Analysis of the Interaction Between Xanthomonas oryzae pv. oryzae and the Rice Cultivars IR24 and IRBB21

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ABSTRACT


Xanthomonas oryzae pv. oryzae causes bacterial blight of rice, and interactions between this pathogen and its host occur in a gene-for-gene manner. In contrast to other resistance genes, the dominant locus Xa-21 confers resistance to all Indian and Philippine races of X. o. oryzae that have been examined. In this study, multiplication of X. o. oryzae and subsequent disease development were comparable in cultivar IRBB21 possessing Xa-21 and in the susceptible rice cultivar IR24 when plants were inoculated prior to 21 days postemergence. Resistance conveyed by Xa-21 was evident in 21-day-old plants, and the level of resistance increased with plant maturity. Cultivar IR24, which is used as a susceptible control in screening trials, displayed resistance to bacterial blight that was expressed only in adult plants. DNA homologous to the avirulence gene avrBs2 from Xanthomonas campestris pv. vesicatoria was present in all Philippine races of X. o. oryzae. Inactivation of the avrBs2-homologous region by transposon mutagenesis did not affect the fitness of X. o. oryzae and did not alter the response elicited by this bacterium on IRBB21, indicating that this locus does not confer avrXa21 activity.

Bacterial blight of rice, caused by Xanthomonas oryzae pv. oryzae, has become an increasingly important disease problem in rice production. Genetic resistance is the most effective and economical measure for the control of this disease, and breeding for bacterial blight resistance is an important component of rice improvement programs. Resistance to bacterial blight has been identified in several cultivars, and examples of both quantitative and major gene resistance to X. o. oryzae have been described for rice. Molecular genetic analyses of the X. o. oryzae–rice interaction have demonstrated that the resistance conferred by single dominant loci in the host occurs in a race-specific manner. An incompatible plant response is elicited when a rice cultivar that possesses a specific resistance gene is inoculated with a strain of X. o. oryzae that carries the corresponding avirulence gene. Recently, a dominant gene for resistance to bacterial blight, designated Xa-21, was identified in the wild rice Oryza longistaminata and was introduced into the rice cultivar IR24 by backcrossing. In contrast to previously identified bacterial blight resistance genes, Xa-21 conveys resistance to all Indian and Philippine races of X. o. oryzae that have been evaluated.

Although many avirulence genes are restricted to particular races of a given bacterial species, the avirulence gene avrBs2 is present and highly conserved among all strains of Xanthomonas campestris pv. vesicatoria that have been examined. In addition to its role in the elicitation of a resistance response in pepper cultivars possessing the Bs2 resistance gene, avrBs2 also contributes to the fitness of this bacterium in association with its plant host. Kearney and Staskiwicz demonstrated that mutations in the avrBs2 locus resulted in the inability of X. c. vesicatoria to multiply to wild-type levels in normally susceptible pepper cultivars. Pepper cultivars carrying the Bs2 resistance locus are resistant to all strains of X. c. vesicatoria because of the distribution of avrBs2 throughout the population of this bacterial pathogen.

In addition to conservation of the avrBs2 locus among strains of X. c. vesicatoria, homologous sequences are distributed among several plant-pathogenic members of the genus Xanthomonas, including X. o. oryzae. Although functional copies of this avirulence gene are present in several X. campestris pathovars, the activity of the avrBs2 homologue in X. o. oryzae and its distribution in the population of this pathogen have not been examined. On the basis of previous reports that Xa-21 confers resistance to all Philippine and Indian strains of the bacterial blight pathogen (1) as well as the widespread presence of avrBs2-homologous sequences among pathovars of X. campestris (9), our initial objective was to determine whether or not the avrBs2 homologue in X. o. oryzae conveyed avrXa21 activity. During our initial experiments, the response of rice cultivars to inoculation with X. o. oryzae suggested that the resistance conferred by Xa-21 may not be functional under certain experimental conditions. Therefore, additional investigations were conducted to determine the effect of plant age on the interaction between X. o. oryzae and the rice cultivar IRBB21.

