Hindawi
International Journal of Agronomy
Volume 2023, Article ID 6464153, 11 pages
https://doi.org/10.1155/2023/6464153

Research Article

Investigating Rice Blast Resistance Gene Distribution among Landrace Rice Varieties in Lower Northern Thailand for Improving Rice Cultivars

Sittichai Urtgam,[1] **Kawee Sujipuli**,[2] **Urasri Suyasunanont**,[3] **Nontaporn Rattanachak**,[1] **Jirapas Jongjitwimol**,[4,5] **and Touchkanin Jongjitvimol**[1]

[1]*Biology Program, Faculty of Science and Technology, Pibulsongkram Rajabhat University, Phitsanulok 65000, Thailand*
[2]*Department of Agricultural Science, Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Phitsanulok 65000, Thailand*
[3]*Department of Biology, Faculty of Science, Naresuan University, Phitsanulok 65000, Thailand*
[4]*Department of Medical Technology, Faculty of Allied Health Sciences, Naresuan University, Phitsanulok 65000, Thailand*
[5]*Centre of Excellence in Biomaterials, Faculty of Science, Naresuan University, Phitsanulok 65000, Thailand*

Correspondence should be addressed to Jirapas Jongjitwimol; jirapasj@nu.ac.th and Touchkanin Jongjitvimol; touchkanin@psru.ac.th

Received 1 March 2023; Revised 24 April 2023; Accepted 21 June 2023; Published 1 July 2023

Academic Editor: Francesca Degola

Rice blast disease caused by the fungus *Pyricularia oryzae* is considered as one of the severe diseases, leading to reduce tremendous rice productivity in its cultivated areas of Thailand. Due to the rapid evolution and high genetic diversity of the pathogen, the innate rice resistance (*R*) genes associated with defense mechanisms are significantly considered as the most important for rice breeding program to create new rice varieties, resistant to blast disease. This study aimed to investigate the rice blast *R* genes (*Pi9*, *Pib*, and *Pi-ta*) in 98 landrace rice germplasms collected from three different provinces in lower northern Thailand, Phichit (PCT), Phitsanulok (PLK), and Sukhothai (STI) through PCR assay. The results showed that the *Pi-ta* gene was presented in 29 different varieties, making it the most widespread, whereas the *Pi9* and *Pib* genes were found in 28 and 25 varieties, respectively. The distribution percentage of studied genes in PLK and STI germplasms is higher than in PCT germplasms. Interestingly, only eight landrace rice varieties (varieties no. 46, 47, 48, 51, 66, 76, 81, and 90) collected from PLK and STI germplasms contain all of these three resistance genes. This finding provided the genetic information and diversity of the *R* genes across landrace rice varieties in the lower north of Thailand. Moreover, these *R* genes could be useful as genetic resources for rice improvement with resistance to blast disease through breeding program in the future.

1. Introduction

Pyricularia oryzae (*Ascomycota,* syn. *Magnaporthe oryzae*) [1] is one of the major fungal pathogens causing rice blast disease (RBD) worldwide [2]. Symptomatic RBD is appeared in many parts of rice seedlings (*Oryza sativa* L.), such as leaf, stem, and node, resulting in a severe reduction of growth development and yield of rice [3]. Generally, the RBD management in rice field could be controlled by using antifungal agents such as azoxystrobin and tricyclazole [4]. However, the application of these agents has been still an ineffective strategy and leads to the environmental pollution [5]. Notwithstanding, the pathogenic *Pyricularia* is highly divergent in genetic patterns [6], causing the rapid outbreak and transmission in rice cultivation areas to other adjacent areas. To prevent outbreak and transmission of pathogenic *Pyricularia* in rice for long-term control of RBD, the rice varieties with RBD resistance would be an effective strategy for long-term control of this disease [7].

Three major resistance (*R*) genes associated with RBD were known as *Pia*, *Pii*, and *Pik*. Over the past decade, more than 100 *R* genes had been identified across rice chromosomes, except for chromosome 3 [8, 9]. Of these, the *R* genes were categorized into 5 groups based on their encoding proteins: (1) nucleotide-binding site leucine-rich repeat (NBS-LRR), e.g., *Pib* located on chromosome 2, *Pi9* located on chromosome 6, *Pi-ta* located on chromosome 12; (2) coiled-coil-nucleotide-binding site leucine-rich repeat (CC-NBS-LRR), e.g., *Pit* located on chromosome 1, *Pi25* located on chromosome 6, *Pia* located on chromosome 11; (3) proline-rich metal binding protein, e.g., *pi21* located on chromosome 4; (4) B-lectin receptor kinase, e.g., *Pi-d2* located on chromosome 6; and (5) atypical protein with an armadillo repeat, e.g., *Ptr* located on chromosome 12 [9].

The rice blast resistance gene is involved in plant cell defense mechanisms against the fungal pathogen invasion. There are five main functions of the *R* genes occurred via their encoding proteins. For example, the rice blast resistance *Pi9* gene encodes the NBS-LRR protein [9] which is involved in upregulating the transcriptional activation of kinases, JA-ET hormones, chitinases, glycosyl hydrolases, lipid biosynthesis, pathogenesis, and secondary metabolism-related genes to trigger the cell signaling pathway for preventing the infection [10]. Rice blast resistance *Pi-ta* gene encoded a protein, cytoplasmic membrane receptor protein containing NBS-LRR domains [11], functioning via the direct interaction of the LRR domain to another protein, neutral zinc metalloprotease encoded by an avirulent-*Pi-ta* (*AVR-Pi-ta*) [12], and then triggering the resistance pathway to respond to the blast fungal infection [13].

The previous study demonstrated that biomarkers could provide the efficiency to investigate the blast resistance gene using the specific DNA marker to the *R* genes. The specific primers corresponding to *R* genes were designed, e.g., *Pi9*, *Pib*, *Pi-ta*. The rice blast resistance *Pi9* gene confers to resist more than 43 isolates of rice blast fungal samples collected from 13 countries, and the DNA marker of *Pi9* was designed based on the sequence of the gene located 2.8cM from RFLP marker RG64 on chromosome 6 [14]. The specific primers of *Pi9* were pB8-F (5′ CCCAATCTCCAATGACCCATAAC 3′) and pB8-R (5′ CCGGACTAAGTACTGGCTTCGATA 3′) for detecting the resistant allele of the *Pi9* gene [15] with the sample of its resistance allele sequence presented in GenBank accession MZ327711. The *Pib* primers were developed based on the sequence of the cloned *Pib* blast resistance gene [16] with Pibdom marker (PibdomF; 5′ GAA CAATGCCCAAACTTGAGA 3′ and PibdomR; 5′ GGG TCCACATGTCAGTGAGC 3′) corresponding to bases 8699-9063 in GenBank accession AB013448 and to specifically amplify a particular fragment of the *Pib* gene [17]. The rice blast resistance *Pi-ta* gene was found as resistant and susceptible alleles with the difference as only one nucleotide, and thus, the specific primer of the resistant allele was developed to locate the middle region of *Pi-ta* gene. DNA primers YL155/YL87 (F; 5′ AGCAGGTTATAAGCTAGG CC 3′ and *R*; 5′ CTACCAACAAGTTCATCAAA 3′) were designed and showed the specific amplification only the resistant allele corresponding to bases 4409-5450 in GenBank accession no. AF207842 [18].

In Thailand, landrace rice is the most important resource of genetic diversity containing responsive genes to abiotic and biotic stress [7, 19]. More recently, rice blast resistance genes were characterized in landrace rice from various locations such as Bangladesh, China, Japan, Malaysia, Thailand, and Vietnam. From these, more than 30 *R* genes were identified in Asian landrace rice, i.e., *Pi-36*, *Pi9*, *Pib, Pigm(t), Pik-p, Pik-h, Pi-ta, Piz* [7, 20–23]. Of these 30 *R* genes, the *Pi9, Pib,* and *Pi-ta* were commonly found in resistance rice cultivars grown in Malaysia [20] and Thailand [7].

