Distribution of *Xanthomonas oryzae* pv. *oryzae* Strains Virulent to *Xa21* in Korea

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ABSTRACT

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Strains of *Xanthomonas oryzae* pv. *oryzae* that are virulent to rice lines carrying the *Xa21* resistance gene were widely distributed in Korea. A total of 105 strains collected during 1987 to 1996 in Korea was characterized by pathogenicity tests and restriction fragment length polymorphism analysis of the *XorII* methyltransferase (*xorIIM*) and *avrXa10* genes. Although the lesion lengths on rice line IRBB21, which carries *Xa21*, decreased as plant age increased, resistance and susceptibility of the plants to 31 strains were clearly differentiated at the seedling (14, 21, and 28 days old), maximum tillering, and flag leaf stages. The resistance or susceptibil-

ity of seedlings was correlated with bacterial populations within an inoculated leaf. There was a significant change in the population structure of *X. oryzae* pv. *oryzae* with regard to virulence to *Xa21* over the last 10 years; this change in population was confirmed by genome analysis. Lineage I, which is avirulent to *Xa21* and does not have a genomic *xorIIM* homolog, was the predominant lineage found between 1987 and 1989, while lineage II, which is virulent to *Xa21* and contains the *xorIIM* homolog, was predominant in strains collected between 1994 and 1995. Our results demonstrate that introduction of *Xa21* into commercial rice should be based on the regional structure of *X. oryzae* pv. *oryzae* populations and suggest that *Xa21* will not be useful in Korea.

Additional keywords: bacterial blight.

Xanthomonas oryzae pv. *oryzae*, the causal agent of bacterial blight (BB), is responsible for severe losses to rice (*Oryza sativa* L.) in tropical, subtropical, and warm temperate regions of the world (25). Host resistance has been used to control the disease, but *X. oryzae* pv. *oryzae* strains show extensive pathogenic differentiation and many pathotypes have been identified in major rice-growing countries (17,19,31). Temporal and spatial variations of the pathogen population have been regarded as factors determining an outbreak of the disease (17,19), which suggests that characterization of the pathogen population is a prerequisite for practical application of host resistance and other control strategies.

Host resistance is the only effective control method for the disease; therefore, breeding for resistance has been important in many countries (18,22,24). More than 21 BB resistance genes have been identified and characterized (22–24). Recently, a new resistance gene, designated *Xa21*, was identified in the wild species *O. longistaminata* and was introduced into cultivated rice (IR24) to generate the isogenic line IRBB21 (10,24). The *Xa21* gene was cloned from IRBB21 and transformed into other rice cultivars (27,29). Genetic analysis of the transgenic plants with *Xa21* confirmed that *Xa21* alone conferred resistance to 29 diverse strains from eight countries that were shown to be genetically distinct based on pathotype and lineage analysis (2,13,21,29). Resistance conferred by *Xa21* to six races from the Philippines was inherited in a dominant manner and functioned at the same level in both *japonica* and *indica* rice genotypes as it did in *O. longistaminata* (29).

All previous information suggested that *Xa21* would be a very promising resistance gene for protection of cultivated rice. However, we previously identified a few Korean *X. oryzae* pv. *oryzae* strains that were virulent on IRBB21; these strains were used by Wang et al. (29) to investigate the resistance spectrum of the *Xa21* gene. *Xa21* has not been used in commercial rice in any country; therefore, we sought to determine the origin and distribution of virulence in *X. oryzae* pv. *oryzae* to *Xa21*. In this article, we evaluate the virulence of *X. oryzae* pv. *oryzae* strains collected during the last 10 years in Korea and present evidence that many strains are virulent to the *Xa21* gene in spite of the fact that this gene has not been deployed in Korea. The temporal change of the Korean *X. oryzae* pv. *oryzae* population for virulence to *Xa21* was investigated by virulence analysis and restriction fragment length polymorphism (RFLP) of genomic DNA. For RFLP studies, clones of the *XorII* methyltransferase (*xorIIM*) (5) and *avrXa10* (7) genes were used as probes. These have previously been used to characterize *X. oryzae* pv. *oryzae* populations (6,12,21).

