Identification of Rice Blast Resistance Genes in South Central Coast of Vietnam Using Monogenic Lines under Field Condition and Pathogenicity Assays

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Abstract: In the present study, the authors studied the interaction between 26 monogenic differentials carrying 26 blast resistance genes with 15 isolates of *Pyricularia oryzae* under field conditions and pathogenicity assays. The result in field conditions showed that area under disease progress curves (AUDPC) of monogenic lines was found to range from 0 to 142.3. Lijiangxintuanheigu (LTH) was susceptible in all of regions. The monogenic lines carrying *Pik*, *Pik-p*, *Pik-h*, *Piz*, *Pi1*, *Pi7(t)*, *Pik-m*, *Pi4(t)*, *Pish*, *Pi9(t)* and *Pita* were highly resistant to blast in all of regions. In plastic house net conditions, the percentage of virulent reactions of monogenic lines to 15 isolates was found to range from 23.1% to 84.6%. LTH was susceptible to all 15 isolates. All 26 monogenic lines were resistant to at least 3 isolates of *P. oryzae*, and the frequency of resistant reactions of the monogenic lines carrying *Pik*, *Pik-p*, *Pik-h*, *Piz*, *Pi4(t)*, *Pish*, *Pi1*, *Pi7(t)*, *Pi9*, *Pik-m* and *Pita* were 80.0%, 93.3%, 93.3%, 80.0%, 73.3%, 86.7%, 73.3%, 80.0%, 80.0%, 80.0% and 80.0%, respectively. These findings suggest that *Pik*, *Pik-p*, *Pik-h*, *Piz*, *Pi4(t)*, *Pish*, *Pi1*, *Pi7(t)*, *Pi9*, *Pik-m* and *Pita* may be important *R* genes for preventing blast disease in South Central Vietnam. Based on these data, a useful strategy for managing rice blast disease by stacking pyramiding blast *R* genes against pathogenic *P. oryzae* isolates at hotspot in South Central Coast of Vietnam was proposed.

Key words: Rice, *P. oryzae*, blast rice, monogenic lines, resistance genes.

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important staple food crops for more than half of the world's population [1]. Rice blast, caused by *Pyricularia oryzae* Cav. (*P. oryzae*), is the most devastating rice disease that occurs all over the world [2]. The yield loss caused by blast vary widely depends on agro-systems and can reach several million tons, 10% to 30% of crops [3]. Under certain environmental

conditions and cultural practices, 100% loss was observed [4]. Cultural practices, fungicides and genetic resistance are interchangeably used among different rice production areas worldwide to prevent blast disease [5-8]. Extensive and uncontrolled use of fungicides poses a significant concern for human health and environmental safety. Hence, utilization of multiple *R* genes with over lapped resistance spectra is one of the most powerful strategies for manage blast disease [9-11]. To date, 96 major blast *R* genes and quantitative trait locus (QTLs) have been identified and mapped using DNA markers [12]; 18 of these

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genes have been cloned and used for crop protection [13]. However, the management of rice blast caused by *P. oryzae* through the use of disease resistant cultivars has become difficult because of the highly variable nature of the pathogen as well as favorable environmental conditions for infection during the crop season. The virulence of rice blast pathogen isolates in a population tends to shift from avirulence to virulence, rather than virulence to avirulence, on a given host [14]. Such variability has made it difficult to identify strategies for selecting or breeding rice cultivars that show durable resistance to blast populations in a given area. Most of the blast resistant cultivars of rice succumb to the disease within 2-3 years of their introduction into a disease-prone area [15, 16]. Therefore, determining the effectiveness of blast *R* genes in a certain area is important in assisting the development of rice breeding programs, which generate rice with long lasting effective genetic resistance [17].

