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Genetics of Resistance in Rice Cultivar Sateng to Philippine and Japanese Races of Bacterial Blight Pathogen

Tsugufumi OGAWA[1, 3], Tsuyoshi YAMAMOTO[1], Gurdev S. KHUSH[2] and Twng-Wah MEW[2]

[1]*Tropical Agriculture Research Center,* 1-2 Owashi, Tsukuba, Ibaraki, 305
[2]*International Rice Research Institute,* P.O.Box 933, Manila, Philippines

Rice cultivar Sateng was analyzed for resistance to bacterial blight of rice (BB) caused by *Xanthomonas campestris* pv. *oryzae,* using Japanese and Philippine BB races. Sateng had been reported to have a recessive gene, *xa-9.* However, it showed reactions similar to those of Chugoku 45, Java 14, and Zenith against Japanese and Philippine races in our preliminary experiment. Therefore, Sateng was subjected to an allele test with the above cultivars which have *Xa-3* gene, using Japanese and Philippine races. The reaction of the F_2 population from the cross Toyonishiki/Sateng to Japanese race IIIA showed that Sateng has one dominant gene. All F_2 plants of Chugoku 45/Sateng and Sateng/Zenith were resistant with browning around the lesions. The reaction of F_1 and F_2 populations from the cross of IR24/Sateng and Toyonishiki/Sateng also showed that Sateng has one dominant gene for resistance to Philippine races 1 to 4. Lesion length of the F_2 plants was somewhat variable due to segregation of minor genes. These results indicated that Sateng has a dominant gene which is allelic to *Xa-3.* Analyis of the F_3 progenies from the cross Java 14/Sateng using Philippine races confirmed these results.

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

Introduction

In order to establish an international set of rice varietal differential for identifying races of bacterial blight (BB) caused by *Xanthomonas campestris* pv. *oryzae,* we are genetically reanalyzing various cultivars used as differentials in Japan and at IRRI (International Rice Research Institute) as well as resistant cultivars which are not used as differentials.

SINGH *et al.* (1983) reported that rice cultivars Khao Lay Nhay and Sateng have one recessive gene allelic to each other but which is non-allelic to and independent of *Xa-3, Xa-4, xa-5, Xa-7,* and *xa-8.* They designated this gene as *xa-9.* We initiated crossing work with resistant cultivars having different resistance genes on the one hand and susceptible cultivars on the other. The F_1 hybrids of Toyonishiki/Sateng and IR 24/Sateng showed higher level of resistance to Philippine races than those of the susceptible cultivars Toyonishiki and IR 24. Furthermore, Sateng showed resistance to Japanese races IA to IIIB and Philippine races 1 to 4 with browning around the lesion (OGAWA and YAMAMOTO 1987b). The reaction was very similar to those of cultivars carrying *Xa-3.* Thus, we re-analyzed the resistance of Sateng to BB, using Japanese and Philippine races.

This study is also part of a collaborative research on BB resistance between IRRI and MAFF (Ministry of Agriculture, Forestry and Fisheries), Japan.

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3)Present address: *National Agriculture Research Center,* 3-1-1 Kannondai, Tsukuba, Ibaraki, 305

Mateirals and Methods

The rice cultivar Sateng was analyzed the resistance to BB, using Japanese and Philippine races. The seeds used in this experiment came from IRGC (International Rice Germplasm Center), IRRI. In a preliminary experiment, Sateng was tested for uniformity in plant type, heading date, and resistance to four Philippine races. A suitable plant was selected and then multipled for this study. The seeds of IR24 and Toyonishiki which are susceptible to Japanese and Philippine races were obtained from the National Agriculture Research Center, Japan. The seed sources of cultivars with *Xa-3,* e.g. Chugoku 45, Java 14, Zenith, and Cempo Selak which were used as materials for the allele tests with Sateng are described in the companion paper of this series (OGAWA *et al.* 1990a, b). The crosses were made at IRRI during 1982 and 1983 by the use of vacuum emasculator (JENNINGS *et al.* 1979).

Hybrid progenies from the crosses IR24/Sateng, Toyonishiki/Sateng, Chugoku 45/Sateng, Java 14/Sateng, Sateng/Zenith and Cempo Selak/Sateng were analyzed, using four Philippine races at IRRI. On the Other hand, hybrids Toyonishiki/Sateng, Chugoku 45/Sateng and Sateng/Zenith were analyzed using Japanese race IIIA (T7133) at TARC (Tropical Agriculture Research Center), Japan. Other experimental procedures followed were based on the standard methods of the previous work (OGAWA *et al.* 1990a).

