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Linkage Analyses of Resistance Genes to Bacterial Blight in Rice

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Abstract

Three resistance genes to bacterial blight (BB), *xa-5*, *Xa-7* and *xa-8*, were genetically analyzed to identify their linkage groups and the loci. The gene xa-5 is known to be located on chromosome 5. The gene *Xa-7* is also known not to be located only on chromosomes 3 and 11. Thus, the linkage analyses of *xa-5* and *Xa-7* were carried out with the marked genes located on chromosomes 5 and 3, respectively. The results showed that the *xa-5* gene was linked with *v-10* and *spl-7* genes at recombination values of 37.7% and 8.8%, respectively. Similarly, the *Xa-7* gene was linked with chl-3 and *fc-1* genes at recombination values of 28.0% and 38.9%, respectively. On the other hand, the locus of *xa-8* was estimated to be located on chromosome 2 or 3 by trisomic analysis. The allelic relationships between a mutant resistance gene, *xa-15*, and the other two mutant resistance genes, *xa-19* and *xa-20* were investigated. The reactions of F_1 plants to the BB races suggested that the *xa-15* gene is nonallelic to both *xa-19* and *xa-20* genes.

Discipline: Plant breeding
Additional key words: *Xanthomonas campestris*, trisomics marker gene, recombination value

Introduction

Bacterial blight (BB), caused by *Xanthomonas campestris* pv. *oryzae*, is one of the most serious rice diseases in the world. Genetic studies on the resistance to BB have been conducted mainly in Japan and at the International Rice Research Institute (IRRI) since they were initiated by Nishimura[6]. Nineteen genes have been identified to date. Nine genes, *Xa-1*[15], *Xa-2*[15], *Xa-3*[1], *Xa-11*[8], *Xa-12*[9], *xa-15*[5], *Xa-16*[7], *Xa-17*[12] and *Xa-18*[20] were identified in Japan and ten genes, *Xa-4*[14], *xa-5*[14], *Xa-7*[16], *xa-8*[16], *Xa-10*[22], *xa-13*[10], *Xa-14*[17], *xa-19*[18], *xa-20*[19] and *Xa-21*[4] were identified at IRRI. In contrast, six races of BB have been identified in the Philippines: PXO61 (race 1), PXO86 (race 2), PXO79 (race 3), PXO71 (race 4), PXO112 (race 5) and PXO99 (race 6). Each resistance gene reacts to each race.

Yoshimura et al.[21] indicated that the *xa-5* gene was located on chromosome 5 by trisomic analysis. Ogawa et al.[11] also reported that the *xa-5* gene was closely linked with the gene *gl-1* (glabrous leaf blade-1) located on chromosome 5. However, the locus of *gl-1* has not been confirmed in the linkage group which is located on chromosome 5. On the other hand, Yoshimura et al.[23] also reported that *Xa-7* was not located on chromosomes 5, 6, 7, 8, and 12 by trisomic analysis. Then, Ogawa et al.[11] indicated that *Xa-7* was also not located on chromosomes 1, 2, 3, 9, and 10, and suggested that *Xa-7* might be located on chromosome 4 or 11.

Thus, to identify the loci of *xa-5* and *Xa-7*, the linkage relationships were examined with some marker genes located on chromosomes 5 and 4, respectively. Trisomic analysis for *xa-8* was also carried out to identify the chromosome location. Two

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recessive mutant genes, *xa-19*[18] and *xa-20*[19], of XM5 and XM6 which are derived from N-methyl-N-nitrosourea-treated progenies of IR24 are resistant to six races of BB from the Philippines. Another mutant, M41, which was induced in Japan by thermal neutron irradiation of japonica variety Harebare is also resistant to the six races[5]. M41 harbors a recessive gene, *xa-15*. Allele tests between *xa-15* and both *xa-19* and *xa-20* were carried out to clarify the allelic relationships among these three mutant genes. In this report, the numbering system of chromosomes and trisomics is based on the agreed system[3].

Materials and methods

1) Linkage analysis for xa-5 and Xa-7 using marker genes

To identify the loci of *xa-5* and *Xa-7*, IR-BB105 and IR-BB7 were crossed with the linkage testers with marker genes located on chromosomes 5 and 3, respectively. IR-BB105 and IR-BB7 are the near-isogenic lines harboring the *xa-5* and *Xa-7* genes with Toyonishiki and IR24 genetic background, respectively[13]. The F_2 plants were transplanted with their parents on the bed in the screenhouse for protecting them from attacks by insect pests and from virus diseases. When they were at the booting stage, they were inoculated by the clipping method using race 2 (PXO86). The length of the lesions of the uppermost three leaves of each individual was measured at 21 days after inoculation. The marker characters of F_2 plants were also observed in the screenhouse.

