Dynamics of *Xanthomonas oryzae* pv. *oryzae* Populations in Korea and Their Relationship to Known Bacterial Blight Resistance Genes

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ABSTRACT

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Developing resistant cultivars requires an understanding of the dynamics of the pathogen populations as well as the genetics of host resistance. Bacterial leaf blight (BB), caused by the vascular pathogen *Xanthomonas oryzae* pv. *oryzae*, has become one of the most devastating diseases of rice. We demonstrate here the quantitative analyses of responses of near-isogenic lines carrying various BB resistance (*R*) genes and *R*-gene combinations against 16 *X. oryzae* pv. *oryzae* isolates repre-

senting Korean BB pathotypes. The estimated main effects of each *R* gene against the 16 isolates identified prominent differences in BB pathotypes between Korea and other countries. Three major aspects of our quantitative observations and statistical analysis are (i) strong and broad resistance of *xa5*; (ii) independent and additive genetic actions of *Xa4*, *xa5*, and *Xa21* under digenic or trigenic status; and (iii) a strong quantitative complementation effect contributed by the functional alleles of *Xa4* and *Xa21*. We conclude that the pyramid line containing genes *Xa4*, *xa5*, and *Xa21* would be the most promising and valuable genotype for improving Korean japonica cultivars for BB resistance.

Additional keywords: marker-aided selection, pathogenicity.

Bacterial leaf blight (BB), caused by the vascular pathogen *Xanthomonas oryzae* pv. *oryzae*, has become one of the most devastating diseases of rice because of the extensive cultivation of nitrogen-responsive modern rice cultivars (23). Plants are infected at the maximum tillering stage, resulting in a yield reduction from 20 to 40% (23). Early BB infection also causes severe yield reduction up to 50% (28). In Korea, southwestern coastal plain areas are hot spots for BB pathogen evolution and new variants of the pathogen spread into the mid-northern plains. In 2003, a serious BB epidemic occurred in the southwestern coastal areas with significant yield loss due to the emergence of a new race, K3a (26).

A number of *X. oryzae* pv. *oryzae* genes involved in virulence have been found and it has been demonstrated that *X. oryzae* pv. *oryzae* virulence is complex. However, after extensive study, the mechanisms related to virulence of *X. oryzae* pv. *oryzae* have been well characterized compared with other bacterial diseases (2, 13, 16, 31, 42, 46). More than 25 major BB resistance (*R*) genes of rice have been identified, and some are tagged with molecular markers (18, 30, 44). Recently, the genes *Xa4*, *Xa7*, and *xa13* have been physically mapped and *Xa1*, *xa5*, *Xa21*, *Xa26*, and *Xa27* have been cloned (6, 9, 29, 32, 36–38, 45). These advances offer the possibility of introducing effective *R* genes into susceptible cultivars more efficiently (33, 35). Although host-plant resistance is the most economical approach for disease control, understanding of the population biology of the pathogen and the genetics of resistance allows the judicious use of genetic resources. Studies using molecular genotyping methods on diversity, distribution of

X. oryzae pv. *oryzae*, and the relationship between phylogeny and virulence have provided better information on population structures and their evolution and have indicated that BB pathogen populations from different Asian countries were distinct (1, 4, 14, 25).

Korean BB isolates have been grouped into five races (K1 to K5) by using five rice cultivars as an *X. oryzae* pv. *oryzae* differential system. Many of the Korean BB isolates are grouped into three main races, K1, K2, and K3, and races K4 and K5 have been reported rarely (17, 26). DNA fingerprinting analysis using *xorII* methyltransferase (*xorIIM*) and *avrXa10* genes as probes on 105 Korean isolates, collected during 1987 to 1996, grouped the isolates into four haplotypes, clustered into two lineages (19). Virulence reactions of 66 field-collected *X. oryzae* pv. *oryzae* isolates during 1982 to 1992 against Korean, Japanese, and International Rice Research Institute (IRRI) differential cultivars suggested that the population structure of Korean *X. oryzae* pv. *oryzae* races is markedly distinct from that of the Philippines and Japan, for which 64 and 58% of tested Korean *X. oryzae* pv. *oryzae* isolates could not be classified into Japanese and IRRI pathotypes, respectively (3). Recent pathotyping results indicated that Korean race K1 showed a decreasing trend by the spread of rice cultivars with the *Xa1* and *Xa3* genes. However, races K2 and K3 have increased in Korea (26). The Korean *X. oryzae* pv. *oryzae* population evolves at a faster rate even in the absence of *R* genes (19).

It is difficult to accurately identify the actual genetic diversity of natural pathogen populations because of the co-evolution of pathogens and host plants (5). Therefore, the effective use of genetic resources for BB resistance requires efficient characterization of pathogen populations and careful selection of *R* genes with suitable deployment strategies. Testing *X. oryzae* pv. *oryzae* isolates from each lineage or haplotype defined by DNA finger-

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printing of *X. oryzae* pv. *oryzae* against near-isogenic lines (NILs) with single and multiple *R* genes may allow the identification of suitable resistance genotypes and deployment strategies as well as efficient characterization of the resistance spectra of the genes in relation to pathogen strains representing the population diversity of *X. oryzae* pv. *oryzae*.

In this study, we demonstrate a new strategy based on quantitative analyses of responses of NILs carrying various BB resistance genes and gene combinations against the 16 *X. oryzae* pv. *oryzae* isolates representing Korean BB pathotypes. We also discuss practical monitoring schemes for changes in the *X. oryzae* pv. *oryzae* population structure in paddy fields as well as various types of *R* gene actions against the corresponding avirulent *X. oryzae* pv. *oryzae* isolates.

MATERIALS AND METHODS

Plant materials. Five sets of rice breeding lines were used in the study. The first set consisted of five Korean differential lines (KDLs) that classified *X. oryzae* pv. *oryzae* isolates into five pathotypes, K1 to K5 (Table 1). The second set included nine NILs with a single *R* gene in an IR24 background (Table 1). The NILs were developed by more than seven generations of backcrossing and have identical phenotypes except for their reactions to the differential *X. oryzae* pv. *oryzae* races (8). The third set included five Korean japonica cultivars susceptible to all Korean *X. oryzae* pv. *oryzae* races (Table 1). The fourth set contained 11 NILs carrying four *R* genes (*Xa4*, *xa5*, *xa13*, and *Xa21*) in all possible combinations in an IR24 background (Table 2). The

TABLE 1. Quantitatively expressed pathogenicity levels (lesion length in centimeters) of two differential systems and highly susceptible Korean leading cultivars against each of 16 Korean *Xanthomonas oryzae* pv. *oryzae* isolates²