Resistance of rice to BB has usually been evaluated quantitatively (3,8,11,22). Recently, the resistant reaction of 12 resistance genes (*Xa1* through *Xa12*) was classified into three infection types, but *Xa21* was not included in this study (9). Mazzola et al. (16) reported that the resistance of IRBB21 to Filipino race 6 strain PXO99A was expressed moderately at 21 days after emergence and that the level increased with plant maturity; 17-day-old plants were not resistant. Determination of the appropriate growth stage for evaluation of resistance genes is important in breeding programs; therefore, the resistance of IRBB21 also was characterized in this study by symptom severity and bacterial multiplication in planta at three different growth stages using a large number of *X. oryzae* pv. *oryzae* strains collected from different locations in different years throughout Korea.

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MATERIALS AND METHODS

Bacterial strains, plasmids, and rice cultivars. All strains of *X. oryzae* pv. *oryzae* used in this study were collected from farmers' fields in Korea during 1987 to 1989 and 1994 to 1996 (Fig. 1). All strains collected were first tested for pathogenicity using susceptible Korean rice cv. Milyang 23 (M23) (32). Pure culture of all strains were maintained at the Plant Pathology Division, National Institute of Agricultural Science and Technology, RDA, Suwon, Korea. Bacterial strains were maintained as suspensions in 10% sterile glycerol at -80°C and retrieved by plating on peptone sucrose agar (PSA) medium (28). Fresh cultures revived from the stock were not transferred more than two or three times before testing pathogenicity.

Plasmids pE7.0 and pBSavrXa10 were used as probes to analyze the genome of *X. oryzae* pv. *oryzae*. pE7.0 carries the *xorIIM* gene, a very short patch repair endonuclease gene (*vsr*), and approximately 5 kilobases (kb) of genomic DNA upstream of *vsr* (4, 5). pBSavrXa10 (obtained from J. E. Leach, Department of Plant Pathology, Kansas State University) carries a 3.2-kb fragment of the *avrXa10* gene cloned into pBluescript II KS+ (7). The *X. oryzae* pv. *oryzae* genome contains multiple copies of DNA homologous to the *avrXa10* gene, but only a single copy of the fragment represented by pE7.0 (4,5,7).

Seed of rice line IR24 and the isogenic line IRBB21 that contains *Xa21* (24) was obtained from the International Rice Research Institute, Los Baños, Philippines. Korean rice cv. M23 was used as a susceptible check. M23 is a Korean BB differential variety that has no resistance to Korean strains of *X. oryzae* pv. *oryzae* (32).

Plant inoculation and bacterial enumeration in planta. Plants were grown in plastic pots ($5 \times 15 \times 10$ cm; five plants per pot) in the greenhouse for evaluation of resistance at the seedling stage. In the field, plants were transplanted at 30 days after seeding at a spacing of 21×27 cm for evaluation of resistance at the maximum tillering and flag leaf stages. Fertilizer was applied at 220-70-80 (N-P₂O₅-K₂O) kg/ha.

Inoculum was prepared by suspending bacteria, which had been grown on PSA for 2 to 3 days at 28°C , in sterile distilled water. Inoculum density was adjusted to 10^9 CFU/ml. Plant inoculation was carried out by clipping the tip (about 1 to 2 cm) of the fully expanded uppermost leaf with scissors that had been dipped into the inoculum (25).

Plants were 14, 21, and 28 days old at the time of inoculation. Lesion lengths on leaves were measured at 10 days after inoculation (DAI) for 14-day-old and at 14 DAI for 21- and 28-day-old plants. At both maximum tillering and flag leaf stages, the lesion length was measured at 21 DAI. Each cultivar-strain combination was replicated three to five times, and each replicate consisted of five leaves. The plant reaction was rated according to lesion length as resistant (<3 cm), moderately resistant (3.1 to 5.0 cm), moderately susceptible (5.1 to 7.0 cm), and susceptible (≥ 7.1 cm). In some cases, responses were simply categorized as resistant (≤ 5.0 cm) and susceptible (≥ 5.1 cm).

