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Isolation and Disease Assessment of *Xanthomonas oryzae* pv. *oryzae* from Java Island and Pathogenic Assay on Near Isogenic Lines with Different Resistant Genes (Isolasi Bakteri *Xanthomonas oryzae* pv. *oryzae* dari P. Jawa dan Pengujian Penyakit pada Galur Isogenik dengan Gen Ketahanan Berbeda)

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ABSTRACT

Bacterial leaf blight disease caused by *Xanthomonas oryzae* pv *oryzae* (*Xoo)* is one of the important bacterial diseases, which is very destructive to rice plant. In this study, seventy isolates of *Xoo* were collected from several regions in West Java, Central Java, Yogyakarta and East Java provinces and ten isolates from ICABIOGRAD collection. The aim of the study was to survey variation and distribution of the pathogen and to study the interactions between the isolates and near isogenic lines carrying specific genes for bacterial leaf blight resistance. Twenty *Xoo* isolates were randomly chosen to assess the differential characteristics of ten near-isogenic rice lines in Indonesia. The results showed that *xa5* resistant gene was the highest effective against the majority of *Xoo* isolate, followed by *Xa21*, and *Xa7* combined with *Xa4* as the background. These findings are useful for rice breeding programs in designing stable broad-spectrum bacterial blight resistant rice cultivars.

Key words: *Xanthomonas oryzae*, resistant gene, Near isogenic line, Java

ABSTRAK

Penyakit hawar daun bakteri yang disebabkan oleh bakteri *Xanthomonas oryzae* pv *oryzae* (*Xoo*) merupakan salah satu penyakit penting yang merusak tanaman padi. Pada penelitian ini telah diisolasi sebanyak 70 isolat bakteri *Xoo* dari empat provinsi di pulau Jawa meliputi Jawa Barat, Jawa Tengah, DI Yogyakarta dan Jawa Timur dan 10 isolat koleksi BB-BIOGEN. Penelitian ini bertujuan untuk melihat variasi dan distribusi pathogen *Xoo* dan interaksi antara isolat *Xoo* dengan galur isogenik yang membawa gen tunggal ketahanan terhadap penyakit hawar daun bakteri. Sebanyak sepuluh isolat baru dan sepuluh isolat koleksi BB-BIOGEN secara acak telah dipilih dan diuji karakteristik diferensialnya pada sepuluh galur isogenik dan varietas elit. Hasil menunjukkan bahwa gen ketahanan *xa5* dari galur IRBB5 dan Angke masih efektif untuk melawan penyakit hawar daun bakteri, diikuti gen *Xa21* dari galur IRBB21 dan *Xa7* dari galur IRBB7 dan Code dan digabungkan dengan *Xa4* yang berasal dari IR64 sebagai *background*. Informasi ini sangat berguna dalam program pemuliaan padi untuk mendapatkan kultivar yang memiliki ketahanan penyakit hawar daun bakteri berspektrum luas dan lestari.

Kata kunci: *Xanthomonas oryzae*, Gen ketahanan, Galur Padi Isogenik, Jawa

INTRODUCTION

Rice production is constrained by several fungal, bacterial and viral diseases. Bacterial leaf blight (BLB) of rice caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the oldest known serious diseases of rice in tropical lowland rice environment (Yamamoto *et al.* 1977). Climate change; resulting in changes in rainfall patterns, affects shifting of seasons and fluctuations in temperature and humidity and thus cropping patterns will be capable of stimulating BLB growth and development. BLB is favored by rain, high levels of fertilizer, high humidity, and warm temperatures (Ou 1985).

Planting resistant rice cultivar is the most economically feasible and environmentally safe method to control plant disease such as BLB. Many resistant cultivars have been developed, but rapid changes in pathogen variability have led to the instability of plant resistance. Host plant population in plant pathogen ecosystem influences the genetic variability of pathogen population (Leach & White 1990), hence the deployment of genes for resistance to *Xoo* in commercial rice cultivars is necessary. The introduction of these resistance genes into rice is correlated with a change in the pathogenic diversity of *Xoo* populations. Evolution of the pathogen is able to break down the resistant genes. New races of the pathogen emerge and overcome deployed resistance (Mew *et al.* 1992). This was first reported in Indonesia by Oka (1972) when he observed that IR8 and IR5 were resistant while other rice cultivars such as Arias, Sintha, and Kortina were susceptible.