Lines, cultivars	Race K1									Race K3			Ambiguous races			
	KXO740	KXO1010	KXO85	KXO1003	KXO831	KXO595	KXO710	KXO169	KXO91	KXO97	KXO578	KXO605	KXO112	KXO82	KXO400	KXO21
KDL																
LSD _{0.05}	1.02	0.81	1.04	0.80	1.21	1.41	1.44	1.49	1.34	2.21	2.68	2.89	1.38	2.20	1.69	3.75
Milyang23	6.5 a	13.5 a	13.3 a	15.5 a	17.7 a	19.3 a	20.4 a	19.4 a	20.8 a	17.3 b	22.0 b	22.5 a	14.1 a	16.6 b	22.6 a	24.3 a
Cheongcheongbyeo	0.3 c	0.3 c	0.8 c	0.5 d	0.8 c	1.6 bc	0.3 c	2.0 bc	1.0 c	22.3 a	30.2 a	14.0 b	10.2 b	19.8 a	1.5 c	23.8 a
Pungsanbyeo	0.6 bc	0.3 c	0.8 c	0.3 d	0.9 c	0.4 c	0.7 c	0.7 c	1.0 c	15.5 b	21.4 b	16.2 b	6.5 c	12.2 c	1.7 c	21.0 a
Hangangchalbyeo	1.4 b	1.5 b	0.7 c	1.7 c	1.5 bc	1.6 bc	2.3 b	2.3 b	1.5 c	3.2 d	3.5 c	0.8 d	4.0 d	4.7 d	1.0 c	3.9 c
Milyang42	0.7 bc	2.1 b	2.6 b	3.2 b	2.6 b	2.6 b	3.3 b	2.5 b	3.9 b	5.6 c	4.8 c	4.2 c	9.8 b	15.8 b	6.9 b	10.9 b
IRBB-SR																
LSD _{0.05}	1.48	0.88	1.10	1.10	1.80	1.36	1.56	1.97	2.80	2.65	1.47	1.92	1.32	1.07	1.58	1.50
IR24	8.3 b	4.5 b	10.2 a	10.4 a	12.9 a	12.1 b	16.8 a	18.6 a	17.8 b	12.3 b	16.7 b	15.6 a	9.9 b	11.4 ab	11.3 c	14.3 ab
IRBB1 (<i>Xa1</i>)	0.6 f	0.2 d	0.2 e	0.2 f	0.2 f	0.2 e	0.2 e	0.2 e	0.4 f	12.7 b	16.9 b	2.1 f	6.8 c	12.1 a	4.0 f	12.9 b
IRBB3 (<i>Xa3</i>)	3.2 cd	2.7 c	3.8 c	3.5 d	2.1 de	6.1 d	7.0 c	5.6 d	6.1 d	17.2 a	14.2 c	4.1 e	4.7 d	12.5 a	5.9 e	9.7 c
IRBB4 (<i>Xa4</i>)	2.7 de	2.8 c	7.2 b	4.8 c	3.3 cd	5.3 d	6.4 c	4.0 d	2.2 ef	1.3 d	5.3 e	7.7 d	7.2 c	7.2 c	5.9 e	3.4 d
IRBB5 (<i>xa5</i>)	1.5 ef	0.8 d	1.3 de	2.0 e	2.0 e	0.3 e	0.8 de	1.8 e	1.9 ef	0.3 d	2.0 fg	0.7 f	1.2 e	2.2 e	2.3 gh	1.1 ef
IRBB7 (<i>Xa7</i>)	0.5 f	3.5 c	0.7 de	0.3 f	3.8 c	0.3 e	0.8 de	0.3 e	2.0 ef	0.3 d	2.3 f	0.8 f	0.3 e	1.3 ef	3.3 fg	1.7 ef
IRBB8 (<i>Xa8</i>)	2.4 de	0.8 d	1.3 d	1.6 e	0.5 f	1.0 e	1.8 d	1.5 e	3.5 de	3.9 c	7.1 d	1.3 f	5.4 d	5.8 d	1.6 h	2.6 de
IRBB10 (<i>Xa10</i>)	9.3 b	6.5 a	10.8 a	9.4 a	7.5 b	14.0 a	17.5 a	16.0 a	14.7 c	12.6 b	17.8 a	15.8 a	12.1 a	10.9 b	18.2 a	14.2 ab
IRBB13 (<i>xa13</i>)	17.2 a	6.1 a	10.6 a	7.6 b	7.6 b	11.8 b	13.8 b	13.8 b	20.8 a	12.5 b	18.8 a	13.8 b	12.0 a	11.7 ab	14.9 b	15.3 a
IRBB21 (<i>Xa21</i>)	3.9 c	3.4 c	7.6 b	9.8 a	6.5 b	7.6 c	12.4 b	11.5 c	1.0 e	1.2 d	0.8 g	9.7 c	1.2 e	1.1 f	8.9 d	0.9 f
Susceptible																
Hwasungbyeo	19.5	17.6	17.7	25.5	17.2	17.5	23.1	18.1	22.4	18.6	17.8	21.9	15.8	15.3	18.0	21.3
Jinbubyeo	10.6	10.2	11.3	9.2	10.3	14.7	19.6	21.3	18.5	13.9	16.3	16.9	9.3	11.9	16.3	14.9
Geumobyeo	3.5	5.0	10.0	6.8	7.5	8.0	10.5	7.1	8.5	7.4	9.0	10.8	7.3	7.9	9.3	12.1
Milyang95	17.7	15.8	16.8	18.5	20.2	17.6	24.3	20.7	19.9	15.4	18.8	23.5	14.3	12.5	22.0	18.4
Mangeumbyeo	0.3	5.7	0.3	0.3	0.3	7.9	0.3	0.3	0.3	9.3	12.9	10.0	6.6	8.7	10.9	16.2

² KDL = Korean differential lines; Susceptible = susceptible elite lines; means followed by the same letter are not significant at the 5% significance level by the least significant difference test (LSD_{0.05}).

TABLE 2. Quantitatively expressed pathogenicity levels (lesion length in centimeters) of resistance (*R*)-gene-pyramided IR24 near-isogenic lines against each of 16 Korean *Xanthomonas oryzae* pv. *oryzae* isolates²

