ASSESSMENT OF BACTERIAL LEAF BLIGHT DISEASE RESISTANCE OF INDONESIAN RICE GERMPLASMS USING SSR MARKERS

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Abstract

Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a serious disease in rice plants worldwide. Yield losses caused by *Xoo* can be as high as 50% in some parts of Asia. *Xa7* gene can potentially confer a broad resistance to BLB. Evaluation of disease resistance characteristics in early breeding generations of rice is important to develop varieties with better resistance. This study reports the evaluation of 167 Indonesian rice germplasms against three BLB isolates/pathotypes in a green house setting and the genotyping of 56 Indonesian rice germplasm using 12 SSR markers linked to *Xa7* BLB resistance gene. The majority of the indigenous rice germplasms was found to be susceptible to three BLB isolates/pathotypes tested. Sate Liko from Bantul, Yogyakarta, Horeg from Cirebon, West Java and Sijem from Malang, East Java revealed consistent resistance to three isolates/pathotypes tested based on BLB evaluation in a greenhouse, UPGMA analysis, and genotyping. Pathotype XII displayed more virulence to Indonesian rice varieties tested compared to pathotypes VI and V. The association analysis using the General linear model identified six markers associated with BLB resistance and two markers were highly associated (RM20589 and RM20590). This information will be useful for future studies of BLB resistance in rice plants.

Keywords: Bacterial leaf blight, Molecular marker, SSR*, Xanthomonas oryzae, Xa7*

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Introduction

BLB caused by *Xanthomonas oryzae* pv. *Oryzae (Xoo),* is a serious disease affecting rice plants in tropical lowland environment. This disease is not only widespread throughout Asia but also occurring in Australia, the United States and in several rice growing countries of Latin America and Africa. Yield losses caused by *Xoo* typically range from 20 to 30% and can be as high as 50% in some parts of Asia (Adhikari *et al.,* 1995).

Hifni and Kardin (1998) reported that there are 12 pathotypes of *Xoo* based on IRRI differential varieties. Pathotype V, the most dominant pathotype (46.23%), can overcome at least seven virulence genes (*Xa1, Xa2, Xa3, Xa4, Xa10, Xa11,* and *Xa14*), while Pathotype VI as the second dominant pathotype (11,32%) can overcome at least eight virulence genes (*Xa1, Xa2, Xa3, Xa4, Xa10, Xa11*, *Xa14* and *Xa21*). Furthermore, the most virulent one, Pathotype XII can overcome at least 10 virulence genes (*Xa1, Xa2, Xa3, Xa4, xa5, Xa7, Xa10, Xa11, Xa14,* and *Xa21*).

Genetic diversity is a key factor in sustaining agricultural productivity. In order to preserve and make this diversity available for crop improvement, tremendous efforts have been made in the collection, maintenance, and classical characterization of germplasm. Generating a new crop variety with certain desirable traits requires a germplasm collection with a wide genetic diversity. The germplasm collection may be of local, introduced, or breed varieties (Syam and Hermanto, 1995).

In order to develop BLB resistant varieties, selection of appropriate donor parents that show a broad spectrum of resistance to other *Xoo* pathotypes is important for the breeding

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program. Thus, evaluation of introduced and indigenous rice germplasms for their BLB resistance profile is one of the primary tasks for rice genetic resources management and utilization.

Molecular markers have shown a tremendous potential for characterizing genetic diversity. The availability of DNA markers linked to genes for BLB resistance would accelerate breeding programs. Furthermore, PCR-based markers closely linked to BLB resistance genes would be very useful for an efficient marker-assisted selection.

Recently, 45 BLB resistance genes (*Xa*) from cultivated rice and wild species have been identified and mapped (Neelam *et al.,* 2020). In Indonesia, *xa5, Xa7*, and *Xa21* are relatively effective against the majority of the *Xoo* isolates/pathotypes, so that these resistance genes can be incorporated into our rice breeding program (Hifni and Kardin, 1998; Tasliah *et al.,* 2013; Fatimah *et al*., 2014). Due to the lower percentage of *Xoo* pathotypes having the ability to overcome *Xa7*, subsequent addition of virulence genes that can overcome *Xa7* into the *Xoo* population occurred after the integration of the virulence genes that can overcome *xa5* and *Xa21* (Hifni and Kardin, 1998).

Located in chromosome 6, *Xa7* is a dominant resistance gene directed against *Xoo* and was originally identified in the rice cultivar DV85, IRRI accession number 8839 (Sidhu *et al.,* 1978). The gene was transferred to cultivar IR24 and near isogenic line IRBB7, where *Xa7* was integrated by recurrent backcrossing (Ogawa *et al.,* 1991). *Xa7* is an example of an R-gene that is directed against an avirulence gene family and is a potential source of broad resistance to BLB affecting rice plants (Cruz *et al.,* 2000).

The objective of this study is to evaluate BLB resistance of 167 Indonesian rice germplasms in a green house against three BLB isolates/pathotypes and to perform genotyping on 56 Indonesian rice germplasms using 12 SSR markers linked to *Xa7* BLB resistance gene. These information will be useful for future studies on BLB resistance in rice plants.

Materials and Methods

Plant Materials. One hundred and sixty-five accessions of Indonesian rice germplasm provided by Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD) and two IRRI varieties were used for BLB resistance evaluation. IRBB7 and Conde for containing *Xa7* resistance gene were used as the resistant controls and IR24 and Kencana Bali as a susceptible control (Table 1). The *Xoo* isolates/pathotypes provided by ICABIOGRAD were used for BLB evaluation (Tasliah *et al.,* 2013) (Table 2).

Disease Assesment of Rice Germplasms.

Rice plants were grown under greenhouse conditions. These rice germplasms were first sown in plastic boxes, and 14 days later, the seedlings were transplanted into a container box containing natural paddy soil. The experimental units consisted of nine rows with a spacing of 6 cm. Each rice germplasm was transplanted in one row of five plants. The seedlings were watered twice a day using a sprinkle system to maintain the proper humidity and the optimum conditions for the growth of *Xoo*.

The bacterial suspension for inoculation was prepared using the two-day old culture of each isolate in 20 ml of sterilized distilled water adjusted of 10^8 CFU/ml. To test the virulence of the strains, the fully expanded leaves were inoculated by the leaf cutting method (Kauffman *et al.,* 1973).

Bacterial inoculation was done when rice plants were 40 days old. Evaluation for resistance of each germplasm was done at 14 DAI (days after inoculation) according to the Standard Evaluation System for Rice. Ten randomly selected leaf clippings of each line-isolate combination were rated for their percent ratio of lesion length to entire leaf length. The symptoms were scored as resistant if the average attack intensity was 25% or less (score 1 - 4) and as susceptible if the average attack intensity was greater than 25% (score 5 to 9) (Chaudry, 1996).

Molecular Marker Analysis.

SSR genotyping of twelve SSR markers (Table 3) on chromosome 6 that are linked to *Xa7* as previously reported by Chen *et al.* (2008) were employed to identify the *Xa7* gene on randomly selected 56 rice accession (no.111-167), which included the resistant and susceptible accessions. Total genomic DNA was extracted using young leaves at seedling

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stage as described previously (Dellaporta, 1983) for each individual accession. Genomic DNA concentration was measured by spectrophotometer and the DNA samples were diluted to 10ng/ul using sterilized distilled water and stored in microfuge tubes at 4°C for further use.

