イネ品種Dewiratih,Sigadis及びTKM－6のイネ白葉枯病菌に対する反応

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Reactions of Rice Cultivars Dewiratih, Sigadis and TKM-6 to Bacterial Leaf Blight Caused by *Xanthomonas campestris* pv. *oryzae*

Hisatoshi KAKU and Toshihiko KIMURA

Contents

I Introduction

Bacterial leaf blight caused by *Xanthomonas campestris* pv. *oryzae* continues to be a serious problem in many rice-growing countries in Asia. Use of resistant rice cultivars has been one of the most important control methods against the disease because of lack of highly effective bacteriocides.

In Japanese differential system, rice cultivars have been classified into Kinmaze, Kogyoku, Rantai Emas, Wase Aikoku, Java, Elwee and Heen Dikwee groups, and isolates of the causal bacterium into pathogenic groups I, II, III, IV and V, on the basis of their differential interactions[1,23,26]. Of these cultivar groups, Wase Aikoku group is resistant to bacterial groups I, II and III, which are prevalent in Japan. Therefore, certain cultivars belonging to the group have been employed in some breeding programs as donor parents to introduce their resistance[21].

We demonstrated that resistance reaction of Wase Aikoku group cultivars was devided into two types; browning type and non-browning one. The latter type of reaction ranged from symptomless to limited yellow lesion. EZUKA et al[3] showed that the resistance of Wase Aikoku 3, which is representative of Wase Aikoku group, to bacterial groups I, II and III is

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controlled by a single dominant gene *Xa-w*. They also reported that three other cultivars belonging to Wase Aikoku group or Java group, Nagomasari, Java 14 and Kuntulan, carried *Xa-w* gene or another gene linked very closely with *Xa-w*. We revealed that all the cultivars with *Xa-w* gene showed browning reaction to incompatible pathogenic groups of *X. campestris* pv. *oryzae*[10,11]. In addition, Wase Aikoku group cultivars TKM-6 and Nakashin 120, whose resistance appeared to be controlled by resistance gene or genes different from *Xa-w*, did not show browning type of resistance reaction. In a previous paper, reaction pattern of a Wase Aikoku group cultivar Lead Rice showing non-browning type of resistance reaction was compared with that of Wase Aikoku 3 using representative isolates of bacterial groups I, II, III, IV and V[15]. The result showed that reactions of the former were MR, MR, MR, MR and MR, respectively, while those of the latter were R, R, R, S and S, respectively. Three other cultivars Dewiratih, Sigadis and TKM-6 were also proved to show non-browning type of resistance reaction[11]. Thus, cultivars of the same group might include various genotypes.

The study reported here was designed to investigate the reactions of the three cultivars Dewiratih, Sigadis and TKM-6 to Japanese and Indonesian isolates of *X. campestris* pv. *oryzae*. On the basis of the results, the nature of their resistance to bacterial leaf blight was discussed.

II Materials and Methods

Plant materials: Three rice cultivars Dewiratih, Sigadis and TKM-6 were used throughout this study. Additional cultivars, Lead Rice and a representative cultivar of Wase Aikoku group, Chugoku 45, were used for the comparison. Seeds were sown and grown in 1/5,000 a plastic pots under the greenhose conditions. Fertilizers were applied according to the normal standard.

Pathogen materials: The isolates of *X. campestris* pv. *oryzae* used in this study were T7174, T7156, T7177, Q6808, X-82 (bacterial group I), T7147, T7142, H5809, N5861-1$^+$, H8201 (bacterial group II), T7133, T7167, H8208, Q6803, Q6809 (bacterial group III), H75373, Xo-7330, Xo-7435, Xo-7439 (bacterial group IV) and Xo-7306 (bacterial group V). Of these twenty isolates, all were used for the inoculation of adult plants, thirteen were used for inoculation of 75-day-old plants, and fourteen for that of 40-day-old seedlings. Isolates Xo-7330, Xo-7435, Xo-7439 and Xo-7306 were isolated in Indonesia by Yamamoto et al[26]. The cultures of all isolates were subcultured from the stock cultures maintained in vacuum-dried ampoules.

Inoculation: Plants were inoculated 40, 75 and 140 days after sowing, respectively. The bacterial suspension used as inoculum was prepared by washing bacterial cells from culture slant and by diluting with sterilized distilled water. The concentration of the suspension was adjusted at approximately 5×10^8 cfu per ml using turbidimeter and it was checked by agar plate count. Uppermost full-opened leaves were inoculated by the clipping inoculation technique[16]. Inoculated plants were kept in the greenhouse and were observed periodically.

III Results

1 Reactions of adult plants

Typical symptom expression on adult plants of cultivars Dewiratih, Sigadis and TKM-6 was critically observed and compared with that on cultivar Chugoku 45 representative of Wase Aikoku group after inoculation with five bacterial isolates representative of bacterial groups I to V. As these cultivars were late in heading under normal conditions, they were inoculated when flag leaves of cultivar Dewiratih and TKM-6 were fully opened. The length of typical lesions on the twenty inoculated leaves was recorded three weeks after inoculation.

