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Pathogenicity of *Pyricularia oryzae* on elite rice cultivars and geographical distribution of avirulence genes causing blast disease in Thailand

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Abstract *Pyricularia oryzae* is a plant pathogenic fungus causing rice blast disease. In Thailand, there is very little information about pathogenicity and avirulence gene of rice blast pathogen. Each of the 50 blast isolates obtained from blast disease samples collected from 19 provinces was investigated for avirulence gene on 31 near isogenic lines (NILs) and their pathotype on elite rice cultivars was determined using 24 Thai elite rice cultivars. Among the 50 isolates, *Avr-Pita-2* was discovered mostly in isolates from the North (83%), *Avr-Pikp* and *Avr-Pi7(t)* were discovered in isolates from the North East (94%), while 8 avirulence genes including *Avr-Pikp, Avr-Pikh, Avr-Pi1, Avr-Pi7(t), Avr-Piz, Avr-Pi12, Avr-Pi19* and *Avr-Pi20* were discovered in isolates from the South (88%) and *Avr-Pi9* was discovered in isolates from the Central region of Thailand (71%). Isolates BRM60001 that carried the most numbers of Avr gene was unable to infect any elite rice cultivars, while isolates that carried as less as 2 Avr genes could infect few elite rice cultivars. Isolate CRI59004.1 carried *Avr-Pik (Avr-Pikh, Avr-Pikm, Avr-Pi7(t)), Avr-Pi9, Avr-Pish, Avr-Pib, Avr-Piz-5, Avr-Pita, Avr-Pita-2* and *Avr-Pi19*, could infect the greatest numbers of elite rice cultivars. The information regarding the distribution of Avr gene in *P. oryzae* will be useful for forecasting rice blast and disease resistance breeding programs.

Keywords: Rice blast disease, *Pyricularia oryzae*, Elite rice cultivars, Near isogenic lines, Geographical distribution of avirulence gene of blast fungi

Introduction

Pyricularia oryzae is a plant pathogenic fungus which cause rice blast disease. The fungi can heavily manage susceptible rice varieties and cause low rice productivity. The most practical and economical method to control this disease is by using blast resistant cultivars. However, using resistant varieties

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has limited success due to the breakdown of resistance genes by the blast fungus that is highly variable and often can overcome the resistant cultivars within a few years (Wang and Valent, 2009). The blast fungus has adaptive mechanisms which could be due to many factors such as the changing of nucleotide at gene positions associated with pathogenicity to produce new genes to avoid detection by resistance genes in rice and blast pathogen could overcome resistance in rice cultivars (Chiapello *et al.,* 2015). The genetic diversity of *P. oryzae* was higher in the South, East and Southeast Asia than in other regions (Zeigler, 1998). Genetically diverse population of rice blast fungus could undergo both sexual and asexual reproductions, genetic and biological evidences of past or present sexual reproduction was noted in several regions including South China (Yunnan), Laos and North Thailand, thus designated as the putative center of origin of *P. oryzae* strains pathogenic on rice (Saleh *et al.,* 2014). Moreover, the geography of each growing region is another important factor that caused genetic diversity of rice blast populations (Gallet *et al*., 2015).

Improvement of blast disease resistant should be a continuing operation together with appropriate blast isolates for breeding programs. Therefore, the study about pathogenicity and avirulence gene of rice blast pathogen is necessary to succeed in rice blast resistance breeding programs. Pathogenicity of blast fungi has been widely studied in Brazil (Prabhu *et al*., 1992), Thailand (Mekwatanakarn *et al.,* 2000), Tanzania (Chuwa *et al.,* 2013), West Africa (Odjo *et al.,* 2014), Cambodia (Fukuta *et al.,* 2014), Benin (Akator *et al*., 2014), China (Lei *et al*., 2013; Xing *et al*., 2017). Furthermore, the geographic distribution of avirulence genes of the rice blast fungus has been studied in Philippines (Lopez *et al*., 2019) and Thailand (Mekwatanakarn *et al*., 2000). As there was only few information about pathogenicity and avirulence genes of rice blast pathogen in Thailand, the purpose of this study were to determine avirulence gene of *P. oryzae* and their pathotype on elite rice cultivars. The monitoring of Avr gene distribution of *P. oryzae* will be useful for forecasting rice blast and for rice blast disease resistance breeding programs.