Results and Discussion

The plant type of the tested lines, except one showed only little variation while heading date varied from line to line. Lesion length upon inoculation with BB races was slightly longer in every line than those observed in Zenith and Cempo Selak, reported by OGAWA *et al.* (1990b) (Table 1). We selected the 8th line, which showed the shortest lesion when inoculated with four Philippine races, for further analysis.

Table 1. Date of heading and lesion length in 10 lines of Sateng when inoculated with four Philippine races of BB pathogen. IRRI, 1983

Line no.	Heading date	Philippine race 1	2	3	4
1	7. 3	0.7-5.6-11.0	2.0-6.7-15.0	0.2-1.8-10.2	2.2-5.6-11.6
2	7.16	2.0-8.8-16.0	2.0-7.4-12.0	0.2-2.4-16.0	4.3-7.5-12.5
3	7.10	1.2-6.5-13.0	1.9-7.4-17.5	0.2-2.7- 7.1	3.4-9.9-14.5
4	7.10	2.5-5.7-11.5	2.5-4.8-10.0	0.1-2.1-10.0	2.5-4.2- 6.5
5	7. 9	0.7-4.5-13.0	1.5-5.1- 8.8	0.2-1.6-10.0	0.2-4.5- 9.5
6	7. 9	0.2-3.5- 7.7	0.5-2.9- 8.0	0.1-1.2- 6.0	1.5-3.6- 6.5
7	7.12	1.2-4.2- 6.8	1.2-3.3- 7.0	0.2-0.9- 4.0	0.5-3.9- 8.7
8	7.10	0.6-2.5- 5.5	0.2-1.3- 5.0	0.1-0.5- 3.5	0.1-1.9- 5.0
9	7.12	0.8-3.9- 7.0	0.2-2.4- 8.0	0.1-0.8- 5.0	0.7-2.4- 5.2
10	7.12	(segregated)			

Heading date: the date when 50% plants of each line headed.
Score is (minimum-average-maximum) lesion length (cm) at 14 days after inoculation.

1) Analysis of resistance to BB in Sateng using a Japanese race

The F_2 population of Toyonishiki/Sateng showed clear differences between resistant and susceptible plants when inoculated with the Japanese race IIIA at TARC (Fig. 1). The lesion development in resistant plants stopped at around 21 days after inoculation (DAI) and the lesion length was less than 8 cm at 18 DAI. There was a browning reaction around the lesion. The lesions on susceptible plants continued to developed beyond 18 DAI, lesion length was more than 6 cm long at 18 DAI. A few plants which had similar lesion lengths at 18 DAI. A few plants which had similar lesion lengths at 18 DAI (Fig. 1) could be distinguished at later stages as the lesion length development continued in susceptible plants after length measurement. As a result, the segregation ratio in the F_2 population of Toyonishiki/Sateng showed a good fit of 3R (resistant) : 1S (susceptible) (299R : 98S plants, $x^2 = 0.021$, p : 0.8-0.9).

The F_2 plants of Chugoku 45/Sateng showed lesions less than 6 cm in length (Fig. 1) and all the plants (356) had browning around the lesion. As reported by OGAWA *et al.* (1990a), resistant plants of hybrids carrying the *Xa-3* gene usually showed lesions less than 7 cm in length when inoculated with the Japanese race at TARC. Therefore, all F_2 plants were considered resistant on the basis of range of lesion length as well as visual observations. We have already reported (OGAWA *et al.* 1990a) that Chugoku 45 has *Xa-3* originally identified in Wase Aikoku 3 by EZUKA *et al.* (1975). These results showed that the resistance genes of Sateng, Wase Aikoku 3 and Chugoku 45 are alellic.