The results are presented in Fig. 1. As obviously seen in this figure, cultivars Dewiratih, Sigadis and TKM-6 showed reaction patterns different from that of cultivar Chugoku 45. This suggests that they are not or might not be included in Wase Aikoku group. These three cultivars showed stable resistance to T7174 (I) and Xo-7306 (V). In Dewiratih and Sigadis, small limited lesions appeared after inoculation with T7147 (II), T7133 (III) and H75373 (IV).

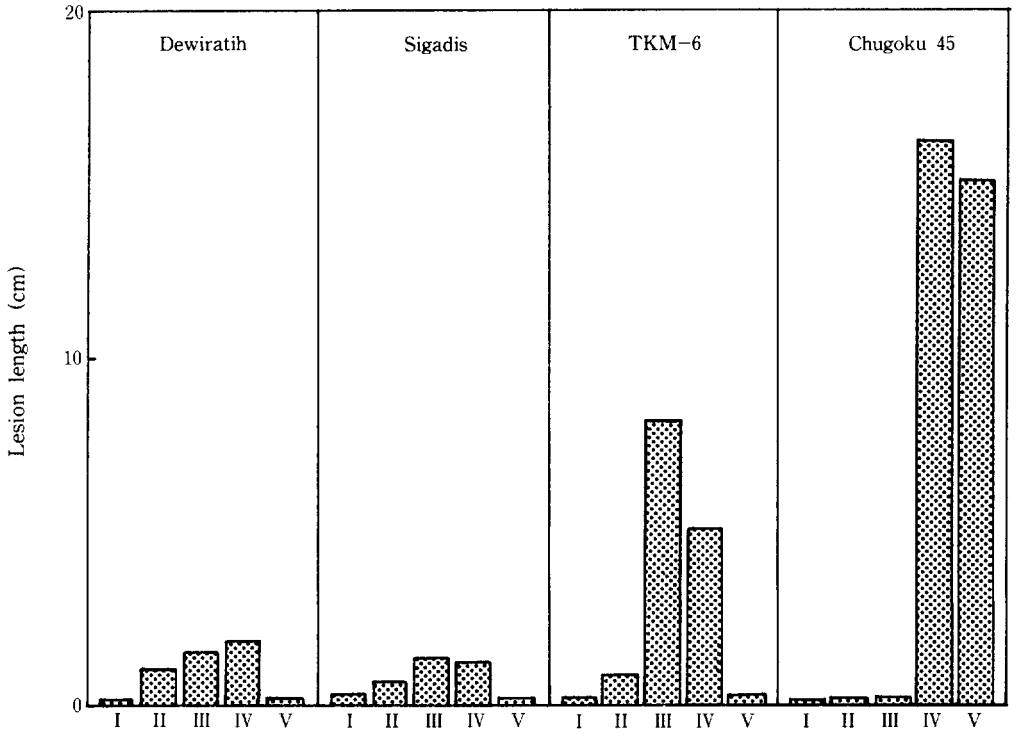

Fig. 1. Reactions of rice cultivars Dewiratih, Sigadis, TKM-6 and Chugoku 45 to representative isolates of bacterial groups I, II, III, IV and V of *Xanthomonas campestris* pv. *oryzae*.