	Race K1									Race K3			Ambiguous races			
IRBB-MR	KXO740	KXO1010	KXO85	KXO1003	KXO831	KXO595	KXO710	KXO169	KXO91	KXO97	KXO578	KXO605	KXO112	KXO82	KXO400	KXO21
LSD _{0.05}	0.82	0.85	1.49	0.67	0.70	0.96	0.82	0.65	0.35	0.18	0.34	0.99	0.45	0.40	1.08	0.37
IRBB50 (<i>Xa4</i> + <i>xa5</i>)	1.3 ef	0.3 g	0.9 c	0.9 ef	0.5 f	0.3 d	0.3 h	0.7 de	0.5 e	0.3 d	0.5 d	0.4 f	1.6 c	1.0 cd	0.8 de	0.8 c
IRBB51 (<i>Xa4</i> + <i>xa13</i>)	2.4 c	2.8 b	4.5 b	5.3 b	2.7 bc	3.2 b	3.4 c	4.4 a	4.0 a	1.9 a	3.2 a	3.0 bc	6.6 a	5.6 a	3.8 bc	3.6 a
IRBB52 (<i>Xa4</i> + <i>Xa21</i>)	1.8 cd	2.3 bc	3.9 b	4.1 c	1.6 de	1.8 c	2.9 cd	3.0 b	0.3 e	0.3 d	0.3 d	2.1 cd	0.3 e	0.3 g	2.9 c	0.3 d
IRBB53 (<i>xa5</i> + <i>xa13</i>)	1.5 de	2.1 bc	1.4 c	1.9 d	2.1 cd	0.7 d	2.0 ef	1.5 c	2.5 b	0.6 bc	2.1 b	1.6 de	3.0 b	2.6 b	1.7 de	1.6 b
IRBB54 (<i>xa5</i> + <i>Xa21</i>)	2.3 c	2.0 cd	1.5 c	1.6 de	1.5 de	0.3 d	1.8 ef	1.2 d	0.3 e	0.3 d	0.6 d	0.5 f	0.3 e	0.3 g	1.2 de	0.3 d
IRBB55 (<i>xa13</i> + <i>Xa21</i>)	8.4 a	6.4 a	6.2 a	9.4 a	5.0 a	9.7 a	13.8 a	ND	1.1 c	0.7 b	1.3 c	10.8 a	0.8 d	0.5 ef	12.5 a	1.4 b
IRBB56 (<i>Xa4</i> + <i>xa5</i> + <i>xa13</i>)	0.9 f	0.5 fg	0.9 c	0.5 f	0.5 f	1.9 c	1.5 f	1.3 cd	0.9 cd	0.5 c	0.5 d	1.0 ef	0.9 d	1.3 c	0.8 de	0.3 d
IRBB57 (<i>Xa4</i> + <i>xa5</i> + <i>Xa21</i>)	1.0 ef	1.6 de	1.5 c	1.5 de	1.5 de	0.3 d	2.5 de	0.5 e	0.5 e	0.3 d	0.6 d	0.5 f	0.3 e	0.6 e	1.2 de	0.3 d
IRBB58 (<i>Xa4</i> + <i>xa13</i> + <i>Xa21</i>)	3.4 b	2.6 b	4.3 b	5.0 b	2.8 b	3.0 b	4.8 b	4.0 a	0.6 de	0.3 d	0.5 d	3.9 b	0.7 de	0.9 d	4.5 b	0.7 c
IRBB59 (<i>xa5</i> + <i>xa13</i> + <i>Xa21</i>)	1.5 de	0.8 fg	0.9 c	1.5 de	2.0 d	0.3 d	1.3 fg	1.1 d	0.5 e	0.3 d	0.5 d	0.5 f	0.5 de	0.8 de	0.9 de	0.5 cd
IRBB59 (<i>Xa4</i> + <i>xa5</i> + <i>xa13</i> + <i>Xa21</i>)	1.6 d	1.3 ef	1.3 c	1.1 ef	1.0 ef	0.3 d	0.5 gh	0.7 de	0.5 e	0.3 d	0.3 d	0.3 f	0.3 e	0.5 fg	0.6 ef	0.5 cd

² Means followed by the same letter are not significant at the 5% significance level by the least significant difference test (LSD_{0.05}); ND = not determined.

multiple-*R*-gene NILs (IRBB-MR) were developed by crossing the single-*R*-gene NILs (IRBB-SR) followed by marker-aided selection (8). The fifth set contained four rice cultivars, (Jang-sungbyeon, R.Emas, Chugoku 69, and Calrose) possessing known *R* genes; these cultivars have been used as genetic sources for improving the BB resistance of japonica cultivars by Korean breeders (34), and were included to estimate current resistance spectra against tested *X. oryzae* pv. *oryzae* isolates. The seed of IR24 and the NILs were obtained from the Genetic Resources Center of the IRRI, Los Baños, Philippines, and the Korean rice germ plasm was obtained from the Genetics and Breeding Division of the National Institute of Crop Science (NICS), Rural Development Administration (RDA), Suwon, Republic of Korea.

Bacterial blight inoculation and evaluation. Rice breeding lines were evaluated for their reactions to *X. oryzae* pv. *oryzae* isolates in the field during 2002 at NICS, RDA, Suwon, under a randomized complete block design (RCBD). Plants were transplanted into two experimental plots in which three plants were in a row per line, and managed using standard cultural practices. At the maximum tillering stage, three leaves of each of the two plants in a plot were inoculated with the *X. oryzae* pv. *oryzae* isolates by the leaf clipping method with a bacterial suspension of 10^9 cells/ml and lesion lengths of the 12 inoculated leaves (six leaves from two plants per plot) were measured 14 days after inoculation following the method of Kauffman et al. (12). The *X. oryzae* pv. *oryzae* isolates used in the study were selected based on the phenogram developed from DNA fingerprinting results of 105 isolates of *X. oryzae* pv. *oryzae* collected during 1987 to 1996 as well as 394 isolates collected during 1999 to 2004 (7) in Korea using the genes of *xorII*M and *avrXa10* as probes (19). We chose 16 isolates from the determined haplotypes as the representative *X. oryzae* pv. *oryzae* strains of Korea (Table 2). The new race, K3a (26), also was used to evaluate the resistance level of a selected three-*R*-gene combination (*Xa4* + *xa5* + *Xa21*).

Data analysis. Data sets of measured lesion lengths from 12 observations were used for analyses. Analysis of variance (ANOVA) was performed to test homogeneity between plots and evaluate variance portions of lines, isolates, and their interactions. To designate race categories of each *X. oryzae* pv. *oryzae* isolate based on the reactions against the Korean differential lines (17), a mean lesion length of <5 cm was considered as resistant. Genetic effects of *R* genes in single-*R*-gene NILs due to the substitution of the susceptible allele by the resistance allele at the *R*-gene locus were estimated in terms of deviations from the lesion length of IR24 for each *X. oryzae* pv. *oryzae* isolate (residual effect of *R* genes). Pairwise *t* tests were performed for all possible comparisons between rice lines for lesion length differences. The least significant difference (LSD) and Duncan's multiple range test methods were used for multiple mean comparisons. To evaluate and compare the discriminating powers of Korean differential lines and single-*R*-gene NILs on the *X. oryzae* pv. *oryzae* isolates, dendrograms were constructed by Ward cluster analysis (40) based on the reactions (lesion lengths) of each line against the tested isolates. Disease severity patterns of the susceptible Korean lines and IR24 against 16 *X. oryzae* pv. *oryzae* isolates were

compared by using correlation analysis. Computations were performed by using PROC GLM, PROC CORR, and PROC CLUSTER of the SAS statistical software package (version 8.2; SAS Institute, Cary, NC).

RESULTS

Pathogenicity and race differentiation. ANOVA on lesion length indicated differences between KDLs and IRBB lines in terms of explainable total variance by five differential lines (41.7%) and 20 NILs, including IR24 (70%) (Table 3). The magnitude of variance of *X. oryzae* pv. *oryzae* isolates for KDLs was 31.9%, but only 2.3, 8.4, and 3.6% was observed for IRBB, IRBB-SR, and IRBB-MR, respectively. Although four *R* genes (*Xa4*, *xa5*, *xa13*, and *Xa21*) were used to generate 11 multiple-*R*-gene NILs, the higher portion of total variance explained by IRBB-MR (76.6%) than by KDLs (41.7%) suggested the uniqueness of each *R*-gene combination as well as its effectiveness for the *X. oryzae* pv. *oryzae* isolates tested. Therefore, our results indicated that the portion of total variance by genotypes relied on the number of effective *R* genes and their combinations that have resistance spectra unique to the corresponding *X. oryzae* pv. *oryzae* isolates.

