RESEARCH ARTICLE
J. Crop Sci. Biotech. 10 (4) : 265 ~ 276

Identification of Major Blast Resistance Genes in Korean Rice Varieties (*Oryza sativa* L.) Using Molecular Markers

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Abstract

The 13 major blast resistance (*R*) genes against *Magnaporthe grisea* were screened in a number of Korean rice varieties using molecular markers. Of the 98 rice varieties tested, 28 were found to contain the *Pia* gene originating from Japanese *japonica* rice genotypes. The *Pib* gene from BL1 and BL7 was incorporated into 39 Korean *japonica* varieties, whereas this same gene from the IRRI-bred *indica* varieties was detected in all *Tongil-type* varieties. We also found that 17 of the *japonica* varieties contained the *Pii* gene. The *Pii* gene in Korean rice varieties originates from the Korean *japonica* variety Nongbaeg, and Japanese *japonica* varieties Hitomebore, Inabawase, and Todorokiwase. The *Pi5* gene, which clusters with *Pii* on chromosome 9, was identified only in Taebaeg. Thirty-four varieties were found to contain alleles of the resistance gene *Pita* or *Pita-2*. The *Pita* gene in *japonica* varieties was found to be inherited from the Japanese *japonica* genotype Shimokita, and the *Pita-2* gene was from Fuji280 and Sadominori. Seventeen *japonica* and one *Tongil-type* varieties contained the *Piz* gene, which in the *japonica* varieties originates from Fukuhikari and 54BC-68. The *Piz-t* gene contained in three *Tongil-type* varieties was derived from IRRI-bred *indica* rice varieties. The *Pi9(t)* gene locus that is present in Korean *japonica* and *Tongil-type* varieties was not inherited from the original *Pi9* gene from wild rice *Oryza minuta*. The *Pik*-multiple allele genes *Pik*, *Pik-m*, and *Pik-p* were identified in 24 of the varieties tested. In addition, the *Pit* gene inherited from the *indica* rice K59 strain was not found in any of the Korean *japonica* or *Tongil-type* varieties tested.

Key words: rice, *japonica* and *Tongil-type*, blast, resistance gene, molecular marker

Introduction

Rice is a staple food in Korea and continuous research efforts have therefore been made to produce varieties with improved levels of disease resistance. Since 1960, 46 *Tongil-type* varieties derived from the crosses between *indica*- and *japonica-type* subspecies, and a further 160 *japonica* varieties have been developed in Korea. In the early 1970s, *Tongil-type* varieties were released for commercial use and occupied more than 75% of the total rice cultivation areas by the end of 1970s. Several commercial varieties of *japonica* rice were also developed in the mid-1980s and were adopted for cultivation due to the needs of farmers and consumers for high yield and good eating quality.

The proliferation of diverse genotypic varieties and hybrids of rice with enhanced disease resistance, however, has triggered genetic mutations leading to the differentiation of the blast fungus *Magnaporthe grisea*. This in turn has led to break down and loss of blast resistance in *Tongil-type* rice varieties. As a result, only a limited area was cultivated with the *Tongil-type* varieties in Korea since 1990. Significantly, the same virulent blast isolates also caused great damage to *japonica* varieties and in 1999, *japonica* rice varieties including Daesan, Dongan, and Ilmi, which were all developed from the same parent Milyang95, were found to frequently lose their resistance to leaf and neck blast isolates (Han et al. 2001).

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Little is currently known about blast resistance genes contained among Korean rice varieties as only a few strains have so far been analyzed for the presence of these genes (Ahn et al. 1997; Cho et al. 2004a, 2004b; Kwon et al. 2002; Yi et al. 2004) or their haplotypes (Hwang et al. 2004). Most of the developed Korean *japonica* varieties have been found to be derived from Japanese *japonica* parents that contain the blast resistance genes, *Pia, Pib, Pii, Pik* multiple alleles, and *Pita* based on the pedigree of the breeding lines (Choi et al. 1989). Our current study was undertaken to expand upon the existing knowledge in this area by identifying the major resistance (*R*) genes to *M. grisea* from Korean *japonica* and *Tongil-type* varieties using molecular markers.

Materials and Methods

Plant materials

A total of 98 varieties and elite germplasms of rice were analyzed in this study consisting of 88 *japonica* and 10 *Tongil-type* varieties. Most of the *japonica* varieties are categorized as good grain quality lines in terms of palatability. Among these *japonica* varieties, nine are glutinous (waxy) varieties and a further 12 are anther culture-derived varieties. Twenty-four monogenic lines containing single major blast resistance genes were used as standard check variety (Tsunematsu et al. 2000).

The primary donors analyzed with 98 Korean varieties were: Aichi37, Asominori, Fuji269, Fuji280, Jinheung, and Kimmaze for *Pia*; BL1, BL7, IR8, IR24, and IR29 for *Pib*; Hitomebore, Inabawase, Todorokiwase, and Nongbaeg for *Pii*; Fuji280, Sadominori and Shimokita for *Pita (Pita-2)*; Fukuhikari and 54BC-68 for *Piz*; Hokuriku109 and Akitsuho for *Pik*. The *Pia* gene from Kanto100 and Kuiku90, and the *Piz-t* gene from IR2061 and IR4445 were inferred from the references (Imbe et al. 1997; Kiyosawa 1972a; Rice Genetics Cooperative 1998).

Evaluation of blast disease resistance by nursery screening

Blast nursery screening of the 98 test varieties of rice used in this study was performed in 14 local experimental plots in Korea from 2003 to 2006. The incidence of blast disease was scored from 0 (no lesions) to 9 (necrosis of all leaves and sheaths) using IRRI standard evaluation method. Varieties with scores of 0-3 in over 70% of the plots screened were assigned to the resistance (R) group, and those with scores of 0-3 in 50-70% of the plots and of 4-6 in the remaining plots were placed in a resistant moderately (RM) group. Varieties with scores of 0-3 or 4-6 in over 80% plots were assigned to medium resistance (M). Varieties with scores of 7-9 in less than 40% of the plots and of 0-3 and/or 4-6 scores in the remaining plots were scored as moderately susceptible (MS), and those with scores of 7-9 in over 40% of the plots were included in the susceptibility (S) group.

DNA molecular markers

A total of eight SNP markers and 12 SCAR and STS markers were used for the detection of the 13 major leaf blast resistance genes. The *Pia*-specific PCR primer set, referred as to yca72, was developed to amplify a 905-bp fragment and is based upon the genomic sequence of the BAC clone OSJNBa44D15 on chromosome 11 (Table 1). Restriction digestions with *Hinf* I were performed to discriminate between the PCR products amplified by the primer set yac72, and the variety having 635-bp band was concluded to have *Pia* gene (Cho et al. unpublished data). The *Pib*-specific PCR primer set, NSb, was developed to yield a 629-bp amplicon based on its genomic sequence on chromosome 2 (GenBank accession No.AB013448) (Table 1). Two major rice blast resistance genes *Pii* and *Pi5* located at the resistance loci on chromosome 9 were commonly detected using three primer sets, JJ80-T3, JJ81-T3, and JJ113-T3 (Jeon et al. 2003; Yi et al. 2004). The *Pi5*-specific PCR primer set JJ817 was developed using the genomic sequence of this resistance locus (S-K Lee and J-S Jeon unpublished data) and can distinguish the *Pi5* resistance gene from the *Pii* gene (Table 1). The major

Table 1. Gene-specific PCR primers used in the identification of the indicated *M. grisea* resistance genes in rice.

