RESEARCH ARTICLE
J. Crop Sci. Biotech. 2008 (September) 11 (3) : 205 ~ 214

Haplotype Diversity and Durability of Resistance Genes to Blast in Korean *Japonica* Rice Varieties

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<Received September 3, 2008 / Accepted September 18, 2008>

Abstract

Blast disease caused by the fungal pathogen, *Magnaporthe oryzae*, is one of the most damaging diseases in rice. The use of resistant varieties is an effective measure to control the disease, however, many resistant varieties were broken down to their resistance effects by the differentiating of new virulent isolates. This study was done to analyze the haplotypes of 31 microsatellite markers linked to five major *R* genes and two QTLs and to identify the alleles for the putatively novel genes related to durable resistance to blast in 56 Korean *japonica* and four *indica* varieties. The 31 microsatellite markers produced 2 to 13 alleles (mean = 5.4) and had PIC*i* values ranging from 0.065 to 0.860 (mean = 0.563) among the 60 rice accessions. Cluster analysis based on allele diversities of 31 microsatellite markers grouped into 60 haplotypes and ten major clusters in 0.810 genetic similarity. A subcluster IV-1 grouped of early flowering varieties harboring *Piz* and/or *Pi9(t)* on chromosome 6 and *Pita/Pita-2* gene on chromosome 12. The other subcluster V-1 consisted of four stable resistance varieties Donghae, Seomjin, Palgong and Milyang20. The analysis of putative QTLs associated with seven blast resistance genes using ANOVA and linear regression showed high significance to blast resistance across regions and isolates in the markers of two genes *Piz* and/or *Pi9(t)* and *Pita/Pita-2*. These results illustrate the utility of microsatellite markers to identify rice varieties is likely carrying the same *R* genes and QTLs and rice lines with potentially novel resistant gene.

Key words: blast, durability, haplotype diversity, *Magnaporthe oryzae*, resistance gene, rice

Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops in the world and the rice blast disease caused by the fungal pathogen, *Magnaporthe oryzae*, is one of the most damaging diseases of rice (Zeigler et al. 1994). Rice blast disease follows a classical gene-for-gene system (Silue et al. 1992), in which a pathogen strain expressing an avirulence (*AVR*) gene triggers the corresponding resistance (*R*) gene-mediated defense response. Incorporation of individual *R* genes into existing rice cultivars has not achieved reliable, long-lasting resistance to blast disease because of the high potential for *AVR* gene variation in the pathogen (Valent 1997). Pyramiding resistance genes for achieving effective blast control require molecular markers for introducing diverse genes into agronomically useful cultivars by marker-assisted selection (MAS). Thus far, more than 40 major blast resistance genes and numerous quantitative trait loci (QTL) have been identified and many of these have been associated with molecular markers (Hayashi et al. 2004, 2006; Hittalmani et al. 2000; Jeung et al. 2007; Rybka et al. 1997; Yi et al. 2004; Zeigler et al. 1994).

Disease resistant genes can often be differentiated using differential isolates of the pathogen, the chromosomal location in the host genome, allelism tests or DNA-based markers. Physiological races in *Magnaporthe oryzae* have not been identified to date, so blast resistance genes cannot be distinguished by different pathogen isolates. DNA-based markers can be used to access genetic diversity across an entire genome or at specific chromosome regions. These approaches make use of previous mapping

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information and have the potential to rapidly differentiate germplasm with different blast resistant genes. This study was to compare haplotypes of microsatellite markers linked to major resistant genes and QTLs and to identify the putatively novel blast resistance genes in good eating-quality Korean *japonica* varieties.

Materials and Methods

Plant materials

Sixty rice accessions were used in this study (Table 1). 56 Korean *japonica* varieties were consisted as 14 early, 18 medium and 24 mid-late maturing varieties in Korea. Also, highly susceptible varieties, Nagdong and Hopyeong, and four varieties, Dongan, Daesan, Ilmi and Ilpum, that broken down of the resistance to blast were included. Two varieties, Palgong and Seomjin, have been known as long-lasting resistance (Han et al. 2001; Roh et al. 2007). Two Korean *Tongil-types (indica/japonica)* Milyang23 and Taebaeg and two foreign cultivars, Moroberekan and Zenith of highly resistance, were used to compare the haplotype to the alleles of loci to blast resistance.

Phenotyping for blast nursery and isolates

The resistance at blast nursery was tested in 14 regions of regional adaptability test (RAT) from 2003 to 2006. The incidence scores at blast nursery test ranged from 0 (no lesion) to 9 (necrosis of all leaves and sheaths) by the standard evaluation method of International Rice Research Institute (IRRI). The reaction grouping was followed to the method of previously report (Cho et al. 2007).

