

STUDY ON THE USE OF COMBINATION RESISTANCE GENES IN RICE LINES AGAINST *Xanthomonas oryzae* pv *oryzae* IN CUULONG RIVER DELTA

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ABSTRACT

In Cuulong Delta, bacterial leaf blight of rice caused by Xanthomonas oryzae pv. oryzae has been severely increasing in recent years. The use of single resistance genes was low effective in disease management due to a great variation of disease pathogens. Eleven near isogenic rice lines carrying 2 to 5 resistance genes were used for testing against to six races of bacterial collected from Cuulong Delta. The results showed that most of the NILs exhibited high level of resistant reaction to many races in Cuulong Delta. Only some lines were moderately susceptible to the disease. The pyramiding line IRBB 65 carrying 4 resistance genes Xa-4, Xa-7, xa-13 and Xa-21 was resistance to all the races in the delta and the line IRBB 54 carrying 2 resistance genes xa-5 and Xa-21 exhibited its resistance to 5 races too, except the race A. This line was effective to the breeding program to develop the rice varieties with the wide spectrum disease resistance .

INTRDUCTION

Bacterial blight (BB) of rice, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the oldest known diseases and was first noticed by the farmers of Japan in 1884. It is one of the most destructive rice diseases and can reduce yield by 20 to 30%, and in some areas of Asia it can reduce crop yield by up to 50%. (Singh et al. 1977, Khush et al. 1989). Though Bordeaux mixture, antibiotics, and other chemical compounds were used in the early 1950s, environmentally safe and stable chemical control agents rendering control at very low concentrations have yet to be developed. Today, the exploitation of host resistance appears to be the only reliable method of disease management (Narayanan 2002).

Numerous resistance genes have been identified and used for disease management so far. Unfortunately, studies on pathogen variation have revealed that breeding with single major genes for resistance, may be ineffective due to resistance breakdown. Thus, pyramiding of multiple resistance genes into rice varieties is one way to

develop durable resistance to Bacterial blight (Gnanamanickam 1999, Sanchez 2000).

In the Cuulong Delta, the bacterial blight was not serious from 1980 - 1995. Recently, severity has increased year by year. Most of leading varieties become susceptible, especially Jasmine 85, OMCS 2000, OM2517, ... which are grown in large-scale area (Loan 2007). The previous results showed that there were 6 bacterial blight races in CRD, only genes *xa5*, *Xa7*, *xa13* and *Xa21* were incompatible with some races of pathogen (Dinh 2008).

In this study, we use some resistance genes pyramided in near isogenic lines of rice and to determine their reaction with the pathogen races in Cuulong Delta.

MATERIALS AND METHODS

Rice lines: Eleven pyramiding lines were provided by Dr. H Leung and CM Vera Cruz from International Rice Research Institute (IRRI), and IR 24 was used as a susceptible check.

Table 1: Near isogenic lines of rice carrying two to five resistance genes

STT	NILs	Resistance genes
1	IRBB 51	<i>Xa-4 + xa-13</i>
2	IRBB 54	<i>xa-5 + Xa-21</i>
3	IRBB 55	<i>xa-13 + Xa-21</i>
4	IRBB 57	<i>Xa-4 + xa-5 + Xa-21</i>
5	IRBB 60	<i>Xa-4 + xa-5 + xa-13 + Xa-21</i>
6	IRBB 61	<i>Xa-4 + xa-5 + Xa-7</i>
7	IRBB 62	<i>Xa-4 + Xa-7 + Xa-21</i>
8	IRBB 63	<i>xa-5 + Xa-7 + xa-13</i>
9	IRBB 64	<i>Xa-4 + xa-5 + Xa-7 + Xa-21</i>
10	IRBB 65	<i>Xa-4 + Xa-7 + xa-13 + Xa-21</i>
11	IRBB 66	<i>Xa-4 + xa-5 + Xa-7 + xa-13 + Xa-21</i>

Bacterial races: Six races of *Xanthomonas oryzae* pv. *oryzae* collected from CRD (Dinh, 2008) were stored in 10% Skim milk medium at -80°C, and maintained in Wakimoto + FeSO₄ – Potato (WF-P) slant at 4°C for routine work.