R genes	Chrs.	Markers	Sequence		Annealing Temp. (°C)	Exp. Size (bp)
			Forward (5'-3')	Reverse (5'-3')		
Pia	11	yca72*	aggagaagaagccaccaagg	gagctgccacatcttcctt	60	635
Pib	2	NSb	atcaactctgccacaaaatcc	cccatatcaccacttgttcccc	57	629
Pi5	9	JJ817	gatatggttgaaaagctaatctca	atcattgtccttcatattcagagt	60	1450

*These PCR products were digested with the *Hinf* I restriction enzyme.

blast resistance genes *Pita* and *Pita-2* on chromosome 12 was detected using three primer sets (Jia et al. 2002, 2004): YL100/YL102 and YL155/YL87 amplify 403- and 1042-bp products, respectively, from the *Pita*-allele. YL183/YL87 was used to amplify a 1042-bp fragment of the susceptible *pita*-allele. The *Piz* and *Piz-t* genes on chromosome 6 were identified based on the four previously described SNP markers, z4792 and z60510, and zt4792 and zt6057, respectively (Hayashi et al. 2004). The identification of the *Pi9* gene on chromosome 6 was determined by the use of three established PCR primers, pBA14 (480 bp) (Liu et al. 2002), NBS2-O/NBS2-U (928 bp), and 195-1F/195-1R (2.0 kb) (Qu et al. 2006). Digestions with *Hin*f I were performed to discriminate between the amplicons generated by the NBS2-O/NBS2-U primer set. The SNP markers used to identify the other resistance genes were: k6415 for the *Pik* gene, k6441 for *Pik-m*, k39575 for *Pik-p* on chromosome 11, and t256 for the *Pit* gene on chromosome 1 (Hayashi et al. 2006).

PCR analysis of genomic DNA

Total genomic DNAs was extracted from 4-week-old fresh leaf tissue according to the slightly modified potassium acetate method described previously (Dellaporta et al. 1983). For the analysis of dominant SNP allele-specific markers (z4792, zt4792, z60510, zt6057, k6415, k6411, k39575, and 6256), 40 ng of genomic DNA was used in a 20 μL PCR reaction containing 2 μL 10x PCR buffer, 1.6 μL 2.5 mM dNTPs, 0.5 μL each of an allele-specific forward primer (10 pM) and a reverse primer (10 pM), and 0.1 μL Hot Start *Taq* polymerase (5U/μL) (Takara, Japan). The PCR amplification program consisted of an initial denaturation at 96 ℃ for 5 min followed by 30 cycles of 96 ℃, for 40 s; 60 ℃, for 40 s; 72 ℃, for 90 s, and a final extension at 72 ℃ for 10 min. For the detection of other markers, the PCR reactions (20 μL) contained 40 ng of genomic DNA, 2 μL 10x PCR buffer, 1.6 μL 2.5 mM dNTPs, 0.5 μL each of an allele-specific forward primer (10 pM) and a reverse primer (10 pM), and 0.1 μL *Taq* polymerase (5U/μL) (Neurotics Inc., Korea). The PCR amplification program in this instance consisted of 35 cycles of 1 min at 95 ℃, 1 min at 50-60 ℃, and 2 min at 72 ℃, and a final extension at 72 ℃ for 10 min. Each of the PCR reactions was performed using a PTC-200 (Bio Rad, Germany). PCR products were separated in 1.5-2% agarose gels in 0.5 x TBE buffer. These experiments were repeated at least three times.

Table 2. *M. grisea* resistance genes and their donors based on molecular marker analysis of 98 Korean rice varieties.

R genes	*Donors*[♪]		Major varieties*	No. of varieties (%)
	Primary	Secondary		
Pia	Aichi37, Asominori, Fuji269, Fuji280, Jinheung, Kanto100, Kimmaze, Kuiku90	Dongjin, Mangeum, Sangpung, Seomjin, Milyang20, Milyang71, Milyang96	Dongjin, Mangeum, Moonjang, Palgong, Saesangju, Samgwang, Seomjin, Sinseonchal	29 (29.6)
Pib	BL1, BL7, IR8, IR24, IR29	Bongkwang, Samnam, Seolag, Seomjin, Milyang20, Suweon345	Daepyeong, Daesan, Dongan, Dongjin1, Geuman, Gopum, Hwayeong, Ilpum, Junam, Palgong, Samgwang, Sangju, Sangmi, Seomjin, Sindongjin, *Gaya, Milyang23, Anda, Dasan*	49 (50)
Pii	Nongbaeg, Hitomebore, Inabawase, Todorokiwase,	Jinmi, Mangeum	Gopum, Hopyeong, Ilpum, Jinpum, Manchu, Nampyeong, Seoan, Sobi, Taebong	17 (17.3)
Pi5			*Taebaeg*	1(1.0)
Pita, Pita-2	Shimokita (*Pita*)	Cheolweon29, Iri390, Suweon362, Sangpung	Sambaeg, Gopum, Nampyeong, Seojin, Sampyeong, Sangmi, Dongjinchal, Sangjuchal, *Gaya*	34 (34.7)
	Fuji280 (*Pita-2*), Sadominori (*Pita-2*)	Daeseong, Dongjin,	Gru, Moonjang, Saesangju, Dongjin, Gyehwa, Ilmi, Jungsan	
Piz	Fukuhikari, 54BC-68	Jinbu, Sambaeg	Gopum, Hwanam, Moonjang, Saesangju, Taeseong, *Gaya*	18(18.4)
Piz-t	IR2061, IR4445	Daeseong, Dongjin, Samnam, Seolak	*Baegunchal, Hangangchal, Samgang*	3(3.1)
Pi9(t)[§]			*Anda,* Undoo, Gru, Moonjang, Sangju, Saesangju, Sambaeg, Sangmi, Goun, Jinbu, Unbong, Geumo	21 (21.4)
Pik	Hokuriku109, Akitsuho	Jinbu	Odae, Jinbu, Tamjin, Gru, Jungsan, Gopum, Goun, Moonjang, Junghwa, Seogan	17 (17.3)
Pik-m			Seogan	1(1.0)
Pik-p			Sangnambat, *Tongil, Milyang23, Taebaeg*	8(8.2)
Pit				0(0)

[♪] Primary donors are the first sources that introduced the *R* genes into Korean rice varieties or lines, and the secondary donors developed from the primary donors are contributed to develop major commercial rice vatieties having specific *R* genes.

* The varieties listed in italics are *Tongil-type* developed from the crosses between *indica* and *japonica*.

[§] The *Pi9(t)* locus is distinct from the original *Pi9* resistance locus.

Results

Screening of the major blast resistance genes to M. grisea in Korean rice varieties

Among the 98 varieties analyzed in this study, 87 (88.8%) were found to have at least one of the 13 major *R* genes analyzed (Tables 2-5). The *Pia* gene on chromosome 11 was identified in 29 *japonica* rice varieties. The primary source of this gene was found principally to be the seven Japanese *japonica* rice genotypes, Aichi37, Asominori, Fuji269, Fuji280, Kanto100, Kimmaze, and Kuiku90, and a Korean *japonica* variety Jinheung (Table 2). The seven Korean rice varieties, Dongjin, Mangeum, Sangpung, Seomjin (Iri353), Milyang20, Milyang71, and Milyang96 (Yeongnam) were used as the secondary donors for the *Pia* gene. This gene was not identified among the 10 *Tongil-type* varieties.

We further found that 49 of the varieties tested in this study harbored the *Pib* gene on chromosome 2. The primary sources of this gene among the *japonica* group included two Japanese isogenic lines, BL1 and BL7. In contrast, the *Pib* gene detected in the *Tongil-type* varieties was found to originate from the IRRI-bred *indica* varieties IR8, IR24, and IR29. Six varieties, Bongkwang, Samnam, Seolag, Seomjin, Milyang20, and Suweon345 were used as the secondary donors for the *Pib* gene. It should be noted that all of the *Tongil-type* cultivars examined in our current study harbor the *Pib* gene. The major *japonica* varieties containing *Pib* were Gopum, Dongjin1, Ilpum, Junam, Palgong, Samgwang, Sangju, Seomjin, and Sindongjin.

