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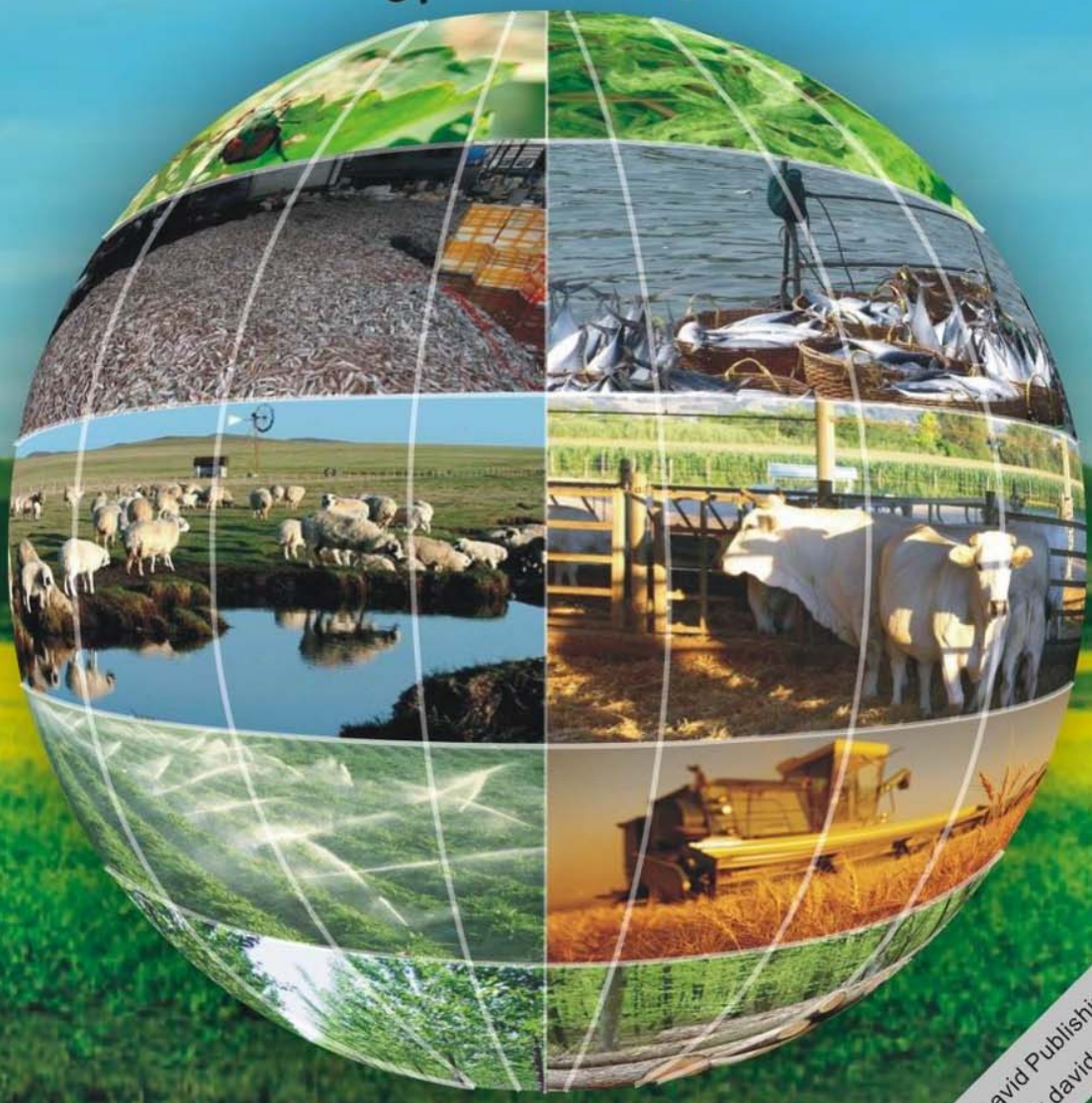

