

イネ白葉枯病抵抗性遺伝子による抵抗性発現の差異(1)

Xa-1主働遺伝子に支配される抵抗性の発現

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Difference in Resistance Expression of Rice to *Xanthomonas campestris* pv. *oryzae* as Controlled by Resistance Genes 1. Resistance Expression Controlled by Resistance Gene *Xa-1*

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Abstract

Resistance expression controlled by resistance gene *Xa-1* was investigated in adult plants of Kogyoku group rice cultivars after inoculation with bacterial group I isolates of *X. campestris* pv. *oryzae*. Kogyoku group cultivars with resistance gene *Xa-1* showed symptomless type of resistance reaction to bacterial group I isolates irrespective of their aggressiveness. Periodical population study showed that there was distinct difference in growth patterns between compatible and incompatible isolates in the leaf tissues of cultivar Kogyoku. Compatible isolate T7147 of bacterial group II grew logarithmically and reached its maximum level above 10^8 cfu per inoculation site. In contrast, incompatible isolates T7174 and Q6808 of bacterial group I multiplied at a much lower rate than compatible T7147, and reached their maxima at less than 10^6 and 10^5 cfu per inoculation site, respectively. In Kogyoku, population levels of six isolates of bacterial group I 12 days after inoculation ranged approximately from 10^5 to 10^6 cfu per inoculation site. The degree of multiplication of isolates T7174 and Q6808 in nine Kogyoku group cultivars ranged approximately from 10^5 to 10^6 and 10^4 to 10^5 cfu per inoculation site, respectively, 12 days after inoculation.

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Key words : rice, *Xanthomonas campestris* pv. *oryzae*, resistance expression.

Introduction

Bacterial leaf blight caused by *Xanthomonas campestris* pv. *oryzae* is one of most serious diseases of rice in many rice-growing countries in Asia. The most effective control method has been use of resistant cultivars.

In Japanese differential system, rice cultivars have been classified into seven groups on the basis of their reactions to five pathogenic groups (races) of the causal bacterium^{3,12,21,22}. Genetical aspect of the resistance has also been studied, and four resistance genes *Xa-1*, *Xa-2*, *Xa-w* and *Xa-kg* have been reported in Japan^{4,13,16}.

We demonstrated that, in rice — *X. campestris* pv. *oryzae* interaction, resistance reactions ranged from symptomless to brown necrosis or small yellow lesion⁷. In addition, these types of resistance reactions appeared to be controlled by resistance genes mentioned above^{6-8,10}.

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The present investigation was designed to clarify the biological aspect of resistance expression controlled by resistance gene *Xa-1*, in special reference to the bacterial population changes and their relations to symptom expression.

Materials and Methods

Plants. Ten rice cultivars of Kogyoku group with resistance gene *Xa-1* used in this study were Kogyoku, Norin 27, Asakaze, Daiyoshi, Aka Shinriki, Tokai 12, Tokai 28, Zensho 26, Sigadagabo and Maratelli (A)S. Of these cultivars, Kogyoku was used as a representative of the cultivar group. Cultivar Kinmaze, which is susceptible to all known bacterial groups of *X. campestris* pv. *oryzae* in Japan, was applied for comparison. Plants were grown in 1/5,000 a plastic pots for approximately 120 days under greenhouse conditions.

Bacterial culture. Isolates of *X. campestris* pv. *oryzae* used were T7174, T7101, T7156, T7177, T7123 and Q6808 belonging to bacterial group I, and T7147, T7142 and H5809 of bacterial group II. They were cultured on Wakimoto's potato semi-synthetic agar slant at 25 C for 48 hr.

Inoculation. Bacterial suspension was prepared by washing bacterial cells from culture slant and diluting it with sterilized distilled water. The concentration was adjusted at approximately 5×10^8 colony-forming unit (cfu) per ml spectrophotometrically. Fully-opened flag or penultimate leaves were inoculated by the single-needle pricking method or the clipping inoculation technique.