Lower northern Thailand is one of the most important main areas for rice production. In 2021, farmers from the three provinces including Phichit, Phitsanulok, and Sukhothai could produce the high yields of rice production by 216.4–248.0 kg/acre [24]. However, rice blast disease outbreaks and causes rice yield losses in the areas. Due to the fungal infection, there were from 10% to 30% of rice harvest losses each year [25], posing a serious danger to Thailand and global rice production, including economic value. Therefore, this study aimed to investigate the profiles of three RBD-resistance genes (*Pi9*, *Pib*, and *Pi-ta*) among landrace rice provided by lower northern Thailand germplasms, located in Phichit, Phitsanulok, and Sukhothai provinces. The findings could identify the rice blast resistance genes in Thai landrace rice and could be essential benefits for rice improvement against blast resistance disease through the crossbreeding program.

2. Materials and Methods

2.1. Plant Materials. Ninety-eight varieties of landrace rice (Table 1) were provided by germplasm of the Phitsanulok Rice Research Center, collected from the three different provinces of Phichit (PCT), Phitsanulok (PLK), and Sukhothai (STI) (Figure 1). The rice seeds were placed on tissue soaking with sterilized water for 7 days, and then, young seedlings were transferred into a pot containing soil for 7–14 days.

2.2. Genomic DNA Extraction. The young leaf was washed with distilled water prior to genomic DNA extraction using the acetyl trimethyl ammonium bromide (CTAB) method according to slightly modified by Doyle and Doyle [26]. Briefly, young rice leaf (1 g) was individually ground into powder in liquid nitrogen and transferred into lysis buffer containing 2% CTAB, 1.4 M NaCl, 100 mM Tris-HCl, 20 mM ethylene diamine tetra-acetic acid (EDTA), 1% polyvinylpyrrolidone (PVP), and 1% sodium dodecyl sulfate (SDS). The mixture was gently homogenized and incubated at 65°C for 30 min. After centrifugation at 12,000 rpm for 10 min, the upper phase was transferred to a new tube, and then, an equivalent volume of chloroform : isoamyl alcohol (24 : 1) was added. The mixture was stored at room temperature for 5–10 min and then centrifuged at 12,000 rpm for 10 min. The supernatant solution was collected for DNA precipitation by adding 2 volumes of isopropanol and then

Table 1: Ninety-eight landrace rice varieties used in blast resistance gene investigation.

Code no.	Varieties	Location*
01	Khao Bai Si	PCT
02	Takosin	PCT
03	Nimnuan	PCT
04	Khao Ko Diao Bao	PCT
05	Khao Pramun	PCT
06	Khao Ko Nak	PCT
07	Khao Ko	PCT
08	Khao Khat Bao	PCT
09	Lueang On	PCT
10	Luang Prathan Nak No. 1	PCT
11	BaiSi No. 1	PCT
12	BaiSi No. 2	PCT
13	BaiSi No. 3	PCT
14	Pom No. 1	PCT
15	Khao Ta Haeng No. 1	PCT
16	Khao Ta Haeng No. 2	PCT
17	Khao Yuan	PCT
18	Chet Ruang No. 1	PCT
19	Chet Ruang No. 2	PCT
20	Hom Mali	PCT
21	Khao Kaset No. 1	PCT
22	Khao Kaset No. 2	PCT
23	Khao Ko Diao Nak	PCT
24	Niao Phan Nak	PCT
25	Hin Kong	PCT
26	Taphao Lom	PCT
27	Dok Du	PCT
28	Yuang Khanun	PCT
29	U Taphao	PCT
30	Khao Chaloem	PCT
31	Luang Prathan Bao 2	PCT
32	Khao Akat 2	PCT
33	Luang Prathan Nak 4	PCT
34	Pom 3	PCT
35	Thong Ma Eng 3	PCT
36	Thong BaiSi 3	PCT
37	Khao Kaset 3	PCT
38	Chet Ruang 3	PCT
39	Khao Ta Haeng 4	PCT
40	Yuan 4	PCT
41	Yuan 5	PCT
42	Khao Chalo 2	PCT
43	Khao Chalo 3	PCT
44	Ta Haeng	PLK
45	Chek Kradot	PLK
46	Phong	PLK
47	Khao Loi Yai	PLK
48	Lamyai	PLK
49	Niao Phama	PLK
50	Na Lao	PLK
51	Kwian Hak/Rot Lak	PLK
52	Niao Nak	PLK
53	Lueang Nakhon Thai	PLK
54	Samo Khae	PLK
55	Khao Phuangmalai	PLK
56	Lueang Chamlong	PLK
57	La Hang Malet Yai	PLK
58	Chai Nam	PLK
59	Ton Dip	PLK
60	No. 1	PLK
61	Mayang	PLK
62	Niao	PLK
63	Bua Luang	PLK
64	Lueang Champa	PLK
65	Laplae	PLK
66	Pin Thong	PLK
67	Phuang Tani	PLK
68	KonKaeo	PLK
69	Phuangmalai	PLK
70	Nan Khlui	PLK
71	Malet Yai	PLK
72	Khao Ta Chuang	PLK
73	Phuang Thong	PLK
74	Thot	PLK
75	Lao	PLK
76	Kaen Chan	PLK
77	Hom Chan	PLK
78	Sao Kot	PLK
79	Phan Kilo	STI
80	Ta Prem	STI
81	Chi Ma O	STI
82	Sangkha	STI
83	Son Malet	STI
84	Malet Yao	STI
85	Nangngam	STI
86	Rachini	STI
87	Phrae	STI
88	Phuang Hang Ma	STI
89	Lueang Thong Nak	STI
90	Mueang Khaek	STI
91	Lueang Thong Thi Nueng	STI
92	Lueang Bai Lek	STI
93	Mae Paet Bao	STI
94	Rahaeng	STI
95	Mali Lueai	STI
96	Dok Ta Baek	STI
97	Rak Haeng	STI
98	On Si	STI

Note. *PCT, PLK, and STI abbreviate to Phichit, Phitsanulok, and Sukhothai provinces, respectively.

incubated at −20°C overnight. The genomic DNA (gDNA) was precipitated by centrifuging at 12,000 rpm for 10 min and washed twice with 70% ethanol. The pellet was air-dried at room temperature for 5–10 min. The gDNA was dissolved in 1x TE buffer, and RNA contamination was eliminated by adding 50 μg/ml RNase A (1 μl) and incubated at 65°C for 30 min. The quality, quantity, and integrity of the gDNA sample were determined in a 1% TBE agarose gel electrophoresis system, strained with 1x SYBR Safe DNA gel stain (Invitrogen, USA), and visualized and photographed under UV light with a gel documentation system (Bio-Rad, USA).

