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Infection Types in Rice-*Xanthomonas campestris* pv. *oryzae* Interaction

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Abstract
The resistance reactions of rice varieties to bacterial leaf blight caused by *Xanthomonas campestris* pv. oryzae were grouped into three types: symptomless, browning and small yellow lesions. In the resistance reaction controlled by the resistance genes Xa-*1*, Xa-*7* and Xa-*10* no symptoms were observed, while the reaction controlled by *Xa-2*, *Xa-4*, *Xa-4*^{a}, *xa-5* and *xa-8* was characterized by small yellow to limited yellow lesions. The resistance reaction controlled by *Xa-3*, *Xa-4*^{b}, *Xa-6*, *xa-9* was characterized by brown necrosis. The population levels of the incompatible strain were lower than 10^6 cfu per inoculated leaf in the symptomless and browning type of resistance reactions. In the resistance reaction with small yellow lesions, the bacterium multiplied to the level of 10^7 cfu per inoculated leaf. Histological observation showed that multiplication and translocation of the incompatible strains were very limited compared with those of the compatible ones. It is concluded that the infection types in the host-parasite interaction are caused by the degree of multiplication of the bacterium in the host tissues and ability of the host tissues to undergo necrosis.

Discipline: Plant pathology
Additional key words: resistance gene, resistance reaction, rice bacterial leaf blight

Introduction

Bacterial leaf blight caused by *Xanthomonas campestris* pv. *oryzae* is one of the most destructive diseases of rice in many rice-growing countries, especially in Southeast Asia. The use of varietal resistance is a promising method of control against the disease. Therefore, breeding of resistant varieties has been a major objective for the control of the disease. As the basis of breeding, genetic analysis of the resistance has been studied in Japan, at IRRI and in other countries. As a result, more than 20 resistance genes have been reported[6,15,21).

Up to date, however, the function of these resistance genes has not been analyzed, and varietal resistance has been evaluated quantitatively. As a disease is the product of the interaction between two organisms, host and parasite, the interaction should also vary qualitatively. In fact, a hypersensitive reaction (HR) has been reported in many bacterial diseases[10). In fungal diseases, infection types have been reported, especially in cereal rust[12).

In this paper, therefore, the variation of the resistance expression in the host-parasite interaction is described with special reference to its relation to the resistance genes involved.

Infection types

The resistance reactions of rice varieties to strains of *X. campestris* pv. *oryzae* were compared using varieties divided into Kogyoku, Rantai Emas, Wase Aikoku and Java groups by Ezuka et al.[2).

As a result, the types of the resistance reaction were grouped into three categories: symptomless, browning and small yellow lesions (Plate 1). In addition, such types were closely related to the resistance genes. Based on the reference of the resistance reaction to resistance genes, resistance reaction controlled by the resistance genes *Xa-1*, *Xa-2*, *Xa-3*, *Xa-kg* (*Xa-12*) were characterized as follows: symptomless, small yellow lesions, browning and symptomless to small yellow lesions, respectively[8,9).

Resistance of the variety Kogyoku, representative of Kogyoku group, is controlled by a single dominant gene *Xa-1*[4,16,20]. The variety showed the symptomless type of resistance reaction to race I at the flag leaf stage, while limited yellow lesions commonly appeared at the younger stage. No additional symptoms were observed around the point of inoculation. Sakaguchi[20] showed that the resistance of 20 Kogyoku group varieties including Kogyoku is controlled by *Xa-1*. All these varieties showed the same type of resistance reaction as Kogyoku.

The resistance of Te-tep, representative of Rantai Emas group, to race II is controlled by a single dominant gene *Xa-2*[4,6,20]. The variety showed a resistance reaction consisting of small yellow lesions to race II, while the inoculated leaves remained symptomless after inoculation with race I. Sakaguchi[20] reported that the resistance of 7 varieties of Rantai Emas group to races I and II was controlled by the resistance genes *Xa-1* and *Xa-2*, respectively. All the varieties belonging to Rantai Emas group showed small yellow lesions as resistance reaction to race II, while they remained symptomless after inoculation with race I. The resistance of the Rantai Emas group varieties to race I was very stable, and symptoms did not appear even at the seedling stage. Thus the resistance reaction of the Rantai Emas group varieties to race II characterized by small yellow lesions.