Sixty rice accessions were screened to three isolates 90-008, 97-277 and 03-177. At 21 days after seeding (DAS), the single-race spore suspension was adjusted to a cell count of 20 - 30 spores per visual field under 100 × magnifications and inoculated by spray method. The inoculated seedlings were kept inside the incubation chamber at 26±1°C with saturated humidity for 24 hours and then transferred to the greenhouse until scoring time. The incidence degree to each race was evaluated at 7 days after inoculation (DAI). Disease reactions were scored on a scale from 0 (no lesion) to 9 (coalescence of > 5 mm lesions or 80% of the leaves killed) in accordance with IRRI standard evaluation method.

Genotyping for DNA markers

The 31 SSR and STS markers linked tightly to five major genes, *Pia, Pib, Pii, Piz* and *Pita* and two QTLs, *qBL1* on chromosome 1 and *qBL4.2* on chromosome 4, were analyzed to detect allele diversity. Markers with simple banding patterns and high polymorphic information content (PIC*i*) values were selected for this study. The PIC*i* values were calculated with the following formula (Botstein et al. 1980):

$$PIC_i = 1 - \sum_{j=1}^{n} Pij^2$$

where n is the number of marker alleles for marker *i*, and *Pij* is the frequency of the *j*th allele for marker *i*.

The PCR reactions were performed in a total volume of 20 μl containing 40 ng of template DNA, 2 μl PCR buffer, 1.6 μl of 2.5 mM dNTP, 0.4 μl each of a microsatellite forward primer (10 pM) and a reverse primer (10 pM), and 0.1 μl *Taq* polymerase (5U/ μl) (Neurotics Inc., Daejeon, Korea). The PCR amplification program in this instance consisted of 35 cycles of 1 min at 95°C, 1 min at 55°C, and 2 min at 72°C, and a final extension at 72°C for 10 min. Each of the PCR reactions was performed using a PTC-200 (Bio-RAD, Hercules, CA, USA). The fragment sizing for PCR amplicons of each primer were resolved using the QIAxcel of capillary gel electrophoresis (QIAGEN, Irvine, USA).

Statistical analysis

The analysis of QTLs for SSR markers associated with five major *R* genes and two QTLs was performed using ANOVA (one-way analysis of variance) and linear regression from SAS procGLM v.6.08. (SAS Institute 2002) Pearson correlation coefficients among the regions of blast nursery test and isolates were evaluated in SAS program using the degree of blast resistance reaction.

NTSYSpc version 2.11a (Exeter Software, Setauket, NY, USA) was used for cluster analysis. The SIMQUAL module was used to calculate similarity coefficients between 60 rice accessions. The SHAN module was used for cluster analysis with the unweighted pair group method with arithmetic mean (UPGMA). The generated DNA bands were analyzed by scoring as present (1) or absent (0) for each alleles.

Results

Table 1 presents the ecotypes, pedigrees and resistant genes, and the reactions to blast by field infection of *M. oryzae* at the blast nursery of 60 rice varieties were listed. Six out of 56 *japonica* rice varieties did not harbor any gene of thirteen *R* genes *Pia, Pib, Pii, Pik, Pik-m, Pik-p, Pit, Piz, Piz-t, Pita, Pita-2, Pi5* and *Pi9(t)* and twenty-nine (48.3%) harbored only one or two *R* genes (Table 1, Cho et al. 2007). The *japonica* varieties

Table 1. Species, ecotype, pedigree, and resistance genes and reaction to blast of 60 rice varieties.