The monogenic lines have been important assets to identify blast *R* genes [18]. The first international rice blast differential system was developed in the 1960 to determine physiological races of *P. oryzae* [19]. Makill and Boman [20] developed a set of near-isogenic lines (NILs) derived from CO39 with 14 *R* genes, including *Pish*, *Pib*, *Piz-5*, *Pz-t*, *Pi5(t)*, *Piks*, *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, *Pi1*, *Pi7(t)*, *Pita* and *Pita-2*. In China, a set of NILs were developed with five blast *R* genes *Pib*, *Pik*, *Pik-m*, *Pik-p* and *Pita-2*, using a susceptible japonica-type genetic background, Lijiangxintuanheigu (LTH) [21]. A set of monogenic lines, containing 24 major *R* genes *Pia*, *Pii*, *Pik*, *Pik-s*, *Pik-m*, *Pik-h*, *Pik-p*, *Piz*, *Piz-5*, *Piz-t*, *Pita*, *Pita-2*, *Pib*, *Pit*, *Pish*, *Pi1*, *Pi3*, *Pi5(t)*, *Pi7*, *Pi9*, *Pi11*, *Pi12(t)*, *Pi19* and *Pi20(t)* in the blast-susceptible recurrent japonica variety LTH, were developed by the International Rice Research Institute (IRRI) [22]. This differential set is excellent materials that have been used to identify blast *R* genes worldwide [12, 23].

In Vietnam, rice blast disease is one of the most

destructive diseases limiting rice production, because the fungal pathogen can infect and cause lesions on almost all organs of the plant [24]. In 2012, the total area of infection rice blast disease was 366,412 ha, of which 11,400 ha are severe infections, 8 ha are full lost, and reduction in milling yield is about 10%-25% [25]. The South Central Vietnam is within the “hotspot” areas of Vietnamese rice. Blast disease has been found in more than 2,054 ha in South Central Vietnam [25]. Although the major blast genes *Pita*, *Pib* and *Pik-m* are widely used, the effectiveness of these *R* genes at Central Vietnam has not been determined yet.

The present study was conducted to evaluate disease reactions of selected field blast isolates on 26 monogenic differentials and provide useful information in formulating strategies for improving blast resistance.

2. Materials and Methods

2.1 Plant Materials

A set of blast differential varieties, consisting of 26 monogenic lines with 26 *R* genes (*Pia*, *Pii*, *Pik-s*, *Pik*, *Pik-p*, *Pik-h*, *Piz*, *Pi2(t)*, *Piz-t*, *Pi4(t)*, *Pib*, *Pit*, *Pish*, *Pi1*, *Pi3*, *Pi5(t)*, *Pi7(t)*, *Pi9*, *Pi12(t)*, *Pi19*, *Pik-m*, *Pi20*, *Pita2*, *Pita*, *Pi11(t)* and *Piz5*) developed under IRRI were used. The recurrent parent was the blast-susceptible variety LTH, which was used as a susceptible control.

2.2 Field Experiment

2.2.1 Experimental Design

The experiment was conducted in farmer's field at the hotspot in Vietnam South Central Coast, including, Quang Nam (QN), Quang Ngai (QNG) and Binh Dinh (BD) province (Fig. 1). The chosen locations have a long history of strong blast disease presence and are mainly at poor alluvial soil.

The trial was laid down in a randomized complete block design. Each genotype was planted in two rows of 5 m long as an experimental unit to evaluate qualitative

Fig. 1 Different location of field trials at South Central Vietnam.

resistance to blast; distance between rows was 20 cm.

The fertilizers were applied at the rate of 100 kg, 50 kg and 50 kg per ha of N, P_2O_5 and K_2O , respectively. All P_2O_5 were used as basal at the time of sowing. The 30 kg N and 25 kg K_2O were uniformly broadcasted at 12 days after sowing (DAS), 40 kg N was used at 20 DAS and the rest on 45 DAS.