The *Pii* and *Pi5* gene regions on chromosome 9 were identified from 17 (17.3%) of the varieties in our study cohort, which displayed positive bands using the primer sets JJ80-T3, JJ81-T3, and JJ113-T3. A positive band for the *Pi5*-specific dominant marker JJ817 was produced only from *Tongil-type* variety Taebaeg (Fig. 1). Hence, the remaining 17 varieties having positive bands for the three markers, JJ80-T3, JJ81-T3, and JJ113-T3, but not for the marker JJ817, were classified as containing the *Pii* gene. Taebaeg was classified as having the *Pi5* gene as it produced positive bands for four primer sets. The primary donors of the *Pii* resistance gene were found to be Nongbaeg from a Korean japonica variety, and Hitomebore, Inabawase, and Todorokiwase from Japanese *japonica* varieties. The secondary donors were determined to be Jinmi and Mangeum. The major *japonica* cultivars harboring the *Pii* gene were found to be Gopum, Hopyeong, Ilpum, Sobi, and Taebong. From our results, we speculate that the *Pi5* gene in the Taebaeg (*Tongil-type*) variety might have been inherited from IRRI-bred *indica* rice.

For the screening of the *Pita* and *Pita-2* genes on chromosome 12, we found that 34 (34.7%) of the 98 rice varieties under study produced positive bands of 403-bp and 1042-bp with the two primer sets for the resistant *Pita*-allele, but the 1042-bp band corresponding to the primer set for the susceptible *pita*-allele was not amplified (Fig. 2). We were not able to discriminate between the *Pita* and *Pita-2* genes using these three primer sets. The primary donor of the *Pita* gene among the Korean *japonica* rice varieties was found to be from Shimokita, and *Pita-2* gene was from Fuji280 and Sadominori. The secondary donor varieties for *Pita-2* developed using the primary donors are Daeseong, Dongjin, Samnam, Seonam, and Seolak, and the secondary donors for *Pita* were Cheolweon29, Iri390, Suweon362, and Sangpung. Interestingly, the three varieties Dongjinchal, Gopum, and Suweon480 produced positive bands with all of the primer sets specific for the resistant *Pita*-alleles and the susceptible *pita*-allele. The *Pita* gene-alleles in Gopum and Suweon480 were introduced from Shimokita and Fuji280, respectively, but the origin of this gene in Dongjinchal could not be

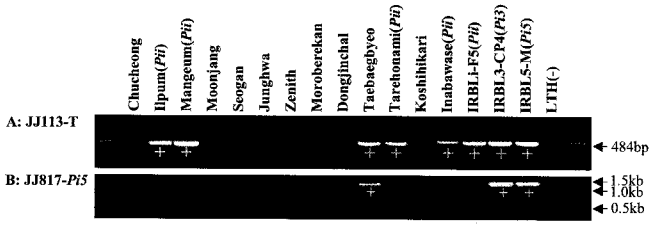

Fig. 1. PCR profiles from genomic DNA amplified by the dominant *Pii*- and *Pi5*-specific primers. The primer JJ113-T produced positive band to the lines harboring *Pii*, *Pi3* and *Pi5* genes. The JJ817-*Pi5* primer was positive only to the rices only having *Pi3* and *Pi5* genes. Three lines, IRBLi-F5, IRBL3-CP4 and IRBL5-M were the monogenic lines of *Pii*, *Pi3* and *Pi5*, respectively.

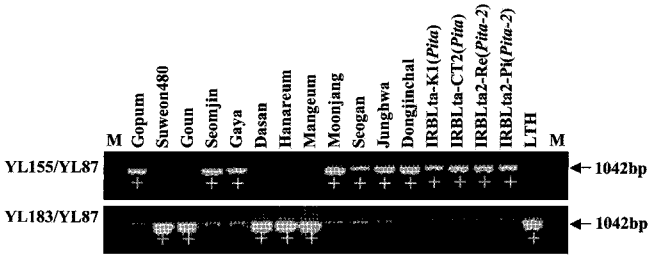

Fig. 2. PCR profiles generated using *Pita* and *Pita-2*-specific primers. (**Top**) The primer set YL155/YL87 is specific for the *Pita*-allele and produced positive bands in the varieties Gopum, Seomjin, Gaya, Moonjang, Seogan, Junghwa and Dongjinchal with four monogenic lines for *Pita* and *Pita-2*. (**Bottom**) The primer set YL183/YL87 that is specific for the *pita*-allele generates a positive band in each of the lines that did not show an amplified product for the YL155/YL87 primer set for the *Pita*-allele.

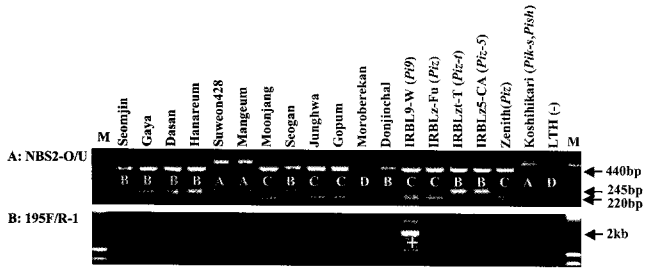

Fig. 3. PCR profiles from genomic DNA amplified by two dominant primers for *NSB2-Pi9* (Qu et al. 2006). (A) The PCR products that produced using *NBS2*-O/U primer set were digested by the restriction enzyme *Hinf* I and classified into four types, type A of 500-460-240bp bands, type B of 460-240bp bands, type C of 460-220bp bands, and type D of null band, respectively. (B) 195F-1/R-1 primer for NSB2-Pi9 candidate gene produced the positive band for IRBL9-W.

inferred from the parent lines. Of the 10 *Tongil-type* varieties screened, only Gaya was positive for the *Pita*-allele markers.

The *Piz* and *Piz-t* genes on chromosome 6 were identified from the 21 Korean varieties (21.4%) in our study cohort. The *Piz* gene using these Japanese *japonica* varieties as primary donors was introduced into Korean *japonica* varieties. The major secondary donors were found to be two early-maturing varieties, Jinbu and Sambaeg. Three Korean *Tongil-type* varieties, Baegunchal, Hangangchal and Samgang, contained *Piz-t* gene inherited from the IRRI-bred *indica* lines, IR2061 and IR4445.

The Korean *japonica* and *Tongil-type* varieties were grouped into four types: Koshihikari-type, *Piz-t* and *Piz-5*-type, *Piz* and *Pi9*-type, and null-type, respectively, based on the allele types of two *Pi9*-specific markers, pBA14 and NBS2-O/U (Fig. 3). The 21 varieties of *Piz* and *Pi9*-type were not positive to 195-1 marker, but the monogenic line IRBL9-W of *Pi9* gene was positive. We designated this locus as *Pi9(t)* in 21 Korean rice varieties because it might not be *Pi9* gene but possibly a member of a multigene family of resistance loci.

Out of the *Pik*-multiple alleles, *Pik*, *Pik-p*, and *Pik-m*, the *Pik-p* gene was identified in a *japonica* variety Sangnambat, and seven *Tongil-type* varieties. The *Pik* gene was identified in 17 *japonica* varieties but not in *Tongil-type* varieties, and the *Pik-m* gene was detected only in a *japonica* Seogan. However, we were not able to deduce the donor parents for these *Pik-m* and *Pik-p* genes in *japonica* varieties without the use of an allelism test. The *Pik-p* gene identified in seven *Tongil-type* varieties could be inherited from the IRRI-bred *indica* lines. None of the Korean rice varieties analyzed in the present study were found to be positive for the SNP marker of the *Pit* gene, t256, which is inherited from an *indica* rice strain K59.