Varieties	Species	Eco*	Eco*	*R* genes#	Reaction§	Origins
Goun	Japonica	E	Jinbu10/Jinbu17	*z,k,9(t)*	M	Korea
Gru	"	E	Suweon313/Cheolweon42	*b,ta2,k,9(t)*	R	"
Jinbu	"	E	Fukuhikari/Hokuriku109	*z,ta,k,9(t)*	RM	"
Jungsan	"	E	Sambaeg/Milyang107	*b,ta,z,k*	MS	"
Moonjang	"	E	Sangsan/Suweon397	*a,ta2,k,9(t)*	R	"
Odae	"	E	Akitsuho/Fuji269	*k*	MS	"
Saesangju	"	E	Junghwa/Sambaeg	*a,ta2,z,9(t)*	R	"
Sambaeg	"	E	Koshihikari/YR2406-2-1-1 //Hokuriku115/Cheolweon29	*a,ta,z,9(t)*	RM	"
Sangju	"	E	Cheonma/Odae	*a,b,z,9(t)*	M	"
Sangjuchal	"	E	YR4117-99-1-1-2-4/YR4200-2-3-2-2	*ta,z,i,k,9(t)*	M	"
Sangmi	"	E	Sambaeg/Oou316	*b,ta,9(t)*	R	"
Taebong	"	E	SR13390-13-3-5-2/Jinbu10	*b,z,i,k,9(t)*	RM	"
Taeseong	"	E	Cheolweon49/Jinbu10	*z,k,9(t)*	RM	"
Undoo	"	E	Odaebyeo/Jinbu13	*b,z,9(t)*	RM	"
Daepyeong	"	M	HR14028-AV5/Milyang122	*b*	M	"
Daesan	"	M	Milyang95/Suweon366	*a,b,ta*	M	"
Donghae	"	M	Milyang20/Nagdong	*a*	RM	"
Geuman	"	M	SR11878-14-4-1/Suweon345	*b,ta2*	RM	"
Gopum	"	M	SR10252-32-2-2-2/Suweon366// SR15140-58-2-2-3	*b,ta,z,i,k*	RM	"
Gwangan	"	M	Namyang7/SR14779-HB234-31	*a,i*	MS	"
Hwabong	"	M	Milyang95/Iri390//Milyang101/Iri390	*a,ta*	MS	"
Hwaseonchal	"	M	Milyang64/Sinseonchal	-	MS	"
Hwaseong	"	M	Aichi37/Samnam	*a*	S	"
Hwayeong	"	M	Chukei830/YR4811Acp8	*b*	M	"
Juan	"	M	Seolag/Koshihikari//Samnam	-	S	"
Palgong	"	M	HR1591-43-2-2-2/YR6542B-16-3-B	*a,b*	RM	"
Sangok	"	M	Milyang101/YR8697Acp19	*a,ta*	RM	"
Seoan	"	M	Suweon224/Inabawase//Seolag	*b,i*	M	"
Seogjeong	"	M	Namyang7/SR11340-30-4-1-3-2	*a*	S	"
Sinseonchal	"	M	Milyang20/Hiyokumochi	*a*	RM	"
Sura	"	M	Suweon345/Kanto PL4//Suweon345	-	MS	"
Suweon365	"	M	Seonam/Iri353	*a,b,ta2*	RM	"
Chucheong	"	ML	Bandainishiki//Wakaba/Kinmaze	*a*	S	"
Daean	"	ML	Oseto/Seomjin	-	M	"
Dongan	"	ML	Milyang95/HR5119-12-1-5	*a,b,ta2*	M	"
Dongjin	"	ML	HR1276(Kinmaze/M.15)/Sadominori	*a,ta2*	M	"
Dongjin 1	"	ML	Hwayeong/HR12800-AC21	*b*	M	"
Dongjinchal	"	ML	Milyang95//SR11155-4-2/Toyonishiki	*ta*	S	"
Hopyeong	"	ML	Hitomebore/Hwajin	*i*	S	"
Ilmi	"	ML	Milyang96//Milyang95/Seomjin	*ta*	M	"
Ilpum	"	ML	Suweon295-sv3/Inabawase	*b,i*	S	"
Junam	"	ML	Hwayeong//Sangju/Ilpum	*b*	M	"
Mangeum	"	ML	Milyang71/Saikai PL1	*a,b,i*	MS	"
Nagdong	"	ML	Norin6/Mineyudaka	-	MS	"
Nampyeong	"	ML	Iri390/Milyang95	*b,ta,i*	M	"
Saegyehwa	"	ML	Ilpum//Mangeum/Chukei830	*b*	M	"
Samgwang	"	ML	Suweon361/Milyang101	*a,b*	M	"
Sampyeong	"	ML	Suweon345/SR11340-46-5-4-1	*b,ta*	RM	"
Seogan	"	ML	Suweon224/Inabawase//Seolag	*ta2,k,km*	RM	"
Seomjin	"	ML	Milyang20/Asominori	*a,b,ta,z*	M	"
Seopyeong	"	ML	Hwayeong/HR11752-11-1-4-3	*b*	M	"
Sindongjin	"	ML	Hwayeong/YR13604Acp22	*b*	M	"
Tamjin	"	ML	HR1591-43-2-1-2-4/HR1590-92-4-4-4-4	*ta,k*	M	"
Milyang20	"	ML	YR91-24-7/Saikai128	*b*	RM	"
Milyang95	"	ML	Chukei1016/YR3477-54-B-2	-	MS	"
Suweon345	"	ML	Suweon224/Inabawase//Cheolweon21	*b*	M	"
Taebaeg	*Tongil*	M	IR24*2/IR747	*b,5,kp*	R	"
Milyang23	"	M	Suweon232/IR24	*b,ta,kp*	RM	"
Zenith	*Indica*	M	-	*a,z*	RM	USA
Moroberekan	*Javanica*	L	-	*5,7,44(t)*	R	West Africa

*Eco.: E, early maturing; M, medium maturing; ML, Mid-late maturing; L, late maturing

#*R* genes: a, *Pia*; b, *Pib*; i, *Pii*; k, *Pik*; kp, *Pik-p*; km, *Pik-m*; ta, *Pita*; ta2, *Pita-2*; z, *Piz*; zt, *Piz-t*; 9(t), *Pi9(t)*.

§Reaction: R, resistance; RM, moderately resistance; M, medium resistance; MS, moderately susceptible; S, susceptible

Table 2. Data summary of the seven blast resistance genes/QTLs intervals and 29 SSR and 2 STS markers.

