

Resistance Gene of Rice Cultivar, Taichung Native 1 to Philippine Races of Bacterial Blight Pathogens

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In the inoculation tests on rice cultivars using Philippine races of bacterial blight (BB) caused by *Xanthomonas campestris* pv. *oryzae*, we found that Taichung Native 1 (TN1) was resistant only to race 5 (PXO112) and susceptible to races 1, 2, 3, 4, and 6. This kind of reaction pattern to BB pathogens has not been found in any other rice cultivar resistant to BB in Japan and at the International Rice Research Institute in the Philippines. Therefore, it was suggested that TN1 has a new gene for resistance to BB.

The mode of inheritance of resistance in TN1 to race 5 was studied. The analysis of the F₁ plants, the F₂ populations, and the backcross progeny from the crosses between TN1 and susceptible cultivars revealed that the resistance of TN1 was controlled by a single dominant gene. Thus this new resistance gene was designated as *Xa-14*.

KEY WORDS: *Oryza sativa*, *Xanthomonas campestris* pv. *oryzae*, resistance gene, disease resistance.

Introduction

Genetic studies on resistance to bacterial blight (BB) caused by *Xanthomonas campestris* pv. *oryzae* were conducted mainly in Japan and at the International Rice Research Institute (IRRI) in the Philippines. Four resistance genes, *Xa-1*, *Xa-2*, *Xa-3*, and *Xa-11*, were identified using Japanese BB races in Japan (SAKAGUCHI 1967, EZUKA *et al.* 1975, OGAWA and YAMAMOTO 1986). On the other hand, *Xa-4*, *xa-5*, *Xa-7*, *xa-8*, *Xa-10*, and *xa-13* were identified using Philippine BB races at IRRI (PETPISIT *et al.* 1977, SIDHU *et al.* 1978, YOSHIMURA *et al.* 1983, OGAWA *et al.* 1987). Moreover, *Xa-12* was identified using an Indonesian race in Japan (OGAWA *et al.* 1978).

During the inoculation tests using Philippine BB races, we found that Taichung Native 1 (TN1) is resistant only to race 5 (PXO112) but is very susceptible to races 1, 2, 3, 4, and 6. Thus we compared the reaction of TN1 with those of the other rice cultivars carrying known resistance genes to six Philippine races, and then analyzed the mode of inheritance for resistance of TN1 to race 5.

This study was carried out under a collaborative research between IRRI and the Japanese Ministry of Agriculture, Forestry, and Fisheries.

Materials and Methods

To compare the disease reaction to the BB pathogens, TN1 and resistant cultivars

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carrying known resistance genes were inoculated with Philippine BB races. The cultivars and their resistance genes used for the inoculation test are shown in Table 1. Isolates used were PXO61 for race 1, PXO86 for race 2, PXO79 for race 3, PXO71 for race 4, PXO112 for race 5, and PXO99 for race 6.

TN1 was crossed with susceptible cultivars, Milyang 23 and Toyonishiki, and with a Japanese differential cultivar, Te-tep, which shows similar reaction as TN1 to Japanese races (OGAWA and YAMAMOTO 1987a, OGAWA and YAMAMOTO 1987b). The F₁ plants and the F₂ populations from the crosses were tested for BB reaction to races 1, 2, 4, and 5. In addition, the backcross progeny of IR24/TN1//IR24 was also tested.

Resistant cultivars, the F₁ plants, and the F₂ populations were grown under standard management in a greenhouse covered with fine net. Before inoculation, tillers of each individual plant were divided into the required numbers of bacterial races with different colored vinyl ties, one color for each race.

Each BB race was transferred to semi-synthetic agar media (WAKIMOTO 1954) whenever inoculum was needed for inoculation and incubated at 28°C for 48 hours. The inoculum was diluted with distilled water. Using a spectrophotometer, the absorbance of the inoculum was adjusted to A=0.05 (620 nm). This value corresponds to a concentration of about 10⁸ cells/ml. Inoculation was done during booting to flowering stage. The fully extended top leaves of two or three tillers were cut off following the clipping inoculation method developed by KAUFFMAN *et al.* (1973). The lesion length caused by each race was measured in three leaves of each plant at 18 days after inoculation. Mean lesion length of the three leaves was used to determine the reaction of each plant. Disease reaction was evaluated based on lesion length and symptom of the lesion. In this study, a lesion length below 5 cm and/or aborted lesion development was evaluated as resistant, 5 to 10 cm as moderately resistant, 10 to 15 cm as moderately susceptible, and above 15 cm as susceptible.

