Epidemic Development of Bacterial Blight on Rice Carrying Resistance Genes *Xa-4*, *Xa-7*, and *Xa-10*

I. Oña, Entomology and Plant Pathology Division, International Rice Research Institute, 1099 Manila, Philippines; C. M. Vera Cruz, Department of Plant Pathology, University of the Philippines, Los Baños, Laguna; Rebecca J. Nelson, Centro Internacional de la Papa, Lima, Peru; Jan E. Leach, Department of Plant Pathology, Kansas State University, Manhattan 66506-5502; and T. W. Mew, Entomology and Plant Pathology Division, International Rice Research Institute, Philippines

ABSTRACT

Oña, I., Vera Cruz, C. M., Nelson, R. J., Leach, J. E., and Mew, T. W. 1998. Epidemic development of bacterial blight on rice carrying resistance genes *Xa-4*, *Xa-7*, and *Xa-10*. Plant Dis. 82:1337-1340.

Epidemic development of bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* on three near-isogenic lines (IRBB lines) carrying bacterial blight resistance genes *Xa-4* (IRBB4), *Xa-7* (IRBB7), and *Xa-10* (IRBB10) was studied in two farmers' fields located in Calauan and Mabitac, Philippines, during 1993, 1994, and 1995. The plots were planted continuously during dry and wet seasons, with disease assessments done during the wet seasons, when weather was conducive to bacterial blight development. Disease incidence (number of hills infected) and severity (diseased leaf area) were assessed and area under the disease progress curve (AUDPC) values were computed. Mean disease incidence and severity in Calauan (AUDPC = 3,241.5 and 716.7, respectively) were significantly higher than in Mabitac (AUDPC = 2,431.2 and 558.0, respectively). In Calauan, AUDPC values for disease incidence and severity on IRBB4, IRBB10, and IR24 did not differ significantly during the three years of testing. In Mabitac, the AUDPC values for disease incidence and severity were not significantly different among IRBB4, IRBB10, and IR24 in 1993 and in 1995. However, in 1994, disease incidence (AUDPC = 3,037.7) and severity (AUDPC = 891.0) on IRBB4 were significantly lower than on IRBB10 and IR24. Bacterial blight was lowest on IRBB7 at both sites and throughout 1993 to 1995, indicating that this genotype is effective in suppressing the disease even with the presence of an indigenous virulent population of *X. oryzae* pv. *oryzae*.

Additional keywords: epidemiology, host resistance

Host resistance is an effective management strategy for bacterial blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* (19). Several race-specific resistance genes have been identified and utilized in the development of near-isogenic lines (16,22). In the Philippines, the bacterial blight resistance gene *Xa-4* was first incorporated into improved rice varieties and field tested during the early 1970s (8). Since then, rice varieties containing this gene have been continually used by farmers. Although there was a shift in the pathogen population since the introduction of this resistance gene into modern rice varieties, no major epidemics have occurred on these varieties (11,12).

In an effort to develop effective deployment strategies for the different bacterial blight resistance genes, the population

structure of the pathogen was analyzed in relation to the host genotype (2,5,20,21). Little is known, however, about the effectiveness of the different resistance genes against bacterial blight epidemics in the field. Progression of bacterial blight in the field was first studied on rice varieties developed at the International Rice Research Institute (IR varieties), carrying either the *Xa-4* or *Xa-3* gene, that were artificially inoculated with two isolates of race 2 (1). Significant reductions in disease severity and yield loss were observed among these varieties, particularly in IR1695 (with *Xa-3* gene) in comparison with the susceptible check, IR24.

In a separate experiment conducted in Calauan and Mabitac for 2 years, Ahmed et al. (3) compared the effect of pure lines, mixed stands, and two-gene pyramids of near-isogenic lines containing *Xa-4*, *xa-5*, and *Xa-10* on bacterial blight epidemics. They found that bacterial blight was less severe in two-gene combinations than in pure lines (single gene). The *Xa-4/xa-5* pyramid had the lowest bacterial blight infection. This gene combination (*Xa-4/xa-5*) was also found to be most resistant to the eight races of *X. oryzae* pv. *oryzae* in greenhouse tests (22).