Bacterial populations in plants were enumerated by viable cell counts at 0, 1, 5, and 10 DAI for 14-day-old seedlings and 0, 1, 4, 8, 12, and 16 DAI for 28-day-old seedlings. Individual leaves were ground in 3 ml of sterile distilled water and sterile sand using a mortar and pestle. The suspensions were then appropriately diluted and 20- μl aliquots were spotted on duplicate PSA plates. After incubation for 48 h at 28°C , the number of colonies formed on each plate was counted. Each treatment was replicated three times, and each replicate consisted of one leaf.

Analysis of genomic DNA. Genomic DNA of *X. oryzae* pv. *oryzae* was isolated as described by Leach et al. (15). Plasmid DNA was extracted by the alkaline lysis method (20). Restriction enzymes were purchased from Promega Biotech (Madison, WI) and reactions were performed as described by the manufacturer. Electrophoresis and transfer to nylon membranes were as described (12). DNA for probes was labeled using a nonradioactive digoxigenin-dATP labeling and detection kit, and hybridization and subsequent washing were conducted according to the manufacturer's specifications (cat. no. 1363514; Boehringer Mannheim Biochemicals,

Fig. 1. Map of the Korean peninsula and collection sites of *Xanthomonas oryzae* pv. *oryzae* strains from farmers' fields during 1987 to 1996. ● = Collection sites during 1987 to 1989; and □ = collection sites during 1994 to 1996. E128° and N36° indicate longitude and latitude, respectively.

TABLE 1. Number of Korean strains of *Xanthomonas oryzae* pv. *oryzae* producing different lesion lengths on rice cvs. IRBB21 and Milyang 23 at the flag leaf stage^a

Collection year	Milyang 23				IRBB21			
	$\leq 3.0^b$	3.1–5.0	5.1–7.0	≥ 7.1	≤ 3.0	3.1–5.0	5.1–7.0	≥ 7.1
1987	0	0	0	7	6	0	0	1
1988	0	0	0	11	9	0	1	1
1989	0	0	0	8	7	0	0	1
1994	0	0	0	4	0	0	3	1
1995	0	0	0	57	3	0	5	49
1996	0	0	0	18	0	0	2	16
Total	0	0	0	105	25	0	11	69
MLL ^c	...	...	...	13.2	0.5	...	6.4	8.7

^a Leaves were inoculated by clipping method.

^b Lesion length (cm) was the average of five plants and was assessed 21 days after inoculation.

^c Mean lesion length (cm) of the strains at each group.

Indianapolis IN). Restriction fragments that hybridized to the probe DNA were detected by color reaction or on X-ray film.

The presence of *xorII*M was determined by hybridization of probe pE7.0 with genomic DNA. Polymorphism of genomic DNA detected by hybridization with pBSavrXa10 was recorded as described (2), and the data were converted into binary data (1 and 0) representing the presence or absence of a fragment. The genetic similarity among strains was calculated from the hybridization patterns by the unweighted pair group method with arithmetic means using NTSYS-pc (26). The robustness of the clusters was tested by bootstrap analysis using the WinBoot program (30).

RESULTS

Predominance of *X. oryzae* pv. *oryzae* strains virulent to *Xa21* in Korea. Between 1987 and 1989, 26 strains of *X. oryzae* pv. *oryzae* were collected from 13 districts, and between 1994 and 1996, 79 strains were collected from 31 districts (Fig. 1 and Table 1).

The reactions of IRBB21 and M23 to 105 Korean strains was classified into four groups based on symptom severity (Table 1). The Korean rice cv. M23 was susceptible to all 105 strains (13.2 cm in average lesion length). Out of 26 strains, only 4 (15.4%) collected during 1987 to 1989 were compatible with IRBB21, while 76 strains out of 79 (96.2%) collected during 1994 to 1996 were virulent to IRBB21. This suggests that strains of *X. oryzae* pv. *oryzae* that are virulent to *Xa21* are widely distributed in Korea.

Differential reaction of IRBB21 to *X. oryzae* pv. *oryzae* at different plant growth stages. To investigate the effect of plant age on the resistant reaction conferred by *Xa21* to *X. oryzae* pv. *oryzae*, rice plants of cvs. IR24 and IRBB21 (with *Xa21*) were inoculated at seedling (14-, 21-, and 28-day-old plants), maximum tillering (73-day-old plants), and flag leaf (112-day-old plants) stages with four Korean strains (KXO19, KXO20, KXO377, and KXO400).

