Detection of Bacterial Leaf Blight Resistance Genes in Indigenous Glutinous Rice Landraces

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Abstract. Bacterial leaf blight disease (BLB) caused by *Xanthomonas oryzae* pv is one of the most widespread devastating diseases of rice worldwide. In this study, a total of 86 indigenous glutinous rice landraces were examined for BLB resistant genes included *Xa4*, *xa5*, and *Xa7* by using molecular markers. The results indicated that 37 samples carrying resistant genes, in which *Xa4* was found in 11 samples, *xa5* determined in 6 samples, *Xa7* was in 19 samples, respectively. However, both of *Xa7* and *Xa4* were observed in only one sample. The resistant level against 10 bacterial strains carrying the resistant genes was also evaluated. We found that the number 6, 9, and 8 out of the 10 bacterial strains were resistant to the landraces which carried the *Xa4*, *xa5*, and *Xa7*. The bacterial strain number 5 was revealed highly toxic, causing infection of all samples. The agronomical traits, yield and yield components of 37 rice landraces included the resistant genes (*Xa4*, *xa5* and *Xa7*) were evaluated. Our findings may provide useful genetic sources in indigenous glutinous rice landraces to further develop BLB resistant rice lines via molecular breeding program.

Introduction

Bacterial leaf blights (BLB) disease in rice caused by *Xanthomonas oryzae* pv. *oryzae* bacterial, and is one of the most widespread devastating diseases in all rice-cultivating areas of the world. Under the pressure of bacteria infestation, the rice leaves were blighted, severely infected leaves tend to dry promptly, and then the chlorophylls will be non-synthesis, leading to reduce productivity up to 80%. If the disease becomes serious, rice yield can be totally lost. In rice producing countries in Asia, millions of hectares of rice paddies have seriously influenced yearly by infestation of BLB.

Rice (*Oryza sativa* L.) is the main food and providing daily meals for over 90 million people in Vietnam. According to the report of MARD (2015) [1] BLB was caused severe infestation across the country up to 11.921 ha in both Mekong and Red River Deltas. More seriously, total BLB affected areas in south provinces were 7.069 ha, increased 2.129 ha comparing in 2016 and 110 ha were severely lost [2] (MARD, 2017). Currently, there is no specific treatment for this disease, other synthetic pesticides are being used only to prevent the disease infestation. Therefore, using the resistant varieties are considered as the most feasible solution, which could help for environmental-friendly and possibly produce safety products instead of using agrochemical pesticides. In order to successfully improve BLB resistant rice varieties, the genetic sources of rice landraces play a key role in controlling this disease.

To date, total 42 resistant genes to BLB have been reported. Among them, there are 14 recessive genes [3, 4]. In Vietnam, 4 resistant genes including *Xa4*, *xa5*, *Xa7*, and *Xa21* have been reported as the significant resistant genes to pathogenic bacterial strains in the northern Vietnam. These genes have had important role in rice breeding for resistance to BLB. Some markers tightly linked with BLB resistant genes have been identified [5]. Therefore, application of molecular markers to detect genetic resources becomes the way of simple and accurate. Therefore, the objective of this study was to examine a total of 86 Vietnamese indigenous glutinous rice varieties for resistant genes *Xa4*, *xa5*, and *Xa7* by molecular markers. Subsequently, resistant levels of rice varieties carrying the resistant genes were evaluated by applying infection of the 10 bacterial strains. Besides, some major agronomical traits were evaluated to select the best varieties for glutinous rice for molecular breeding.

Materials and Methods

Plant Materials

The 86 rice landraces were kindly provided by the Center for Conservation and Development of Crop Genetics Resources (CCD-CGR), Vietnam National University of Agriculture were used in this study. The 10 pathogenic bacterial strains of BLB were collected in the northern Vietnam were used in this study as shown in Table 1.

Table 1. List of pathogenic bacterial strains of BLB.

No.	Strain	Code	Isolated	Gathering location
1	Strain 1	HUA 01043	TN 13-4	Soc Son, Ha Noi
2	Strain 2	HUA 0020131-1	Khangdan	Dong Trieu, Quang Ninh
3	Strain 3	HUA 0020131-2	Khangdan	Dong Trieu, Quang Ninh
4	Strain 4	HUA 020361	Neptan	Thuan Chau, Son La
5	Strain 5	HUA 02012	Tethom	Dong Anh, Ha Noi
6	Strain 6	HUA 010081	Bacthom7	Binh Giang, Hai Duong
7	Strain 7	HUA 020020-2	Nep87	Xuan Truong, Nam Dinh
8	Strain 8	HUA 020083	Bacthom7	Quynh Luu, Nghe An
9	Strain 9	HUA 020020-1	Nep87	Xuan Truong, Nam Dinh
10	Strain 10	HUA 020131-3	Khangdan	Dien Chau, Nghe An

The molecular markers were used to detect the resistant genes *Xa4*, *xa5*, and *Xa7* including the information of sequence, genes, markers and chromosomes were shown in Table 2.

Table 2. The list of genes and markers were used in this study.

Linkage gene	Marker	Chr.	Sequences 5'-3'	Author
<i>Xa4</i>	Npb181	11	F: ATCGATCGATCTTCACGAGG R: GTGCTATAAAAGGCATTTCGGG	[6]
<i>xa5</i>	RM122	5	F: GAGTCGATGTAATGTCATCAGTGC R: GGAAGGAGGTATCCGCTTTGTTGGAC	[7]
<i>Xa7</i>	P3	6	F: CAG CAA TTC ACT GGA GTA GTG GTT R: CAT CAC GGT CAC CAC CAT ATC GGA	[8]

Methods

Application of molecular marker to detect resistant genes *Xa4*, *xa5* and *Xa7*

DNA extraction

Total DNA extraction was carried out according to the process of Zheng et al. [9]. The young leaves were chopped at 2 cm and crushed in 800 µl of mixed solution (200mM Tris-HCl pH 8.0 buffer; 25 mM EDTA; 250 mM NaCl, 0.5 SDS) in order to break the cells. Fine hundred µl of

crushing solution were transferred into the test tube (1.5 ml) and added 700 µl of mixture (with the ratio of phenol:chloroform:isoamyl alcohol are 25:24:1, respectively). The mixture was slightly shaken and centrifuged for 5 min at 13.000 rpm and 4°C. The supernatant was then transferred into the new test tube, added 800 µl of ethanol 100%, and centrifuged for 5 min for 13.000 rpm at 4°C. The precipitated DNA extracts were collected, cleaned by ethanol 70% and naturally dried. The precipitated DNA extracts were dissolved in 50 µl TE and stored at -20°C for next use.

PCR performance

PCR performance was carried out with the volume of 20 µl including: 10 µl PCR master mix (2X) provided by Promega company, 1 µl Primer Forward (1.0 pmol/ul), 1 µl Primer Reverse (1.0 pmol/ul), 1 µl total DNA and 7 µl nuclease-free water. PCR Performances of genes *Xa4*, *xa5*, and *Xa7* were performed as follows: 94°C for 4 min, 30 cycles: 94°C for 1 min, 56°C for 1 min, 72°C for 2 min, and 72°C for 7 min. Electrophoresis of PCR products were performed on agarose gel 1.5%, 100V for 45 min. Gel was dyed with Ethidium Bromide 0.5 µg/ml and observed by UV scanner.

Evaluation of the agronomical traits

Investigation of rice landraces

The rice samples were grown in the paddy field according to the conventional methods. Each landrace was cultivated in an area of 5 m² with the density of 11 cm x 20 cm x 30 cm. The agronomic traits were evaluated following the method of IRRI [10] and QCVN 01-55: 2011/BNNPTNT such as: number of panicles per plant (NPP), filled grains per panicle (FGP), grain weight (GW-P1000), plant height (PH), days to heading (DTH) and some characteristics involving in quality were also recorded.

Experiment of artificial infection

Artificial infection was carried out at the time that rice plant started booting stage by cutting at 3-5 cm of leaf. The bacteria were cultured on Wakimoto medium for 48 h. The concentration of bacterial solution was about 10⁸ - 10⁹ cells/ml. Whole green leaves of each plant were also cut. Resistant ability of each variety was evaluated by measuring the lesion length after 20 days of infection following the IRRI standards [10] as follows: lesion length <8cm: resistance (R); from 8-12 cm: medium (M); and >12 cm: susceptibility (S).

Results and Discussion

Detection of resistant gene *Xa4*

The indigenous rice landraces have been cultivated and undergone a long time for domestication. Therefore, they have had many valuable agronomic characteristics such as: high yield potential (grains per panicle), high quality, strong tolerance to abiotic and biotic stresses, especially resistance to pests and diseases. In order to exploit them for rice breeding, it is initially examine for BLB resistant genes. In this research, we investigated the occurrence of *Xa4*, *xa5* and *Xa7* in the 86 rice landraces. According to report of Ton et al. [5], *Xa4* gene was determined the resistance to 6/10 leaf blight bacterial strains in the northern Vietnam. This gene is located on the chromosome 11 which is tightly linked with the marker Npb181 and marker MP [6], respectively. Npb181 marker was used to detect *Xa4* gene. PCR product was electrophoresised on agarose gel 1.5%. Rice landraces containing *Xa4* gene showed a DNA band with the size of 150 base pair (bp). Otherwise, the landraces with non-carrying *Xa4* gene which displayed a DNA band with the size of 120bp fragment as corresponding to the negative line IR24. The result of electrophoresis of PCR products disclosed that 11 glutinous rice varieties which had *Xa4* gene in the total of 86 samples as shown in Fig. 1. Recently, Ullah et al [11] reported that *Xa4* was detected in Basmati-385 and Basmati 2000 cultivars by using STS markers, which was agreed with our current study. In Vietnam, there are sporadic work examining BLB resistant genes of rice landraces. In much

effort has been paid on developing the BLB resistant cultivars since 1970 by using *Xa4* gene. Some cultivars carrying *Xa4* were reported such as CR203, MT [12].

Figure 1. Electrophoresis of PCR-products to detect *Xa4* gene using Npb181 marker
Lane 1: IR24 (Negative control); lane 2: IRBB4 (Positive control); Lane 3-15: glutinous rice varieties.

Detection of resistant gene *xa5*

The recessive gene *xa5* is considered as the best resistant gene against BLB. This gene involves in the resistance to 9/10 leaf blight bacterial strains existing in the North of Vietnam [5]. Two markers were previously identified that tightly linking with this gene including RG556 [13] and RM122 [7]. In order to identify the resistant gene *xa5* using RG556 marker, PCR products must be cut by restriction enzymes *DraI*. Otherwise *xa5* can be detected directly from PCR products using RM122 marker. Basically, two markers have the same accuracy. Therefore, to save time and cost in this study, RM122 marker was used to detect *xa5* gene. The results have shown that the samples carrying the *xa5* gene which appears the band with the size of 240 bp, the samples with non-containing the *xa5* gene displayed a band with the size of 220 bp, respectively (Fig. 2). Overall, 6 out of 86 rice landraces carrying the *xa5* gene were identified.

Figure 2. Electrophoresis of PCR products to detect *xa5* gene
Lane 1: IR24 (negative control); lane 2: IRBB7 (positive control); Lane 3-15: glutinous rice varieties.

Detection of resistant *Xa7* gene

The *Xa7* gene is dominant gene and considered as responsible for greater resistance level to 8/10 bacterial strains in the northern Vietnam [5]. Some markers have been found that tightly linking with this resistant gene such as P3 marker [8], RM5509 marker [13], M5 marker [14]. In Vietnam, P3 marker is widely used to screen and detect the *Xa7* resistant gene. By using this marker, PCR products of the landraces containing *Xa7* gene which were shown a band with the size of 262 bp after electrophoresis, the varieties with non-containing this gene appears a band with the

size of 297 bp (Fig. 3). The result of electrophoresis has shown that 19/86 glutinous rice varieties having *Xa7* gene. Only one rice landrace, Pe con Lam (lua nep) carrying both *Xa4* and *X7* genes (Table 3). Previously, some single dominant genes codes for resistance to *Xo*, were reported in some varieties such as Di Huong HD, Dai Duc 65, Canh Nong HB, Mai Khua TB, IR20 and IR22 [12].

Figure 3. Electrophoresis of PCR products to detect *Xa7* gene
Lane 1: IR24 (Negative control); lane 2: IRBB24 (Positive control);
Lane 3-15: glutinous rice varieties.

Table 3. Results of PCR performance to detect *Xa4*, *xa5* and *Xa7* resistant genes to BLB.

No.	landrace	<i>Xa4</i>	<i>xa5</i>	<i>Xa7</i>	No.	Landrace	<i>Xa4</i>	<i>xa5</i>	<i>Xa7</i>
1	Nep Van	-	-	-	44	Nep pelanh (2)	-	-	+
2	Nep hoa vang	-	-	-	45	Lua nep bong tron	+	-	-
3	Bong tranh	+	-	-	46	Nep, Khau bong tron	-	+	-
4	Lua Thua	-	-	-	47	Nep Cam (suoi thin)	-	-	-
5	Lua rang do	-	-	-	48	Nep Cam (Chieng pa)	-	-	-
6	Lua re do	-	-	+	49	Nep Som (Khau Vang)	-	-	-
7	Lua se	-	-	+	50	Nep Cam (ta luong)	-	-	-
8	Nam vang	-	-	-	51	Nep thom hat to	-	-	-
9	Nep ca ro	-	-	-	52	Nep Thom hat nho	-	-	+
10	Nep co pa	-	-	-	53	Nep Vang	-	-	+
11	Nep do	-	-	+	54	Nep Nati	-	-	+
12	Nep mo	-	-	+	55	Nep ve	+	-	-
13	Nep mui	-	+	-	56	Pe con lam (lua nep)	+	-	+
14	Nep muong	-	-	-	57	Pe lon (lua nep)	-	-	+
15	Nep non tre	-	-	-	58	Nep Plenl cau	-	-	+
16	Nep quan	-	-	+	59	Plenl lam	-	+	-
17	Nep ruoi	+	-	-	60	Planh meo	-	-	-
18	Nep sap	-	-	-	61	Plenl do	+	-	-
19	Nep som	-	-	-	62	Lua loc nep cam	+	-	-
20	Nep thap	-	-	-	63	Nep cam (Phu Yen)	-	-	-
21	Nep trang	-	-	-	64	Pau cam	-	-	-
22	Nep oc	-	-	+	65	Khau cam	-	-	-
23	Nep xap	-	-	+	66	Khau cam pi	-	-	-
24	Nep cai Hai Duong	-	-	-	67	Lo cam	-	-	+
25	Nep den	-	+	-	68	Nep cam nuong	-	-	-
26	Nep hom	-	-	-	69	Khau cam panh	+	-	-
27	Nep nut	-	-	-	70	Nep cam den	-	+	-
28	Lua ngoi	-	-	-	71	Nep cam (ban cam)	-	-	-
29	Nep Cam tim	+	-	-	72	Nep cam (ruot tim)	-	-	-
30	Nep cai hoa trang	-	-	-	73	Nep cam (vo tim)	-	-	-

31	Deo dang	+	-	-	74	Nep cam (Vo trang)	-	-	-
32	Nep rau	-	-	+	75	Khau cam pung	+	-	-
33	Nep loc nuong	-	-	+	76	Beo ta cam	-	-	-
34	Nep hat may	-	-	+	77	Nep nuong (Son La)	-	-	-
35	Khau tan pom	+	-	-	78	Nep 44	-	-	-
36	Khau tan hang	-	-	-	79	Nep TK90	-	-	-
37	Khau tan nuong	-	-	+	80	Nep 97	-	-	-
38	Nep ran	-	+	-	81	Nep 87	-	-	-
39	Dang chiem	-	-	-	82	Nep 352	-	-	-
40	Lua ma	-	-	-	83	Nep nhung	-	-	-
41	Nep chin som	-	-	-	84	Nep lang lieu	-	-	-
42	Lua nep Tang San	-	-	-	85	Nep cau	-	-	-
43	Lua nep, Pelenh	-	-	+	86	Nep qua vai	-	-	+

Note: “+”: Landrace containing resistant gene; “-”: Landrace non-containing resistant gene

Evaluation of BLB resistant samples

Currently, there were over ten existing bacterial strains in the north of Vietnam [3]. These strains are being stored at Faculty of Biotechnology, Vietnam National University of Agriculture. In this research, 10 bacterial strains were used to examine resistant level to BLB. The results demonstrated that the landraces containing *Xa4* gene had similar resistant level to IRBB4 control variety (containing *Xa4* gene). These landraces had resistant activity against 6/10 bacterial strains including the strains number 2, 5, 6, 7, 9, and 10, respectively. The other landraces containing *xa5* resistant gene which had similar resistant level to control variety (carrying *xa5* gene). These landraces were resistant against 9/10 bacterial strains except the bacterial strain number 5. The containing *Xa7* resistant gene also had similar resistant ability to control variety (carrying *Xa7* gene). These varieties were resistant to 8/10 bacterial strains, they were infected by only the two strains including number 4 and 5, respectively.

In general, almost the rice landraces with non-containing resistant genes to BLB were seriously infected by all the 10 bacterial strains or some strains out of the 10 studied strains (Figs. 4A, 4B, 4C and 4D). Additionally, the bacterial strain number 5 had the strongest toxicity, causing infection to all samples which carried three resistant genes. It noted that only carrying *xa5* gene landraces showing resistant to the number 4 bacterial strains. Our results have been agreed with the previous of Ton et al. (2013) who reported that the number 4 and 5 bacterial strains were shown highly toxic

Figure 4A. The symptom of control rice variety IR24 under the infection of the 10 bacterial strains.

Figure 4B. The response of the rice variety containing *Xa4* under the infection of 10 bacterial strains.

Figure 4C. The response of the rice variety containing *xa5* under the infection of 10 bacterial strains.

Figure 4D. The response of the rice variety containing *Xa7* under the infection of 10 bacterial strains.

Agronomic traits of some landraces carrying resistant genes

In order to effectively exploit some major agronomical characteristics of each genetic source were evaluated including some major traits: days to heading (DTH), plant height (PH), a number of panicle per plant (NPP), number of grains per panicle (NGP), grain weight (GW-P1000), individual yield (IY) were evaluated. The results showed that the 37 rice landraces containing resistant genes to BLB, then were evaluated their agronomic traits in spring season crop, 2016 to provide further comprehensive information for effective exploitation these genetic resources as shown in Table 4.

Table 4. Agronomic traits of the glutinous rice landraces carrying resistant genes to BLB.

No.	Landrace	GD (days)	PH (cm)	NPP	TG	PFGP (%)	GW (g)	IY (g)	CG
1	Bong tranh	138	140.5±4.0	5.0	139.8	76.9	28.0	13.6	<i>Xa4</i>
2	Lua re do	132	108.2±2.3	4.6	102.6	60.7	24.0	9.5	<i>Xa7</i>
3	Lua se	133	101.0±2.4	4.2	137.6	76.9	28.5	13.5	<i>Xa7</i>
4	Nep do	131	100.5±2.8	4.4	143.6	63.2	32.0	10.5	<i>Xa7</i>
5	Nep mo	132	132.0±4.1	4.6	85.8	55.1	32.0	11.3	<i>Xa7</i>
6	Nep mui	133	148.6±4.5	5.0	81.2	66.5	32.5	11.4	<i>xa5</i>
7	Nep ruoi	136	132.4±3.6	6.4	190.4	76.9	29.0	11.5	<i>Xa4</i>
8	Nep oc	139	145.6±6.1	5.0	126.2	48.5	28.5	9.4	<i>Xa7</i>
9	Nep xap	132	138.8±3.7	6.2	148.2	69.8	32.5	14.6	<i>Xa7</i>
10	Nep Den	133	142.0±5.2	6.4	116.6	65.9	28.0	13.5	<i>xa5</i>
11	Nep Cam tim	133	154.8±5.5	5.8	184.8	65.7	28.0	15.7	<i>Xa4</i>
12	Deo dang	146	143.5±4.3	4.4	171.4	78.3	24.3	12.4	<i>Xa4</i>
13	Nep rau	143	120.2±4.7	4.2	116.0	63.8	40.3	12.9	<i>Xa7</i>
14	Nep loc nuong	144	145.8±6.2	5.8	97.6	69.7	21.3	7.5	<i>Xa7</i>
15	Nep hat may	144	156.4±5.5	5.4	187.2	71.0	21.6	9.6	<i>Xa7</i>
16	Khau tan pom	143	124.2±3.6	4.8	159.4	75.9	20.6	10.6	<i>Xa4</i>
17	Khau tan nuong	132	133.6±2.3	5.6	178.0	77.0	24.5	14.2	<i>Xa7</i>
18	Nep ran	132	136.6±3.4	5.2	170.2	78.1	25.5	11.5	<i>xa5</i>
19	Lua nep, Pelenh	142	128.6±3.9	4.2	208.6	51.3	24.4	11.5	<i>Xa7</i>
20	Nep pelanh (2)	136	141.8±4.7	6.2	124.8	71.6	15.4	8.8	<i>Xa7</i>
21	Lua nep bong tron	138	157.2±5.1	6.8	107.2	87.7	28.5	12.6	<i>Xa4</i>
22	Khau bong tron	135	163.6±5.5	4.8	118.8	76.1	28.8	10.1	<i>xa5</i>

23	Nep Thom hat nho	137	155.8±2.5	6.4	132.6	86.4	20.8	11.7	<i>Xa7</i>
24	Nep Vang	134	140.2±3.8	7.0	166.4	75.1	27.9	16.8	<i>Xa7</i>
25	Nep Nati	135	108.2±2.4	4.0	170.6	59.6	28.5	10.3	<i>Xa7</i>
26	Nep ve	132	123.5±3.2	5.4	186.4	69.6	29.5	17.2	<i>Xa4</i>
27	Pe con lam	132	132.5±3.5	4.0	210.5	64.8	32.5	11.5	<i>Xa4/Xa7</i>
28	Pe lon (lua nep)	142	143.9±4.0	7.6	127.2	68.2	32.5	17.5	<i>Xa7</i>
29	Nep Plenh cau	146	136.2±4.4	5.4	144.4	75.9	28.7	14.2	<i>Xa7</i>
30	Plenh lam	140	131.8±4.6	5.0	105.8	67.7	36.4	14.5	<i>xa5</i>
31	Plenh do	147	126.6±2.6	5.8	152.5	83.2	24.4	15.3	<i>Xa4</i>
32	Lua loc nep cam	146	104.5±2.5	4.6	113.2	79.5	36.5	13.5	<i>Xa4</i>
33	Lo cam	134	136.1±3.2	6.2	209.6	56.6	28.1	17.3	<i>Xa7</i>
34	Khau cam panh	140	152.5±4.5	5.0	173.6	74.2	28.5	13.6	<i>Xa4</i>
35	Nep cam den	142	142.6±4.5	4.8	149.4	76.0	32.3	13.5	<i>xa5</i>
36	Khau cam pung	142	135.8±5.1	4.6	60.2	67.4	32.4	10.1	<i>Xa4</i>
37	Nep qua vai	135	134.5±3.9	5.3	117.3	76.8	24.3	9.8	<i>Xa7</i>

GD: Growth Duration, PH: Plant height, NPP: Number of panicle per plant, TG: Total grain, PFGP: Percentage of full-filled grain per plant, GW: Grain weight, IY: Individual yield, CG: Containing gene

Regarding the growth duration, the growth duration of 37 samples was ranged from 131 to 146 days. In which, 17 landraces were grouped into short growth duration (115 to 135 days). The other landraces were grouped into medium growth duration (136 to 160 days). For plant height, there were 7 rice landraces in medium height (90 to 125 cm). The other landraces were rather high with the height of more than 125 cm. The number of panicles per plant was ranged from 4.0 to 7.6 panicles. The number of grains per panicle was from 60.2 to 210 grains/panicle. Some rice landraces had extremely big panicles such as: Lua nep Pelenh, Pe con lam, Lo cam and nep roi. For the total grains (P1000), the glutinous rice landraces mostly had very high weight, some samples had P1000 up to over 36 g, especially was 40.3 g found in Nep rau. In the case of individual yield, it was ranged from 7.5 g to 17.3 g, which was shown significantly high. This is an extremely valuable material sources, which should be exploited and managed for future rice breeding program.

Conclusions

Among the total of 86 rice landraces, 11 samples carrying *Xa4* gene, 6 landraces were detected *xa5* gene, 19 landraces possessed *Xa7* gene and 1 rice landrace carried both of the *Xa4* and *Xa7* genes were identified by using molecular markers. The evaluation of resistant level of rice varieties to BLB using 10 bacterial strains indicated that *Xa4* gene involved in resistance to 6/10 strains, *Xa5* gene involved in resistance to 9/10 strains and *Xa7* gene involved in resistance to 8/10 strains of bacteria. The bacterial strain number 5 had the strongest toxicity. The evaluation of agronomic traits of the 37 samples included the resistant genes rice landraces disclosed that they are precious source of materials for glutinous rice breeding program of resistance to BLB disease.

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