Table 2: The components of media used for storage and maintaining bacterial blight pathogen

Components of Skim milk medium	
Skim Milk (non-fat milk)	10.0 g
Na glutamate	1.5 g
dH ₂ O	100 ml
Components of WF-P medium	
Sucrose	20.0 g
Bacto peptone	5.0 g (strickly)
Calcium Nitrate	0.5 g
Sodium Phosphate	0.82 g
Ferrous Sulfate	0.05 g
Agar	17.0 g (strickly)
Distilled water	1,000 ml

Inoculum preparation and inoculation

Xanthomonas oryzae pv. *oryzae* races were transferred to WF-P slants and incubated at 25°C for 48 h. A two days old culture of each race was used to prepare inoculum. Inoculum was prepared by suspending the bacterial cells in 20 ml of sterile distilled water and adjusting to a concentration of 10⁹ CFU/ml prior to inoculation.

Rice lines were seeding and planting in pots at density of 3 plants per pot with three replications in greenhouse condition. Nine fully top expanded

leaves of plants in each pot were clip-inoculated (Kaufman et al.1973) with each race at 40 days after sowing.

Disease assessment

Lesion length from the cut leaf tip was measured in centimeters (cm) at 18 days after inoculation. Disease reactions were categorized according to lesion length. The lesion length was 0 to 6 cm classified as resistance (R) and more than 6 cm as susceptible (S) (Sanchez et al., 2000). Lesion length data for each line-race was analyzed for variance (ANOVA).

RESULTS

Lesion length of rice lines carrying 2 to 5 resistance genes were showed in table 3, the

difference of lesion lengths were clearly between NILs and susceptible variety IR 24.

Table 3: Reaction of Near-Isogenic Lines carrying different resistance genes against rice bacterial leaf blight.

NILs	Lesion length (cm)					
	Race A	Race B	Race C	Race D	Race E	Race F
IRBB 51	9.83 b	4.36 efg	9.17 c	8.98 cd	6.59 d	8.81 c
IRBB 54	9.39 b	2.47 ghi	5.93 def	2.69 g	2.90 h	5.94 d
IRBB 55	9.50 b	4.42 efg	6.67 de	4.54 f	5.56 def	6.10 d
IRBB 57	7.18 c	8.81 c	4.14 f-i	3.22 fg	4.88 efg	4.30 ef
IRBB 60	10.00 b	13.96 b	7.18 d	7.51 de	3.70 gh	5.28 de
IRBB 61	7.23 c	9.20 c	3.50 hi	3.97 fg	4.66 efg	3.80 f
IRBB 62	5.90 cd	7.28 cd	7.20 cd	4.18 fg	3.62 gh	6.21 d
IRBB 63	5.79 cd	1.38 i	2.63 ij	9.48 c	11.21 c	3.00 f
IRBB 64	1.30 e	4.81 ef	1.25 j	12.72 b	5.71 de	3.28 f
IRBB 65	4.80 d	3.41 fgh	3.95 ghi	2.82 fg	3.72 gh	3.57 f
IRBB 66	0.87 e	2.39 hi	5.51 d-g	4.49 fg	2.89 h	6.14 d
IR24	21.13 a	20.52 a	19.34 a	22.02 a	21.80 a	22.42 a
CV (%)	11.6	15.6	17.5	14.8	13.2	11.9

Most of the rice lines carrying 2 to 5 resistance genes have the incompatible with the *Xoo* races in CRD. Some rice lines were moderate susceptible to some *Xoo* races, with the lesion length from 6 to 13 cm. While the lesion length of susceptible line IR24 were about 20 cm.

High level of resistance against to race A were observed in rice lines IRBB 64 (Four-gene combination including: *Xa-4*, *xa-5*, *Xa-7* and *Xa-21*) and IRBB 66 (Five-gene combination including: *Xa-4*, *xa-5*, *Xa-7*, *xa-13* and *Xa-21*) the LL were 1.30 cm and 0.87 cm respectively.

In the column of race B showed that there were four rice lines have high level of resistance such as IRBB 54 (*xa-5*, *Xa-21*), IRBB 63 (*xa-5*, *Xa-7*, *xa-13*), IRBB 65 (*Xa-4*, *Xa-7*, *xa-13*, *Xa-21*), IRBB 66 (*Xa-4*, *xa-5*, *Xa-7*, *xa-13* and *Xa-21*). Similarly, the reaction of rice lines to other *Xoo* races in table 3, there were three rice lines which were high level resistance to race C, four lines to race D, five lines to race E and four lines to race F.