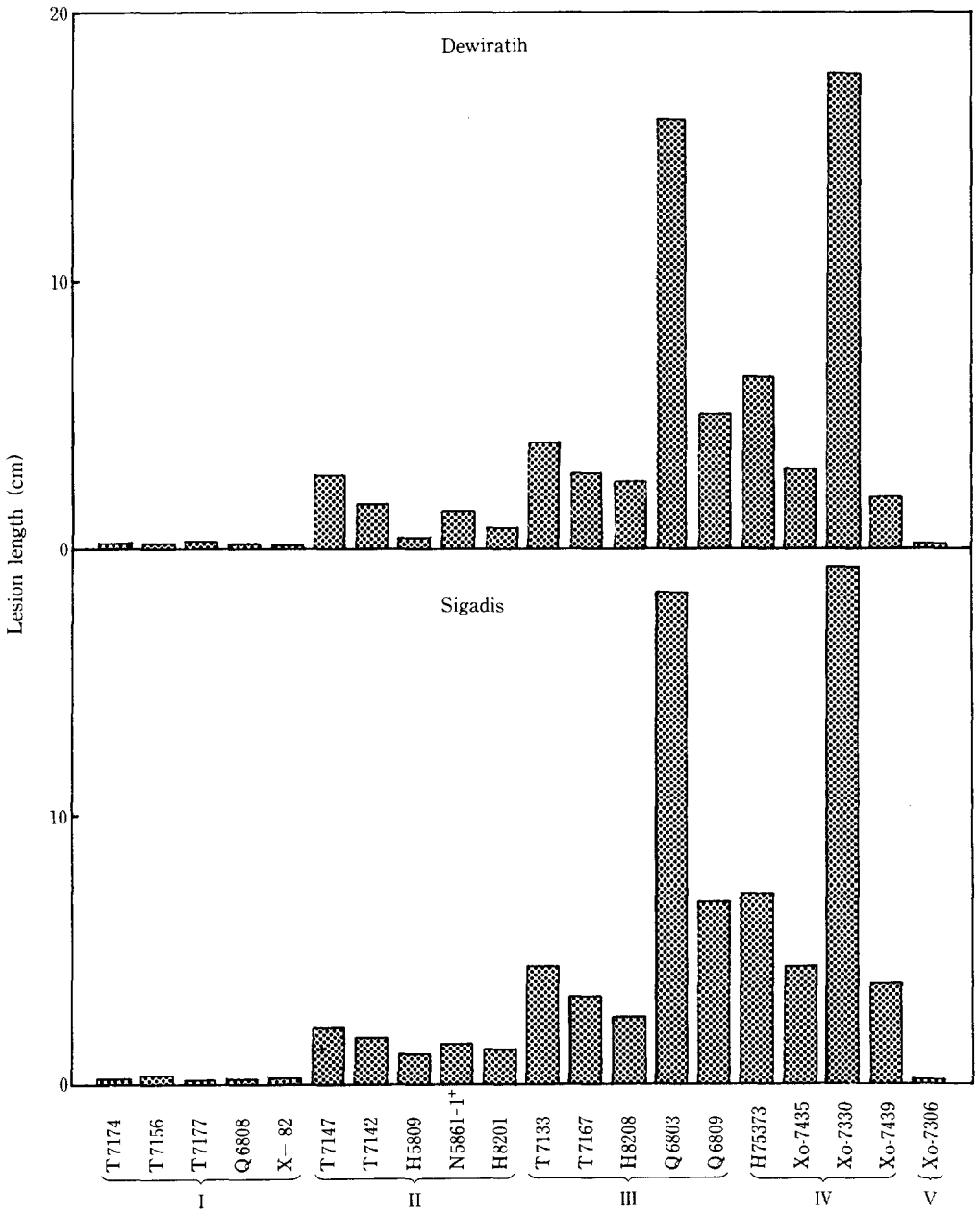

Fig. 2. Reactions of adult plants of rice cultivars Dewiratih and Sigadis to twenty isolates belongig to bacterial groups I, II, III, IV and V of *Xanthomonas campestris* pv. *oryzae*.

