Inheritance of Resistance to Bacterial Blight in Ten Rice (*Oryza sativa* L.) Cultivars

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Abstract

Ten rice cultivars from the International Rice Germplasm Centre originating from Bangladesh were analysed for their genetics of resistance to bacterial blight. The test cultivars were selected on the basis of their high level of resistance to races IV & VI of ***Xanthomonas oryzae pv. oryzae****. The mode of inheritance was studied by crossing the test cultivars with Taichung Native 1 (TNI) which is highly susceptible to the races 1,2,3,4 & 6 of X. Oryzae pv. oryzae. The genetic analysis revealed that the test cultivars have two recessive genes conferring resistance to races 1 (PX 061) and 6 (PX 079) of* ***X. oryzae pv*** *oryzae, respectively. The allelic relationship of genes conferring resistance with xa-5 and xa-13 was studied in progenies derived from crosses between the cultivars and IRBB5 and IRBB13 which are near isogenic lines carrying genes xa-5 and xa-13 respectively. The Chi-square analysis of* F_2 *populations revealed that resistance in these cultivars to race 1 was conferred by xa-5. Resistance to race 6 was found to be conferred by another gene which was non-allelic to xa-13.*

Keywords: *Oryza sativa*, rice, *Xanthomonas oryzae* pv. *oryzae*, bacterial blight, races.

Introduction

Bacterial pathogens cause several diseases of rice. Among these, *Xanthomonas oryzae* pv oryzae (*Xoo*) causing bacterial blight (BB), is the most destructive disease in many rice growing countries. The incidence of this disease increased because of the introduction of modern improved cultivars which require intensive agronomic practices such as the use of high rates of nitrogenous fertilizers, close spacing and continuous cropping (Khush & Virmani, 1985). The disease occurs throughout Asia, in several Latin American countries, Northern Australia, the Sahelian region and other parts of Africa and in the United States (Mew, 1989).

The most effective and economic method of controlling bacterial blight of rice is by using host plant resistance. Breeding for resistance to *Xoo* has, therefore, become an integral part of rice improvement in many countries (Mew, 1987). Variability within strains of *Xoo* has been reported by various workers (Tagami and Mizukami, 1962, Kusaba *et al.* 1966, Ou *et al.* 1971, Buddenhagen and Reddy 1972, Ezuka and Horino, 1974, Mew *et al.*, 1982). Such pathogen variability has made breeding for BB disease resistance difficult. A number of studies have been made to classify rice cultivars for resistance to BB (Ezuka & Sakaguchi, 1978, Mew *et al.* 1982, Ogawa *et al.* 1986, 1991). Twenty one genes for resistance to *Xoo* have been identified in rice (Khush *et al.*, 1990, Taura *et al.*, 1992). On the other hand, seven races of the pathogen have been described (Noda and Ohichi, 1989).

Allelic relationships between the identified resistance genes in rice have been studied at the International Rice Research Institute, Philippines, Japan, India and in several other countries (Sahu and Khush, 1989, Taura *et al.*, 1992, Goel and Saini, 1997, Xu *et al.* 1998).

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Ogawa *et al.* (1989) identified two recessive genes conferring resistance to the varieties of BJI group. The new gene conferring resistance to race 6 was designated as *xa*-13. This gene was linked with *xa*-5 and the two genes have complementary effect on imparting resistance to race 4 of *Xoo*. Sidhu *et al.* (1978) identified gene *xa*-8 in cultivar PI 231129 which segregates independently of *Xa*-4, *xa*-5 and *Xa*-6. Ikeda *et al.* (1995) reported that *xa*-8 was located in chromosome 5, while *xa*-8 locus as located in chromosome 2 or 3.

The availability of genes conferring resistance to different races of *Xoo* would enable a breeder to use diverse resistance donors in a breeding program. Such an approach will be supported by the systematic identification of resistance genes in breeding materials. Some of the identified resistance genes have been used in pyramiding bacterial blight resistance genes in rice by using DNA marker-assisted selection (Huang *et al.*, 1997). This study was undertaken to identify genes for resistance to *Xoo* in certain rice cultivars from the International Rice Research Institute (IRRI) germplasm bank.

Materials and Methods

The study was conducted between 1990-1992 in the Division of Plant Breeding, Genetics and Biochemistry of the IRRI, Los Banos, Laguna, Philippines, located at latitude 14' 10" N and longitude 121' 15" E and 39 metres above sea level (m.a.s.l.).

Testers and Test Varieties

Ten cultivars from the International Rice Germplasm Centre of IRRI, which were reported to be resistant to the Philippine races 1,2,3,4 & 6 of *Xoo,* were, used in the study. Taichung Native I (TNI), which is highly susceptible to races 1, 2, 3, 4 and 6, was used as the susceptible parent. Two near-isogenic lines, IRBB5 and IRBB13, were crossed with all the selected test cultivars. These isogenic lines had the *xa*-5 and *xa*-13 resistant genes, respectively.

Seeds of the test rice cultivars were sown along with testers in seed boxes. The seedlings were transplanted in one-row plots in concrete beds. Each row consisted of 12 plants spaced at 30 cm using one seedling per hill.