Results and Discussion

The results of the inoculation tests using six Philippine races of BB are given in Table 1. The susceptible cultivars, IR24 and Milyang 23, showed long lesions in reaction to the six Philippine BB races, about double time lesion lengths that developed in Toyonishiki. As expected, TN1 was resistant only to race 5, but susceptible to races 1, 2, 3, 4, and 6. Kinmaze, a susceptible check cultivar in Japan, was susceptible to the Philippine BB races.

The Japanese differential cultivars, Te-tep, Chugoku 45, Java 14, and Kogyoku have a combination of *Xa-1*, *Xa-2*, *Xa-3*, and *Xa-11* resistance genes. Te-tep, which has *Xa-1* and *Xa-2* (SAKAGUCHI 1967), and TN1 showed similar reaction patterns to Japanese BB races (OGAWA and YAMAMOTO 1987b), but Te-tep is susceptible to the six Philippine BB races, Chugoku 45, which has *Xa-3* (OGAWA *et al.* 1986), is moderately susceptible to race 6 but resistant to races 1 to 5. Its reaction was different from that of TN1. Java 14, which has *Xa-1*, *Xa-3*, and *Xa-12* (OGAWA *et al.* 1978), showed a reaction pattern similar to that of Chugoku 45. According to HORINO *et al.* (1980), Kogyoku, a Japanese differential cultivar, which has *Xa-1* and *Xa-12* (OGAWA *et al.* 1978), is susceptible to Philippine BB races. These data suggested that *Xa-1*, *Xa-2*, and *Xa-12* do not convey resistance to Philippine BB races.

Therefore, the resistance genes of Japanese differential cultivars were different from that of TN1.

Table 1. The reaction of rice cultivars and F₁ plants to the Philippine races of bacterial blight pathogens at booting stage

Cultivar ¹⁾ and cross	Lesion length ²⁾ (cm) and reaction ³⁾											
	Race 1		Race 2		Race 3		Race 4		Race 5		Race 6	
IR24 (none)	25.7	S	35.6	S	32.5	S	31.6	S	22.7	S	36.0	S
Milyang 23 (none)	15.3	S	26.5	S	28.5	S	31.3	S	24.9	S	43.5	S
Toyonishiki (none)	8.3	MR	17.0	S	16.2	S	11.6	MS	12.9	MS	21.2	S
Taichung Native 1	19.6	S	35.2	S	37.4	S	31.5	S	4.9	R	30.7	S
Kinmaze (none)	15.2	S	18.0	S	21.0	S	21.7	S	17.1	S	20.4	S
Te-tep (<i>Xa-1</i> , <i>Xa-2</i>)	14.5	MS	30.7	S	29.1	S	26.5	S	17.0	S	33.1	S
Chugoku 45 (<i>Xa-3</i>)	0.4	R	1.9	R	2.2	R	1.3	R	3.5	R	11.5	MS
Java 14 (<i>Xa-1</i> , <i>Xa-3</i> , <i>Xa-12</i>)	0.6	R	2.3	R	1.4	R	2.9	R	6.9	MR	24.4	S
IR8 (<i>Xa-11</i>)	17.0	S	25.6	S	28.7	S	14.9	MS	13.6	MS	19.5	S
IR20 (<i>Xa-4</i>)	6.9	MR	22.5	S	24.5	S	9.7	MR	3.9	R	18.3	S
IR1545-339 (<i>xa-5</i>)	1.2	R	2.6	R	1.1	R	17.9	S	4.4	R	30.3	S
DV85 (<i>xa-5</i> , <i>Xa-7</i>)	0.4	R	0.4	R	0.5	R	6.0	MR	2.0	R	6.5	MR
Cas 209 (<i>Xa-10</i>)	24.2	S	3.5	R	45.3	S	40.0	S	1.7	R	50.4	S
BJ1 (<i>xa-5</i> , <i>xa-13</i>)	0.6	R	3.8	R	2.8	R	4.7	R	0.6	R	3.7	R
PI231129 (<i>xa-8</i>)	3.5	R	8.0	MR	6.5	MR	12.2	MS	5.5	MR	4.5	R
Milyang 23/TN1	16.9	S	32.9	S	— ⁴⁾	—	29.5	S	2.7	R	—	—
TN1/Te-tep	12.1	MS	25.2	S	—	—	29.2	S	1.0	R	—	—
Toyonishiki/TN1	15.3	S	30.0	S	—	—	25.5	S	3.3	R	—	—