When leaves of 14-day-old rice seedlings were inoculated, IR24 showed curling and water-soaked lesions at 5 DAI. The lesions were continuous and extended to the leaf base. Lesion lengths on IR24

Fig. 2. Lesion length and number of bacteria (CFU per leaf) in rice plants inoculated with Korean strains of *Xanthomonas oryzae* pv. *oryzae* at different growth stages. Plants were inoculated by the leaf clipping method (25). **A and B**, 14-day-old plants; **C and D**, 28-day-old plants; **E**, maximum tillering (MT) stage; and **F**, flag leaf (FL) stage. □-□ = KXO19; ○-○ = KXO20; ■-■ = KXO377; and ●-● = KXO400. Bars represent the standard deviation of three to five replicates.

ranged from 10 to 11 cm at 10 DAI, and there were not any significant differences among strains (Fig. 2A). However, there was a distinct difference in symptom severity among strains when IRBB21 was inoculated. Lesion lengths on IRBB21 were similar to those on IR24 when plants were inoculated with KXO377 (10.7-cm lesions) and KXO400 (11-cm lesions). In contrast, no leaf curling or water-soaking occurred when IRBB21 was inoculated with strains KXO19 and KXO20; however, yellow to brown lesions <4 cm in length developed (Fig. 2A). Bacterial populations in IR24 increased continuously until 5 DAI and reached 10^8 to 10^9 CFU per leaf (Fig. 2B). There was no difference among strains in bacterial multiplication rates and the final populations in IR24. However, major differences among strains were found in resistant cv. IRBB21. We observed a rapid increase in bacterial cell numbers in the leaves with KXO377 and KXO400; the multiplication rate and final numbers were the same as seen in the susceptible cv. IR24. The total number of bacteria per leaf in plants inoculated with KXO19 and KXO20 was less than one-tenth that observed in those inoculated with KXO377 and KXO400 at 5 DAI (Fig. 2B).

When 21-day-old seedlings were inoculated, lesion lengths observed on the susceptible cv. IR24 ranged from 11.8 to 15.1 cm, while those on IRBB21 averaged 1.7, 3.9, 10.2, and 11.0 cm when inoculated with KXO19, KXO20, KXO377, and KXO400, respectively (data not shown). The reactions of 28-day-old plants of IR24 and IRBB21 were similar to those of 14- and 21-day-old seedlings in symptom severity and bacterial cell multiplication in planta (Fig. 2C and D). A differential reaction of IRBB21 was also observed at the maximum tillering and flag leaf stages to the same strains used for seedling experiments. Although symptom severity on IRBB21 at maximum tillering and flag leaf stages was not severe, the re-

TABLE 2. Lesion length on IRBB21 when inoculated with 31 Korean strains of *Xanthomonas oryzae* pv. *oryzae* at three different growth stages