2.3. Detection of Blast Resistance Genes in Rice. The gDNA samples (50–80 ng) were used as templates for the detection of blast resistance gene amplification using the polymerase chain reaction (PCR) method [27] with gene-specific

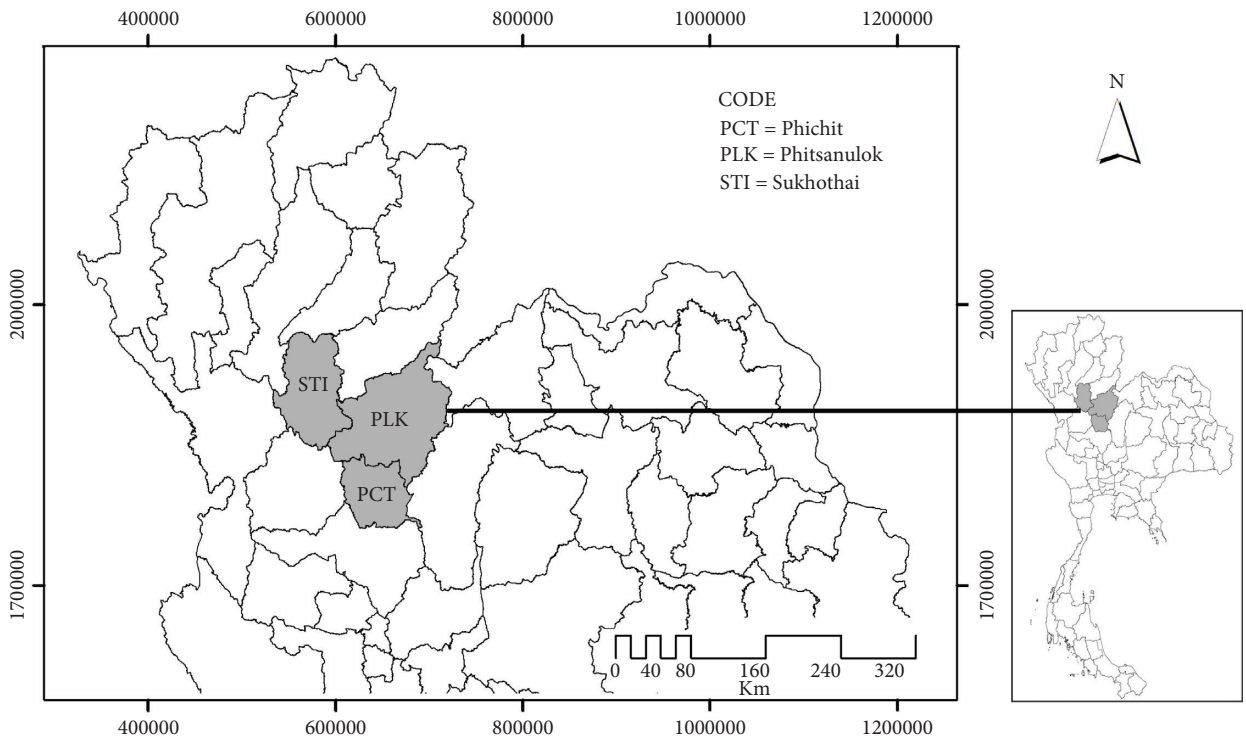

Figure 1: Map of Thailand to display the location of three provinces: Phichit (PCT), Phitsanulok (PLK), and Sukhothai (STI) in lower northern Thailand.

primers (Table 2). A PCR reaction (25 μl) was performed by 2.5 μl of 10x *Taq* buffer, 3 mM $MgCl_2$, 1 unit of *Taq* DNA polymerase (Invitrogen, USA), and 200 nM of each dNTP, 200 μM of individual gene-specific primer pair of *Pi9*, *Pib,* and *Pi-ta* (listed in Table 2). The PCR amplification was carried out in T100™ Thermal Cycle (Bio-Rad, USA) under an optimal condition: predenaturation 1 cycle at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 45 seconds, annealing at an optimal temperature of each gene for 45 seconds and extension at 72°C for 90 seconds, and final extension for 1 cycle of 72°C for 5 min. The PCR product was analyzed on 2.5% TBE agarose gel electrophoresis together with 100 bp DNA ladder (GeneDireX, Taiwan), containing 1x SYBR Safe DNA gel stain (Invitrogen, USA), and visualized under UV light with Gel documentation system (Bio-Rad, USA). The rice *actin* gene, a housekeeping gene, was used as a reference gene to determine the PCR amplification using a gene-specific primer (ACT-F; 5′ ATGAAGATCAAGGTGGTCGC 3′ and ACT-R; 5′ GTACTCAGCCTTGGCAATCC 3′) with the appropriated condition [28].

2.4. Data Analysis. The amplicon of *actin* gene with 200 bp long was indicated for the reference gene in each landrace rice varieties prior to the *R* gene determination. The presence of amplicon size on gel, corresponding to 466, 365, or 1,042 bp long, was indicated for the specific profile of target gene either *Pi9*, *Pib*, or *Pi-ta*, respectively, across studied rice varieties. All experiments were performed as triplicates. The data analysis was performed using the SPSS statistics program (Version 17.0; SPSS Inc., Chicago). Descriptive statistics, e.g., frequency and percentage, was used to describe the distribution of each *R* gene in different landrace rice.

3. Results and Discussion

3.1. Distribution of Resistance Genes among Rice Varieties. The presence of selected blast resistance genes (*Pi9, Pib,* and *Pi-ta*) was examined in 98 landrace rice varieties (listed in Table 1), using PCR assay with the gene-specific primers. The amplicons of the reference gene, *actin* gene, were determined in all of 98 landrace rice varieties with 200 bp long (Figure 2). The *Pi9, Pib,* or *Pi-ta* primer enabled to generate PCR amplicon size, corresponding to approximately 466 (Figure 3), 365 (Figure 4), and 1,042 bp long (Figure 5), respectively, according to previous works reported by Liu et al. [15], Fjellstrom et al. [17], and Jia et al. [18]. Among the landrace rice varieties studied, the examined genes displayed slightly different distribution. The *Pi-ta* gene was identified in the highest proportion from 29 varieties, accounting for 29.59%. The *Pi9* gene was found in 28 varieties or 28.57%. In contrast, the *Pib* gene exhibited the lowest occurrence, representing 25.51% across 25 varieties (Figure 6).

The distribution of the resistance genes across studied rice varieties was explored that *Pi9* gene gave highest in STI germplasms (45.00%), followed by PLK germplasms (31.43%), and 18.60% in PCT germplasms, respectively. The findings in this study correlated with the other studies on landrace rice. The previous study reported that almost all Thai landrace rice carried at least one *R* gene, and more than

Table 2: Specific primers for blast resistance gene investigation.

R Genes	Primer sequence (5′⟶3′)	Tm (°C)	Amplicon size (bp)	References
Pi9	F; 5′ CCCAATCTCCAATGACCCATAAC 3′ R; 5′ CC GACTAAGTACTGGCTTCGATA 3′	57	466	[15]
Pib	F; 5′ GAACAATGCCCAAACTTGAGA 3′ R; 5′ GGGTCCACATGTCAGTGAGC 3′	50	365	[17]
Pi-ta	F; 5′ AGCAGGTTATAAGCTAGGCC 3′ R; 5′ CTACCAACAAGTTCATCAAA 3′	52	1,042	[18]