¹⁾ Genes for resistance are in parentheses

²⁾ The mean of lesion length from 15 inoculated leaves (3 leaves from 5 plants) at 18 days after inoculation

³⁾ R = resistant; MR = moderately resistant; MS = moderately susceptible; and S = susceptible

⁴⁾ — = not tested.

The resistance genes of IR8, IR20, IR1545-339, Cas 209, and PI231129 are *Xa-11*, *Xa-4*, *xa-5*, *Xa-10*, and *xa-8*, respectively (OGAWA and YAMAMOTO 1986, PETPISIT *et al.* 1977, YOSHIMURA *et al.* 1983, SIDHU *et al.* 1978). The reaction patterns of the five cultivars, due to the resistance genes *Xa-11*, *Xa-4*, *xa-5*, *Xa-10*, and *xa-8*, were different from that of TN1.

DV 85 has a recessive gene *xa-5* and a dominant resistance gene *Xa-7*. SIDHU *et al.* (1978) reported that the F₁ plants from the cross of TN1/DV85 were resistant to race 1 (PXO61) at flowering stage. It indicates that the dominant resistance gene *Xa-7* is resistant to race 1. Therefore, the reaction of resistance gene *Xa-7* was different from that of resistance gene of TN1.

BJ1 has two recessive resistance genes *xa-5* and *xa-13*, and shows resistance to six Philippine BB races (OGAWA *et al.* 1987). As resistance gene *xa-5* in IR1545-339 shows susceptibility to race 6, resistance gene *xa-13* conveys resistance to race 6. The reaction of *xa-13* was also different from that of TN1.

From the above inoculation tests, the resistance of TN1 to Philippine race 5 appeared to

be controlled by an unknown gene(s).

The reaction of the F_1 plants from the crosses of Milyang 23/TN1, TN1/Te-tep, and Toyonishiki/TN1 to four Philippine BB races are shown in Table 1. They were resistant to race 5 and susceptible to races 1, 2, and 4, which is similar to the reaction of TN1. Therefore, it is concluded that resistance of TN1 to race 5 is controlled by a dominant gene(s).

The disease reaction of the F_2 populations from the three crosses of Milyang 23/TN1, TN1/Te-tep, and Toyonishiki/TN1 to Philippine BB races 1, 2, 4, and 5 are shown in Table 2. The F_2 populations from the crosses of Milyang 23/TN1 and TN1/Te-tep segregated into 200 SSSR:70 SSSS and 208 SSSR:57 SSSS plants, respectively (the combined capitals stand for the reaction to races 1, 2, 4, and 5, respectively; R=resistant, S=susceptible) and fitted the expected ratio of 3:1. This means that the resistance of TN1 to race 5 is due to a

Table 2. The reaction of the F_1 plants and the F_2 populations from the crosses of TN1 with three cultivars to four Philippine races (races 1, 2, 4, and 5) of the bacterial blight pathogens

Cross	Reaction of F_1 plants	Reaction of F_2 populations			χ^2 3:1	P
		SSSR ¹⁾	SSSS	Total		
Milyang 23/TN1	SSSR	200	70	270	0.12	0.70~0.80
TN1/Te-tep	SSSR	208	57	265	1.72	0.10~0.20
Toyonishiki/TN1	SSSR	185	84	269	5.56	0.01~0.02

¹⁾ R = resistant; S = susceptible, The combined capitals stand for the reaction to races 1, 2, 4, and 5, respectively.