To better understand the effect of resistance genes on the epidemics of bacterial blight and to develop a basis for the development of a resistance gene deployment strategy for the disease, we determined the relative effectiveness of three race-specific resistance genes, *Xa-4*, *Xa-7*, and *Xa-10*, on the progress of bacterial blight in two farmers' fields where the disease was reported to occur naturally in the past 10 years.

MATERIALS AND METHODS

General. Field studies were conducted in 1993, 1994, and 1995 in two farmers' fields located in Calauan and Mabitac, Laguna (situated in the southern part of Luzon, Philippines), where bacterial blight is endemic. Mabitac is about 40 km from Calauan. The near-isogenic lines IRBB4, IRBB7, and IRBB10, carrying the resistance genes, *Xa-4*, *Xa-7*, and *Xa-10*, respectively, were evaluated for their effectiveness against bacterial blight under field conditions and compared with the recurrent parent IR24. *Xa-4* is resistant to race 1 but susceptible to races 2 and 3; *Xa-7* is resistant to races 1, 2, and 3; *Xa-10* is resistant to race 2 but susceptible to races 1 and 3. A previous survey revealed the presence of races 1, 2, and 3 in both sites, but race 2 was the dominant population during 1992.

Experimental layout. In Calauan, the experiment was laid out in a randomized complete block using 10- by 10-m plots (treatments) bordered by 5 m of variety IR 54 during 1993, 1994, and 1995. In Mabitac, a Latin square design was used, with each treatment plot measuring 25 by 25 m. Each plot was bordered by 5 m of cv. RC10 in 1993 and IR 54 in 1994 and 1995. IR 54 has *Xa-4* and partial resistance against the indigenous population of *X. oryzae* pv. *oryzae* at both sites and was used as border plants between plots. The experiment was replicated four times at both sites.

Seedlings (21 days old) were transplanted in January during the dry season and in July (Calauan) or August (Mabitac) during the wet season. The farmer's routine cultural practices were followed in both locations, including split application of 100 kg N/ha at 21, 50, and 70 days after transplanting (DAT) and weeding at 30 and 40 DAT.

Corresponding author: T. W. Mew
E-mail: T.Mew@cnet.com

Accepted for publication 25 August 1998.

Fig. 1. Progress curves for (A and C) bacterial blight incidence and (B and D) severity during 1994 wet season in (A and B) Calauan and (C and D) Mabitac. ◆ = IRBB4, × = IRBB7, □ = IRBB10, and Δ = IR 24.

Fig. 2. Area under disease progress curve (AUDPC) for bacterial blight incidence in (A) Calauan and (B) Mabitac during 1993 to 1995 wet seasons.

Disease assessments. Under field conditions, the disease commonly occurs during the wet season when there are frequent rains and strong winds (13,17). The disease was monitored only during the wet season, although the plots were planted continuously during the dry season.

Disease assessments were made weekly for 7 to 8 weeks, starting from 1 week after visual appearance of leaf symptoms. A partial replacement technique for repeated sampling was used to assess the amount of disease from five sampling areas per plot, with each sampling area containing 9 hills in Calauan and 16 hills in Mabitac (6).

Severity was assessed by estimating the percentage of leaf area infected from 9 randomly selected leaves per plant. The incidence was obtained by counting the number of hills infected with bacterial blight and dividing it by the total number of hills in the sampling area. Area under the disease progress curve (AUDPC) for incidence and severity were calculated according to the equation (18):

$$AUDPC = \sum_{i=1}^N [(y_i + y_{i+1}) / 2] [t_{i+1} - t_i]$$

where y_i = bacterial blight severity or incidence at the i th observation, N = number of observations, and t_i = time (days) of the i th observation.

AUDPC values were transformed to base-10 logarithm prior to analysis of variance. Homogeneity of variances among years and between sites were determined by Bartlett's test. Combined analysis of variance across years and sites was done by using a split-split-plot analysis with the sites as mainplot, the isolines as subplot, and the years as sub-subplot.

RESULTS

Disease incidence. In both sites, the disease was visually observed at 30 to 40 DAT. Disease incidence was significantly higher in Calauan (AUDPC = 3,241.5) than in Mabitac (AUDPC = 2,431.2) during 1993 and 1994.

In Calauan, bacterial blight spread rapidly among hills on IRBB4, IRBB10, and IR24 (Fig. 1A). During 1993 and 1994, almost all hills were infected 60 DAT. The bacterial blight incidence was about 60% on these genotypes at 43 and 41 DAT during 1993 and 1994, respectively. During 1995, the incidence was 20% observed at 41 DAT, after which the disease spread gradually between hills with the maximum disease incidence of 100% observed at 80 DAT (data not shown). AUDPC for disease incidence on IRBB4, IRBB10, and IR24 was not significantly different in the 3 years (Fig. 2A). IRBB7 had the lowest disease incidence throughout 1993 to 1995 ($P < 0.01$).

In Mabitac, progression of disease incidence on IRBB4, IRBB10, and IR24 was similar except during 1994, when disease incidence at the first observation (37 DAT)

was 60% on IRBB10 and IR24 and 15% on IRBB4 (Fig. 1C). A gradual increase in disease incidence was observed on IRBB4, IRBB10, and IR24 during 1993 and 1995 (*data not shown*). Disease incidence increased from 18 to 100% at 58 to 91 DAT in 1993 and 36 to 65 DAT in 1995. AUDPC was significantly lower for IRBB4 than for IRBB10 and IR24 during 1994 (Fig. 2B). AUDPC was lowest during 1993 ($P < 0.01$). The lowest disease incidence was observed on IRBB7 in all years ($P < 0.01$).

Disease severity. In Calauan, bacterial blight was most severe during 1993 but was lowest in 1995 ($P < 0.01$). The shapes of the progress curves for IRBB4, IRBB10, and IR24 were similar in each year (Fig. 1B). Disease severity among IRBB4, IRBB10, and IR24 was not significantly different from each other during both high (as in 1993) and low (as in 1995) disease pressure (Fig. 3A).

Severity was the lowest on IRBB7 ($P < 0.01$) throughout the 3 years (Fig. 3A). Bacterial blight lesions were observed on this line at early tillering stage (25 to 35 days after sowing). However, as the plants matured, lesions became less visible. At maximum tillering stage (45 to 55 days after sowing), the age of rice plants when bacterial blight is most commonly observed, the symptoms on IRBB7 were hardly discernible. In contrast, the disease continued to progress on IRBB4 and IRBB10 until maturity.

Bacterial blight severity in Mabitac was the lowest in 1993 and the highest in 1994 ($P < 0.01$). Bacterial blight developed early and fast on IRBB10 and IR24 in all three years. However, disease development was more gradual on IRBB4, particularly in 1994 (Fig. 1D). Severity was significantly lower on IRBB4 in 1994 and 1995 (Fig. 3B). IRBB7 had the lowest disease level (<5%) over the 3-year period ($P < 0.01$).

DISCUSSION

Significant variation in the development of bacterial blight epidemic was observed in the Calauan and Mabitac sites for 3 years. Disease pressure was the highest in Calauan and Mabitac in 1993 and 1994, respectively. Rainfall data in the 1993 to 1995 wet season were taken from the weather station 2 to 3 km from the Calauan experimental site (*data not shown*). However, no correlation was observed between rainfall amount and number of days with rain to the amount of disease in each year. Rainfall was highest during 1995 when disease pressure was low. This may suggest that the inoculum of *X. oryzae* pv. *oryzae* was a limiting factor.

The relative effectiveness of the resistance gene in IRBB4, IRBB7, and IRBB10 was clearly observed under both high and low disease pressure. Although differences were slight, IRBB10 always had the highest rate of disease increase in all 3 years in

both locations. IR24 ranked next to IRBB10, followed by IRBB4. IRBB4 (carrying *Xa-4*) was relatively effective in Mabitac, particularly during 1994, even with the presence of a virulent indigenous bacterial population (20).