MATERIALS AND METHODS

Bacterial strains, plasmids, bacteriophage, and media. The bacterial strains, plasmids, and phage used in this study are listed in Table 1. X. o. oryzae was cultured in peptone-sucrose (PS) medium (22) at 28 C. For isolation of X. o. oryzae from plant tissue, 1.5% agar was added to PS medium. Strains of Escherichia coli were cultured in Luria-Bertani medium (13) or terrific broth (21). Antibiotics were used in selection media at the following concentrations (µg/ml): cephalixin, 30; cycloheximide, 100; kanamycin, 50; nalidixic acid, 50; rifampicin, 30; spectinomycin, 100; and streptomycin, 100.

Recombinant DNA techniques. Isolation of plasmid DNA from E. coli was performed as described by Birnboim and Doly (5). Restriction endonuclease digestions were performed as recommended by the supplier (Bethesda Research Laboratories, Gaithersburg, MD). Isolation of genomic DNA, transfer of DNA to nitrocellulose, labeling of DNA with 32P-dCTP by nick translation, hybridization, electrophoresis, transformation of E. coli, and conjugative transfer of plasmids to X. o. oryzae were conducted essentially as described by Maniatis (14) or Ausubel et al (1). Colony
blots were performed as described by Maas (13) and were washed after hybridization under high stringency conditions (11).

Identification of sequences homologous to avrB2 in X. o. oryzae. The plasmid pB1533 contains a 2.35-kb SpH1 DNA fragment from X. c. vesicatoria that has avrB2 activity (9). A 1.9-kb HindIII fragment that is internal to avrB2 can be used as a specific probe for the gene (B. Staskawicz, personal communication). Plasmid pB1533 was digested with HindIII, and fragments were separated on a 0.7% agarose gel. The 1.9-kb HindIII fragment was cut from the agarose gel and electroeluted into TE (10 mM Tris and 1 mM EDTA) buffer. The DNA was labeled with 32P-dCTP by nick translation and used in colony hybridizations to probe a genomic library of X. o. oryzae PX086 constructed in the cosmids phMI (6). Plasmid DNA was isolated from E. coli strains that hybridized with the avrB2-specific probe in colony blots. Plasmid DNA was digested with Sphl and probed with the 1.9-kb HindIII fragment in Southern hybridizations to confirm the presence of X. o. oryzae DNA homologous to avrB2.

Tn5 mutagenesis. Clones of X. o. oryzae DNA in phMI were mutagenized in E. coli strain TBI with λB200::Tn5 (20). Conjugative transfer of mutated plasmids to E. coli strain C1210 was conducted by triplication of the mating with strains possessing presumptive Tn5 insertions in cosmids clones as the donor strains and pRK2073 as the helper plasmid. Transconjugants were selected on Luria-Bertani agar containing kanamycin, nalidixic acid, and spectinomycin. Plasmid DNA was isolated from pooled transconjugants and transformed into E. coli strain S17.1. Plasmid DNA was isolated from individual colonies and digested with HindIII or Sphl, separated by agarose gel electrophoresis, and probed with Tn5 or the 1.9-kb HindIII fragment specific for avrB2. Mutagenized clones possessing a Tn5 insertion within the avrB2-homologous region were utilized in marker-exchange

Marker-exchange mutagenesis. Mutated plasmids were introduced into X. o. oryzae strains PX086R and PX099A in biparental matings. Transconjugants were grown for several generations in PS broth without selection for resistance to spectinomycin, and then cultures were plated onto PS agar containing kanamycin.

Individual colonies were selected and replica plated onto PS agar amended with kanamycin or spectinomycin. Total DNA was isolated from kanamycin-resistant, spectinomycin-sensitive colonies. DNA was digested with HindIII, separated by agarose gel electrophoresis, transferred to nitrocellulose, and probed with pHM1, the 1.9-avrB2-specific DNA fragment, and Tn5 to ensure that the mutated avrB2-homologous sequence had integrated into the chromosome of X. o. oryzae.

Plant materials and inoculation. The rice cultivar IR24 is susceptible to all Philippine races of X. o. oryzae. Cultivars IRRB10 and IRBB21 are near-isogenic lines of IR24 and possess the Xa-10 and Xa-21 resistance loci, respectively (7,10,23). Cultivar IRRB10 is resistant to races of X. o. oryzae that possess the avirulence gene avrXa10 (6) and was used as the resistant control in inoculations with strain PX086R.