2.2.2 Disease Assessment

Ten plants were randomly (diagonally) selected from each plot (4 m × 2 m) and tagged. Disease scoring

started from the 15th day after seeding and periodic survey was made one time every seven days. The disease severity was calculated as disease severity (%), calculated as Eq. (1):

$$\text{Disease severity (\%)} = \frac{(\text{sum of all numerical rating})}{(\text{total number of leaves or plants observed}) \times \text{maximum rating}} \times 100 \quad (1)$$

Disease assessment was scored based on the reaction of the differential varieties/cultivars. The lesions were scored as follows:

0 = no lesion observed;

1 = small brown specks off pinpoint size or large brown specks without sporulation center;

3 = small, roundish to slightly elongated necrotic sporulation spots, about 1-2 mm in diameter with a distinct brown margin or yellow halo;

5 = narrow or slightly elliptical lesions, 1-2 mm in breadth, more than 3 mm long with a brown margin;

7 = broad spindle-shaped lesion with yellow, brown, or purple margin;

9 = rapidly, coalescing small, whitish, grayish, or bluish lesions without distinct margins.

Symptom scores were attributed as average symptom observed in each experimental unit [26].

Area under disease progress curves (AUDPC) was calculated based on the Eq. (2) used by Das et al. [27]:

$$\text{AUDPC} = \sum_{i=1}^{n-1} [(Y_{i+1} + Y_i) / 2] \times (t_{i+1} - t_i) \quad (2)$$

where Y_i is percentage of disease at the i th observation, t_i is time at the i th observation, and n is total number of observation. AUDPC was calculated for each rice cultivar.

2.3 Pathogenicity Assays

2.3.1 Collection, Isolation and Culture Maintenance

A total of 200 samples of leave and panicle neck having blast rice symptom were collected from experimental fields of rice production areas of three provinces at South Central Vietnam (QN, QNG and BD) from 2014 to 2015. Single lesions were placed on glass rods in Petri dishes with wet filter paper and

incubated at 25 °C until sporulation. With a pointed capillary tube, spores will be picked from a single lesion under dissecting microscope and spread onto water agar in Petri dishes. A single spore was transferred on potato dextrose agar slant under a dissecting microscope and incubated from 8 d to 10 d at 26 °C. For maintenance of the isolates obtained from single spore culture, the colony was grown in sterilized filter paper, which was put on potato dextrose agar and incubated for 10 d. After that, only the paper pieces were transferred onto empty Petri plate for drying at 37 °C for 3 d; after drying, they were stored in small bottle with silica gel in a freezer at -10 °C [28]. List of blast isolates is presented in the Table 1.

2.3.2 Test Cultivars

Seedlings of 26 monogenic lines resistance to *P. oryzae* and one susceptible variety LTH were grown in the plastic house net in either plastic pots (25 × 25 × 30 cm) containing 4 kg of soil fertilized with N-P-K (20-20-10 g pot). Ten seeds of each line/cultivar were planted in a plastic pot. Plants were grown plastic house net at 25-30 °C for four weeks, until they reached the four leaf stage. The experiment was conducted in a randomized complete block design with three replications. Four-week-old plants (4-leaf

stage) were inoculated with spore suspensions as described in the study of Berruyer et al. [29].

2.3.3 Inoculation and Disease Assessment

Five strains of *P. oryzae* selected in each province were used as representative monoconidial isolates for the present study. Four-week-old plants at the 4-5 leaf stage in each pot were inoculated by spraying 20 mL aqueous spore suspension (1×10^5 spores/mL) onto the leaves. Disease reactions were scored at the 7th days after inoculation on a scale from 0 to 5, in which 0 = no evidence of infection; 1 = brown specks smaller than 0.5-1 mm in diameter; 3 = roundish to elliptical lesions about 1-3 mm in diameter with gray centers and brown margins; 4 = typical spindle-shaped blast lesion, 3 mm or longer with little or no coalescence of lesions; 5 = same as 4, but half of one or more leaves killed by coalescence of lesions. Plants rated 0-3 were considered resistant, and those rated 4-5 were considered susceptible [20, 30]. For blast screening, the scores of five plants for each line were averaged and an actual infection means scales was used in analysis.

3. Results

3.1 Field Experiments

Table 2 showed AUDPC of leaf disease severity in the different monogenic line and at the different regions of the study. AUDPC figures varied among the different cultivars and were different for the three regions.