In commercial IR varieties carrying the *Xa-4* gene, bacterial blight epidemic can be significantly reduced in the field. This was attributed to the background resistance of these varieties (11). Quantitative resistance was further observed in a number of IR varieties carrying the *Xa-4* gene (9,10). The residual effects of a "defeated" resistance gene was also observed in a winter wheat-powdery mildews pathosystem (14). However, according to Anderson (4), the residual effects of resistance genes against certain pathogens could not be totally attributable to these genes because unidentified unlinked genes could also be responsible. Results of crosses made between cultivars moderately resistant to bacterial blight indicated that, other than the presence of the *Xa-4* gene, these cultivars carry several factors for quantitative resistance (9).

During the 3 years of evaluation, bacterial blight infection remained the lowest on IRBB7 in the two sites. The low final disease severity showed that bacterial blight epidemic was effectively suppressed on

this line when compared to their development on IRBB10 and IRBB4. *X. oryzae* pv. *oryzae* race 9 (virulent on IRBB4, IRBB7, and IRBB10) has been detected in high frequency (>80%) in both sites (20). Although there existed a race virulent to IRBB7, the disease remained at a significantly low level on this line. How this virulent race of *X. oryzae* pv. *oryzae* survived in the rice field during fallow periods, particularly during the dry season, is not yet understood.

Avirulence genes present in bacterial pathogens served as elicitors of the incompatible response (resistance) in plants carrying the corresponding resistance genes. In the bacterial spot of tomato and pepper pathosystem, mutation in the *avrBs1* gene enabled the pathogen to persist longer in the soil and dead leaf tissue (15). A number of avirulence genes, including *avrXa7* and *avrXa10* (corresponding to *Xa-7* and *Xa-10*, respectively), were isolated from *X. oryzae* pv. *oryzae* (7). The effect of these *avr* genes on virulence and survival of the different races of *X. oryzae* pv. *oryzae* in the field has not been investigated.

The relative effectiveness of IRBB7 on the progress of bacterial blight was consistent in both sites and across years. Likewise, the gene *xa-5* provided significant reduction in bacterial blight severity

Fig. 3. Area under disease progress curve (AUDPC) for bacterial blight severity in (A) Calauan and (B) Mabitac during 1993 to 1995 wet seasons.

in Calauan and Mabita (3). The effect of gene combinations involving *Xa-7* on bacterial blight epidemics is still unknown.

