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Broad-Spectrum Resistance and Monogenic Inheritance of Bacterial Blight Resistance in an Indigenous Upland Rice Germplasm ULR207

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Abstract: Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *Oryzae* (*Xoo*) is a serious disease of rice worldwide that can reduce crop yield and affect food insecurity. A rice resistance variety is an alternate way to solve this problem. The broad-spectrum resistance (BSR) of ULR207 is important for durable resistance to several of the *Xoo* isolates. However, the inheritance of this resistance gene in ULR207 must be known before it can be utilized. Thus, this study aimed to survey the BB resistance gene with reference to the BB resistance gene for identification of non-analogous or analogous genes and confirmation of a broad-spectrum resistance, to investigate the gene effect, the number of genes, and the heritability of the BB resistance gene in the ULR207 variety. Six populations of two crosses (Maled Phai × ULR207 and RD6 × ULR207), i.e., ULR207 (Donor parent), Maled Phai and RD6 (Recurrent parent), F_1, F_2, BC_1P_1, and BC_1P_2 were constructed. These materials were evaluated for BB resistance by clipping methods under greenhouse conditions using a virulence isolate of a pathogen in Thailand. The results showed that ULR207 exhibited the strongest against BB with 0.8 of BSR with low area under the disease progress curve (AUDPC). Molecular screening for surveying of the BB resistance gene in ULR207 revealed a non-analogous resistance gene with resistance check varieties. The phenotype of the disease lesion length of F_2 and BC_1P_2 populations exhibited a ratio of 1:3 and 1:1 (resistant: susceptible), respectively, revealing a single recessive gene in both crosses. The scaling test parameters A, B, and C were non-significant ($p < 0.01$), indicating that variation in data was sufficiently explained by additive (d) and dominance (h) components. The gene action of ULR207 was controlled by additive gene action. Heritability of the two crosses, Maled Phai x ULR207 and RD6 x ULR207, exhibited high values with 0.817 and 0.716, whereas the numbers of the genes were 1.4 and 1.2, respectively. The result indicated that the breeding strategy could be employed in early generations when using ULR207 as a new source of bacterial blight resistance.

Keywords: generation mean analysis; gene action; recessive gene; heritability; clipping method

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1. Introduction

Bacterial blight disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a serious disease that affects rice yield loss up to 20–80% under suitable climates [1]. To solve the problem, several ways have been used such as chemical application, but toxicity and environmental-friendliness are serious concerns. Moreover, the *Xoo* isolates in Thailand have been documented which are highly diversified on both regional and national scales due to continuous mono-cropping with the susceptible rice variety [2,3]. The utilization of genetic resource is a better way to solve this problem. To date, numerous amounts of BB resistance genes have been reported over the last 50 years [4]. These were reported at different loci on chromosomes of resistance varieties [5–7]. The BB resistance variety IRBB21 carrying *Xa21* on chromosome 11 has been reported to be strong against bacterial blight and durable in Northern Vietnam and India [8,9]. Although this is a high potential

exotic source for improvement of the Thai elite variety, it always comes with a genetic linkage dragging and a non-specific resistance to the pathogen race [10].

In Thailand, BB resistance variety IR62266 carrying *xa5* gene was reported as having a high bacterial blight resistance with a broad-spectrum resistance found in central parts of Thailand [11,12]. The *Xa4* resistance gene was found to have a moderate resistance to the Thai *Xoo* isolate in at least one isolate test [13]. BB resistance rice varieties in Thailand have been recommended such as Suphan Buri 1, Suphan Buri 2, Suphan Buri 60, Suphan Buri 90, and RD31 [14]. However, the mutation and genetic diversity of the *Xoo* isolate could cause resistance breaking in these varieties. In addition, the use of the same resistance gene in large cultivation areas frequently causes loss of resistance by the selective pressure of the pathogen. Thus, the identification of new indigenous sources for breeding against disease is more compromising.

Although numerous resistant varieties have been reported, some of them have lost their effectiveness due to resistance breaking. Thus, evaluation to identify new resistance sources is most needed. On screening Thai indigenous lowland rice germplasm, it was found that five varieties; LLR023, LLR134, LLR137, LLR205, and LLR207 out of those ten resistant varieties were high performance in agronomic traits compared with KDML105, the most famous cultivar [15]. Furthermore, Thai indigenous upland rice germplasm was also evaluated for BB resistance and ULR207 was identified as the strongest variety [16]. However, that evaluation was practiced with only few *Xoo* isolates, which might not be sufficient for defining durable resistance. To be utilized as a donor for BB resistance, ULR207 is thus needed to confirm broad-spectrum resistance with various *Xoo* isolates. In addition, ULR207 cannot be accomplished since its inheritance has not been uncovered. Inheritance knowledge is essential for breeding program planning leading to the selection of suitable breeding methods [17].

To date, molecular techniques have been used as a tool to identify the R gene in rice germplasm and assist in improving rice cultivar with single and multiple genes [13,18–20]. Also, numerous research studies have been reported utilizing molecular markers for searching for the BB resistance gene in germplasm such as *Xa4* and *Xa7* resistance genes in Pakistani rice germplasm [21,22]—*Xa4*, *xa5*, *Xa7*, and *xa13* resistance genes in basmati rice [23] and *Xa4*, *xa5*, *Xa7* and *xa13* resistance genes in Thai rice germplasm [14]. The identification of BB resistance gene using molecular markers has been practiced and found to be a quick way to identify R gene in germplasm. The BB resistance gene was identified as the analogous gene or non-analogous gene, and could be used as the reported molecular marker for improving the elite rice. Hence, the identification of R gene by a molecular marker is needed.

Generation mean analysis (GMA) is an approach to understand the effect of genes (additive effect, dominant effect, and epitasis) in breeding programs [24,25]. This method has been employed worldwide due to its simplicity and cost effectiveness as it can estimate from means of six generations. GMA has been used to study the gene effects of quantitative traits, for example in chickpea [26], cotton [27], barley [28], and corn [29]. As for rice, cooking characteristics were studied and resulted in exhibits controlled by additive and dominance gene actions for the selected crosses involving aromatic rice [30]. In cowpea, seed resistance to *Callosobruchus chinensis* and *C. maculatus* resistance in TVu 2027 was studied which revealed additive and additive x dominance gene effects, which suggested that the selection of breeding lines should be performed in the advanced generation [31]. The knowledge of gene effect is beneficial for selecting an appropriate breeding method [32].

Therefore, the study was aimed (1) to survey the BB resistance gene with reference to the BB resistance gene for identification of non-analogous or analogous genes, (2) to confirm broad-spectrum resistance of the indigenous upland rice ULR207, and (3) to verify the inheritance of bacterial blight resistance in indigenous upland rice ULR207.

2. Materials and Methods

2.1. Confirmation of Broad-Spectrum Resistance for Bacterial Blight Disease

2.1.1. Plant Materials

To confirm resistance, ULR207 and sixteen check varieties were evaluated under greenhouse conditions during the dry season of 2019 at the Agronomy Field Crop Station, Khon Kaen University, Thailand (Table 1). The eleven reference varieties carrying the resistance gene and five check varieties were compared with ULR207 to identify the resistance gene against virulent isolate. This experiment was laid out using a completely randomized design (CRD) with 3 replications. The seeds of each variety were planted in a plastic tray and kept in the greenhouse at a temperature of 25–30 °C (min–max temperature) and 90% RH. Fertilizer was applied at 14 and 20 days after planting, with 40 kg/ha of N, P_2O_5, and K_2O.

Table 1. List and genetic background of indigenous upland rice, reference, and check varieties.

Varieties	Sources	*BB Resistance Genes*
ULR207	Indigenous, Thailand	Unknown
IRBB1	IRRI	*Xa1*
IRBB3	IRRI	*Xa3*
IRBB4	IRRI	*Xa4*
IRBB5	IRRI	*xa5*
IRBB7	IRRI	*Xa7*
IRBB8	IRRI	*xa8*
IRBB10	IRRI	*Xa10*
IRBB11	IRRI	*Xa11*
IRBB13	IRRI	*xa13*
IRBB14	IRRI	*Xa14*
IRBB21	IRRI	*Xa21*
IR62266	IRRI	Resistance check
IR21	IRRI	Resistance check
RD6	Department of rice, Thailand	Susceptible check
KDML105	Department of rice, Thailand	Susceptible check
Maled Phai	Indigenous, Thailand	Susceptible check

2.1.2. Bacterial Isolates and Inoculum Preparation

Ten different isolates of *Xanthomonas oryzae*. pv. *oryzae* collected from different parts of Thailand (Table 2) were used in this study. These isolates were purified by 3D cross streak and subsequently cultured on nutrient agar (NA) under ambient temperature with a dark condition for 48–72 h. The cultured bacteria was dissolved in sterile water and the concentration adjusted to OD600 = 0.6 by spectrophotometer [33].

Table 2. List of ten isolates of *Xanthomonas oryzae* pv. *oryzae* in Thailand.

Isolates	Sources	
	Provinces	Part of Thailand
UT2-1	Uthai Thani	Central
CM4-1	Chiang Mai	Northern
CM3-1	Chiang Mai	Northern
NB7-7	Nonthaburi	Central
PR5-1	Prachinburi	Eastern
NB7-8	Nonthaburi	Central
CN2-1	Chainat	Central
NY1-1	Nakhon Nayok	Central
SP1-1	Suphan Buri	Central
MS1-2	Maha Sarakham	Northeastern

2.1.3. The Inoculation of Bacterial Blight

A sterile scissor was used to cut 2 upper leaves of each around 2 cm from the leaf tip. Ten isolates were inoculated individually at 21 DAS with approximately 4 leaves by the clipping method [34]. The infected seedlings were kept in a moist chamber at 25–35 °C and over 85% relative humanity. Afterwards, the disease lesion length of an individual leaf was measured at 17 days after inoculation. Disease reaction was classified following the standard of [35] based on disease lesion length on leaf (cm), i.e., as follows: lesion length of 0–5 cm (resistance: R), more than 5.1–10 cm (moderate resistance; MR), more than 10.1–15 cm (moderate susceptible: MS), more than 15.1–20 cm (susceptible: S) and more than 20 cm (highly susceptible: HS) (Figure 1).

Figure 1. Bacterial blight disease symptom in the seedling stage of rice.

Broad spectrum resistance (BSR) was calculated following the method of Ahn [36]. The BSR ranged from 0 to 1; a BSR of 0 indicates that the rice variety is susceptible to all isolates. Meanwhile a BSR of 1 indicates that the rice variety is resistant to all isolates [37]. The disease lesion length was also used to calculate the severity index by Formula (1):

$$SI\ (\%) = (LLD/HLLD) \times 100 \quad (1)$$

When, LLD is the disease lesion length of each variety, HLLD is the highest lesion length disease of each day after inoculation time. The severity index was consequently used for analysis of the area under the disease progress curve (AUDPC) as Formula (2) following the method of Madden et al. [38]:

$$AUDPC = \sum I\ (SI\ (DAIi) + SI\ (DAIi + 1)/2) \times (ti + 1 - ti), \quad (2)$$

When SI (DAI) = severity index of each DAI, t = days after inoculation, i = 3 7 14 . . . 30 days after inoculation.

2.1.4. Data Analysis

The disease lesion length of the individual plant was analyzed by analysis of variance. This was performed by statistix10 software.

2.1.5. The Identification of BB Resistance Gene in Indigenous Upland Rice ULR207

The indigenous upland rice ULR207 and sixteen check varieties were surveyed for possessing eleven BB resistance genes including *xa1*, *Xa3*, *Xa4*, *xa5*, *Xa7*, *Xa8*, *Xa10*, *Xa11*, *Xa13*, *Xa14*, and *Xa21* by molecular markers presented in Table 3. The fresh leaves were used for DNA extraction. DNA samples were diluted to a concentration of 25 ng/µL, and 1 µL of each sample was used for PCR. The PCR samples amplified with BB resistance

gene were separated on 6% polyacrylamide gels (Himedia; Kennett Square, PA, USA.), and subsequently resolved using silver staining. The amplification profiles and the molecular sizes (bp) were determined based on the migration relative to the Phix DNA marker (Promega, Madison, WI, USA). Each allele was identified as resistant or susceptible when visually compared with standard bands.

Table 3. Details of primers used for identification of BB resistance gene.

Gene	Marker Name	Type of Marker	Forward (5′-3′)	Reverse (3′-5′)
Xa1	Xa1	Gene specific	ACTGCCCTCTTGCACACGCCTTTGG	CCGGTACATCAGTATTGTCCATCGG
Xa3	RM113	SSR	CACCATTGCCCATCAGCACAAC	TCGCCCTCTGCTGCTTGATGGC
Xa4	RM224	SSR	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG
xa5	PAxa5	Gene specific	CTGGAAGAAGCTCTTAATTT	GATTCCTTTAGCAAGGTGTG
Xa7	Xa7	Gene linked	CGATCTTACTGGCTCTGCAACTCTGT	GCATGTCTGTGTCGATTCGTCCGTACGA
xa13	xa13	Gene specific	AGCTCCAGCTCTCCAAATG	CATTGCTACTGGTGATGAAGG
xa8	RM214	SSR	CTGATGATAGAAACCTCTTCTC	AAGAACAGCTGACTTCACAA
Xa10	RM206	SSR	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG
Xa11	RM1350	SSR	CGCCCTAGTAGATAGGTAATTG	AAATCAGCAAGAAAGCTCTG
Xa14	RM303	SSR	GCATGGCCAAATATTAAAGG	GGTTGGAAATAGAAGTTCGGT
Xa21	RM21	SSR	ACAGTATTCCGTAGGCACGG	GCTCCATGAGGGTGGTAGAG

2.2. Genetic Analysis of Bacterial Blight Resistance in ULR207

2.2.1. Population Construction

To verify inheritance, six populations including P_1, P_2, F_1, F_2, BC_1P_1, and BC_1P_2 derived from two crosses (Maled Phai x ULR207 and RD6 x ULR207) were constructed. The indigenous upland rice Maled Phai (high anthocyanin and good eating quality but susceptible to bacterial blight) was crossed with ULR207 (resistance to bacterial blight) to obtain F_1, F_1 and was then crossed back to P_1 (Recurrent parent, Maled Phai) and P_2 (Donor parent, ULR207) to generate BC_1P_1 and BC_1P_2 population, respectively. F_1 individual was also self-pollinated to produce F_2 population (Figure 2a). To confirm inheritance of ULR207, RD6 cultivar (high aromatic, soft and good cooking quality but susceptible to bacterial blight) was crossed with ULR207 to obtain F_1 of the second population. The backcross and F_2 population were constructed the same as Maled Phai x ULR207 (Figure 2b). In this study, six populations of both crosses, Maled Phai x ULR207 and RD6 x ULR207, were employed to assess inheritance and heritability.

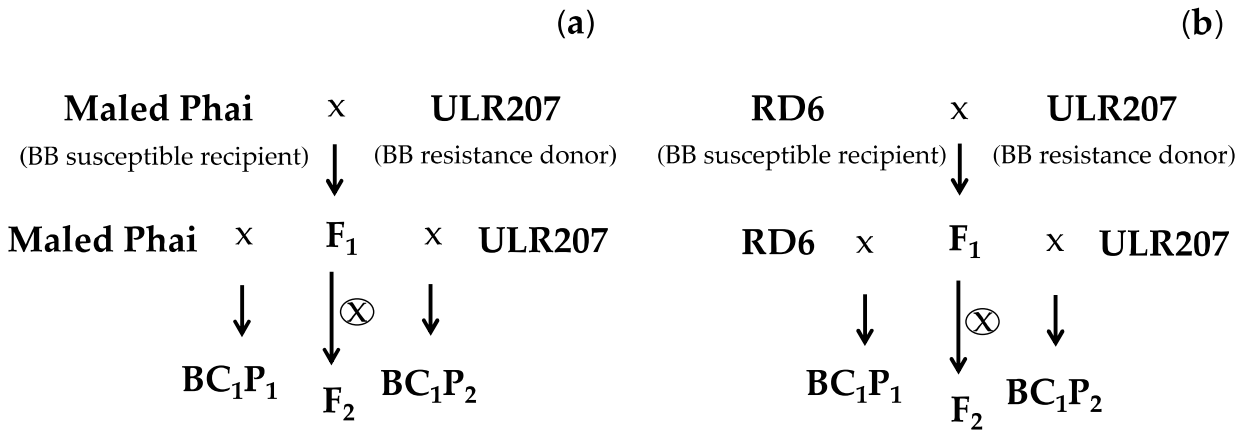

Figure 2. Schematic of six developed populations of both crosses Maled Phai x ULR207 (**a**) and RD6 x ULR207 (**b**).

2.2.2. Pathogenic Assay of BB Resistance

Six populations of both crosses were evaluated under greenhouse condition at the Agronomy Field Crop, Khon Kaen University, Thailand. Thirty seeds of P_1, P_2, and 2

check varieties including KDML105, IR62266, and fifty seeds of F_1, sixty seeds of BC_1P_1, BC_1P_2 as well as two hundred of F_2 populations were sown in a 72-holes tray. At 14 days after sowing (DAS), the seedling was applied with N fertilizer at the rate of 15 kg/ha and subsequently maintained under greenhouse conditions until 21 DAS for further inoculation. Inoculums of pathogen isolates were applied to individual plants of each population by artificial inoculation using the previous protocol mentioned above. Finally, isolate SP1-1 was identified as the most virulent that could distinguish the two parental lines. Hence, SP1-1 was cultured on nutrient agar (NA) by the cross-streak plate method for isolate purification under dark conditions for 72 h at ambient temperature. The cultured bacteria was dissolved with sterile water and the concentration adjusted to OD600 = 0.6 by spectrophotometer [33].

The inoculation was practiced by the clipping method [34]. The disease lesion length was recorded 14 days after inoculation. Classification of disease reaction followed the standard evaluation system of IRRI [39]. The assessment of disease lesion length by which BB lesion length of less than 5 cm was classified as resistant, while more than 5 cm was identified as susceptible [11].

2.2.3. Statistical Analysis

The chi-square test of goodness of fit was performed to assess the correspondence to the Mendelian pattern following [40,41] Formula (3):

$$x^2 = (\sum (Oi\text{-}Ei)^2)/Ei \quad (3)$$

The scaling test used for the adequacy of the additive–dominance model followed the method described by [42]. In addition, the generation mean analysis was estimated for gene effect as Formula (4) following the method described by Mather and Jink [43]:

$$Y = m + \alpha[d] + \beta[h] + \alpha^2\,[i] + 2\alpha\beta[j] + \beta^2\,[l] \quad (4)$$

where:

Y = the mean of one generation
m = the mean of all generation
d = the sum of additive effects
h = the sum of dominance effects
i = the sum of additive x additive interaction (complementary)
l = the sum of dominance x dominance interaction (duplicate)
j = sum of additive x dominance and α, $2\alpha\beta$, and β^2 are the coefficients of genetic parameters

The genetic parameters (m, [d], [h], [I], [j], [1]) were tested for significance using a *t*-test.

Heritability of broad sense and narrow sense were calculated as Formulas (5) and (6) following Warner [44]:

$$\text{Broad sense } (H_b^2) = [V_{F2} - (V_{P1} + V_{P2} + V_{F1})/3]/V_{F2} \quad (5)$$

$$\text{Narrow sense } (H_n^2) = [2V_{F2} - (V_{BC1P1} + V_{BC1P2})]/V_{F2} \quad (6)$$

When:

V = variance in population

Number of gene (N) was estimated by the equation in Formula (7) reported by Poehlman [45]:

$$N = (m1 - m2)^2/\{8(V^2_{F2} - V^2_{F1})\} \quad (7)$$

When
N = number of gene to control resistance
m1 = mean of P_1
m2 = mean of P_2
V_{F2} = variance in population F_2
V_{F1} = variance in population F_1

3. Results

3.1. Confirmation of Broad-Spectrum Resistance for Bacterial Blight Disease

The disease lesion lengths among varieties/lines were significantly different in all isolates (Table 4). The susceptible varieties including KDML105, RD6, and Maled Phai were highly susceptible to all isolates except CM3-1, PR5-1, CN2-1, and NY1-1. RD6 showed to be highly susceptible to NB7-7, NY1-1, SP1-1, and MS1-2, while Maled Phai showed to be susceptible to NB7-7, SP1-1, and MS1-2. Of most isolates that were moderately virulent, ULR207 exhibited a resistant reaction against CM3-1 and NB7-8 while moderate resistance to UT2-1, NB7-7, PR5-1, CN2-1, SP1-1, and MS1-2. Moreover, ULR207 performed the same resistance reaction as resistance varieties including IRBB1, IRBB3, IRBB5, IRBB8, and IRBB7. However, ULR207 was defeated by CM4-1 and NY1-1 isolates as it exhibited more than 10 cm of lesion length. Based on the broad-spectrum resistance (BSR), ULR207 revealed a high of 0.8 which was the same as reference varieties IRBB1, IRBB3, IRBB5, IRBB8, and IRBB7, whilst susceptible varieties, Maled Phai and RD6, showed a low of BSR. These results indicated that ULR207 provided effective resistance against the predominant *Xoo* isolates in Thailand. Moreover, SP1-1 was identified as the most virulent isolate with a high potential to distinguish disease reaction between ULR207, Maled Phai, and RD6 (Table 4). SP1-1 isolate was thus subsequently used for evaluation of six populations in the inheritance study.

Table 4. Disease lesion length of 17 rice varieties at 17 DAI against 10 *Xoo* isolates under greenhouse conditions.

Varieties	Isolates Code/Disease Lesion Length (cm)											
	Genes	UT2-1	CM4-1	CM3-1	NB7-7	PR5-1	NB7-8	CN2-1	NY1-1	SP1-1	MS1-2	BSR
ULR207	-	6.58	11	3.96	8.66	5.53	4.81	5.85	12.73	6.09	8.18	0.8
Maled Phai	-	13.31	13.79	11.02	15.18	12.72	11.87	11.18	12.56	17.51	17.71	0
RD6	-	17.28	19.92	16.88	21.34	16.27	19.64	12.01	20.69	21.34	22.33	0
KDML105	-	21.84	21.8	18.55	26.81	19.14	24.49	16.54	17.7	25.96	22.32	0
IRBB21	*Xa21*	16.19	18.53	13.89	16.31	13.96	15.96	10.21	13.13	17.05	17.96	0
IRBB1	*Xa1*	3.03	8.94	0.76	7.18	8.64	4.89	5.38	7.4	7.5	7.87	1.0
IRBB3	*Xa3*	6.28	8.77	6.94	6.23	3.59	5.27	3.04	4.02	5.58	6.17	1.0
IRBB14	*Xa14*	15.25	16.26	12.65	15.22	13.98	19.14	10.88	15.97	14.92	16.99	0
IRRBB13	*Xa13*	18.36	15.78	12.74	16.64	16.01	12.98	10.82	15.13	15.98	16.37	0
IR21	-	18.35	16.74	13.46	19.07	14.48	17.63	9.77	15.99	12.72	20.87	0.1
IRBB11	*Xa11*	13.41	9.88	11.09	15.73	12.94	16.03	9.72	11.11	12.32	13.43	0.2
IRBB4	*Xa4*	15.64	4.87	7.71	16.52	13.93	17.97	10.89	5.82	12.48	18.04	0.3
IRBB5	*Xa5*	4.08	1.61	1.79	2.19	1.89	2.46	2.14	3.31	1.11	1.63	1.0
IR62266	*Xa21*	13.86	2.38	2.03	9.32	12.88	10.18	8.32	1.77	11.64	12.02	0.7
IRBB8	*Xa8*	8.98	6.15	7.39	7.88	6.15	5.09	4.53	10.45	7.12	7.26	1.0
IRBB10	*Xa10*	16.17	14.07	13.19	12.98	13.3	18.75	9.67	15.21	16.84	18.75	0
IRBB7	*Xa7*	1.85	0.9	0.28	2.33	2.31	0.61	0.47	0.26	0.14	2.07	1.0
F-test		**	**	**	**	**	**	**	**	**	**	
C.V.%		18.52	17.61	12.41	16.75	23.41	18.38	19.68	39.73	23.75	20.72	

** Significant at $p = 0.01$.

The AUDPC of indigenous rice ULR207 individually infected by 10 *Xoo* isolates showed the lowest value range from 408.43 to 1170 among all isolates. Compared with

resistance check variety IRBB5, IRBB3, IRBB1, and IRBB7 from 43.88–172.35, 437.81–987.46, 242.97–983.25, and 582.41–977.17, respectively, ULR207 was slightly higher. On the other hand, the susceptible varieties KDML105, RD6, and Maled Phai exhibited a high AUDPC value in the range of 1582.49–2426.14, 1153.42–2042.72, and 942.19–1432.66, respectively which showed a high disease development, indicating that ULR207 and resistance check varieties were lower in disease development than the susceptible check varieties KDML105, RD6, and Maled Phai. However, the AUDPC of other resistance check varieties including IRBB21, IRBB14, IRBB13, IRBB11, IRBB4, and IRBB10 revealed a high value (Figure 3). This result demonstrated that indigenous upland rice ULR207 reacted against 10 isolates in a similar pattern to resistance check varieties. In addition, it showed a closely similar AUDPC with the reference varieties containing the *Xa1* and *Xa3* gene, IRBB1 and IRBB3, respectively.

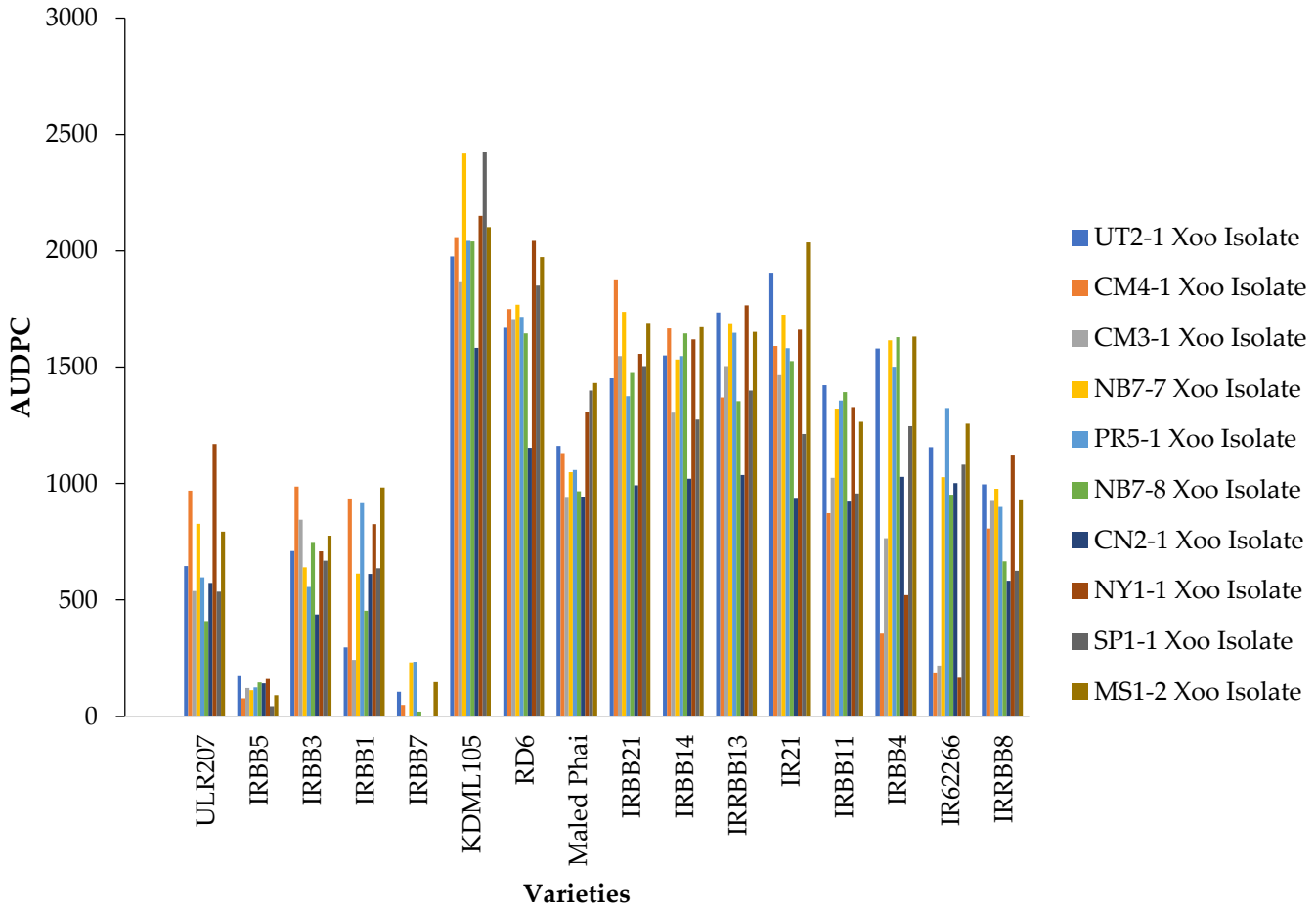

Figure 3. Area under disease progress curve of indigenous upland rice ULR207 and sixteen check varieties with 10 *Xoo* isolates.

Survey of R Gene

The amplicon length polymorphism is utilized for verifying the candidate resistance gene. The SSR marker RM224, used to screen the indigenous upland rice ULR207 for *Xa4*, was polymorphic between the positive control (IRBB4) and negative control (IR24), which delivered the amplicons of ~200 bp and ~180 bp, respectively. The indigenous upland rice ULR207 was polymorphic from the positive control (IRBB4). For *xa5*, *Xa7*, *Xa10*, *Xa11*, *Xa14*, and *Xa21* identification was conducted using gene specific marker PAxa5, RM206, RM1350, RM303, and RM21, respectively. The positive control IRBB4, IRBB8, IRBB10, IRBB14, and IRBB21 produced amplicons with ~200, ~120, ~200, ~220, and ~180 bp, respectively, while ULR207 was absent to the positive control. For *xa5*, *Xa7*, and *Xa11*, the indigenous upland

rice ULR207, and KDML105 produced amplicons of ~221, 1100, and ~220 bp, respectively, while the positive control IRBB5, IRBB7, and IRBB11 showed different amplicons from those. However, the amplifications of ULR207 for *Xa1*, *Xa3*, and *xa13* were monomorphic with either positive control or negative control (Table 5). These indicated that BB resistance in the indigenous upland rice ULR207 was the non-analogous gene with the 3 BB reference genes.

Table 5. Amplicon size of ULR207 and reference varieties amplified by markers associated/specific with 11 BB resistance genes.

Variety	Disease Reaction	Amplicon Length Polymorphism (bp)										
		Xa1	*Xa3*	*Xa4*	*xa5*	*Xa7*	*xa8*	*Xa10*	*Xa11*	*xa13*	*Xa14*	*Xa21*
		Gene Specific	RM114	RM224	PAxa5	Gene Linked	RM214	RM206	RM1350	RM224	RM303	RM21
ULR207	R	600 [-]	180 [-]	150 [-]	221 [-]	1100 [-]	180 [-]	180 [-]	220 [-]	500 [-]	210 [-]	200 [-]
KDML105	S	600 [-]	220 [-]	250 [-]	221 [-]	1100 [-]	120 [+]	190 [-]	220 [-]	500 [-]	240 [-]	180 [+]
Positive control	R	IRBB1 (600) [-]	IRBB3 (190) [+]	IRBB4 (200) [+]	IRBB5 (134) [+]	IRBB7 (300) [+]	IRBB8 (120) [+]	IRBB10 (200) [+]	IRBB11 (210) [+]	IRBB13 (500) [-]	IRBB14 (220) [+]	IRBB21 (180) [+]
Negative control	S	IR24 (600) [-]	IR24 (200) [-]	IR24 (180) [-]	IR24 (221) [-]	IR24 (1000) [-]	IR24 (120) [+]	IR24 (180) [-]	IR24 (220) [-]	IR24 (500) [-]	IR24 (240) [-]	IR24 (200) [-]

R = Resistance, S = Susceptible, bp = Base pairs, + or - = Presence or absence of respective genes.

3.2. Genetic Analysis

The averages of recurrent varieties Maled Phai and RD6 were 15.82 cm and 17.07 cm, respectively, and were revealed as highly susceptible (Figure 4). In contrast, the donor variety ULR207 in both crosses Maled Phai x ULR207 and RD6 x ULR207 were 1.21 cm and 1.89 cm, respectively, showed high resistance in disease lesion length (Figure 3). The distribution of F_2 population revealed a recessive hypothesis with 1 resistance and 3 susceptible. Meanwhile, the distribution of F_1 population showed susceptible in Maled Phai x ULR207 cross, and the distribution of backcross population BC_1P_1 was skewed toward susceptible. Meanwhile, the BC_1P_2 population skewed toward resistance in both crosses Maled Phai x ULR207 (Figure 4a). The results showed t signaling to the recessive hypothesis. However, the size of backcrossing in RD6 x ULR207 crosses have a few plants which show unclear segregation for explanation of the Mendelian hypothesis. For chi-square, the F_2 population of Maled Phai x ULR207 exhibited segregation of 47 resistant and 132 susceptible plants which respect the 1:3 ratio (p = 0.96). Meanwhile, BC_1P_2 population showed segregation of 13 resistant and 22 susceptible plants with 1:1 ratio (p = 0.49). Similarly, the cross RD6 x ULR207, F_2 population exhibited segregation of 37 resistance and 122 susceptible plants with 1:3 ratio (p = 0.98). BC_1P_2 population showed segregation of 0 resistance and 3 susceptible plants with 1:1 ratio (p = 0.39) (Table 6). Again, the result indicated that bacterial blight resistance in ULR207 was controlled by a single recessive gene.

Table 6. Chi-square test for frequency of F_2 and BC_1P_2 segregating lines of the two populations classified to two categories: resistant and susceptible plants.

Cross	Population	No. of Plants	No. of Plants *				Ratio	Chi-Square	*p*-Value
			Resistant		Susceptible				
			O	E	O	E			
Maled Fai x ULR207	F_2	179	47	44	132	135	1:3	0.24	0.96
	BC_1P_2	33	13	17	22	17	1:1	2.41	0.49
RD6 x ULR207	F_2	159	37	39	122	120	1:3	0.14	0.98
	BC_1P_2	3	0	1.5	3	1.5	1:1	3.00	0.39

* O = observed, E = expected.

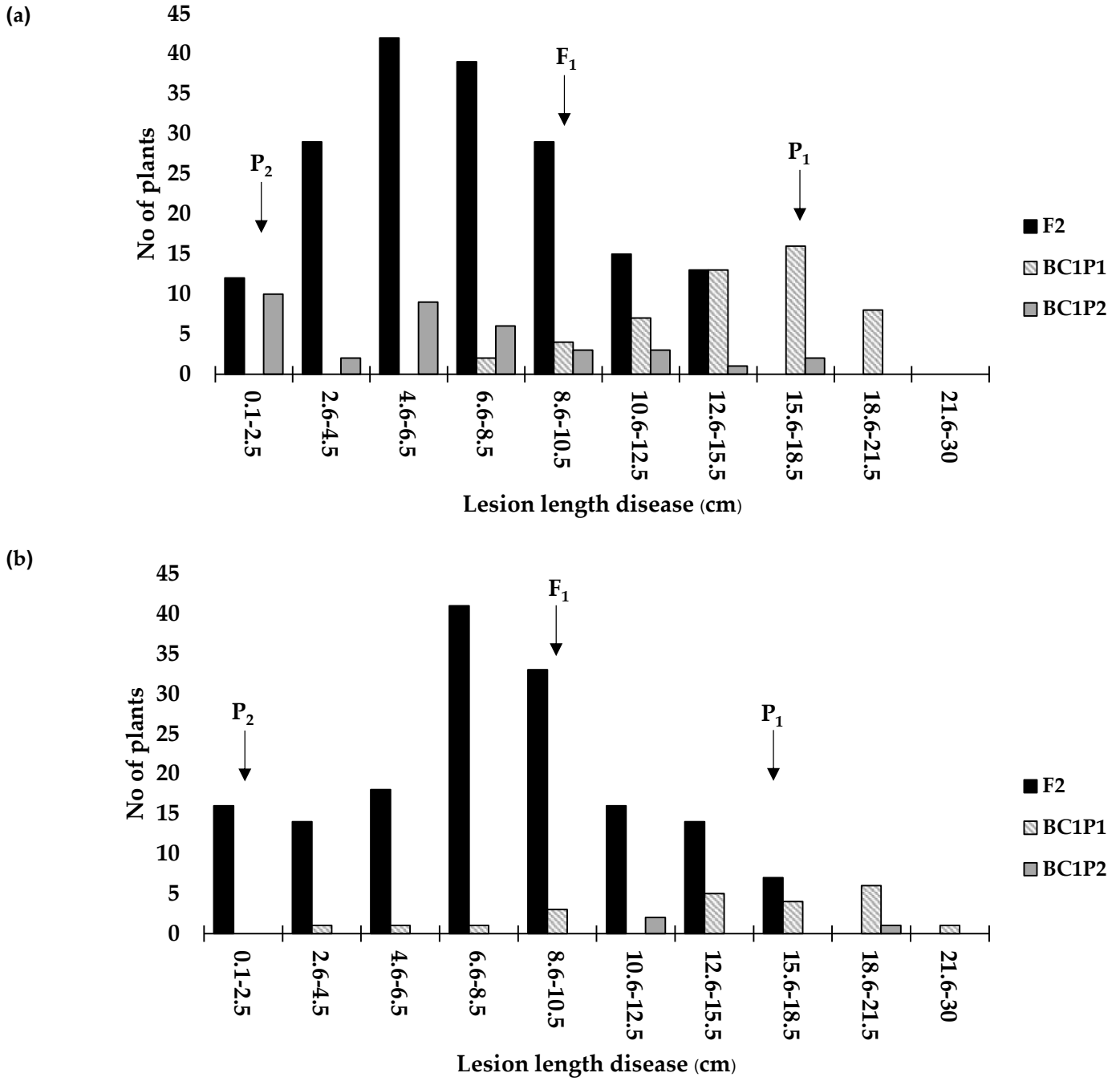

Figure 4. Frequency distribution of disease lesion length of Maled Phai x ULR207 (**a**) and RD6 x ULR207 (**b**) in P_1, P_2, F_2, F_1, BC_1P_1, and BC_1P_2 populations.

The scaling test analysis showed all scales, A B and C were not significant, indicating that the absence of non-allelic gene interactions or epistasis explains the variation of genetic value for the disease lesion length in both crosses (Table 7). Thus, the additive–dominance model is adequate for explaining the inheritance of disease lesion length traits. The mean parameter of both crosses, Maled Phai x ULR207 and RD6 x ULR207 for disease lesion length, exhibited a significant additive gene action (a) with 7.30 and 7.59, respectively. The gene action of dominant (h), additive × additive (i), dominant × dominant (l) and additive × dominant (j) were not significant in both crosses (Table 7). which indicated that the bacterial blight resistance in indigenous upland rice ULR207 is controlled by additive gene action.

Table 7. Scaling test and estimated gene effect for disease lesion length in six populations derived from both crosses Maled Phai x ULR207 and RD6 x ULR207.

Gene Action/Scaling Test	Crosses	
	Maled Phai x ULR207	**RD6 x ULR207**
A	36.56 ns	0.68 ns
B	1.40 ns	22.05 ns
C	35.74 ns	43.11 ns
m (mean)	65.63 **	49.57 **
d (additive)	7.30 **	7.59 **
h (dominant)	17.24 ns	46.18 ns
i (additive × additive)	4.65 ns	13.77 ns
l (dominant × dominant)	3.51 ns	−17.58 ns
j (additive × dominant)	−10.97 ns	−32.72 ns

A, B, C = additive × additive gene interaction, additive × dominance gene interaction and dominance × dominance gene interaction, respectively. ns, ** = non-significant difference and highly significant difference, respectively.

Broad-sense heritability of lesion length in estimates of both crosses were high at 0.817 and 0.716, respectively. Moreover, the number of genes in both crosses, Maled Phai x ULR207 and RD6 x ULR207, were estimated and demonstrated a single gene with 1.4 and 1.2, respectively. Likewise, the two crosses showed high narrow-sense heritability with 0.709 and 0.621. The result indicated that the BB resistance of ULR207 could be introgressed to a breeding progeny well (Table 8). The result of this study demonstrates that the genetics of bacterial blight resistance in ULR207 is a single recessive gene with additive gene action.

Table 8. Heritability and number of gene for disease lesion length in the two rice crosses.

Crosses	Heritability		No. of Gene
	Broad Sense	**Narrow Sense**	
Maled Fai x ULR207	0.817	0.709	1.4
RD6 x ULR207	0.716	0.621	1.2

4. Discussion

4.1. Local Rice Germplasm and Broad-Spectrum Resistance in Bacterial Blight Disease

In the present study, resistance check varieties are exotic and exhibited as susceptible to some isolates such as IRBB21, IRBB13, and IRBB14. In a previous study, IRBB21 carrying *Xa21* gene was susceptible to *Xoo* isolate in Bangladesh [46], indicating that, the BB resistance gene is race-specific against different *Xoo* isolates. The reported resistance cultivars IRBB21, IR62266, and SR1 exhibited susceptibility to the mixed isolate inoculation and natural infection experiment. The result indicated bacterial blight resistance cultivars might lose resistance ability when faced with specific *Xoo* isolates [9,14–16]. The specific of *Xoo* isolate was reported as able to lose bacterial blight resistance and led to ineffectiveness against an evolved *Xoo* [47,48]. Nonetheless, the present study showed the strength of IRBB5, IRBB3 IRBB7, and IRBB8 against 10 *Xoo* isolates with 1.0 of broad-spectrum resistance. IRBB5 variety carrying *xa5* gene has been used as a broad-spectrum donor in Thailand [12,13]; IR62266 also possesses *xa5* gene. It was used to breed RD6, the Thai glutinous rice cultivar for BB resistance in the seedling stage [37,49]. The result suggested that the *xa5* gene is appropriate for the introgression of BB resistance gene in Thai cultivar. Although these exotic sources, including IRBB5, IRBB3, IRBB7, and IRBB8 showed broad-spectrum resistance which could be utilized as a resistance parental line for Thai cultivar improvement, the utilization of an exotic source has always come with genetic linkage dragging [37,49,50]. and non-specific resistance to local *Xoo* isolate. Moreover, the utilization of exotic sources was concerned with the adaptability to local climate conditions. Adoption of an exotic source might lead

to poor agronomic performance and rice productivity. Using local indigenous rice is most likely seen as a good alternative way to accomplish the breeding program.

Evaluation of germplasm leads to identifying the source of resistance for the rice development program. In a previous study, ULR207 was evaluated as bacterial blight resistant with mixed 5 *Xoo* isolates by the clipped method under greenhouse conditions. ULR207 was reported to have high resistance to the mixed five diverse isolates of Thailand (BSA = 0.8) under the greenhouse experiment [16]. In addition, broad spectrum resistance is important to maintain durability against disease pathogen. However, ULR207 was not strong against CM4-1 and NY1-1 isolates due to gene-pathogen specificity. This indicated that resistance breaking in ULR207 is possible as previously reported [9,14–16]. In another way, the indigenous rice ULR207 could be used to reduce aggressiveness of pathogens due to the immediate resistance in ULR207 which can decrease pressure to pathogens. Leonard [51] showed that the selection pressure on the pathogen leads to a rapid increase in the frequency of the gene for virulence. Thus, the utilization of ULR207 to unextreme BB resistance is to enable a durable resistance in the breeding program.

AUDPC depicted the same slow disease development as in IRBB1 and IRBB3. As mentioned, *Xa1* [52,53] and *Xa3* [54–56] are dominant genes. The response of ULR207 is non-analogous with those two genes. In the present study, the disease progression of indigenous rice ULR207 was faster than the resistance check varieties IRBB5 and IRBB7. In another way, the slow disease progress of IRBB5 and IRBB7 occurred due to the strong selective pressure of a pathogen and resulted in overcoming of susceptibility in the plants [57]; this indicated that the resistance check varieties were intended to lose efficiency against bacterial blight. The demonstration of the present study is that rice ULR207 could be able to cause a decrease of the selective pressure of the pathogen which will result in a durable resistance contribution.

Surveying the BB resistance gene with the BB reference gene is important for understanding the resistance gene in ULR207. A previous study reported that the released rice varieties and landrace collected from eastern and northeastern India identified the presence of ten R genes of which 31% of the released rice varieties and 7% of the landrace rice were carrying R genes [58]. Also, 155 Thai germplasm rice varieties out of germplasm reported the presence of four resistance genes (*Xa4*, *xa5*, *Xa7*, and *xa13*) [13]. In addition, *xa21*, *xa13*, *xa5*, *xa4*, and *xa2* were identified in 10 local Malaysian rice varieties [59]. The upland rice ULR207 shows different amplicons with BB resistance genes from IRRI reference varieties. Since R gene *xa1*, *xa3*, *Xa8*, and *xa13* were monomorphic, it depicts the limitation of molecular techniques to identify the R gene. In this study, in the survey of BB resistance gene in indigenous upland rice, ULR207 is a non-analogous gene to reference resistance varieties. Perhaps, the bacterial blight resistance gene in ULR207 is the novel gene. The study found a single recessive gene in ULR207. For example, *xa5* gene was reported to be strong against *Xoo* in Thai isolate and used to introgress the BB resistance in elite cultivars [13]. Recessive genes have a limitation in the conventional breeding program in which the recessive gene could not express a phenotype in heterozygous generation. To advance the breeding population, it needs, in each heterozygous generation, to identify plants carrying a target recessive allele which is time consuming. Thus, to utilize this gene introgression through maker assisted backcrossing, QTL and marker associate with BB resistance is urgent and most needed.

4.2. Genetic Resistance of Bacterial Blight in ULR207

The distribution of the BC_1P_2 population exhibited a similar disease reaction with resistance variety ULR207 that indicated progress of the backcross population. This study, on the frequency of backcross population, showed a genetic contribution of bacterial blight resistance in the progeny of Maled Phai x ULR207 cross; this indicated that the backcross method is suitable for improvement with a high opportunity of success [60]. The backcrossing method has been widely used in rice improvement for introgression or substitution of a target gene from a donor parent to a recurrent parent [21]. However,

the RD6 x ULR207 population exhibited unclear segregation due to a lowerr number of population affected progeny segregations. Similar to that reported in [31], the frequency distribution of the backcross population in *C. chinensis* resistance, based on PDS (percentage of damaged seeds) and AUDPC, exhibited high resistance to *Callosobruchus chinensis* and *C. maculatus* in only a few plants and led to express unclear transgressive segregation of the segregated population. The results of this study indicated that the population size was not large enough to explain the transgressive resistance gene; the segregation pattern requires further confirmation.

For the chi-square test, the result of both crosses, Maled Phai x ULR207 and RD6 x ULR207, indicated that the bacterial blight resistance in ULR207 possessed a single recessive gene; this suggests that as [61] reported that the Bangladesh cultivar Kali Mekri 745 and Aus 295 exhibited resistance to race 4 and 6 of the Philippines race which were controlled by a single recessive gene. Similarly, Lee et al [62] reported that inheritance of bacterial blight resistance in cultivar Nep Bha Bong was controlled by a single recessive gene. In the previous studies, bacterial blight resistance controlled a single recessive gene in Bangladesh cultivars Kali Mekri745, Aus 295 [63] and Nep Bha Bong cultivar from Vietnam, Indonesia cultivar Latu [64]. The recessive gene of bacterial blight resistance has been broadly reported including *xa5*, *xa8*, *xa9*, *xa13*, *xa15*, *xa19*, *xa20*, *xa24*, *xa26b*, *xa28*, *xa31*, *xa32*, *xa34*, *xa41*, and *xa42* [63–65]. The individual gene has its race specificity that contributes to BSA [66], indicating that these recessive genes were differently expressed to pathogens while may be specific to local pathogens. For Thai rice cultivar improvement, the recessive gene in ULR207 leads to achieving durable resistance through the pyramiding gene in Thai rice cultivars.

The six-parameter analysis of both crosses, Maled Phai x ULR207 and RD6 x ULR207, revealed an additive (d) gene effect for the disease lesion length. Previous studies found additive and dominance gene effects in HUR-917 [67] as well as the epistasis effect which was unlikely reported for leaf infection [68]. The present study, resistance in ULR207 was mostly governed by additive gene action since the additive variation was larger than others. Similarly, additive gene rather contributed resistance than susceptibility in LRA-5166 [69], suggesting that bacterial blight resistance is simply to inherit progeny through the breeding approach. Additionally, to improve resistance, early generation selection in the breeding program is very possible [32,70,71].

Heritability of disease lesion length in both crosses, Maled Phai x ULR207 and RD6 x ULR207, exhibited a high value. Higher heritability values indicate the relative stronger selection effectiveness for a particular character [72]. On the other hand, a low heritability value indicate the selection is less effective due to the phenotypic variance being mostly influenced by environmental factors [73]. Thus, the breeding lines could be improved by a simple selection leading to being effective in early generation advancement [74–76]. The number of genes in both crosses, Maled Phai x ULR207 and RD6 x ULR207, exhibited a smaller number of genes. A previous study reported a lower number of genes are easy to manipulate for resistance breeding [32,77]. This indicated that this trait is governed by additive gene action and could simply be selected through phenotypic selection [78].

5. Conclusions

In conclusion, the bacterial blight resistance of ULR207 was identified as a non-analogous gene with reference BB resistance gene and exhibited broad-spectrum resistance to 10 Thai isolates. Also, the resistance in ULR207 was identified as a single recessive gene with additive gene action and the heritability exhibited a high value. The outcome of this study is the new BB resistance source which could be employed as a new donor parent for a breeding program. However, the suggestion of this study for future utilization is the design of a molecular marker associated with the BB resistance in ULR207 for a marker assisted selection program.

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