Gene/ QTL	Marker	Chr.	Location on chromosome (cM)	Number of alleles	Number of haplotypes	PIC*i*	Amplicon size range (bp)
qBL1	RM297	1	132.0	7	33	0.742	150-195
	RM302		135.8	5		0.334	116-156
	RM265		140.5	4		0.607	128-134
	RM315		143.7	3		0.527	154-162
qBL4.2	RM3217	4	100.7	3	36	0.371	186-196
	R746		101.8	5		0.679	256-305
	RM255		102.7	3		0.466	160-166
	S20518		122.9	6		0.572	212-250
	RM131		123.8	4		0.453	194-202
	RM127		129.6	5		0.391	135-157
Pia	RM5704	11	28.6	8	45	0.761	146-201
	RM120		30.8	4		0.606	179-186
	RM6894		31.6	4		0.577	120-128
	RM441		32.1	5		0.684	192-201
Pib	RM208	2	154.1	5	49	0.762	166-180
	RM406		155.5	6		0.621	270-284
	RM482		155.5	4		0.562	184-194
	RM207		156.3	12		0.770	118-156
	RM48		157.9	10		0.841	226-249
	RM138		157.9	2		0.445	243-257
Pii	RM296	9	34.9	3	13	0.065	143-149
(Pi5)	RM105		40.1	4		0.396	131-138
	RM524		41.9	2		0.180	185-188
	RM460		45.2	4		0.544	269-277
Pita	RM101	12	48.2	13	41	0.860	279-356
(Pita-2)	OSR32		48.2	3		0.479	156-176
	RM155		49.3	5		0.626	273-292
	RM1337		50.4	9		0.765	180-211
Piz	RM527	6	56.3	3	24	0.428	214-229
(Pi9)	RM5850		58.7	7		0.660	166-214
	RM541		67.7	8		0.684	144-172

[a] Based on a consensus of TIGR pseudomolecule assembly release 4 of IRGSP

[b] PIC*i* are polymorphic information content by $1 - \sum_{j=1}^{n} P_{ij}^{2}$

harboring three, four and five *R* genes were ten, eight and three, respectively. Thirteen out of fourteen varieties of early maturing had 3 to 5 R genes with a total average of 3.7 *R* genes. The *japonica* varieties of 18 medium and 24 mid-late maturing had 1.6 and 1.5 *R* genes in the average, respectively. Two *Tongil-type* varieties Taebaeg and Milyang23 harbored 3 *R* genes. Two foreign rice varieties Zenith and Moroberekan contained two and three *R* genes, respectively.

The reactions to *M. oryzae* among the 56 Korean *japonica* 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 from 2003 to 2006 in 14 regions throughout Korea. Among the 56 *japonica* varieties tested, 19 varieties were classified as resistant, 21 as medium resistance and 16 as susceptible (Table 1). The 19 varieties of resistant group consisted of 9 early (64.3%), 7 medium (38.9%) and 3 mid-late maturing varieties (12.5%). While the varieties of medium resistance included 3 early, 4 medium and 14 mid-late maturing, the susceptible varieties were 2 early, 7 medium and 7 mid-late maturing, respectively.

Thirty-one microsatellite markers detected 2 to 13 alleles (mean = 5.4) and had PIC*i* values ranging from 0.065 to 0.860 (mean = 0.563) among the 60 rice accessions (Table 2). The markers linked to *Pib, Pita* and *Piz* genes produced more alleles than those of other genes. PIC*i* values of markers related to *Pia, Pib* and *Pita* genes were higher than those of other genes. A total of 60 haplotypes were detected to 31 microsatellite markers among 60 rice accessions. The haplotypes by the allele types of SSR markers linked to *Pia, Pib* and *Pita* resistance genes were detected more 45, 49 and 41, respectively than 13 - 36 haplotypes by the markers linked to two QTLs, *qBL1* and *qBL4.2*, and two genes, *Pii* and *Piz*. The locus of *Pii* on chromosome 9 had