Table 3. Major *R* genes present in *japonica* rice varieties of resistance at blast nursery test.

Varieties	Ecotype*	Line no.	Cross combinations	*R* genes[♪]	Reaction
Gru	*E*	Suweon416	Suweon313/Cheolweon42	*b, ta-2, k, 9(t)*	R
Moonjang	*E*	Sangju21	Sangsan/Suweon397	*a, ta- 2, k, 9(t)*	R
Saesangju	*E*	Sangju24	Junghwa/Sambaeg	*a, ta-2, z, 9(t)*	R
Samcheon	*E*	Unbong13	Unbong/Fukei126	*a, z, 9(t)*	R
Sangmi	*E*	Sangju19	Sambaeg/Oou316	*b, ta, 9(t)*	R
Undoo	*E*	Jinbu25	Odae/Jinbu13	*b, z, 9(t)*	RM
Jinbu	*E*	Jinbu10	Fukuhikari/Hokuriku109	*z, ta, k, 9(t)*	RM
Manchu	*E*	Iksan448	Jinmi/Unbong12	*ta-2, i, k*	RM
Sambaeg	*E*	Sangju12	Koshihikari/YR2406-2-1-1//Hokuriku115/CH.29	*a, ta, z, 9(t)*	RM
Suweon365	*E*	Suweon365	Seonam/Iri353	*a, b, ta-2*	RM
Taebong	*E*	Cheolweon5	SR13390-13-3-5-2/Jinbu10	*b, i, z, k, 9(t)*	RM
Taeseong	*E*	Cheolweon61	Cheolweon49/Jinbu10	*z, k, 9(t)*	RM
Donghae	*M*	Yeongdeog5	Milyang20/Nagdong	*a*	RM
Geuman	*M*	Suweon462	SR11878-14-4-1/Suweon345	*b, ta-2*	RM
Gopum	*M*	Suweon479	SR10252-32-2-2-2/Suweon366//SR15140-58-2-2-3	*b, ta, z, i, k*	RM
Manweol	*M*	Milyang173	Milyang120/Hwayeong	*a, b*	RM
Palgong	*M*	Milyang80	HR1591-43-2-2-2/YR6542B-16-3-B	*a, b*	RM
Sampyeong	*M*	Suweon444	Suweon345/SR11340-46-5-4-1	*b, ta*	RM
Sangnamba	*M*	Milyang93	YR153-12-1-2/Norin mochi 1	*b, k-p, 9(t)*	RM
Sangok	*M*	Milyang182	Milyang101/YR8697Acp19	*a, ta*	RM
Seogan	*L*	Namyang6	Suweon224/Inabawase//Seolak	*ta-2, k, k-m*	RM
Sinseoncha	*L*	Iri355	Milyang20/Hiyokumochi	*a*	RM
Sujin	*L*	Milyang156	Milyang95/Milyang96//Milyang106	*b, ta-2*	RM

*Ecotype: *E*, early maturing; *M*, medium maturing; *L*; mid-late maturing.

[♪] *R* genes: *a, Pia; b, Pib; i, Pii; k, Pik; k-m, Pik-m; k-p, Pik-p; ta, Pita; ta-2, Pita-2; z, Piz; 9(t), Pi9(t).*

Table 4. Major *R* genes present in *japonica* rice varieties of moderate resistance at blast nursery test.

Varieties	Ecotype*	Line no.	Cross combinations	*R* genes[♭]	Reaction
Goun	*E*	Jinbu36	Jinbu10/Jinbu17	*z, k, 9(t)*	M
Jinbuchal	*E*	Jinbu9	SR4085/Todorokiwase//Wasetoramochi	*z, i*	M
Joan	*E*	Suweon478	Jinmi/Jinbu10	*a, b, i, k, 9(t)*	M
Junghwa	*E*	Sangju15	Etsnan126/Bongkwang//Daeseong	*b, ta-2, k, 9(t)*	M
Sangju	*E*	Sangju10	Cheonma/Odae	*a, b, z, 9(t)*	M
Sangjuchal	*E*	Sangju18	YR4117-99-1-1-2-4/YR4200-2-3-2-2	*ta, z, i, k, 9(t)*	M
Sinunbong	*E*	Unbong7	37-A/Akayudaka	*z*	M
Suweon345	*E*	Suweon345	Suweon224/Inabawase//Cheolweon21	*b*	M
Unbong	*E*	Unbong1	Fukei104/Fuji269	*a, z, 9(t)*	M
Haepyeong	*M*	Yeongdeog26	Milyang101/Akichikara	*b, 9(t)*	M
Seoan	*M*	Namyang6	Suweon224/Inabawase//Seolak	*b, i*	M
Sobi	*M*	Iksan435	Hwayeong/YR13604Acp22	*i*	M
Yeonghae	*M*	Yeongdeog19	Milyang101/Chucheong	*b*	M
Daepyeong	*L*	Iksan450	HR14028-AV5/Milyang122	*b*	M
Daesan	*L*	Milyang142	Milyang95/Suweon366	*a, b, ta*	M
Dongan	*L*	Iksan418	Milyang95/HR5119-12-1-5	*a, b, ta-2*	M
Dongjin	*L*	Iri348	Kinmaze/Milyang15//Sadominori	*a, ta-2*	M
Dongjin 1	*L*	Iksan444	Hwayeong/HR12800-AC21	*b*	M
Gyehwa	*L*	Gyehwa3	Dongjin/Saikai145	*a, ta-2*	M
Hojin	*L*	Iksan436	Hwayeong//Dongjin/Milyang95	*b, ta-2*	M
Hwajung	*L*	Suweon387	Sasanishiki/Cheonma	*a*	M
Hwanam	*L*	Milyang115	Milyang95/Tamjin	*ta*	M
Hwasin	*L*	Iri407	Iri390/Milyang110	*i*	M
Hwayeong	*L*	Milyang101	Chukei830/YR4811Acp8	*b*	M
Ilmi	*L*	Milyang122	Milyang96//Milyang95/Seomjin	*ta-2*	M
Junam	*L*	Milyang165	Hwayeong//Sangju/Ilpum	*b*	M
Nampyeong	*L*	Iri416	Iri390/Milyang95	*b, ta, i*	M
Saegyehwa	*L*	Gyehwa19	Ilpum//Mangeum/Chukei830	*b*	M
Samgwang	*L*	Suweon474	Suweon361/Milyang101	*a, b*	M
Seomjin	*L*	Iri353	Milyang20/Asominori	*a, b, ta, z*	M
Seopyeong	*L*	Gyehwa22	Hwayeong/HR11752-11-1-4-3	*b*	M
Sindongjin	*L*	Iksan438	Hwayeong/YR13604Acp22	*b*	M
Tamjin	*L*	Iri373	HR1591-43-2-1-2-4/HR1590-92-4-4-4-4	*ta, k*	M

*Ecotype: *E*, early maturing; *M*, medium maturing; *L*; mid-late maturing.

