Inheritance of Resistance to Bacterial Blight in Rice Cultivar Cas 209

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Accepted for publication 2 May 1983.

ABSTRACT

Yoshimura, A., Mew, T. W., Khush, G. S., and Omura, T. 1983. Inheritance of resistance to bacterial blight in rice cultivar Cas 209. Phytopathology 73:1409-1412.

The mode of inheritance of resistance in rice cultivar Cas 209 to bacterial strain PX086, a representative of race II of *Xanthomonas campestris* pv. *oryzae* in the Philippines, was studied. The analysis of F_1, F_2, and backcross populations from the crosses of Cas 209 with susceptible cultivars revealed that Cas 209 resistance was controlled by a single dominant gene. Tests for linkage relationships indicated that this newly identified dominant gene was linked with *Xa*-4 with a recombination value of 27.4%, but was independent of *xa*-5. This new gene for bacterial blight resistance was designated *Xa*-10.

Additional key words: disease resistance, *Oryza sativa*, races.

Four dominant genes in rice (*Oryza sativa* L.), *Xa*-1, *Xa*-2, *Xa*-3 (*Xa*-*w*), and *Xa*-*kg*, for resistance to Japanese isolates of *Xanthomonas campestris* pv. *oryzae* (Ishiyama, 1922) Dye 1978, the cause of bacterial blight, have been identified (1,11,14). Six additional genes, *Xa*-4, *xa*-5, *Xa*-6, *Xa*-7, *xa*-8, and *xa*-9, for resistance to Philippine isolates of the bacterium were identified at the International Rice Research Institute (IRRI) (12,13,15–17). Two alleles at the *Xa*-4 locus are known. *Xa*-4^a confers resistance at tillering and postbooting stages, and *Xa*-4^b confers resistance only at postbooting (6). Cultivars with *xa*-5 show resistance at all plant growth stages and cultivars with *Xa*-6 show resistance at booting and postbooting (12,15). *Xa*-7 confers resistance at flowering as well as at later growth stages. Cultivars with *xa*-8 show resistance at flowering (15) and those with *xa*-9 show resistance at postbooting (17).

Four race groups of the bacteria in the Philippines were identified based on interactions with IRRI differential rice cultivars (8,9). Cultivar IR8 is susceptible to all races, IR20 is resistant only to race I, Cas 209 is resistant only to race II, IR1545-339 (hereafter, IR1545) is resistant to races I, II, and III, and DV85 is resistant to all races (Table 1). To date, only the bacteria of race I have been

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TABLE 1. Reactions of four rice cultivars and F_1 progeny of crosses between them to four strains of *Xanthomonas campestris* pv. *oryzae*

Parents and F_1	Plants tested (no.)	Lesion length (cm) PX061 (race I)	PX086 (race II)	PX079 (race III)	PX071 (race IV)
TN1	3	21.7	19.0	22.3	19.3
IR20	3	2.0	10.3	13.2	5.8
Cas 209	3	22.8	1.7	22.3	21.5
IR1545	3	1.0	2.3	1.8	6.3
TN1/Cas 209	6	24.5	4.5	26.3	20.8
IR20/Cas 209	3	5.7	4.2	23.5	11.5
IR1545/Cas 209	2	24.5	9.5	29.3	27.8

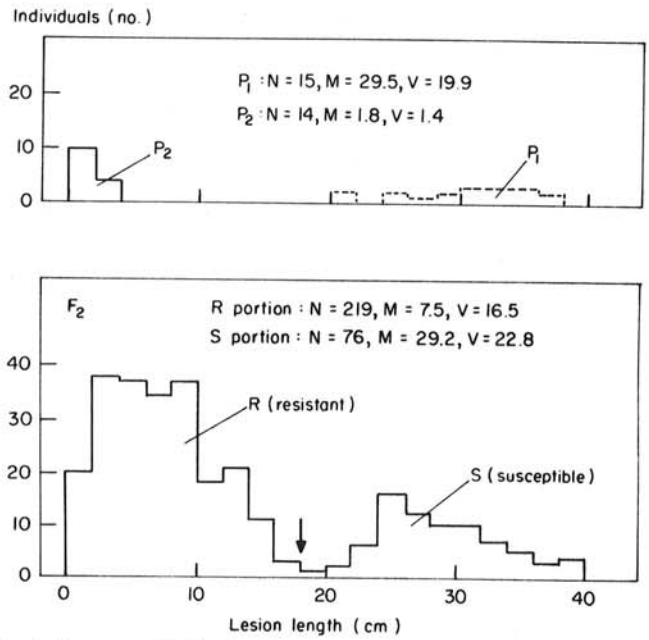

Fig. 1. Frequency distributions for resistance to PX086 in the F_2 population from the cross of TN1 (P_1)/Cas 209 (P_2) and the parents. N = number of individuals, M = mean lesion length (cm), and V = variance.

used in studying the genetics of resistance. IR20 has *Xa*-4[a], IR 1545 has *xa*-5, and DV85 possesses *xa*-5 and *Xa*-7.