At present, most of the existing commercial rice varieties are susceptible to the *Xoo* isolates from Indonesia (Kadir 2009). To solve such problems, the deployment of a strategy of varietal resistance needs to be established. Therefore, comprehensive information on the development and distribution of *Xoo* in Indonesia is needed. In this study, we used BLB samples collected from different areas in four provinces in Java island (West Java, Central Java, DI Yogyakarta and East Java) and from ICABIOGRAD collection to determine variation and distribution of BLB pathogen, and their interactions between the isolates and several near isogenic lines carrying specific genes for BLB resistance.

MATERIALS AND METHODS

Randomly choosen 10 isolates obtained from field collection in Java Island (between isolate no #1 to #70) and ten *Xoo* isolates from the collection of ICABIOGARD (isolate no #71 to 80) were used to test ten isogenic rice lines with different single resistant genes (*Xa1, Xa2, Xa3, Xa4, xa5, Xa7, Xa10, Xa11, Xa14,* and *Xa21)* obtained from IRRI (International Rice Research Institute), the Phillipines. Ciherang, INPARI13, IR64 and Cisadane were ellite varieties, Code (*Xa4*+*Xa7*) and Angke (*Xa4*+*xa5*) as resistant control having two resistance genes, and Kencana Bali and IR24 were used as susceptible control (Table 1).

Diseased rice leaves showing typical BLB symptoms were collected from various rice fields from four provinces in Java Island (West Java, Central Java, DI Yogyakarta and East Java) during the 2013 planting seasons. Samples were collected based on random sampling method from rice fields at heading stages approaching mature stages, as the disease usually develop well in these growth stages. Diseased leaves were detached and put into paper envelopes labeled with plant varieties, sampling locations and sampling dates, and then kept in plastic boxes. The samples were taken into the laboratory and kept in the refrigerator for further process.

Tabel 1. Lists of near isogenic lines and control varieties

No.	varieties	remarks
1	Code	*Xa7*
2	Angke	*xa5*
3	Ciherang	Elite Variety
4	Inpari 13	Elite Variety
5	IR64	Elite Variety
6	Cisadane	Elite Variety
7	Kencana Bali	Susceptible check
8	IRBB1	*Xa1*
9	IRBB2	*Xa2*
10	IRBB3	*Xa3*
11	IRBB4	*Xa4*
12	IRBB5	*xa5*
13	IRBB7	*Xa7*
14	IRBB10	*Xa10*
15	IRBB11	*Xa11*
16	IRBB14	*Xa14*
17	IRBB21	*Xa21*
18	IR24	Susceptible check

Diseased leaves were taken out from the envelope, washed with tap water and then air-dried. The samples were then cut into small pieces of about 1cm x 1cm in size and put in a shallow-well sterile plates containing 70% ethanol for about 3 minutes. The samples were then washed twice in sterile distilled water in new plates for 1 minute each. After the washing the samples were placed into new plates wounded using a sterilized sharp tip bamboo stick. The bamboo stick was then streak onto petridish containing Wakimoto's medium (WF-P; 20 g sucrose, 5 g peptone, 0.5 g [NO3] $_2Ca{\cdot}_4H_2O$, 1.82 g $Na_2HPO_4{\cdot}_7H_2O$, 0.05 g $FeSO_4{\cdot}_7H_2O$, 18 g bacto agar for 1 L Media) (Ou 1985). The plates were incubated at room temperature (28-30 °C) for 3 to 4 days. Single yellow, round and smooth margin, non flat, mucous colonies were selected and transferred into slant WF-P medium as pure culture and were used for this study.

Bacterial isolates from lyophilized cultures were revived on Wakimoto agar medium and incubated at 28 °C for 48h. A-2-day-old culture of each isolate was used to prepare inoculums suspensions. The bacterial colonies were suspended with 20 ml sterile distilled water and adjusted to 10^8 $CFUml^{-1}$ concentration prior to incoculation. To test the virulence of the strains, the fully expanded leaves were inoculated by the leaf cutting method (Kauffman *et al.* 1973).