[♭] *R* genes: *a, Pia; b, Pib; i, Pii; k, Pik; k-m, Pik-m; k-p, Pik-p; ta, Pita; ta-2, Pita-2; z, Piz; 9(t), Pi9(t).*

Classification of rice varieties based on nursery screening for blast resistance

The reactions to *M. grisea* among the 88 *japonica* and 10 *Tongil-type* varieties under study were classified into three categories: a) resistant (R, RM); b) medium resistant (M); and c) susceptible (MS, S) based upon a four-year nursery test undertaken between 2003 and 2006 in 14 regions throughout Korea. Among the 88 *japonica* varieties that we tested, 23 varieties were classified as resistant, 33 as moderately resistant, and 21 as susceptible (Tables 3-5). The 23 varieties of resistant group consisted of 12 early maturing, 8 medium, and 3 mid-late varieties, while the varieties of medium resistance included 9 early, 4 medium and 20 mid-late maturing, and the susceptible varieties were 5 early, 9 medium and 7 mid-late maturing. The remaining 11 *japonica* varieties were not classified into any of these three groups since they do not contain any of the 13 major blast resistant genes (Table 6). Ten *Tongil-type* varieties were classified as resistant (R and RM) at the blast nursery screening (Table 7). Three NPTs of *Tongil-type* showed resistant moderately (RM) with scores of 0-3 in most of the plots or of 4-5 in a few plots.

R genes associated with the resistant group

The results obtained with the resistant group consisting of 23 *japonica* rice varieties (Table 3) suggested that 19 of these varieties had two to four *R* genes. Two varieties, Donghae and Sinseonchal contained one *R* gene *Pia* and *Pii* respectively. Taebong of early maturing harbored five *R* genes, *Pib, Pii, Piz, Pik,* and *Pi9(t)* while Gopum variety of good palatability and medium maturity had five *R* genes, *Pib, Pita, Piz, Pik,* and *Pi9(t)*. Four early-maturing varieties had four *R* genes each; Gru (*Pib, Pita, Pik, Pi9(t)*), Moonjang (*Pia, Pita, Pik, Pi9(t)*), Saesangju (*Pia, Pita, Piz, Pi9(t)*), Jinbu (*Piz,*

Pita, Pik, and Pi9(t)), and Sambaeg (*Pia, Pita, Piz, Pi9(t)*). Among the 23 *japonica* varieties belonging to the resistant group, 12 (52.2%) harbored the *Pib* gene, 14 (60.1%) contained the *Pita* or *Pita-2* genes. The *Pia, Pii, Piz, Pik, Pik-m*, and *Pik-p* genes were identified from 10, 3, 8, 8, 1, and 1 of these varieties, respectively. The *Pi9(t)* gene, a putative member of the multiple *R* gene family, was identified in 11 (47.8%) out of 23 resistant *japonica* varieties.

R genes contained in the moderately resistant group

Thirty-three *japonica* varieties were assigned to the moderately resistant group at blast nursery test (Table 4). The resistance genes in this group were *Pia* in 10 varieties, *Pib* in 20, *Piz* in 7, *Pii* in 7, *Pik* in 5, and *Pita* or *Pia*-2 in 12 further varieties. Sixteen varieties contained a single *R* gene, whereas 13 varieties contained two to three resistance genes. Two varieties, Joan and Sangjuchal contained five *R* genes, and two other varieties, Junghwa and Sangju harbored four *R* genes. Among the varieties containing a single *R* gene, *Pia* was found in two, *Pib* in nine, *Pita* and *Pii* in two of these strains, and *Piz* in one variety. A putative *R* gene of the multigene family of the *Pi9(t)* locus was identified in seven of the *japonica* varieties in this moderately blast resistant group. Two varieties, Palgong and Seomjin were evaluated as the durable resistance varieties from the result of long-term blast nursery screening and sequential planting method (Han et al. 2001; Kim et al. 2004b). Palgong was found to have two *R* genes, *Pia* and *Pib*, whereas Seomjin harbored four R genes *Pia, Pib, Pita*, and *Piz*.

R genes present in the blast susceptible group

Among the 88 *japonica* varieties of rice that we analyzed in this study, 21 were assigned to the blast susceptible group based on their nursery screening score (Table 5). Among the resistance genes that were contained in the varieties of this group, *Pia* was found in 9 varieties, *Pib* in 7, *Piz* in 2, *Pii* in 7, PIK in 4, and *Pita* or *Pita-2* in 6 varieties. Furthermore, 19 of 21 varieties in this group contained either a single or two *R* genes. A Mangeum was found to contain three *R* genes, *Pia, Pib,* and *Pii* with one further variety Jungsan harboring four *R* genes, *Pib, Pik, Pita*, and *Piz*. Two varieties, Geumo and Jungan contained a locus corresponding to the *Pi9*-multigene family.

Non-*R* gene containing group

Eleven (11.2%) of the *japonica* varieties analyzed appeared not to contain any of the 13 *R* genes screened in this study (Table 6). These varieties were consisted of one early maturing, five medium, and five mid-late. Five varieties were grouped to moderate resistance, another five varieties were moderately susceptible, and the last variety Juan was

Table 5. Major *R* genes present in *japonica* rice varieties of different degrees of susceptibility at blast nursery test.

Varieties	Ecotype*	Line no.	Cross combinations	*R* genes†	Reaction
Geumo	*E*	Suweon313	Akitsuho/Fuji269	*z, 9(t)*	MS
Hwadong	*E*	Suweon409	Daegwan/SR13345-20-1	*a b, k*	MS
Jinmi	*E*	Suweon349	Inabawae/SR4084-5-4-6	*b, i*	S
Jungsan	*E*	Sangju22	Sambaeg/Milyang107	*b, ta-2, z, k*	MS
Odae	*E*	Suweon303	Akitsuho/Fuji269	*k*	MS
Gwangan	*M*	Suweon429	Namyang7/SR14779-HB234-31	*a, i*	MS
Hwaan	*M*	Suweon447	Suweon362/SR10778-2-2	*ta*	MS
Hwabong	*M*	Milyang138	Milyang95/Iri390//Milyang101/Iri390	*a, ta*	MS
Hwajin	*M*	Suweon346	Milyang64/Iri353	*a*	MS
Hwaseong	*M*	Suweon330	Aichi37/Samnam	*a*	S
Jinpum	*M*	Suweon434	SR14703-60-5-GH1/Suweon353	*b, i*	MS
Jungan	*M*	Suweon438	Namyang7/Hapcheonaengmi1*2	*k, 9(t)*	S
Seogjeong	*M*	Namyang26	Namyang7/SR11340-30-4-1-3-2	*a*	S
Seojin	*M*	Namyang17	Aichi37/Sangpung	*ta*	S
Dongjinchal	*L*	Iksan425	Milyang95//SR11155-4-2/Toyonishiki	*ta*	S
Hopyeong	*L*	Iri401	Hitomebore/Hwajin	*i*	S
Hwamyeong	*L*	Suweon423	Milyang101/SR14779-HB234-32	*a*	MS
Ilpum	*L*	Suweon355	Suweon295-sv3/Inabawase	*b, i*	S
Jongnam	*L*	Milyang169	Milyang96/YR12734-B-B-22-2	*a, ta-2*	MS
Mangeum	*L*	Iri390	Milyang71/Saikai PL1	*a, b, i*	MS
Suweon480	*L*	Suweon480	Suweon379/SR12191-38-2-1-1-3	*b, i*	S

*Ecotype: *E*, early maturing; *M*, medium maturing; *L*; mid-late maturing.

† *R* genes: *a, Pia; b, Pib; i, Pii; k, Pik; ta, Pita; ta-2, Pita-2; z, Piz; 9(t), Pi9(t).*

Table 6. *Japonica* rice varieties containing none of the *R* genes under study.