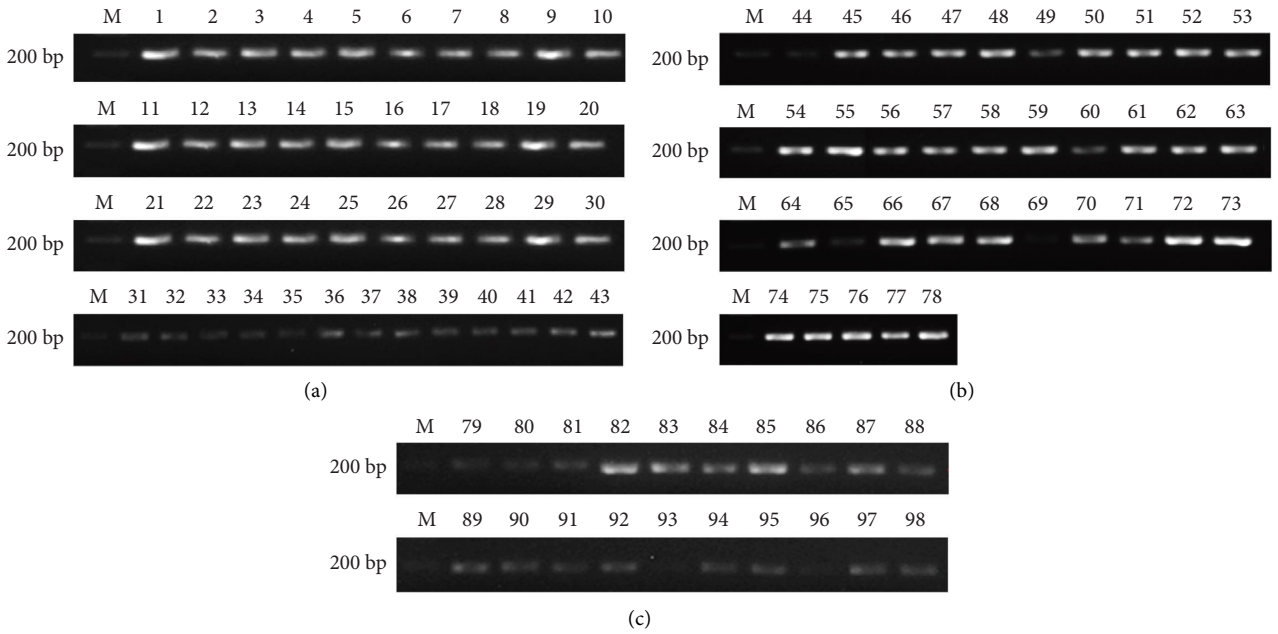

Figure 2: Visual inspection of the positive PCR product (200 bp long) amplified from *actin* gene with individual rice DNA template on agarose gel electrophoresis system. Lane M represents the 100-bp DNA ladder (GeneDireX, Taiwan), and other lanes represent the PCR products in PCT germplasms (a), PLK germplasms (b), and STI germplasms (c), respectively. The numbers labelled in each lane indicate rice varieties related to the list in Table 1.

80% carried at least three or more *R* genes. Especially, the *Pid3* is the most frequently *R* gene found in rice. The *Pi9* is one of the major *R* gene found in international rice [29]. In addition, the *Pi9* distribution was various in the different locations of Thailand which is the highest frequency in the north-eastern of Thailand compared to the other parts [30, 31].

Recently, a previous study had been reported that the *Pi9* gene located on chromosome 6, encoding the nucleotide-binding site and leucine-rich repeat (NBS-LRR) protein [9]. It plays an important role for upregulated genes involved in the transcriptional activation of kinases (e.g., WRKY, MYB, ERF transcription factors), JA-ET hormones, chitinases, glycosyl hydrolases, lipid biosynthesis, pathogenesis, and secondary metabolism to prevent the blast disease in rice, Pusa-Basmati-1 [10]. This might be suggested that the *Pi9* gene was one of the major regulatory genes for rice blast defense mechanisms.

Additionally, the *Pib* gene was found the greatest distribution in PLK germplasms (40.00%), followed by STI germplasms (30.00%) and PCT germplasms (11.63%), respectively (Figure 7). The *Pib* gene is only one *R* gene located on chromosome 2 at the distal end of the long arm, which shows high resistance to a broad spectrum of many Japanese blast races [32–34]. Based on molecular genetics, the NBS region in the N-terminal of the *Pib* protein was modified by adding the kinase 1a, 2, and 3a motifs, and eight cysteine residues were clustered in the middle of the LRRs which have not been reported for other *R* genes [16].

Another *R* gene, the *Pi-ta*, was found the highest distribution in PLK germplasm (40.00%), followed by STI germplasms (35.00%) and PCT germplasms (18.60%), respectively. The *Pi-ta* gene, located near the centromere on chromosome 12 of rice, encodes a cytoplasmic membrane receptor protein with NBS-LRR domains [11]. These domains enable directly interact with neutral zinc metalloprotease, translated from an avirulent-*Pi-ta* (*AVR-Pi-ta*) gene [12], leading to disease resistance responses [13]. Other publications had been reported that the *Pi-ta* gene was linked to other resistance genes which often found a broad spectrum of blast resistance in *Pi-ta* rice varieties [11, 35–37]. Correspondingly, this study found that *Pi-ta* varieties show other *R* genes as 64.28% (18 of 28 *Pi-ta* varieties) (Figure 7). A similar trend of other *R* gene

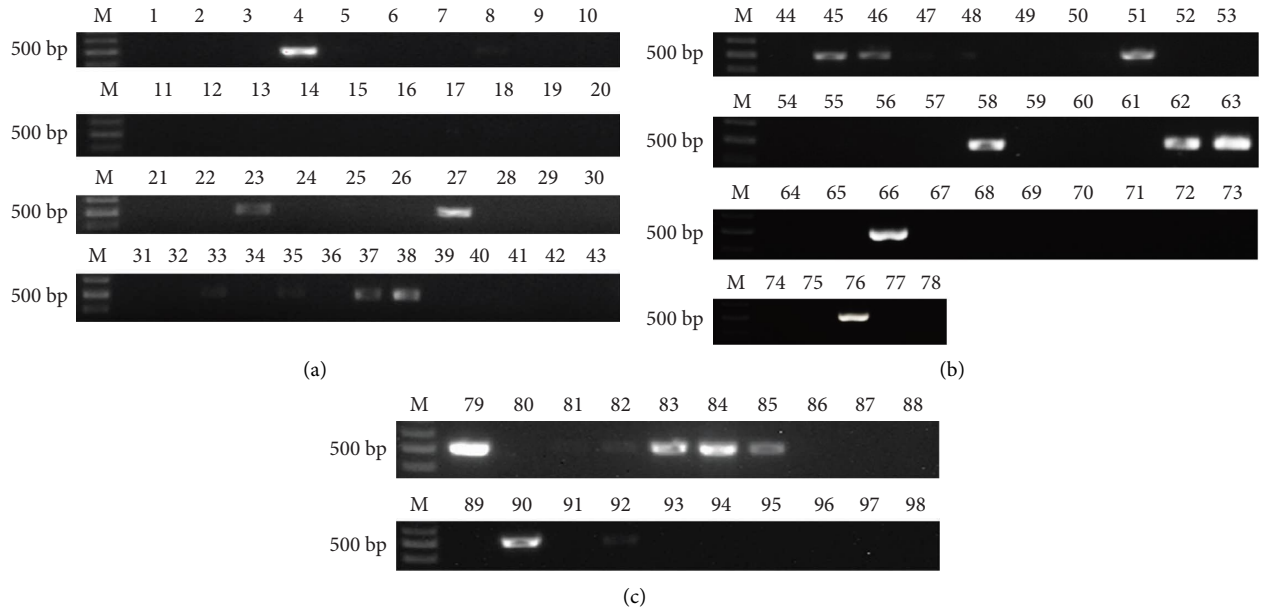

Figure 3: Visual inspection of the positive PCR product (466 bp long) amplified from *Pi9* gene with individual rice DNA template on agarose gel electrophoresis system. Lane M represents the 100-bp DNA ladder (GeneDireX, Taiwan), and other lanes represent the PCR products in PCT germplasms (a), PLK germplasms (b), and STI germplasms (c), respectively. The numbers labelled in each lane indicate rice varieties related to the list in Table 1.