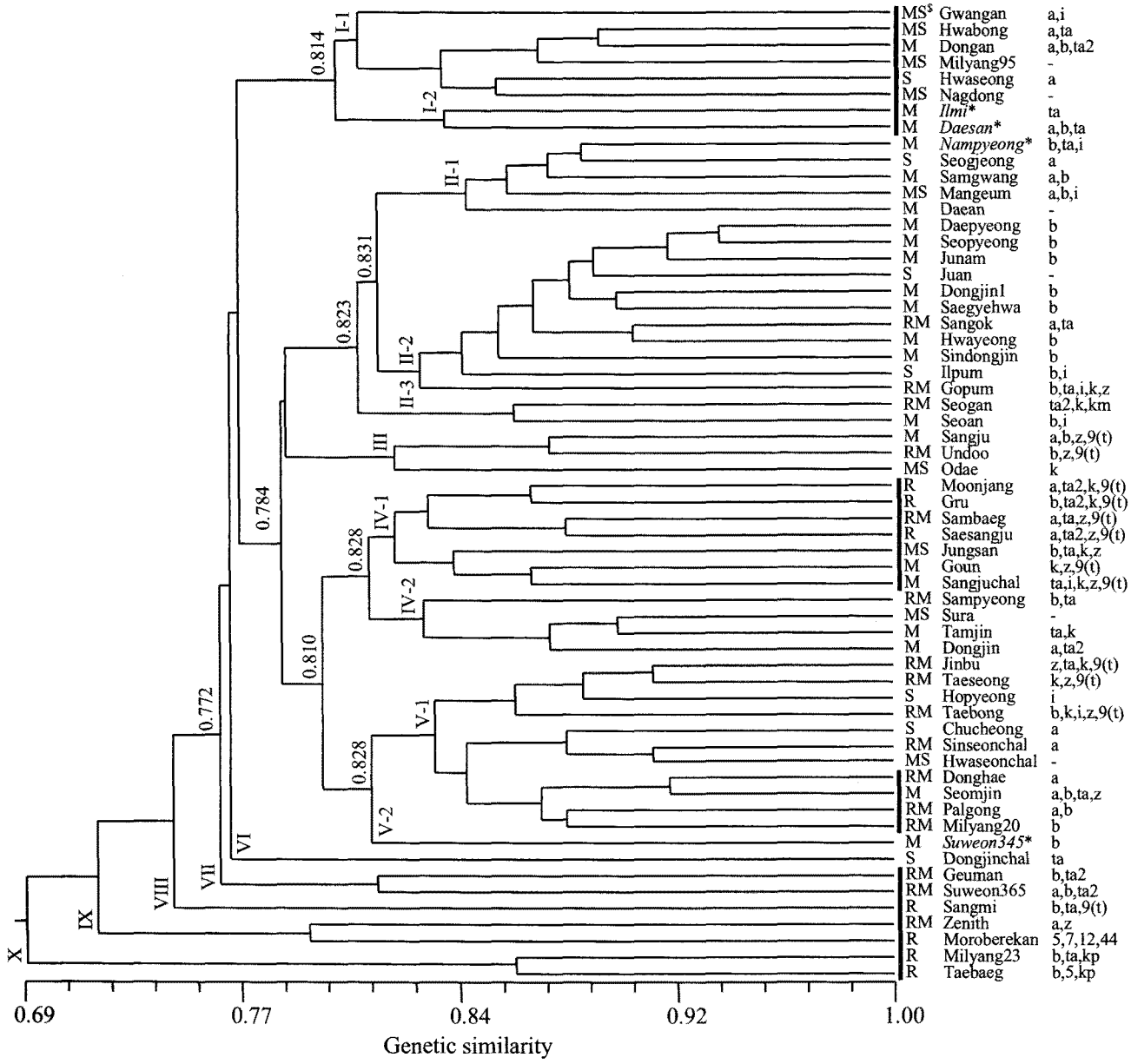

Fig. 1. Dendrogram resulting resulting from the genetic distance matrix for good eating-quality *japonica* rice varieties showing different resistance reaction to blast (*Magnaporthe grisea*) using UPGMA as the clustering method. The asterisk marked varieties are break-downed and/or highly susceptible to leaf and neck blast.
[$]R, RM: resistance, M: medium resistance, MS: moderately susceptible, S: susceptible.

relatively fewer haplotypes relative to the other six chromosome regions and the PIC*i* value of the microsatellite RM460 marker was similar to the mean value for the microsatellite markers (Table 2).

Based on allele diversities of 31 microsatellite markers, mapping near two QTLs and five major *R* genes to blast, cluster analysis, grouped into the same 60 haplotypes with the number of rice varieties assayed (Fig. 1). The varieties were grouped into ten major clusters within 0.810 genetic similarity based on the diversity of markers linked to resistant genes. The first grouped cluster consisted of eight varieties, Milyang95, four varieties, Daesan, Dongan, Hwabong, and Ilmi that developed from the crosses to Milyang95, and three susceptible varieties, Kwangan, Hwaseong and Nagdong. The second cluster consisted of eighteen varieties of medium and mid-late flowering. Thirteen varieties of them harbored *Pib* gene and eleven and four varieties had single and two *R* genes, respectively. The rest four varieties, Nampyeong, Mangeum, Gopum and Seogan, had 3 to 5 *R* genes. The group III was consisted to three early flowering varieties, Sangju, Undoo and Odae. Undoo harboring three

Table 3. ANOVA analysis of DNA markers located at the chromosome regions of two QTLs and five major genes of resistance to rice blast