Ezuka et al.[4] performed an F_2 analysis with special emphasis on the resistance of the Wase Aikoku group varieties. They showed that the resistance of Wase Aikoku 3 to races I, II and III is governed by a single dominant gene designated by them as *Xa-w* (later redesignated as *Xa-3*). The variety Wase Aikoku 3 showed a browning type of resistance reaction to races I, II and III, while typical symptoms of yellowing or withering appeared after inoculation with compatible race IV.

The varieties Nagomasari, Java 14 and Kuntulan belonging to the same group also showed the same type of resistance reaction to races I, II and III. Resistance expression of the Wase Aikoku group varieties is unstable at the seedling stage, and they often show a susceptible reaction[3,5]. In such cases, browning seldom appeared, or appeared partially. Thus, the appearance of browning corresponded to the resistance expression in the varieties harboring *Xa-3*.

A series of genetic studies on the resistance of

Table 1. Infection types[a)] controlled by resistance genes *Xa-4*, *xa-5*, *Xa-6*, *Xa-7*, *xa-8*, *xa-9* and *Xa-10* in rice-*Xanthomonas campestris* pv. *oryzae* interaction

Variety	Resistance gene	Reaction[a)]					
		Race 1 (PXO61)	Race 2 (PXO86)	Race 3 (PXO79)	Race 4 (PXO71)	Race 5 (PXO111)	Race 6 (PXO99)
IR20	*Xa-4*	SL	TS	MR	MR	MR	TS
IR-BB 4	*Xa-4*	SL	TS	MR	TS	SL	TS
IR-BB 204	*Xa-4*	SL	TS	MR	TS	MR	TS
Semora Mangga	*Xa-4*[b]	BR	BR	BR	BR	BR	TS
IR1545-339	*xa-5*	SL	SL	SL	SL	SL	MR
BJ1	*xa-5*	SL	SL	SL	SL	SL	SL
IR-BB 5	*xa-5*	SL	SL	-	SL	SL	SL
IR-BB 205	*xa-5*	SL	SL	--~SL	SL	SL	SL
Zenith	*Xa-6*	BR	BR	BR	BR	BR	TS
DV85	*xa-5*, *Xa-7*	-	SL	SL	SL	SL	SL
IR-BB 7	*Xa-7*	TS	-	-	TS	-	TS
IR-BB 207	*Xa-7*	TS	-	-	TS	-	TS
PI231129	*xa-8*	SL	SL	SL	TS	SL	SL
IR-BB 8	*xa-8*	MR	MR	SL	TS	MR	MR
IR-BB 208	*xa-8*	MR	MR	SL	TS	MR	MR
Sateng	*xa-9*	MR	TS	MR	MR	MR	MR
Cas209	*Xa-10*	TS	-	TS	TS	TS	TS
IR-BB 10	*Xa-10*	TS	-	TS	TS	--~SL	TS
IR-BB 210	*Xa-10*	TS	-	TS	TS	--~SL	TS

a): -; Symptomless, BR; Browning, SL; Small yellow lesions (less than 3 cm in length), MR; Moderate reaction (3-6 cm in length), TS; Typical symptoms.

many indica varieties to bacterial leaf blight was conducted at IRRI in the Philippines[11,13,14,17,18]. At IRRI, the resistance genes *Xa-4*, *xa-5*, *Xa-6*, *Xa-7*, *xa-8*, *xa-9* and *Xa-10* were identified.

The reactions of the varieties and the near-isogenic lines carrying the genes to six races of the Philippines were observed and the results were summarized in Table 1.

Of the resistance genes identified, *Xa-4* was detected in many IRRI varieties. The resistance gene *Xa-4* is an incompletely dominant gene, and it controls the resistance to race 1 of the Philippines. The resistance gene *xa-5* is a recessive gene involved in the resistance of IR1545-284, RP291-7, BJ1, Kele and other varieties.