Race categories of the 16 *X. oryzae* pv. *oryzae* isolates were determined based on the reactions against five KDLs (Table 1). Nine isolates were virulent on only the first KDL, Milyang23, and three isolates were virulent on the first, second, and third differential lines (Milyang23, Cheongcheongbyeon, and Pungsanbyeon) and were designated as races K1 and K3, respectively. The other four isolates cannot be classified and were called "ambiguous." The ambiguity arose because of the higher susceptibility of Milyang42 than Hangangchalbyeon (Table 1). All 12 isolates, belonging to races K1 and K3, also were more virulent on Milyang42, except for KXO740. Multiple mean comparisons (LSD) of the 16 isolates on Hangangchalbyeon indicated that the aggressiveness of each isolate could be separated into two groups: >3.2 cm (KXO97) and <2.3 cm (KXO169) (data not shown). Under this statistical judgment, five isolates (KXO97, KXO578, KXO112, KXO82, and KXO21) should belong to race K5, which never was reported in Korea.

***R*-gene effects of IR24 NILs.** The main effects of each of the nine *R* genes on each *X. oryzae* pv. *oryzae* isolate were estimated using the deviations of the lesion length of IR24 (Table 1; IRBB-SR). IR24 did not show incompatibility against any of the 16 *X. oryzae* pv. *oryzae* isolates; however, the aggressiveness of each isolate ranged from 4.5 cm for KXO1010 to 18.6 cm for KXO169 (overall mean = 12.7 cm). The NILs containing *xa5* and *Xa7* genes conferred resistance to all isolates tested, but *Xa10* and *xa13* were highly susceptible. Hypersensitivity of the genotypes with *Xa1* was very clear for isolates belonging to race K1, and the resistance of *xa5* and *Xa7* genes also was due to hypersensitivity for most of the *X. oryzae* pv. *oryzae* isolates, although the level of resistance (as measured by lesion length) they exhibited was less than that of *Xa1*. The *R* gene *xa8* exhibited a broad spectrum of resistance against *X. oryzae* pv. *oryzae* isolates except for KXO578, KXO112, and KXO82, which belong to race K3 and ambiguous

TABLE 3. Analysis of variance results of lesion length data for Korean differential lines (KDL), IR24 near-isogenic lines (IRBB), IR24 monogenic lines (IRBB-SR + IR24), and IR24 pyramided near-isogenic lines (IRBB-MR+IR24) caused by 16 Korean *Xanthomonas oryzae* pv. *oryzae* isolates

Source	KDLs				IRBB				IRBB-SR + IR24				IRBB-MR + IR24			
	df	SS	F	R ² (%)	df	SS	F	R ² (%)	df	SS	F	R ² (%)	df	SS	F	R ² (%)
Set	80	65,464.1	153.3	93.3	335	93,299.3	111.6	91.0	160	61,189.5	90.4	89.2	191	33,305.4	122.8	91.8
Line	4	27,302.2	1279.0	41.7	20	65,271.3	1,307.1	70.0	9	38,405.1	1,008.7	62.8	11	25,497.5	1,632.9	76.6
Isolate	15	20,889.6	261.0	31.9	15	2,177.0	58.1	2.3	15	5,152.2	81.2	8.4	15	1,183.8	55.6	3.6
Line × isolate	60	17,268.8	53.9	26.4	299	25,847.4	34.6	27.7	135	17,624.6	30.9	28.8	164	6,623.7	28.5	19.9
Plot	1	3.6	0.7	0.0	1	3.6	1.4	0.0	1	7.5	1.8	0.0	1	0.4	0.3	0.0
Residual	879	4,691.0		6.7	3,684	9,198.0		9.0	1,759	7,441.4		10.8	2,100	2,981.0		8.2

racess. In contrast, the resistance spectrum of the *Xa21* gene showed a somewhat different reaction from that of *xa8*. The *Xa21* gene did not confer clear resistance to race K1 except for isolate KXO91, and it showed susceptibility to one isolate (KXO605) of race K3 and an isolate (KXO400) of an ambiguous race.

Complex genetic interactions between *R* genes and pathotypes also were illustrated by IRBB-SR (Table 1). The NIL containing the *Xa4* gene was moderately effective against avirulent isolates, and also showed a very strong negative residual effect (shorter lesions than IR24) against the virulent isolates (overall mean = 4.8 cm). The NIL with the *Xa3* gene exhibited a type of resistance similar to that of *Xa4* for race K1. Reaction patterns against the isolates of race K3 and ambiguous races, however, exhibited isolate specificity. The NILs with the *Xa10* and *xa13* genes were not effective against any isolate and exhibited all possible directions of residual effects against virulent isolates. These *R* genes were similar to each other in that they both exhibited insignificant (statistically the same with IR24: KXO97, KXO605, KXO82, and KXO21) or significant positive residual effects (longer lesions than IR24: KXO578, KXO112, and KXO400) against isolates of race K3 and ambiguous races (Table 1). However, their reaction patterns against race K1 were variable (KXO740, KXO831, and KXO91, for example).

Diversity and discrimination of *X. oryzae* pv. *oryzae* isolates mediated by KDLs and single-*R*-gene NILs. Cluster analysis indicated that more informative estimation of the diversity of *X. oryzae* pv. *oryzae* isolates could be obtained with quantitatively determined aggressiveness of each representative isolate (Fig. 1). Genetic structures estimated by KDLs and IRBB-SRs were consistent with each other and lineage I was dominant for isolates of race K1 (marked in bold) and diverse for those of race K3 and ambiguous races (lineage II). However, an estimation of popula-

tion structure using KDLs (Fig. 1A) tended to underestimate the genetic relationships of isolates within a group (lineage) and exaggerate the distance between groups when it was compared with the results from IRBB-SR (Fig. 1B). Clustering results from IRBB-SR exhibited “deep-rooted” branches for isolates within a lineage and a closer relationship between lineages than KDLs (Fig. 1), and also suggested that the Korean *X. oryzae* pv. *oryzae* isolates had continuous genetic variations; therefore, they might have had unique hierarchical evolutionary pathways for their pathogenicity.

A graphical comparison of lesion lengths of the isolates on IR24 NILs illustrated that significant genetic diversity of *X. oryzae* pv. *oryzae* isolates is possible even within a race determined by KDLs (Fig. 2A). The aggressiveness of KXO595 and KXO91 isolates belonging to race K1 on IRBB4 and IRBB21 clearly is distinct. Reactions between KXO97 and KXO605 (race K3) on monogenic lines also were variable (Fig. 2A). Therefore, the degree of “deep-rooted” branches represented the overall capacity of monitoring lines (KDLs and IRBB-SRs) in terms of diverse *R* genes against several possible mutations of avirulence (*avr*) genes of the *X. oryzae* pv. *oryzae* population (Fig. 1).