Table 1. List of rice germplasms used in this study

No.	Acc.No.	Accession Name	Origin
1	1430	Putih Ampat Angkek	West Sumatera
2	3983	Reli	West Kalimantan
3	3995	Seng Kumang	West Kalimantan
4	3996	Mingkai	West Kalimantan
5	4043	Rijah	Central Java
6	4050	Aluh Kuranji	West Sumatera
7	4071	Kuning Biaro	West Sumatera
8	4084	Gondak Kiah	West Sumatera
9	4141	Si Rendah Cogok	West Sumatera
10	4206	Tromas	Central Java
11	4213	Goter	East Java
12	4214	Untup	East Java
13	4256	Mayor	East Java
14	4305	Baliman Putih	South Kalimantan
15	4310	Biduin	South Kalimantan
16	4311	Randah Pala	South Kalimantan
17	4315	Lalantik Bamban	South Kalimantan
18	4316	Raden Pulatar	South Kalimantan
19	4324	Siam Parapuk	South Kalimantan
20	4491	Sara Kasa	Central Sulawesi
21	5593	Pingkan	Central Sulawesi
22	5594	Pimpin	Central Sulawesi
23	6859	Bengkongang	Central Java
24	6967	Cicih Buleleng	Bali
25	7237	Angkong	West Java
26	9151	Padi Book	South East Sulawesi
27	9154	Longandobu	South East Sulawesi
28	9155	Padi Ana-Ana	South East Sulawesi
29	9173	Anambar	South East Sulawesi
30	9186	Wulu Mata	South East Sulawesi
31	9458	Ingsa Cendana	Bali
32	9467	Ingsa Bondol	Bali
33	10613	Banja Ili	South East Sulawesi
34	12270	Rejuno	Tanjung Jabung, Jambi
35	12307	Abang	Pekalongan, Central Java
36	12308	Segon Borondol	Kodya Bogor, West Java
37	12335	Mojang	Garut, West Java
38	12338	Rangkong	Tangerang, West Java
39	12344	Cere Mentik/Toray	Purwakarta, West Java
40	12352	Hawara Batu	Cianjur, West Java
41	12368	Muncang	Cianjur, West Java
42	12571	Gondok	Tanah Datar, West Sumatera
43	12574	Laila	Tanah Datar, West Sumatera
44	12674	Padi Belanak Kosambi	Bangli, Bali
45	12968	Padi Jambai	Kampar, Riau
46	14866	Terotel	Sawahlunto, West Sumatera
47	14895	Wuri Bura	West Nusa Tenggara
48	14903	Samada	West Nusa Tenggara
49	14915	Dendak	Sumbawa, West Nusa Tenggara
50	14986	Lumbuk	Central Java
51	18960	Secangkir	South Kalimantan
52	19100	Thapora	Philippine
53	19101	Tuma	Philippine
54	19125	Seratus Malam	Kodya Bogor, West Java
55	19228	Brown Gora	Philippine
56	19229	Dular	Philippine
57	19250	Sibentar	Karo, North Sumatera
58	19251	Siremut	Karo, North Sumatera
59	19661	Buhbolon	Kodya Bogor, West Java
60	19673	Danau Atas	Kodya Bogor, West Java
61	19677	Poso	Kodya Bogor, West Java
62	19701	Engkoran	West Kalimantan
63	19703	Baung	Sanggau, West Kalimantan
64	19708	Banjar Sawan	Sanggau, West Kalimantan
65	19710	Kail	West Kalimantan
66	19757	Payak Tembakau	Sambas, West Kalimantan
67	19768	Ribun	Kapuas Hulu, West Kalimantan
68	19777	Bonti	Kapuas Hulu, West Kalimantan
69	20227	Idi	Aceh
70	20228	Syair	Aceh
71	20241	Rangkoh	Aceh
72	20244	Si Reguek	Aceh
73	20245	Putih Panjah	Aceh
74	20256	Bho	Aceh
75	20513	Lemo	Central Kalimantan
76	20673	Si Motung	North Sumatera
77	20678	Si Anak Bogor	North Sumatera
78	20679	Si Lotik	North Sumatera
79	20682	Si Pulo Angkola	North Sumatera
80	20683	Si Pulo Manda iling	North Sumatera
81	20688	Putri Manis	North Sumatera
82	20694	Kuala Deli	North Sumatera
83	20708	Ramos Batu	North Sumatera

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No.	Acc.No.	Accession Name	Origin
84	20746	Mama Laka	East Nusa Tenggara
85	20759	Wajo Kuning	East Nusa Tenggara
86	20765	Sera	East Nusa Tenggara
87	20766	Keakubi	East Nusa Tenggara
88	20819	Sehan	North Maluku
89	20848	Ardas	North Sulawesi
90	20851	Apel	North Sulawesi
91	20878	Ndangan Cantik 1	South Sulawesi
92	20906	Lokal buntu Sangala 2	South Sulawesi
93	20908	Pare Pulunglia	South Sulawesi
94	20962	Cere Gelas	West Java
95	20967	Kembang Ading	West Java
96	20974	Limar	West Java
97	20977	Sasak Jalan	East Kalimantan
98	20982	Popot	East Kalimantan
99	21073	Kembang Singkan	East Kalimantan
100	21074	Ketan Siam	East Kalimantan
101	21096	Uyun	Jambi
102	21116	Kwatik Tinggi	Jambi
103	21119	Rumbai ayam	Jambi
104	21120	Padi Rantau Undik	Bengkulu
105	21123	Cinta Kasih	Bengkulu
106	21124	Padi Bugis	Bengkulu
107	21126	Surya	Bengkulu
108	21181	Beras merah	West Java
109	21183	Slereng	West Java
110	6329A	Pae Laguh	South East Sulawesi
111	10065	Ketan Jambruk	Bantul, Yogyakarta
112	10077	Sate Liko	Bantul, Yogyakarta
113	10221	Pulu Bolong	Bone, South Sulawesi
114	10479	Pare Solo	Kolaka, North Sulawesi
115	10578	Pulut Tomene	South East Sulawesi
116	15016	Horeg	Cirebon, West java
117	11720	Majair	Lampung, North Lampung
118	11731	Kalimis	Lampung, North Lampung
119	11920	Termas	Lampung, Central Lampung
120	12074	DR (Daya Itoh Rice) 4	Lampung
121	11984	Si Gudang	North Sumatera
122	12052	Cempo	Deli Serdang, North Sumatera
123	12122	Gata	Bogor, West Java
124	12167	Ringgit	Tanjung Jabung, Jambi
125	12276	Tampay	Semarang, Central Java
126	12296	Nolo kario	Semarang, Central Java
127	12297	Lumbu	Semarang, Central Java
128	12315	Sri Makmur	Bateng, Central java
129	12341	Fajar	Garut West Java
130	12564	Kodok Putih	Tanah Datar, West Sumatera
131	12723	Cempo Abang ner	Cirebon, West java
132	13102	Teratai	Ketapang, West Kalimantan
133	15005	Banda	South Sulawesi
134	3571	Betonan	East Java
135	4065	Lumut	50 Kota, West Sumatera
136	4087	Sunting Beringin	Tanah datar, West Sumatera
137	4117	Siak Simpur	Sambas, West Kalimantan
138	4137	Bendang Bujur	Agam, West Sumatera
139	4139	Kuku Balam	Agam, West Sumatera
140	4141	Sirandah Tjogok	Solok, West Sumatera
141	4153	Empat	Solok, West Sumatera
142	4176	Padi Rasi	Aceh
143	4224	Djula Djuli A	Banyuwangi, East Java
144	4225	Hoing	Banyuwangi, East Java
145	4227	Makmur	Banyuwangi, East Java
146	4231	Revolusi	Banyuwangi, East Java
147	4240	Gropak Serung	Lumajang, East Java
148	4242	Itun	Lumajang, East Java
149	4257	Nangka Bosok	Malang, East Java
150	4260	Bengawan	Malang, East Java
151	4268	Sijem	Malang, East Java
152	4285	Sampa kiring	Kota Waringin, Central Kalimantan
153	12287	Pelopor	Semarang, Central Java
154	12293	Cempo Slamet	Semarang, Central Java
155	12302	Ketan Uis	Bandung, West Java
156	12303	Loyang	Bandung, West Java
157	12353	Jerah	Cianjur, West java
158	12354	Ketan Wangi	Tangerang, Banten
159	12366	Marus	Cianjur, West java
160	12372	Koneng Gundil	Cianjur, West java
161	12563	Sirandah Lunto	Tanah datar, West Sumatera
162	19625	Cisadane	Bogor, West Java
163	19629	Semeru	Bogor, West Java
164	C1	IRBB7	IRRI
165	C2	IR24	IRRI
166	C3	Conde	ICABIOGRAD
167	C4	Kencana Bali	ICABIOGRAD