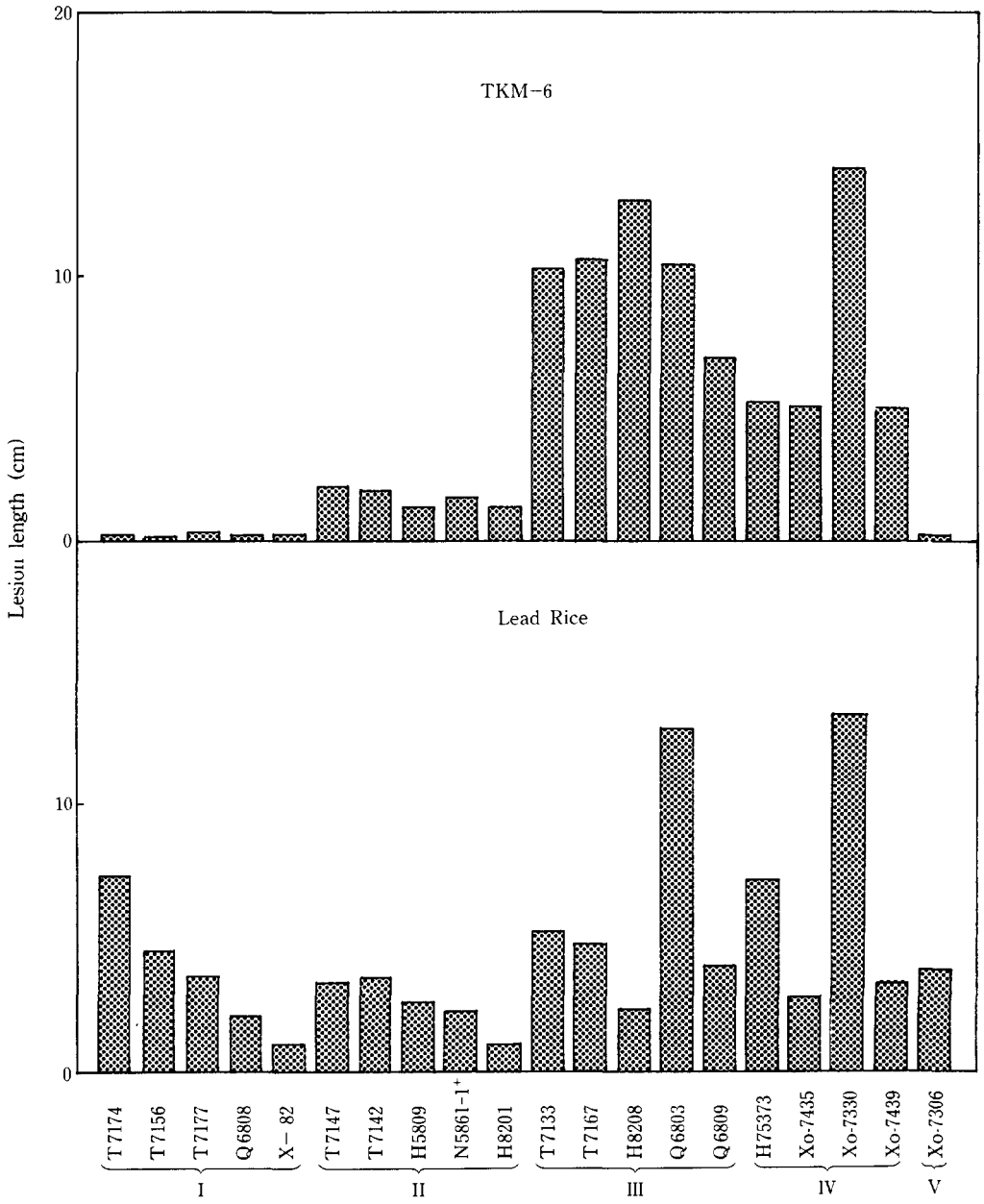

Fig. 3. Reactions of adult plants of rice cultivars TKM-6 and Lead Rice to twenty isolates of bacterial groups I, II, III, IV and V of *Xanthomonas campestris* pv. *oryzae*.

In TKM-6, similar reaction was observed after inoculation with T7147 (II), but somewhat larger lesions developed by inoculation with T7133 (III) and H75373 (IV). On the other hand, Chugoku 45 showed very stable resistance to T7174 (I), T7147 (II), T7133 (III), and did typical susceptible reaction to H75373 (IV) and Xo-7306 (V). In addition, brown necrosis appeared at the site of inoculation in Chugoku 45 after inoculation with incompatible isolates, while such reaction was not observed in Dewiratih, Sigadis and TKM-6.

Based on the reactions to the five representative isolates of bacterial groups I to V, resistance of Dewiratih, Sigadis and TKM-6 appeared to be qualitative to bacterial groups I and V, and to be quantitative to bacterial groups II, III and IV. Their reactions were therefore investigated further against twenty isolates of *X. campestris* pv. *oryzae*. Reaction of cultivar Lead Rice to the same twenty isolates was additionally observed and was compared with that of the three cultivars, since it showed similar reaction to bacterial group II, III or IV in our previous study[15].

The results are summarized in Fig. 2 and in Fig. 3. The outline of reaction patterns of Dewiratih and Sigadis was very similar to each other. They showed stable resistance to all the isolates belonging to bacterial groups I and V. No visible symptoms were observed except necrosis induced by clipping after inoculation with bacterial groups I and V. Against most of the isolates of bacterial groups II, III and IV, they showed MR reaction of Kauffman's standard for evaluation of resistance to bacterial leaf blight by the clipping inoculation technique. The size of typical yellow lesions ranged from 0.6 to 6.5 cm in length, depending on aggressiveness of isolates inoculated. Of the isolates tested, however, Q6803 and Xo-7330 induced large typical lesions on the leaves of the two cultivars. Isolate Xo-7330 was identified as bacterial group IV pathogenic to all five cultivar groups, and its aggressiveness is known to be high. On the other hand, isolate Q6803 has been known as a weakly aggressive isolate of bacterial group III pathogenic to Kinmaze, Kogyoku and Rantai Emas groups. This suggests that the aggressiveness of the latter isolate is not weak by nature, but its pathogenicity is different from that of bacterial group III.

Reaction pattern of TKM-6 was similar to that of Dewiratih or Sigadis in the response to isolates belonging to bacterial groups I, II, IV and V. In the response to bacterial group III isolates, however, fairly large lesions were observed three weeks after inoculation. All the isolates belonging to bacterial group III induced lesions around 10 cm in length. The lesions further developed thereafter.

In Lead Rice, which had shown MR reaction to five representative isolates of bacterial groups I to V, the outline of the reaction pattern to the twenty isolates was different from that in Dewiratih or Sigadis. It showed MR reaction to bacterial groups I and V as well as to bacterial groups II, III and IV. In other words, reaction pattern of Lead Rice appeared to be that lacking the stable resistance of Dewiratih and Sigadis to bacterial groups I and V.

2 Reactions of 75-day-old plants

It has been reported that the resistance in rice plants to bacterial leaf blight is affected by growth stage[2,4,5,6,18,19]. Usually rice plants become more resistant to the disease as they get