In this study, we investigated the inheritance of resistance in rice cultivar Cas 209. The cultivar is a useful differential for distinguishing bacteria belonging to race groups II and III. It is resistant to race II, but susceptible to races I, III, and IV (9). Taichung Native 1 (TN1), instead of IR8, was used as a susceptible check. It is equally susceptible as IR8 to all four races in the Philippines, and has been used previously in the gene analysis in rice cultivars to bacterial blight.

MATERIALS AND METHODS

Cultivar Cas 209 was crossed to rice cultivars TN1 (Taichung Native 1), IR20, and IR1545. The F_1 and F_2 progenies of these crosses were tested for bacterial blight reaction. Progenies from the backcrosses TN1*2/Cas 209 and TN1*2/Cas 209/2/TN1 were also tested.

X. campestris pv. *oryzae* strains PX061 of race I, PX086 of race II, PX079 of race III, and PX071 of race IV were used to inoculate the parental and segregating populations. The inoculum of each strain was prepared by incubating the bacteria on potato semisynthetic agar (18) in slants of 30 C for 3 days. Inoculum was prepared by suspending each pure culture in sterile distilled water and adjusting the inoculum for about 10^9 cells per milliliter.

Hybrid populations and each parent were grown in the screenhouse or in the greenhouse under standard management. Tillers of each hill were divided equally according to the number of bacterial strains used. The plants were inoculated when the maximum number of tillers had formed or about 50–55 days after seeding. Inoculation of fully expanded leaves was by the leaf clipping method (5). More than two leaves of each hill of the parent F_1 and F_2 populations were inoculated with each bacterial strain. In the backcross populations, at least six leaves of each hill were inoculated with one bacterial strain.

Disease scores were taken 14 days after inoculation. Inoculated leaves were rated by measuring the lesion length in centimeters. Two leaves from the parents, and the F_1 and F_2 populations inoculated with bacterial strains were randomly taken for lesion length measurement. In the backcross populations, five inoculated leaves were randomly taken for lesion length measurement. The mean lesion length was used to determine the reaction of each hill.