The varieties carrying *Xa-4* showed a resistance reaction characterized by small yellow lesions to the strains of race 1, while those with *xa-5* also showed such type of resistance reaction to all the six races. Small yellow lesions characterizing the resistance reaction controlled by *Xa-4* or *xa-5* were confirmed using near-isogenic lines carrying the two genes. In the near-isogenic lines with *xa-5*, however, the symptomless type of resistance reaction was observed to certain strains. Librojo et al.[11] reported that the resistance gene of Semore Mangga was located at the same locus as *Xa-4*, but the resistance was expressed only at the adult plant stage. Therefore, they designated the resistance gene of Semora Mangga as $Xa\text{-}4^b$. The reaction of the variety to the six races of the Philippines was observed after inoculation by the single-needle pricking method. The results showed that the reaction of Semora Mangga belonged to the browning type and was different from that controlled by *Xa-4*. In addition, the reaction pattern to the six races was also different between *Xa-4* and $Xa\text{-}4^b$. Similar results were obtained in the varieties carrying the resistance gene *Xa-6*. The gene is linked to *Xa-4* with a crossover value of 26%. The variety Zenith carrying *Xa-6* showed the browning type of resistance reaction to the five races. Similarly, the resistance reactions controlled by *Xa-7*, *xa-8*, *xa-9* and *Xa-10* which were observed using the near-isogenic lines, included the following types: symptomless, small yellow lesions, browning reaction and symptomless, respectively. At IRRI, however, Sateng showed a moderate reaction to most of the Philippine races.

The relationship between the resistance genes and the reactions controlled by them is summarized in Table 2.

Table 2. Known resistance genes and their relation to reaction to incompatible strains of *X. campestris* pv. *oryzae*

Resistance gene	Reaction	Representative varieties
Xa-1	Symptomless	Kogyoku, Java 14, IR20
Xa-2	Small yellow lesions	Rantai Emas 2, Te-tep
Xa-3	Browning	Wase Aikoku 3, Chugoku 45, Kuntulan, Java 14
Xa-4	Small yellow lesions	IR20, TKM6, IR22
$Xa\text{-}4^b$	Browning	Semora Mangga
xa-5	Small yellow lesions	IR1545-339, DZ192, BJ1
Xa-6	Browning	Zenith
Xa-7	Symptomless	DV85
xa-8	Small yellow lesions	PI231129
xa-9	Browning	Sateng
Xa-10	Symptomless	Cas209
Xa-12	Symptomless to small yellow lesions	Kogyoku, Java 14

Comparative expression of resistance

As described above, the resistance reaction varied depending on the resistance genes. Population studies and histological observations were conducted to clarify the biological aspect of resistance expression in rice to *X. campestris* pv. *oryzae*. For the purpose, resistance expression controlled by *Xa-1* and *Xa-3* were studied as models.

Resistance reaction controlled by *Xa-1* belonged to the symptomless type, though symptoms appeared at the seedling stage. Population trends of the two incompatible strains of race I, T7174 and Q6808, were investigated in leaf tissues of Kogyoku which is representative of Kogyoku group. Among the race I strains, T7174 and Q6808 were highly and weakly aggressive, respectively. The compatible strain T7147 of race II was also inoculated for comparison.

The results are illustrated in Fig. 1A. Immediately after inoculation, the number of bacterial cells recovered ranged from approximately 10^2 to 10^3 cfu per inoculated leaf, when a suspension of 5×10^8 cfu