Inoculum preparation and inoculation

Bacterial strains maintained by the Division of Plant Breeding, Genetics and Biochemistry at IRRI were used for the study. Each strain was cultured in modified Wakimoto medium (WF-p) (Ou, 1985). Inoculum was prepared by suspending each pure culture in sterile distilled water. Using a spectrophotometer, the absorbance of the inoculum was adjusted to a concentration of approximately 10^8 colony forming units (CFU) per ml. The parents and hybrid populations, along with the testers, were inoculated at booting stage (60 days after seeding) by clipping the leaves 2-3 cm from the tip using a pair of sterilized scissors which had been dipped in bacterial suspension (Kauffman *et al.*, 1973). Disease reaction was assessed 14-18 days after inoculation using the standard evaluation system for rice (IRRI, 1988).

Inheritance study

Genetic analyses were conducted by crossing the test cultivars with TNI. The allelic relationship of genes conferring resistance in the rice test varieties with *xa*-5 and *xa*-13 was studied in progenies derived from cross between the test cultivars and IRBB5 and IRBB13, respectively. The testers were grown in three plantings at weekly intervals to synchronize with the flowering of the test cultivars. Emasculation and pollination were done according to the procedure outlined by Jennings *et al.* (1979). Crosses were made in the screenhouse where test rice cultivars were used as male parents for easy identification of selfed progenies.

The F_1 and F_2 progenies originating from the crosses were inoculated with races 1, 4 and 6 of *Xoo* to determine the mode of inheritance as well as the allelic relationships with *xa*-5 and *xa*-13. Inoculation of parents and hybrids was done using the clipping method described by (Kauffman *et al.*, 1973).

Data analysis

Chi-square analysis was performed to analyze and determine the mode of inheritance in the F_2 populations using the formulae and procedures given by Gomez & Gomez (1984).

Results

Inheritance of resistance

All test cultivars were resistant to the 5 races of *Xoo* used (Table 1). The F_1 plant populations from the crosses with TNI were all susceptible to races 1, 4 & 6 (Table 2). When the F_2 populations of each of the crosses were inoculated with the three races of the pathogen, the plants segregated into IR:3S ratio (Table 2). This indicated that a single recessive gene governs resistance in the test cultivars.

Allelic relationship with *xa*-5

The F_1 plants from all the crosses between test cultivars and IRBB5 carrying *xa*-5 gene were resistant to race 1 but showed varied reaction to race 4, ranging from resistant to susceptible (Table 3). F_1 plants from the same crosses were susceptible to race 6 (Table 3). All the F_2 plant populations screened tested with race 1 of the pathogen were resistant with no segregation for susceptibility. The results indicated that these varieties have a recessive gene for resistance which is allelic to *xa*-5.

Allelic Relationship with *xa*-13

The F_1 plants of crosses between test cultivars and IRBB13 were all susceptible to Xoo races 4 and 6 of *Xoo* (Table 4). The reaction of the F_2 populations, segregated into 7 Resistant = 9 susceptible plants when they were inoculated with rate 6. This is a typical inheritance pattern of two recessive genes. The results indicated that the single recessive genes governing resistance to race 6 in all the test cultivars are different from and are inherited independently of *xa*-13.

Table 1: Reaction of rice cultivars and testers used in the study to the five races of *Xanthomonas oryzae* pv. *oryzae*.

Cultivars	IRRI Accession No.	Origin	Reaction to *Xoo* race				
			1 (P X 061)	2(PX 086)	3(PX 079)	4(PX 071)	6(PX 099)
DV 204-1	8818	Bangladesh	R	R	R	R	R
Chiknal	25848	Bangladesh	R	R	R	R	R
Gambir	25853	Bangladesh	R	R	R	R	R
Bora Diga	26439	Bangladesh	R	R	R	R	R
Mathia	26726	Bangladesh	R	R	R	R	R
Birmadla	27531	Bangladesh	R	R	R	R	R
Chakila	27540	Bangladesh	R	R	R	R	R
Aus 155	28998	Bangladesh	R	R	R	R	R
Aus 255	29047	Bangladesh	R	R	R	R	R
Kalonchi	31836	Bangladesh	R	R	R	R	R
TNI		Philippines	S	S	S	S	S
IRBB5		Philippines	R	R	R	R	R
IRBB13		Philippines	S	S	S	R	R

R = Resistant, S = Susceptible

Discussion

Host plant resistance is the most effective and economic method of controlling rice diseases (Khush & Virmani, 1985, Mew, 1989). In general, plant bacterial diseases are known to be difficult to control by using chemicals. Al-

Table 2: Reaction of F_1 and F_2 populations from crosses of the tested rice, cultivars with TN 1 to *Xanthomonas oryzae* pv. *oryzae* races 1, 4 and 6