Gene/ QTLs	Markers	Chr.	Blast nursery test								Isolates					
			Jecheon		Cheolwon		Suwon		Namyang		03-177		97-227		90-008	
			Sig.	$R^{2\#}$	Sig.	R^2	Sig.	R^2	Sig.	R^2	Sig.	R^2	Sig.	R^2	Sig.	R^2
qBL1	RM302															
	RM297	*1*									**	27.7	*	21.8		
	RM265															
	RM315															
qBL4.2	RM3217															
	R746		*	19.2									*	16.5		
	S20518	*4*	***	20.4	*	10.4										
	RM255															
	RM131		*	14.1												
	RM1272															
Pia	RM5704						*	25.3							*	26.1
	RM120	*11*														
	RM6894						**	23.6								
	RM441		*	17.8												
Pib	RM208				*	18.2							***	31.8		
	RM406															
	RM482	*2*														
	RM207						*	19.2					**	32.6		
	RM48		***	43.7			*	25.9								
	RM138		**	15.8			**	11.7					*	9.9		
Pii	RM296															
(Pi5)	RM105	*9*														
	RM524															
	RM460															
Pita	RM101		**	15.8	**	31.9	****	48.5	**	35.1	**	33.1			***	42.4
(Pita-2)	OSR32				***	27.5	*	10.7	****	39.8	***	25.2			****	34.6
	RM155	*12*			*	12.9	*	13.2								
	RM1337						*	16.2							**	27.9
Piz/	RM527				***	23.5			***	22.4	****	39.4	*	12.5	***	26.4
Pi9(t)	RM5850	*6*	**	26.8	**	27.9			*	24.4	***	33.5			**	22.5
	RM541		*	25.8	*	22.4					*	23.1	****	40.5	*	18.9

[§]Sig: *, **, *** and **** mean significant at 5%, 1%, 0.1% and 0.01%, respectively.
[#]R^2(%): percentage explained for a total of phenotypic variation.

genes, *Pib, Piz* and *Pi9(t)*, showed resistant reaction to leaf blast, but highly susceptible to neck blast (Shin et al. 2003). The fourth cluster grouped of 7 early flowering in subcluster IV-1 and four medium and mid-late flowering in subcluster IV-2, respectively. Six early flowering varieties, Moonjang, Gru, Sambaeg, Saesangju, Goun and Sangjuchal of subcluster IV-1, showed resistance to leaf blast and harbored 3 to 5 *R* genes. They had *Piz* and/or *Pi9(t)* on chromosome 6 and *Pita/Pita-2* gene on chromosome 12 in common. Four varieties, Sampyeong, Sura, Tamjin and Dongjin of subcluster IV-2, did not have *Piz* or *Pi9(t)* and were mid-late flowering in common. The cluster V grouped 12 varieties consisting of three early, four medium and five mid-late flowering. Three early flowering varieties, Jinbu, Taeseong and Taebong, were resistant moderately and had 3 to 5 *R* genes with *Piz* and/or *Pi9(t)* on chromosome 6 in common. A subcluster V-1 consisted of four stable resistant varieties, Donghae, Seomjin, Palgong, and Milyang20. Two varieties, Seomjin and Palgong, having *Pia* and *Pib* genes in common showed resistance to leaf blast over 20 years and the rest two Donghae and Milyang20 also showed stable resistance to leaf blast for long time. The other groups from VI to X included seven varieties consisting of four *japonica*, an USA *indica* Zenith, a tropical *japonica* Moroberekan and two *Tongil-type (indica/japonica)*, Milyang23 and Taebaeg. They had 2 to 4 *R* genes and all of them showed resistance to leaf blast. Two foreign rice varieties Zenith and Moroberekan showed stable resistance at blast nursery in Korea.

The analysis of putative QTLs associated with seven blast resistance genes was performed using ANOVA and linear regression (Table 3). The marker RM297 in a QTL, *qBL1* was

significant to isolates 03-177 and 97-227. A QTL *qBL4.2* showed significant to leaf blast in Jecheon and Cheolwon and an isolate 97-227. A few marker loci of *Pia* gene showed significant to leaf blast in Jecheon and Suwon and an isolate 90-008. A marker RM48 of *Pib* gene was significant to leaf blast in Jecheon and Suwon and explained 43.7% and 25.9% of total phenotypic variation, respectively. For isolate 97-227, a marker RM208 was significant and explained 31.8% of total phenotypic variation. Any marker linked with *Pii* gene on chromosome 9 was not significant to leaf blast. The markers located at the regions of two genes, *Piz* on chromosome 6 and *Pita* on chromosome 12, were high significant to blast resistance across regions and isolates. Merely, three markers of *Piz* gene region did not show any significant to leaf blast in Suwon and four markers of *Pita* gene region were not any significant to isolate 97-227.