Varieties	Ecotype*	Line no.	Cross combinations	*R* genes	Reaction
Namil	*E*	Suweon472	Ilpum/Namyang7	-	MS
Geumo 1	*M*	Milyang125	Chukei830///Kanto PL5/Milyang79/Aichi65	-	M
Hwaseonchal	*M*	Suweon384	Milyang64/Sinseonchal	-	MS
Juan	*M*	Suweon383	Seolak/Koshihikari//Samnam	-	S
Samdeog	*M*	Yeongdeog32	YR12733-B-B-5-1/Milyang101	-	M
Sura	*M*	Suweon427	Suweon345/Kanto PL4//Suweon345	-	MS
Anjung	*L*	Suweon362	Chugoku69/Sangpung	-	M
Daean	*L*	Suweon396	Oseto/Seomjin	-	M
Hwasam	*L*	Milyang123	Milyang101/Iri389	-	M
Nagdong	*L*	Milyang15	Norin6/Mineyudaka	-	MS
Milyang95	*L*	Milyang95	Chukei1016/YR3477-54-B-2	-	MS

*Ecotype: *E*, early maturing; *M*, medium maturing; *L*; mid-late maturing.

grouped to susceptible. Nagdong, a highly susceptible Korean *japonica* variety compatible to most Korean blast isolates, was grouped to moderately susceptible at blast nursery test, and not positive for any of the *R* gene-specific markers, as it did not contain any *R* gene source from its parents, Norin6 and Mineyudaka. Although these varieties did not contain any of the blast resistance genes tested, the possibility that they harbored other as yet unknown resistance gene(s) could not be discounted. Other varieties of this group were developed by using most parents having any specific *R* genes, but we suggested that the *R* genes flowed out through the breeding.

R genes present in *Tongil-type (indica/japonica)* varieties

All 10 *Tongil-type* rice varieties in this study harbored 1 to 3 *R* genes. *Pib* gene was identified in all *Tongil-type* varieties (Table 7). Three *R* genes, *Pik-p, Pita,* and *Piz-t* genes inherited from the *indica* rice genotypes were identified from 7, 2, and 3 of these varieties, respectively. The *R* gene *Piz* was identified only in Gaya. Two varieties, Dasan and Hanareum, of three new high-yielding *Tongil-types* developed from the crosses with IRRI-bred NPT types (New Plant type) were found to contain the *Pib* gene and the other, Anda, contained three *R* genes, *Pib, Pik-p*, and *Pi9(t)*. The *Pi5* gene was identified only in Taebaeg.

Discussion

In our current study, 13 blast resistance genes for *M. grisea* were successfully screened from Korean *japonica* and *Tongil-type* varieties of rice using specific molecular markers. Our results for the 13 *R* genes analyzed were found to be largely consistent with the genetic background by genealogical tracking of varieties. A large number of Korean rice varieties were found to contain between 1-5 blast resistance genes, and showed degrees of resistance to the *M. grisea* pathogen ranging from highly resistant to susceptible. All of the *Tongil-type* varieties contained 1-3 *R* genes with *Pib* as the common gene.

The four *R* genes *Pia, Pii, Pik*, and *Pik-m* of 13 resistance genes analyzed originated from *japonica* rices, and identified only from *japonica* varieties in our current analyses. Three genes *Pia, Pii*, and *Pik* were originally inherited from the Japanese *japonica* varieties Aichi Asahi, Fujisaka5, and Kanto51, respectively (Yamasaki and Kiyosawa 1966), and *Pik-m* gene originated from the Japanese *japonica* variety Tsuyuake (Kiyosawa 1978).

The other eight genes, *Pib, Pi5, Pita, Pita-2, Piz, Piz-t, Pik-p*, and *Pit* in this study originated from *indica* rices, and a *R* gene *Pi9* was from wild rice. The *Pib* gene on chromosome 2 is an *indica*-derived resistance gene, and introgressed independently from two Indonesian and two Malaysian varieties (Yokoo et al. 1978). The donor isogenic lines for *Pib* gene in Korean *japonica* rice varieties were BL1 from an Indonesian *indica* variety Tjahaja, and BL7 derived from the Malaysian *indica* Milek Kuning strain, respectively. Two *R* genes *Pita* and *Pita-2* on chromosome 12 were introgressed from a common donor, the *indica* Philippine variety Tadukan into various Japanese *japonica* rices (Shigemura and Kitamura 1954). *Pita* was mapped to the position overlapping *Pita-2* by graphical genotype analysis of an NIL with a very narrow introgressed region, Shimokita (Rybka et al. 1997). This result was consistent in that these functionally related genes are allelic or at least very closely located, and may be derived from a common ancestral gene. The *Pita* and *Pita-2* genes in Korean *japonica* varieties were from Shimokita, and Fuji280 and Sadominori, respectively.

The *Piz* gene derived from the USA indica rice Zenith was introduced into the Japanese *japonica* rice varieties Fukuhikari and 54BC-68 (Kiyosawa 1967), and this gene in

Table 7. Major *R* genes present in Korean *Tongil-type* rice varieties under study.

Varieties	Ecotype*	Line no.	Cross combinations	*R* genes♪	Reaction
Baegunchal	*E*	Iri344	Milyang20/IR29	*b, z-t, k-p*	RM
Taebaeg	*E*	Suweon287	IR24*2/IR747	*b, 5, k-p*	R
Gaya	*M*	Milyang54	Milyang21/IR32//Milyang23/Milyang30	*b, ta, z*	R
Hangangchal	*M*	Suweon290	IR2061/KR51	*b, z-t, k-p*	R
Samgang	*M*	Milyang46	Milyang30/IR4445	*b, z-t, k-p*	R
Tongil	*M*	Suweon213	IR8//Yukara/Taichung Native 1	*b, k-p*	R
Anda♫	*M*	Suweon431	SR11532-4/SR14502F2	*b, k-p, 9(t)*	RM
Dasan	*M*	Suweon405	Suweon332/Suweon333	*b*	RM
Hanareum	*M*	Milyang181	Milyang103/Suweon405	*b*	RM
Milyang23	*L*	Milyang23	Suweon232/IR24	*b, ta, k-p*	RM

*Ecotype: *E*, early maturing; *M*, medium maturing; *L*; mid-late maturing.
♪ *R* genes: *b, Pib; 5, Pi5; Pik; k-p, Pik-p; ta, Pita; z, Piz; z-t, Piz-t; 9(t), Pi9(t).*
♫ Varieties of italic are New Plant Type (NPT) of *Tongil-type.*

Korean *japonica* varieties was from Fukuhikari and 54BC-68. The *Piz-t* gene in the IRRI-bred *indica* lines originated from the Indian *indica* varieties, TKM1 and TKM6 (Ebron et al. 2004), and this gene in *Tongil-type* was derived from TKM6 based on pedigree tracking.

The *Pik-p* gene which was first reported in the west Pakistani *indica* rice variety Pusur (Kiyosawa 1969), could be inherited into seven *Tongil-type* varieties from the IRRI-bred *indica* lines. The resistance gene *Pit* on chromosome 1 was inherited from an *indica* rice strain K59 (Kiyosawa 1972b). However, this *R* gene was not identified in Korean *japonica* and *Tongil-type* varieties in this study. The *Pi5* gene was identified from a cross between CO39 and Moroberekan (Wang et al. 1994). In this study, this gene was only identified in *Tongil-type* variety Taebaeg. In DNA-gel blot analysis, the *Pi5* gene was not inherited from the West Africa upland rice, Moroberekan, but from the *indica* rice, and the genomic structure of three *indica* varieties, IR72, Jahangdo and Taebaeg was most similar to that of *Pi5* (Yi et al. 2004). Consequently, our finding that *japonica* varieties were not positive at the *Pi5* gene was consistent with the suggestion that this gene would be inherited from an *indica* rice.