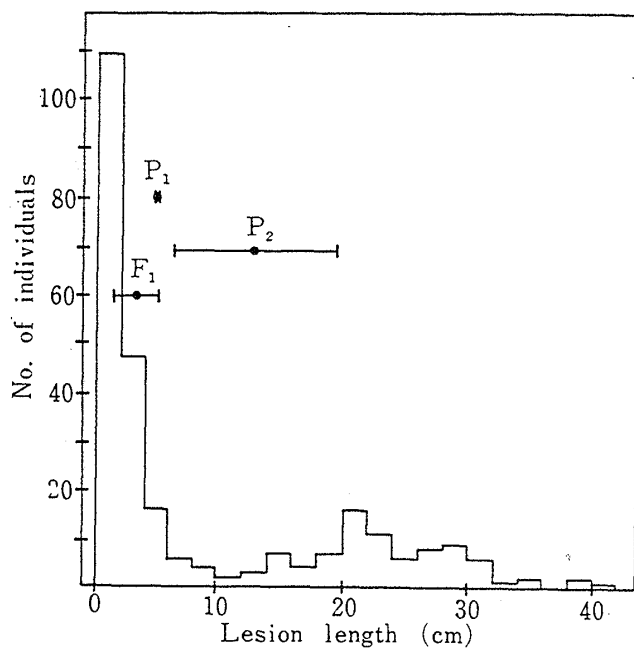


Fig. 1. Frequency distribution for lesion length F_2 population from the cross of Toyonishiki (P_2)/TN1(P_1) to race 5 (PXO 112) of the bacterial blight pathogen.

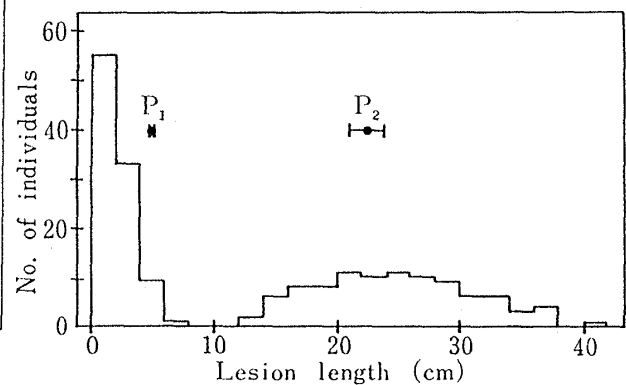


Fig. 2. Frequency distribution for lesion length of backcross progeny from the cross of IR24 (P_2)/TN1 (P_1)//IR24 to race 5 (PXO 112) of the bacterial blight pathogen.

single dominant resistance gene. The F₂ population from the cross of Toyonishiki/TN1 to Philippine BB races 1, 2, 4, and 5 segregated into 185 SSSR:84 SSSS plants, but this segregation was significantly different from the expected ratio of 3 SSSR:1 SSSS at 5% level. The number of resistant plants in F₂ population was less than its expected value based on segregation of a single dominant gene. We considered that the segregation distortion was caused by cross combination between *japonica* and *indica* rice plants. In addition, poor growth of *japonica* rice plants under short-day condition in low latitude influenced the expression of resistance in some F₂ plants.

The frequency distribution for lesion length of the F₂ population from the cross of Toyonishiki/TN1 to race 5 is shown in Fig. 1. The group of F₂ plants had less than 10 cm lesion length was considered resistant and while those F₂ plants with over 10 cm lesion length was classified as susceptible. The frequency distribution for lesion length of the other two F₂ populations to the races was also observed and similar results were obtained.

The backcross progeny of IR24/TN1//IR24 to races 1, 2, 4, and 5 segregated into 98 SSSR and 97 SSSS plants (Fig. 2). The segregation fitted a ratio of 1:1 ($\chi^2=0.005$, $0.98 < P < 0.99$).

Thus, we concluded that the resistance of TN1 is governed by a single dominant gene, and we designated it as *Xa-14* following the rule of the Rice Genetic Cooperative (TAURA *et al.* 1987).