Seed were planted and incubated in the greenhouse at a day-night temperature regime of 30 and 25°C without supplemental lighting. X. o. oryzae strains PX099A and PX086R were grown in PS broth for 24 h. Cells were collected by centrifugation, washed with sterile water, and resuspended in a volume of sterile water to obtain a cell density of approximately 10^5 cfu/ml. Plants were inoculated with the leaf-clip method (8) or by localized infiltration of X. o. oryzae cell suspensions with a needleless syringe into leaves of 10-day-old rice plants as described by Reimer and Leach (18). Lengths of lesions induced by X. o. oryzae after leaf-clip inoculation of 11-, 17-, 21-, 31-, 41-, 51-, 61-, and 71-day-old plants were assessed 14 days postinoculation. Twenty leaf samples were assayed per cultivar-strain combination. Infiltrated leaves of 10-day-old rice plants were scored for water-soaking (susceptible) or the hypersensitive (resistant) responses 48 h postinoculation. Experiments were repeated twice.

Colonization of leaf tissue by X. o. oryzae. The effect of plant age on the multiplication of X. o. oryzae strain PX099A in leaves of the rice cultivars IR24 and IRBB21 was assessed. Plants were inoculated at 11, 21, 41, or 61 days postemergence with the leaf-clip method as described above. For each rice cultivar, three inoculated leaves were randomly harvested at 0, 4, 8, 12, and 16 days postinoculation. The leaves were ground individually with

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### TABLE 1. Bacterial strains, plasmids, and phage used in this study

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<th>Strain/Plasmid</th>
<th>Relevant characteristic</th>
<th>Source or reference</th>
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<tr>
<td>Xanthomonas oryzae pv. oryzae</td>
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<tr>
<td>PX086R</td>
<td>Race 2, Rif^R</td>
<td>This study</td>
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<td>PX086R::pMM31.10 marker-exchange mutant, Km^R</td>
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<td>SI7-1</td>
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<td>TB1</td>
<td>JM383, lacZ, hisR</td>
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<td>Plasmids</td>
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<td>pB1533</td>
<td>pUC18 clone containing a 2.35-kb SpH1 fragment from Xanthomonas campestris pv. vesicatoria with avrB2 activity</td>
<td>B. Staskawicz, University of California, Berkeley</td>
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<td>pHM1</td>
<td>cos, parA, lacW, Sp^R, Sm^R</td>
<td>R. Innes, Indiana University</td>
</tr>
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<td>pRK2073</td>
<td>Tra^R, Sp^R</td>
<td>This study</td>
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<td>pXO15-10</td>
<td>pHM1 clone containing X. o. oryzae DNA homologous to avrB2 from X. c. vesicatoria</td>
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<td>pXO16-43</td>
<td>pHM1 clone containing X. o. oryzae DNA homologous to avrB2 from X. c. vesicatoria</td>
<td>This study</td>
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<td>pMM31.10</td>
<td>pXO15-10 containing a Tn5-lac insertion in the avrB2 homologous region</td>
<td>This study</td>
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<tr>
<td>pMM69.11</td>
<td>pXO15-10 containing a Tn5-lac insertion in the avrB2 homologous region</td>
<td>This study</td>
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<td>Phage</td>
<td>Tn5-B20</td>
<td>lac fusion, Km^R</td>
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*Rif^R = rifampicin resistant; Km^R = kanamycin resistant; Na^+ = nalidixic acid resistant; Sm^R = streptomycin resistant; Te^R = tetracycline; Sp^R = spectinomycin resistant.*

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a mortar and pestle. The resulting leaf homogenate was serially
inoculated with sterile water and plated onto PS agar containing
cycloheximide and cephalaxin. The plates were incubated at 28 C,
and colonies were counted 3 days after plating. All experiments
were conducted two times.

The activity of the avrBs2-homologous sequence in X. o. oryzae
was determined by monitoring the multiplication of strains
PX099A and PX086R and their respective mutants in the cultivar
IR24 after leaf-clipping inoculation. Population densities of X. o.
oryzae were determined as described above at 0, 1, 3, 6, and
9 days after inoculation of 51-day-old rice plants. Plant response
to bacterial infection of 10-day-old rice plants was assessed
48 h postinoculation. In addition, lengths of lesions induced by
PX099A and mutants possessing insertions in the avrBs2-
homologous region were assessed 14 days after leaf-clipping
inoculation of IR24 and IRBB21.