Strain	Lesion length (cm) at each stage ^a		
	SD	MT	FL
KXO18	5.6 ± 1.5	0.9 ± 0.4	0.2 ± 0.2
KXO19	5.4 ± 1.1	1.4 ± 1.2	0.5 ± 0.1
KXO21	5.0 ± 1.0	0.9 ± 0.4	0.5 ± 0.0
KXO42	3.8 ± 1.2	1.1 ± 0.4	0.5 ± 0.1
KXO78	4.3 ± 1.7	1.0 ± 0.2	0.5 ± 0.1
KXO82	1.6 ± 0.3	1.0 ± 0.3	0.5 ± 0.1
KXO83	4.0 ± 0.5	0.5 ± 0.1	0.5 ± 0.2
KXO84	6.7 ± 2.0	1.0 ± 0.2	0.5 ± 0.1
KXO87	4.2 ± 0.8	0.6 ± 0.4	0.2 ± 0.2
KXO88	3.0 ± 1.1	0.5 ± 0.1	0.5 ± 0.1
KXO89	2.3 ± 0.7	0.7 ± 0.7	0.5 ± 0.1
KXO90	14.4 ± 1.0	16.5 ± 2.8	10.4 ± 1.9
KXO91	5.2 ± 1.3	1.0 ± 0.2	0.5 ± 0.1
KXO92	5.9 ± 2.2	0.5 ± 0.1	0.5 ± 0.1
KXO93	1.9 ± 0.2	0.5 ± 0.1	0.2 ± 0.1
KXO95	7.7 ± 1.9	1.2 ± 2.1	0.3 ± 0.2
KXO97	3.3 ± 1.2	0.4 ± 0.1	0.5 ± 0.1
KXO99	2.7 ± 0.8	1.2 ± 1.5	0.5 ± 0.1
KXO102	3.9 ± 0.8	1.1 ± 0.5	0.5 ± 0.2
KXO103	8.1 ± 2.4	1.9 ± 0.9	0.5 ± 0.1
KXO112	4.1 ± 0.7	1.0 ± 0.3	0.5 ± 0.1
KXO131	1.8 ± 1.0	1.0 ± 0.4	0.5 ± 0.1
KXO138	2.5 ± 0.8	0.8 ± 0.4	0.5 ± 0.1
KXO140	4.7 ± 0.3	1.4 ± 0.8	0.5 ± 0.1
KXO169	17.7 ± 1.0	13.3 ± 2.7	7.1 ± 2.1
KXO171	5.7 ± 1.5	0.9 ± 0.4	0.5 ± 0.1
KXO172	3.6 ± 1.1	0.9 ± 0.2	0.5 ± 0.1
KXO342	14.0 ± 1.8	13.2 ± 4.0	6.4 ± 1.1
KXO377	16.2 ± 2.2	11.0 ± 1.9	6.3 ± 1.3
KXO378	15.5 ± 1.3	10.2 ± 3.5	6.3 ± 1.7
KXO400	13.8 ± 3.5	12.3 ± 3.5	10.7 ± 1.7

^a Leaves were inoculated with a bacterial suspension (10^9 CFU/ml) by the clipping method (25). SD = seedling stage, 21 days after seeding (DAS); MT = maximum tillering stage, 73 DAS; and FL = flag leaf stage, 112 DAS. Lesion length was assessed 14 days after inoculation at the SD stage, and 21 days after inoculation at the MT and FL stages. Lesion lengths are the average of five plants.

sistant reaction caused by KXO19 and KXO20 was distinct from the susceptible reaction caused by KXO377 and KXO400 (Fig. 2E and F).

The reactions of IRBB21 at three different growth stages was repeated with 28 additional Korean strains collected during 1987, 1988, 1989, and 1994 (Table 2). The average lesion length at the seedling stage when inoculated with the six virulent strains (KXO90, KXO169, KXO342, KXO377, KXO378, and KXO400) was 15.3 cm, while that at the maximum tillering and flag leaf stages was 12.8 and 6.7 cm, respectively. Lesion lengths at the seedling, maximum tillering, and flag leaf stages were 4.3, 0.9, and 0.5 cm, respectively, when inoculated with the other 25 strains. The differential reaction of IRBB21 to the six virulent strains compared with the other 25 strains at all three growth stages (Table 2) indicated that resistance in IRBB21 conferred by the *Xa21* gene was expressed well at all the growth stages.

Characterization of the *X. oryzae* pv. *oryzae* strains virulent to *Xa21* by genome analysis. A total of 87 Korean *X. oryzae* pv. *oryzae* strains was differentiated into two groups based on hybridization with pE7.0. In some strains, two *EcoRV* fragments of genomic DNA hybridized with pE7.0 (Fig. 3A). In other strains, no hybridization was observed (Fig. 3A). Only a single 13- to 15-kb *EcoRI* fragment hybridized with probe pE7.0 (data not shown; 5,6). This suggests that the multiple *EcoRV* fragments hybridizing with the probe (Fig. 3A) were parts of a contiguous segment of DNA that contained *xorIIM*, *vsr*, and flanking DNA upstream of the *vsr* or downstream of *xorIIM*.