Amplification reactions were carried out in 20 uL reaction volumes containing 50 ng genomic DNA, 1.0 µM each of primer, 12.5 µM each of dATP, dCTP, dGTP and dTTP, 1 unit of Taq DNA Polymerase, 1X Taq polymerase buffer and 2.5 mM MgCl2. DNA amplification was performed in a Tetrad MJ Research Thermal Cycler programmed as follows: an initial denaturation of 4 min at

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94°C; 35 cycles of 94°C for 1 min (denaturation), 50/55°C for 1 min (depending on optimal annealing temperature of each primer), and 72°C for 1 min (extension). One additional cycle of 10 min at 72°C was used for final extension. Amplified products were separated by electrophoresis in either 3% agarose gels run in 0.5X TBE or 8% polyacrylamide gel at 100 V (Dual Triple-Wide Mini-Vertical System, C.B.S. Scientific, CA, USA), visualized under UV light following addition of ethidium bromide using a gel documentation system (BioRad).

Table 2. List of BLB isolates/pathotypes used in this study

No.	Isolates	Host	Province	Pathotype
1	Xoo1110	Ciherang	Cianjur, West Java	VI
2	Xoo1122	Kuriak Putiah	Maninjau, West Sumatera	V
3	Xoo1130	Kuriak Putiah	Maninjau, West Sumatera	XII

Table 3. List of SSR primers used in this study.

No.	Primer	Sequence	Repeat Motif	size (bp)
1	RM20573	F:ggctattcctttcctcctctcc R:aatcttcacgtgtgcgtaactagc	(CT)10	197
2	RM20580	F:cgtcacttcaccagcctgtagcc R:gtccatcaatgcccatccatcc	(CT)10	99
3	RM20582	F:agagcgtcgtccttcaccatcc R:ggccaatacgacgatacattacacg	(TCT)7	83
4	RM20589	F:catgtatttgtgtgcacgtaccg R:acctttcttgggcctttcttgg	(AC)22	263
5	RM20590	F:ttcgatgagcacctttccttgtcc R:gcctcgccgattcacttatgc	(AT)28	343
6	RM20591	F:tcgtctgcgcgaatatttagagagg R:atctgcatcggagtcagcaacg	(TGGA)6	195
7	RM20593	F:aaggtacacttgctctgacggtagc R:agacctcagtggcaaatcctacg	(CT)12	315
8	RM20595	F:aacttcctttccaggctttcagc R:ttcactgagcctgaacacattgc	(TA)10	169
9	RM20601	F:ggagtgaaactgaggctcctatcg R:tcgttctccctgcaagttaatgg	(TA)13	406
10	RM20603	F:tacaaatcaacagccaccacagc R:ccatttggaacagattggacttgg	(CAA)8	101
11	RM20608	F:ttcgatcagtcagatagtcacg R:tcttgcttcagtctgctacacc	(GA)17	145
12	RM20612	F:tgtctctcgatacctcccatacc R:gcccacctctcttgtcctatcc	(AG)13	152

Data Analysis.

The disease extent of rice germplasms was assessed and grouped based on their resistance using statistical analysis software program NTSYSpc 2.11p (Rohlf, 2005). Resistance or susceptibility was coded in a binary form of 1 or 0, respectively. The Dice Coefficient (SIMQUAL) and UPGMA method (*Unweighted Pair Group Method Arithmetic*) were used to cluster the varieties and visualize their genetic relatedness to each other.

The bands of *Xa7* allele were noted from the polymorphic band patterns of the PCR products. The bands of *Xa7* allele were standardized using the amplified PCR products of IRBB7 and IR24 used as control. SSR analysis was conducted based on their allele size. The genetic distance was calculated using *Dc* implemented by Cavalli and Edwards (1967) in PowerMarker V3.23 (Liu and Muse, 2004). The germplasms were grouped based on their SSR profile using statistical analysis software program NTSYSpc 2.11p (Rohlf, 2005). The Dice Coefficient and UPGMA method used to cluster the varieties and visualize their genetic relatedness to each other. Tassel v.3.0 (Bradbury et al., 2007) was used to determine the association testing between SSR markers and BLB resistance. The P-value determined whether a QTL is associated with the marker and the R^2-marker evaluated the magnitude of the QTL effects.

Results

Disease Assessment of Rice Germplasms.

BLB is a vascular disease that spreads through the xylem vessels. Lesions usually begin at the margin, a few centimeters from the tip, as water-soaked stripes. It can occur at any stages of the rice plants' growth. At the seedling stage, the symptoms first appeared as tiny water-soaked spots at the margin of the rice leaf blade. Then, it will enlarge and the rice plants turn yellow and wither. The symptoms of the disease at the seedling stage is known in local language (Bahasa Indonesia) as *kresek*.

The plants' reactions to pathogen infection varied. Symptoms such as pale green or grayish green patches on the leaves indicated infected germplasms. In the spots, oozing milky-white bacteria could be seen on the surface of the leaves in the morning. In resistant cultivars IRBB7 and Conde (Figure 1: leaf no. A1, A2, B1, B2, C1 and C2) and resistant germplasms (Figure 1: leaf no. 5, 6, 7, 8), the spots developed into a yellowish-white color on leaves with a wavy tip. On the other hand, in the susceptible cultivars Kencana Bali

and IR24 (Figure 1: leaf no. A3, A4, B3, B4, C3 and C4) lesions were observed in all parts of the infected leaves, accompanied by a color change to slightly white or grey, and the leaves became dry and died.