With respect to AUDPC of leaf disease severity, LTH (susceptible control) showed the highest percentage of spots, ranged from 203.8% to 352.6%, of which the highest is at BD province and the lowest at QN province. LTH was followed by the lines containing *R* genes: *Pia*, *Pii*, *Pik-s*, *Piz-t*, *Pib*, *Pit*, *Pi12(t)*, *Pi11(t)*, *Pi3*, *Piz5* and *Pi20(t)* which showed high disease severity in all of regions, with values ranging between 24.3% and 142.3%. However, in each location, a single blast *R* gene can be effective in preventing blast disease, for example, *Pib* and *Piz-t* in

Table 1 *P. oryzae* strains from center of Vietnam used in this study.

Isolate	Origin	Cultivar
QN49	Quang Nam	BC15
QN95	Quang Nam	Xi23
QN125	Quang Nam	OM4900
QN186	Quang Nam	SV181
QN239	Quang Nam	KD18
QNG63	Quang Ngai	Thom 1
QNG109	Quang Ngai	KD18
QNG158	Quang Ngai	Q5
QNG193	Quang Ngai	BC15
QNG249	Quang Ngai	Thom 1
BD38	Binh Dinh	BC15
BD92	Binh Dinh	Q5
BD128	Binh Dinh	KD28
BD183	Binh Dinh	BC15
BD258	Binh Dinh	KD28

Table 2 AUDPC for monogenic lines resistance to *P. oryzae* in the field of Central Vietnam during the spring season of 2014.

Rice line	Gene name	AUDPC (%)		
		Quang Nam	Quang Ngai	Binh Dinh
IRBLa-A	<i>Pia</i>	104.6	142.3	105.3
IRBLi-F5	<i>Pii</i>	98.3	91.3	95.2
IRBLks-F5	<i>Pik-s</i>	93.2	132.7	92.7
IRBLk-ka	<i>Pik</i>	0.0	0.0	0.0
IRBLkp-K60	<i>Pik-p</i>	0.0	0.0	0.0
IRBLkh-K3	<i>Pik-h</i>	0.0	0.0	0.0
IRBLz-Fu	<i>Piz</i>	0.0	0.0	0.0
IRBLz5-CA	<i>Pi2(t)</i>	78.1	72.4	0.0
IRBLzt-T	<i>Piz-t</i>	104.2	94.2	0.0
IRBLta-K1	<i>Pi4(t)</i>	28.4	15.8	19.5
IRBLb-B	<i>Pib</i>	77.3	82.5	0.0
IRBLt-K59	<i>Pit</i>	95.4	92.5	60.4
IRBLsh-S	<i>Pish</i>	19.4	21.6	18.5
IRBL1-CL	<i>Pi1</i>	0.0	0.0	0.0
IRBL3-CP4	<i>Pi3</i>	64.2	68.3	94.6
IRBL5-M	<i>Pi5(t)</i>	28.4	82.4	0.0
IRBL7-M	<i>Pi7(t)</i>	0.0	0.0	0.0
IRBL9-W	<i>Pi9(t)</i>	29.3	27.3	32.4
IRBL12-M	<i>Pi12(t)</i>	95.2	87.2	24.3
IRBL19-A	<i>Pi19</i>	62.4	68.3	31.5
IRBLkm-Ts	<i>Pik-m</i>	0.0	0.0	0.0
IRBL20-IR24	<i>Pi20(t)</i>	97.3	132.8	57.3
IRBLta2-Pi	<i>Pita2</i>	74.3	72.5	0.0
IRBLta-CP1	<i>Pita</i>	22.4	19.3	0.0
IRBL11-Zh	<i>Pi11(t)</i>	123.7	27.6	48.5
IRBLz5-CA	<i>Piz5</i>	101.7	123.8	112.9
LTH		203.8	310.4	352.6