Measurement of bacterial population in vivo. Number of the bacterial cells in leaf tissues inoculated by the single-needle pricking method was determined by dilution plate technique. Six leaf samples were collected and were homogenized in a mortar with 10 ml of sterilized distilled water. The resulting suspension was diluted with sterilized distilled water in a standard logarithmic series and appropriate dilutions were plated out on Suwa medium. Colonies were counted after 5 day incubation at 25 C, and the number of the bacterial cells in inoculated tissues was estimated from the data obtained.

Results

Macroscopic reactions of Kogyoku group cultivars to bacterial group I isolates of X. campestris pv. oryzae

Macroscopic reactions of nine Kogyoku group cultivars with resistance gene *Xa-1* were observed 21 days after inoculation with isolates T7174, T7177 and Q6808 bacterial group I, and T7147, T7142 and H5809 of bacterial group II by the clipping inoculation technique. All these cultivars with resistance gene *Xa-1* showed symptomless type of resistance reaction to the three isolates of bacterial group I irrespective of their aggressiveness (Table 1). No visible symptoms were observed except necrosis induced by clipping. In contrast, typical symptom of yellowing developed in these cultivars after inoculation with the compatible three isolates of bacterial group II. In Kinmaze without

Table 1. Reactions of nine Kogyoku group cultivars and cultivar Kinmaze to bacterial group I (left three) and bacterial group II (right three) isolates of *Xanthomonas campestris* pv. *oryzae*

Cultivar	Lesion length by each isolates ^{a)}					
	T7174	T7177	Q6808	T7147	T7142	H5809
Kogyoku	0	0	0	10.3	10.5	4.7
Norin 27	0	0	0	13.9	15.5	4.8
Asakaze	0	0	0	11.5	14.8	3.5
Tokai 12	0	0	0	12.4	11.1	3.9
Tokai 28	0	0	0	8.5	8.6	2.0
Aka Shinriki	0	0	0	11.1	12.6	5.3
Zensho 26	0	0	0	13.0	9.6	4.0
Sigadagabo	0	0	0	18.7	19.8	4.8
Maratelli (A) S	0	0	0	22.5	22.8	5.7
Kinmaze	19.6	6.8	4.2	17.2	15.8	6.6

a) Average lesion length (cm) of twenty inoculated leaves.

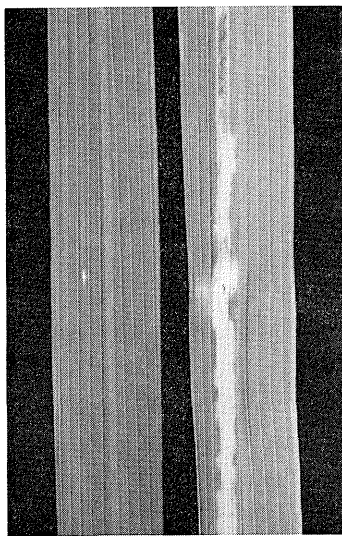


Fig. 1. Reactions of cultivar Kogyoku to isolate T7174 of bacterial group I (left) and isolate T7147 of bacterial group II (right) of *Xanthomonas campestris* pv. *oryzae* 16 days after inoculation by the single needle pricking method.

resistance gene *Xa-I*, such typical lesions appeared after inoculation with the isolates of bacterial group I as well as those of bacterial group II. In both cases, typical symptom started to develop around 8 days after inoculation and the sizes of the lesions varied among cultivar-isolate combinations.

Symptomless type of resistance reaction was confirmed in Kogyoku after inoculation with isolate T7174 by the single-needle pricking method (Fig. 1).

Population changes of bacterial group I isolates of *X. campestris* pv. *oryzae* in leaf tissues of cultivar Kogyoku

Population trends of two incompatible isolates T7174 and Q6808 of bacterial group I were investigated in leaf tissues of Kogyoku, and they were compared with population trend of compatible isolate T7147 of bacterial group II in the same cultivar. Among bacterial group I isolates, T7174 and Q6808 are highly and weakly aggressive ones, respectively, to compatible cultivar Kinmaze as shown in Table 1.