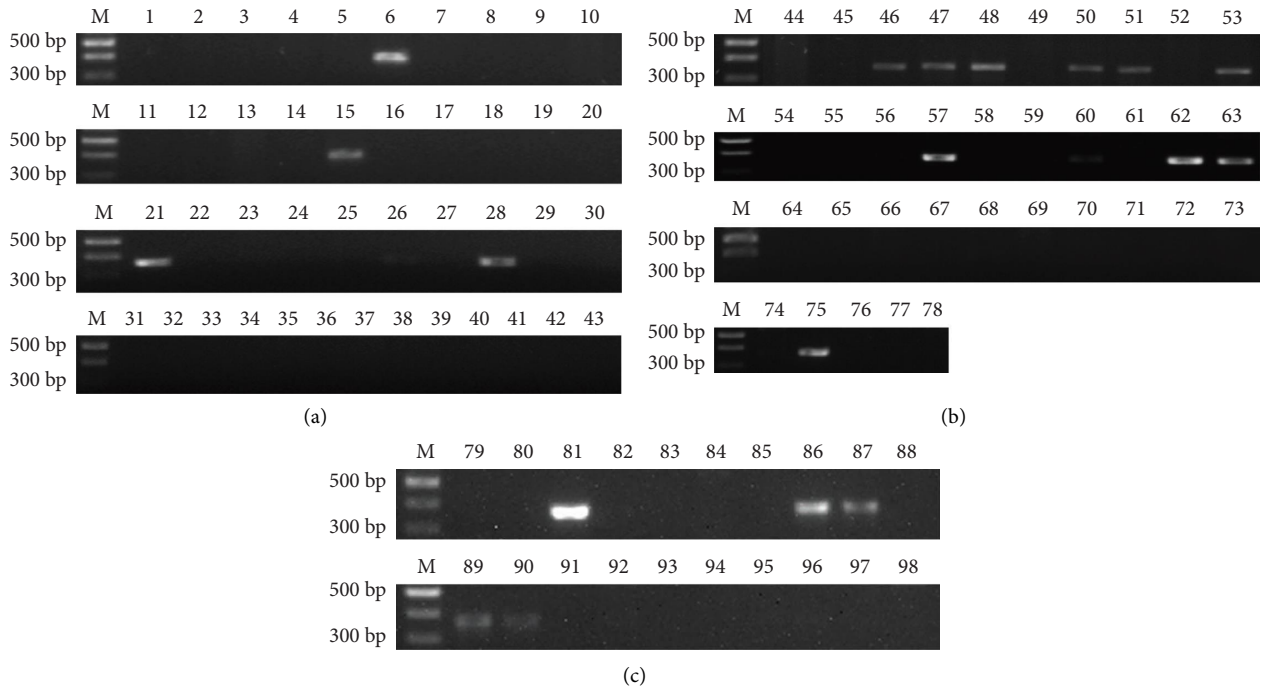

Figure 4: Visual inspection of the positive PCR product (365 bp long) amplified from *Pib* gene with individual rice DNA template on agarose gel electrophoresis system. Lane M represents the 100-bp DNA ladder (GeneDireX, Taiwan), and other lanes represent the PCR products in PCT germplasms (a), PLK germplasms (b), and STI germplasms (c), respectively. The numbers labelled in each lane indicate rice varieties related to the list in Table 1.

distribution might be found in these landrace rice. Therefore, the *Pi-ta* gene might be used as the first gene marker for the preliminary screening of rice blast resistance gene in rice breeding program.

The summarised *R* gene distributions found that the *R* genes were different distributions that relied on rice germplasms (Figure 7). The rice cultivars collected from PLK and STI germplasms were higher distribution of the blast

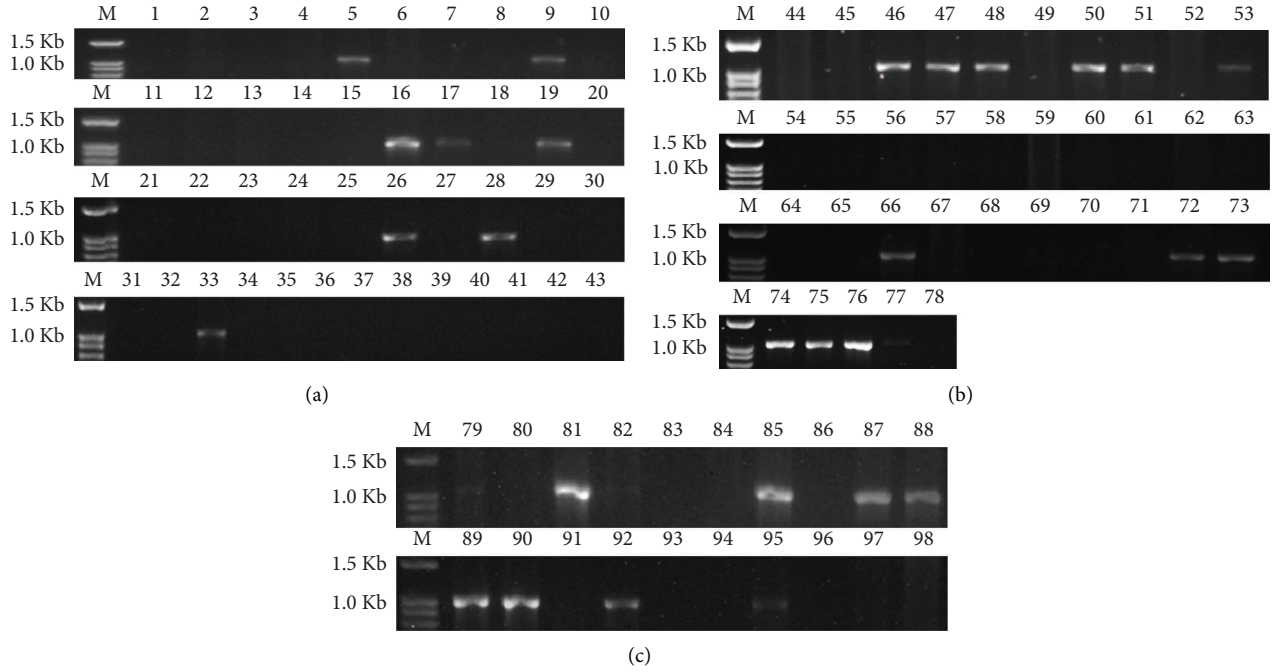

Figure 5: Visual inspection of the positive PCR product (1,042 bp long) amplified from the *Pi-ta* gene with individual rice DNA templates on an agarose gel electrophoresis system. Lane M represents the 100-bp DNA ladder (GeneDireX, Taiwan), and other lanes represent the PCR products in PCT germplasms (a), PLK germplasms (b), and STI germplasms (c), respectively. The numbers labelled in each lane indicate rice varieties related to the list in Table 1.

resistance genes than the PCT germplasms. The three areas are individually isolated because of physical natural barriers, e.g., rivers, great distances, mountains. These physical barriers prevent them from regularly mating, which leads to a process of divergence. One possible explanation is that the *R* genes might assist their survival and natural selection of land rice under different environmental conditions [38]. According to Vejchasarn et al. [39], the rice population structure varied because of a special ecological system, genetic makeup, and agronomic features.

This was supported by observations that the landraces had a long history of coevolution between plants and pathogens [3, 40, 41], and the distribution of resistance genes and rice blasts was closely related [42]. The evolution of the *R* genes correlated together with the pathogenic variability [2]. However, the *R* gene mutations were offend found in *Oryza sativa* such as the additional mutation in *Pi54* gene of the most *japonica* and some *indica* cultivars, resulting in the loss function of the nucleotide-binding site and leucine-rich repeat (NBS–LRR) domains [41]. Moreover, the single amino acid altered in the *Pi-ta* resistance protein [11, 43] might lead to the disruption of the protein-protein interaction between the *Pi-ta* protein and its associated protein [35]. It was denoted that the genetic diversity source of the *R* gene is required in supported by observations for sustainably new cultivars. The high variation of the *R* genes found in PLK and STI germplasms would be helpful for *R*-gene evolution study and rice breeding program to prevent the rapid breakdown of rice blast pathogens and to create rice resistance cultivation to rice blast disease [2].