BD region. The cultivars that showed medium susceptibility were *Pi2(t)*, *Pi19*, *Pita2* and *Pi5(t)* with values ranging from 31.5%-82.4%. Among them, blast *R* genes are still effective in preventing blast disease in some regions, such as *Pi2(t)*, *Pi5(t)* and *Pita2* in BD province (Table 2). *Pi4(t)*, *Pish*, *Pi9(t)* and *Pita* showed significantly resistance to *P. oryzae*. The highly resistant level to leaf blast in both two season and at all regions was observed in the monogenic lines carrying *Pik*, *Pik-p*, *Pik-h*, *Piz*, *Pi1*, *Pi7(t)* and *Pik-m* which with value of zero. This result suggested that effective genes for blast resistance in rice production areas in South Central Vietnam included *Pik*, *Pik-p*, *Pik-h*, *Piz*, *Pi1*, *Pi7(t)*, *Pik-m*, *Pi4(t)*, *Pish*, *Pi9(t)* and *Pita*.

3.2 Pathogenicity Assays

3.2.1 Evaluation of Useful Blast *R* Genes

All monogenic lines were resistant to at least one isolate, indicating that the *R* genes they carry will have some impact in preventing blast disease. However, the recurrent parent LTH was susceptible to all tested isolated. Among 26 monogenic lines, monogenic lines carrying *Pik*, *Pik-p*, *Pik-h*, *Piz*, *Pi4(t)*, *Pish*, *Pi1*, *Pi7(t)*, *Pi9*, *Pik-m* and *Pita* showed strong resistance, with the percentages of resistant reactions were 80.0%, 93.3%, 93.3%, 80.0%, 73.3% 86.7%, 73.3%, 80.0%, 80.0%, 80.0% and 80.0%, respectively (Fig. 2). These results suggest that *Pik*, *Pik-p*, *Pik-h*, *Piz*, *Pi4(t)*, *Pish*, *Pi1*, *Pi7(t)*, *Pi9*, *Pik-m* and *Pita* are highly effective against blast disease in South Central Vietnam. Furthermore,

Fig. 2 Percentage of the resistant reaction of 26 monogenic lines containing an *R* gene to isolates of *P. oryzae*.

in each location, a single blast *R* gene can be effective in preventing blast disease. *Pik-h* in QN province; *Pik-p* and *Pish* in QNG province and *Pik*, *Pik-p*, *Piz*, *Pik-h*, *Pi4(t)*, *Pish*, *Pi1*, *Pi7(t)*, *Pi9*, *Pik-m*, *Pita2* and *Pita* in BD province may be adequate to prevent blast disease (Table 3). This result is similar to the result of experiments in the field.

3.2.2 Virulence of *P. oryzae* Isolates

All isolates were virulent to one or more monogenic lines. The frequency of virulence was found to be from 23.1% to 84.6%. The frequency of virulence for isolate BD92 and BD258 was the lowest, which was 23.1%, and the greatest was QN186 which was 84.6% (Fig. 3). Overall, one or two isolates in each region were very virulent on the tested monogenic lines with *R* genes. Notably, the pathogenicity of isolates from QN province was higher than those of the isolates in the other province (Table 3). The most virulent isolates (QN186) also were found in the QN province.

3.2.3 A Strategy for Pyramiding Blast *R* Genes

In the present study, monogenic differentials

carrying 26 major blast *R* genes (*Pia*, *Pii*, *Pik-s*, *Pik*, *Pik-p*, *Pik-h*, *Piz*, *Pi2(t)*, *Piz-t*, *Pi4(t)*, *Pib*, *Pit*, *Pish*, *Pi1*, *Pi3*, *Pi5(t)*, *Pi7(t)*, *Pi9*, *Pi12(t)*, *Pi19*, *Pik-m*, *Pi20*, *Pita2*, *Pita*, *Pi11(t)* and *Piz5*) were used to test the efficacy of resistance to 15 isolates from three provinces of South Central Vietnam. As a result, it can be better understand the resistance mediated by a specific *R* gene. Such as, the monogenic variety carrying the *Pi4(t)* gene was demonstrated to prevent infections by *P. oryzae* isolates in the BD rice growing regions, but not in the QN and QNG region. In addition, it was able to identify the effective major *R* gene(s) for different rice growing regions. Based on disease reactions, it is proposed that *Pik*, *Pik-p*, *Pik-h*, *Piz*, *Pi4(t)*, *Pi1*, *Pi7(t)*, *Pi9*, *Pish*, *Pik-m* and *Pita* can be used as *R* gene donors for improved blast resistance in South Central Vietnam (Fig. 2). Furthermore, *Pik-p* and *Pik-h* appear to be resistant to most isolates and can be used as these blast *R* genes to aid in breeding rice variety resistance of blast disease in South Central Vietnam. A combination of *Pik-p* or *Pik-h* with one of

Table 3 Disease reaction of 26 rice monogenic lines with 15 isolates of *P. oryzae*.