The results are illustrated in Fig. 2A. Moment after inoculation, bacterial cells recovered ranged from approximately 10^2 to 10^3 cfu per inoculation site, when suspension of 5×10^8 cfu per ml was applied as inoculum. No noted changes in populations were observed 24 hr after inoculation.

Two days after inoculation, population level of compatible T7147 was slightly higher than that of incompatible T7174 or Q6808. Thereafter, compatible T7147

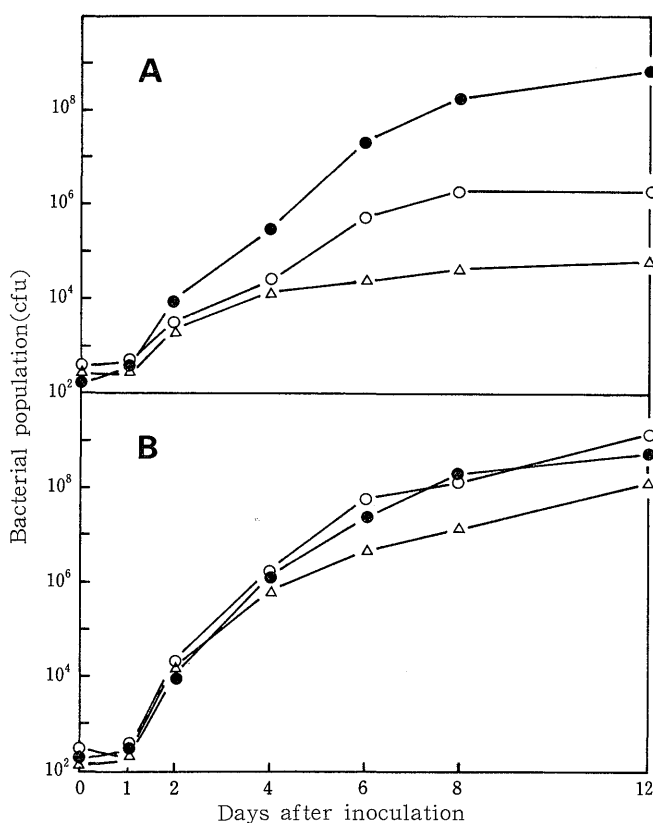


Fig. 2. Comparative population trends of two bacterial group I isolates T7174 (○-○) and Q6808 (△-△), and a bacterial group II isolate T7147 (●-●) of *Xanthomonas campestris* pv. *oryzae* in the leaf tissues of cultivars Kogyoku (A) and Kinmaze (B).

multiplied at a logarithmic rate up to 8 days after inoculation. After 8 days following inoculation, the multiplication rate of T7147 slowed down, but it still continued to multiply at a lower rate. Typical symptom appeared 8 days after inoculation, when population level of the isolate exceeded 10⁸ cfu per inoculation site. On the other hand, maximum population levels of incompatible isolates T7174 and Q6808 were much lower than that of compatible isolate T7147. The significant difference in multiplication rate between compatible and incompatible isolates became apparent around 4 days after inoculation. In addition, such difference was also observed between highly aggressive T7147 and weakly

aggressive Q6808 of incompatible bacterial group I. Multiplication of Q6808 ceased earlier than that of T7174, and the final population level of the former was much lower than that of the latter.

Similar experiment was made using cultivar Kinmaze, which lacks resistance to all known bacterial groups in Japan. The results are presented in Fig. 2B. The growth patterns of isolates T7174 and T7147 were very similar to each other and to that of the latter in the leaf tissue of Kogyoku. The multiplication rate of isolate Q6808 in the leaf tissue of Kinmaze was much higher than that in incompatible Kogyoku, though it was somewhat lower than that of T7174 or T7147 in Kinmaze.