Discussion

Out of 56 Korean *japonica* rice varieties in this study, six and eighteen varieties were identified to have no and single *R* gene, respectively, and 32 varieties harbored 2 to 5 *R* genes. The 31 microsatellite markers linked to five *R* genes, *Pia, Pib, Pii (Pi5), Pita (Pita-2)* and *Piz (Pi9(t))*, and two QTLs, *qBL1* and *qBL4.2*, produced 2 to 13 alleles (mean = 5.4) and had PIC*i* values ranging from 0.065 to 0.860 (mean = 0.563) among 60 rice accessions (Table 2). The markers linked to *Pib, Pita* and *Piz* genes produced more alleles than those of other genes. PIC*i* values of markers related to *Pia, Pib* and *Pita* genes were higher than those of other genes. Also, more haplotypes at the *Pia, Pib* and *Pita (Pita-2)* were identified, but fewer haplotypes were identified at the *Pii* and *Piz* locus. The *Pia* gene was introduced into Korean *japonica* varieties from many Japanese *japonica* varieties. Two genes, *Pib* and *Pita (Pita-2)*, were first introduced into *japonica* from *indica* rices and then after, they were introduced into Korean *japonica* varieties. However, two genes, *Pii* and *Piz*, were introduced into Korean *japonica* varieties from the simple Japanese *japonica* donor varieties (Cho et al. 2007). As a result, the DNA markers linked to *Pia, Pib* and *Pita (Pita-2)* could produce higher haplotype diversities than those linked to *Pii* and *Piz* genes.

Sixty varieties were grouped into ten major clusters within 0.810 genetic similarity based on the diversities of markers linked to resistant genes (Fig. 1). The varieties of each cluster were grouped into resistant genes and ecotypes of early and mid-late maturing. The cluster I grouped to susceptible five varieties, Gwangan, Hwabong, Hwaseong, Milyang95 and Nagdong, and three varieties, Dongan, Daesan and Ilmi that broken-down their resistance (Han et al. 2001). Two subclusters, IV-1 of Goun, Gru, Jungsan, Moonjang, Saesangju, Sambaeg and Sangjuchal and V-1 of Donghae, Milyang20, Palgong and Seomjin, were resistant varieties showing stable resistance for long time. Most varieties of subcluster IV-1 contained *Pita/Pita-2, Piz* and *Pi9(t)* and they showed stable resistance in the field. Especially, two varieties, Seomjin and Palgong of subcluster V-1, were known to the durable resistance varieties in Korea (Han et al. 2001; Roh et al. 2007).

In QTLs analysis for microsatellite markers associated with five major *R* genes and two QTLs by ANOVA and linear regression, only SSR markers linked to *Pita (Piat-2)* and *Piz (Pi9(t))* showed significant across regions and isolates (Table 3). A gene, *Pia*, and two QTLs, *qBL1* and *qBL4.2*, were significant only to a few markers for one to two specific regions and an isolate 97-227. The markers linked to *Pii* were not significant to any region and isolate. These results were related with *R* genes

Table 4. Grouping of varieties for five major *R* genes based on resistance reaction at blast nursery.

R genes	Reaction at blast nursery		
	R, RM	M	MS, S
Pia	Donghae, Moonjang, Palgong, Saesangju, Sangok, Sambaeg, Sinseonchal, Suweon365, *Zenith*[z] (9)*	Daesan, Dongan, Dongjin, Samgwang, Sangju, Seomjin (6)	Chucheong, Gwangan, Hwabong, Hwaseong, Mangeum, Seogjeong (6)
Pib	Geuman, Gopum, Gru, Palgong, Sangmi, Taebong, Undoo, Milyang20, Suweon365, *Taebaeg, Milyang23* (11)	Daepyeong, Daesan, Dongan, Dongjin1, Hwayeong, Junam, Nampyeong, Saegyehwa, Samgwang, Sampyeong, Sangju, Seoan, Seomjin, Seopyeong, Sindongjin, Suweon345 (16)	Ilpum, Jungsan, Mangeum (3)
Pii	Taebong, Gopum (2)	Nampyeong, Sangjuchal, Seoan (3)	Gwangan, Hopyeong, Ilpum, Mangeum (4)
Pita (Pita-2)	Geuman, Gopum, Gru, Jinbu, Moonjang, Saesangju, Sambaeg, Sampyeong, Sangjuchal, Sangok, Sangmi, Seogan, Suweon365, *Milyang23* (14)	Daesan, Dongan, Dongjin, Ilmi, Nampyeong, Seomjin Tamjin (7)	Hwabong, Jungsan (2)
Piz	Gopum, Goun, Jinbu, Saesangju, Sambaeg, Taebong, Taeseong Undoo, *Zenith* (9)	Sangju, Sangjuchal, Seomjin (3)	Jungsan (1)
Pi9(t)	Gru, Jinbu, Moonjang, Saesangju, Sambaeg, Sangmi, Taebong, Taeseong, Undoo (8)	Goun, Sangju, Sangjuchal (3)	(0)

*The number of parenthesis are the number of varieties.
[z]The varieties of italic are *Tongil-type*.