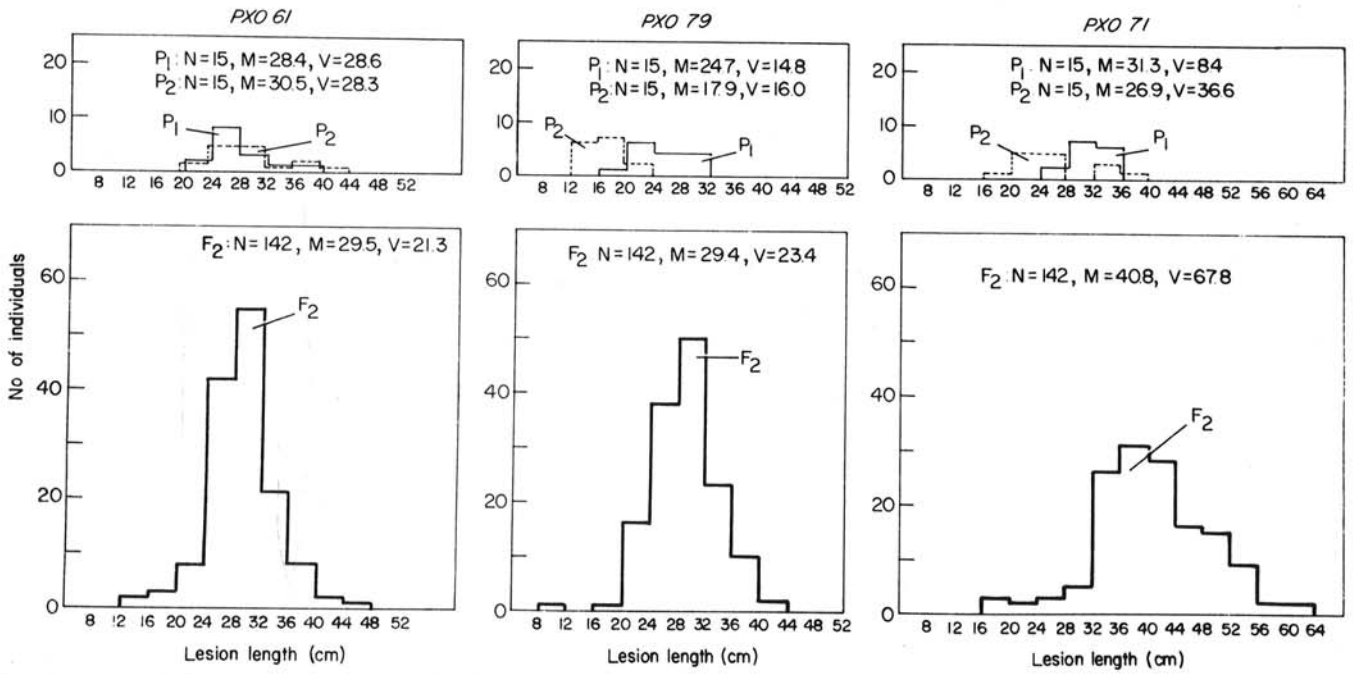

Fig. 2. Frequency distributions for resistance to PX061, PX079, and PX071 in the F_2 population from the cross of TN1 (P_1)/Cas 209 (P_2) and the parents. N = number of individuals, M = mean lesion length (cm), and V = variance.

Disease reactions of F_1 plants inoculated at flowering stage were evaluated by visual scores using the Standard Evaluation System for Rice (4) to confirm reaction at maximum tillering stage.

RESULTS

Inheritance of resistance. The reactions of the parents were similar to those reported by Mew et al (9) (Table 1). The response of F_1 plants from the cross of TN1/Cas 209 to PX061, PX086, PX079, and PX071 were susceptible (S), resistant (R), S, and S, respectively; F_1 plants of IR20/Cas 209 were R, R, S, and S. The reactions of IR1545/Cas 209 to the four strains were S, moderately resistant (MR), S, and S. Similar reaction patterns were observed in F_1 plants from these three crosses at flowering stage. These reactions indicate that the resistance of Cas 209 to PX086 is governed by dominant gene(s).

The segregation of the F_2 population from the cross of TN1/Cas 209 showed a wide range of response to PX086 with the lesion length varying from 1 to 40 cm and forming a clear-cut bimodal distribution (Fig. 1). Therefore, the F_2 plants were classified into two groups using an 18-cm lesion length as the dividing point. One group included resistant and moderately resistant plants, the other group included susceptible plants. This distribution fits a ratio of 3:1($\chi^2 = 0.028$, $0.90 > P > 0.75$). Reactions of the F_2 to the three other strains were continuously and unimodally distributed (Fig. 2). No plant was resistant to any of the strains, indicating that Cas 209 has no gene for resistance to PX061, PX079, nor PX071. The frequency distribution for scores of reaction to PX086 in the F_2 population of IR20/Cas 209 is shown in Fig. 3. When inoculated with PX086, the F_2 population showed two distinct modes of lesion length (Fig. 3). The F_2 population could be classified into two groups, using a 10-cm lesion length as the dividing line and fit a 3:1 ratio ($\chi^2 = 1.684$, $0.25 > P > 0.10$). The F_2 resistant and moderately resistant class mean lesion length was smaller for IR20/Cas 209 than for TN1/Cas 209. The backcross populations from the cross TN1*2/Cas 209 and TN1*2/Cas 209/2/TN1 were also tested (Fig. 4). Both populations segregated in a ratio of 1 resistant:1 susceptible ($0.75 > P > 0.50$ for B_2F_1, $0.50 > P > 0.25$ for B_2F_1) thus confirming the conclusion drawn from the reaction of F_2 populations.

Test for allelism and linkage relationships with *Xa*-4 and *xa*-5. To investigate linkage relationships between the newly identified dominant gene of Cas 209 and the *Xa*-4 and *xa*-5 loci, F_2 populations from the crosses of IR20/Cas 209 were also inoculated with PX061 of race 1. Resistance donors possessing these two genes are widely used in IRRI's breeding program (10). IR20 carries *Xa*-4[a]. With a 16-cm lesion length dividing line, there were 203 resistant and 68 susceptible plants in the F_2 of IR20/Cas 209. Data fit the expected 3:1 ratio ($\chi^2 = 0.001$, $P > 0.90$) and confirmed that resistance to PX061 in IR20 is controlled by a single dominant gene, *Xa*-4 (13). The F_2 population was also inoculated with PX086. With respect to reaction to both isolates, the F_2 population did not segregate in the expected ratio of 9RR:3RS:3SR:1SS ($\chi^2 = 20.286$), suggesting that two loci are linked (Table 2). The recombination value was estimated to be 27.4% by the use of the maximum likelihood method (7).

