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GENETICS OF RESISTANCE TO BACTERIAL BLIGHT IN RICE (*ORYZA SATIVA* L.)

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ABSTRACT

The mode of inheritance and allelic relationship among genes for resistance to five different races of bacterial blight (*Xanthomonas campestris* pv *oryzae*) in three cultivars, resistant at all growth stages, was studied. The F_1 and F_2 progenies from crosses of the test cultivars with TN 1, when inoculated at seedling, maximum tillering and flowering stages, revealed that two recessive genes control resistance in them. Tests for allelism indicated that one of the genes is xa-5, which confers resistance to races I, II and III at the seedling stage. The other gene confers resistance at seedling stage to races IV and VI. This newly identified recessive gene segregates independently of Xa-3, Xa-4, Xa-7 and Xa-10 but is linked to xa-5 with the crossover value of about 9.68%. Thus the cultivars Long Grain, Kali Mekri 77-5, and Aus 295 have a second recessive gene besides xa-5, which may be allelic to xa-13.

Key words: *Xanthomonas campestris* pv *oryzae* race VI, seedling resistance, allelic relationship.

Among the sixty-odd diseases attacking the rice crop, bacterial blight caused by *Xanthomonas campestris* pv *oryzae*, is one of the most destructive disease in most of the rice growing regions [1]. The disease has attained greater prevalence with the spread of high yielding varieties cultivated under improved agronomic practices.

Twelve resistance genes have so far been identified, of which five dominant genes, viz. Xa-1, Xa-2, Xa-3 (Xa-w), Xa-kg, and Xa-11 were identified using Japanese isolates of the bacterium [2–5]. Seven genes, viz. Xa-4, xa-5, Xa-6, Xa-7, xa-8, Xa-9 and Xa- 10, were identified for resistance to the Philippine isolates at IRRI [6–9]. However further analysis carried out at IRRI, led to the conclusion that Xa-6 and xa-9 are allelic to Xa-3 [10].

Among these resistance genes, Xa-1, Xa-2, Xa-kg and Xa-11 do not confer resistance to any of the Philippine races of bacterial blight. Xa-4 and xa-5 impart only moderate level of resistance to the Philippine race IV, that too at the adult plant stage only. Though Xa-3 causes

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high level of resistance to this race, it is not very stable at the seedling stage. Besides, none of these genes confer resistance to Philippine race VI. In a tropical country like Philippines resistance at the seedling stage is very important as high population density of the bacterial blight pathogen is encountered at the seedling stage. The present study aims to identify genes conferring satisfactory level of resistance to races IV and VI, so that they could be utilized in IRRI's breeding program.

MATERIALS AND METHODS

Forty six varieties from eight rice growing countries of the world, were screened for resistance to five races of bacterial blight at different growth stages. Three of these varieties, viz., Kali Mekri 77-5 (IRRI Acc. No. 6613), Aus 295 (IRRI Acc. No. 29083) and Long Grain (IRRI Acc. No. 35023), found resistant at all growth stages to races I, II, III, IV and VI, were selected for further study. The results of their genetic analysis are presented.

The cultivars were crossed with Taichung Native 1 (TN 1), R29-13 (homozygous for Xa-3), IR 22 (homozygous for Xa-4), IR 1545-339 (homozygous for xa-5), S-1-5-78-1-1 (homozygous for Xa-7) and RI 72-11 (homozygous for Xa-10), to determine the mode of inheritance and allelic relationships of the bacterial blight resistance genes carried by them. The parental, F_1 and F_2 populations were studied at various growth stages for their reaction to different Philippines races of bacterial blight.

Representative strains PXO 61, PXO 86, PXO 79, PXO 71 and PXO 99 of races I, II, III, IV and VI, respectively, were used for inoculation. They were cultured on potato-semisynthetic agar medium for two or three days at 27°C, suspended in sterile distilled water with concentration adjusted to about 10^9 cells/ml.

For seedling inoculation, the parents, differentials, and F_2 populations were seeded in wooden boxes filled with garden soil. Separate sets of seedlings (20–25 seedlings for parents and differentials and 250–300 seedlings for F_2 populations) were grown for each race. Twenty-one-day old seedlings were clip- inoculated with a pair of scissors frequently dipped in freshly prepared inoculum. At maximum tillering stage (about 50–55 days after seeding), tillers of each plant were equally divided by binding with different coloured vinyl ties, one colour for each race. Similar procedures were adopted for inoculation at the flowering stage when only the flag leaves were inoculated. Plants grown in the field were inoculated with garden clippers attached with a bottle of inoculum, at maximum tillering and flowering stages.

Disease reaction was assessed visually two weeks after inoculation. Leaves infected by clipping were scored according to the Standard Evaluation System (SES) for rice [10].