RESULTS

Identification and activity of avrBs2-homologous sequences in
X. o. oryzae. Total DNA from Philippine strains of X. o. oryzae,
representing races 1-8, was probed with the 1.9-kb HindIII
fragment from X. c. vesciatoria specific for avrBs2 in Southern
hybridizations. In each case, the avrBs2-specific probe hybridized
to a fragment from X. o. oryzae that was similar in size to the
1.9-kb fragment from X. c. vesciatoria (Fig. 1). Two overlapping
cosmid clones, pXO15-10 and pXO16-43, from the X. o. oryzae
strain PX086 genome library possessed sequences homologous
to avrBs2, as determined by colony blots and Southern hybrid-
ization analysis.

The cosmid clone pXO15-10 was subjected to transposon muta-
genesis, and the positions of the insertions were determined by
restriction enzyme mapping (Fig. 2). Several mutated clones
containing insertions in the 1.9-kb HindIII fragment were identi-
fied and used in marker exchange mutagenesis to introduce the
Tn5 into the chromosomes of strains PX099A and PX086R
(Fig. 3). Insertion of the mutated clone into the chromosome of
X. o. oryzae was confirmed by the absence of a hybridization
signal when mutants were probed with pHM1. In addition, while
the avrBs2-specific probe hybridized with a HindIII fragment of
approximately 1.9 kb from PX099A, insertion of Tn5 into the
avrBs2-homologous region created HindIII fragments of approxi-
amately 2.1 and 4.9 kb in strains PX099-67.1, PX086-13.19, and
PX086-31.10 and fragments of 2.9 and 4.1 kb in strain PX099-
69.11, which hybridized with this probe (Fig. 3). The faint signal
resulting from hybridization of the 1.9-kb avrBs2-specific probe
to the 4.1-kb HindIII fragment of strain PX099-69.11 may result
from the fact that this DNA fragment possesses less than 100
bp of DNA from the avrBs2-homologous region in X. o. oryzae.
The mutants and their respective parental strains were used in
inoculations of 10- and 51-day-old plants of the rice cultivars
IR24 and IRBB21. A water-soaking (susceptible) response was
observed within 48 h postinoculation on both IR24 and IRBB21
after infiltration of 10-day-old plants with PX099A, PX086R,
or their respective mutants. Lengths of lesions produced by
PX099A, PX086R, and derivatives possessing Tn5 insertions in
the avrBs2-homologous region were not significantly different
on either rice cultivar after inoculation of 51-day-old plants (Table
2). Likewise, multiplication of the parental strains and of their
corresponding mutants was similar on the rice cultivar IR24 (Fig. 4).

Disease symptom development. A water-soaking reaction was
observed on all rice cultivars examined when leaves of 10-day-
old seedlings were infiltrated with a cell suspension of PX099A
and on cultivars IR24 and IRBB21 inoculated with PX086R.
Infiltration of PX086R into leaves of IRBB10 resulted in the
induction of a hypersensitive resistance response that was detected
within 48 h postinoculation.

The rice cultivars IR24, IRBB21, and IRBB10 responded differen-
tially to leaf-clipping inoculation with X. o. oryzae strain PX099A,
and the response, in terms of lesion lengths, by any given cultivar
varied with plant age. Lengths of lesions induced by strain PX099A
on 11- and 17-day-old plants did not differ significantly among
the three rice cultivars. However, the development of a resistant
reaction in the cultivar IRBB21 was apparent for plants that were
inoculated at 21 days postemergence; lesion lengths on this cultivar

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Fig. 1. Southern blot analysis of HindIII-digested genomic DNA from
Philippine strains of Xanthomonas oryzae pv. oryzae probed with a
32P-labeled 1.9-kb HindIII fragment from Xanthomonas campestris pv.
vescitoria that is specific for avrBs2. Lane 1, 1-kb ladder; lane 2, avrBs2-
specific 1.9-kb HindIII fragment from X. c. vescitoria; lane 3, X. o.
oryzae strain PXO132 (race 1); lane 4, X. o. oryzae strain PX086 (race
2); lane 5, X. o. oryzae strain PX079 (race 3); lane 6, X. o. oryzae
strain PX0176 (race 3); lane 7, X. o. oryzae strain PX071 (race 4);
lane 8, X. o. oryzae strain PX080 (race 5); lane 9, X. o. oryzae strain
PX099 (race 6); lane 10, X. o. oryzae strain PX054 (race 7); and lane
11, X. o. oryzae strain PX0211 (race 8).