Similarly, almost of F_2 plants from the cross Sateng/Zenith also had lesions which were less than 6 cm long after inoculation with the Japanese race IIIA and showed browning around the lesions. A few F_2 plants showed 6 to 8 cm long lesions (Fig. 1), but the lesion

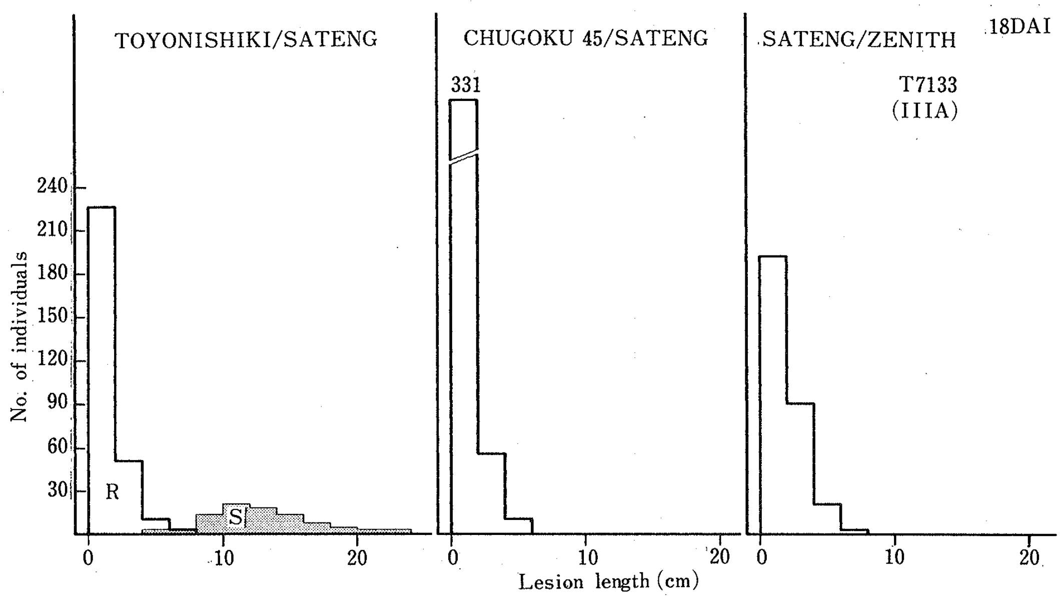

Fig. 1. Frequency distribution of lesion length of the F_2 populations from the crosses Toyonishiki/Sateng, Chugoku 45/Sateng and Sateng/Zenith at booting to flowering stages. TARC, 1985.
R: resistant, S: susceptible.

development stopped at around 21 DAI. Therefore, all plants of this F_2 population were classified as resistant to Japanese race IIIA. These results show that Sateng and Zenith have the same gene for resistance. In the previous paper (OGAWA *et al.* 1990b), we reported that Zenith, Chugoku 45, and Java 14, have *Xa-3* for resistance.

2) Analysis of resistance in Sateng using Philippine races

Sateng was inoculated along with F_1 and F_2 progenies of its cross with IR24. It showed long lesions with four Philippine races, although the lesions were somewhat shorter than those of IR24 (Fig. 2). However, the lesions of Sateng had browning border around it and the development of the lesion stopped at about 21 DAI. The reaction of Sateng to the four Philippine races was similar to those of Chugoku 45 and Java 14, although Sateng's lesions were longer than those of Chugoku 45 and Java 14.