and resistant reactions to blast of varieties (Table 4). The resistance reactions to blast of varieties having *Pia* and *Pii* genes were evenly distributed into R & RM, M and MS & S groups. The varieties having *Pib* gene were distributed into 37% R & RM, 53% M and 10% MS & S groups, respectively. These distributions were resulted in the significant resistant effects only to two regions and one isolate. The region of *Pia* gene on chromosome 11 was clarified to single gene or two genes, *Pia* and *Pi-CO39(t)*. The *Pi-CO39(t)* gene was inherited from an *indica* rice CO39 of low resistant effect (Chauhan et al. 2002). Two genes *Pii* and *Pi5* on chromosome 9 were tightly linked to together or would be the cluster gene family (Jeon et al. 2003; Yi et al. 2004). *Pii* gene identified in 17 varieties out of 88 Korean *japonica* rice varieties, but *Pi5* was only in a *Tongil-type* variety Taebaeg (Cho et al. 2007). The monogenic line IRBL5-M of *Pi5* gene showed to stable resistant effect at blast nursery and in the field (Cho et al. 2005), but this gene was not introduced into Korean *japonica* rice varieties. As a result, the microsatellite markers linked to two genes, *Pia* and *Pii*, were not shown any significant for the regions and isolates.

The varieties having *Pita (Pita-2)*, *Piz* and *Pi9(t)* genes were distributed into 60.9 - 72.7% R & RM, 23.1 - 30.4% M, and 0 - 8.7% MS & S groups, respectively (Table 4). The *Piz* and *Pi9(t)* loci on chromosome 6 were known as the region of multi-gene family of *Piz-t, Pi2 (Piz-5), Pi40, Pigm(t)* and so on (He et al. 2007; Jeung et al. 2007; Liu et al. 2002; Qu et al. 2006). Actually, the 76-kb region of *Pi9* gene was sequenced and led to identification of six tandemly arranged resistance-like genes with a nucleotide-binding site (NBS) and leucine-rich repeats (LRRs) (*Nbs1-Pi9~Nbs1-Pi9*) (Qu et al. 2006). The *Pita* and *Pita-2* genes commonly used in rice breeding programs worldwide have originated from several traditional *indica* cultivars, including Tetep from Veitnam and Tadukan from the Philippines. The *Pita-2*-containing rice varieties, such as Reiho, uniformly contain *Pita* as well as a second *R* gene identified by a second *AVR-vir* mutant pair of strains lacking *AVR-Pita* homology altogether (Bryan et al. 2000). Each of two varieties, 'Katy' and 'Drew', which are both reported to contain *Pita-2* (Moldenhauer et al. 1992), contain this same additional *R* gene identified in *Pita-2* cultivars studies previously (Bryan et al. 2000). Also, the region of *Pita/Pita-2* gene on chromosome 12 was known as the multi-gene family located to *Pi4, Pi6(t), Pi20, Pi31(t)* and *Pi157* (Kiyosawa 1972; Mackill and Bonman 1992; Naqvi and Chattoo 1996; Rybka et al. 1997; Sallaud et al. 2003; Yu et al. 1996). Thus, our data continue to suggest that the *Pita-2* specificity spectrum results from action of at least two *R* genes by the region of *Pita (Pita-2)* gene of the multi-gene family.

The breakdown of resistance in three varieties Daesan, Dongan and Ilmi that developed by the common parent Milyang95, was resulted from the differentiation of new virulent races, KI-1117a and KI-1113a (Han et al. 2001). The third allele of RM254 and the second allele of OSR32 in the varieties that developed by the common parent Milyang95, respectively, were related to the resistance to KI-1113 and KI-105 and KI-1117a (Hwang et al. 2004). The specific allele-types for microsatellite markers linked to resistance genes were related to resistant and susceptible varieties for the specific blast isolates (Suh et al. 2008). Particularly valuable alleles in haplotype analysis are functional polymorphisms that are directly responsible for the resistance or susceptibility phenotypes. Each polymorphism differentiating susceptibility and resistance alleles provides an opportunity for developing reliable molecular markers for incorporating major *R* genes into advanced breeding lines. The present study was thorough analysis of allele diversity of microsatellite markers linked to five *R* genes and two QTLs across 56 good eating-quality Korean *japonica* varieties and four resistance germplasms. This study illustrated the utility of microsatellite markers to identify rice varieties likely carried the same *R* genes and QTLs and rice lines with potentially novel resistance. Rice varieties with a number of alleles in common with any specific resistance haplotype might have a similar blast *R* gene. Also, understanding the natural diversity at the specific gene is important for incorporation of specific *R* gene using DNA marker into rice breeding program (Jia et al. 2003). Based on our results, we could suggest that two *R* genes, loci *Piz (Pi9, Pi40)* (Chr. 6) and *Pita/Pita-2* (Chr. 12), would be effective to develop the durable resistance *japonica* rice varieties in Korea. Two alleles of 214 bp and 174 bp by RM527 and RM5850 markers at *Piz* gene and alleles, 285 bp and 156 bp by RM101 and OSR32 markers in *Pita/Pita-2* gene, respectively, were related with the haplotypes of resistant varieties in rice breeding program. