACTGGCTTCGATA	56	500	Liu <i>et al.</i> , 2002
<i>Pi54</i>	11	Pi54 MAS	F-CAATCTCCAAAGTTTTTCAGG R- GCTTCAATCACTGCTAGACC	55	261	Ramkumar <i>et al.</i> , 2011
<i>Pia</i>	11	-	F-GAGCAATGCCCAATCTCCAG R- TTTACCGTTCACCTGACGCAG	60	906	-
<i>Pi50</i>	6	-	F-CTTGACATCCAAACCGCACC R- TAGGCCTAGCCAATTTTTGCC	60	1172	Xiao <i>et al.</i> , 2017
<i>Pigm(t)</i>	6	S2974 7	F-CAGTGAAACGAACGCTATG R- AATAGGAAGGGTTGATGTTG	56	555	Deng <i>et al.</i> , 2006
<i>Pi-ta</i>	12	Pi-ta	F-AGCAGGTTATAAGCTAGGCC R- CTACCAACAAGTTCATCAAA	58	1024	Jia <i>et al.</i> , 2002
<i>Pik</i>	11	-	F-GGAAAGCTGATATGTTGTCG R- ACTCGGAGTCGGAGAGTCAG	58	1144	-
<i>Pib</i>	2	NSb	F-ATCAACTCTGCCACAAAATCC R- CCCATATCACCCTTGTTC	57	629	Cho <i>et al.</i> , 2007

## Results

### *Identification of R gene in 40 Thai elite rice varieties and indigenous Huai variety*

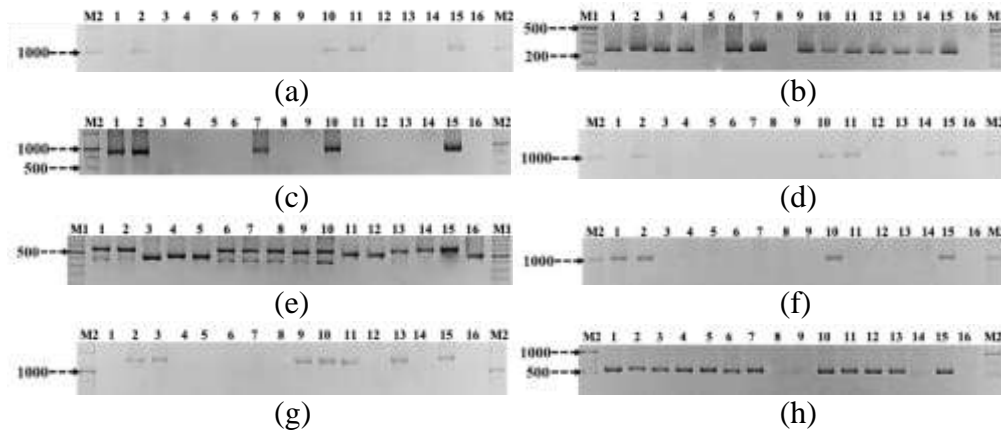
The presence and absence of *R* genes in rice varieties and control varieties were confirmed using PCR technique and gene specific primers and PCR products were visualized. The estimation of *Pib* on chromosome 2 and *Pi-ta* on chromosome 12 were determined through visualization of the presence of DNA size 629 bp and 1024 bp, respectively. The rice blast *R* genes on chromosome 6 included *Pi9*, *Pi50* and *Pigm(t)* could be detected by an appearance of 500, 1272 and 555 bp fragment size, respectively. For the screening of the *Pi54*, *Pia* and *Pik* genes on chromosome 11, the PCR product sizes of 261, 906 and 1144 bp were compiled as positive detection (Figure 1).

Among the 40 Thai elite rice varieties analyzed in this study, at least one of the eight blast *R* gene was detected. The gene profiling of *Pib* was found in the maximum number, 38 rice varieties (95%), while the *Pi50* was presented in the least, 6 rice varieties (15%) (Figure 2, Table 3). It was found that the variety Hahng Yi 71 and Sunpatong 1 contained the highest number of the *R* genes as total of 7 genes included *Pi54*, *Pia*, *Pi50*, *Pigm(t)*, *Pi-ta*, *Pik* and *Pib* while RD31 and RD29 contained only a single gene *Pib* and *Pi54*, respectively. The