RFLP analysis of the same 87 strains was performed by hybridization of *EcoRI*-digested genomic DNA with pBSavrXa10. Differences among strains were obvious in the fragments between 2.6 and 10 kb (Fig. 3B). For example, strains KXO85, KXO169, KXO377, KXO400, and KXO635, which contained the *xorIIM* homolog, have the 6.4- and 7.8-kb fragments in common. In contrast, the *xorIIM*-negative strains, for example KXO19, KXO21, KXO112, KXO140, and KXO599, did not have the 6.4- and 7.8-kb fragments, but instead contained 4.0- and 6.7-kb fragments. Therefore, *xorIIM*-positive strains were distinguishable from *xorIIM*-negative strains by the lack of a 6.7-kb fragment and the presence of 6.4- and 7.8-kb frag-

Fig. 3. Restriction fragment length polymorphism of genomic DNA from Korean strains of *Xanthomonas oryzae* pv. *oryzae*. DNA was **A**, digested with *EcoRV* and hybridized with plasmid pE7.0 or **B**, digested with *EcoRI* and hybridized with pBSavrXa10. Plasmid pE7.0 contains the *XorIIM* methyltransferase gene (*xorIIM*), a very short patch repair endonuclease gene (*vsr*), and 5 kb of DNA upstream of *vsr* (4,5). Plasmid pBSavrXa10 contains internal fragments of the *avrXa10* gene (7). Strains KXO19, KXO112, and KXO140 were collected in 1988 and KXO21, KXO85, and KXO169 in 1989. KXO377 and KXO400 were collected in 1994, and KXO599 and KXO635 in 1995. All strains were collected from different counties.

ments (Fig. 3A and B). However, significant variation was present among the genomes of strains within the *xorIIM*-negative or -positive groups. For example, *xorIIM*-positive strains showed variation in the presence of 2.6-, 2.8-, 4.0-, 4.9-, 9.1-, and 10.2-kb fragments, while *xorIIM*-negative strains varied in the occurrence of 4.9-, 5.4-, and 9.1-kb fragments (Fig. 3A and B).

Genetic similarity calculated from the RFLP data produced by hybridization of *EcoRI*-digested genomic DNA with the *avrXa10* probe is shown in Figure 4. The confidences for the dendrogram groupings were 74.1 and 76.0% for lineage I and lineage II, respectively, but subgroups within these lineages showed low confidences that ranged from 22.6 to 39.3% (Fig. 4). Strains in *avrXa10*-RFLP lineage I did not contain the genomic region containing *xorIIM* and *vsr*, while strains in *avrXa10*-RFLP lineage II contained the region. The strains named with numbers lower than 300 in lineage I were collected during 1987 to 1989, while those with numbers larger than 300 in lineage II were collected during 1994 to 1995 (Fig. 4). A perfect correlation between presence of a *xorIIM* homolog and pathogenicity to *Xa21* in recent lineage II strains suggested that the current Korean *X. oryzae* pv. *oryzae* population is genetically distinct from the *xorIIM*-negative population (lineage I) that was predominant during the 1980s in Korea (Fig. 4).

Fig. 4. Dendrogram displaying the genetic relationships among 87 Korean strains of *Xanthomonas oryzae* pv. *oryzae* collected during 1987, 1988, 1989, 1994, and 1995. Similarity coefficients were calculated from the restriction fragment length polymorphism data produced by hybridization of *EcoRI*-digested genomic DNA with pBSavrXa10. Strains were grouped by the unweighted pair group method with arithmetic means. Bootstrap analysis with 1,000 iterations was performed using the WinBoot program (30); numbers on the branches are percentage of confidence. The strains with numbers lower than 300 were collected from 1987 to 1989. Four strains (KXO342, KXO377, KXO378, and KXO400) were collected in 1994. Strains with numbers larger than KXO576 were collected in 1995. The second column indicates the presence (+) or absence (-) of an *xorIIM* homolog. The right column indicates virulence (+) or avirulence (-) to rice cv. IRBB21.

DISCUSSION

This article reports virulence in Korean strains of *X. oryzae* pv. *oryzae* to rice carrying the *Xa21* gene, which has been reported to confer broad-spectrum resistance to BB (29). We characterized a large number of these strains by pathogenicity tests and genotypic analysis. The *X. oryzae* pv. *oryzae* strains virulent to *Xa21* were distributed over a wide region and have been recovered at a high frequency in Korea since 1994 (Fig. 1 and Table 1), even though the *Xa21* gene has not been used in commercial rice in Korea. These findings pose questions concerning the origin and increase of strains virulent to *Xa21*.