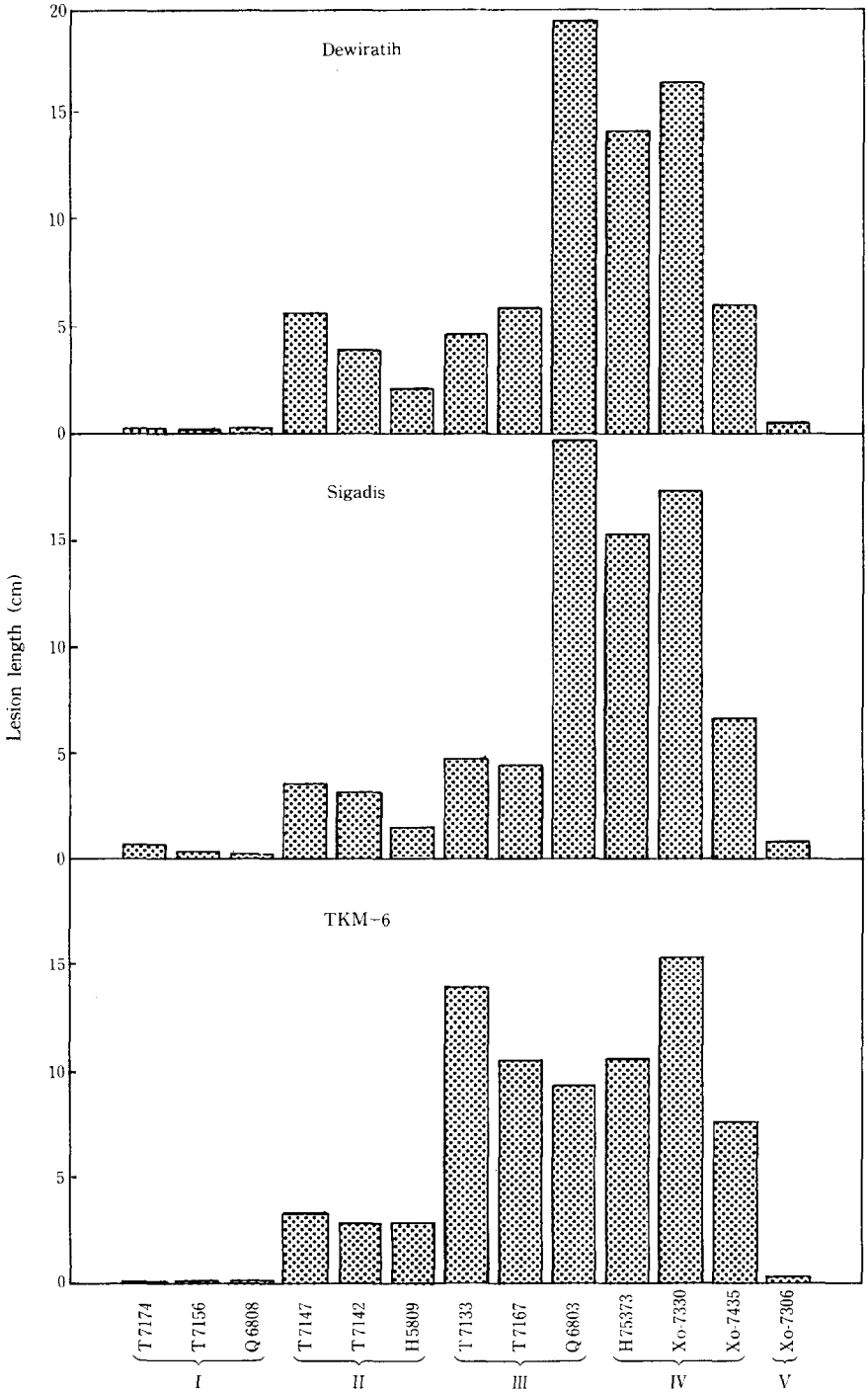

Fig. 4. Reactions of 75-day-old plants of rice cultivars Dewiratih, Sigadis and TKM-6 to thirteen isolates belonging to bacterial groups I, II, III, IV and V of *Xanthomonas campestris* pv. *oryzae*.

older. Reactions of younger plants of cultivars Dewiratih, Sigadis and TKM-6 were therefore observed after inoculation with thirteen isolates of *X. campestris* pv. *oryzae*, and were compared with those in adult plants. Plants were inoculated 75 days after sowing, and the lesion length was recorded 16 days after inoculation.

The results are presented in Fig. 4. In the same way as in adult plants, cultivars Dewiratih and Sigadis showed very similar reaction patterns to each other, and cultivar TKM-6 did a little different reaction pattern from them.

As expected, lesion size at this stage was generally larger than that in adult plants, though the period after inoculation was shorter. These three cultivars showed stable resistance to bacterial groups I and V just like that in adult plants. No macroscopic typical lesions appeared on them after inoculation with such isolates except necrosis induced by clipping.

Dewiratih and Sigadis showed MR reaction to isolates belonging to bacterial group II. They showed the same reaction to isolates T7133 and T7167 of bacterial group III and Xo-7435 of bacterial group IV. On the other hand, isolates Q6803 of bacterial group III and, H75373 and Xo-7330 of bacterial group IV, induced large typical lesions on these cultivars. Thus,the outline of reactions of these cultivars to *X. campestris* pv. *oryzae* was similar between 75-day-old plants and adult ones.

Another cultivar TKM-6 also showed stable resistance to bacterial group I and V. It showed MR reaction to all isolates of bacterial group II and isolate Xo-7435 of bacterial group IV. By inoculation with all three isolates of bacterial group III, large typical lesions appeared. Isolates of bacterial group IV, H75373 and Xo-7330, also induced the same type of reaction. TKM-6 appears to be more compatible with bacterial group III isolates than Dewiratih and Sigadis.

3 Reactions of 40-day-old seedlings

Resistance of rice seedlings to bacterial leaf blight has been reported using various rice cultivar-bacterial isolate combinations[2,4,5,6,18,19]. In general, resistance expression in rice seedlings is not so stable as that in adult plants, especially in Wase Aikoku group cultivars carrying resistance gene *Xa-w*. In this experiment, stability of resistance in cultivars Dewiratih, Sigadis and TKM-6 to fourteen isolates of *X. campestris* pv. *oryzae* was investigated and was compared with that in adult plants.

The results showed that the outline of reaction patterns in seedlings of the cultivars was very similar to that of 75-day-old plants (Fig. 5). Dewiratih and Sigadis showed similar reaction patterns to each other against thirteen isolates tested. In the two cultivars, isolates Q6803 and H75373 induced larger lesions than the other isolates used here. Their resistance expression to bacterial groups I and V was stable even in seedlings, while they showed intermediate reaction to other isolates.

Reaction pattern of TKM-6 was similar to that of Dewirath or Sigadis except that to bacterial group III isolates.

The length of the lesions in seedlings of the three cultivars was shorter than that in 75-day-old plants and adult ones. This was due to the shorter period after inoculation

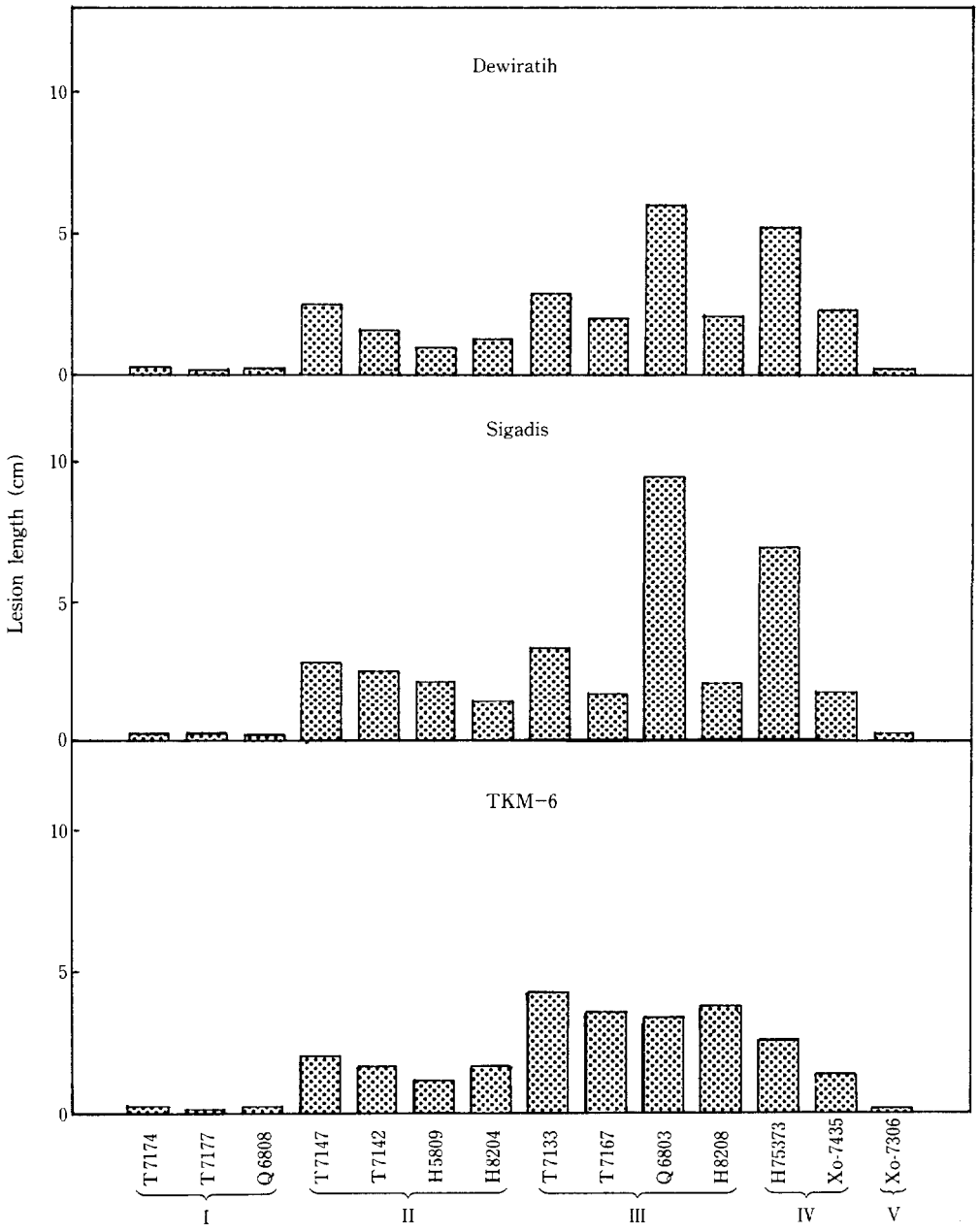

Fig. 5. Reactions of 40-day-old seedlings of rice cultivars Dewiratih, Sigadis and TKM-6 to fourteen isolates belonging to bacterial groups I, II, III, IV and V of *Xanthomonas campestris* pv. *oryzae*.

(14 days) and to relatively low temparature that prevailed during the period of experiment.