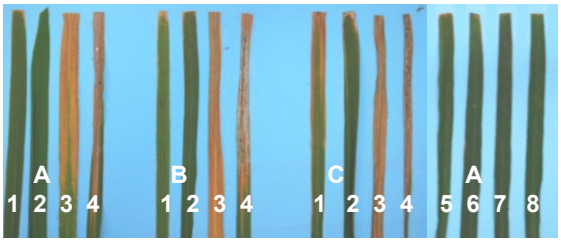

Figure 1. Lesions on leaves detected 14 days after inoculation with (A) Xoo1110, (B) Xoo1122, (C) Xoo1130. From left to right: Leaf no 1) IRBB7, 2) Conde, 3) Kencana Bali and 4) IR24, (5) Payak Tembakau (6) Padi Bugis, (7) Surya, and (8) Pare Puluglia.

The BLB intensity in resistant varieties (IRBB7 and Conde having the *Xa7* resistance gene) against Xoo1110 (Pathotype VI) and Xoo1122 (Pathotype V) when measured as lesion size were less than 5%, whereas for the Xoo1130 (Pathotype XII), it was higher than 10%. It suggested that Xoo1130 was more virulent than the other two isolates/pathotypes and could overcome the *Xa7* resistance gene in IRBB7 and Conde varieties. The BLB intensity in susceptible varieties, IR24 and Kencana Bali, were higher than 30% in terms of lesion size against the three BLB isolates/pathotypes tested. These two isolates showed a moderate susceptibility against the three BLB isolates/pathotypes tested (Figure 2).

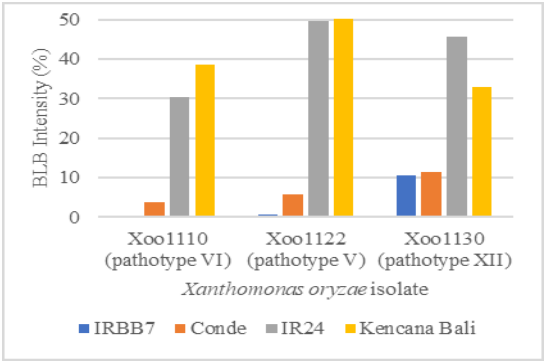

Figure 2. The BLB intensity of resistant controls (IRBB7 and Conde) and susceptible controls (IR24 and Kencana Bali) against the three isolates/pathotypes tested.

The BLB scores on 167 indigenous rice germplasms revealed that more than 68% rice accessions displayed a medium susceptibility (score 5) to high susceptibility (score 8) against the three BLB isolates/pathotypes tested (Suppl. 1). There were 54 (32.3%) rice accessions found to be resistant to Xoo1110 (Pathotype VI), 41 (24.5%) were resistant to Xoo1122 (pathotype V), and 22 (13.2%) were resistant to Xoo1130 (pathotype XII) (Figure 3). Payak Tembakau, Pare Pulunglia, Sate Liko, Pulu Bolong, Horeg, and Sijem cultivars were resistant to all three isolates/pathotypes tested (Table 4).

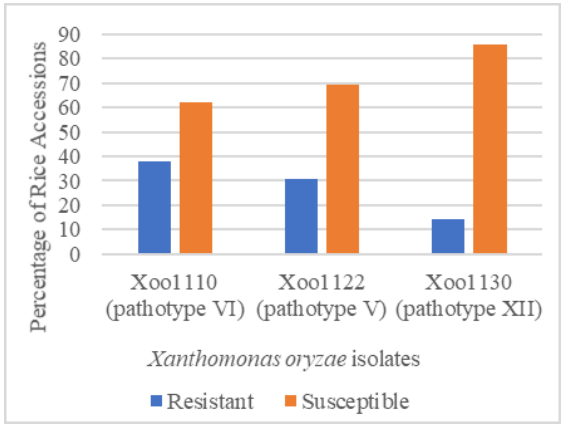

Figure 3. The percentages of resistant and susceptible Indonesian rice germplasms to the three isolates/pathotypes tested.

Table 4. The resistance profiles of Indonesian rice germplasms against the three isolates/pathotypes tested.

No. of resistance occurrence	Xoo Isolates /pathotypes	Resistant Accessions
1	Xoo1110 /pathotype VI	Biduin, Abang, Terotel, Wuri Bura, Secangkir, Jhapara, Tuma, Seratus Malam, Dular, Sibentar, Siremut, Buhbolon, Danau Atas, Poso, Baung, Banjar Sawan, Kail, Bonti, Putri Manis, Keakubi, Ketan Jambruk, Pare Solo, Pulut Tomene, Sri Makmur, Empat, Hoing, Makmur, Revolusi, Gropak Sarung, Nangka Bosok, Sampa Kiring, Pelopor, Cempo Slamet, Jerah, SiRandah Lunto, Semeru.

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	Xoo1122 /pathotype V	Putih Ampat Angkek, Aluh Kuranji, Untup, Mayor, Randah Pala, Lalantik Bamban, Siam Parapuk, Sara Kasa, Pimpin, Si Pulo Angkola, Kuala Deli, Wajo Kuning, Ardas, Ndangan Cantik 1, Lokal Buntu Sangala 2, Cere Gelas, Uyun, Kwatik Tinggi, Padi Rantau Undik, Cempo.
	Xoo1130 /pathotype XII	Idi, Si Motung, Angkong, Kembang Ading, Kembang Singkan, Pae Laguh, Kalimis.
2	Xoo1110 + Xoo1122	Brown Gora, Lemo, Si Pulo Mandailing, Cinta Kasih, Majair, Nolo Kario, Lumbu, Sera
	Xoo1110 + Xoo1130	SiRuguek, Cisadane
	Xoo1122 + Xoo1130	Goter, Surya, Daya Itoh Rice 4, Padi Bugis, Si Gudang
3	Xoo1110 + Xoo1122 + Xoo1130	Payak Tembakau, Pare Pulunglia, Sate Liko, Pulu Bolong, Horeg, Sijem

Cluster analysis calculated from BLB scoring (resistant: score 1-4 and susceptible: score 5-9) was constructed. A cutoff value of 0.65 was used for genetic similarity among all varieties as the threshold for UPGMA clustering, which resulted in four major groups. Group 1 represented accessions displaying resistance to all three BLB isolates/pathotypes. This group included the IRBB7 and Conde varieties and consisted of 10 accessions (6%): Horeg, Pare Pulunglia, Sijem, Sate Liko, Pulu Bolong, Payak Tembakau, Padi Bugis, Goter, Surya, and Daya Itoh Rice 4. Group 2 represented accessions resistant to only one BLB isolate/pathotype (Xoo1130), Group 3 represented accessions susceptible to all three BLB isolates/pathotypes, and Group 4 represented accessions resistant to one or two BLB isolates/pathotypes (Suppl.2).

Molecular Marker Analysis.