Disease severity on susceptible lines. Quantitative observations of the four susceptible Korean japonica cultivars (Table 1) were compared with those of cvs. Milyang23 (Tong-il) and IR24 (indica). Cv. Mangeumbyeo has been reported as susceptible to all BB races. However, strong resistance to the isolates of race K1 indicated the presence of unknown *R* genes and this cultivar was excluded from further analysis. The pattern of susceptibility in decreasing order among the cultivars was Hwasungbyeo > Milyang95 > Milyang23 > Jinbubyeo > IR24 > Geumbyeo. However, very complicated reaction patterns against *X. oryzae* pv. *oryzae* isolates were observed, especially for the isolates of race K1 (Fig. 2B). Although the susceptibility spectra of Milyang23 and IR24 moderately correlated with each other ($r = 0.7$, $P = 0.002$), Jinbubyeo exhibited the strongest correlation with IR24 ($r = 0.84$, $P < 0.001$) and was moderately correlated with Milyang23 ($r = 0.68$, $P = 0.004$). The wide spectrum of resistance in Geumbyeo was difficult to explain because it showed significant correlation with Milyang23 ($r = 0.79$, $P < 0.001$) and moderate correlation with IR24 ($r = 0.55$, $P = 0.026$). Otherwise, the most susceptible line (Hwasungbyeo) did not show any correlation with other lines tested except for Milyang95 ($r = 0.57$, $P = 0.022$). The disease severity of susceptible cultivars suggested that each cultivar might have a unique genetic mechanism against pathogen invasion even within Korean japonica lines, for which very narrow genetic diversity had been reported (4,11).

Genetic effects of combined *R* genes. The IRBB-MRs containing *R* genes (*Xa4*, *xa5*, *xa13*, and *Xa21*) in different combinations exhibited higher resistance levels than IRBB-SRs (Table 2). These increased resistance levels might be due to the synergistic effects of the *R* genes, and each *R*-gene combination expressed a unique genetic effect against the corresponding *X. oryzae* pv. *oryzae* isolates as predicted during the ANOVA test (Table 3). Quantitative complementation (21) was prominent in the *R*-gene pair *Xa4* + *Xa21*, especially when they had significant negative residual effects against the corresponding compatible *X. oryzae* pv. *oryzae* isolates, such as KXO85, KXO595, KXO710, KXO605, and KXO400 (Table 2; Fig. 3A). Significant reductions in lesion length were not frequently observed between the gene pairs *xa5* and *Xa4* or *Xa21*, probably because of the effective resistance spectrum of *xa5* against all tested *X. oryzae* pv. *oryzae* isolates (Table 2; Fig. 3B). Therefore, in the gene pairs *Xa4* + *xa5*, *Xa4* + *Xa21*, and *xa5* + *Xa21*, the *R* genes interacted with each other independently and additively, with a few exceptions, such as the isolates KXO 112 for *Xa4* + *xa5* and KXO740 and KXO710 for *xa5* + *Xa21*, in which the genetic effects of *xa5* were quantitatively compensated for by the other defeated *R* genes; however, this was not statistically significant (Table 2).

Fig. 1. Cluster analysis on reactions of *Xanthomonas oryzae* pv. *oryzae* isolates on **A**, Korean differential lines and **B**, IRBB monogenic lines. Genetic distance of the tested 16 Korean bacterial leaf blight isolates was estimated depending on their reactions. Dendrograms were constructed using the “Ward” method. The isolates indicated in bold letters belong to race K1.

The NIL containing the *xa13* gene was not effective against all 16 isolates and even showed bidirectional residual effects as well as insignificance from the lesion lengths of IR24 (Table 1). However, the *R* genes *Xa4*, *xa5*, and *Xa21*, in combination with the *xa13* gene, exhibited divergent genetic effects on the *X. oryzae* pv. *oryzae* isolates within the same reaction categories determined by directions of residual effects (Table 4). Generally, the *R*-gene combination of *Xa4* + *xa13* maintained resistance levels against the isolates incompatible with *Xa4* (KXO740, KXO1010, KXO21, and KXO831), or showed quantitative complementation against the common isolate (KXO710) virulent on *Xa4* and *xa13*. Al-

though quantitative compensation between *Xa4* and *xa13* also was detected, the degree of resistance was not significant (Table 2). The *R*-gene pairs involving *xa13* and *xa5* or *Xa21* suggested that the genetic effects of the *R*-gene pairs were specifically dependent on each tested isolate (Table 4). Reactions of two *R*-gene pairs (*xa13* + *xa5* and *xa13* + *Xa21*), for example, showed an opposite reaction against the isolates having more compatibility to the NIL with the *xa13* gene than to IR24, in which quantitative compensation was significant for *xa13* + *xa5* and *xa13* + *xa21* against KXO91 and KXO740, respectively. However, the resistance levels of NILs with *xa5* and *Xa21* in combination with *xa13* genes were

Fig. 2. Graphical explanation of **A**, the low discriminating power of the Korean differential system and **B**, disease severity patterns of six rice breeding lines susceptible to isolates tested. Mean lesion lengths of each Korean differential line and IRBB line compared with the corresponding isolates are plotted on vertical bars.

maintained against KXO740 and KXO91 isolates, respectively. Quantitative complementation was observed only for *xa13* + *Xa21* with the isolate KXO831, but quantitative compensation was clear for others. For example, the level of moderate resistance of *Xa21* against KXO740 was defeated by the quantitative compensation of the *xa13* gene (Fig. 3C).

Resistance spectra of selected *R*-gene pyramids against BB isolates. IR24 NILs were tested for their effectiveness and durability against the 16 representative *X. oryzae* pv. *oryzae* isolates of Korea (Tables 1 and 2). NILs with *R* genes in a monogenic status did not show a broad spectrum of resistance against the 16 isolates tested except for *xa5* and *Xa7*. The NILs with the *Xa10* and *xa13* genes were highly susceptible to all isolates and the NILs with *Xa1*, *Xa3*, *Xa4*, *xa8*, and *Xa21* genes exhibited race- or isolate-specific susceptibility. The multiple-*R*-gene NILs in all combinations having *xa13* were excluded from our consideration because of their undesirable quantitative compensation, especially

when it was combined with the *Xa21* gene (Table 4). Inoculation experiments on IR24 NILs with a newly evolved field isolate of K3a showed high susceptibility to bacterial blight in IRBB4 (*Xa4*) and IRBB7 (*Xa7*) and moderate resistance to IRBB21 (*Xa21*), whereas IRBB50 (*Xa4* + *xa5*), IRBB54 (*xa5* + *Xa21*), and IRBB57 (*Xa4* + *xa5* + *Xa21*) expressed strong resistance (Fig. 4A).

With results on three major aspects of our quantitative observations and statistical interpretation—(i) strong and broad resistance of the *xa5* gene, (ii) independent and additive genetic actions of the *Xa4*, *xa5*, and *Xa21* genes under digenic status, and (iii) a strong quantitative complementation effect between the *Xa4* and *Xa21* genes—we deduced that the *R*-gene pyramid of *Xa4*, *xa5*, and *Xa21* would be the most prospective genotype for improving Korean japonica cultivars for BB resistance. A comparison of resistance spectra between the NILs with *Xa4* + *xa5* + *Xa21* gene combinations and rice lines with known *R* genes that have been used as genetic sources for improving BB resistance by Korean breeders also confirmed the effectiveness of the three-*R*-gene pyramid against the BB population of Korea represented by the 16 *X. oryzae* pv. *oryzae* isolates (Fig. 4B).