Based on the gene-for-gene concept, virulence to *Xa21* should result from the absence or inactivation of a postulated bacterial avirulence gene (*avrXa21*), which is complementary to the host resistance gene *Xa21* (14). Avirulence of most southeast Asian strains (10,29) and many Korean strains of *X. oryzae* pv. *oryzae* (Fig. 4) on *Xa21* lines implies that *avrXa21* is widely distributed in Asian populations of *X. oryzae* pv. *oryzae*. However, we do not know whether the virulence of Korean strains to *Xa21* results from a mutation in *avrXa21* or from the absence of this avirulence gene.

Geographic migration patterns of *X. oryzae* pv. *oryzae* in Asia have been proposed based on genome RFLP analysis and the presence or absence of restriction-modification systems of the bacteria (6). Based on the organization of the *xorIIM* locus and flanking region, the Korean *X. oryzae* pv. *oryzae* strain KXO169 (formally JW89011 in literature citations 5, 6, and 29), which is virulent to *Xa21* (Fig. 4), or its parental lineage might have migrated to Korea from southeast Asia (6). This is consistent with the recent identification of virulence in a few Filipino strains to *Xa21* (M. R. S. Madamba, C. M. Vera Cruz, and H. Leung, *personal communication*). However, all of the tested Filipino strains with the *xorIIM* homolog were avirulent (6,29), while all of the Korean strains having *xorIIM* homolog, including KXO169, are virulent (Fig. 4). Thus, the virulence of *X. oryzae* pv. *oryzae* to *Xa21* that we observed in this study probably originated in Korea.

Particular rice genotypes are known to apply selection pressure to the *X. oryzae* pv. *oryzae* population structure (19). In the Philippines, the population structure changed from a predominance of race 1 (avirulent to *Xa4*) to races that are virulent to rice with *Xa4* after the release of rice cultivars with the *Xa4* gene (19). However, the rapid change of the Korean population from avirulence to virulence to *Xa21* could not be due to a direct selection pressure of resistance gene *Xa21*, because this gene has not been used in Korean rice cultivars. The *Xa10* gene has also never been used in commercial rice in Korea, but all of the *X. oryzae* pv. *oryzae* strains collected from 1987 to 1996 in Korea were highly virulent to the rice cv. IRBB10, which contains *Xa10* (S. H. Choi, *unpublished data*). These examples of the absence of *Xa10* and *Xa21* and the wide distribution of virulence to these genes in the Korean *X. oryzae* pv. *oryzae* population suggest that a structural change of pathogen population is driven by factors other than the major resistance genes in commercial cultivars. Bacterial populations are clonal in nature; therefore, strong selection for any fitness trait would concomitantly increase the frequency of all traits associated with that lineage, so that virulent pathotypes could increase in the absence of the related resistance genes. Both the wide distribution of virulent strains and their rapid increase in Korea (Table 1 and Fig. 1) suggest that the buildup of indigenous lineage II strains could have occurred simultaneously at many locations.

The resistant reaction conferred by *Xa21* occurred at all growth stages tested, including 14-, 21-, and 28-day-old seedlings (Fig. 2), with 31 different *X. oryzae* pv. *oryzae* strains (Table 2). These findings demonstrate that assessment of resistance can be carried out as early as 14 or 21 days after planting, even though a previous study by Wang et al. (29) used 6- to 8-week-old plants.

The wide distribution of *X. oryzae* pv. *oryzae* strains virulent to *Xa21* in Korea, Nepal (1), and the Philippines (M. R. S. Madamba,

C. M. Vera Cruz, and H. Leung, *personal communication*) cautions that introduction of the *Xa21* gene into cultivated rice should be based on a detailed characterization of the *X. oryzae* pv. *oryzae* population in a given region. For example, this gene would not be useful in Korea, because more than 96% of the recent (during 1994 to 1996) Korean strains were virulent to IRBB21 (Table 1).

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