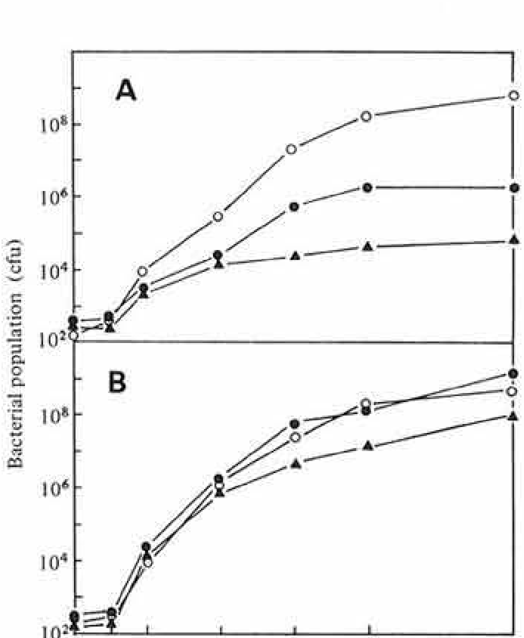

Fig. 1 Comparative population trends of two race I strains T7174 (●—●) and Q6808 (▲—▲), and a race II strain T7147 (○—○) of *X. campestris* pv. *oryzae* in the leaf tissues of rice varieties Kogyoku (A) and Kinmaze (B)

per m*l* was applied as inoculum. No appreciable changes in the population levels were observed 24 hr after inoculation. Two days after inoculation, the population level of the compatible strain T7147 was slightly higher than that of the incompatible strains T7174 and Q6808. Thereafter, the compatible strain T7147 multiplied at a logarithmic rate up to 8 days following the inoculation. Eight days after inoculation, the multiplication rate slowed down, but the strain still continued to multiply at a lower rate. Typical symptoms appeared 8 days after inoculation, when the population level of the strain exceeded 10^8 cfu per inoculated leaf. On the other hand, maximum population levels of the two incompatible strains T7174 and Q6808 were much lower than that of the compatible strain T7147. Significant differences in the multiplication rate between the compatible and incompatible strains became apparent around 4 days after inoculation. In addition, such differences were also observed between the highly aggressive T7174 and weakly aggressive Q6808. Multiplication of Q6808 ceased earlier than that of the former, and the final population level of the former was markedly lower than that of the latter.

A similar experiment was conducted using the variety Kinmaze, which lacks resistance genes to all known races in Japan. The results are presented in Fig. 1B. The growth patterns of the strains T7174 and T7147 were very similar to each other. They were almost the same as that of T7147 in the leaf tissues of Kogyoku. The multiplication rate of the weakly aggressive strain Q6808 in Kinmaze was much higher than that in incompatible Kogyoku, though it was somewhat lower than that of T7174 or T7147 in Kinmaze.

As mentioned above, the resistance reaction controlled by the resistance gene *Xa-3* was characterized by the browning reaction, while typical symptoms appeared after inoculation with compatible strains in the varieties carrying the gene. The population trends of the incompatible strains of races I, II and III were investigated in Chugoku 45 which was shown to carry *Xa-3* together with the compatible race IV strain. The strains used were T7174 (race I), T7147 (race II), T7133 (race III) and Xo-7435 (race IV). The growth patterns of the incompatible strains T7174, T7147 and T7133 were very similar to that of T7174 in the variety Kogyoku. On the other hand, population change of the compatible strain Xo-7435 was almost the same as that of the incompatible strain T7147 in Kogyoku. The multiplication rate of the incompatible three strains decreased at around 4 days after inoculation. In addition, the decrease corresponded to the development of browning.

Histopathological study was conducted to compare the distribution of the bacterium and the changes of the inoculated tissues between compatible and incompatible interactions using Kogyoku carrying *Xa-1* and Kuntulan carrying *Xa-3*. Histological observation showed that the translocation of the incompatible strains was very limited compared with that of the compatible ones (Plate 2-A, B, C). Even in incompatible combinations, however, the inoculated bacterial cells multiplied to some extent around the point of inoculation, irrespective of the resistance genes (Plate 2-B, C). In a compatible variety-race combination, the bacterial mass was abundant in the lumen of xylem vessels. Invasion of the bacterial cells into the parenchymatous tissues was not observed except that into the epithem below hydathodes