The lesion length of high level resistance of these lines was shorter than 4 cm.

The rice line IRBB 54 (*xa-5*, *Xa-21*) was resistance to five *Xoo* races from B to F and have high level resistance to three races such as races B, D and E. The rice line IRBB 65 (*Xa-4*, *Xa-7*, *xa-13*, *Xa-21*) was resistance to all races in CRD and have the high level resistance to five races except races A with the lesion length of 4.08 cm. (table 3).

DISCUSSION

One of the major goals of rice improvement programs has been to develop rice cultivars with stable resistance to *Xanthomonas oryzae* pv. *oryzae* (Khush, 1989). In this study, Near Isogenic lines of rice having several different resistance genes recently developed by IRRI, which were two-genes, three-genes, four-gene and five-gene combination, were used to analyze virulent of *Xoo* races in CRD. In our earlier studies, none of the single resistance gene can be expected to control

all races of bacterial blight in CRD, since of the great variation of bacterial blight pathogen (Dinh 2008).

Breeding lines with two, three and four resistance genes were developed and tested for resistance to the bacterial blight pathogen (*Xanthomonas oryzae* pv. *oryzae*), and the pyramid lines showed a wider spectrum and a higher level of resistance than lines with only a single gene (Huang et al. 1997). Yoshimura et al. (1995) combined resistance genes in pairs (*Xa-4/xa-5*, and *xa-5/Xa-10*) and showed that plants with two genes can have a higher level of resistance to *Xoo* than would be expected from the sum of the parental levels. The two-gene combination of *Xa-4* and *xa-5* were more resistance than the *Xa-4* or *xa-5* gene alone (Adhikari et al. 1999).

The information gained in this study has significant implications for regional resistance gene deployment. Because of the pathogen's ability to rapidly overcome major genes (Mew 1987), one of the most challenging and important areas of research will be to develop a sound strategy for deployment of R-gene-containing lines in order to maximize the durability of resistance in a particular location. This study clearly shows the potential for gene pyramiding to develop cultivars with durable resistance to bacterial leaf blight disease. Thus, using the genes combination for management bacterial blight disease on rice in CRD is important and necessary.

CONCLUSION

The near isogenic lines of rice with the two-to five resistance genes combinations have the high level of resistance to most of the races of bacterial blight pathogen in Cuulong Delta.

The line IRBB 65 carrying 4 resistance including *Xa-4*, *Xa-7*, *xa-13* and *Xa-21* were resistance to all races of *Xanthomonas oryzae* pv. *oryzae*. The line IRBB 54 with two-gene combination resistance to five races can be used for the cross program to develop rice cultivars with wider spectrum resistance to bacterial blight pathogen.

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**Nghiên cứu sử dụng các dòng lúa kết hợp nhiều gen kháng phòng trừ vi khuẩn
Xanthomonas oryzae pv. *oryzae* ở vùng ĐBSCL**

Bệnh bạc lá lúa do vi khuẩn *Xanthomonas oryzae* pv *oryzae* gây ra đang có chiều hướng gây hại nặng trên lúa cho vùng ĐBSCL trong những năm gần đây. Việc sử dụng gen kháng đơn có hiệu quả thấp do sự đa dạng về nòi của kí sinh gây bệnh. 11 dòng lúa có mang từ 2 đến 5 gen kháng bệnh được sử dụng cho thí nghiệm kháng bệnh với 6 nòi vi khuẩn được thu thập ở ĐBSCL. Kết quả hầu hết các giống có phản ứng kháng cao với các nòi vi khuẩn trong vùng. Một số giống có biểu hiện nhiễm nhẹ với bệnh. Dòng lúa IRBB 65 có 4 gen kháng là *Xa-4*, *Xa-7*, *xa-13* và *Xa-21* kháng với tất cả các nòi vi khuẩn trong vùng. Dòng IRBB 54 có chứa 2 gen kháng *xa-5* và *Xa-21* cũng có phản ứng kháng tốt với 5 nòi vi khuẩn ngoại trừ nòi A, dòng này có thể sử dụng tốt cho chương trình lai tạo giống kháng rộng với kí sinh gây bệnh bạc lá lúa..