Eight out of twelve SSRs used for genotyping showed good polymorphism and were able to be measured in 56 Indonesian rice germplasms (Figure 4). Polymorphic analysis revealed the genetic variations that exist, namely 31 alleles with an average of four alleles per locus – the range was two (RM20595 and RM20603) to six alleles (RM20590). Polymorphism Information Content (PIC) values averaged at 0.34 and ranged from a low of 0.06 (RM20573) to a high of 0.53 (RM20582). RM20573 showed the highest frequency allele, whereas RM20612 showed the lowest frequency allele (Table 5). The presence of 31 alleles in the 56 accessions indicated a low genetic diversity within the BLB *Xa7* resistance gene locus and a low PIC value.

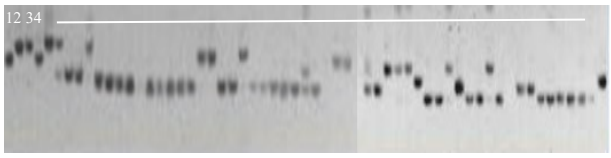

Figure 4. Polymorphic analysis of Indonesian rice germplasms using SSR marker RM20582. Lane 1: IRBB7, lane 2: IR64, lane 3: IR24, lane 4-53 Indonesian rice germplasms.

Table 5. Statistical summary of molecular marker analysis of 56 rice accessions

Marker	Size Range (bp)	Allele Number	Allele Frequency	PIC
RM20573	197 - 310	3	0.9649	0.0672
RM20582	80 - 83	4	0.5614	0.5320
RM20589	235 - 600	5	0.7895	0.3393
RM20590	280 - 400	6	0.7895	0.3545
RM20591	190 - 195	4	0.5965	0.4972
RM20595	50 - 169	2	0.8246	0.2475
RM20603	101 - 105	2	0.8772	0.1922
RM20612	152 - 160	5	0.4561	0.5118
Average		4	0.7324	0.3427

Cluster analysis was performed on similarity coefficient matrices calculated from the molecular markers to generate a dendrogram. When a cutoff value of 0.48 was used for genetic similarity among all varieties as the threshold for UPGMA clustering, two major groups were observed. Group 1 represented susceptible rice varieties consisting of 44 accessions and included the IR24 variety. Group 2 represented the resistant varieties, which included the IRBB7 and Conde varieties, and consisted of Sate Liko, Pulu Bolong, Horeg, Sijem, Majair, Daya Itoh Rice 4, Lumbu, Nolokario, Si Gudang and Cempo varieties (Suppl. 3). The dendrogram

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suggested that there was no geographical segregation of the rice accessions based on the obtained data.

Disease assessment of 167 rice germplasms against three BLB isolates/pathotypes analyzed using BLB scoring (Table 4), UPGMA cluster analysis (Suppl. 2), and molecular marker analysis (Suppl. 3) revealed that three germplasms displayed a consistent resistance profile: Sate Liko, Horeg and Sijem. A summary of results for those germplasms can be seen in Figure 5.

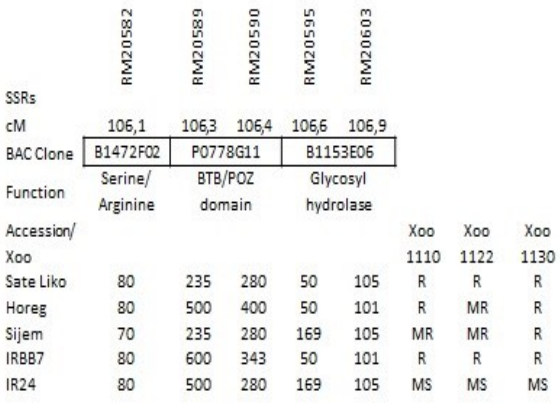

Figure 5. Genotyping of the region containing *Xa7* gene for each rice accession displaying consistent resistance profiles. SSRs = simple sequence repeats, R=resistant, MR=Medium Resistance and MS=Medium Susceptible. Centimorgan (cM) refers to relative distances along the Nipponbare BAC.

Association Analysis.

Association analysis performed using a General linear model has identified marker-trait associations ($P < 0.05$) of BLB resistance (Xoo1130/pathotype XII). Evaluation of six out of eight markers revealed a significant association between BLB resistance and markers (Table 6).

Table 6. Association (R^2) of SSR markers with resistance to BLB

Trait	SSR Marker[a]	Position (Mb)	P	R^{2b}
BLB Resistant	RM20582	27.91	0.0141	0.2708
	RM20589	27.93	0.0004	0.4820
	RM20590	28.01	0.0000	0.6211
	RM20595	28.08	0.0097	0.2093
	RM20603	28.15	0.0029	0.2668
	RM20612	28.29	0.0065	0.4113

[a] Only SSR markers with a significant marker-trait association are reported

[b] R^2 indicates the percentage of the total variation explained

Discussion

Natural selection has generated landraces with highly diverse quality, quantity and disease resistance traits-controlling loci. It is important to identify and maintain polymorphisms to broaden the genetic base of commercially cultivated varieties and to reduce pathogen pressure (Das *et al.,* 2014). Assessing diversity from large germplasm collections pose significant challenges, therefore this study concentrated on a small group of representative accessions before extending to a broader range of varieties.

Previous studies mentioned that *xa5, Xa21*, and *Xa7* resistance genes are effective against the majority of the *Xoo* isolates/pathotypes in Indonesia. (Fatimah *et al.,* 2014; Tasliah *et al.,* 2013; Hifni and Kardin, 1998). Hifni and Kardin (1998) reported that based on IRRI differential varieties, the population of *Xoo* isolates of pathotypes V and VI are the dominant pathotypes in Indonesia. Pathotype V could not overcome rice varieties with *xa5, Xa7,* and *Xa21* resistance genes, whereas pathotype VI could not overcome rice varieties with *xa5* and *Xa7* resistance genes. Pathotype XII was found to be the most virulent *Xoo* isolate and could overcome the rice varieties with more than ten resistance genes, but fortunately the population of *Xoo* isolates with pathotype XII is relatively low (Hifni and Kardin, 1998). However, in recent years pathotype XII was the most dominant pathotype found in 15 out of 22 districts in Central Java Province (Yuliani *et al.,* 2018) and several rice growing areas in South Sulawesi showed a shift to a larger proportion of pathotype XII (Asysyuura *et al.,* 2017).

Looking at 165 rice accessions and two resistant control varieties having *Xa7* resistance gene (IRBB7 and Conde varieties), the results of this study found that Xoo1130 (pathotype XII) was the most virulent compared to Xoo1110 (Pathotype VI) and Xoo1122 (pathotype V) (Figure 3). Implications of this study are that potentially, pathotypes V and VI could be used for selection of breeding lines with three resistance genes (*xa5, Xa7* and *Xa21*), while pathotype XII could be used to evaluate rice germplasm collection as a new source of resistance gene against *Xoo*.

The disease assessment of Indonesian rice germplasms by screening with three BLB

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isolates/pathotypes and genotyping using SSR primer linked to *Xa7* resistance gene revealed three out of 167 accessions that displayed a consistent resistant profile, i.e: Sate Liko, Horeg, and Sijem. It indicated that these three rice germplasms with a high degree of resistance could be a new source of resistance gene against BLB disease and can potentially be used to diversify the genetic base of core breeding sets.

Analyzing the phenotype-genotype association after an actual disease inoculation is a prerequisite for confirming BLB resistance with allele identification. Fine mapping of *Xa7* resistance gene has been previously constructed (Chen *et al*., 2008; Zhang *et al.,* 2009). The *Xa7* gene is located in an approximately 200-kb segment in the subtelomeric region of chromosome 6 (Zhang *et al.,* 2009). Several candidate genes that have been identified include BTB/POZ and Nramp6, known to be involved in plant resistance (Chen *et al.,* 2008). Those genes are located within the 84 kb region comprising the SNPs detected in GWAS (Dilla-Ermita *et al.,* 2017).

In this study, among the six markers evaluated in Indonesian rice germplasms, two markers (RM20589 and RM20590) revealed a high association with isolate Xoo1130 (pathotype XII) (Table 6), indicating that RM20589 and RM20590 were the closest markers to the *Xa7* resistance gene. These primers were located on BAC clone P0778G11 of Loc_Os06g46240 in region 28,007,285-28,017,490 bp (10.2 kb) on rice plant genome. This region is known as the BTB/POZ domain-containing protein, putative, expressed (MSU Rice Genome Annotation Project). These results are similar to an earlier study (Fatimah *et al.* 2018) that screened $BC_{1:2}F_2$ individuals with 35 primers and revealed the significance of primer Xa7LD37 with *Xa7* resistance gene. This primer was located between primer RM20589 and RM20590.

These results demonstrated the application of SSR primers in elucidating resistance to BLB in Indonesian rice germplasms that would enable discovery of significant SSR markers for marker-assisted selection. Efficient tracking of *Xa7* genes in the breeding pipeline provides breeders an insight that it is feasible to combine multiple genes (gene pyramiding) having complementary resistance spectra into a single plant genotype. This approach can provide a broad-spectrum of resistance that ensures maintenance of durability of *Xa* genes deployed in the field. The feasibility of this approach was demonstrated by Fatimah *et al.* (2015) where gene pyramiding on three BLB resistant genes *xa5, Xa7* and *Xa21* from Angke, Conde, and IRBB21 varieties into Ciherang and Inpari 13 elite varieties were performed and combined in a single BLB resistant gene *Xa4* background.

In conclusion, the majority of the indigenous Indonesian rice germplasms were susceptible to the three BLB isolates/pathotypes tested. Sate Liko from Bantul, Yogyakarta, Horeg from Cirebon, West Java and Sijem from Malang, East Java showed consistent resistance based on BLB evaluation in greenhouse using three isolates/pathotypes tested, UPGMA analysis, and genotyping using SSR markers linked to *Xa7* resistance gene. For future studies, these indigenous rice germplasms are potential donors that can confer resistance to multiple pathotypes and therefore are useful to be used in breeding and improvement program for BLB resistant-varieties in Indonesia.

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Supplement

Table 1. BLB Scoring of rice accession against BLB isolates used in this study.

No.	Accession Name	Xoo 1110		Xoo 1122		Xoo 1130	
		B	Cat.	B	Cat	B	Cat.
1	Putih Ampat Angkek	5	MS	4	MR	5	MS
2	Reli	6	S	5	MS	5	MS
3	Seng Kumang	5	MS	5	MS	5	MS
4	Mingkai	6	S	5	MS	5	MS
5	Rijah	6	S	5	MS	6	S
6	Aluh Kuranji	6	S	4	MR	5	MS
7	Kuning Biaro	5	MS	5	MS	5	MS
8	Gondak Kiah	5	MS	5	MS	5	MS
9	Si Rendah Cogok	6	S	5	MS	5	MS
10	Tromas	5	MS	5	MS	5	MS
11	Goter	5	MS	4	MR	4	MR
12	Untup	5	MS	4	MR	5	MS
13	Mayor	6	S	4	MR	5	MS
14	Baliman Putih	5	MS	5	MS	5	MS
15	Biduin	4	MR	5	MS	5	MS
16	Randah Pala	6	S	4	MR	6	S
17	Lalantik Bamban	5	MS	4	MR	5	MS
18	Raden Pulatar	5	MS	5	MS	5	MS
19	Siam Parapuk	6	S	4	MR	5	MS
20	Sara Kasa	5	MS	4	MR	5	MS
21	Pingkan	5	MS	5	MS	5	MS
22	Pimpin	5	MS	4	MR	6	S
23	Bengkongang	5	MS	5	MS	5	MS
24	Cicih Buleleng	5	MS	5	MS	5	MS
25	Angkong	5	MS	5	MS	4	MR
26	Padi Book	5	MS	5	MS	5	MS
27	Longandobu	5	MS	5	MS	5	MS
28	Padi Ana-Ana	5	MS	5	MS	5	MS
29	Anambar	5	MS	5	MS	5	MS
30	Wulu Mata	5	MS	5	MS	5	MS
31	Ingsa Cendana	5	MS	5	MS	5	MS
32	Ingsa Bondol	5	MS	5	MS	5	MS
33	Banja Ili	5	MS	5	MS	5	MS
34	Rejuno	5	MS	5	MS	5	MS
35	Abang	4	MR	5	MS	5	MS
36	Segon Borondol	5	MS	5	MS	5	MS
37	Mojang	5	MS	5	MS	6	S
38	Rangkong	5	MS	5	MS	5	MS
39	Cere Mentik/ Toray	S	S	5	MS	5	MS
40	Hawara Batu	5	MS	5	MS	5	MS
41	Muncang	5	MS	6	S	6	S
42	Gondok	6	S	5	MS	6	S
43	Laila	5	MS	6	S	6	S
44	Padi Belanak Kosambi	5	MS	5	MS	6	S
45	Padi Jambai	5	MS	5	MS	5	MS
46	Terotel	4	MR	6	S	5	MS
47	Wuri Bura	4	MR	5	MS	5	MS
48	Samada	5	MS	5	MS	5	MS
49	Dendak	5	MS	5	MS	5	MS
50	Lumbuk	5	MS	5	MS	5	MS
51	Secangkir	3	R	5	MS	5	MS
52	Jhapara	2	R	5	MS	5	MS
53	Tuma	2	R	5	MS	5	MS
54	Seratus Malam	2	R	5	MS	5	MS
55	Brown Gora	4	MR	4	MR	5	MS
56	Dular	4	MR	5	MS	5	MS
57	Sibentar	3	R	5	MS	5	MS
58	Siremut	4	MR	5	MS	5	MS
59	Buhbolon	3	R	5	MS	5	MS
60	Danau Atas	2	R	6	S	5	MS
61	Poso	2	R	5	MS	5	MS
62	Engkoran	5	MS	6	S	6	S
63	Baung	2	R	5	MS	5	MS
64	Banjar Sawan	4	MR	6	S	6	S
65	Kail	4	MR	5	MS	5	MS
66	**Payak Tembakau**	**3**	**R**	**R**	**R**	**4**	**MR**
67	Ribun	5	MS	5	MS	5	MS
68	Bonti	4	MR	5	MS	5	MS
69	Idi	5	MS	5	MS	4	MR
70	Syair	5	MS	5	MS	5	MS
71	Rangkoh	5	MS	5	MS	5	MS
72	Si Reguek	4	MR	5	MS	4	MR
73	Putih Panjah	5	MS	5	MS	5	MS
74	Bho	5	MS	5	MS	5	MS
75	Lemo	4	MR	4	MR	5	MS
76	Si Motung	5	MS	5	MS	4	MR