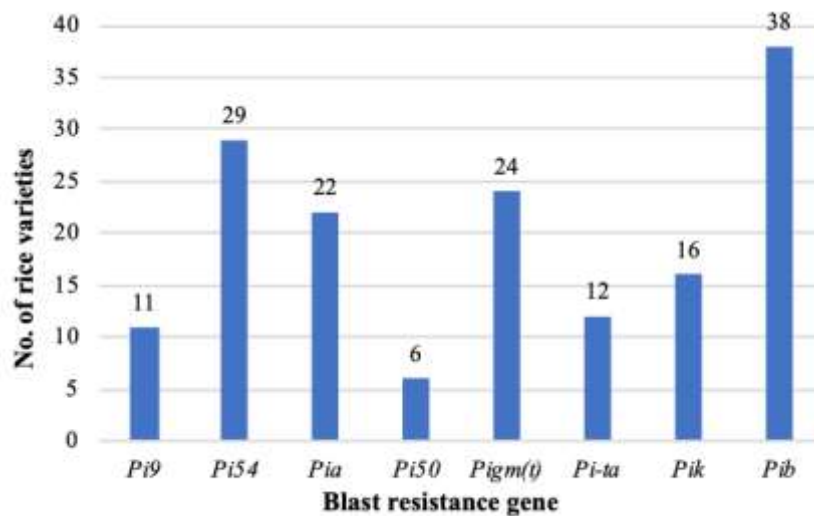
study of gene resistance to rice blast disease in Huai variety revealed that this broad-spectrum resistance variety contained only 2 out of tested 8 *R* genes included *Pi54* and *Pigm(t)* (Figure 3).

**Table 3.** Rice blast resistance genes found in 40 Thai elite rice plus Huai varieties using PCR technique and gene specific primers

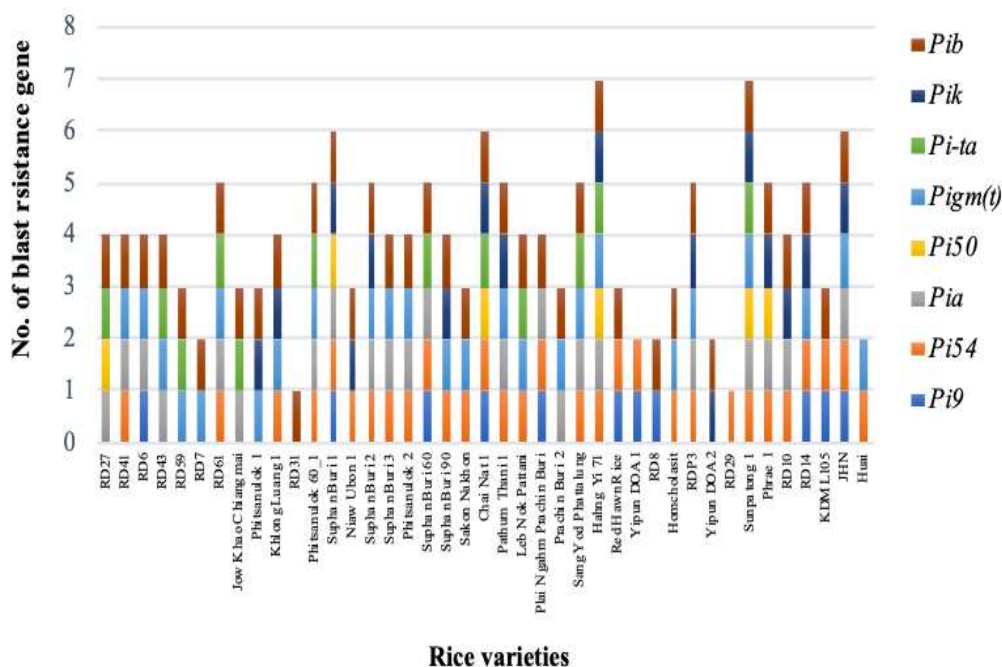
<i>R gene</i>	Chrs	Varieties	No. of varieties (%)
<i>Pi9</i>	6	RD6, Suphan Buri 1, Suphan Buri 60, Chai Nat 1, Plai Ngahm Prachin Buri, Red Hawn Rice, Yipun DOA1, RD8, RD14, KDML105 and Jao Hom Nin	27.5
<i>Pi54</i>	11	RD41, RD61, Khlong Luang 1, Phitsanulok 60_1, Suphan Buri 1, Niaw Ubon 1, Suphan Buri 2, Suphan Buri 3, Phitsanulok 2, Suphan Buri 60, Suphan Buri 90, Sakon Nakhon, Chai Nat 1, Pathum Thani 1, Leb Nok Pattani, Plai Ngahm Prachin Buri, Sang Yod Phattalung, Hahng Yi 71, Red Hawn Rice, Yipun DOA1, Homcholasit, RDP3, RD29, Sunpatong 1, Phrae 1, RD10, RD14, KDML105, Jao Hom Nin and Huai	72.5
<i>Pia</i>	11	RD27, RD41, RD6, RD43, Jow Khao Chiangmai, Phitsanulok 60_1, Suphan Buri 1, Suphan Buri 2, Suphan Buri 3, Phitsanulok 2, Suphan Buri 60, Pathum Thani 1, Plai Ngahm Prachin Buri, Prachin Buri 2, Sang Yod Phattalung, Hahng Yi 71, RDP3, Sunpatong 1, Phrae 1, RD10 and Jao Hom Nin	55
<i>Pi50</i>	6	RD27, Suphan Buri 1, Chai Nat 1, Hahng Yi 71, Sunpatong 1 and Phrae 1	15
<i>Pigm(t)</i>	6	RD41, RD6, RD43, RD59, RD7, Phitsanulok 1, Khlong Luang 1, Phitsanulok 60_1, Suphan Buri 2, Suphan Buri 3, Phitsanulok 2, Suphan Buri 90, Sakon Nakhon, Pathum Thani 1, Leb Nok Pattani, Prachin Buri 2, Sang Yod Phattalung, Hahng Yi 71, Homcholasit, RDP3, Sunpatong 1, RD14, Jao Hom Nin and Huai	60
<i>Pi-ta</i>	12	RD27, RD43, RD59, RD61, Jow Khao Chiangmai, Phitsanulok 60_1, Suphan Buri 60, Chai Nat 1, Leb Nok Pattani, Sang Yod Phattalung, Hahng Yi 71 and Sunpatong 1	30
<i>Pik</i>	11	Phitsanulok 1, Khlong Luang 1, Suphan Buri 1, Niaw Ubon 1, Suphan Buri 2, Suphan Buri 90, Chai Nat 1, Pathum Thani 1, Hahng Yi 71, RDP3, Yipun DOA2, Sunpatong 1, Phrae 1, RD14 and Jao Hom Nin	40
<i>Pib</i>	2	RD27, RD41, RD6, RD43, RD59, RD7, RD61, Jow Khao Chiangmai, Phitsanulok 1, Khlong Luang 1, RD31, Phitsanulok 60_1, Suphan Buri 1, Niaw Ubon 1, Suphan Buri 2, Suphan Buri 3, Phitsanulok 2, Suphan Buri 60, Suphan Buri 90, Sakon Nakhon, Chai Nat 1, Pathum Thani 1, Leb Nok Pattani, Plai Ngahm Prachin Buri, Prachin Buri 2, Sang Yod Phattalung, Hahng Yi 71, Red Hawn Rice, RD8, Yipun DOA1, Homcholasit, RDP3, Sunpatong 1, Phrae 1, RD10, RD14, KDML105 and Jao Hom Nin	95



**Figure 1.** The detection of eight rice blast resistant genes; *Pi9* (a), *Pi54* (b), *Pia* (c), *Pi50* (d), *Pigm(t)* (e), *Pi-ta* (f), *Pik* (g) and *Pib* (h) using gene specific primers. (M1 = 100 bp DNA ladder; M2 = 1 kb DNA ladder; 1 – 14 are samples of Thai elite rice varieties; Sang Yod Phattalung, Hahng Yi 71, Red Hawn Rice, Yipun DOA1, RD8, Homcholasit, RDP3, Yipun DOA2, RD29, Sunpatong 1, Phare, RD10, RD14, and Huai variety, respectively; 15 = Possitive control for each correspondind *R* gene; 16 = Negative control was LTH )



**Figure 2.** Number of 8 blast resistance genes distributed in 40 Thai elite rice plus Huai varieties



**Figure 3.** Blast resistance genes found in 40 Thai elite rice varieties and Huai variety

### Discussion

Investigation of rice blast resistant genes in 40 Thai elite rice varieties and indigenous rice Huai variety was carried out by using 8 gene specific primers. Gene specific primers described above has been used as gene markers to detect those resistance genes among rice varieties. The result in this study was similar with the report of Mahender *et al.* (2012) which proceeded the screening of rice blast disease resistance genes by using 8 specific marker genes (*Pib*, *Piz*, *Pizt*, *Pi9*, *Pi40*, *Pi5*, *Pia*, and *Pita*) for characterizing the various accessions of Manipur, India and found the varieties containing 2 – 7 blast resistance genes. The report also indicated the richness of blast diversity in landraces rice. Furthermore, Phaitreejit *et al.* (2011) reported the screening of Thai landrace rice for blast resistance gene *Pi9*, *Pi36* and *Pigm(t)* using DNA markers and found that 203 cultivars have at least one blast resistant gene and 42 cultivars have all the three blast resistant genes. In the present study, the

finding that Huai variety contained *Pi54* and *Pigm(t)*, which located on chromosome 11 and 6 respectively, was accorded to the reported that Huai variety showed a broad-spectrum and one of the blast resistance genes was mapped on chromosome 11 (Parinthawong *et al.*, 2015). Poonsin and Parinthawong (2017) reported that Huai variety was high resistant to 24 blast isolates except isolate PLK40.4 from Phitsanulok province. However, it has been reported that PLK40.4 contains *Avr-Pib* (Jaihom, 2015) which therefore agreed with result of this study where *Pib* resistance gene was untraceable in Huai variety. Therefore, adding the blast resistance gene *Pib* to Huai variety should enhance resistance phenotype to the rice, in another words, *Pib* is probably a good resistance gene candidate for sustaining and broad-spectrum blast resistance breeding program.

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