2) Trisomic analysis for xa-8

To determine on which chromosome *xa-8* is located, IR-BB8 was crossed with 10 trisomic lines (triplo lines). IR-BB8 is the near-isogenic line harboring the *xa-8* gene with IR24 genetic background[13]. The triplo lines were developed from the original triplo lines with IR36 background[2] through backcrossing with IR24 at least five times, because IR36 harbors the *Xa-4* gene[11]. The F_1 plants, derived from the crosses of IR-BB8 with the triplo lines were evaluated morphologically. With chromosome 11, however, it was difficult to distinguish the trisomics from disomics based on the morphological characters, because triplo 11 was a pseudonormal type. Then, the F_1 plants from the cross of triplo 11 with IR-BB8 were tested under the microscope at the metaphase of the first meiotic division of pollen mother cells to determine whether they were trisomics. The F_2 populations derived from the trisomic F_1 plants were transplanted with their parents on the bed in the screenhouse. Then, they were inoculated

Table 1. Linkage relationships among *xa-5*, *v-10* and *spl-7* genes in the cross GM23/IR-BB105

BB (*xa-5*)	(*v-10*)		
	+	a	Total
Sus.	108	18	126
Res.	35	16	51
Total	143	34	177

Goodness of fit			
Item	Ratio	d.f.	x^2
BB (*xa-5*)	1:3	1	1.373
a (*v-10*)	1:3	1	3.166
Total	9:3:3:1	3	9.968*
R.V. (%) = 37.7 ± 3.68			

BB (*xa-5*)	(*spl-7*)		
	+	b	Total
Sus.	124	2	126
Res.	13	38	51
Total	137	40	177

Goodness of fit			
Item	Ratio	d.f.	x^2
BB (*xa-5*)	1:3	1	1.373
b (*spl-7*)	1:3	1	0.544
Total	9:3:3:1	3	113.180***
R.V. (%) = 8.8 ± 1.81			

(*spl-7*)	(*v-10*)		
	+	a	Total
+	118	19	137
b	25	15	40
Total	143	34	177

Goodness of fit			
Item	Ratio	d.f.	x^2
a (*v-10*)	1:3	1	3.166
b (*spl-7*)	1:3	1	0.544
Total	9:3:3:1	3	12.901**
R.V. (%) = 32.5 ± 3.42			

at the booting stage by the clipping method using four races, race 1 (PXO61), race 2 (PXO86), race 3 (PXO79), and race 4 (PXO71). The classification of disomics and trisomics in each F_2 population was based on whether the F_2 plants showed the morphological characters of each trisomic parent. Only the F_2 plants from the cross with triplo 11 were tested under the microscope to confirm the presence of disomics or trisomics as mentioned above.

3) Allelic relationships among resistance genes in mutants

The resistant mutant M41 was crossed with XM5 and XM6. The F_1 plants of two crosses, M41/XM5 and M41/XM6, were transplanted with their parents in the screenhouse. They were inoculated at the booting stage by the clipping method using four races, race 1 (PXO61), race 2 (PXO86), race 4 (PXO71) and race 6 (PXO99).

Results

1) Linkage analysis for xa-5 and Xa-7 using marker genes

Linkage relationship between *xa-5* and the marker genes located on chromosome 5, *v-10* (virescent-10) and *spl-7* (spotted leaf-7) was examined in the cross of GM23 (with *v-10*, *spl-7*)/IR-BB105(*xa-5*). As shown in Table 1, the segregation of each recessive gene in this cross fitted very well

Table 2. Linkage relationships among *Xa-7* and some marker genes located on chromosome 3

(1)	(*chl-1*)			Goodness of fit			
BB (*Xa-7*)	+	a	Total	Item	Ratio	d.f.	x^2
Res.	109	43	152	BB (*Xa-7*)	3:1	1	1.212
Sus.	40	7	47	a (*chl-1*)	1:3	1	0.002
Total	149	50	199	Total	9:3:3:1	3	3.515

(2)	(*chl-3*)			Goodness of fit			
BB (*Xa-7*)	+	b	Total	Item	Ratio	d.f.	x^2
Res.	131	19	150	BB (*Xa-7*)	3:1	1	2.038
Sus.	33	29	62	b (*chl-3*)	1:3	1	0.629
Total	164	48	212	Total	9:3:3:1	3	31.857***
				R.V. (%) = 28.0 ± 2.91			