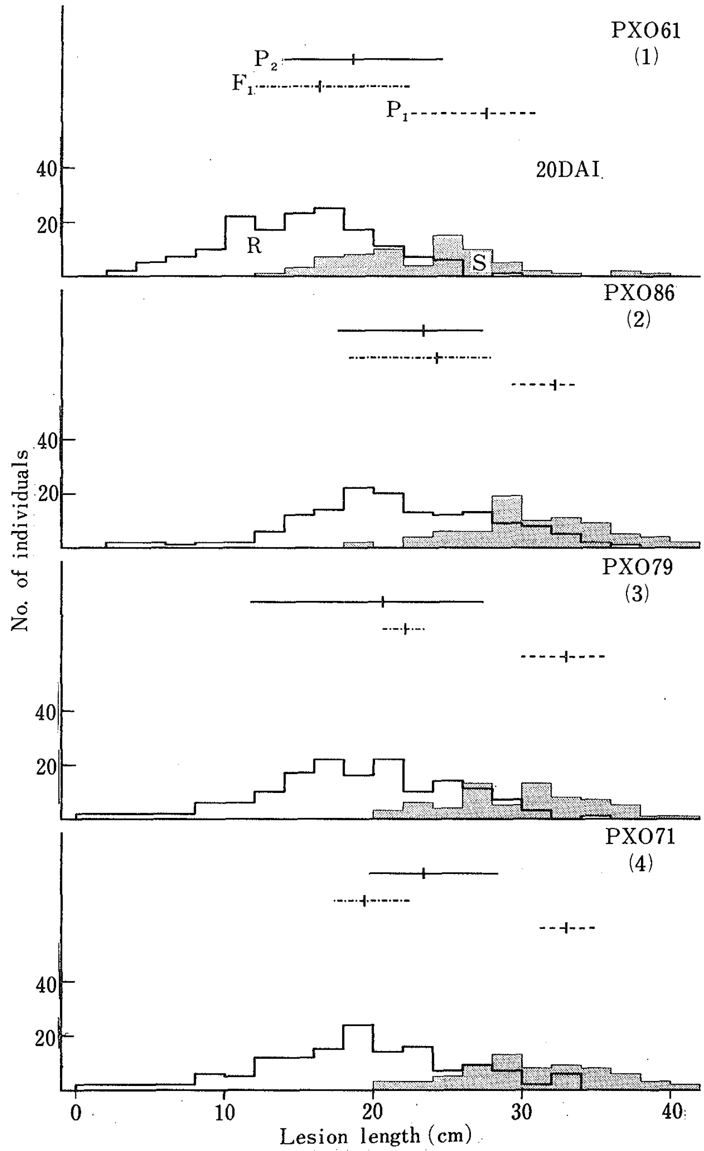

Fig. 2. Frequency distribution of lesion length of the F_2 population from the cross of IR24 (P_1)/Sateng (P_2) at booting to flowering stages. IRRI, 1983.
R: resistant, S: susceptible.

F_1 hybrids of IR 24/Sateng showed reaction similar to those of Sateng and the lesion length was similar to that of Sateng when inoculated with Philippine races (Fig. 2). These results indicated that Sateng has a dominant gene for resistance. The F_2 population of IR 24/Sateng showed segregaton pattern similar to those of F_2 populations of IR 24/Zenith and IR 24/Cempo Selak (OGAWA *et al.* 1990b); that is, the lesion length of the F_2 plants of IR24/Sateng had a wide distribution and it was difficult to evaluate all plants using lesion length alone. Through continuous observation, however, reaction of each plant could be estimated. The resistant plants showed browning reaction around the lesion. Those in which the lesion development stopped at about 21 DAI were evaluated resistant, while those which lesions developed continously even after 21 DAI were considered susceptible. The frequency distribution of lesion length of the F_2 plants of IR 24/Sateng is shown in Fig. 2. The lesion lengths of resistant and susceptible plants overlapped but the peak of distribution of two groups corresponded mostly to the average lesion length of Sateng (or F_1 hybrids) and

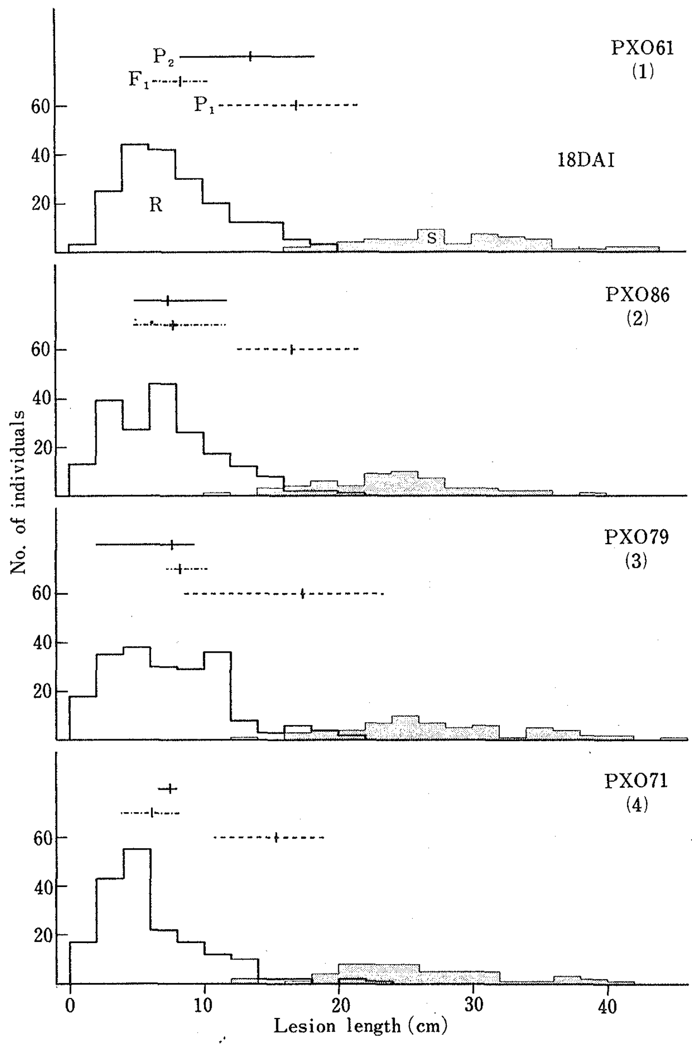

Fig. 3. Frequency distribution of lesion length of the F_2 population from the cross of Toyonishiki (P_1)/Sateng (P_2) at booting to flowering stages. IRRI, 1984. R: resistant, S: susceptible.