Materials and methods

Rice variety

Thirty-one near isogenic lines (NILs) and a susceptible cultivar, Lijiangxintuanheigu (LTH), were used to investigate the avirulence gene of each rice blast fungal isolate. The NILs rice used in this research was obtained from IRRI (International Rice Research Institute, Philippines), each variety

contained only 1 known resistance (R) gene/allele. The selected Thai elite rice cultivars used in the pathogenicity test were 24 varieties. The resistant rice Jao Hom Nin (JHN), IR64 and susceptible KDML105 were used as standard check varieties in all experiments.

Fungal isolation and spore suspension preparation

Rice with leaf or neck blast symptoms were collected from blast infected fields in Thailand in the year 2016 and 2017. Fungal spores were isolated from rice leaves and necks using single spore isolation technique and grown on Rice Flour Agar (RFA, 20 g rice flour, 2 g yeast extract, 20 g agar in 1000 ml) where a filter paper was put on top of medium. The mycelium was stored at -20◦C until further use as described by Sirithunya *et al*. (2007). The filter paper containing fungal mycelium was placed on RFA medium for 4 days. The edge of mycelium was transferred to new RFA medium. The sporulation was induced by scraping 8 to 10-day-old mycelia and allowed to growth for another 48 hr. Spores were harvested and the concentration of the spores was adjusted to 10^5 spores/mL in 0.5% gelatin.

Inoculation and disease assessment

Seeds of each of the rice varieties were sown individually in plastic trays (33 x 11 x 11 cm.) half-filled with soil and fertilized with ammonium sulphate (5 g/tray). The control check varieties, JHN, IR64 and KDML105, were planted at both sides of the end and central rows of each trays. Ammonium sulphate (1 g) was added to each tray 7 days prior to inoculation. Inoculation of the blast isolates was performed following the method described by Sreewongchai *et al*. (2009). The inoculum was sprayed onto 14-day-old seedlings using an air-brush pressure pump. The inoculated seedlings were placed in a high-humidity chamber for 18 hr at 25℃ and were then transferred to a greenhouse. The degree of infection of each seedling was evaluated 7 days after inoculation by a standard reference scale for rice blast, scoring 0 (resistant) to 6 (susceptible) (Roumen *et al.,* 1997). The experiment was repeated twice with 4 plants each.

Results

Isolation

Blast samples were collected from 19 provinces with blast disease epidemics in Thailand. A total of 50 isolates were obtained from Thai rice

varieties which were damaged by blast disease during the years 2016 and 2017 and covered all 5 regions of Thailand. Eighteen isolates were from the Northern region (Chiang Rai, Phrae and Mae Hong Son), 16 isolates from the North East (Buri Ram, Nong Bua Lam Phu, Nong Khai, Nakhon Ratchasima, Roi Et, Sakon Nakhon, Surin, Udon Thani and Ubon Ratchathani), 7 isolates from the Central region (Lop Buri, Phetchabun and Uttaradit), 8 isolates from the South (Krabi, Nakhon Si Thammarat and Phatthalung) and 1 isolate from the Western region (Ratchaburi) (Table 1).

Table 1. Source and host variety of 50 blast isolates collected in year 2016 and 2017

No.	Isolates	Host	Province	Region	Years
1	BAG2.4	KDML105	Ubon Ratchathani	Northeast	2016
2	BAG20.4	KDML105	Ubon Ratchathani	Northeast	2016
3	BAG44.2	KDML105	Roi Et	Northeast	2016
4	CRI59001	RD6	Chiang Rai	North	2016
5	CRI59002.1	RD14	Chiang Rai	North	2016
6	CRI59004.1	Sanpatong 1	Chiang Rai	North	2016
7	CRI59005	-	Chiang Rai	North	2016
8	LBR59003.1	-	Lop Buri	Central	2016
9	PNB59001.4	-	Phetchabun	North	2016
10	PNB59003.1	-	Phetchabun	North	2016
11	PNB59003.2	-	Phetchabun	North	2016
12	PNB59003.3	-	Phetchabun	North	2016
13	PRE59001	Suphanburi 1	Phrae	North	2016
14	PRE59003	Phitsanulok 2	Phrae	North	2016
15	PRE59004.1	RD53	Phrae	North	2016
16	PRE59006.2	RD6	Phrae	North	2016
17	PRE59008.1	KDML105	Phrae	North	2016
18	RBR59001	-	Ratchaburi	West	2016
19	UTD17002	-	Uttaradit	North	2016
20	BRM60001	KDML105	Buri Ram	Northeast	2017
21	BRM60012.2	KDML105	Buri Ram	Northeast	2017
22	BRM60011.1	KDML105	Buri Ram	Northeast	2017
23	CRI60001.1	RD6	Chiang Rai	North	2017
24	CRI60003.2	RD6	Chiang Rai	North	2017
25	CRI60004.1	RD6	Chiang Rai	North	2017
26	CRI60007	KDML105	Chiang Rai	North	2017
27	KBI60001	Pathumthani 1	Krabi	South	2017
28	MSN60001	Hahngyi 71	Mae Hong Son	North	2017
29	MSN60002	Sanpatong 1	Mae Hong Son	North	2017
30	MSN60006.1	RD6	Mae Hong Son	North	2017
31	MSN60009.1	RD15	Mae Hong Son	North	2017
32	MSN60012	RD15	Mae Hong Son	North	2017
33	NBP60001	RD6	Nong Bua Lam Phu	Northeast	2017
34	NBP60002	RD6	Nong Bua Lam Phu	Northeast	2017

Table 1. (continued)

No.	Isolates	Host	Province	Region	Years
35	NKI60001.1	RD6	Nong Khai	Northeast	2017
36	NKI60001.2	RD6	Nong Khai	Northeast	2017
37	NMA60001	-	Nakhon Ratchasima	Northeast	2017
38	NRT60001	RD41	Nakhon Si Thammarat	South	2017
39	NRT60002	RD41	Nakhon Si Thammarat	South	2017
40	PL60001	RD41	Phatthalung	South	2017
41	PL60004	KDML105	Phatthalung	South	2017
42	PL60009	Sungyod	Phatthalung	South	2017
43	PL600010	Sungyod	Phatthalung	South	2017
44	PL600011	Riceberry	Phatthalung	South	2017
45	PNB60001	-	Phetchabun	North	2017
46	SKN60001.1	-	Sakon Nakhon	Northeast	2017
47	SKN60003	-	Sakon Nakhon	Northeast	2017
48	SRN60001	RD15	Surin	Northeast	2017
49	UDN60001.1	-	Udon Thani	Northeast	2017
50	UDN60005	RD6	Udon Thani	Northeast	2017

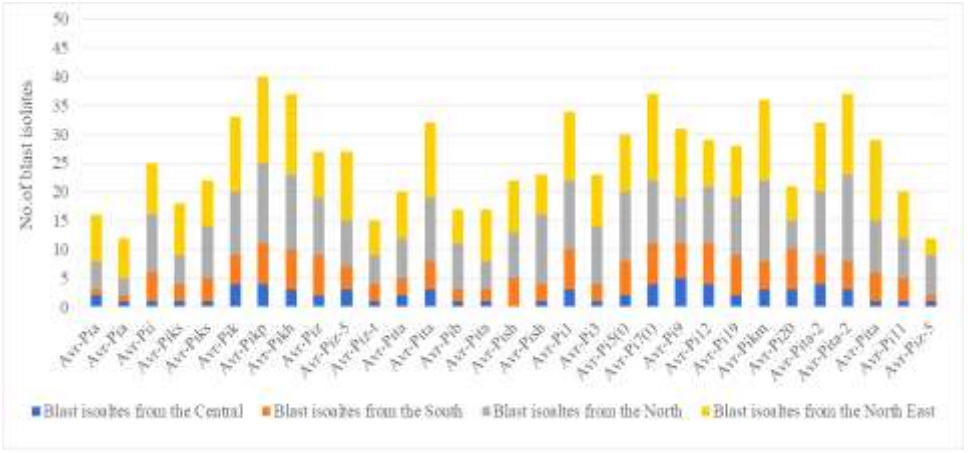

Figure 1. The distribution of avirulence genes analyzed from 50 blast isolates from the North, the North East, the South and the Central region of Thailand