LITERATURE CITED

1. Adhikari, T. B., Mew, T. W., and Teng, P. S. 1996. Progress of bacterial blight on rice cultivars carrying different *Xa* genes for resistance in the field. *Plant Dis.* 78:73-77.
2. Adhikari, T. B., Vera Cruz, C. M., Zhang, Q., Nelson, R. J., Skinner, D. Z., Mew, T. W., and Leach, J. E. 1995. Genetic diversity of *Xanthomonas oryzae* pv. *oryzae* in Asia. *Appl. Environ. Microbiol.* 61:966-971.
3. Ahmed, H., Finckh, M. R., Alfonso, R., and Mundt, C. C. 1996. Epidemiological effect of gene deployment strategies on bacterial blight of rice. *Phytopathology* 87:66-70.
4. Anderson, M. G. 1982. Interpreting residual effects of "defeated" resistance genes. *Phytopathology* 72:1383-1384.
5. Ardales, E. Y., Leung, H., Vera Cruz, C. M., Mew, T. W., Leach, J. E., and Nelson, R. J. 1996. A hierarchical analysis of spatial variation of the rice bacterial blight pathogen across diverse ecosystems in the Philippines. *Phytopathology* 86:241-252.
6. Gomez, K. A., and Gomez, A. A. 1984. Statistical Procedures for Agricultural Research. 2nd Ed. Wiley, New York.
7. Hopkins, C. M., White, F. F., Choi, S. H., Guo, A., and Leach, J. E. 1992. Identification of a family of avirulence genes from *Xanthomonas oryzae* pv. *oryzae*. *Mol. Plant-Microbe Interact.* 6:451-459.
8. Khush, G. S., Mackill, D. J., and Sidhu, G. 1989. Breeding rice for resistance to bacterial blight. Pages 207-218 in: *Bacterial Blight of Rice*. International Rice Research Institute, Los Baños, Laguna, Philippines.
9. Koch, M., and Parlevliet, J. 1991. Genetic analysis of, and selection for, factors affecting quantitative resistance to *Xanthomonas campestris* pv. *oryzae* in rice. *Euphytica* 53:235-245.
10. Koch, M., and Parlevliet, J. 1991. Residual effects of the *Xa-4* resistance gene in three rice cultivars when exposed to a virulent isolate of *Xanthomonas campestris* pv. *oryzae*. *Euphytica* 55:187-193.
11. Mew, T. W., and Vera Cruz, C. M. 1988. Background resistance to bacterial blight (BB) hill and leaf infection. *Int. Rice Res. Newsl.* 13 (2):11-12.
12. Mew, T. W., Vera Cruz, C. M., and Medalla, E. S. 1992. Changes in the race frequency of *Xanthomonas oryzae* pv. *oryzae* in response to rice cultivars planted in the Philippines. *Plant Dis.* 76:1029-1032.
13. Mizukami, T., and Wakimoto, S. 1969. Epidemiology and control of bacterial leaf blight of rice. *Annu. Rev. Phytopathol.* 7:51-72.
14. Nass, H. A., Pedersen, W. L., MacKenzie, D. R., and Nelson, R. R. 1981. The residual effects of some "defeated" powdery mildews resistance genes in isolines of winter wheat. *Phytopathology* 71:1315-1318.
15. O'Garro, L. W., Gibbs, H., and Newton, A. 1997. Mutation in the *avrBs1* avirulence gene of *Xanthomonas campestris* pv. *vesicatoria* influences survival of the bacterium in soil and detached leaf tissue. *Phytopathology* 87:960-966.
16. Ogawa, T., Tabien, R. E., Yamamoto, T., Busto, G. A., and Ikeda, R. 1990. Breeding for near-isogenic lines resistant to bacterial blight of rice. *Rice Genet. Newsl.* 7:10.
17. Ou, S. H. 1985. *Rice Diseases*, 2nd Ed. Commonwealth Mycological Institute, Kew, England.
18. Shaner, G., and Finney, R. E. 1977. The effect of nitrogen fertilisation on the expression of slow-mildewing resistance in Knox wheat. *Phytopathology* 67:1051-1056.
19. Swings, J., Van der Mooter, M., Vauterin, L., Hoste, B., Gillis, M., Mew, T. W., and Kersters, K. 1990. Reclassification of the causal agents of bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) and bacterial leaf streak (*Xanthomonas campestris* pv. *oryzicola*) of rice as pathovars of *Xanthomonas oryzae* (ex Ishiyama 1922) sp. nov., nom. ret. *Int. J. Syst. Bacteriol.* 40:302-311.
20. Vera Cruz, C. M. 1996. Host selection influences the population structure of the rice bacterial blight pathogen, *Xanthomonas oryzae* pv. *oryzae*. Ph.D. dissertation. Kansas State University, Manhattan.
21. Vera Cruz, C. M., Ardales, E. Y., Skinner, D. Z., Talag, J., Nelson, R. J., Louws, F. J., Leung, H., Mew, T. W. and Leach, J. E. 1996. Measurement of haplotypic variation in *Xanthomonas oryzae* pv. *oryzae* within a single field by rep-PCR and RFLP analyses. *Phytopathology* 86:1352-1359.
22. Yoshimura, S., Yoshimura, A., Iwata, N., McCouch, S. R., Abenes, M. L., Baraoidan, M. R., Mew, T. W., and Nelson, R. J. 1995. Tagging and combining bacterial blight resistance genes in rice using RAPD and RFLP markers. *Mol. Breeding* 1:375-387.