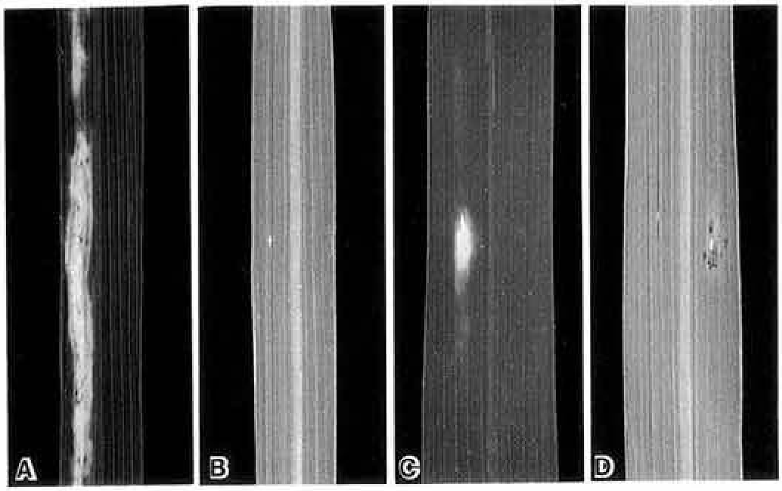

Plate 1.
Infection types in rice-*Xanthomonas campestris* pv. *oryzae* interaction
A: Susceptible reaction,
B: Symptomless reaction,
C: Small yellow lesion reaction,
D: Browning reaction.

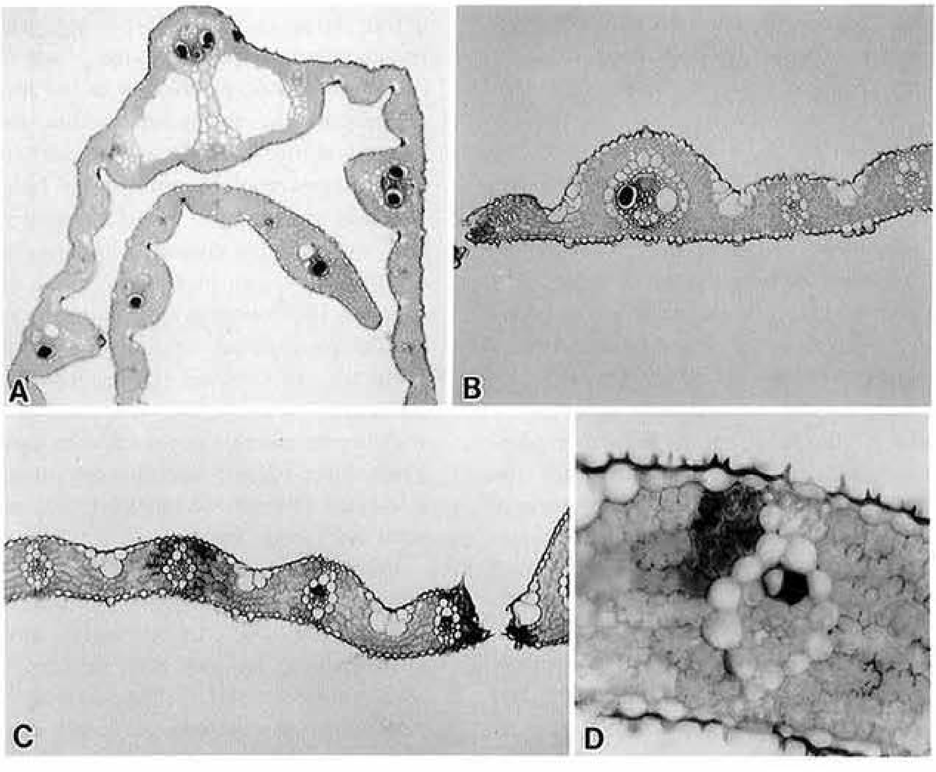

Plate 2. Histopathological characteristics of resistant and susceptible reactions in rice cultivars to *X. campestris* pv. *oryzae*
A: Transverse section of typical symptom in Kinmaze,
B: Transverse section of the leaf of Kogyoku harboring *Xa-1* showing symptomless type of reaction,
C: Transverse section of the leaf of Kuntulan harboring *Xa-3* showing browning type of resistance reaction,
D: Nectrotic cells in parenchymatous tissue surrounding vascular bundle in Kuntulan.