Investigation of avirulence gene of rice blast fungus in Thailand using near isogenic lines (NILs)

Investigation of avirulence gene of each isolate was conducted using 31 near isogenic lines (NILs) and susceptible cultivar, Lijiangxintuanheigu (LTH). The result of this study confirmed "gene-for-gene" interactions, each NIL has 1 R gene and a pathogen has a corresponding Avr gene. The plant can recognize the pathogen (compatible reaction) and induce defense responses. Thirty NILs showed compatible reaction with Avr gene in blast fungi that were isolated

from rice-field areas in the North, the North East and the South except the IRBLsh-S which carried *Pish* gene that showed incompatible reaction to an isolate from rice-field area in the Central region. The most discovered Avr genes in Thailand were *Avr-Pik* (*Avr-Pikp, Avr-Pikh, Avr-Pikm, Avr-Pi7(t)*) and *Avr-Pita-2*. These Avr genes were found in more than 35 blast isolates (Figure 1). *Avr-Pita-2* was discovered in isolates from the North (83% of blast isolates from the North), *Avr-Pikp* and *Avr-Pi7(t)* were discovered in isolates from the North East (94% of blast isolates from the North East), while 8 avirulence genes including *Avr-Pikp, Avr-Pikh, Avr-Pi1, Avr-Pi7(t), Avr-Piz, Avr-Pi12, Avr-Pi19* and *Avr-Pi20* were discovered in isolates from the South (88% of blast isolates from the South) and *Avr-Pi9* was discovered in isolates from the Central region of Thailand (71% of blast isolates from the Central). The compatible reaction between R gene in NILs and isolates PNB59003.3 was not shown (Figure 3).

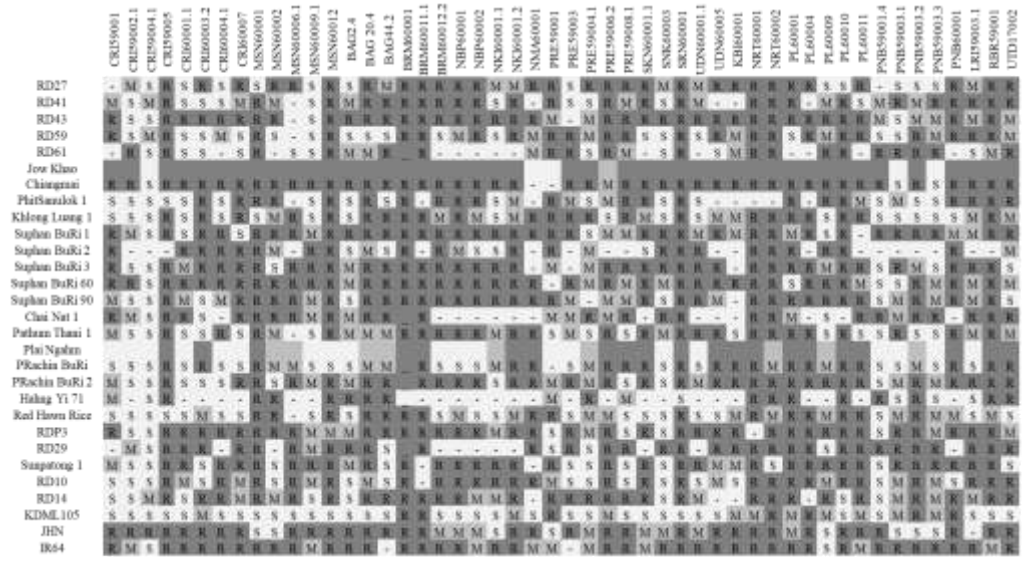

Figure 2. Pathogenicity assessment of 50 blast isolates on 24 elite rice cultivars. R is Resistant (score 0-2), M is modulated resistant (score 3-4), and S is susceptible (score 5-6)