IR24, respectively (Fig. 2). Thus, among the F_2 plants of IR24/Sateng, 139 were resistant and 58 were susceptible to Philippine races 1 to 4. This segregation suggested a ratio of 3 : 1, but the x^2 value was high ($x^2=6.804$, P : 0.01-0.001).

During analysis of the F_2 population of IR 24/Sateng, 12% of the F_2 plants could not be scored because of infection with virus diseases. This may be the reason for high x^2 value. However, the results suggest that Sateng has one dominant gene for resistance to Philippine races 1 to 4.

To confirm the results of the F_2 analysis of IR24/Sateng, hybrids of Toyonishiki/Sateng were analyzed using four Philippine races, because Toyonishiki appeared to have higher quantitative resistance to BB than IR24 (OGAWA and YAMAMOTO 1987a). The F_1 hybrid of Toyonishiki/Sateng showed resistance to all four Philippine races, while Toyonishiki showed moderate susceptibility and Sateng showed resistance with browning reaction around the lesions. It was easier to classify the F_2 plants of Toyonishiki/Sateng as compared to those of IR24/Sateng. Most of the resistant plants showed lesion lengths less than 20 cm (Fig. 3). As a result, 172 F_2 plants were considered resistant and 51 susceptible to Philippine races 1

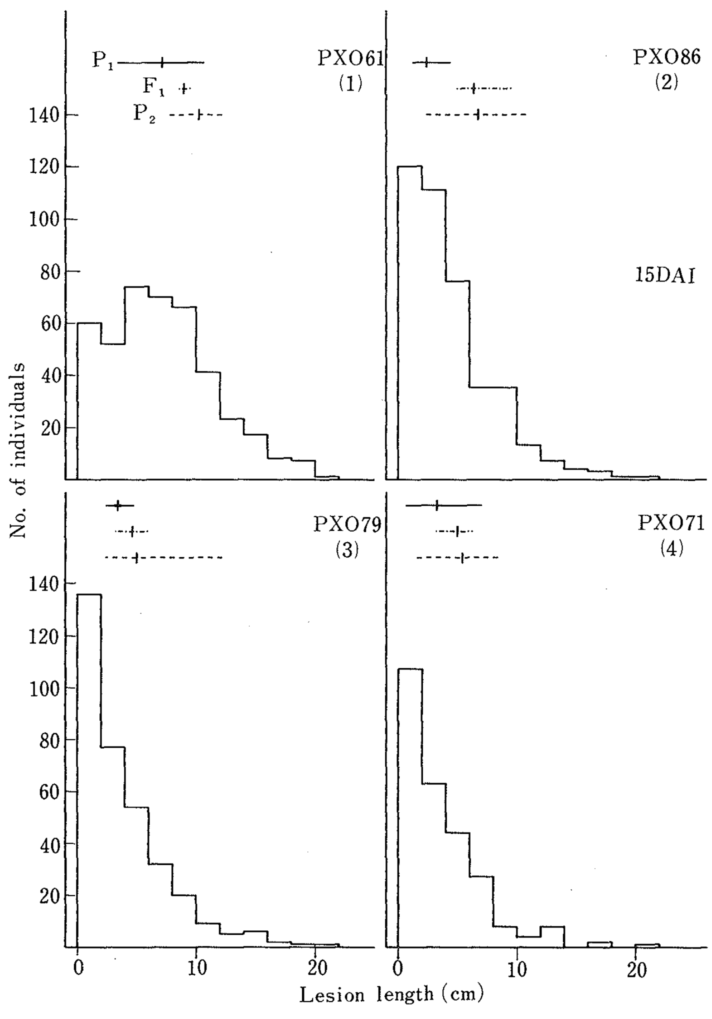

Fig. 4. Frequency distribuiton of lesion length of the F_2 population from the cross of Chugoku 45 (P_1)/Sateng (P_2) at booting to flowering stages. IRRI, 1984.

to 4. This segregation gave a good fit of 3:1 ratio ($x^2=0.540$, P: 0.3-0.5).