The *Pi9* gene locus was discovered in *Oryza minuta*, a tetraploid wild species of the *Oryza* genus (Amante-Bordeos et al. 1992). The Korean *japonica* and *Tongil-type* varieties were grouped into four types: Koshihikari-type, *Piz-t* and *Piz-5*-type, *Piz* and *Pi9-type*, and null-type, respectively, from two markers, pBA14 and NBS2-O/U. The 21 varieties of *Piz* and *Pi9-type* were not positive to the 195-1 marker, but the monogenic line IRBL9-W of *Pi9* gene was positive. As a result, the *Pi9(t)* gene locus from the Korean *japonica* and *Tongil-type* varieties differs from the original *Pi9* gene, indicating that it may be a member of a multi *R* gene family. Twelve out of the 21 varieties tested that contain the *Pi9(t)* gene cluster were found to contain the *Piz* gene. The loci of this cluster are located on chromosome 6 and form a region containing the *Pi9, Piz* and *Piz-t* genes, of which the *Pi9* and *Piz-t* genes are closely related in sequence and structure to the multiple gene family members at their corresponding loci (Qu et al. 2006; Zhou et al. 2006). However, the alleles identified in this locus of *Pi9(t)* gene in Korean rice varieties must be clarified based on allelic test with *Pi9, Piz*, and *Piz-t* genes.

In the screening of 26 monogenic lines of *R* genes to blast, the genes originating from *japonica* genotypes were not effective to the blast isolates, and a few genes, *Piz-5, Piz-t, Pi5,* and *Pi9*, derived from *indica* and wild rice showed stable resistance (Cho et al. 2005). Although the *Pib* and *Pik-p* resistance genes identified in *Tongil-type* varieties might confer resistance to a number of blast isolates, these genes could still be vulnerable to naturally-occurring virulent mutants (Yokoo 2005), as well as to artificial mutant isolates (Kim et al. 2004a). Kiyosawa (1976) reported that in the field the *Avr* gene corresponding to the *R* gene, *Pib*, could be clearly categorized into compatible isolates to the *Pib* gene. These findings indicate that since 1978, the blast resistance of the *Tongil-type* varieties having *Pib* gene in common has been lost due to the differentiation of new virulent isolates that are compatible with *Tongil-type* as well as *japonica* varieties of rice.

In 77 *japonica* varieties having over at least one *R* gene, 26 early maturing varieties had an average of 3.2 *R* genes in each, 21 medium maturing had 1.8, and 30 mid-late maturing contained 1.7, respectively. The resistance varieties to blast was 46.2% in early maturing, 38.1% in medium maturing, and 10% in mid-late maturing varieties, respectively. These phenotypes did not always appear to depend on the number of *R* genes present in a particular variety. Of three ecotypes, most early-maturing varieties harbored more *R* genes than the medium and mid-late maturing ones, and showed a medium to high resistance. Fifteen out of the 17 *japonica* varieties that were found to harbor the *Piz* gene in our present report

are early maturing varieties that have been adapted to mid-mountainous or mountainous areas such as Jinbu, Sangju, and Unbong in Korea. Only two *japonica* varieties, Gopum and Seomjin, which contain the *Piz* gene are classified as medium and mid-late maturing, respectively. It is known that the *Piz* gene has been linked to the major photoperiod sensitivity gene *Se1*, which is responsible for late heading and located on chromosome 6 (Yokoo, 2005). Interestingly, most varieties having *Piz* and/or *Pi9(t)* genes on chromosome 6 showed more stable resistance.

The areas cultivated with 18 good palatability *japonica* varieties reached over 95% of the total areas cultivated with rice in Korea, 2006; notably, the eco-friendly cultivation using good palatability *japonica* varieties is gradually increasing. We experienced the breakdown of the resistance to blast in about 10-year cycles since 1978 with the rapid increase of cultivation areas of varieties of similar genetic background such as *Tongil-type* varieties. Also, new isolates of the enhanced virulence by differentiation are compatible with both *Tongil-type* and *japonica* varieties. Our present results from screening for different rice blast isolates may not be conclusive as yet but make an important contribution to our understanding of the particular *R* genes that are present in specific Korean rice varieties (Ebron et al. 2004; Jin et al. 2007). Thus, the findings of our current study will be useful information to enhance blast resistance in rice breeding programs in the future.

The available molecular markers that are linked to the major blast resistance genes were also found to be useful in confirming and identifying these specific genes (Campbell et al. 2004; Chen et al. 1999; Cho et al. 2003; Hayashi et al. 2004, 2006; Jia et al. 2002, 2004; Liu et al. 2002; Naqvi et al. 1996; Qu et al. 2006; Wang et al. 1994; Yi et al. 2004). In our present study, we were not able to identify the origins of some of the *R* genes based only on the genealogy of the varieties. In addition, the relationship between the number of resistance gene(s) and the reaction of blast resistance, and also the determination of whether resistance had been lost for particular *R* gene(s), could not be completely clarified in our present experiments. In this regard, genetic approaches based on the allelism test will be necessary in future experiments to confirm the presence of predicted or masked *R* gene(s).

Acknowledgements

This work was supported in part by a Basic Fund of the NICS and Biogreen21 program, RDA, and by the Crop Functional Genomics Center, the 21st Century Frontier Program, Korea.

References

Ahn SN, Kim YK, Hong HC, Han SS, Choi HC, McCouch SR, Moon HP. 1997. Mapping of genes conferring resistance to Korean isolates of rice blast fungus using DNA markers. Korean J. Breed. 29: 416-423

Amante-Bordeos A, Sitch LA, Nelson R, Dalmacio RD, Oliva NP, Aswidinnoor H, Leung H. 1992. Transfer of bacterial blight and blast resistance from the tetraploid wild rice *Oayza minuta* to cultivated rice, *Oryza sativa*. Theor. Appl. Genet. 84: 345-354

Campbell MA, Chen D, Ronald PC. 2004. Development of co-dominant amplified polymorphic sequence markers in rice that flank the *Magnaporthe grisea* resistance gene Pi7(t) in recombinant inbred line 29. Phytopathology 94: 302-307

Chen DH, dela Vina M, Inukai T, Mackill DJ, Ronald PC, Nelson RJ. 1999. Molecular mapping of the blast resistance gene, Pi44(t), in a line derived from a durably resistant rice cultivar. Theor. Appl. Genet. 98: 1046-1053

Cho YC, Choi IS, Baek MK, Suh JP, Hong HC, Kim YG, Koizumi S, Jena KK, Choi HC, Hwang HG. 2003. Resistant genes and their effects to rice blast in isogenic lines of genetic background of Chucheongbyeo and Suweon345. Rice Genet. News. 20: 101-105

Cho YC, Choi IS, Baek MK, Yanoria MJT, Suh JP, Roh JH, Kim YG, Jena KK, Choi HC, Hwang HG. 2004a. Analysis of resistant genes to blast (*Magnaporthe grisea*) in the *japonica* rice Ilpumbyeo. Korean J. Breed. 36: 81-89

Cho YC, Baek MK, Suh JP, Kwon SJ, Choi IS, Kim YG, Han SS, Cho HC, Hwang HG. 2004b. Mapping of quantitative resistant genes to leaf blast in a *japonica* rice cultivar, Suweon365. Korean J. Breed. 36: 125-132

Cho YC, Roh JH, Kim BR, Choi IS, Kim MK, Han SS, Fukuta Y, Hwang HG, Kim YG. 2005. Reaction of resistance genes of monogenic lines to rice blast (*Magnaporthe grisea*). Korean J. Breed. 37: 155-161