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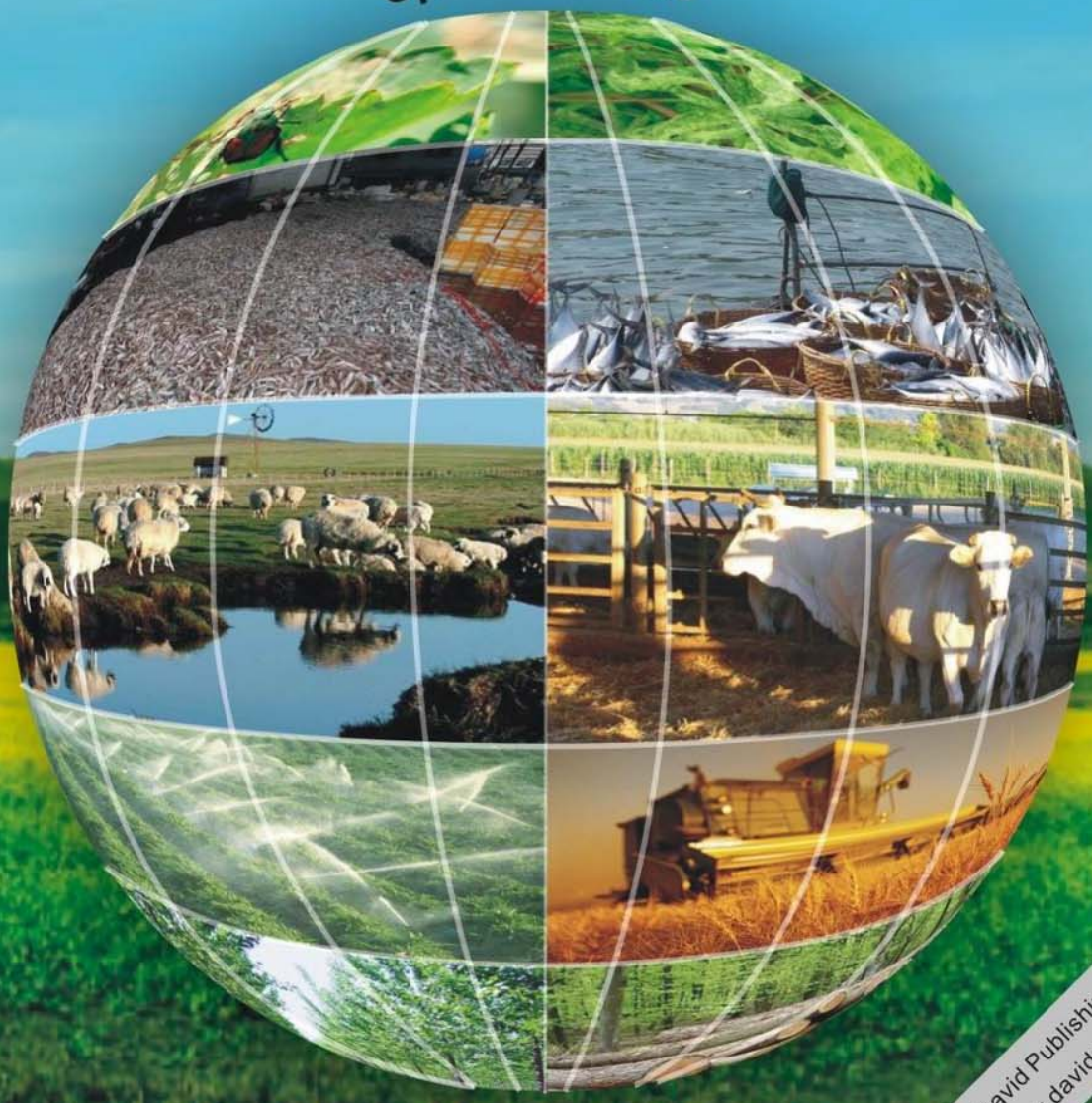

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Identification of Rice Blast Resistance Genes in South Central Coast of Vietnam Using Monogenic Lines under Field Condition and Pathogenicity Assays

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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 was 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, was 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 was 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 was 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 7 days. The disease severity was calculated as disease severity (%), calculated as Eq. (1):

Disease severity (%) = (sum of all numerical rating)/(total number of leaves or plants observed) × maximum rating × 100 (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 ith observation, t_i is time at the ith 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].

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

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 [30, 20]. For blast screening, the scores of five plants for each line were averaged and an actual infection means scales were 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 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	*Pia*	104.6	142.3	105.3
IRBLi-F5	*Pii*	98.3	91.3	95.2
IRBLks-F5	*Pik-s*	93.2	132.7	92.7
IRBLk-ka	*Pik*	0.0	0.0	0.0
IRBLkp-K60	*Pik-p*	0.0	0.0	0.0
IRBLkh-K3	*Pik-h*	0.0	0.0	0.0
IRBLz-Fu	*Piz*	0.0	0.0	0.0
IRBLz5-CA	*Pi2(t)*	78.1	72.4	0.0
IRBLzt-T	*Piz-t*	104.2	94.2	0.0
IRBLta-K1	*Pi4(t)*	28.4	15.8	19.5
IRBLb-B	*Pib*	77.3	82.5	0.0
IRBLt-K59	*Pit*	95.4	92.5	60.4
IRBLsh-S	*Pish*	19.4	21.6	18.5
IRBL1-CL	*Pi1*	0.0	0.0	0.0
IRBL3-CP4	*Pi3*	64.2	68.3	94.6
IRBL5-M	*Pi5(t)*	28.4	82.4	0.0
IRBL7-M	*Pi7(t)*	0.0	0.0	0.0
IRBL9-W	*Pi9(t)*	29.3	27.3	32.4
IRBL12-M	*Pi12(t)*	95.2	87.2	24.3
IRBL19-A	*Pi19*	62.4	68.3	31.5
IRBLkm-Ts	*Pik-m*	0.0	0.0	0.0
IRBL20-IR24	*Pi20(t)*	97.3	132.8	57.3
IRBLta2-Pi	*Pita2*	74.3	72.5	0.0
IRBLta-CP1	*Pita*	22.4	19.3	0.0
IRBL11-Zh	*Pi11(t)*	123.7	27.6	48.5
IRBLz5-CA	*Piz5*	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,

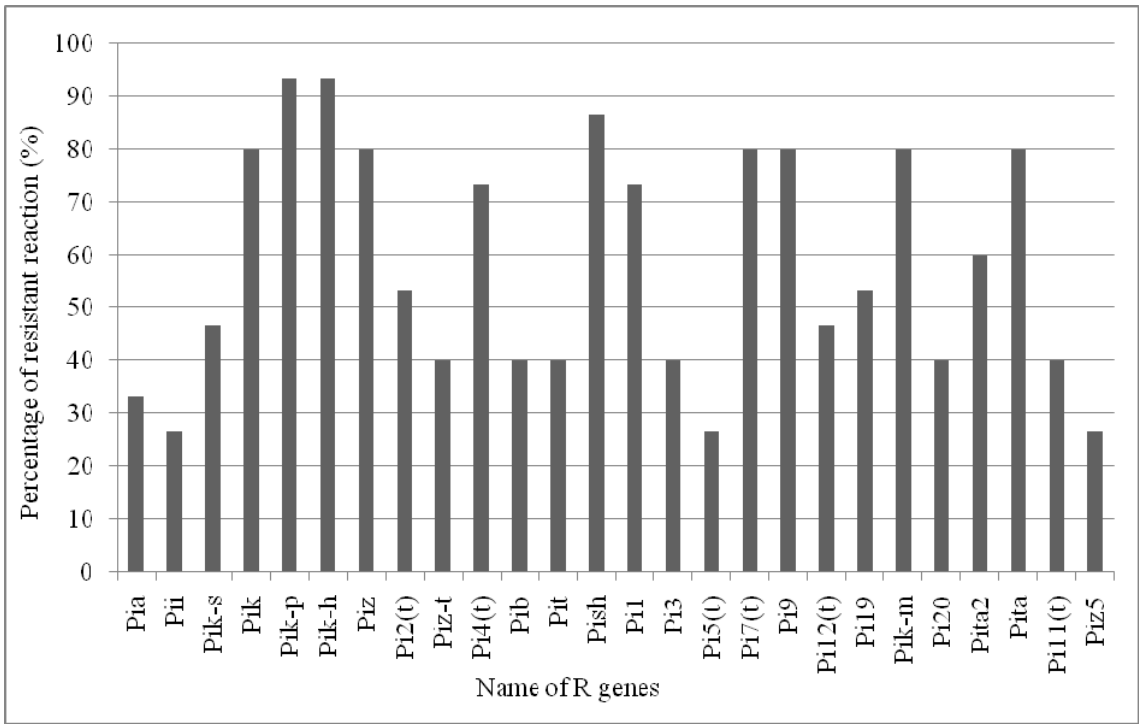

Fig. 2 Percentage of the resistant reaction of 26 monogenic lines containing an *R* gene to isolates of *P. oryzae*.

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*, *Pk-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). Futhemore, *Pik-p* and *Pik-h* appears 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*.

R 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 (%)
Pia	R	S	S	R	S	S	R	S	S	S	S	S	R	S	R	5	33.3
Pii	S	S	S	S	S	S	S	R	S	R	S	R	S	S	R	4	26.7
Pik-s	S	R	S	S	R	S	R	S	R	S	R	S	R	R	S	7	46.7
Pik	S	R	R	S	R	R	R	R	R	S	R	R	R	R	R	12	80.0
Pik-p	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	14	93.3
Pik-h	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	14	93.3
Piz	R	R	R	S	S	R	R	R	R	S	R	R	R	R	R	12	80.0
Pi2(t)	S	R	S	S	R	R	S	S	S	R	R	S	R	R	S	8	53.3
Piz-t	S	R	S	S	R	R	S	S	R	S	S	R	S	S	R	6	40.0
Pi4(t)	S	S	R	R	R	R	S	R	R	S	R	R	R	R	R	11	73.3
Pib	R	S	S	S	S	S	R	S	S	S	R	R	R	R	S	6	40.0
Pit	S	S	R	S	S	R	S	S	S	R	S	R	S	R	R	6	40.0
Pish	S	R	R	R	S	R	R	R	R	R	R	R	R	R	R	13	86.7
Pi1	R	S	R	S	S	R	R	R	R	S	R	R	R	R	R	11	73.3
Pi3	S	S	S	S	S	R	R	S	S	S	R	R	R	R	S	6	40.0
Pi5(t)	S	S	R	R	S	S	R	S	S	S	S	S	R	S	S	4	26.7
Pi7(t)	R	S	R	S	R	R	R	R	R	S	R	R	R	R	R	12	80.0
Pi9	R	R	R	S	S	R	R	R	S	R	R	R	R	R	R	12	80.0
Pi12(t)	S	S	R	S	R	R	S	S	R	S	S	R	R	S	R	7	46.7
Pi19	S	R	R	S	S	S	R	S	R	R	S	R	S	R	R	8	53.3
Pik-m	R	R	S	S	R	R	R	R	R	S	R	R	R	R	R	12	80.0
Pi20	S	R	S	S	R	S	S	S	R	R	R	S	S	R	S	6	40.0
Pita2	S	S	R	S	R	R	S	S	R	S	R	R	R	R	R	9	60.0
Pita	R	R	R	S	R	R	S	R	R	S	R	R	R	R	R	12	80.0
Pi11(t)	S	S	R	S	R	S	R	S	R	S	S	R	S	S	R	6	40.0
Piz5	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 [31, 11]. 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

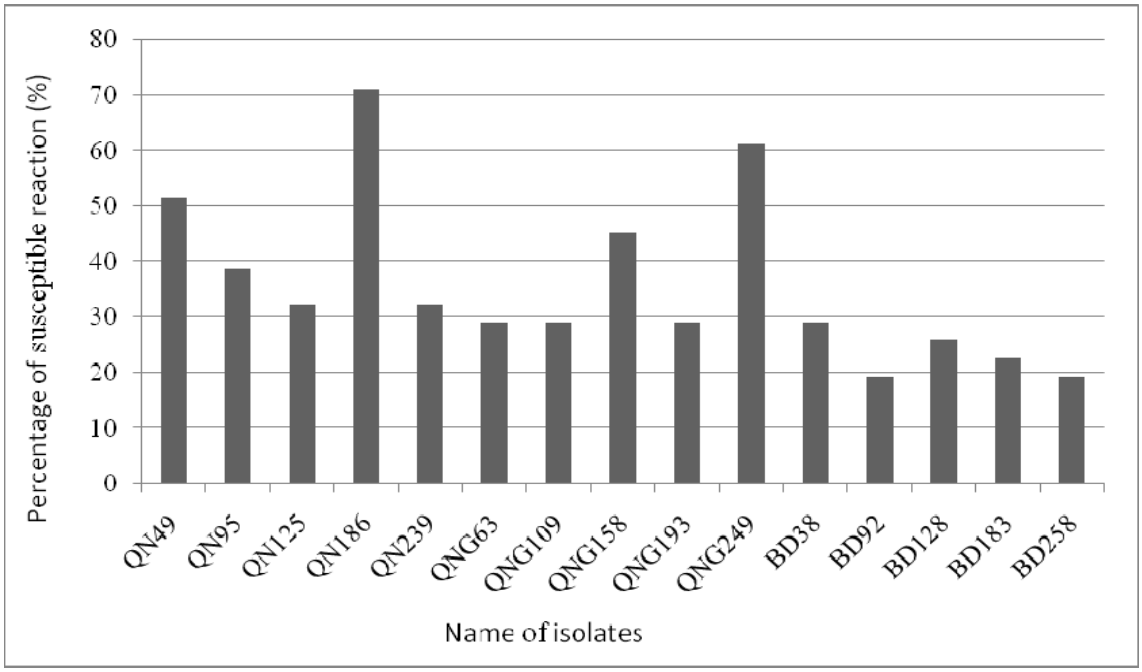

Fig. 3 Virulence frequency of the 15 *P. oryzae* isolates.

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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Evaluation on Agronomical Characteristics of F1 Hybrid Tomato Lines in Spring-Summer Season 2015 in Thua Thien Hue

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Abstract: The four F1 hybrid tomato lines (CT2011, CW2011, TS2011, and CLN2011) and two commercial F1 hybrid tomato cultivars used as control (TN52 and TN561) were evaluated for yield and yield components. The efforts were made to minimize reliance on the import of hybrid seed. The experiment was laid out in randomized complete block design (RCBD) with three replications in spring-summer season 2015. Data was recorded on five plants for each replication. The results indicated that all of F1 tomato hybrid lines grew well under Thua Thien Hue condition. CT2011 had the highest plant height, followed by TS2011 and CLN2011. CT2011 is a cherry type and obtained the highest percentage of fruit setting (82.87%) and the highest of Brix (4.97). This line also had high yield (70.91 tons/ha). TN52 had the highest yield (80.69 tons/ha) among the processing tomato lines, and TN561 had the biggest fruit (90.1 g). All of F1 hybrid tomato lines had high resistant level to late blight and bacterial wilt diseases.

Key words: Hybrid, bacterial wilt, late blight, *Solanum lycopersicum*, Thua Thien Hue.

1. Introduction

Tomato is one of important *Solanum* crops occupying 14% of world vegetable production in 2010 [1]. It is popular fruit vegetable and gives high fresh consumption in many countries, including Vietnam, because of its high nutrition and good adaptability under different agro-climatic conditions. Rick [2] indicated that in the US, tomatoes are ranked the first among fruits and vegetables by abundant sources of vitamins and minerals. In addition, tomatoes are rich of lycopene, a phytochemical that protects cells from oxidants [3]. Tomato is one of the most expected vegetables, not only rich nutrients but also high economic efficiency allows be cultivating and investing commonly in the central provinces of Vietnam, including Thua Thien Hue.

Due to high demand of tomatoes during entire year, enhancing tomato production and productivity are needed. Moreover, consumers are concerning about food safety due to pesticide residues on products. Thus, to restrict abuse of pesticides, the disease resistance cultivars are needed. Thus, many breeding programs aim to select F1 lines having good characteristics as well as resistant ability, high yield and good quality. Choudhary et al. [4] emphasized extensive utilization of heterosis to increase tomato production. In addition, Yordanov [5] indicated that the heterosis manifestation in tomato is in the form of the faster growth and development and increased productivity. However, farmers are used to use old cultivars or import new hybrid tomatoes for sowing next season, which caused qualitative decreasing,

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degeneration and high cost. In this study, the authors evaluated four F1 hybrid tomato lines, which were made at National Institute of Horticultural and Herbal Science (NIHHS), Korea, for their agronomical characteristics under Thua Thien Hue condition.

2. Materials and Methods

2.1 Materials

The four F1 hybrid tomato lines, namely CT2011, CW2011, TS2011 and CLN2011, which were made at NIHHS, Korea in 2011, and two commercial F1 tomato cultivars as control, namely TN52 and TN561, which are commonly used in Thua Thien Hue, were used in this study (Table 1).

2.2 Methods

The experiment was conducted during spring-summer season from January to May in 2015 at Agronomy Faculty of Hue university of Agriculture and Forestry. The experiment was laid out in a randomized complete block design (RCBD) with three replications. Each plot was 1.2 m^2 containing six plants for each replication. Tomato population was obtained of 50,000 plants/ha. The distance between row to row was 50 cm and the distance between plant to plant was 40 cm. Seeds were sown in a plastic tray. When the seedlings grew to reach 5-6 true leaves, they were ready to transplant into experimental field. Taking care methods were applied regularly and uniformly to keep plant health. According to the guidelines of the national technical regulations QCVN01-63:2011/BNNPTNT of the Ministry of Agriculture and Rural Development, the relevant field techniques for this study were applied.

Table 1 List of tomato lines used.

No.	Name of hybrid	Place of collection
01	CT2011	NIHHS
02	CW2011	NIHHS
03	TS2011	NIHHS
04	CLN2011	NIHHS
05	TN52	Thua Thien Hue
06	TN561	Thua Thien Hue

The data parameters were saved on quantitative and qualitative characteristics in open field. Data included day to finish growth stages (day); vegetative characteristic, such as growth type, plant height, number of nodes and height from foot to the first flower, length and width of leaf (cm); morphological traits on leaf and fruit, as shape, color or firmness, flower number per bunch, percentage of flowering, fruit size (length, diameter, wall thickness…); yield components and pest situation. The data of five plants per experimental plot were recorded regularly. The Statistic 10.0 trial version was used to analyze analysis of variance (one-way ANOVA) and compare differences between means values by Duncan's test at $P < 0.05$ [6].

3. Results and Discussion

3.1 Morphological and Vegetative Characteristic of Plant

Depending on the ability of growth and branching, growth types of tomatoes expressed different as indeterminate, determinate or semi-determinate. Growth habit of the F1 hybrids tomato lines was indeterminate or semi-determinate. CT2011, TS2011 and CLN2011 had indeterminate growth, and the others had semi-determinate (Table 2). Leaf shape included two types; potato leaf was found in CT2011, TN52 and TN561 hybrids, and regular leaf was found in the remains. Leaf color of tomato lines changed from green to dark green. Leaves of CT2011 and CW2011 had dark green color, TS2011 and CLN2011 had grey green color, and TN52 and TN561 had green color.

Significant difference in plant height was observed among F1 tomato hybrid lines. The highest plant height was obtained in CT2011 (277.13 cm), whereas the shortest plant height was observed in CW2011 (134.7 cm). The plant height of the remains ranged from 149.80 cm to 263.73 cm. Singh et al. [7] had evaluated of tomato hybrid (*Lycopersicon esculentum* Mill.) in Allahabad agro-climatic conditions during

Table 2 Vegetative traits of F1 hybrid tomato lines.

No.	Hybrids	Growth type	Leaf shape	Leaf color	Plant height (cm)	No of nodes from foot to 1st flower (node)	Height from foot to 1st flower (cm)	Leaf length (cm)	Leaf width (cm)
01	CT2011	I	PL	DG	277.13[a]	11.80[a]	52.47[b]	41.80[ab]	27.96[a]
02	CW2011	S-D	RL	DG	134.70[c]	11.13[ab]	64.67[a]	44.84[a]	31.60[a]
03	TS2011	I	RL	GG	263.73[a]	10.00[bc]	57.40[b]	43.28[ab]	28.41[a]
04	CLN2011	I	RL	GG	216.37[ab]	10.65[ab]	58.70[ab]	43.80[a]	29.51[a]
05	TN52	S-D	PL	Gr	149.80[bc]	9.60[bc]	45.73[c]	40.28[ab]	33.27[a]
06	TN561	S-D	PL	Gr	171.00[bc]	8.47[c]	39.80[c]	37.27[b]	30.65[a]
$LSD_{0.05}$					80.36	1.70	6.25	6.42	6.33

I = indeterminate, S-D = semi-determinate, PL = potato leaf, RL = regular leaf, DG = dark green, GG = grey green, Gr = green.
[a, b, c] Different letters in each columns indicate significantly different means (Duncan's test, $P < 0.05$).

Table 3 Flower and fruit characteristics of F1 tomato hybrid lines.

No.	Hybrid	Inflorescence type	No. of flowers per bunch (flower)	Percentage of fruiting (%)	No. of seeds
01	CT2011	Compound dichasium	8.61[a]	82.87[a]	78.87[abc]
02	CW2011	Compound dichasium	7.67[ab]	41.57[b]	82.63[ab]
03	TS2011	Compound dichasium	7.40[bc]	38.27[b]	30.37[d]
04	CLN2011	Compound dichasium	7.12[bc]	45.98[b]	51.13[cd]
05	TN52	Compound dichasium	7.31[bc]	41.07[b]	89.17[a]
06	TN561	Compound dichasium	6.45[c]	46.80[b]	55.27[bcd]
$LSD_{0.05}$			1.09	13.88	30.23

[a, b, c] Different letters in each columns indicate significantly different means (Duncan's test, $P < 0.05$).

the winter (rabi) season of 2011, and found that the best on the basic of plant height was 83.67 cm.

Number of nodes to first flower was significantly different between CT2011 and TN561 and expressed narrow variation. CT2011 had the highest number of nodes (11.80 nodes), whereas TN561 had the lowest number of node (8.47 nodes). There was significant difference in height from foot to first flower among F1 hybrid tomato lines. CW2011 had the longest (64.67 cm), and the shortest was observed in TN561 (39.80 cm). Leaves of CW2011 were the longest (44.84 cm), and the shortest one was control TN561 (37.27 cm). There was no significant difference about leaf width among F1 tomato hybrids lines. It ranged from 27.96 cm to 33.27 cm (Table 2).

3.2 Flowering Ability

Inflorescence type of all tomato lines was compound dichasium (Table 3). Significant differences were detected in number of flowers per bunch among F1 tomato hybrid lines. It ranged from 6.45 to 8.61 flowers per bunch. The highest number of flowers was found in CT2011 (8.61 flowers) and significantly different from the others, except CW2011 (7.67 flowers). The number of flowers of control TN561 was observed to be the lowest (6.45 flowers), followed by CLN2011, TN52 and TS2011 hybrids with 7.12, 7.31 and 7.40 flowers, respectively. The highest fruiting rate was observed in CT2011 (82.87%) and obviously different significance from the remains. Percentage of fruiting of TN561 was 46.80%, which was similar to CLN2011 (45.98%). The lowest percentage of fruiting was observed in TS2011 (38.27%). The analyzed data showed large variation on number of seeds. TN52 revealed the highest number of seeds per fruit (89.17 seeds), next were CW2011 (82.63 seeds) and CT2011 (78.87 seeds). Number of seeds per fruit of the remains ranged from 30.37 seeds to 55.27 seeds.

3.3 Fruit Morphological and Qualitative Characteristics

The morphological characteristics were described by one time observation and recorded in Table 4. Almost fruits of the F1 hybrid tomato lines had medium firmness, excepted for CT2011, which had soft fruits. Color of fruit shoulder changed from light green to dark green among F1 hybrids tomato lines. Fruits of CW2011, TS2011 and TN52 had light green shoulder before ripening. Dark green was observed in TN561. Fruits of CT2011 and CLN2011 had green shoulder. At maturity stage, all of F1 hybrid tomato lines revealed red fruit color. However, fruit flesh color was different between lines. Red color was observed in CT2011 and CLN2011, whereas orange-yellow was in CW2011, TS2011 and TN52. Fruit of TN561 had pink fruit flesh. The fruit shape is dependent on genetic and may distinguish between varieties. Recorded data described four type of fruit bottom shape, such as flat, point, pointless and depress. TN52 and TN561 had depressed fruit bottom, whereas CT2011 and CLN2011 had flat fruit bottom. Fruit bottom was pointed in CW2011 and pointed less in TS2011. Wetness of flesh and firmness of fruit are related. Only CT2011 obtained soft fruit, because it had wet flesh. Therewith, fruits of TS2011 and TN52 had wet flesh. Mild dry was found in the remains. Tasty of fruits of CT2011 was mild sour, and sour in TS2011, CLN2011 and TN52. Tasteless was found in CW2011 and TN561.

Fruit quality was described by parameters, including diameter, length, pericarp thickness, number of locules and Brix, and presented in Table 5. There was significant difference in fruit diameter among F1 tomato hybrid lines. TS2011 had the widest fruit (5.53 cm), followed by CLN2011 (5.47 cm), TN52 (5.44 cm) and TN561 (5.19 cm). The smallest fruit width was observed in CT2011 (2.84 cm). While Singh et al. [7] had reported that the tomato hybrid (*Lycopersicon esculentum* Mill.) line that well adapted in Allahabad agro-climatic conditions during the winter (rabi) season of 2011 had fruit diameter 6.47-6.65 cm (polar and radial), Farooq et al. [8] had reported that fruit diameter of tomato hybrid line ranged from 5.49 cm to 7.89 cm. Thus, significant differences in fruit diameter were observed in this study and previous studies [8, 9].

Table 4 Fruit morphological traits.

No.	Hybrid	Firmness	Color of fruit shoulder	Fruit color at maturity	Fruit flesh color	Shape of bottom fruit	Wetness (flesh)	Tasty
01	CT2011	Soft	Green	Red	Red	Flat	Wet	Mild sour
02	CW2011	Medium	Light green	Red	Orange-yellow	Point	Mild dry	Tasteless
03	TS2011	Medium	Light green	Red	Orange-yellow	Point less	Wet	Sour
04	CLN2011	Medium	Green	Red	Red	Flat	Mild dry	Sour
05	TN52	Medium	Light green	Red	Orange-yellow	Depress	Wet	Sour
06	TN561	Medium	Dark green	Red	Pink	Depress	Mild dry	Tasteless

Table 5 Fruit qualitative characteristics.

No.	Hybrid	Fruit diameter (cm)	Fruit length (cm)	Fruit firmness (mm)	No. of locules (locular)	Brix
01	CT2011	2.84^{c}	2.57^{c}	3.00^{c}	2.40^{d}	4.97^{a}
02	CW2011	5.09^{b}	4.89^{b}	4.73^{b}	4.43^{a}	2.95^{d}
03	TS2011	5.53^{a}	5.27^{a}	6.02^{a}	3.90^{abc}	3.35^{bc}
04	CLN2011	5.47^{ab}	5.11^{ab}	6.12^{a}	4.43^{a}	3.18^{cd}
05	TN52	5.44^{ab}	5.22^{a}	5.99^{a}	3.60^{bc}	3.45^{bc}
06	TN561	5.19^{ab}	5.06^{ab}	6.25^{a}	3.23^{c}	3.51^{b}
$LSD_{0.05}$		0.41	0.27	0.71	0.81	0.27

[a, b, c] Different letters in each columns indicate significantly different means (Duncan's test, $P < 0.05$).

Fruit length had wide variation among tomato lines ranged from 2.57 cm to 5.27 cm. TS2011 produced the longest fruit length (5.27 cm) and CT2011 had the shortest fruit length (2.57 cm). Fruit length of CLN2011, TN52 and TN561 lines were alike by 5.11, 5.22 and 5.06 cm, respectively.

Fruit firmness varies significantly among the hybrids. Fruit firmness of CT2011 was the lowest (3.00 mm), next was CW2011 (4.73 mm) and they are significantly different from the others. The fruit firmness of the remains ranged from 5.99 mm to 6.25 mm. The fruit firmness in this study was higher than those in study of Farooq et al. [8], similar to fruit firmness of Chaudhry et al. [10] and correlated with the studies of Kanno and Kanno [11] and Hall [12] who also observed significant differences in fruit firmness in different cultivars of tomato.

A significant difference was observed among tomato hybrids for number of locules. Number of locules showed wide variation from 2.40 locules to 4.43 locules. CW2011 and CLN2011 had the highest number of locules (4.43), whereas CT2011 had the lowest number of locules (2.40). The result in this study is similar with those in study of Farooq et al. [8] and correlated with the study of Kartofel [13] who obtained varied number of locules in different tomato cultivars.

There was significant difference in Brix among F1 tomato hybrid lines. The results indicated the highest Brix was found in CT2011 (4.97) and significantly different from the others. The lowest Brix was observed in CW2011 (2.95).

3.4 Yield Components and Yield

Yield and yield components of pepper were influenced by conditions of growth and cultivation, and potential of cultivar. Fruit weight and number of fruits per plant are relevant components to use as selection criteria for improving pepper yield. Fruits of almost F1 hybrid tomato lines were obviously different (Table 6). Fruits of CT201 were the smallest (16.47 g), which is belonged to cherry tomato. The remains ranged from 74.41 g to 90.1 g, which are belonged to processing tomato. TN561 had the biggest fruits (90.10 g), next was TN52 (87.64 g) and TS2011 (82.96 g). Chaudhry et al. [14] evaluated 10 tomato hybrids under plastic tunnel. The maximum fruit weight of 167.11 g was observed in Carmello. Chaudhry et al. [15] evaluated six indeterminate tomato hybrids under plastic tunnel, and Carmello gave the highest fruit weight of 163.33 g. Farooq et al. [8] had evaluated 17 tomato hybrids under plastic tunnel and got the result that the maximum individual fruit weight of 170.63 g was recorded in NTT-05-08. Singh et al. [7] had reported that fruit weight of the tomato hybrid (*Lycopersicon esculentum* Mill.) line which was well adapted in Allahabad agro-climatic conditions during the winter (rabi) season of 2011 was 72.27 g. The fruit weight of tomato hybrid line in this study is similar with previous studies.

There was significant difference in number of fruit per plant between CT2011 and the others. Only cherry type tomato hybrid CT2011 produced the highest number of fruits per plant (82.87 fruits), whereas the

Table 6 Yield and yield components.

No.	Hybrid	Fruit weigh (g)	No. of fruit per plant	Yield (ton/ha)
01	CT2011	16.47^{e}	82.87^{a}	70.91^{ab}
02	CW2011	74.41^{d}	41.57^{b}	39.03^{bc}
03	TS2011	82.96^{c}	38.27^{b}	38.39^{bc}
04	CLN2011	75.09^{d}	45.97^{b}	22.67^{c}
05	TN52	87.64^{b}	41.07^{b}	80.69^{a}
06	TN561	90.10^{a}	46.80^{b}	40.95^{bc}
$LSD_{0.05}$		2.40	13.88	36.92

[a-e] Different letters in each columns indicate significantly different means (Duncan's test, $P < 0.05$).

Table 7 Level of damage by diseases and insect.

No.	Hybrids	Percentage of wilted plant by bacteria wilt disease (%)	Index of late blight disease (%)	Percentage of harmed fruit by fruitworm (%)
01	CT2011	20.00[a]	25.33[b]	48.00[a]
02	CW2011	6.67[ab]	26.67[b]	6.67[b]
03	TS2011	0.00[b]	18.67[b]	9.33[b]
04	CLN2011	6.67[ab]	29.33[b]	20.67[b]
05	TN52	20.00[a]	78.67[a]	18.67[b]
06	TN561	20.00[a]	72.00[a]	56.67[a]
$LSD_{0.05}$		18.75	16.09	22.91

[a, b] Different letters in each columns indicate significantly different means (Duncan's test, $P < 0.05$).

remains had number of fruit per plant ranged from 38.27 to 46.80. Chaudhry et al. [16] evaluated 12 indeterminate tomato hybrids and reported that cherry hybrid gave significantly higher number of fruits per plan. While Singh et al. [7] had reported that the tomato hybrid (*Lycopersicon esculentum* Mill.) line that well adapted in Allahabad agro-climatic conditions during the winter (rabi) season of 2011 had 22 of fruits per plant, Farooq et al. [8] reported that fruit number of tomato hybrid ranged from 11.75 to 30.26. Thus, fruit number in this study is in agreement with previous studies.

TN52 had the highest yield (80.69 tons/ha), followed by CT2011 (70.91 tons/ha). Yield of the remains ranged from 22.67 tons/ha to 40.95 tons/ha, and were statistically at par with each other. CLN2011 produced the lowest actual yield (22.67 tons/ha), followed by TS2011 (38.39 tons/ha). Thus, among processing tomato type, control hybrid variety TN52 have the highest yield (80.69 tons/ha), followed by control TN561 (40.95 tons/ha), but there is no significant difference with the rest of hybrid tomato lines. Farooq et al. [8] have evaluated 17 tomato hybrids which had yield ranging from 34.75 tons/ha to 71.85 tons/ha. It is thus concluded that CT2011 had high yield, good quality and should be grown under local condition for cherry tomato.

3.5 Pest Situation

All of four new F1 hybrid tomato lines had high resistant level to bacteria wilt and late blight disease. Bacterial wilt caused by *Ralstonia solanacearum* is one of the most damaging soilborne diseases of many important crops worldwide. The results in Table 7 showed that almost F1 hybrid tomato lines were affected slightly by bacteria wilt disease, except for TS2011. CT2011, TN52 and TN561 had 20% of infected plants, whereas CW2011 and CLN2011 had 6.67% of infected plants. The winter-spring season is considered as appropriate environmental conditions (temperature, humidity, sun…) for late blight (*Phytopthora infestan*) development. Late blight disease was observed in all of F1 tomato hybrid lines. Index of late blight disease ranged from 18.67% to 78.67%. TN52 and TN561 had the highest susceptible level to late blight, up to 78.67% and 72.00%, respectively. The remains were infected at low level as 29.33% in CLN2011, 26.67% in CW2011 and 25.33% in CT2011. Fruitworm (*Helicoverpa zea*) damages tomato at fruiting development stages. Percentage of harmed fruit by fruitworm ranged from 6.67% to 56.67% among F1 tomato hybrid lines. Of these, TN561 had the highest damaged level (56.67%), followed by CT2011 (48%).

4. Conclusions

All F1 tomato hybrid lines grew well under Thua Thien Hue conditions. CT2011, TS2011 and TN561 lines were considered as displaying good growth and development ability. Fruits of TS2011, CLN2011 and TN52 lines were suit for transporting and process. CT2011 is a cherry tomato, which had small fruit and high Brix, and good for fresh food and decoration. TS2011 had higher predicted yield than TN52 and

TN561. CT2011 produced higher actual yield than TN561. Almost new F1 hybrid tomato lines had high resistant level to bacteria wilt and late blight diseases. The F1 tomato hybrid lines gave good characteristics and should be designed in next experiments in different seasons and ecological areas to assess their potentiality to complete crop structure.

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