3.2. Rice Varieties Containing All Tested R Genes. Of 98 tested rice varieties, 34 varieties contained one *R* gene, and 11 varieties contained two *R* genes while three *R* genes (*Pi9, Pib,* and *Pi-ta*) were presented in 8 varieties including 6 PLK varieties: No. 46 (Phong), 47 (Khao Loi Yai), 48 (Lamyai), 51 (Kwian Hak/Rot Lak), 66 (Pin Thong), and 76 (Kaen Chan), and 2 STI varieties: No. 81 (Chi Ma O) and 90 (Mueang Khaek) (Figure 6). This suggests that the rice germplasms, carrying all three *R* genes, might be useful for the rice breeding program due to providing the combined function of invasive defense against blast pathogens in rice. In order to control the disease, it has been recommended that developing rice cultivars resistant to blast disease is an alternative strategy [44]. According to Jiang et al. [45], the presence of multiple *R* genes results in a higher efficiency of pathogen control. Their study found a positive correlation between the number of *R* genes and the improved resistance to rice blast disease. However, all of those three *R* genes were absent in 44 rice varieties (44.90%), observed in 24 PCT varieties, 13 PLK varieties, and 7 STI varieties (Figure 5). The results revealed that the undetected *R* genes by the specific primers in these specimens simultaneously occurred in landrace rice germplasms, indicating that the *R* genes in rice might be missing to inherit during the evaluation process such as propagations and selections. This result also showed that the *R* genes were highly distributed in more than half of the STI varieties (65.00%) and in the PLK varieties (62.86%) whereas the presence of the *R* gene was low in the PCT varieties (44.19%). In agreement with previous findings, the genetic diversity of rice blast resistance gene within *indica*, *japonica,* and wild rice revealed that the *Pib* and *Pi9* genes were the intermediate diversity whereas the

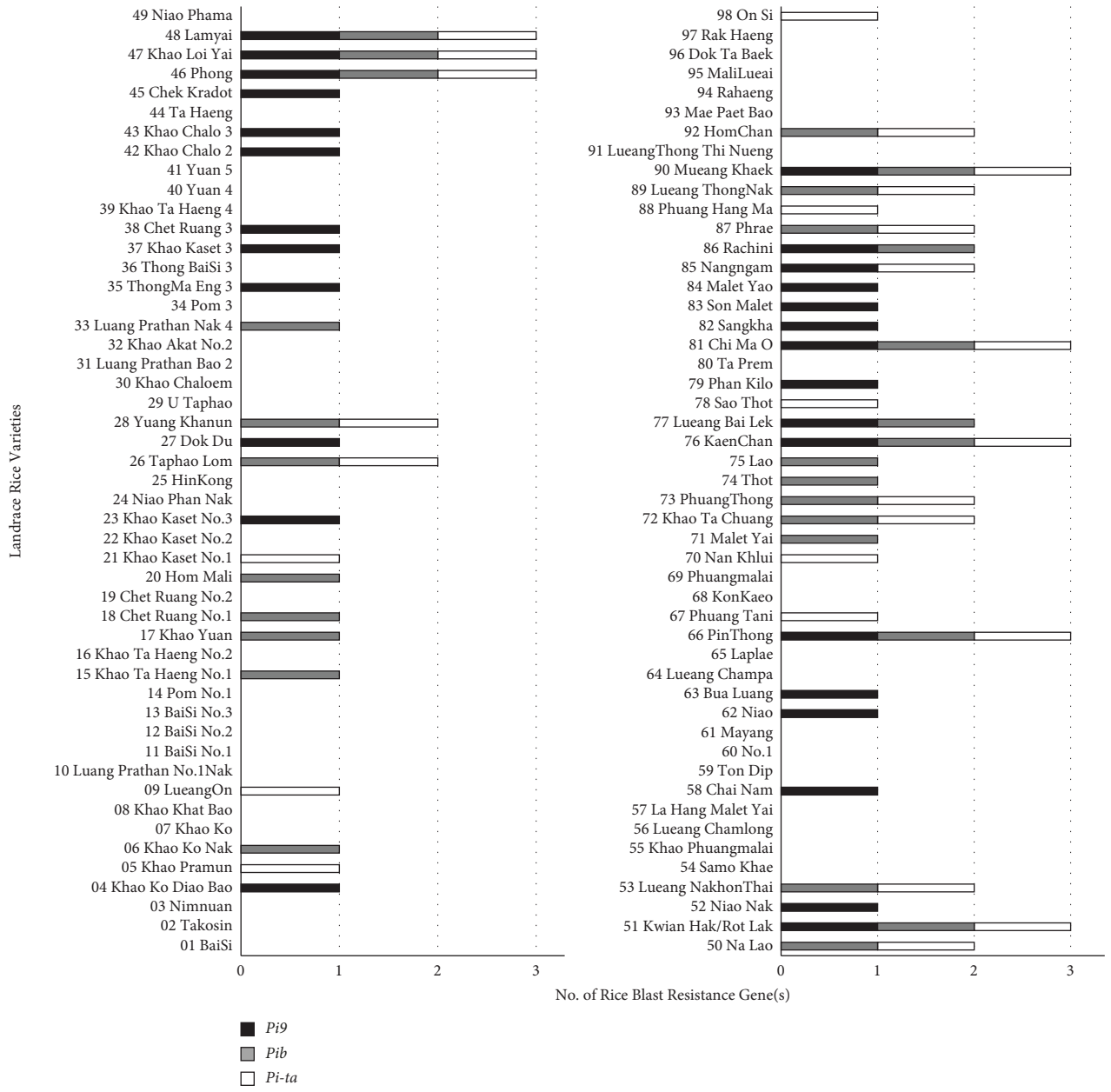

Figure 6: Number of rice blast resistance genes (*Pi9*, *Pi-ta*, and *Pib* gene) presented in individual landrace rice varieties of Phichit (PCT), Phitsanulok (PLK), and Sukhothai (STI). Note: The presence of the *Pi9*, *Pib*, and *Pi-ta* genes in rice verities is related to PCR amplicons in Figures 1–3, respectively. The *Pi9* presents in landrace rice varieties no. 04, 23, 27, 35, 37, 38, 42, 43, 45, 46, 47, 48, 51, 52, 58, 62, 63, 66, 76, 77, 79, 81, 82, 83, 84, 85, 86, and 90 with a total of 28 (28.57%) varieties. The *Pib* presents in landrace rice varieties no. 06, 15, 21, 26, 28, 46, 47, 48, 50, 51, 53, 66, 67, 70, 72, 73, 74, 76, 78, 81, 86, 87, 89, 92, and 98 with a total of 25 (25.51%) varieties. The *Pi-ta* presents in landrace rice varieties no. 05, 09, 17, 18, 20, 26, 28, 33, 46, 47, 48, 50, 51, 53, 66, 71, 72, 73, 74, 75, 76, 77, 81, 85, 87, 88, 89, 90, and 92 with a total of 29 (29.59%) varieties.

Pi-ta gene was relatively conserved [21]. Although these three *R* genes encoded the same types of protein, namely nucleotide-binding site leucine-rich repeat (NBS-LRR) [9], their functions were different in particular disease resistance mechanisms.

In this study, the finding provided *R* genes divergence, associated with blast resistance across landrace rice cultivars in lower northern Thailand. This could be essentially beneficial as genetic resources to improve sustainable rice cultivars with blast resistance in the future.