DISCUSSION

This study shows that the use of the Korean BB differential lines (KDLs) to develop rice cultivars with a broad spectrum of BB resistance may not be effective. The new pathotype, K3a, that had reaction scores identical to those of race K3 against KDLs caused a severe BB epidemic in 2003 (26). Therefore, a simple qualitative interpretation of the reaction patterns of tested isolates on KDLs led to unclear pathotyping. However, the reaction of the 16 *X. oryzae* pv. *oryzae* isolates to IR24 NILs allowed better understanding of the genetic interactions between the host and pathogen and a characterization of pathogen strains in relation to *R* genes. The estimated main effects of the individual *R* gene against the 16 representative isolates of the Korean BB population demonstrated prominent differences in BB pathotypes of Korea and other countries. A high frequency of isolates compatible with the NIL containing the *Xa21* gene was observed in this study as well as reported previously (19). Moreover, the NILs containing the *xa13* gene expressed bidirectional residual effects against the Korean BB isolates in contrast to their resistance against all tested virulent *X. oryzae* pv. *oryzae* races of South and Southeast Asia (21).

The IR24 NILs carrying *Xa1*, *xa5*, and *Xa7* genes expressed a hypersensitive response against some of the Korean BB isolates (22,27). However, the magnitude and directions of residual effects of monogenic NILs as well as cluster analysis of their genetic effects against corresponding *X. oryzae* pv. *oryzae* isolates also imply the quantitative nature of the *R* genes (20,21). The quantitative nature of the *R* genes was manifested more with different combinations of them than at the monogenic level. For example, the NILs with combinations of three *R* genes (*Xa4* + *xa5* + *Xa21*) have increased BB resistance against the Korean *X. oryzae* pv. *oryzae* isolates in a cumulative manner (10). The quantitative complementation effect was identified clearly at a digenic level

Fig. 3. Complex genetic effects of combined resistance (*R*) genes. Quantitatively determined effects of each *R*-gene pair were compared with the lesion lengths for the corresponding monogenic near-isogenic lines and classified into three epigenetic categories: **A**, quantitative complementation (combined *R* genes expressed significantly increased resistance), **B**, qualitative complementation (one *R*-gene effect masks the susceptibility of the defeated *R* gene), and **C**, quantitative compensation (the susceptible defeated *R* gene tends to reduce the *R*-gene effect).

TABLE 4. Statistical evaluation for three unique epistatic interactions under the digenic status of resistance (*R*)-gene combinations (*R* genes)^y

<i>R</i> genes		<i>Xanthomonas oryzae</i> pv. <i>oryzae</i> isolates ^z																	
		Positive residual effect						Insignificant						Negative residual effects					
		KXO740			KXO91			KXO1010			KXO21			KXO831			KXO710		
A	B	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B	A	B	A+B
<i>xa13</i>	<i>Xa4</i>	17.2 a	2.7 b	2.4 b	20.8 a	2.3 b	4.0 b	6.1 a	2.8 b	2.8 a	15.3 a	3.4 b	3.6 b	7.6 a	3.3 ab	2.7 a	13.8 a	6.4 a	3.4 a
<i>xa13</i>	<i>xa5</i>	17.2 a	1.5 b	1.5 b	20.8 a	1.8 b	2.5 b	6.1 a	0.8 c	2.1 c	15.3 a	1.1 b	1.6 b	7.6 a	2.0 bb	2.1 b	13.8 a	0.8 b	2.0 b
<i>xa13</i>	<i>Xa21</i>	17.2 a	3.9 c	8.4 b	20.8 a	1.0 b	1.1 b	6.1 a	3.4 b	6.4 a	15.3 a	0.9 b	1.4 b	7.6 a	6.5 ab	5.1 b	13.8 a	12.4 a	13.7 a

^y Means followed by the same letter are not significant at the 5% significance level by Duncan's multiple range test for the multiple mean comparisons.

^z Isolates were categorized as significant positive and negative residual effects and insignificant based on the directions of residual effects of *xa13* in Table 1.

with combination *Xa4* + *Xa21*, especially when both *R* genes had significant negative residual effects against the corresponding isolate. We also identified another important feature of quantitative interactions among the *R* genes, in which the resistance level of one *R* gene with a strong main effect tended to be compensated for by another *R* gene that was moderately resistant or defeated by the isolate tested. Quantitative compensation, however, appeared to be isolate-specific, and was frequently observed under the digenic conditions of *xa13* and another *R* gene.

Large-scale and long-term cultivation of certain resistant cultivars has been attributed to the shift in the race frequency of *X. oryzae* pv. *oryzae*, and the breakdown of *Xa4* is an example of the instability of a single major *R* gene (8,24). Breeding for durable disease-resistant cultivars following the theory of “stabilizing selection” has been emphasized recently (15,39). The K3a pathotype of Korea has been detected as a new field race compatible with the *Xa7* gene even though it has been speculated that

xa5 and *Xa7* have durable resistance (2,39). Additionally, a high frequency of Korean BB pathotypes compatible with the *Xa21* gene indicated that the co-evolutionary concept was not appropriate for all indigenous relationships between populations of the rice host and the pathogen in the field. It should be mentioned that pathotypes compatible with *Xa7* and *Xa21* have evolved in the absence of their host-gene counterparts for co-evolution in Korean rice fields. Reassortments of *avr* gene copies may be the cause of the host-independent emergence of virulent pathotypes in the field because *X. oryzae* pv. *oryzae* is an *avrBs3* family member having multigene families of avirulence genes (15). Therefore, divergence between the *avr* gene copies could be possible through intragenic or intergenic recombination followed by the evolution of new host specificities (43). Moreover, Yang et al. (41) reported the potential for conversion of *avrXa7* to a virulence effector without *Xa7*-dependent elicitor activities due to the replacement of a portion of the C-terminal coding region of *avrXa7*.

A

B

Fig. 4. A, Reactions of the IR24 near-isogenic lines to the newly evolved field isolate K3a and **B**, expected resistance spectrum of IRBB57 (*Xa4* + *xa5* + *Xa21*) against Korean *Xanthomonas oryzae* pv. *oryzae* isolates. IRBB57: *Xa4* + *xa5* + *Xa21*; IRBB50: *Xa4* + *xa5*; IRBB54: *xa5* + *Xa21*; and IRBB7: *Xa7*. Hwasungbyeo and Jinbubyeo are highly susceptible to 16 tested isolates, and Jangsungbyeo (*Xa1*), R. Emas (*Xa1*), Chugoku69 (*Xa3*), and Calrose (*Xa22*) have been used as *R*-gene sources for Korean japonica cultivars.

The concept of “residual effect” of the defeated major *R* genes has been proposed as a co-evolutionary feature of the relationship between rice and *X. oryzae* pv. *oryzae* populations (21). Our observations on the reaction of IR24 NILs to the Korean BB isolates indicated the importance of residual effects in terms of quantitative genetic interactions between *R* genes and their corresponding pathotypes. Moreover, our results suggest that several allele types are expressing different breeding values at the tested *R*-gene locus even though those allele types within different hosts were defeated by the same pathotypes. Therefore, it is postulated that heterogeneous susceptibility spectra of cultivars highly compatible with *X. oryzae* pv. *oryzae* may have resulted from various combinations of different allele types of the *R*-gene loci along with interactions corresponding to the *avr* genes of the pathogen. In this context, each of the 16 *X. oryzae* pv. *oryzae* isolates was a part of a pathogen population showing continuous distribution and composed of complex qualitative and quantitative interactions between alleles at all *R* loci of the host and alleles at all avirulence loci of the pathogen as proposed earlier (21).