In the F_2 population of IR1545/Cas 209, two distinct modes for the frequency distribution of scores of reaction to PX061 were observed and classification into resistant and susceptible groups was clear. The observed segregation of 65 resistant and 216 susceptible plants fit the expected 1:3 ratio. The results confirmed that a single recessive gene, *xa*-5 in IR1545, was responsible for resistance to PX061 (12,13). The same F_2 population, when scored for resistance to PX086, also formed a clear-cut bimodal

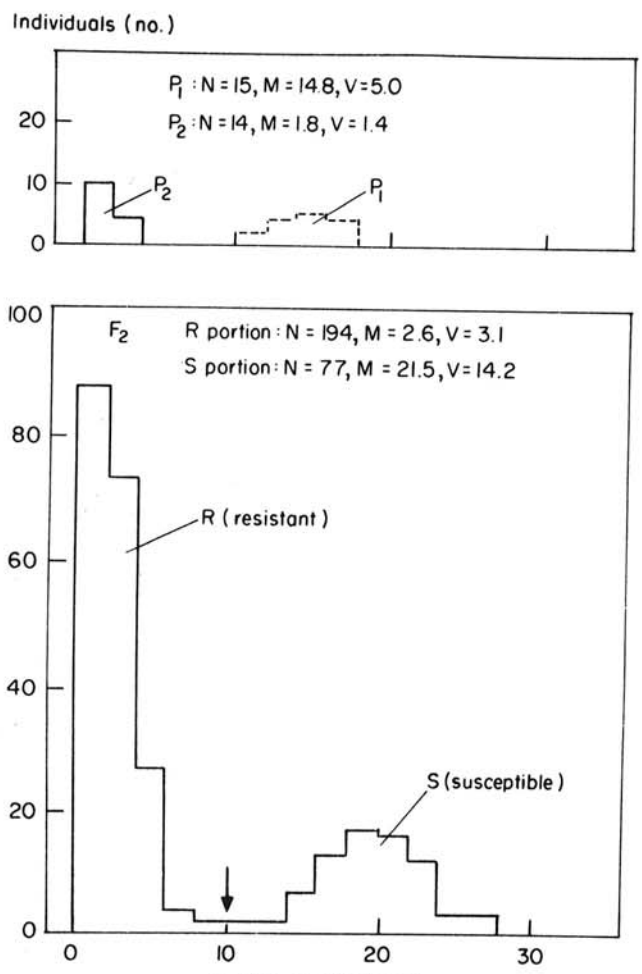

Fig. 3. Frequency distributions for resistance to PX086 in the F_2 population from the cross of IR20 (P_1)/Cas 209 (P_2) and the parents. N = number of individuals, M = mean lesion length (cm), and V = variance.

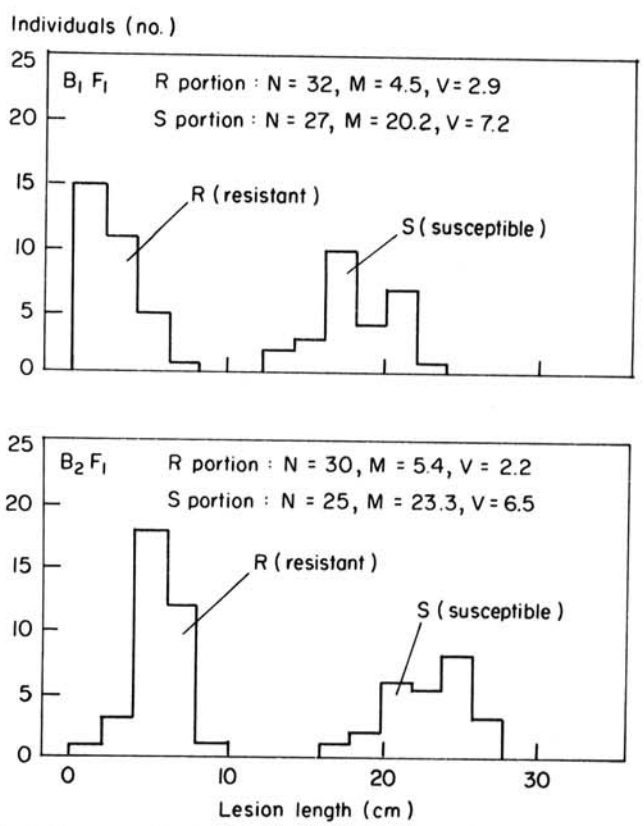

Fig. 4. Frequency distributions for resistance to PX086 in the backcross populations from the crosses of TN1*2/Cas 209 and TN1*2/Cas 209/2/TN1. N = number of individuals, M = mean lesion length (cm), and V = variance.

TABLE 2. Reactions to *Xanthomonas campestris* pv. *oryzae* strains PX061 and PX086 of F_1 and F_2 populations from the crosses of rice cultivar Cas 209 with cultivars IR20 and IR1545

Cross	F_1	Number of F_2 plants in each reaction pattern					χ^2	P	Recombination value (%)
		RR	RS	SR	SS	Total			
IR20/Cas 209	RR[a]	132	71	62	6[b]	271			27.4 ± 5.6
		(140.57)	(62.67)	(62.67)	(5.07)[c]		1.802	0.75–0.50	
IR1545/Cas 209	RR	65	0	163	53[b]	281			
		(70.25)	(0.00)	(158.06)	(52.69)[d]		0.548	0.90–0.75	

[a] R (resistant), MR (moderately resistant), S (susceptible): For combined capital letters, the first letter stands for reaction to PX061; the second, to PX086.
[b] Observed segregation.
[c] Calculated based on recombination value of 27.4%.
[d] Calculated on the basis of 4:0:9:3 ratio.

distribution and was classified into two groups with an 18-cm lesion length dividing line. Of 281 plants, 228 were resistant and 53 susceptible. Data fit to the ratio of 13:3 which would be expected based on two independent gene segregations, one recessive and the other dominant. The results show that the recessive gene for resistance to PX086 in IR1545 is independent of the dominant gene in Cas 209. In the F_2 population of IR1545/Cas 209, all the plants resistant to PX061 were also resistant to PX086, showing that the resistance of IR1545 to these two strains was conferred by *xa*-5. Assuming the two independent genes for resistance were segregating independently in this F_2 population, the ratio of 4RR:0RS:9SR:3SS would be expected when classified for reaction to the two strains. The observed segregation fit to the expected ratio, confirming that the single dominant gene for resistance to PX086 in Cas 209 is independent of *xa*-5.

DISCUSSION

In general, resistance of rice cultivars to bacterial blight at the maximum tillering stage appears to be maintained up to the flowering stage through the effect of the same resistance gene(s). Therefore, in this study, plants of segregating populations were inoculated only at maximum tillering. Cas 209 is resistant to race II from seedling to adult plant (9). The F_1 plants from the crosses of susceptible cultivars with Cas 209 were resistant to PX086 at flowering as well as at maximum tillering (Table 1). Therefore, it is reasonable to conclude that the resistance of Cas 209 to PX086 at flowering is governed by the same dominant gene that conveys resistance at maximum tillering. Recently, Yamada and Horino (19) assessed the resistance of some rice cultivars and indicated that the resistance of IR28, IR29, and IR30 to bacterial groups I and V of Japan at seedling and reproductive stages could be attributed to the pleiotropic effect of resistance genes, although their evidence was indirect.

The present results indicated that the F_2 population frequency distribution could differ considerably depending on differences in the genetic background of parents involved. Consequently, the dividing line where the two groups separate could also change. Nevertheless, it was obvious that F_2 populations derived from the crosses of TN1/Cas 209 and IR20/Cas 209 could be classified into two distinct groups when inoculated with PX086.

The F_1 plants from the crosses of TN1/Cas 209 and IR20/Cas 209 were resistant, suggesting that the resistance of Cas 209 to PX086 is dominant. F_2 populations from both crosses segregated in the ratio of 3 resistant:1 susceptible, demonstrating that the resistance of Cas 209 to PX086 is controlled by a single dominant gene.

The test for allelism and linkage relationships indicates that the dominant gene for resistance in Cas 209 is linked with *Xa*-4 with a crossover frequency of 27%. It segregates independently of *xa*-5. Two genes, *Xa*-6 and *Xa*-7, confer resistance to PX061 at booting and at later growth stages (12,15,16). Cas 209 is susceptible to PX061 at all growth stages. Therefore, the dominant gene of Cas 209 is not *Xa*-6 or *Xa*-7. Two recessive genes, *xa*-8 (15) and *xa*-9 (17), also convey resistance to PX061. Therefore, the dominant gene of Cas 209 appears to be different from four dominant genes identified in Japan because Cas 209 is susceptible to all the bacterial groups of Japan (2). These facts suggest that the dominant gene for resistance in Cas 209 is different from *Xa*-1, *Xa*-2, *Xa*-3 (*Xa*-*w*), *Xa*-*kg*, *Xa*-4, *xa*-5, *Xa*-6, *Xa*-7, *xa*-8, and *xa*-9. Based on the rules of gene nomenclature (3), we designate this gene *Xa*-10.

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