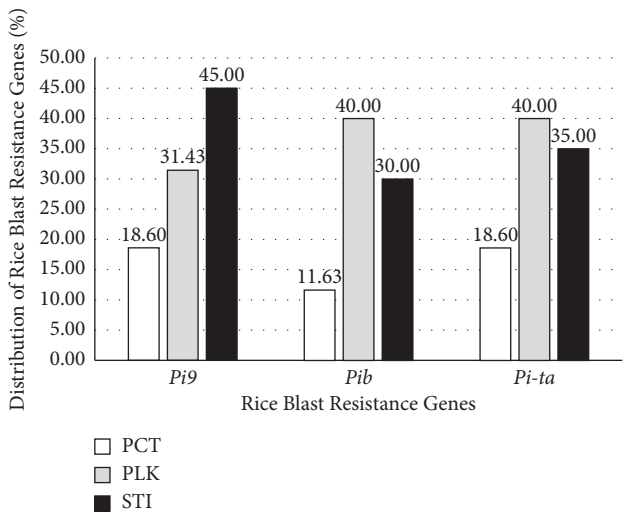

Figure 7: Percent distribution of rice blast resistance genes found in 98 landrace rice germplasms collected from Phichit (PCT), Phitsanulok (PLK), and Sukhothai (STI).

4. Conclusions

This research reports the screening of the *R* genes (*Pi9*, *Pib*, and *Pi-ta*) related to blast disease in 98 landrace rice varieties collected which is the highest number from the lower northern region of Thailand (Phichit, Phitsanulok, and Sukhothai germplasms). The result of positive PCR amplification revealed that of these rice varieties, 34 varieties contained only one *R* gene, and 11 varieties obtained two *R* genes, whereas especially 8 varieties from PLK and STI germplasms carried all three studied *R* genes. This indicated that the landrace rice varieties collected from PLK and STI germplasms could be important genetic resources of the *R* genes for the further rice breeding program of blast resistance cultivar rice.

Although the presence of the *R* genes in the cultivar rice could prevent the pathogen invasion, the rapid revolution of the pathogen might affect the defense mechanisms of the pathogen control. Therefore, these findings could be utilised for selecting landrace rice varieties in rice breeding program to improve blast resistance in rice cultivars worldwide.

Data Availability

The data supporting the current study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

S.U., J.J., and T.J. conceptualized the study; S.U., N.R., J.J., and T.J. provided methodology; S.U., J.J., and T.J. validated the study, did formal analysis, and investigated the study; S.U. and T.J. collected resources; S.U., K.S., U.S., N.R., J.J., and T.J. curated the data; S.U., K.S., U.S., N.R., J.J., and T.J. wrote the original draft; S.U., K.S., U.S., N.R., J.J., and T.J. wrote the manuscript and reviewed and edited the manuscript; S.U., J.J., and T.J. visualized the study, supervised the manuscript, and did project administration; S.U., J.J., and T.J did funding acquisition. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

This research was financially supported by a grant from Research Fund from the Research and Development Institute Pibulsongkram Rajabhat University (Grant nos. RDI-2-63-28, RDI-2-64-37, RDI-2-65-48, and RDI-2-65-47). This research was also partly funded by the Global and Frontier Research University (Grant no. R2566C052), Naresuan University. The authors would like to thank the Phitsanulok Rice Research Center for kindly providing rice samples and the Science Center, Faculty of Science and Technology, Pibulsongkram Rajabhat University.

References

[1] V. Nizolli, C. Pegoraro, and A. Costa de Oliveira, "Rice blast: strategies and challenges for improving genetic resistance," *Crop Breeding and Applied Biotechnology*, vol. 21, 2021.

[2] Z. Ying, W. Tao, Y. Bin et al., "Improving rice blast resistance by mining broad-spectrum resistance genes at *Pik* Locus," *Rice Science*, vol. 29, no. 2, pp. 133–142, 2022.

[3] H. Qi, J. Yang, C. Yin et al., "Analysis of *pyricularia oryzae* and *p. grisea* from different hosts based on multilocus phylogeny and pathogenicity associated with host preference in China," *Phytopathology*, vol. 109, no. 8, pp. 1433–1440, 2019.

[4] A. Kunova, C. Pizzatti, and P. Cortesi, "Impact of tricyclazole and azoxystrobin on growth, sporulation and secondary infection of the rice blast fungus, *Magnaporthe oryzae*: *Magnaporthe oryzae* responses to tricyclazole and azoxystrobin," *Pest Management Science*, vol. 69, no. 2, pp. 278–284, 2013.

[5] T. Ho Thi Thu, C. Nguyen Ngoc Bao, T. Nguyen Phuong, J. Chatchawan, and Q. Nguyen Bao, "Analyzing sequence variation of the avirulence *Avr-Pita1* gene of rice blast isolates, *Magnaporthe oryzae* in vietnam," *Agriculture and Natural Resources*, vol. 53, pp. 20–25, 2019.

[6] S. R. Devi, K. Singh, B. Umakanth et al., "Development and identification of novel rice blast resistant sources and their characterization using molecular markers," *Rice Science*, vol. 22, no. 6, pp. 300–308, 2015.

[7] C. Sooklim, P. Chomnunti, C. Jantasuriyarat, E. Chukeatirote, R. Nilthong, and S. Nilthong, "Genetic diversity and population structure of blast resistance genes in Thai upland rice germplasm," *European Journal of Plant Pathology*, vol. 163, no. 3, pp. 587–599, 2022.

[8] W. Li, M. Chern, J. Yin, J. Wang, and X. Chen, "Recent advances in broad-spectrum resistance to the rice blast disease," *Current Opinion in Plant Biology*, vol. 50, pp. 114–120, 2019.

[9] N. Xiao, Y. Wu, C. Pan et al., "Improving of rice blast resistances in japonica by pyramiding major *R* genes," *Frontiers of Plant Science*, vol. 7, p. 1918, 2016.

[10] P. Jain, P. K. Singh, R. Kapoor et al., "Understanding host-pathogen interactions with expression profiling of NILs carrying rice-blast resistance *Pi9* Gene," *Frontiers of Plant Science*, vol. 8, p. 93, 2017.

[11] G. T. Bryan, K.-S. Wu, L. Farrall et al., "A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene *Pi-ta*," *The Plant Cell*, vol. 12, no. 11, pp. 2033–2045, 2000.

[12] Y. Jia, S. A. McAdams, G. T. Bryan, H. P. Hershey, and B. Valent, "Direct interaction of resistance gene and avirulence gene products confers rice blast resistance," *The EMBO Journal*, vol. 19, no. 15, pp. 4004–4014, 2000.

[13] M. J. Orbach, L. Farrall, J. A. Sweigard, F. G. Chumley, and B. Valent, "A telomeric avirulence gene determines efficacy for the rice blast resistance gene *Pi-ta*," *The Plant Cell*, vol. 12, no. 11, pp. 2019–2032, 2000.

[14] Z. H. Yu, D. J. Mackill, J. M. Bonman, and S. D. Tanksley, "Tagging genes for blast resistance in rice via linkage to RFLP markers," *Theoretical and Applied Genetics*, vol. 81, no. 4, pp. 471–476, 1991.

[15] G. Liu, G. Lu, L. Zeng, and G. L. Wang, "Two broad-spectrum blast resistance genes, *Pi9*(*t*) and *Pi2*(*t*), are physically linked on rice chromosome 6," *Molecular Genetics and Genomics*, vol. 267, no. 4, pp. 472–480, 2002.

[16] Z. X. Wang, M. Yano, U. Yamanouchi et al., "The *Pib* gene for rice blast resistance belongs to the nucleotide binding and leucine-rich repeat class of plant disease resistance genes," *The Plant Journal*, vol. 19, no. 1, pp. 55–64, 1999.