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No.	Accession Name	Xoo 1110		Xoo 1122		Xoo 1130	
		B	Cat.	B	Cat	B	B
77	Si Anak Bogor	5	MS	5	MS	5	MS
78	Si Lotik	5	MS	5	MS	5	MS
79	Si Pulo Angkola	5	MS	4	MR	5	MS
80	Si Pulo Manda iling	4	MR	4	MR	5	MS
81	Putri Manis	4	MR	5	MS	5	MS
82	Kuala Deli	5	MS	4	MR	5	MS
83	Ramos Batu	5	MS	5	MS	5	MS
84	Mama Laka	5	MS	5	MS	5	MS
85	Wajo Kuning	5	MS	4	MR	5	MS
86	Sera	4	MR	4	MR	5	MS
87	Keakubi	3	R	5	MS	5	MS
88	Sehan	5	MS	5	MS	5	MS
89	Ardas	5	MS	4	MR	5	MS
90	Apel	5	MS	5	MS	5	MS
91	Ndangan Cantik 1	5	MS	4	MR	5	MS
92	Lokal buntu Sangala 2	5	MS	3	R	5	MS
93	**Pare Pulunglia**	**4**	**MR**	**3**	**R**	**4**	**MR**
94	Cere Gelas	5	MS	4	MR	5	MS
95	Kembang Ading	5	MS	5	MS	4	MR
96	Limar	5	MS	5	MS	5	MS
97	Sasak Jalan	5	MS	5	MS	5	MS
98	Popot	5	MS	5	MS	5	MS
99	Kembang Singkan	5	MS	5	MS	4	MR
100	Ketan Siam	5	MS	5	MS	5	MS
101	Uyun	5	MS	4	MR	6	S
102	Kwatik Tinggi	5	MS	4	MR	6	S
103	Rumbai ayam	5	MS	5	MS	5	MS
104	Padi Rantau Undik	5	MS	4	MR	5	MS
105	Cinta Kasih	4	MR	4	MR	5	MS
106	Padi Bugis	5	MS	4	MR	4	MR
107	Surya	5	MS	4	MR	4	MR
108	Beras merah	5	MS	5	MS	5	MS
109	Slereng	5	MS	5	MS	5	MS
110	Pae Laguh	5	MS	6	S	4	MR
111	Ketan Jambruk	4	MR	5	MS	5	MS
112	**Sate Liko**	**3**	**R**	**3**	**R**	**3**	**R**
113	**Pulu Bolong**	**4**	**MR**	**4**	**MR**	**4**	**MR**
114	Pare Solo	4	MR	5	MS	5	MS
115	Pulut Tomene	3	R	5	MS	5	MS
116	**Horeg**	**2**	**R**	**4**	**MR**	**3**	**R**
117	Majair	3	R	4	MR	5	MS
118	Kalimis	5	MS	5	MS	4	MR
119	Termas	6	S	5	MS	5	MS
120	Daya Itoh Rice 4	6	S	2	R	4	MR
121	Si Gudang	5	MS	4	MR	4	MR
122	Cempo	5	MS	4	MR	5	MS
123	Gata	5	MS	5	MS	5	MS
124	Ringgit	5	MS	5	MS	5	MS
125	Tampay	5	MS	5	MS	5	MS
126	Nolo kario	4	MR	3	R	5	MS
127	Lumbu	4	MR	3	R	5	MS
128	Sri Makmur	4	MR	5	MS	5	MS
129	Fajar	5	MS	5	MS	5	MS
130	Kodok Putih	5	MS	5	MS	5	MS
131	Cempo Abang ner	5	MS	5	MS	5	MS
132	Teratai	5	MS	5	MS	6	S
133	Banda	5	MS	5	MS	5	MS
134	Betonan	6	S	5	MS	6	S
135	Lumut	6	S	5	MS	6	S
136	Sunting Beringin	6	S	5	MS	5	MS
137	Siak Simpur	5	MS	5	MS	5	MS
138	Bendang Bujur	5	MS	5	MS	5	MS
139	Kuku Balam	5	MS	5	MS	5	MS
140	Sirandah Tjogok	5	MS	5	MS	6	S
141	Empat	4	MR	5	MS	5	MS
142	Padi Rasi	5	MS	5	MS	6	S
143	Djula Djuli A	5	MS	5	MS	6	S
144	Hoing	4	MR	5	MS	5	MS
145	Makmur	4	MR	5	MS	5	MS
146	Revolusi	3	R	5	MS	5	MS
147	Gropak Serung	4	MR	5	MS	5	MS
148	Itun	5	MS	5	MS	5	MS
149	Nangka Bosok	4	MR	5	MS	5	MS
150	Bengawan	5	MS	5	MS	5	MS
151	**Sijem**	**4**	**MR**	**4**	**MR**	**3**	**R**
152	Sampa kiring	4	MR	5	MS	5	MS
153	Pelopor	3	R	5	MS	5	MS

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No.	Accession Name	Xoo 1110		Xoo 1122		Xoo 1130	
		B	Cat.	B	Cat	B	Cat.
154	Cempo Slamet	4	MR	5	MS	6	S
155	Ketan Uis	5	MS	5	MS	5	MS
156	Loyang	5	MS	5	MS	5	MS
157	Jerah	4	MR	5	MS	5	MS
158	Ketan Wangi	5	MS	5	MS	5	MS
159	Marus	5	MS	5	MS	5	MS
160	Koneng Gundil	5	MS	5	MS	5	MS
161	Sirandah Lunto	3	R	5	MS	5	MS
162	Cisadane	3	R	5	MS	4	MR
163	Semeru	4	MR	5	MS	5	MS
164	IRBB7	1	HR	1	HR	3	R
165	IR24	5	MS	5	MS	5	MS
166	Conde	1	HR	2	R	3	R
167	Kencana Bali	5	MS	HS	HS	5	MS

Note: B: BLB Score: 1 – 9 (Resistant: 1 – 4 and Susceptible: 5- 9)
Cat.: Category: HR: Highly Resistant; R: Resistant; MR: Medium Resistant, MS: Medium Susceptible, S: Susceptible; HS: Highly Susceptible