RESULTS

Inheritance of resistance: The reaction of F_1 and F_2 progenies of the crosses of these

cultivars with TN 1 at the seedling and maximum tillering stages, indicated that F_1 progenies of all the crosses were susceptible to races I, II, III and IV at all the growth stages, indicating that resistance is caused by recessive gene(s). The F_2 populations in all these crosses segregated in the ratio of 1R : 3S at the seedling and maximum tillering stages in their reaction to each of the four races considered individually. However, when the reaction to the four races of each individual F_2 plant was considered jointly, they could be grouped into four phenotypic classes, viz., SSSS, SSSR, RRRS and RRRR (Table 1). This analysis indicated that two recessive genes are involved: one causing resistance against races I, II and III and the other against race IV. The data, however, do not fit the digenic ratio of 9:3:3:1, suggesting that the genes do not segregate independently. The frequency of parental types is much higher than expected on the basis of independent assortment. The percentage recombination between these two genes as calculated by the method of minimum discrepency [11] ranges from 9.39 to 10.04.

Table 1. Disease reaction to four races of bacterial blight of the F_1 and F_2 progenies of the crosses of TN 1 with cv. Long Grain, Kali Mekri 77-5 and Aus 295 at maximum tillering stage

Cross	Reaction of F_1 plants	Distribution of F_2 plants in various reaction groups				χ^2 (9:3:3:1)	P	Recombination value (%)
		SSSS	SSSR	RRRS	RRRR			
TN 1 x Long Grain:								
Population I	SSSS	165	18	7	50	136.60	<0.01	9.75
Population II	SSSS	282	32	12	88	241.82	-do-	9.39
TN 1 x Kali Mekri 77-5:								
Population I	SSSS	161	15	8	51	144.74	-do-	9.98
Population II	SSSS	271	27	14	87	244.10	-do-	10.04
TN 1 x Aus 295:								
Population I	SSSS	260	30	11	81	184.24	-do-	9.45
Population II	SSSS	236	26	20	72	196.98	-do-	9.48

SSSS, SSSR, RRRS, RRRR—reaction to races I, II, III and IV, respectively. R—resistant, S—susceptible.

To confirm these results, 79 single plants were harvested at random from the F_2 population of the cross TN 1 x Long Grain. These were grown as F_3 families with 29 plants in each F_3 family and were inoculated with four races at the maximum tillering stage. On the basis of the data recorded on disease reaction, the families were classified into segregating and non-segregating (Table 2). The data reveal that the parental types of families are far in excess than expected on the basis of independent assortment. These data confirm that two recessive genes linked together are involved in conferring resistance in these cultivars.

As all the cultivars has shown resistance to race VI also, the F_2 populations of their crosses with TN 1 were separately inoculated with races IV and VI at the flowering stage. The F_2 population in each of these crosses segregated into 1R : 3S for their reaction to race VI, but no definite segregation pattern could be established for their reaction to race IV (Table 3). However, all the resistant plants to race VI were also resistant to race IV, but the reverse was not true, indicating that the gene causing resistance to race VI, also confers resistance to race IV.

Table 2. Reaction of F_3 families from the cross TN 1 x Long Grain to four races of bacterial blight

Reaction of F_2 plants	Reaction of F_3 families	Frequency of F_3 families	
		observed	expected
SSSS	Not segregating	12	4.9375
	Segregating for all races	29	19.7500
	Segregating for races I, II and III only	3	9.8750
	Segregating for race IV only	2	9.8750
SSSR	Not segregating	4	4.9375
	Segregating for races I, II and III only	7	9.8750
RRRS	Not segregating	0	4.9375
	Segregating for race IV only	9	9.8750
RRRR	Not segregating	13	4.9375
		$\chi^2 = 94.06$	$P < 0.01$

Allelic relationship: The cultivars were then crossed to IR 1545-339. The F_1 progenies of these crosses were resistant to races I, II and III at all growth stages but showed S to MS reaction to race IV even at the flowering stage, the reaction pattern exhibited by cultivars having xa-5 gene for resistance. All the F_2 plants were resistant to races I, II and III (Table 4). For reaction to race IV, no definite segregation pattern was observed. These results suggest that a recessive gene, allelic to xa-5, causes resistance to races I, II and III in these cultivars.

Table 3. Disease reaction to the Philippine race-IV and race VI of bacterial blight of the F_2 population from the crosses of TN 1 with the test cultivars at the flowering stage

Cross	Race	Reaction of F_2 population			χ^2	P
		R-MR	MS-S	ratio		
TN 1 x Long Grain	IV	186	160	7:9	14.08	0.01
	VI	78	268	1:3	1.11	0.25–0.50
TN 1 x Kali Mekri 77-5	IV	226	210	7:9	11.58	0.01
	VI	121	315	1:3	1.76	0.10–0.25
TN 1 x Aus 295	IV	155	137	7:9	10.33	0.01
	VI	62	230	1:3	2.21	0.10–0.25

R—resistant, S—susceptible, MR—moderately resistant, and MS—moderately susceptible.