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Fig. 2. Restriction and physical map of the avrBs2-homologous sequence
of Xanthomonas oryzae pv. oryzae. Sites of Tn5-B20 insertions and the
designation of the corresponding X. o. oryzae mutants are shown. E =
EcoR1, and H = HindIII.

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Fig. 3. Southern blot analysis of genomic DNA from Xanthomonas oryzae
pv. oryzae strain PX099A and derivatives of PX099A and PX086R
containing Tn5 insertions in the avrBs2 homologous region. DNA was
digested with HindIII, and the blot was probed with a 32P-labeled 1.9-kb
HindIII fragment from Xanthomonas campestris pv. vescitoria that is
specific for avrBs2. Lane 1, 1-kb ladder; lane 2, X. o. oryzae strain PX099A;
lane 3, X. o. oryzae strain PX099-67.1; lane 4, X. o. oryzae strain PX099-
69.11; lane 5, X. o. oryzae strain PX086-13.19; and lane 6, X. o. oryzae
strain PX086-31.10.

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were significantly less than those observed on either IR24 or IRBB10 (Fig. 5). The relative susceptibility-resistance of the three cultivars was similar for plants inoculated at 21, 31, or 41 days postemergence. Plants inoculated at 51 days postemergence had significantly smaller lesions than had been observed when 21-, 31-, or 41-day-old plants were inoculated with X. o. oryzae. Lesions induced by strain PXO99A on all cultivars at subsequent inoculation periods were smaller than those detected at the previous inoculation period. The lengths of lesions observed on cultivars IR24 and IRBB10 inoculated at 61 and 71 days postemergence are considered to represent a moderately resistant or resistant reaction to the bacterial blight pathogen on the basis of previous classification schemes (17).

Population dynamics of X. o. oryzae in the cultivars IR24 and IRBB21. As was observed for lengths of lesions induced by X. o. oryzae, plant age had a significant effect on the population dynamics of this bacterial pathogen in rice leaves. When plants were inoculated 11 days postemergence, the population dynamics of strain PXO99A were similar in leaves of both IR24 and IRBB21.

Strain PXO99A multiplied to a maximum population of $10^8$ cfu per leaf within 4 days postinoculation, and thereafter the population remained stable or declined gradually (Fig. 6A). The population dynamics of PXO99A on IRBB21 inoculated 21 days postemergence were similar to those observed in leaves of 11-day-old plants inoculated with X. o. oryzae, multiplying to a maximum population of $10^8$ cfu per leaf and then remaining stable (Fig. 6B). In contrast, strain PXO99A continued to multiply in leaves of IR24 inoculated at 21 days postemergence until approximately 12 days postinoculation, and its population was approximately two orders of magnitude larger than that detected in the cultivar IRBB21. Multiplication of PXO99A in leaves of 41-day-old rice plants was comparable to that observed in 21-day-old plants with the exception that maximum bacterial numbers detected in leaves of IR24 were nearly two orders of magnitude larger than had been observed in 21-day-old plants (Fig. 6C). Multiplication of X. o. oryzae after inoculation of 61-day-old plants was significantly restricted in comparison with that observed in 41-day-old plants. Populations of strain PXO99A in both IR24 and IRBB21 at 16 days postinoculation were three orders of magnitude smaller than had been detected in leaves of 41-day-old plants at 16 days postinoculation (Fig. 6C and D).

**DISCUSSION**

The rice resistance gene Xa-21 was previously reported to convey resistance to all tested Philippine and Indian strains of X. o.