(3)	(*fc-1*)			Goodness of fit			
BB (*Xa-7*)	+	c	Total	Item	Ratio	d.f.	x^2
Res.	130	43	173	BB (*Xa-7*)	3:1	1	0.289
Sus.	30	23	53	c (*fc-1*)	1:3	1	2.130
Total	160	66	226	Total	9:3:3:1	3	9.265*
				R.V. (%) = 38.9 ± 3.30			

(3)	(*v-1*)			Goodness of fit			
BB (*Xa-7*)	+	d	Total	Item	Ratio	d.f.	x^2
Res.	142	31	173	BB (*Xa-7*)	3:1	1	0.289
Sus.	41	12	53	d (*v-1*)	1:3	1	4.300*
Total	183	43	226	Total	9:3:3:1	3	5.158

(3)	(*fc-1*)			Goodness of fit			
(*v-1*)	+	c	Total	Item	Ratio	d.f.	x^2
+	151	32	183	c (*fc-1*)	1:3	1	2.130
d	9	34	43	d (*v-1*)	1:3	1	4.300*
Total	160	66	226	Total	9:3:3:1	3	61.276***
				R.V. (%) = 20.5 ± 2.42			

(1): IR-BB107/GM33(*chl-1*), (2): IR-BB107/GM29(*chl-3*), (3): IR-BB107/GM20(*fc-1*, *v-1*).

to the ratio of 1 : 3, showing that the segregation of each gene was not distorted in the F_2 population. However, segregation of F_2 plants with combined characters for both recessive genes did not fit to the ratio of independent segregation with 9 : 3 : 3 : 1, indicating that these two genes are linked with each other. Accordingly, their recombination values were calculated by using the maximum likelihood formula to be 37.7% between *xa-5* and *v-10*, 32.5% between *v-10* and *spl-7*, and 8.8% between *xa-5* and *spl-7*, respectively.

Similarly, linkage analysis of *Xa-7* with *chl-1* (chlorina-1), *chl-3* (chlorina-3), *fc-1* (fine culm-1), and *v-1* (virescent-1) was carried out in three crosses, IR-BB7 (*Xa-7*)/GM33 (*chl-1*), IR-BB7/GM29 (*chl-3*), and IR-BB7/GM20 (*fc-1*, *v-1*) (Table 2). Out of them, *Xa-7* was linked with only *chl-3* at a recombination value of 28.0%.

2) Trisomic analysis for xa-8

The data given in Table 3 show that the segregation ratios of resistant and susceptible individuals fitted very well to the disomic ratio of 1 : 3 in both disomic and trisomic types for triplo 1, 3, 5, 6, 7, 8, 9, 10, 11, and 12, indicating that the gene *xa-8* is not associated with any of these ten chromosomes.

3) Allelic relationships among resistance genes in mutants

The F_1 plants of both crosses M41/XM5 and M41/XM6 showed longer lesions than their parents against any race (Table 4), indicating that the recessive resistance gene in M41 is nonallelic to genes in both XM5 and XM6.

Table 3. Trisomic analysis of bacterial blight resistance gene, *xa-8*

Parents and cross	Number of chromosomes	F_2 population			x^2 for 1:3
		Res.	Sus.	Total	
Triplo 1	2n + 1	0	10	10	
Triplo 4	2n + 1	0	10	10	
Triplo 5	2n + 1	0	10	10	
Triplo 6	2n + 1	0	10	10	
Triplo 7	2n + 1	0	10	10	
Triplo 8	2n + 1	0	10	10	
Triplo 9	2n + 1	0	10	10	
Triplo 10	2n + 1	0	10	10	
Triplo 11	2n + 1	0	10	10	
Triplo 12	2n + 1	0	10	10	
IR-BB8	2n	15	0	15	
Triplo 1/IR-BB8	2n	38	134	172	0.78
	2n + 1	16	34	50	1.31
Triplo 4/IR-BB8	2n	47	122	169	0.71
	2n + 1	17	49	66	0.02
Triplo 5/IR-BB8	2n	11	30	41	0.09
	2n + 1	6	13	19	0.46
Triplo 6/IR-BB8	2n	52	108	160	4.80*
	2n + 1	17	58	75	0.22
Triplo 7/IR-BB8	2n	17	35	52	1.64
	2n + 1	5	18	23	0.12
Triplo 8/IR-BB8	2n	17	48	65	0.05
	2n + 1	5	13	18	0.07
Triplo 9/IR-BB8	2n	35	96	131	0.21
	2n + 1	27	62	89	1.35
Triplo 10/IR-BB8	2n	61	169	230	0.28
	2n + 1	24	66	90	0.12
Triplo 11/IR-BB8	2n	37	101	138	0.24
	2n + 1	18	47	65	0.25
Triplo 12/IR-BB8	2n	45	124	169	0.24
	2n + 1	24	51	75	1.69