<i>R</i> genes	QN 49	QN 95	QN 125	QN 186	QN 239	QNG 63	QNG 109	QNG 158	QNG 193	QNG 249	BD 38	BD 92	BD 128	BD 183	BD 258	Amount of incompatible isolates	Percent of incompatible isolates (%)
<i>Pia</i>	R	S	S	R	S	S	R	S	S	S	S	S	R	S	R	5	33.3
<i>Pii</i>	S	S	S	S	S	S	S	R	S	R	S	R	S	S	R	4	26.7
<i>Pik-s</i>	S	R	S	S	R	S	R	S	R	S	R	S	R	R	S	7	46.7
<i>Pik</i>	S	R	R	S	R	R	R	R	R	S	R	R	R	R	R	12	80.0
<i>Pik-p</i>	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	14	93.3
<i>Pik-h</i>	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	14	93.3
<i>Piz</i>	R	R	R	S	S	R	R	R	R	S	R	R	R	R	R	12	80.0
<i>Pi2(t)</i>	S	R	S	S	R	R	S	S	S	R	R	S	R	R	S	8	53.3
<i>Piz-t</i>	S	R	S	S	R	R	S	S	R	S	S	R	S	S	R	6	40.0
<i>Pi4(t)</i>	S	S	R	R	R	R	S	R	R	S	R	R	R	R	R	11	73.3
<i>Pib</i>	R	S	S	S	S	S	R	S	S	S	R	R	R	R	S	6	40.0
<i>Pit</i>	S	S	R	S	S	R	S	S	S	R	S	R	S	R	R	6	40.0
<i>Pish</i>	S	R	R	R	S	R	R	R	R	R	R	R	R	R	R	13	86.7
<i>Pi1</i>	R	S	R	S	S	R	R	R	R	S	R	R	R	R	R	11	73.3
<i>Pi3</i>	S	S	S	S	S	R	R	S	S	S	R	R	R	R	S	6	40.0
<i>Pi5(t)</i>	S	S	R	R	S	S	R	S	S	S	S	S	R	S	S	4	26.7
<i>Pi7(t)</i>	R	S	R	S	R	R	R	R	R	S	R	R	R	R	R	12	80.0
<i>Pi9</i>	R	R	R	S	S	R	R	R	S	R	R	R	R	R	R	12	80.0
<i>Pi12(t)</i>	S	S	R	S	R	R	S	S	R	S	S	R	R	S	R	7	46.7
<i>Pi19</i>	S	R	R	S	S	S	R	S	R	R	S	R	S	R	R	8	53.3
<i>Pik-m</i>	R	R	S	S	R	R	R	R	R	S	R	R	R	R	R	12	80.0
<i>Pi20</i>	S	R	S	S	R	S	S	S	R	R	R	S	S	R	S	6	40.0
<i>Pita2</i>	S	S	R	S	R	R	S	S	R	S	R	R	R	R	R	9	60.0
<i>Pita</i>	R	R	R	S	R	R	S	R	R	S	R	R	R	R	R	12	80.0
<i>Pi11(t)</i>	S	S	R	S	R	S	R	S	R	S	S	R	S	S	R	6	40.0
<i>Piz5</i>	S	S	S	S	R	S	R	S	S	S	S	S	R	S	R	4	26.7
Number of virulence isolates	16	12	10	22	10	9	9	14	9	19	9	6	8	7	6		
Percent of virulence isolates (%)	61.5	46.2	38.5	84.6	38.5	34.6	34.6	53.8	34.6	73.1	34.6	23.1	30.8	26.9	23.1		

R = resistant; S = susceptible.

the genes (*Pik*, *Piz*, *Pi4(t)*, *Pi1*, *Pi7(t)*, *Pi9*, *Pik-m* and *Pita*) can also be effective in preventing blast infections in South Central Vietnam (Table 3).