Discussion

The distribution of Avr genes found in the North, the North East and the South of Thailand revealed that the corresponding R genes appeared in rice cultivars growing in those parts of Thailand. The *Avr-Pish* gene was found in all isolates but not in any of those collected from the Central region. However,

the most Avr gene discovered in each region of Thailand was different. *Avr-Pita-2* was discovered in isolates from the North, *Avr-Pikp* and *Avr- Pi7(t)* were discovered in isolates from the North East, while 8 avirulence genes including *Avr-Pikp, Avr-Pikh, Avr-Pi1, Avr-Pi7(t), Avr-Piz, Avr-Pi12, Avr-Pi19* and *Avr-Pi20* were discovered in isolates from the South and *Avr-Pi9* was discovered in isolates from the Central of Thailand. The occurrence of these genes could be due to the adaptive mechanisms of the pathogen brought about by the use of difference rice cultivation system and the favoured rice varieties being chosen by the farmer according to the geography of each province (Gallet *et al*., 2015). The rice varieties being cultivated in Thailand have high genetic diversity (Chakhonkaen *et al*., 2012; Promsomboon and Promsomboon, 2016). The diverse rice varieties that are being planted might cause different Avr genes in isolates of the same province. Isolates BRM60001 that carried the most numbers of Avr gene was unable to infect any elite rice cultivars (Figure 2 and 3). Conversely, isolates that carried the less numbers of Avr genes could infect few elite rice cultivars (Figure 2 and 3), this indicated that many Avr-genes were not necessary for fungal pathogenicity and Avr genes are adequate for infection on a rice cultivar (Liao *et al.,* 2016). Isolate CRI59004.1 carried *Avr-Pik (Avr-Pikh, Avr-Pikm, Avr-Pi7(t)), Avr-Pi9, Avr-Pish, Avr-Pib, Avr-Piz-5, Avr-Pita, Avr-Pita-2* and *Avr-Pi19,* could infect the greatest numbers of elite rice cultivars.

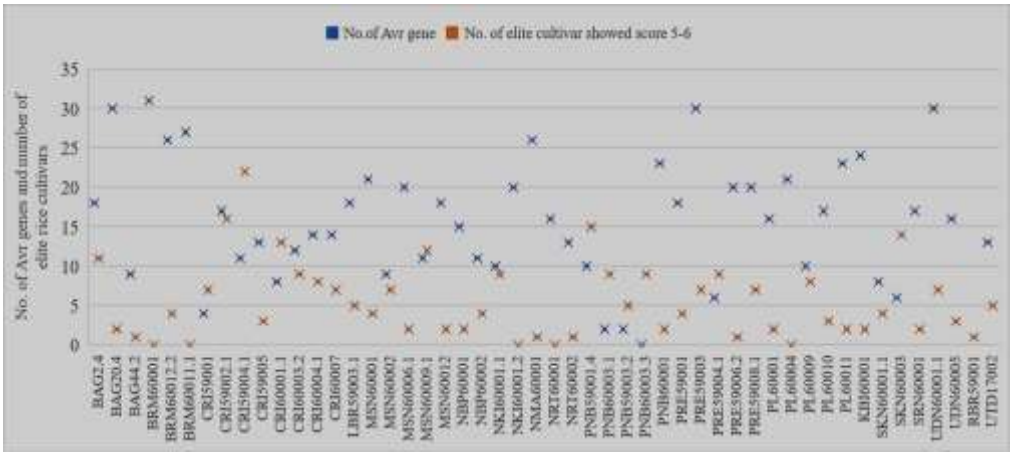

Figure 3. Co-analysis of number of Avr genes and number of elite rice cultivars that was infected by each blast isolate

Therefore, the resistance genes corresponding to these Avr genes were important for rice blast disease resistance breeding programs in Thailand. Similary, Mekwatanakarn *et al*. (2000) reported that the resistance genes *Pi1,*

Piz-5, and *Pita-2* were broadly effective to rice blast population in Thailand. The results in this study is essential for the appropriate rice blast disease resistance breeding programs. Our observation provided information and recommends R genes (*Pik (Pikh, Pikm, Pi7(t)), Pi9, Pish, Pib, Piz-5, Pita, Pita-2* and *Pi19*) to be chosen for developing potential resistant rice to blast disease.

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