The susceptible plants of the F_2 population of Toyonishiki/Sateng had longer lesion than those of Toyonishiki (Fig. 3). The frequency distribution of the lesion length of resistant plants of the F_2 population indicated that the peak is located at a position similar to that of the average lesion length of Sateng and the F_1 hybrids. On the other hand, the range of distribution of lesion length of the susceptible plants of the F_2 population of IR 24/Sateng was similar to those of IR 24 plants (Fig. 2). Therefore, the quantitative resistance of Sateng was considered to be lower than that of Toyonishiki and similar to that of IR 24, although the inoculation conditions were different. The F_2 poulation of IR 24/Sateng was analyzed in the rainy season but that of Toyonishiki/Sateng was studied in the dry season. These results confirm the conclusion that Sateng has a dominant gene for resistance to Philippine races 1 to 4.

The frequency distribution of lesion length at 15-18 DAI of the F_2 populations of Chugoku 45/Sateng (253 plants) and Java 14/Sateng (339 plants) was similar (Fig. 4 and 5). The lesion length of most of the plants of two populations was less than 20 cm with browning reaction around the lesions. Thus, we classified all plants of the two populations to be resistant to four Philippine races. The two populations also showed a lower degree of resistance to Philippine race 1 than those to Philippine races 2 to 4. These results are similar to those of the F_2 populations of Chugoku 45/Java 14, Chugoku 45/Zenith, Java

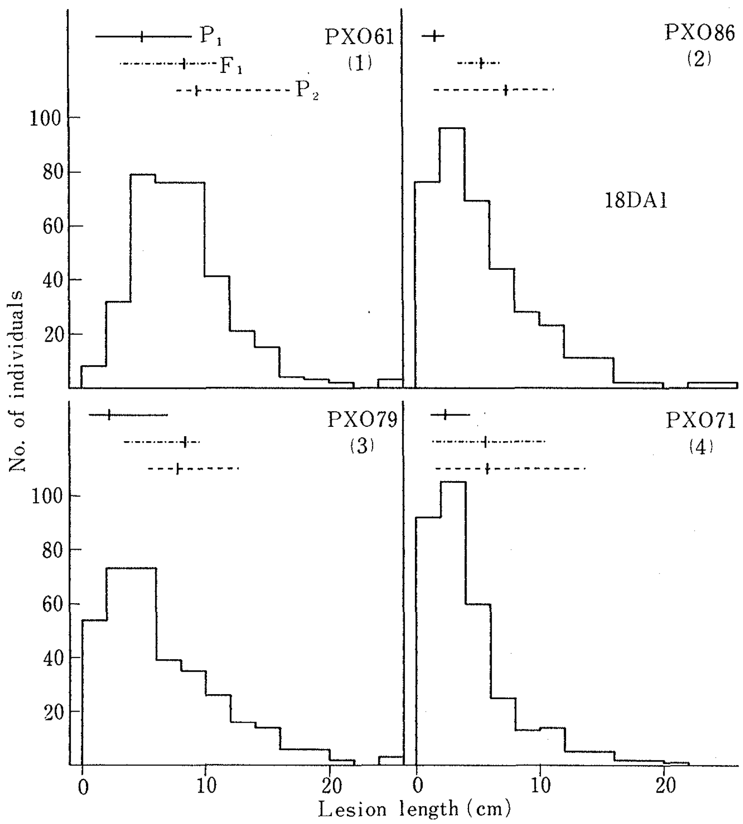

Fig. 5. Frequency distribution of the lesion length of F_2 population from the cross Java 14 (P_1)/Sateng (P_2) at booting to flowering stages. IRRI. 1984.

14/Zenith, and Java 14/Cempo Selak as reported earlier (OGAWA *et al.* 1990a, b). The results of the F_2 analysis of Java 14/Sateng were further confirmed by studying F_3 lines derived from the F_2 plants that showed lesions longer than 13 cm. We alanyzed 21 F_3 lines and all were homozygously resistant. Their lesions on the average were shorter as compared to the original F_2 plants. Thus, there was no correlation between the average lesion length of the F_3 line and the lesion length of the F_2 plant (Fig. 6).