Cross	Race	F_1 plant reaction	Reaction of F_2 plant reaction		X^2 (1:3)
			Resistant	Susceptible	
TNI/D204-1	1	S	129	338	1.58
	4	S	95	235	2.33
	6	S	78	252	0.26
TNI/Chiknal	1	S	115	373	0.46
	4	S	126	358	0.22
	6	S	126	354	4.34*
TNI/Gambir	1	S	131	353	1.17
	4	S	107	364	0.65
	6	S	96	364	3.97*
TNI/Bora Diga	1	S	128	361	0.30
	4	S	124	336	2.11
	6	S	101	359	0.22
TNI/Mathia	1	S	107	366	1.30
	4	S	84	314	3.02
	6	S	86	311	2.18
TNI/Birmadla	1	S	132	355	1.04
	4	S	123	326	1.25
	6	S	99	350	1.93
TNI/Chakila	1	S	133	366	0.64
	4	S	92	331	2.22
	6	S	91	332	2.56
TNI/Aus 155	1	S	110	367	0.86
	4	S	104	350	0.95
	6	S	120	334	0.42
TNI/Aus 755	1	S	105	373	2.19
	4	S	46	163	0.84
	6	S	44	165	153
TNI/Kalonchi	1	S	115	384	0.91
	4	S	92	329	2.06
	6	S	85	336	4.94*

* = significant at " = 0.05 by Chi square test, S = susceptible

though a few chemicals have been reported to be effective, their use is limited by environmental pollution and the increase in the production cost. In addition, the small-holder farmers can not afford to buy chemicals. Moreover, the development of resistance to some chemicals has been reported in some bacteria (Mew, 1992). Therefore, the most effective and economic method of controlling bacterial blight of rice is by using host plant resistance. The identification of several genes conferring resistance would facilitate breeders to use diverse resistant donors in the breeding programme allowing them to pyramid genes controlling such resistance.

The rice varieties screened for resistance to *Xoo* were found to have two recessive genes for resistance to races 1, 2, 3, 4 and 6 of the pathogen. One of the genes was allelic to *xa*-5 and confers resistance to race 1. Another recessive gene, which was found to be non-allelic to

Table 3: Reaction of F_1 plants of crosses of the tested rice cultivars with IRBB5 to *Xanthomonas oryzae* pv. *oryzae* races 1, 4 and 6

	Reaction to race Xoo		
Cross	1	4	6
IR BB5/D 204-1	R	R	S
IRBB5/Chiknal	R	MR	S
IRBB5/Gambir	R	MS	S
IRBB5/Bora Diga	R	MS	S
IRBB5/Mathia	R	MR	S
IRBB5/Birmadla	R	MS	S
IRBB5/Chakila	R	S	S
IRBB5/Aus 155	R	MS	S
IRBB5/Aus 255	R	MS	S
IRBB5/Kalonchi	R	R	S

R = resistant; MR = moderate resistant; MS = moderate susceptible; S = susceptible

Table 4: Reaction of F_1 and F_2 populations from crosses of tested rice cultivars with IRBB13 to *Xanthomonas oryzae* . *oryzae* race 6

		F_2 population		$X^2_{(7:9)}$
Cross	F_1 population	Resistant	Susceptible	
IRBB13/D204-1	S*	188	274	1.63
IRBB13/Chiknal	S	164	174	2.94
IRBB13/Gambil	S	150	201	2.51
IRBB13/Boroga	S	204	300	2.06
IRBB13/Mathia	S	196	283	1.45
IRBB13/Birmadla	S	140	203	1.08
IRBB13/Chakila	S	136	152	1.72
IRBB13/Aus 155	S	226	268	0.72
IRBB13/Aus 255	S	148	168	1.71
IRBB13/Kalonchi	S	138	160	0.64

* S = susceptible

xa-13, confers resistance to race 6. The gene *xa*-5 also confers resistance to all Japanese races and has been reported to be widespread among the Asian rice cultivars (Ogawa and Khush, 1989). This gene also confers moderate resistance to *Xoo* race 4. The gene *xa*-13 is known to confer resistance to race 6 and has a complementary effect with gene *xa*-5 in imparting resistance to race 4 (Ogawa *et al.*, 1989, Ogawa and Khush, 1989). The new gene which confers resistance to the rice cultivars tested to race 6 has not been identified. Mir and Khush (1991) reported that resistance in cultivars Kali Mekri 775 and Aus 295 from Bangladesh to races 4 and 6 was conferred by a recessive gene which was non-allelic but linked to *xa*-5, at a cross over value of 9.68%. Chen (1990), however, reported a recessive gene in rice cultivars Bazael 414 and Bazael 980, which conferred resistance to races 4 and 6, was non allelic to *xa*-13. The allelic relationships of the recessive gene conferring resistance to *xa*-13 and another recessive gene, *xa*-8 identified earlier (Sidhu *et al.* 1978), need to be further investigated in order to confirm that the tested rice varieties

(listed in Table 1) have a new gene for resistance to *Xoo*.

Conclusion

The genetic analysis of the ten rice cultivars used in this study revealed that they possess a recessive gene for resistance to *Xoo* which is allelic to *xa*-5. Moreover, the same cultivars possessed another recessive gene which conferred resistance to *Xoo* race 6 but was found to be non-allelic to *xa*-13. However, the identity of the new gene identified needs to be determined by conducting follow-up studies.

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