Table 4. Allelic relationships of mutant resistance genes in M41, XM5 and XM6

Parents and F_1	Lesion length (cm)			
	Race 1	Race 2	Race 4	Race 6
M41	5.7	6.3	5.4	6.4
XM5	11.3	-	11.4	12.3
XM6	5.0	3.9	5.4	7.5
F_1 (M41/XM5)	16.2	18.0	19.6	20.2
F_1 (M41/XM6)	15.9	18.9	14.4	17.1
IR24 (S check)	25.4	19.6	25.1	22.2

Discussion

The gene *xa-5* is linked with *v-10* and *spl-7* at the recombination values of 37.7% and 8.8%, respectively. Because the loci of *v-10* and *spl-7* are on the site of 54 and 83 on chromosome 5, the locus of *xa-5* is estimated to be on the site shown in Fig. 1. The *Xa-7* gene is estimated to be located on chromosome 3 and to be linked with *chl-3* and *fc-1* as shown in Fig. 2.

To date, it has been determined that the loci of three genes, *Xa-1*, *Xa-2* and *Xa-12*, are located on chromosome 4, and another four genes, *Xa-3*, *Xa-4*, *Xa-10* and *Xa-21*, are located on chromosome 11. In this study, it was confirmed that the *xa-5* gene was located on chromosome 5 and *Xa-7* gene on chromosome 3. The locus of *xa-8* was also estimated

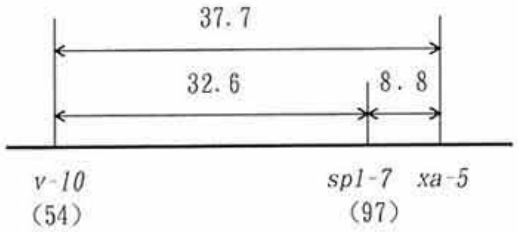

Fig. 1. Linkage of the resistance gene *xa-5* and marker genes located on chromosome 5

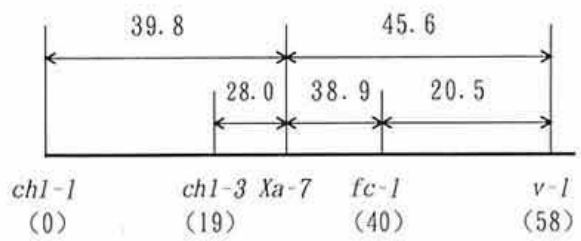

Fig. 2. Linkage of the resistance gene *Xa-7* and marker genes located on chromosome 3

to be located on chromosome 2 or 3. Moreover, *xa-13* is also linked to *xa-5*. These results suggest that the BB resistance genes are located not only on limited chromosomes such as chromosomes 4 and 11 but also chromosomes 3 and 5.

The results of the trisomic analysis indicated that the *xa-8* gene was not located on chromosomes 1, 4, 5, 6, 7, 8, 9, 10, 11, and 12. Therefore, the locus of *xa-8* is considered to be on chromosome 2 or 3. In the near future, the locus of *xa-8* will be identified by linkage analysis using the marker genes and the RFLP markers located on chromosome 2 or 3.

Near-isogenic lines were used to identify the loci of *xa-5*, *Xa-7* and *xa-8* in this study. These isogenic lines were developed for use to monitor the distribution of BB races in each country or location as international differentials. The isogenic lines were more efficient than varieties with each resistance gene for genetic analysis. Especially, since the reaction of the *xa-8* gene to four BB races, 1, 2, 3, and 4 indicated a moderate resistance, the determination of resistance and susceptibility became difficult unlike in the case of *xa-5* or *Xa-7*. It was considered that the utilization of parents with a similar background to that of IR24 was very efficient for trisomic analysis of *xa-8*, because the resistance reactions of the F_2 plants in these crosses were very distinct due to the absence of background effects.

It is concluded that the mutant gene in M41 is nonallelic to the mutant genes in XM5 and XM6, confirming the designation of *xa-15* by Nakai.

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