at the late stage of infection. In the resistant reaction of Kogyoku carrying *Xa-1*, no marked histological changes were observed except for the presence of bacterial cells and reaction materials in the xylem vessels near the point of inoculation (Plate 2-B). In the resistance reaction controlled by *Xa-3*, however, conspicuous changes were observed in the inoculated tissues (Plate 2-C, D). Browning was detected in the xylem vessels, xylem parenchyma, mestom sheath, vascular bundle sheath and surrounding parenchymatous tissues. Fine particles often appeared in the brown cells surrounding vascular bundles, and the protoplasm of the cells eventually became granular (Plate 2-D). The most important feature in this type of reaction was that the bacterial cells were confined to the brown necrotic areas. Various reaction materials were also observed in the xylem vessels after inoculation with the incompatible strain in the varieties carrying *Xa-3*.

Discussion

The resistance reactions to *X. campestris* pv. *oryzae* in the rice varieties were grouped into three types: symptomless, browning and small yellow lesions. In addition, the term "infection types" was used since such variation in the resistance reactions was related to the presence of resistance genes.

The infection types are in agreement with the genetic analysis of the resistance to the disease. Ogawa et al.[17,18] reported that the resistance genes *Xa-4*^{b}, *Xa-6* and *xa-9* are identical with the resistance gene *Xa-3*. In this study, the resistance reaction in the varieties with the genes was characterized by browning. Sateng which harbors *xa-9* showed a moderate reaction to all the races except for race 6 of the Philippines. Under the climatic conditions prevailing at IRRI in the Philippines, premature plants of the variety are inoculated, and the resistance expression of Sateng is incomplete. At Tropical Agriculture Research Center (TARC) in Japan, adult plants of the variety exhibited the browning reaction to the incompatible strains. In addition, the varieties showed the same reaction patterns as Chugoku 45 with *Xa-3*. These facts suggest that the resistance genes *Xa-4*^{b}, *Xa-6* and *xa-9* are identical with *Xa-3*. The infection types in the host-parasite interaction can be ascribed mainly to two factors: the degree of multiplication of the bacterium in the host tissues, and the ability of the host tissues to undergo necrosis. Based on population studies in the host-parasite interaction, the threshold point of typical symptom expression appeared to be 10^6 cfu per inoculated leaf. In the resistance reaction controlled by *Xa-1*, the maximum population level of bacterial growth was less than 10^6 cfu per inoculated leaf, while it was significantly higher than 10^6 cfu per inoculated leaf in the reactions controlled by *Xa-2*. Accordingly, the plants remained symptomless in the former, while small yellow lesions appeared in the latter. In the compatible variety-strain combination, the bacteria could multiply above the level of 10^8 cfu per inoculated leaf. The atypical symptom, browning, controlled by *Xa-3* was induced below the level of 10^5 cfu per inoculated leaf, and the population of incompatible strains reached a peak before the appearance of browning. Thus, each infection type corresponded to the growth of the strains of *X. campestris* pv. *oryzae* in the host tissues.

Based on the histological studies, the browning reaction controlled by *Xa-3* appears to be similar to the hypersensitivity occurring in fungal diseases. Bacterial leaf blight of rice is a typical vascular disease and vascular browning has been described as a typical symptom in many vascular diseases[1]. In contrast, the browning reaction in the rice-*X. campestris* pv. *oryzae* relationship is an atypical symptom. In addition, the incompatible strains of *X. campestris* pv. *oryzae* were confined to the zone with brown necrosis in the varieties carrying *Xa-3*. These facts suggest that the browning reaction is associated with the resistance mechanisms in the varieties harboring *Xa-3*.

Vascular browning results from the oxidation and polymerization of phenolics through the action of polyphenoloxidase. In rice leaves, however, polyphenoloxidase has not been detected. Therefore, comparative analysis of phenolics and the oxidation enzymes among varieties with each resistance gene is necessary to elucidate the resistance mechanisms. Reimers and Leach[19] found that the growth inhibition of incompatible strains of *X. campestris* pv. *oryzae* was correlated with the early accumulation of fluorescent compounds and host cell death. Molecular response of rice to *X. campestris* pv. *oryzae* should be analyzed in relation to such compounds together with various antibacterial compounds reported[7].

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