Comparative population levels of bacterial group I isolates of *X. campestris* pv. *oryzae* in the leaf tissues of Kogyoku group cultivars with resistance gene *Xa-1*

As described above, maximum population levels of the incompatible isolates of bacterial group I were less than 10⁶ cfu per inoculation site of Kogyoku with gene *Xa-1*. In addition, there was much difference in population levels between highly aggres-

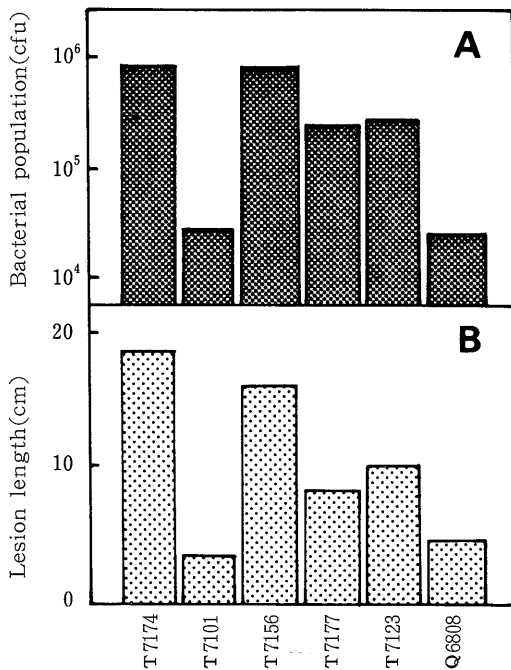


Fig. 3. Relationship between degree of multiplication of six isolates belonging to bacterial group I of *Xanthomonas campestris* pv. *oryzae* in cultivar Kogyoku and their aggressiveness expressed by lesion length. A: Populations of the six isolates in the leaf tissues of Kogyoku 12 days after inoculation, B: Lesion length on the leaves of cultivar Kinmaze inoculated with the six isolates 21 days after inoculation.

Table 2. Comparative populations of bacterial group I isolates T7174 and Q6808, and bacterial group II isolate T7147 of *Xanthomonas campestris* pv. *oryzae* in the leaf tissues of nine rice cultivars belonging to Kogyoku group

Cultivar	Isolate		
	T7174	Q6808	T7147
Kogyoku	5.6×10^5	5.4×10^4	3.2×10^8
Norin 27	9.2×10^5	5.2×10^4	3.8×10^8
Daiyoshi	1.2×10^5	2.5×10^4	6.6×10^8
Tokai 12	6.8×10^5	1.3×10^4	2.2×10^8
Tokai 28	1.2×10^5	9.0×10^3	8.3×10^7
Aka Shinriki	1.1×10^5	4.2×10^4	4.1×10^8
Zensho 26	1.4×10^5	4.6×10^4	6.2×10^8
Sigadagabo	1.8×10^5	4.8×10^4	5.8×10^8
Maratelli (A) S	2.3×10^5	1.2×10^4	2.2×10^8

sive and weakly aggressive isolates of bacterial group I in the cultivar.

To confirm the degree of multiplication of bacterial group I isolates under control of gene *Xa-1*, Kogyoku was inoculated with the six isolates of bacterial group I including T7174 and Q6808 as listed in Fig. 3 and their population levels were investigated 12 days after inoculation.

The results showed that the populations of the six isolates in the leaf tissues of Kogyoku ranged from 2.8×10^4 to 9.2×10^5 cfu per inoculation site (Fig. 3A).

Besides, cultivar Kinmaze was inoculated with the six isolates of bacterial group I to clarify the relationship between the degree of multiplication and their aggressiveness expressed by lesion length. Lesion length on the inoculated leaves recorded 21 days after inoculation was presented in Fig. 3B.

The degree of their multiplications was correspondent to their aggressiveness. Highly aggressive T7174 and T7156 reached level slightly lower than 10^6 cfu per inoculation site, while population levels of weakly aggressive Q6808 and T7101

were much lower than that of T7174 or T7156. Moderately aggressive T7177 and T7123 reached intermediate level between highly and weakly aggressive isolates.

Similar experiment was made using nine Kogyoku group cultivars-isolates T7174, Q6808 and T7147 combinations. The population level of each isolate was almost at the same level as that in Kogyoku; the population levels of T7174, Q6808 and T7147 were approximately 10^5 to 10^6 cfu, 10^4 to 10^5 cfu, and 10^8 to 10^9 cfu per inoculation site, respectively, 12 days after inoculation (Table 2). In Tokai 28, however, the population levels of the three isolates were significantly lower than those in other cultivars with gene *Xa-1*.