The frequency distribution of the lesion length of F_2 plants of Sateng/Zenith (355 plants) and Cempo Selak/Sateng (139 plants) was similar to those of Chugoku 45/Sateng and Java 14/Sateng. The peak of distribution of lesion length with race 1 was located lower than those with races 2 to 4. The lesion development in all F_2 plants of these crosses stopped at around 21 DAI and showed browning reaction around the lesions. Thus, all F_2 plants were considered resistant to four Philippine races. From these results, we concluded that the resistance of Sateng to Japanese and Philippine races is conditioned by the allelic gene with *Xa-3* in Chugoku 45, Java 14, Zenith, and Cempo Selak, but the quantitative resistance of Stateng appears to be lower than those of Cempo Selak, Chugoku 45, Java 14, and Zenith.

The earlier identificaiton of SINGH *et al.* (1983) as Sateng having a recessive gene for resistance was in error which was caused because F_1 and F_2 populations were inculated at

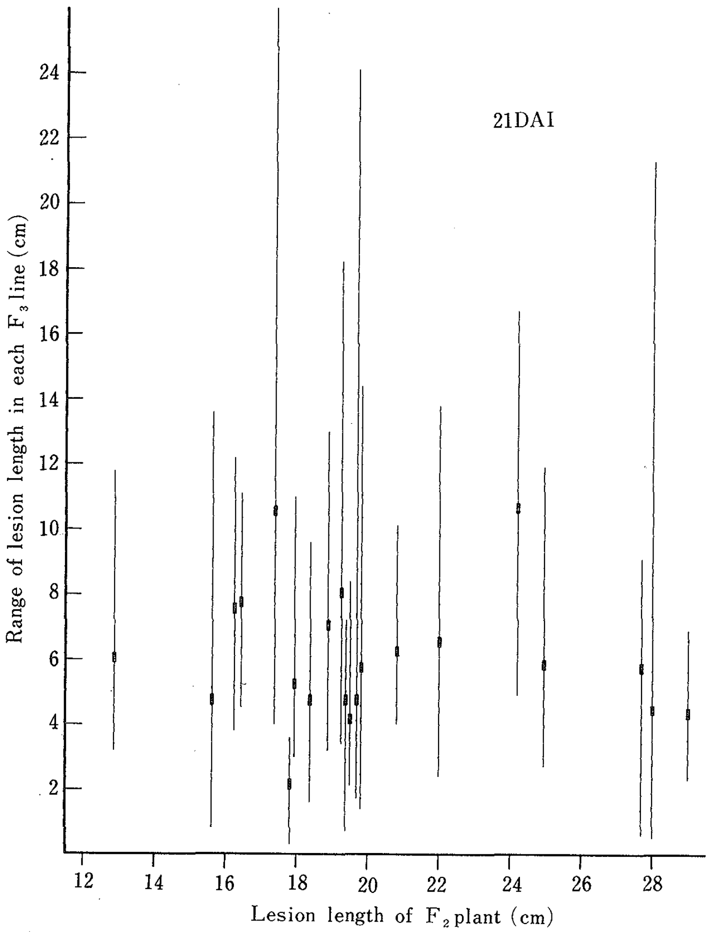

Fig. 6. Lesion length of the F_2 plants and F_3 lines from the cross Java 14/Sateng at booting to flowering stages. IRRI, 1985.

maximum tillering stage rather than booting or flowering stage. Moreover, SINGH *et al.* (1983) used a higher concentration of inoculum (10^9 cells/m*l*) and used only race 1 for inoculation. When plants heterozygous for *Xa-3* are inoculated at maximum tillering stage, they look like susceptible reaction in lesion length. Since the F_1 and F_2 populations of TN1/Sateng studied SINGH *et al.* (1983) were inoculated at maximum tillering stage, the F_1 might be evaluated as susceptible reaction from lesion length and F_2 gave a segregation ratio of 1 resistant to 3 susceptible (heterozygous plants were classified as susceptible). It was, therefore, concluded that Sateng has a recessive gene for resistance. Thus the error might be caused because of wrong stage of inoculation, higher concentration of the inoculum and evaluation criteria for resistance. Moreover, SINGH *et al.* (1983) did not study the browning reaction around the lesions. The results of our analysis clearly show that Sateng has a dominant gene for resistance to 4 races of BB in the Philippines and it is allelic to *Xa-3*.

In our previous paper (OGAWA *et al.* 1990a, b), it was reported that the plants carrying the resistance gene, *Xa-3* (Chugoku 45, Java 14 and Zenith) show lesions less than 7 cm long when inoculated with the Japanese races in Japan and less than 20 cm long lesions when inoculated with four Philippine races at IRRI. The lesion length may be variable because of various factores, but the resistant reaction is always characterized by browning reaction (KAKU and KIMURA 1978) around the lesions before or after lesion development stops. This reaction of plants with *Xa-3* gene can not be evaluated correctly according to the criteria of the IRRI standard scoring system (IRRI, 1980). EZUKA and HORINO (1974) and EZUKA *et al.* (1974), who originally identified the *Xa-3* gene, also reported that the screening for resistance conditioned by *Xa-3* should not be done at young seedling stage but at adult stage. They emphasized that the Wase Aikoku group cultivers did not always show good level of resistance and that careful analysis was necessary to evaluate the resistance of germplasm with *Xa-3*.

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フィリピン産および日本産イネ白葉枯病菌レースに対する イネ品種Satengの抵抗性の遺伝

小川紹文[1,3]・山元　剛[1]・G. S. KHUSH[2]・苗　東花[2]

([1]熱帯農業研究センター，茨城県つくば市，〒305
[2]国際稲研究所，P. O. Box933，マニラ，フィリピン
[3]現　農業研究センター，茨城県つくば市，〒305)

イネ白葉枯病菌レースの国際判別品種を設定するため，抵抗性遺伝子を一つずつもつ準同質遺伝子系統の育成が日本農林水産省とIRRI（国際稲研究所）との共同研究として行われた．その準同質遺伝子系統を育成する前提として，日本とIRRIの判別品種をフィリピン産および日本産白葉枯病菌レースを用いて分析する必要があった．そのため，抵抗性品種Satengの遺伝を，フィリピン産及び日本産白葉枯病菌レースを用いて分析した．イネ品種SatengはIRRIの研究者によって劣性の抵抗性遺伝子*xa-9*を有することが報告されている．日本，IRRI判別品種には*xa-9*を持つ品種は含まれていない．現在同定されている抵抗性遺伝子を，準同質遺伝子系統に組み込むためには，*xa-9*を持つ系統も育成する必要があった．一方，日本産およびフィリピン産白葉枯病菌レースを用いて，この品種に予備的に接種検定をしたところ，その反応は*Xa-3*を持つ中国45号，ジャワNo. 14，Zenith及びCempo Selakとよく似ていた．すなわち，Satengはフィリピン産の4つのレースおよび日本産のIからIIIのレースに抵抗性を示すと共に，病斑の周囲が褐変化した．このことから，Satengの持つ抵抗性遺伝子が優性か劣性かを再検討すると共に中国45号およびジャワNo. 14との抵抗性遺伝子の対立性検定を行うこととした．

まず，日本産白葉枯病菌レースIII Aを供試して，Satengを分析した結果，単一の優性遺伝子によってその抵抗性が支配されていることを確認した．また，中国45号，ZenithとSatengとのF_2では感受性個体が出現せず，これらの品種は日本産白葉枯病菌レースIII Aに対して同一の抵抗性遺伝子を持つと結論された．フィリピン産白葉枯病菌レース1から4を供試して，Satengの抵抗性を分析した場合もこの品種が優性の抵抗性遺伝子を持つことが明らかになった．ただ，トヨニシキを片親にした場合とIR 24を片親にした場合とではF_2の感受性個体の病斑長の頻度分布が異なることから，Satengの量的抵抗性が他の*Xa-3*を持つ中国45号，ジャワNo. 14，Zenithなどに比較して弱いものと推定された．

フィリピン産白葉枯病菌レース1から4を供試して，Satengと*Xa-3*を持つ中国45号，ジャワNo. 14，Zenith及びCempo SelakとのF_2を分析したところ，いずれのF_2でも感受性個体は出現しなかった．

従って，Satengの白葉枯病抵抗性も中国45号・ジャワNo. 14・Zenith及びCempo Selakと同じ座の遺伝子によって支配されていると結論した．

Satengが劣性遺伝子を持つと誤って同定された理由としては(1)Satengの量的抵抗性が弱く主働抵抗性遺伝子*Xa-3*の発現にそれが影響すること，(2)比較的高濃度の接種源が使われたこと，(3)最高分げつ期に接種されたこと，(4)抵抗性と感受性個体の判別を高いレベルの基準で機械的に行ったこと，などが考えられる．