IV Discussion

Rice cultivars have been classified into seven groups in Japan on the basis of their resistance to differential isolates of *X. campestris* pv. *oryzae*. The reaction patterns of three cultivars Dewiratih, Sigadis and TKM-6 were R, MR, MR, MR and R, respectively, to bacterial groups I, II, III, IV and V. This indicates that their reaction patterns are different from those in Wase Aikoku group cultivars carrying resistance gene *Xa-w*, as that of cultivar Lead Rice reported in a previous paper[15]. Their reaction patterns appear to be similar to those of derivatives of Lead Rice. Their resistance to bacterial groups I and V assumed to be qualitative, and to be quantitative to bacterial groups II, III and IV, though the size of lesions induced by bacterial group III was fairly large in TKM-6.

Further observation of their reactions to twenty isolates of *X. campestris* pv. *oryzae* showed that they have a wide spectrum of resistance including MR reaction by KAUFFMAN's standard. Their resistance was very clear-cut to bacterial groups I and V, and limited typical lesions appeared by inoculation with bacterial groups II, III and IV. In addition, Dewiratih and Sigadis showed typical susceptible reaction to isolates Q6803 and Xo-7330. The latter belongs to bacterial group IV, and its aggressiveness is known to be high[26]. Q6803, however, has been known to be a weakly aggressive isolate of bacterial group III[14]. The result obtained in this study indicates that its aggressiveness is not weak, but its pathogenicity is specific.

We have demonstrated that, in rice plant-*X. campestris* pv. *oryzae* interaction, there are various types of resistance reactions ranging from symptomless to browning or limited yellow lesion[11]. These different types of resistance reactions are controlled by different resistance genes[10, 11, 12, 13]. Our critical observation showed that all types of resistance reactions were included in cultivars which had been classified into Wase Aikoku group. It is of great interest that all the cultivars whose resistance was revealed to be conferred by a single dominant gene *Xa-w* showed browning reaction to incompatible isolates of *X. campestris* pv. *oryzae*. On the other hand, Dewiratih, Sigadis and TKM-6, which had been classified into Wase Aikoku or Java group, showed reaction ranging from symptomless to intermediate typical lesion. According to the result obtained here, stable resistance of the three cultivars to bacterial groups I and V suggests that they might be conferred by resistance gene *Xa-1* and *Xa-kg*, respectively.

At IRRI, a number of genetic studies have been conducted on the resistance of many *indica* cultivars to Philippine isolates of *X. campestris* pv. *oryzae*[17, 20]. Of the three cultivars used in this study, Sigadis and TKM-6 were revealed to carry an incompletely dominant gene *Xa4*. Cultivars IR20, IR22, IR26, IR28, IR29, IR30 and IR32 developed at IRRI were also shown to carry resistance gene *Xa4*. HORINO and YAMADA[6] tested their reactions to five pathogenic groups of *X. campestris* pv. *oryzae* existing in Japan, and showed that their resistance to bacterial groups I and V was stable, while small to intermediate lesions appeared after inoculation with isolates belonging to bacterial groups II, III and IV. Their reaction patterns

were very similar to those observed in Dewiratih, Sigadis and TKM-6 in this study. HORINO et al[8] compared Japanese and Philippine differential systems for distinguishing pathogenic groups of *X. campestris* pv. *oryzae*. Up to the present, however, resistance reaction type controlled by resistance gene *Xa4* has not been described, though cultivars carrying the gene appear to a wide spectrum of resistance including moderate resistance to *X. campestris* pv. *oryzae*.

Genetic studies were also conducted on the resistance of cultivars including Lead Rice, Sigadis and TKM-6 in Sri Lanka by WATANABE[22]. According to his results, in ten cross combinations, all the F_1 plants showed mid-parent values of resistance and F_2 plants showed segregation that gave a normal curve with a peak about midway between both plants. He considered that the resistance in those cultivars was governed by minor genes. Genetic studies of resistance in IR26 and IR28 to Japanese bacterial groups have been conducted in Japan[7,9,24,25]. As a result, their resistance to bacterial groups I and V was qualitative and that to bacterial groups II, III and IV was quantitative. Thus, genetic nature above mentioned appears to be coincident with reaction patterns to *X. campestris* pv. *oryzae* in cultivars Dewiratih, Sigadis and TKM-6 as well as IR26-type cultivars.

For the moment, it can only be said that three cultivars Dewiratih, Sigadis and TKM-6 have a broad spectrum of resistance including moderate reaction to *X. campestris* pv. *oryzae*, which is qualitative to some pathogenic groups and quantitative to others.

Acknowledgement

We are grateful to Mr. T. Yamamoto and Mr. T. Noda for their kind supply of the bacterial isolates.

V Summary

Rice cultivars Dewiratih, Sigadis and TKM-6 were investigated for their reactions to Japanese and Indonesian isolates of *Xanthomonas campestris* pv. *oryzae* using clipping inoculation technique.

Their reactions to the isolates representative of bacterial groups I, II, III, IV and V were R, MR, MR, MR and R, respectively, according to the standard by KAUFFMAN et al. They were quite different from reaction pattern of a representative cultivar of Wase Aikoku group, Chugoku 45, whose reactions to bacterial group, I, II, III, IV and V were R, R, R, S and S, respectively.