Ten randomly chosen representative isolates from the 70 *Xoo* isolates (#1 to #10) and ten isolates rejuvenation originated from ICABIOGRAD genebank collections (#71 to #80) were used to assess the differential characteristics of ten near-isogenic rice lines and elite varieties (Table 1). The ten near isogenic and elite varieties seeds were first sown in plastic boxes, 14 days, seedlings were transplanted into plastic boxes containing field paddy soil. Rice plants were grown under greenhouse condition. The experimental unit consisted of 18 rows with a spacing of 3 x 6 cm. Each rice lines were transplanted in one row of five plants. The disease inoculation was done when rice plants were 40 days old.

The disease evaluation was conducted at 7 and 14 days after inoculation. Ten randomly selected clipped leaves of each line-isolate combination were rated for lesion. Lesion lengths of rice line lower than 5 cm were classified as resistant (R), 5-10 cm were medium resistant (MR), 10-15 cm were medium susceptible (MS) and >15 cm were rated as susceptible (S) (IRRI 1996).

The data obtained were input into Microsoft Excel spread sheet program (Microsoft Excel version 2007). Statistical analyses were performed using software program NTSYSpc 2.11p (Exeter Software, Setauket, USA), based on the resistant clasification. The resistant or susceptibility to of Xoo was coded by binary numbers of 1 or 0; where 1 was for resistant (R) and 0 was for MR, MS and S. The Dice Coefficient (SIMQUAL) and UPGMA (*Unweighted Pair Group Method Aritmatic*) methods were used for Xoo clustering analysis.

A total of 70 isolates of *Xoo* were collected from four provinces in Java Island (isolate no #1 to #70) (Table 2). Based on their origin, out of total the isolates proportion inform West Java, Central Java, Yogyakarta and East Java were 27,1%, 44,3%, 7,1% and 21,4%, respectively, but most of them from Pemalang, Central Java (17%) and Subang, West Java (12,8%). Based on varieties, 58,6% of isolates was isolated from Ciherang varieties and 11,4% was IR64. It revealed the existence and dominance of local strain of *Xoo* collected from West Java, Central Java, Yogyakarta and East Java. The commercial rice cultivar, Ciherang and IR64, resulted as susceptible varieties. The deployment of genes for resistance to *Xoo* to these varieties is needed. Out of 70 collected isolates, isolate #1 to #10 and ten isolates from randomly selected ICABIOGRAD collection (isolate #71-80) were evaluated for disease assessment to ellite varieties and near isogenic lines.

Different reaction revealed from tested cultivar to twenty *Xoo*. Based on the percentage of compatible

Table 2. List of *Xanthomonas oryzae* pv *oryzae* used in this study.