[17] R. Fjellstrom, C. A. Conaway-Bormans, A. M. McClung, M. A. Marchetti, A. R. Shank, and W. D. Park, "Development of DNA markers suitable for marker assisted selection of three *Pi* genes conferring resistance to multiple *Pyricularia grisea* pathotypes," *Crop Science*, vol. 44, no. 5, pp. 1790–1798, 2004.

[18] Y. Jia, Z. Wang, and P. Singh, "Development of dominant rice blast *Pi-ta* resistance gene markers," *Crop Science*, vol. 42, no. 6, pp. 2145–2149, 2002.

[19] V. Kanjoo, S. Jearakongman, K. Punyawaew et al., "Co-location of quantitative trait loci for drought and salinity tolerance in rice," *Thai Journal of Genetics*, vol. 4, pp. 126–138, 2011.

[20] L. Goh, E. T.-J. Chong, J. Wong et al., "Molecular identification of blast resistance and pathogenesis-related genes in various traditional paddy varieties from different divisions of Sabah, East Malaysia," *International Food Research Journal*, vol. 25, pp. 626–631, 2018.

[21] X.-Q. Liu, J.-L. Wei, J.-C. Zhang et al., "Genetic variation of rice blast resistance genes in *Oryza sativa* and its wild relatives," *International Journal of Plant Sciences*, vol. 172, no. 8, pp. 970–979, 2011.

[22] N. Thi Thu Thuy, H. Truong, T. Nguyen Long, and N. Hoang Khanh Linh, "Identification of rice blast resistance genes in South central coast of vietnam using monogenic lines under field condition and pathogenicity assays," *Journal of Agriculture, Science and Technology*, vol. 5, pp. 491–500, 2015.

[23] L. Yan, Y. Bai-Yuan, P. Yun-Liang et al., "Molecular scree ning of blast resistance genes in rice germplasms resistant to *Magnaporthe oryzae*," *Rice Science*, vol. 24, no. 1, pp. 41–47, 2017.

[24] S. Songtanasak, *Agricultural Statistics of Thailand 2020; Office of Agricultural Economics*, Ministry of Agriculture and Cooperatives: Bangkok, Bangkok, Thailand, 2021.

[25] P. Skamnioti and S. J. Gurr, "Against the grain: safeguarding rice from rice blast disease," *Trends in Biotechnology*, vol. 27, no. 3, pp. 141–150, 2009.

[26] J. J. Doyle and J. L. Doyle, "A rapid DNA isolation procedure for small quantities of fresh leaf tissue," *Phytochemical Bulletin*, vol. 19, pp. 11–15, 1987.

[27] K. Mullis, F. Faloona, S. Scharf, R. Saiki, G. Horn, and H. Erlich, "Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction," *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 51, pp. 263–273, 1986.

[28] M. Park, H. K. Yim, H. G. Park, J. Lim, S. H. Kim, and Y. S. Hwang, "Interference with oxidative phosphorylation enhances anoxic expression of rice alpha-amylase genes through abolishing sugar regulation," *Journal of Experimental Botany*, vol. 61, no. 12, pp. 3235–3244, 2010.

[29] W. Teerasan, P. Moonsap, A. Longya et al., "Rice blast resistance gene profiling of Thai, Japanese and international rice varieties using gene-specific markers," *Plant Genetic Resources*, vol. 20, no. 1, pp. 22–28, 2022.

[30] K. Phaitreejit, E. O. Srikaew, C. Jantasuriyarat, T. Sriwongchai, and S. Kate-ngam, "Screening Thai landrace rice for blast resistance gene *Pi9, Pi36, Pigm*(*t*) using DNA markers," *Thai Journal of Genetics*, vol. 4, pp. 52–62, 2011.

[31] C. Krainart, R. Siangsai, A. Longya, S. Sinumporn, and S. Talumphai, "Screening for rice blast resistant genes, *Pi9, Pita, Pigm*(*t*) and *Pi54* in landrace rice varieties of north-eastern Thailand," *Journal of Applied Research on Science and Technology*, vol. 21, pp. 122–132, 2022.

[32] K. Rybka, M. Miyamoto, I. Ando, A. Saito, and S. Kawasaki, "High resolution mapping of the indica-derived rice blast resistance genes II. *Pi-ta2* and *Pi-ta* and a consideration of their origin," *Molecular Plant-Microbe Interactions*, vol. 10, no. 4, pp. 517–524, 1997.

[33] L. Monna, A. Miyao, H. S. Zhong et al., "Saturation mapping with subclones of YACs: DNA marker production targeting the rice blast disease resistance gene, *Pi-b*," *Theoretical and Applied Genetics*, vol. 94, no. 2, pp. 170–176, 1997.

[34] H. Shinoda, K. Toriyama, T. Yunoki, A. Ezuka, and Y. Sakurai, "Studies in the varietal resistance of rice to blast. 6. linkage relationship of blast resistance genes (in Japanese with English summary)," *Bulletin of Chugoku National Agriculture Experimental Station: Series A*, vol. 20, pp. 1–25, 1917.

[35] Y. Jia, Z. Wang, R. G. Fjellstrom et al., "Rice *Pi-ta* gene confers resistance to the major pathotypes of the rice blast fungus in the United States," *Phytopathology*, vol. 94, no. 3, pp. 296–301, 2004.

[36] J. Ma, Y. Sun, Y. Yang, J. Li, and X. Minghui, "Distribution of *Pita* gene for rice blast resistance in the rice cultivars (lines) from China Japan and Korea," *Molecular Plant Breeding*, vol. 18, pp. 459–465, 2020.

[37] K. Shi, C. L. Lei, Z. J. Cheng, X. T. Xu, J. L. Wang, and J. M. Wan, "Distribution of two blast resistance genes *Pita* and *Pib* in major rice cultivars in China," *Plant Genetic Resources*, vol. 10, pp. 21–26, 2009.

[38] J. Y. Song, G.-A. Lee, Y.-M. Choi et al., "Blast resistant genes distribution and resistance reaction to blast in Korean landraces of rice (*Oryza sativa* L.)," *Korean Journal of Polar Research*, vol. 27, no. 6, pp. 687–700, 2014.

[39] P. Vejchasarn, J. R. Shearman, U. Chaiprom et al., "Population structure of nation-wide rice in Thailand," *Rice*, vol. 14, no. 1, p. 88, 2021.

[40] S. K. Mutiga, F. Rotich, V. D. Ganeshan et al., "Assessment of the virulence spectrum and its association with genetic diversity in *Magnaporthe oryzae* populations from Sub-Saharan africa," *Phytopathology*, vol. 107, no. 7, pp. 852–863, 2017.

[41] L. Zhang, Y. Nakagomi, T. Endo et al., "Divergent evolution of rice blast resistance *Pi54* locus in the genus *Oryza*," *Rice*, vol. 11, no. 1, p. 63, 2018.

[42] J. Li, D. Q. Li, Y. Sun, and M. Xu, "Rice blast resistance gene *Pi1* identified by MRG4766 marker in 173 yunnan rice

landraces," *Proceedings of the Rice Genomics and Genetics*, vol. 3, pp. 13–18, 2012.

[43] Y. Jia, G. T. Bryan, L. Farrall, and B. Valent, "Natural variation at the *Pi-ta* rice blast resistance locus," *Phytopathology*, vol. 93, no. 11, pp. 1452–1459, 2003.

[44] G. S. Khush and K. K. Jena, "Current status and future prospects for research on blast resistance in rice (*Oryza sativa* L.)," in *Proceedings of the Advances in Genetics*, pp. 1–10, Genomics and Control of Rice Blast Disease, 2009.

[45] H. Jiang, Z. Li, J. Liu et al., "Development and evaluation of improved lines with broad-spectrum resistance to rice blast using nine resistance genes," *Rice*, vol. 12, no. 1, p. 29, 2019.