Reaction patterns in adult plants of Dewiratih, Sigadis and TKM-6 were investigated using twenty bacterial isolates from Japan and Indonesia. To the isolates tested, Dewiratih and Sigadis showed similar reaction patterns to each other. They were highly resistant to isolates belonging to bacterial groups I and V, while they showed moderate resistance to other isolates belonging to bacterial groups II, III and IV except isolates Q6803 and Xo-7330. Isolates Q6803 and Xo-7330 of bacterial groups III and IV, respectively, induced large typical lesions

on the inoculated leaves of the two cultivars. Cultivar TKM-6 showed a reaction pattern slightly different from that in Dewiratih or Sigadis in the response to isolates belonging to bacterial group III. All isolates of the group induced fairly large lesions in TKM-6.

Reaction patterns in 75-day-old plants and 40-day-old seedlings of the three cultivars were almost similar to those in adult plants. In 75-day-old plants, however, the sizes of the lesions were generally larger than those in adult plants. At this stage, reactions in the three cultivars to H75373 of bacterial group IV were MS, while they were MR in adult plants. Reactions of TKM-6 to bacterial group III isolates were also evaluated as MS. In 40-day-old seedlings, relative length to that of inoculated leaves of the three cultivars was larger than that in adult plants. All three cultivars showed high level of resistance to bacterial group I isolates in 40-day-old seedlings as well as 75-day-old plants.

On the basis of the results, three cultivars Dewiratih, Sigadis and TKM-6 showed qualitative resistance to bacterial group I and V, and did high level of quantitative resistance to most isolates belonging to bacterial groups II, III and IV.

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イネ品種 Dewiratih, Sigadis 及び TKM-6 の イネ白葉枯病菌に対する反応

加来久敏・木村俊彦

和文摘要

イネ白葉枯病に対し高度抵抗性を有するとされているイネ品種 Dewiratih, Sigadis 及び TKM-6 の成稲のイネ白葉枯病菌Ⅰ～Ⅴ群菌に対する反応について検討した.

これら3品種にⅠ～Ⅴ群菌の代表菌株を剪葉接種し，接種21日後に反応を観察した結果，これらの品種はⅠ及びⅤ群菌に対して安定した高度抵抗性を示した．一方，Ⅱ，Ⅲ及びⅣ群菌の接種によっては，小型ないし中型の病斑が現われ，特に TKM-6 においてはⅢ及びⅣ群菌の接種により，他の2品種より明らかに伸展した病斑を生じた．これらの品種のⅠ，Ⅱ，Ⅲ，Ⅳ及びⅤ群菌に対する反応は Kauffman らの基準によれば，それぞれ R, MR, MR, MR, R であり，早稲愛国群の代表的品種である中国45号の反応，R, R, R, S, S とは明らかに異なっていた.

次に，日本産及びインドネシア産のイネ白葉枯病菌Ⅰ～Ⅴ群菌，計20菌株を供試し，これらの菌株に対する3品種 Dewiratih, Sigadis 及び TKM-6 の反応を観察した．その結果，Dewiratih と Sigadis は供試20菌株に対し，互によく似た反応型を示した．すなわち，これらはⅠ及びⅤ群に属する菌株に対しては高度の抵抗性を示したが，その他の菌株の接種ではⅢ群菌 Q6803 及び Ⅳ群菌 Xo-7330 を除き，小型の病斑を生じた．2菌株 Q6803 及び Xo-7330 の接種によっては，大型の病斑が生じた．一方，TKM-6 はⅠ及びⅤ群菌に対しては，前2品種と同様，高度の抵抗性を示した．また，Ⅱ及びⅣ群菌に対しては Dewiratih 及び Sigadis と同様な反応を示した．しかしながら，Ⅲ群菌の接種では，Dewiratih 及び Sigadis とは異なり，4菌株いずれの接種によっても 10 cm 前後のかなり大型の病斑が生じた.

Dewiratih, Sigadis 及び TKM-6 の播種75日後の生育段階における日本産及びインドネシア産のイネ白葉枯病菌13菌株に対する反応を調査した結果，いずれの品種も成稲と同様な反応を示した．ただし，病斑長は接種より調査までの日数が少ないにもかかわらず，成稲時より大型となる傾向がみられ，他の報告と同様，本病抵抗性は生育段階が進むにつれて，増高することが認められた．特に，TKM-6 ではⅢ群菌3菌株の接種により 10 cm を越す大型の病斑が生じ，この生育段階では MS（Kauffman らの基準）と判定された．また，いずれの品種においても，Ⅳ群菌 H75373 に対する感受性が高まり，それらの反応も同様に MS と判定された.

また，播種後40日の幼苗についても日本産及びインドネシア産14菌株を接種し，同様な試験を行った．その結果，3品種いずれの反応型も成稲と同様であった．これら3品種のⅠ及びⅤ群菌に対する抵抗性は幼苗においても非常に安定していた.

以上の結果から，3品種 Dewiratih, Sigadis 及び TKM-6 はⅠ及びⅤ群菌に対しては質的抵抗性を，Ⅱ，Ⅲ及びⅣ群菌に対しては一部の菌株を除き，強い量的抵抗性を有すると考えられた.