Isolates Code	Variety	Sub-distric	Distric	Province	Isolates Code	Variety	Sub-distric	Distric	Province
Xoo13-01	Inpari 18	Jatisari	Karawang	West Java	Xoo13-636	Ciherang	Jombang	Jombang	East Java
Xoo13-02	Inpari 18	Cikalong wetan	Karawang	West Java	Xoo13-645	Ciherang	Jombang	Jombang	East Java
Xoo13-03	C4	Gantar	Indramayu	West Java	Xoo13-648	Ciherang	Plaso	Jombang	East Java
Xoo13-04	Mentikwangi	Pabela	Magelang	Central Java	Xoo13-652	Ciherang	Plaso	Jombang	East Java
Xoo13-05	Lokal	Kaliduren	Doroyudan	Central Java	Xoo13-654	Ciherang	Manggalin	Jombang	East Java
Xoo13-06	Ciherang	Kepanjen	Klaten	Central Java	Xoo13-664	Ciherang	Joyorego	Jombang	East Java
Xoo13-07	Ciherang	Sedayu	Bantul	Yogyakarta	Xoo13-668	Ciherang	Bangsel	Mojokerto	East Java
Xoo13-08	Ciherang	Sejati	Mayudan	Central Java	Xoo13-687	Ciherang	Mojosari	Mojokerto	East Java
Xoo13-09	Ciherang	Sleman	Sleman	Yogyakarta	Xoo13-690	Ciherang	Bongsol	Mojokerto	East Java
Xoo13-10	Ciherang	Sukamandi	Subang	West Java	Xoo13-694	Ciherang	Pondohan	Pasuruan	East Java
Xoo13-57	Ciherang	Petarukan	Pemalang	Central Java	Xoo13-699	Situbagendit	Pandoan	Pasuruan	East Java
Xoo13-58	Ciherang	Petarukan	Pemalang	Central Java	Xoo13-704	Ciherang	Bongil	Pasuruan	East Java
Xoo13-59	Sidenuk	Comet	Pemalang	Central Java	Xoo13-707	IR64	Bangil	Pasuruan	East Java
Xoo13-60	Ciherang	Comet	Pemalang	Central Java	Xoo13-718	Ciherang	Wonoasih	Probolinggo	East Java
Xoo13-61	Ciherang	Pemalang	Pemalang	Central Java	Xoo13-732	Ciherang	Kadema meah	Probolinggo	East Java
Xoo13-62	Ciherang	Boden	Pemalang	Central Java	Xoo13-813	Ciherang	Pemalang	Pemalang	Central Java
Xoo13-63	IR64	Kedungwuri	Pemalang	Central Java	Xoo13-1013	Ciherang	Pemalang	Pemalang	Central Java
Xoo13-64	Sidenuk	Kedungwuri	Pemalang	Central Java	Xoo13-4613	Ciherang	Pekalongan	Pekalongan	Central Java
Xoo13-65	Sidenuk	Kedungwuri	Pemalang	Central Java	Xoo13-4813	IR64	Pekalongan	Pekalongan	Central Java
Xoo13-66	Ciherang	Pemalang	Pemalang	Central Java	Xoo13-7613	Sidenok	Pekalongan	Pekalongan	Central Java
Xoo13-224	Ciherang	Belanakan	Subang	West Java	Xoo13-10313	Inpari 10	Batang	Batang	Central Java
Xoo13-240	Ciherang	Binong	Subang	West Java	Xoo13-10713	Inpari 10	Batang	Batang	Central Java
Xoo13-247	Ciherang	Pusakanagara	Subang	West Java	Xoo13-11613	Ciherang	Batang	Batang	Central Java
Xoo13-248	Ciherang	Patokbeusi	Subang	West Java	Xoo13-12813	Sidenok	Kudus	Kudus	Central Java
Xoo13-254	Ciherang	Pamanukan	Subang	West Java	Xoo13-14013	Ciherang	Jepara	Jepara	Central Java
Xoo13-338	Ciherang	Sukasari	Subang	West Java	Xoo13-15813	Ciherang	Jepara	Jepara	Central Java
Xoo13-343	Ketan	Batang	Subang	West Java	Xoo13-16313	Ciherang	Jepara	Jepara	Central Java
Xoo13-363	Mekongga	Kotabaru	Karawang	West Java	Xoo13-18213	Situbagendit	Pati	Pati	Central Java
Xoo13-384	Ciherang	Binong	Subang	West Java	Xoo13-18513	Situbagendit	Pati	Pati	Central Java
Xoo13-389	Muncul	Cilamaya	Karawang	West Java	Xoo13-18813	Ciherang	Pati	Pati	Central Java
Xoo13-409	Muncul	Tegalwaru	Karawang	West Java	Xoo12-231	IR64	Sawahan	Batang	Central Java
Xoo13-463	Ciherang	Haurgeulis	Indramayu	West Java	Xoo12-200	Ciherang	Kalimanggis	Batang	Central Java
Xoo13-483	Mekongga	Anjatan	Indramayu	West Java	Xoo12-176	IR64	Kauman	Batang	Central Java
Xoo13-489	Ciherang	Gantar	Indramayu	West Java	Xoo12-225	IR64	Duwet	Pekalongan	Central Java
Xoo13-517	IR kebo	Losarang	Indramayu	West Java	Xoo12-183	IR64	Duwet	Pekalongan	Central Java
Xoo13-569	IR kebo	Kalasari	Sleman	Yogyakarta	Xoo12-190	Ciherang	Kalimanggis	Batang	Central Java
Xoo13-578	Mekongga	Berban	Sleman	Yogyakarta	Xoo12-198	Ciherang	Kalimanggis	Batang	Central Java
Xoo13-587	Ketan	Prambanan	Sleman	Yogyakarta	Xoo12-207	Ciherang	Kalimanggis	Batang	Central Java
Xoo13-601	Situbagendit	Kalitirto	Suman	Yogyakarta	Xoo12-177	IR64	Kauman	Batang	Central Java
Xoo13-607	Situbagendit	Plered	Bantul	Yogyakarta	Xoo12-195	Ciherang	Kalimanggis	Batang	Central Java