Discussion

In general, plants respond to incompatible phytopathogenic bacteria with hypersensitive reaction (HR)¹¹⁾. In rice cultivars with resistance gene *Xa-1*, however, such reaction was not observed after inoculation with incompatible isolates belonging to bacterial group I. Failure of the cultivars to show HR may partly be due to weak ability of *X. campestris* pv. *oryzae* to induce HR. In addition, resistance reaction with necrosis has not been reported in this host-parasite interaction except cultivars with resistance gene *Xa-w*⁷⁾.

Although adult plants of Kogyoku group cultivars do not show macroscopic symptoms to bacterial group I isolates, the multiplication of inoculated bacteria is not suppressed completely. The population study showed that even weakly aggressive isolate Q6808 could multiply in incompatible cultivar Kogyoku with *Xa-1* gene. The difference between compatible and incompatible isolates was degree of multiplication in host tissues, which is common to other plant-bacterium relationships^{1,2,14,15,17,18)}. Compatible isolates could multiply above the level of 10^8 cfu per inoculation site in host plants, and induce typical symptoms. In contrast, growth of incompatible isolates was suppressed to the level much less than that of compatible one, and inoculated plants remain symptomless. Lower population levels of the three isolates in Tokai 28 appears to be due to high level of quantitative resistance of the cultivar⁹⁾. Thus, development of typical symptoms and their sizes depend on the degree of multiplication of inoculated bacteria in host tissues.

The difference in multiplication rate between compatible and incompatible isolates became apparent from 2 days after inoculation in cultivar Kogyoku with resistance gene *Xa-1*. This suggests that incompatible isolates induce resistance reaction, and that resistance mechanisms start to operate at around 2 days after inoculation. At present, however, what factors play a decisive role in suppression of growth of incompatible isolates in the cultivars is not elucidated, though antibacterial substances or fibrillar material (FM) have been reported^{5,19,20)}.

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和 文 摘 要

加来久敏・木村俊彦：イネ白葉枯病抵抗性遺伝子による抵抗性発現の差異 1. *Xa-1* 主働遺伝子に支配される抵抗性の発現

Xa-1 主働遺伝子に支配されるイネ白葉枯病抵抗性の発現の様相を明らかにするため、同抵抗性遺伝子を有する黄玉群品種にイネ白葉枯病菌 I 群菌を接種し、病徴発現及び葉組織における I 群菌の増殖を調べた。*Xa-1* 主働遺伝子を有する黄玉群 9 品種の成稲は病原力の異なる I 群菌 3 菌株のいずれに対しても無病徴型の抵抗反応を示した。黄玉を代表品種として、単針接種により I 群菌の強病原力菌株 T7174 及び弱病原力菌株 Q6808 の増殖を経時的に調べたところ、両菌株とも接種後 2 日ころから増殖を始めたが、増殖率は親和性菌株に比べて低く、接種 12 日後においても 1 接種箇所当り 10^6 cfu 以下であった。また、黄玉に病原力の異なる I 群菌 6 菌株を単針接種し、接種 12 日後における増殖程度を比較した結果、いずれの菌株も 1 接種箇所当り 10^6 cfu 以下にとどまり、各菌株の増殖程度はそれらの病原力と並行関係にあるようであった。さらに、*Xa-1* 主働遺伝子を有する黄玉群 9 品種に I 群菌 T7174 及び Q6808 を同様に接種し、接種 12 日後の増殖程度を調べたところ、すべての供試品種において、T7174 は 10^5 から 10^6 cfu の範囲に、また Q6808 は 10^4 から 10^5 cfu の範囲にあった。以上のように、*Xa-1* 主働遺伝子に支配される抵抗性が十分に発現した場合、非親和性 I 群菌の増殖程度は典型的病徴の発現限界点 10^6 cfu 以下に抑制され、しかも同抵抗性遺伝子は宿主細胞の壊死を誘導しないため、無病徴型の抵抗反応となるものと推定された。