4. Discussion

In Vietnam, rice blast disease is managed by the use of resistance genes in combination with seed treatments with systemic fungicides and fungicidal foliar sprays. The use of host resistance has proven to be the most effective and economical method for controlling rice blast [11, 31]. It is necessary to know that host resistance, pathogenicity characteristics,

including degree of pathogenicity, sporulation and spectrum of pathogenicity and environmental conditions are the critical factors determining the degree of plant disease [2]. The key step of controlling disease is to know interaction between host resistance and the pathogen. Evaluation of rice germplasm and breeding lines with the selected isolates have been realized worldwide.

The different progenies from various crosses showed the different level of blast disease reaction. Such differential defense responses result from the interaction of dominant and resistant (*R*) gene of host

Fig. 3 Virulence frequency of the 15 *P. oryzae* isolates.

and avirulence (AVR) genes of pathogen as proposed gene-for-gene hypothesis [32]. In rice, the interaction between host plant and pathogen *P. oryzae* is well documented as gene-for-gene system [6]. The resistance and susceptible interaction on rice conferred by single amino acid substitution in *Pi-ta* leucine rich domain (LRD) or in the AVR-*Pi-ta* protease motif, result in loss of resistant in plant. Thus, the different genetic of rice lines used in this study showed different interaction host resistance and pathogen blast rice in South Central Vietnam.

In this study, the differential system consisting of 26 differential *R* genes and a universal susceptible LTH were used for rapid identification of the resistance spectra of *R* genes in field condition and greenhouse conditions in South Central Vietnam. Inoculation and evaluation were repeated three times, and the disease reaction of each variety was based on a standard of IRRI [26] and the method of Bastiaans [30]. The authors' results suggest that *Pik*, *Pik-p*, *Pik-h*, *Piz*, *Pi4(t)*, *Pi1*, *Pi7(t)*, *Pish*, *Pi9*, *Pik-m* and *Pita* are the most effective blast *R* genes in South Central Vietnam.

In the present study, 15 isolates were selected to represent the genetic diversity of rice blast populations at South Central Vietnam. Therefore, these results should provide a systematic way to map *R* genes in breeding parents and assist in the development of appropriate breeding strategies for achieving effective management of the blast disease. To our knowledge, this was the first identification of blast *R* genes for rice breeding in a specific rice growing area in South Central Vietnam.

5. Conclusions

Among 26 monogenic lines carrying 26 *R* genes evaluated on farmers field condition, seven monogenic lines containing genes *Pik*, *Pik-p*, *Pik-h*, *Piz*, *Pi1*, *Pi7(t)* and *Pik-m* are highly resistant, four lines (*Pi4(t)*, *Pish*, *Pi9(t)* and *Pita*) are moderately resistant, four lines are moderately susceptible and 11 lines were susceptible. Similarly, on greenhouse condition, 10 monogenic lines carrying genes *Pik*, *Pik-p*, *Pik-h*, *Piz*, *Pi4(t)*, *Pish*, *Pi1*, *Pi7(t)*, *Pi9*, *Pik-m* and *Pita* were high resistant with the percentages of resistant reactions were 80.0%, 93.3%, 93.3%, 80.0%,

73.3% 86.7%, 73.3%, 80.0%, 80.0%, 80.0% and 80.0%, respectively. These findings suggest that *Pik*, *Pik-p*, *Pik-h*, *Piz*, *Pi4(t)*, *Pish*, *Pi1*, *Pi7(t)*, *Pi9*, *Pik-m* and *Pita* may be useful for preventing blast disease in South Central Vietnam. A concerted effort should be made to incorporate these effective genes into adapted backgrounds and to develop gene combinations that can provide broad-based durable resistance to blast rice.

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