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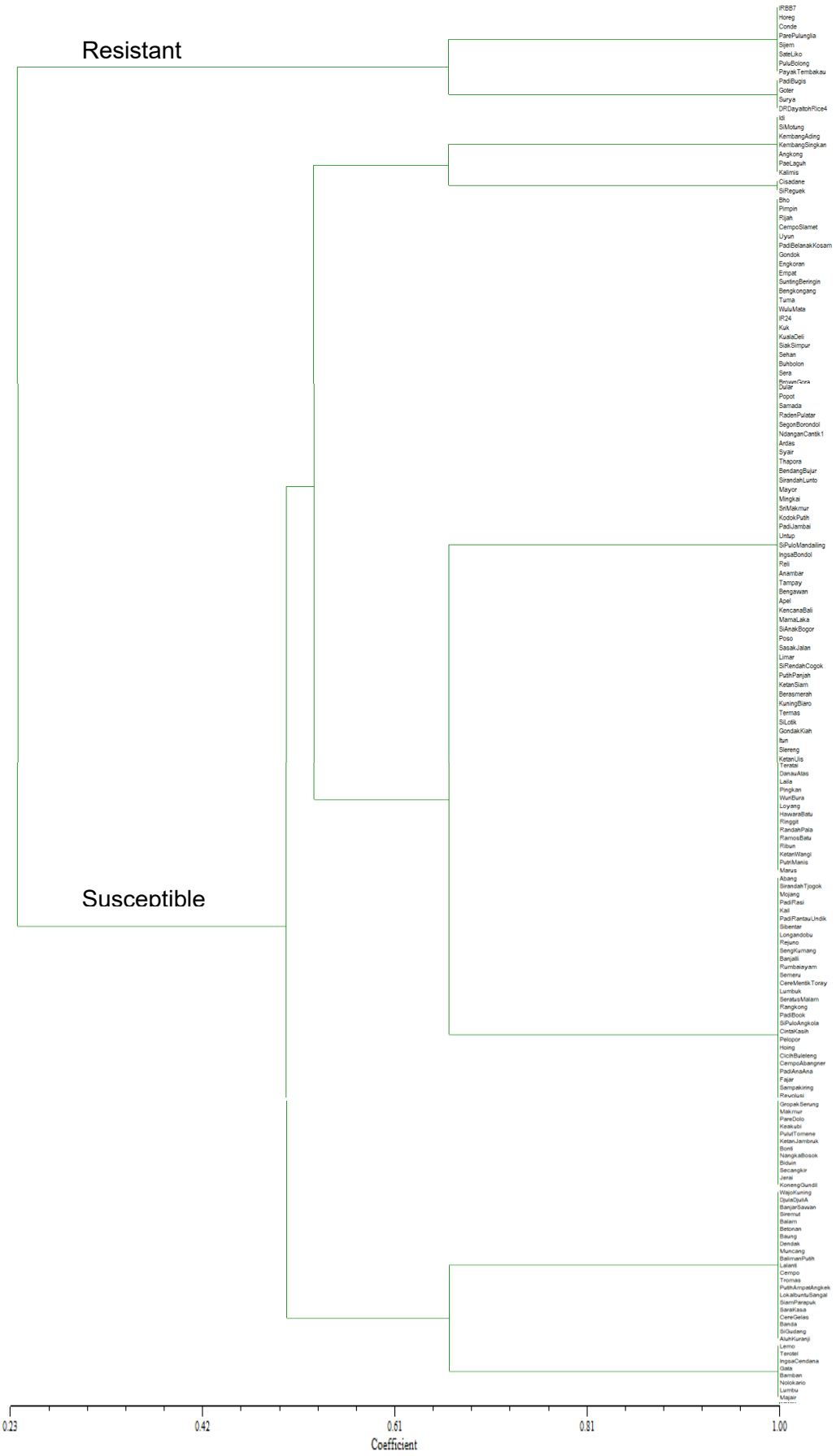

Figure 1. UPGMA Clustering analysis of 167 Indonesian rice germplasms based on their resistance to the three isolates/pathotypes tested.

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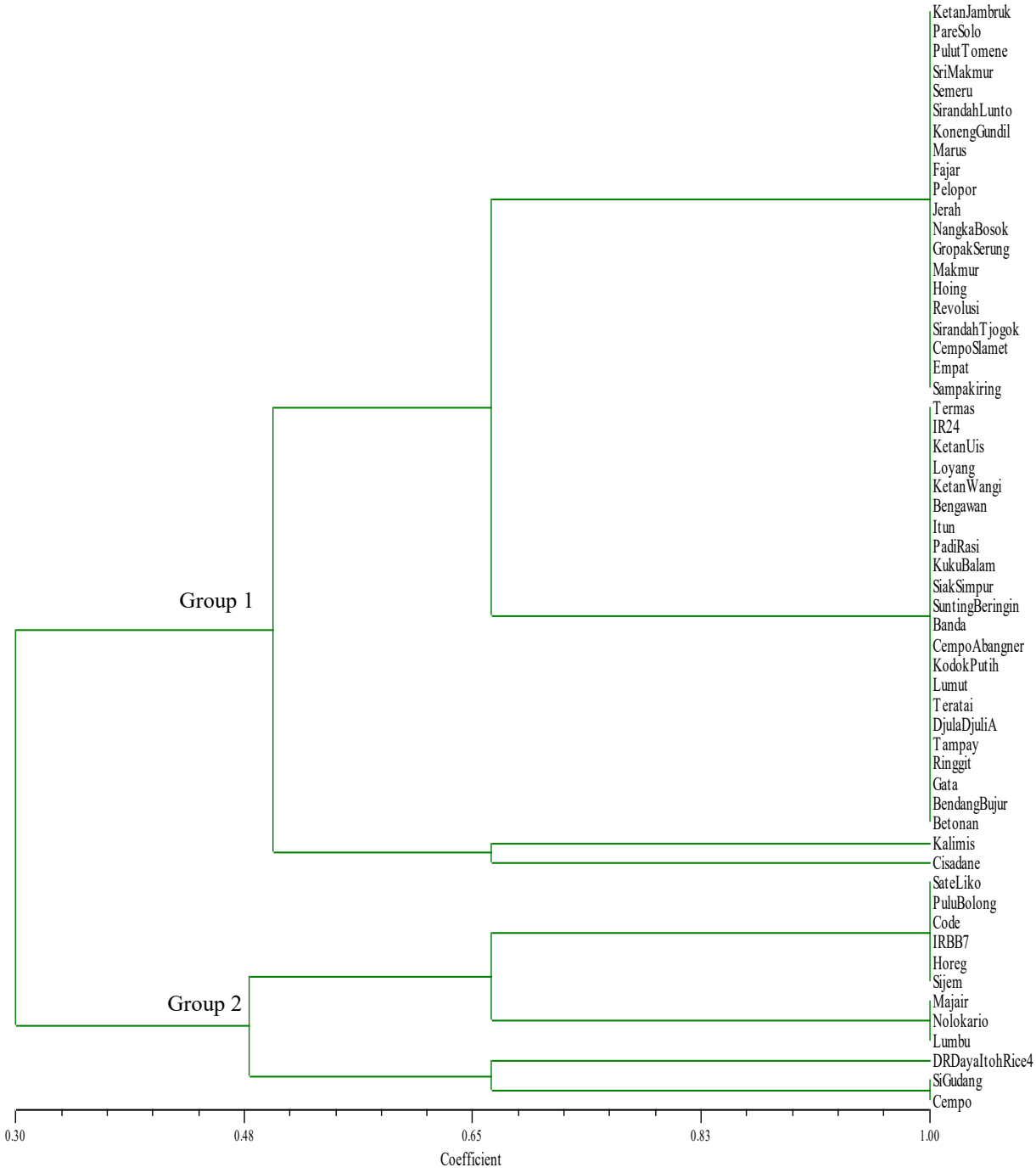

Figure 2. UPGMA tree of 56 Indonesian rice accessions using eight *Xa7* resistance-gene-linked SSR markers on chromosome 6, clustered as two major groups. Group 1 represents the susceptible varieties as indicated by IR24 as the susceptible control (black box). Group 2 represents the resistant varieties indicated by IRBB7 and Conde.