isolates, the tested isolates (>80%) were compatible (host susceptible) on IR24, IR64 and Kencana Bali while the near isogenic lines were IRBB2, IRBB4, IRBB10, IRBB11, and IRBB14 with mean lesion length was more than 9 cm. The moderate reaction was found on Inpari 13, Cisadane and Ciherang varieties while the near isogenic lines were IRBB1 and IRBB3 on 55-75% tested isolates and means lesion length was more than 7 cm. The incompatible isolates (host resistant) on <30% tested isolates and means lesion length was more than 3 cm were found on Code and Angke varieties and the near isogenic lines were IRBB5, IRBB7 and IRBB21 (Figure 1 and 2). Code and Angke carried two resistance genes the *Xa4+Xa7* and *Xa4+xa5*, respectively, were more resistant then lines having single resistance gene of *xa5* (IRBB5), *Xa7* (IRBB7) and *Xa4* (IR64). The *Xa4* derived from IR64 as the background might enhance the resistance of *xa5* and *Xa7* if combined together formed *Xa4+xa5* and *Xa4+Xa7*. Out of ten resistance genes used in this study, only *xa5*, *Xa7*, and *Xa21* were relatively

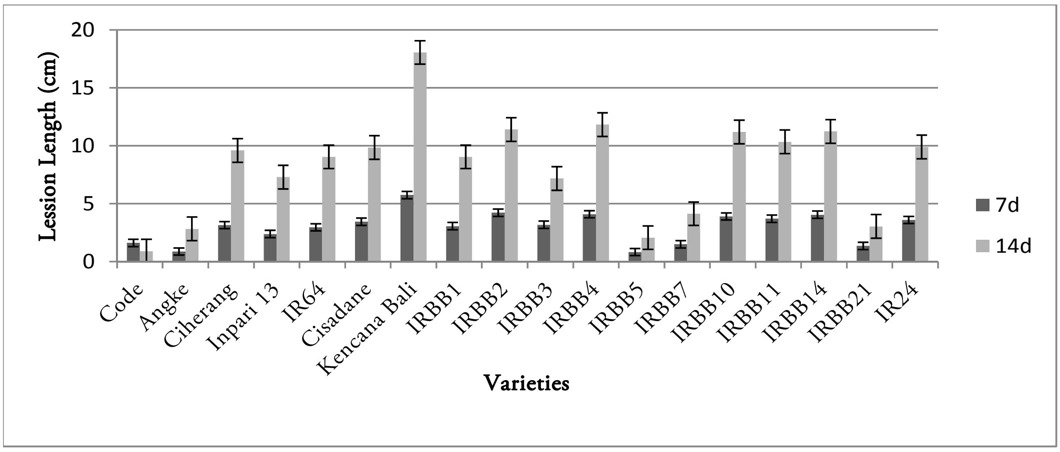

Figure 1. Mean lession length (cm) of 18 rice lines infected by twenty *Xoo*, on 7 and 14 days after inoculation.

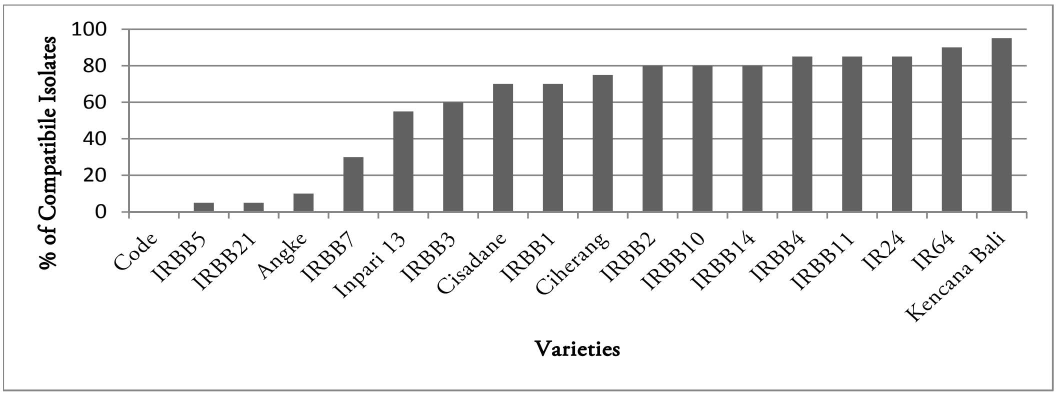

Figure 2. Percentage of compatible isolates on 18 rice lines inoculated with 20 isolates of *Xoo*. The discrimination of 18 rice lines in *Xoo* was evaluated by their susceptible reaction to twenty *Xoo* isolates measured 14 days after inoculation.

effective against the majority of the *Xoo* isolates. The isolate were Xoo13-01, Xoo13-32, Xoo13-04, Xoo13-05, Xoo13-06, Xoo13-07, Xoo13-09, Xoo13-10, Xoo12-225, Xoo12-183, Xoo12-190, and Xoo12-177.

Figure 3 showed the visualization of variation in disease severity of infected leaves of isolate Xoo12-225 at 14 days after inoculation. The reaction of Code, Angke, IRBB5, IRBB7 and IRBB21 were resistant as it developed a yellow stripe appears inside of the margin of the leaf blade with no necrotic lesion for some time but stripes may eventually turn yellow and necrotic. The reaction of susceptible tested cultivars Ciherang, Inpari 13, IR64, Cisadane, Kencana Bali, IRBB1, IRBB2, IRBB3, IRBB4, IRBB10, IRBB11, IRBB14, and IR24, the lesions occured on leaf sheath at the site of an injury. The affected leaves turned yellow, roll up and wilt rapidly that produces tannish-grey to white lesions along the vein.

The ten near isogenic lines in this study were IRBB1, IRBB2, IRBB3, IRBB4, IRBB5, IRBB7, IRBB10, IRBB11, IRBB14 and IRBB21, next we call IRBBN, revealed nine resistance pattern of ten IRBBN tested to twenty *Xoo* isolates. Five isolates or 25% (Xoo12-225, Xoo12-183, Xoo12-190, and Xoo12-177) giving SSSSRRSSSR pattern (R for resistance and S for susceptible) with the R being IRBB5, IRBB7 and IRBB21, respectively. Three isolates or 15% (Xoo1305, Xoo13-09 and Xoo13-10) giving RRRRRRRRRR pattern to ten IRBBN and one isolate or 5% (Xoo12-176) giving SSSSSSSSSR pattern with the R being IRBB21. The other IRBBN resistance pattern was showed in Table 3.

The twenty *Xoo* isolates were submitted to UPGMA analysis based on their reaction of resistance/

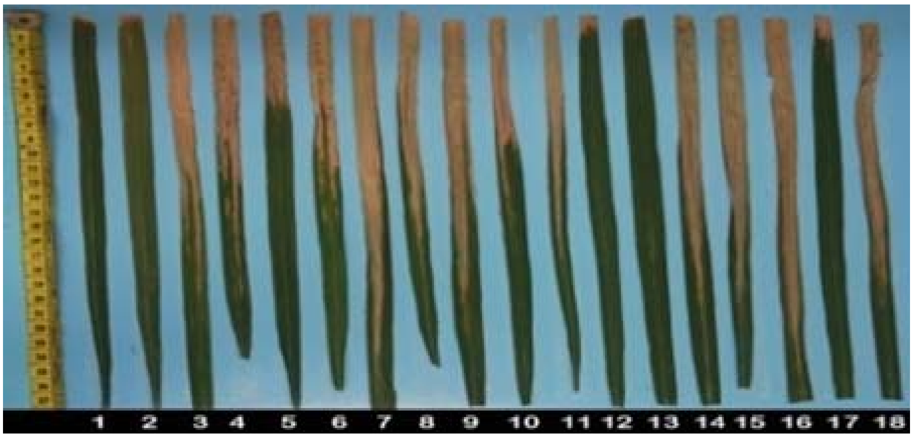

Figure 3. Visualization of variation in disease severity of infected leaves (tannish-grey to white lesions along the vein).of Xoo12-225 from Pekalongan, Central Java at 14 days after inoculation. From left to right Code, Angke, Ciherang, Inpari 13, IR64, Cisadane, Kencana Bali, IRBB1, IRBB2, IRBB3,IRBB4, IRBB5, IRBB7, IRBB10, IRBB11, IRBB14, IRBB21, and IR24, respectively.

Table 3. Resistance pattern of disease severity of twenty *Xoo* on ten near isogenic lines.

Near Isogenic Lines	Resistance pattern								
IRBB1	R	S	S	S	S	R	R	S	S
IRBB2	R	S	S	S	S	S	R	S	S
IRBB3	R	R	R	S	S	R	R	S	S
IRBB4	S	S	S	S	S	S	R	S	S
IRBB5	R	R	R	S	R	R	R	R	R
IRBB7	R	S	R	S	R	R	R	R	S
IRBB10	R	S	S	S	S	S	R	S	S
IRBB11	S	S	S	S	S	S	R	S	S
IRBB14	R	S	S	S	S	S	R	S	S
IRBB21	R	R	R	R	S	R	R	R	R
No.of Isolate	1	1	1	1	2	2	3	5	4
Percentage of Isolate (%)	5	5	5	5	10	10	15	25	20

susceptible to 18 cultivars tested. The results yielded four clusters of major groups at a similarity level of 79%, called GI, GII, GIII and GIV (Figure 4). GI, GII and GIII were consisted of incompatible isolates (host resistant) i.e Xoo13-01; Xoo1305, Xoo13-09, Xoo13-10; Xoo13-02, Xoo13-03, Xoo13-04, Xoo13 -06, respectively, while GIV group was consisted of compatible isolates (host susceptible) i.e. Xoo1307, Xoo13-08, Xoo12-231, Xoo12-225, Xoo12-183, Xoo12-190, Xoo12-177, Xoo12-200, Xoo12-198, Xoo12-195, Xoo12-207, and Xoo12-176.

DISCUSSION

Near isogenic rice lines having ten different major genes for resistance to *Xoo*, developed by IRRI were used to analyze virulence of 20 isolates of *Xoo*. These infection responses were clear and easily classified into the level of resistance on the test rice lines possessing different genes for resistance. We identified nine resistance pattern based on infection responses elicited on rice lines containing single resistant genes and four groups of compatible

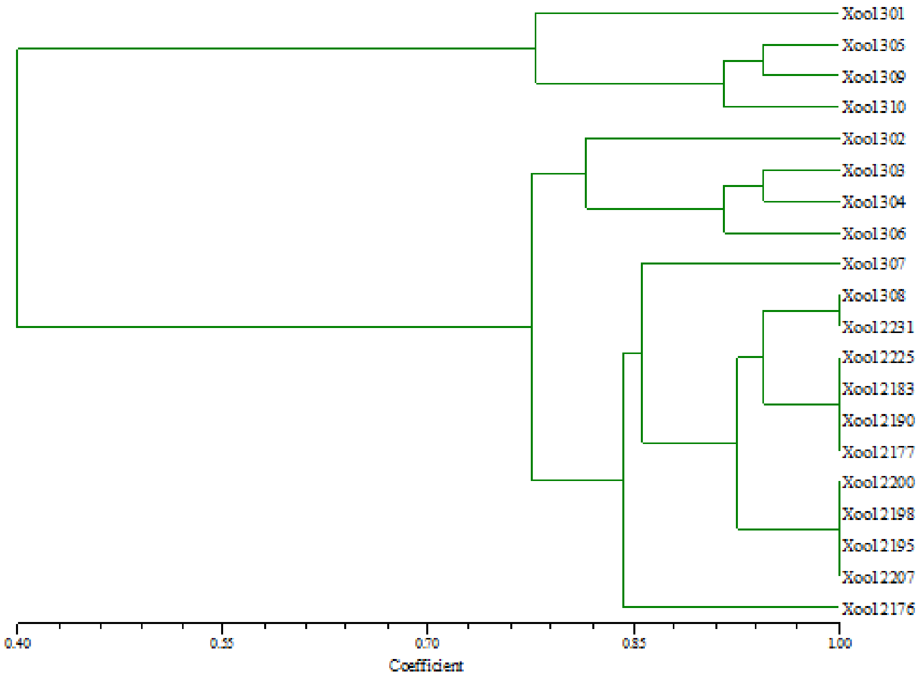

Figure 4. Dendrogram generated after cluster analysis of the R/S reaction to 18 varieties of twenty Xoo.

and incompatible isolates. Out of ten resistance genes used in this study, only *xa5, Xa7*, and *Xa21* and combined *Xa4* would relatively effective against the majority of the *Xoo* isolates. Susanto & Sudir (2012) reported that IRBB64 (*Xa4+xa5+Xa7 +Xa21*) was more resistant in Sukamandi.

Compatible and incompatible interactions of near isogenic lines with bacterial leaf blight isolates were examined in order to determine variation of bacterial leaf blight resistance and the stability of resistance gene (Kim *et al.* 2007). This finding was also reported in *Xoo* isolates in Mekong Delta region, Vietnam by Dinh *et al.* (2008). Moreover, studies to assess differential characteristics of 24 near isogenic rice lines with resistance gene to strains from China have been reported (Liu *et al.* 2007). All subjected isolates were avirulent to IRBB5 (*xa5*) which was the most effective against the majority of the *Xoo* and the next were IRBB21 (*Xa21*), and IRBB7 (*Xa7*). Similar phenomenon was also reported from 106 Indonesian isolates of *Xoo* isolated from diseased plants in Bekasi and Karawang Districts in West Java, Indonesia (Hifni & Kardin 1998), from four regions in China such as south China, south west China, Yangtze Valleys and north-east China (Liu *et al.* 2007), and farmer fields in Gagillapur and Kompally, Andhra Pradesh in India (Reddy *et al.* 2009). It is indicated that for almost 20 years, *xa5, Xa7* and *Xa21* are still effective against the majority of the Xoo isolates in Indonesia (Hifni & Kardin 1998).

However Banito *et al.* (2012) reported that the near isogenic lines IRBB5 was susceptible in Togo, West Africa. Some strains overcome the resistance of the near isogenic line IRBB5 with gene *xa5*. Similar results was also reported by Onasanya *et al.* (2009) who found IRBB5 was susceptible to bacteria strains isolated from 7 African countries. Similarly in rice line IR24 revealed resistance to the 13 strains used. It also confirmed the results found by Onasanya *et al.* (2009) who reported that IR24 was one of the resistant lines tested against 50 strains of *Xoo* from different countries. Mannan *et al.* (2009) reported that none of the lines used was resistant against all *Xoo* strains prevalent in Pakistan were virulent to *Xa21*. Although *Xa21* was reported to be effective and stable against multiple isolates of *Xoo* (Shanti *et al.*

2001), however in this study, two isolates (10%) Xoo13-08 and Xoo12-231 showed the susceptible reaction of IRBB21. This finding was confirmed by Kadir *et al.* (2007) who reported that the reaction of IRBB21 was susceptible to some Indonesian isolates in which the disease severity was up to 48% (on IRBB21).

The information of this study has significant implication for regional gene deployment. In this study, only *xa5, Xa21*, and *Xa7* are relatively effective against the majority of the *Xoo* isolates, and also *Xa4* as the background of IR64, so that these resistance genes can be incorporated into our rice breeding program. A pyramid line containing genes *Xa4*, *xa5, Xa21* and *Xa7* would be the most promising and valuable genotype for improving Indonesian cultivars for bacterial blight resistance. These findings are useful to rice breeding programs designed to develop stable broad-spectrum resistance to bacterial blight in rice cultivars.

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