**TABLE 2. Lengths of lesions induced by Xanthomonas oryzae pv. oryzae strains PXO99A and PXO86R and mutants possessing transposon insertions in the avrB52-homologous region on the rice cultivars IR24 and IRBB21 after leaf-clip inoculation of 51-day-old rice plants**

<table>
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<tr>
<th>Strain</th>
<th>IRBB21</th>
<th>IR24</th>
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<tbody>
<tr>
<td>PXO99A</td>
<td>3.3 a</td>
<td>11.2 a</td>
</tr>
<tr>
<td>PXO99A-69.11</td>
<td>3.8 a</td>
<td>10.4 a</td>
</tr>
<tr>
<td>PXO99A-67.1</td>
<td>3.4 a</td>
<td>10.0 a</td>
</tr>
<tr>
<td>PXO86R</td>
<td>1.7 b</td>
<td>ND</td>
</tr>
<tr>
<td>PXO86-31.10</td>
<td>2.2 b</td>
<td>ND</td>
</tr>
<tr>
<td>PXO86-13.19</td>
<td>1.6 b</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Means in the same column followed by the same letter are not significantly different ($P = 0.05$) based on Ficher's protected least significant difference.

*Not determined.

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**Fig. 4.** Populations of Xanthomonas oryzae pv. oryzae strains A, PXO99A and B, PXO86R and corresponding mutants possessing Tn5 insertions in the avrB52-homologous region in leaves of the rice cultivar IR24. Leaves were clip inoculated with a bacterial suspension of $10^8$ cfu/ml. Values are means from three replications, and standard errors of mean population size are indicated. Similar results were obtained in two independent experiments.

**Fig. 5.** Lengths of lesions induced by Xanthomonas oryzae pv. oryzae strain PXO99A on rice cultivars IR24, IRBB10, and IRBB21. Leaves (20 per cultivar) of 11-, 17-, 21-, 31-, 41-, 51-, 61-, and 71-day-old plants were clip inoculated with a bacterial suspension of $10^8$ cfu/ml. Lesion lengths were assessed 14 days postinoculation. Values are means from three independent experiments. Standard errors of mean lesion length are indicated.
orzyae (7,10). Khush et al (10) indicated that the level of resistance conferred by this locus was very high as determined after leaf clip inoculation of rice plants. In contrast to these studies, we observed that rice plants possessing Xa-21 were susceptible to the bacterial blight pathogen during the initial 2–3 wk following plant emergence. The response of 10-day-old plants to infiltration of leaves with X. o. orzyae PXO99A or PXO86R was comparable for both the susceptible (IR24) and resistant (IRBB21) cultivars; spreading water-soaked lesions were observed on leaves within 48 h postinoculation. Likewise, lengths of lesions induced by X. o. orzyae PXO99A were similar on both cultivars when plants were inoculated prior to 3 wk postemergence. Reimers and Leach (18) demonstrated that resistance-susceptibility to X. o. orzyae could be distinguished among 10-day-old seedlings on the basis of bacterial multiplication in rice leaves. In this study, there were no differences in multiplications of PXO99A in 11-day-old plants of IR24 and IRBB21, which indicates that resistance conferred by Xa-21 is not expressed in rice seedlings.

Barton-Willis et al (3) demonstrated that multiplication of X. o. orzyae in 21-day-old plants of susceptible and resistant rice cultivars was comparable until bacterial numbers reached 10^2 to 10^6 cfu per leaf. Thereafter, bacterial multiplication in resistant plants was negligible in comparison with that in susceptible cultivars. In this study, similar results were obtained when 21-day-old plants were inoculated with X. o. orzyae. Populations of strain PXO99A in the resistant cultivar IRBB21 increased at 10^6 cfu per leaf, while maximum populations of strain PXO99A in leaves of the susceptible cultivar IR24 were approximately 10^10 cfu per leaf at 16 days postinoculation.

As plants matured, the apparent level of resistance conveyed by Xa-21 increased as defined by lesion length and maximum populations of X. o. orzyae at 16 days postinoculation. Plants exhibited a moderate level of resistance when inoculated at 21 and 41 days postemergence, while plants inoculated at 51 days postemergence or later exhibited increased levels of bacterial blight resistance. Similarly, the cultivar IR24 demonstrated a moderate level of adult plant resistance, which was expressed in plants inoculated at 61 days postemergence or later. Maximum populations of X. o. orzyae and lengths of lesions induced by this pathogen in IR24 declined significantly as plants matured. The population dynamics of strain PXO99A in leaves of IR24 inoculated at 61 days postemergence were similar to those observed in the resistant cultivar IRBB21 when inoculated at 41 days postemergence. The results obtained for IRBB10 and IRBB21 also support the existence of mature plant resistance in IR24. These near-isogenic lines were generated with IR24 as the backcross parent, and thus all three cultivars possess a similar genetic background, with the exception of the specific resistance loci Xa-10 and Xa-21. Each of these rice cultivars demonstrated a similar increase in resistance to the bacterial blight pathogen as plants matured, which was expressed as reduced bacterial populations and/or lesion lengths.