Table 4. Disease reaction to four Philippine races of bacterial blight of the F_1 and F_2 progenies from the crosses of IR 1545-339 with the test cultivars at flowering stage

Cross	Race	Reaction of F_1 plants	Reaction of F_2 populations				χ^2	P
			R-MR	MS-S	total	ratio		
IR 1545-339 x Long Grain	I	R	332	0	332	1:0	0.0	1.00
	II	R	332	0	332	1:0	0.0	1.00
	III	R	332	0	332	1:0	0.0	1.00
	IV	MS	232	100	332	—	—	—
IR 1545-339 x Kali Mekri 77-5	I	R	370	0	370	1:0	0.0	1.00
	II	R	370	0	370	1:0	0.0	1.00
	III	R	370	0	370	1:0	0.0	1.00
	IV	MS	226	144	370	—	—	—
	I	R	424	0	424	1:0	0.0	1.00
	II	R	422	0	422	1:0	0.0	1.00
	III	R	422	0	422	1:0	0.0	1.00
	IV	MS	277	147	424	—	—	—

R—resistant, S—susceptible, MR—moderately resistant and MS—moderately susceptible.

The cultivars were also crossed to the breeding lines R29-13 (homozygous for Xa-3), IR22 (homozygous for Xa-4), S-1--5-78-1-1 (homozygous for Xa-7), and R172-11 (homozygous for Xa-10). The F_1 and F_2 populations of these crosses were inoculated with races I, II, III and VI at the flowering stage (Table 5). These data reveal that resistance to race VI in these cultivars is due to a single recessive gene that segregates independently of Xa-3, Xa- 4, Xa-7 and Xa-10.

Table 5. Disease reaction to the Philippine race VI of bacterial blight of the F_1 and F_2 progenies from the crosses of different testers with the resistant cultivars at the flowering stage

Cross	Reaction of F_1 plants	Reaction of F_2 populations		χ^2 (1:3)	P
		R-MR	MS-S		
IR 22 x Long Grain	S	69	255	2.75	0.25–0.50
IR 22 x Kali Mekri 77-5	S	57	227	3.68	0.05–0.10
R 29-13 x Long Grain	S	63	176	0.24	0.50–0.75
R 29-13 x Kali Mekri 77-5	S	125	311	3.13	0.05–0.10
R 172-11 x Long Grain	S	48	192	3.20	0.05–0.10
R 172-11 x Kali Mekri 77-5	S	79	257	0.87	0.50–0.75
S-1-5-78-1-1 x Long Grain	S	79	261	0.56	0.25–0.50
S-1-5-78-1-1 x Kali Mekri 77-5	S	70	246	1.37	0.10–0.25

R—resistant, S—susceptible, MR—moderately resistant and MS—moderately susceptible.

DISCUSSION

The cultivars showed resistance to the five races used for inoculation in these studies at all the growth stages. The F_2 populations of their crosses with TN 1 segregated into 1R : 3S at the seedling stage for all the races. The recessive gene xa-5 causes resistance to races I, II and III at all growth stages but is suceptible to race IV at the seedling stage and to race VI at all growth stages. It is thus clear that resistance to races IV and VI in these cultivars at the seedling stage is not due to gene xa-5 but is caused by another nonallelic recessive gene. However, xa-5 causes moderate level of resistance to race IV at the flowering stage. Thus, at the flowering stage, two recessive genes confer resistance to this race, but the F_2 data from the crosses of these cultivars with IR 1545-339 do not fit the expected 7R : 9S ratio of two complementary nonlinked recessive genes, and thus support the conclusion that these two genes are linked.

None of the resistance genes identified at IRRI so far imparts resistance to race VI at any growth stage. The cultivars Long Grain, Kali Mekri 77-5, and Aus 295 carry another recessive gene besides xa-5, which determines resistance to races IV as well as VI from early growth stages. This recessive gene segregates independently of Xa-3, Xa-4, Xa-7 and Xa-10, but is linked to xa- 5, with an average crossover value of 9.68%. A recessive gene for resistance to race VI has been recently discovered and designated as xa-13 [12]. The recessive gene for resistance in the cultivars Long Grain, Kali Mekri 77-5 and Aus 295 may be allelic to xa-13. Since it is linked to xa-5, it should be easier to transfer both these genes to the breeding lines for developing cultivars with broad-spectrum resistance to bacterial blight in the Philippines.

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