Choi JE, Park JS, Park NK. 1989. Resistance of varieties to rice blast in Korea, I. Japonica type of rice varieties. J. Agri. Sci. Chungnam Natl. Univ. 16: 1-18 (in Korean, English summary)

Dellaporta SL, Wood J, Hick JB. 1983. A plant DNA mini preparation; Version II. Plant Mol. Biol. Rep. 1: 19-21

Ebron LA, Fukuta Y, Imbe T, Kato H, Yanoria JMT,

Tsunematsu H, Khush GS, Yokoo M. 2004. Estimate of genes in blast resistance in elite Indica-type rice (*Oryza sativa* L.) varieties bred at the International Rice Research Institute. Breeding Sci. 54: 381-387

Han SS, Ryu JD, Shim HS, Lee SW, Hong YK, Cha KH. 2001. Breakdown of resistant cultivars by new race KI-1117a and race distribution of rice blast fungus during 1999~2000 in Korea. Res. Plant Dis. 7: 86-92 (in Korean, English summary)

Hayashi K, Hashimoto N, Daigen M, Ashikawa I. 2004. Development of PCR-based SNP markers for rice blast resistance genes at the *Piz* locus. Theor. Appl. Genet. 108: 1212-1220

Hayashi K, Yoshida H, Ashikawa I. 2006. Development of PCR-based allele-specific and InDel marker sets for nine rice blast resistance genes. Theor. Appl. Genet. 113: 251-260

Hwang HG, Kwon SJ, Cho YC, Ahn SN, Suh JP, Moon HP. 2004. Genetic diversity of high-quality rice cultivars based on SSR markers linked to blast resistance genes. Korean J. Crop Sci. 49: 251-255 (in Korean, English summary)

Imbe T, Oba S, Yanoria MJT, Tsunematsu H. 1997. A new gene for blast resistance in rice cultivar, IR24. Rice Genet. Newsl. 14: 60-62

Jeon JS, Chen D, Yi GH, Wang GL, Ronald PC. 2003. Genetic and physical mapping of *Pi5(t)*, a locus associated with broad-spectrum resistance to rice blast. Mol. Genet. Genomics 269: 280-289

Jia Y, Wang Z, Shing P. 2002. Development of dominant rice blast *Pi-ta* resistance gene markers. Crop Sci. 42: 2145-2149

Jia Y, Wang Z, Fjellstrom RG, Moldenhauer KAK, Azam MDA, Correll J, Lee FN, Xia Y, Rutger JN. 2004. Rice *Pi-ta* gene confers resistance to the major pathotypes of the rice balst fungus in the United States. Phytopathology 94: 296-301.

Jin XJ, Lee EJ, Choi JE. 2007. Classification of Korean rice cultivars based on reaction pattern to variable blast pathogen. J. Crop Sci. Biotech. 10: 3-7

Kim BR, Han SS, Roh JH, Choi SH, Lee YH. 2004a. Pathogenic characters of transformants mediated from Magnaporthe grisea, KJ-201(90-014) by ATMT methods. In Proceeding of The 2004 Korean Society of Plant Pathology Annual Meeting & Intl. Sym., Oct. 6-9, Pyeongchang, Korea. pp. 138

Kim BR, Roh JH, Choi SH, Ahn SW, Han SS. 2004b. Durability of rice cultivars to blast in Korea by sequential planting method. Korean J. Breed. 36: 350-356 (in Korean, English summary).

Kiyosawa S. 1967. The inheritance of resistance of the Zenith type varieties of rice to the blast fungus. Japanese J. Breed. 17: 99-107

Kiyosawa S. 1969. Inheritance of blast-resistance in west Pakistani rice variety, Pusur. Japan. J. Breed. 19: 121-128

Kiyosawa S. 1972a. Genetics of blast resistance. In Rice Breeding. IRRI, Manila, Philippines. pp. 203-225.

Kiyosawa S. 1972b. The inheritance of blast resistance transferred from some indica varieties in rice. Bull. Natl. Inst. Agric. Sci. D23: 69-96

Kiyosawa S. 1976. Pathogenic variations of Pyricularia oryzae and their use in genetic and breeding studies. SABRAO J. 8: 53-67

Kiyosawa S. 1978. Identification of blast-resistance genes in some rice varieties. Japan. J. Breed. 28: 287-296

Kwon SJ, Ahn SN, Hong HC, Cho YC, Suh JP, Kim YG, Kang KH, Han SS, Choi HC, Moon HP, Hwang HG. 2002. Identification of DNA markers linked to resistance genes to rice blast (*Pyricularia grisea* Sacc.). Korean J. Breed. 34: 105-110

Liu G, Lu G, Zeng L, Wang GL. 2002. Two broad-spectrum blast resistance genes, Pi9(t) and Pi2(t), are physically linked on rice chromosome. Mol. Genet. Genomics 267: 472-480

Naqvi NI, Chattoo BB. 1996. Development of a sequence characterized amplified region (SCAR) based indirect selection method for a dominant blast-resistance gene in rice. Genome 39: 26-30

Qu S, Liu G, Zhou B, Bellizzi M, Zeng L, Dai L, Han B, Wang G. 2006. The road-spectrum blast resistance gene *Pi9* encodes an NBS-LRR protein and is a member of a multigene family in rice. Genetics 172: 1901-1914

RGC (Rice Genetics Cooperative). 1998. B. Report of the committee on gene symbolization, nomenclature and linkage groups. Rice Genet. Newsl. 15: 13-76

Rybka K, Miyamoto M, Ando I, Saito A, Kawasaki S. 1997. High resolution mapping of the *Indica*-derived rice blast resistance genes II. *Pita2* and *Pita* and a consideration of their origin. Mol. Plant-Microbe Interact. 10: 517-524

Shigemura S, Kitamura E. 1954. Breeding of blast resistant cultivars with crossing of japonica and indica rices. J. Agric. Sci. Tokyo Nogyo Daigaku 9: 321-323. (in Japanese with English summary)

Tsunematsu H, Yanoria MJT, Ebron LA, Hayashi N, Ando I, Kato H, Imbe T, Khush GS. 2000. Development of monogenic lines of rices for blast resistance. Breeding Sci. 50: 229-234

Wang GL, Mackill DJ, Bonman JM, McCouch SR, Champoux CM, Nelson RJ. 1994. RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. Genetics 136: 421-434

Yamasaki Y, Kiyosawa S. 1966. Studies on inheritance of resistance of rice varieties to blast. I. Inheritance of resistance of Japanese varieties to several strains of the fungus. Bull. Natl. Inst. Agric. Sci. D14: 39-69. (in Japanese with English summary)

Yi G, Lee SK, Hong YG, Cho YC, Nam MH, Kim SC, Han SS, Wang GL, Hahn TR, Ronald PC, Jeon JS. 2004. Use of *Pi5(t)* markers in marker-assisted selection to screen for cultivars with resistance to *Magnaporthe grisea*. Theor. Appl. Genet. 109: 978-985

Yokoo M, Kikuchi F, Fujimaki H, Nagai K. 1978. Breeding of blast resistance lines (BL1 to 7) from indica-japonica crosses of rice. Japan. J. Breed. 28: 359-385. (in Japanese with English summary)

Yokoo M. 2005. Introduction of *Piz-t* and *Pib* genes for blast disease resistance from *indica* varieties and their utilization for genetic researches in rice. Japanese Agri. Res. Quarterly 39: 239-245

Zhou B, Qu S, Liu G, Dolan M, Sakai H, Lu G, Bellizzi M, Wang G. 2006. The eight amino-acid differences within three leucine-rich repeats between *Pi2* and *Piz-t* resistance proteins determine the resistance specificity to *Magnaporthe grisea*. Mol. Plant-Microbe Interact. 19: 1216-1228