These findings have significant implications for breeding programs directed toward the development of improved rice cultivars that possess resistance to bacterial blight. Several improved rice cultivars, including IRBB10 and IRBB21, have been generated with IR24 as the recurrent parent. In screening progeny for resistance to X. o. orzyae, IR24 has been utilized as the susceptible host plant. This procedure is suitable provided that screening for resistance is conducted prior to the booting stage when adult plant resistance is expressed in IR24. The results of this study also demonstrate the possible risk of screening for disease resistance during a single phase of plant development rather than at multiple growth stages. While IRBB21 possessed a high level of resistance to X. o. orzyae in adult plants, seedlings were susceptible to bacterial blight, and plants possessed only a moderate level of resistance through the fifth-leaf stage of plant growth (approximately 50 days postemergence) under the conditions of these experiments. Thereafter, IRBB21 expressed a high level of resistance to race 2 and race 6 strains of X. o. orzyae.

The variation in resistance of IRBB21 and IR24 to bacterial blight observed at different growth stages is in agreement with
previous work conducted with other rice genotypes (24). In general, rice cultivars that express a moderate level of resistance to X. o. oryzae at the seedling stage either maintain a similar level of resistance or become more resistant as the plant matures (15). This type of resistance describes that observed in this study for IRBB21 to both race 2 and race 6 strains of X. o. oryzae. An increase in resistance with growth stage is most pronounced in rice cultivars that express adult plant resistance (15). In this study, lesions induced by X. o. oryzae on IR24 inoculated at 71 days postemergence were as much as 72% smaller than those observed on 31-day-old plants. Although adult plant resistance usually is observed at leaf position 10 or 11 (24), resistance in IR24 was generally expressed at leaf position 5 or 7.

In previous studies, sequences homologous to avrBs2 from X. c. vescicatoria have been identified in several other pathovars of X. campestris (9). The avrBs2-homologous sequences from X. c. alfalfae, X. c. malvacearum, X. c. phaseoli, X. c. vignicola, and X. c. vitians were shown to confer avrBs2 activity. In addition, mutations in avrBs2 reduced the ability of X. c. alfalfae to multiply in susceptible host tissues in a manner similar to that observed for X. c. vescicatoria (9). In this study, sequences homologous to avrBs2 were identified in strains of all Philippine races of X. o. oryzae, and no polymorphisms were detected within this locus. All strains possessed similar HindIII and SpH1 fragments of approximately 1.9 and 2.3 kb, respectively, that hybridized with the avrBs2-specific probe from X. c. vescicatoria. The widespread distribution of this sequence and the ability of the Xa-21 locus to convey multirace resistance led to our investigation of whether the avrBs2 homologue in X. o. oryzae conferred avrXa21 activity. Mutations in the avrBs2-homologous sequence, when introduced into PXO99A or PXO86R, had no effect on the ability of these strains to multiply in the susceptible cultivar IR24 and did not alter the development of an incompatible interaction when inoculated onto the resistant cultivar IRBB21. Therefore, the avrBs2-homologous sequence does not confer avrXa21 activity in X. o. oryzae. In addition, unlike several other X. campestris pathovars, the avrBs2 homologue from X. o. oryzae does not appear to have an important role in the fitness of this bacterium during pathogenesis, since it is not required for full virulence on susceptible rice cultivars. Interestingly, the avrBs2-homologous sequence from X. c. holcicola, a pathogen of sorghum, did not have avrBs2 activity (9). Thus, the avrBs2 homologue from these two bacterial pathogens, which infect monocotyledonous plants, do not appear to function in the manner described for seven of eight X. campestris pathovars that are pathogenic toward dicotyledonous plant species. Further experiments involving the replacement of avrBs2 in X. c. vescicatoria with the homologous sequence from X. o. oryzae and X. c. holcicola will be necessary to determine whether these sequences are functional or whether expression of the avrBs2-homologous sequence is suppressed in these bacteria.

LITERATURE CITED