Because of the difficulties of describing the actual pathogen population structure in the field, as well as predicting the durability levels of an *R* gene against pathogen invasion, host-plant improvement for disease resistance has always been challenging. Our results proposed several advantageous aspects of using *R*-gene NILs: (i) testing *R*-gene NILs at multiple locations would be helpful in characterizing local pathotypes from other locations or countries by comparing the reactions of each *R*-gene NIL, (ii) including NILs with an effective number of *R* genes and their combinations for designing resistant genotypes suitable against the current field *X. oryzae* pv. *oryzae* races would be useful, and (iii) continuously surveying the reactions of NILs against the pathogen in epidemic hot spots along with leading cultivars would assist in monitoring changes in the pathogen population followed by prompt development of a new resistant genotype. The collection of newly evolved virulent isolates corresponding to each *R*-gene NIL and comparisons of DNA marker-mediated pathotyping within and among NIL categories would provide practical tools on co-evolutionary features of the pathogen population with the host plants. Information on pathogen diversity and distribution of pathogens across regions or countries would be more useful by sharing the same probes for DNA fingerprinting. Additionally, if *R*-gene NILs of local elite germ plasm were available, this would allow the transfer of effective *R* genes into other local cultivars with less possibility of unfavorable linkage drag from *R*-gene donor lines. Based on our results, we believe that the *R*-gene pyramid of *Xa4* + *xa5* + *Xa21* would be the most effective for improving Korean japonica cultivars for BB resistance. These three *R* genes are being transferred into susceptible elite Korean japonica cultivars Jinbubyeo and Mangeumbyeo via marker-aided and phenotype selection without dispensing with the resistance spectrum.

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LITERATURE CITED

- Adhikari, T. B., Vera Cruz, C. M., Zhang, Q., Nelson, R. J., Mew, T. W., and Leach, J. E. 1995. Genetic diversity of *Xanthomonas oryzae* pv. *oryzae* in Asia. *Appl. Environ. Microbiol.* 61:966-971.
- Bai, J. F., Choi, S. H., Ponciano, G., Leung, H., and Leach, J. E. 2000. *Xanthomonas oryzae* pv. *oryzae* genes contribute differentially and specifically to pathogen aggressiveness. *Mol. Plant-Microbe Interact.* 13:1322-1329.
- Choi, J. E., Kang, H. K., and Lee, D. G. 1996. Classification of Korean isolates of *Xanthomonas oryzae* pv. *oryzae* on the basis of their virulence to Korean, Japanese and IRRI differential varieties. *Kor. J. Plant. Pathol.* 12: 202-208. (In Korean)
- Choi, S. H., Vera Cruz, C. M., and Leach, J. E. 1998. Distribution of *Xanthomonas oryzae* pv. *oryzae* DNA modification systems in Asia. *Appl. Environ. Microbiol.* 64:1663-1668.
- Crute, I. R., and Pink, D. A. C. 1996. The genetics and utilization of pathogen resistance in plants. *Plant Cell* 8:1747-1755.
- Gu, K., Yang, B., Tian, D., Wu, L., Wang, D., Sreekala, C., Yang, F., Chu, Z., Wang, G. L., White, F. F., and Yin, Z. 2005. *R* gene expression induced by a type-III effector triggers disease resistance in rice. *Nature* 435:1122-1125.
- Heu, S. G., Ra, D. S., and Lee, S. D. 2004. Physiological race differentiation of *Xanthomonas oryzae* pv. *oryzae* and development of rice differential varieties. Pages 7-21 in: *Agric. Biol. Res., Rural Development Administration, Korea.* (In Korean with English abstract)
- Huang, N., Angeles, E. R., Domingo, J., Magpantay, G., Singh, S., Zhang, G., Kumaravadeivel, N., Bennett, J., and Khush, G. S. 1997. Pyramiding of bacterial blight resistance genes in rice: marker-aided selection using RFLP and PCR. *Theor. Appl. Genet.* 95:313-320.
- Iyer, A. S., and McCouch, S. R. 2004. The rice bacterial blight resistance gene *xa5* encodes a novel form of disease resistance. *Mol. Plant-Microbe Interact.* 17:1348-1354.
- Jena, K. K., Jeung, J. U., Shin, M. S., Heu, S., Choi, S. H., Hwang, H. G., Vera Cruz, C. M., and Lee, M. H. Pyramiding specific BB resistance genes for improvement of temperate japonica rice in South Korea. In: *Proc. 1st Int. Conf. Bacterial Blight of Rice*, Tsukuba, Ibaraki, Japan. (In press.)
- Jeung, J. U., Hwang, H. G., Moon, H. P., and Jena, K. K. 2005. Fingerprinting temperate japonica and tropical indica rice genotypes by comparative analysis of DNA markers. *Euphytica* 146:239-251.
- Kauffman, H. E., Reddy, A. P. K., Hsieh, S. P. Y., and Merca, S. D. 1973. An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. *Plant Dis. Rep.* 57:537-541.
- Kumar, A., Kumar, S. K., and Sakthivel, N. 2003. Compositional difference of the exopolysaccharides produced by the virulent and virulent-deficient strains of *Xanthomonas oryzae* pv. *oryzae*. *Curr. Microbiol.* 46:251-255.
- Leach, J. E., Rhoads, M. L., Vera Cruz, C. M., White, F. F., Mew, T. W., and Leung, H. 1992. Assessment of genetic diversity and population structure of *Xanthomonas oryzae* pv. *oryzae* using a repetitive DNA element. *Appl. Environ. Microbiol.* 58:2188-2195.
- Leach, J. E., Vera Cruz, C. M., Bai, J., and Leung, H. 2001. Pathogen fitness penalty as a predictor of durability of disease resistance genes. *Annu. Rev. Phytopathol.* 39:187-224.
- Leach, J. E., and White, F. F. 1996. Bacterial avirulence genes. *Annu. Rev. Phytopathol.* 34:153-179.
- Lee, D. K., Seo, J. H., Choi, J. E., Park, K. H., and Bae, S. H. 1986. Pathotypes of *Xanthomonas campestris* pv. *oryzae* in Honam District, Korea. *Kor. J. Plant Pathol.* 2:102-106. (In Korean)
- Lee, K. S., Rasabandith, S., Angeles, E. R., and Khush, G. S. 2003. Inheritance of resistance to bacterial blight in 21 cultivars of rice. *Phytopathology* 93:147-152.
- Lee, S. W., Choi, S. H., Han, S. S., Lee, D. G., and Lee, B. Y. 1999. Distribution of *Xanthomonas oryzae* pv. *oryzae* strain virulent to *Xa21* in Korea. *Phytopathology* 89:928-933.
- Li, Z. K., Luo, L. J., Mei, H. W., Paterson, A. H., Zhao, X. H., Zhong, D. B., Wang, Y. P., Yu, X. Q., Zhu, L., Tabien, R., Stansel, J. W., and Ying, C. S. 1999. A “defeated” rice resistance gene acts as a QTL against a virulent strain of *Xanthomonas oryzae* pv. *oryzae*. *Mol. Gen. Genet.* 261:58-63.
- Li, Z. K., Sanchez, A., Angeles, E., Singh, S., Domingo, J., Huang, N., and Khush, G. S. 2001. Are the dominant and recessive plant disease resistance gene similar?: A case study of rice *R* genes and *Xanthomonas oryzae* pv. *oryzae* races. *Genetics* 159:757-765.
- Mew, T. W. 1987. Current status and future prospects of research on bacterial blight of rice. *Annu. Rev. Phytopathol.* 25:359-382.
- Mew, T. W., Alvarez, A. M., Leach, J. E., and Swings, J. 1993. Focus on bacterial blight of rice. *Plant Dis.* 77:5-12.
- Mew, T. W., Vera Cruz, C. M., and Medella, E. S. 1992. Change in race frequency of *Xanthomonas oryzae* pv. *oryzae* in response to the planting of rice cultivars in the Philippines. *Plant Dis.* 76:1029-1032.
- Nelson, R. J., Baraoidan, M. R., Vera Cruz, C. M., Yap, I. V., Leach, J. E., Mew, T. W., and Leung, H. 1994. Relationship between phylogeny and pathotype for the bacterial blight pathogen of rice. *Appl. Environ. Microbiol.* 60:3275-3283.
- Noh, T. H., Lee, D. K., Kang, M. H., Shin, M. S., and Na, S. Y. 2003. Identification of new race of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) in Korea. (Abstr.) *Phytopathology* 93(suppl.):S66.
- Ogawa, T., and Khush, G. S. 1988. Major genes for resistance to bacterial blight in rice. Pages 177-192 in: *Bacterial Blight of Rice, Proceedings of the International Workshop on Bacterial Blight of Rice*. International Rice Research Institute, Manila, Philippines.