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台中在来1号のフィリピン産イネ白葉枯病菌に対する抵抗性遺伝子

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フィリピン産イネ白葉枯病菌を用いた接種の結果, 台中在来1号(TN1)はレース5(PXO112)に対して抵抗性を, レース1, 2, 3, 4および6に高い感受性を示した。これは今までに見られない特別な反応であった。

そこでTN1と既存の抵抗性遺伝子を有する抵抗性品種のイネ白葉枯病に対する反応を比較するため, それらの品種にフィリピン産菌系, レース1(PXO61), レース2(PXO86), レース3(PXO79), レース4(PXO71), レース5(PXO112)およびレース6(PXO99)の接種を試みた。結果はTable1に示した。日本の判別品種, 金南風はフィリピン産6菌系に対して感受性を示した。抵抗性遺伝子*Xa-1*および*Xa-2*を有するTe-tepは日本産菌系に対してはTN1と同様な反応(OGAWA and YAMAMOTO 1987b)を示したが, フィリピン産6菌系に対しては感受性を示した。*Xa-3*を有する中国45号はレース1~5に対しては抵抗性を示し, レース6に中度感受性を示した。*Xa-1*, *Xa-3*および*Xa-12*を有するJava14は中国45号と同様な反応を示した。HORINO *et al.* (1980)は*Xa-1*および*Xa-12*を有する黄玉がフィリピン産6菌系に対して感受性であると報告していることから日本の判別品種が有する*Xa-1*, *Xa-3*および*Xa-12*はTN1の抵抗性と異なる。

抵抗性品種, IR8, IR20, IR1545-339, Cas209およびPI231129は*Xa-11*, *Xa-4*, *xa-5*, *Xa-10*および*xa-8*のそれぞれ1個の抵抗性遺伝子を有する。これらはTN1のフィリピン産菌系に対する反応と異なった。よって*Xa-11*, *Xa-4*, *xa-5*, *Xa-10*および*xa-8*はTN1の抵抗性と異なる。

抵抗性品種DV85は劣性遺伝子*xa-5*と優性遺伝子*Xa-7*を有する。SIDHU *et al.* (1978)はTN1/DV85のF₁植物はレース1に抵抗性を示すと報告していることから*Xa-7*の抵抗性反応はTN1の反応と異なる。BJ1は2個の劣性遺伝子*xa-5*および*xa-13*を有する。OGAWA *et al.* (1987)は*xa-13*はフィリピン産レース6に抵抗性を示すと報告していることから*xa-13*の抵抗性反応はTN1の反応と異なる。

フィリピン産6菌系の接種試験の結果, 既存の抵抗性遺伝子*Xa-1*, *Xa-2*, *Xa-3*, *Xa-4*, *xa-5*, *Xa-7*, *xa-8*, *Xa-10*, *Xa-11*, *Xa-12*および*xa-13*の反応はTN1の抵抗性反応と異なるものであり, TN1のフィリピン産レース5に対する反応は未知の抵抗性遺伝子によるものである。

TN1の有する抵抗性遺伝子の遺伝様式を調べるため, TN1を感受性品種密陽23号, トヨニシキおよび日本産菌系に対してTN1と同様な反応を示すTe-tepと交配した。それぞれのF₁植物にフィリピン産レース1, 2, 4および5を接種した結果, すべてのF₁植物はTN1の反応と同様な反応を示した(Table1)。これはTN1の有する抵抗性が優性の遺伝子であることを示した。

この3組の組合せから得たF₂集団のフィリピン産レース1, 2, 4および5に対する反応を調べた。密陽23号/TN1およびTN1/Te-tepのF₂集団はレース5に対して1個の優性遺伝子による分離比3:1に適合した。トヨニシキ/TN1のF₂集団は分離比3:1に適合しなかったが他のF₂集団と同様な病斑長の頻度分布を示した(Table2, Fig.1)。さらに, IR24/TN1/IR24の戻し交配のF₁集団におけるフィリピン産レース1, 2, 4および5に対する反応を調べた結果, レース5に対して抵抗性98個体および感受性97個体に分離した(Fig.2)。これは1個の優性遺伝子による分離比1:1に適合した。

フィリピン産イネ白葉枯病菌を用いた接種の結果見出されたTN1のレース5(PXO112)に対して抵抗性を, レース1, 2, 3, 4および6には高い感受性を示す反応は1個の新しい優性遺伝子により制御されたものである。その抵抗性遺伝子をRice Genetics Cooperativeの命名法に従い*Xa-14*と命名した(TAURA *et al.* 1987)。