28. Ou, S. H. 1985. Pages 70-74 in: Rice Diseases. Commonwealth Mycological Institute, Aberystwyth, England.
29. Porter, B. W., Chittoor, J. M., Yano, M., Sasaki, T., and White, F. F. 2003. Development and mapping of markers linked to the rice bacterial blight resistance gene *Xa7*. *Crop Sci.* 43:1484-1492.
30. Rao, K. K., Lakshminarasu, M., and Jena, K. K. 2002. DNA markers and marker-assisted breeding for durable resistance to bacterial blight disease in rice. *Biotechnol. Adv.* 20:33-47.
31. Ray, S. K., Rajeshwari, R., and Sonti, R. V. 2000. Mutants of *Xanthomonas oryzae* pv. *oryzae* deficient in general secretory pathway are virulence deficient and unable to secrete xylanase. *Mol. Plant-Microbe Interact.* 13:394-401.
32. Sanchez, A. C., Ilag, L. L., Yang, D., Brar, D. S., Ausubel, F., Khush, G. S., Yano, M., Sasaki, T., Li, Z., and Huang, N. 1999. Genetic and physical mapping of *xa13*, a recessive bacterial blight resistance gene in rice. *Theor. Appl. Genet.* 98:1022-1028.
33. Sanchez, A. C., Brar, D. S., Huang, N., Li, Z., and Khush, G. S. 2000. Sequence tagged site marker-assisted selection for three bacterial blight resistance genes in rice. *Crop Sci.* 40:792-797.
34. Shin, M. S., Noh, T. H., Lee, J. K., Shin, H. T., and Lee, Y. M. 2000. Breeding of Japonica near-isogenic lines for resistance to bacterial blight in rice. *Kor. J. Breed.* 32:291-295. (In Korean)
35. Singh, S., Sidhu, J. S., Huang, N., Vikal, Y., Li, Z., Brar, D. S., Dhaliwal, H. S., and Khush, G. S. 2001. Pyramiding three bacterial blight resistance genes (*xa5*, *xa13* and *Xa21*) using marker-assisted selection into indica rice cultivar PR106. *Theor. Appl. Genet.* 102:1011-1015.
36. Song, W. Y., Wang, G. L., Chen, L. L., Kim, H. S., Pi, L. Y., Holsten, T., Gardner, J., Wang, B., Zhai, W. X., Zhu, L. H., Fauquet, C., and Ronald, P. 1995. A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. *Science* 279:1084-1086.
37. Sun, X., Cao, Y., Yang, Z., Xu, C., Li, X., Wang, S., and Zhang, Q. 2004. *Xa26*, a gene conferring resistance to *Xanthomonas oryzae* pv. *oryzae* in rice, encodes an LRR receptor kinase-like protein. *Plant J.* 37:517-527.
38. Sun, X., Yang, Z., Wang, S., and Zhang, Q. 2003. Identification of a 47 kb DNA fragment containing *Xa4*, a locus for bacterial blight resistance in rice. *Theor. Appl. Genet.* 106:683-687.
39. Vera Cruz, C. M., Bai, J., Ona, I., Leung, H., Nelson, R. J., Mew, T. W., and Leach, J. E. 2000. Predicting durability of a disease resistance gene based on an assessment of the fitness loss and epidemiological consequences of avirulence gene mutation. *Proc. Natl. Acad. Sci. USA* 97:13500-13505.
40. Ward, J. H. 1963. Hierarchical grouping to optimize an objective function. *J. Am. Stat. Assoc.* 58:236-244.
41. Yang, B., Sugio, A., and White, F. F. 2005. Avoidance of host recognition by alterations in the repetitive and C-terminal regions of *AvrXa7*, a type III effector of *Xanthomonas oryzae* pv. *oryzae*. *Mol. Plant-Microbe Interact.* 18:142-149.
42. Yang, B., and White, F. F. 2004. Diverse members of the *AvrBs3/PthA* family of type III effectors are major virulence determinants in bacterial blight disease of rice. *Mol. Plant-Microbe Interact.* 17:1192-1200.
43. Yang, Y., and Gabriel, D. W. 1995. Intragenic recombination of a single plant pathogen gene provides a mechanism for the evolution of new host specificities. *J. Bacteriol.* 177:4963-4968.
44. Yang, Z., Sun, X., Wang, S., and Zhang, Q. 2003. Genetic and physical mapping of a new gene for bacterial blight resistance in rice. *Theor. Appl. Genet.* 106:1467-1472.
45. Yoshimura, S., Yamanouchi, U., Katayose, Y., Toki, S., Wang, Z. X., Kono, I., Kuruta, N., Yano, M., Iwata, N., and Sasaki, T. 1998. Expression of *Xa1*, a bacterial blight resistance gene in rice, is induced by bacterial inoculation. *Proc. Natl. Acad. Sci. USA* 95:1663-1668.
46. Zhu, W., Magbanua, M. M., and White, F. F. 2000. Identification of two novel hrp-associated genes in hrp gene cluster of *Xanthomonas oryzae* pv. *oryzae*. *J. Bacteriol.* 182:1844-1853.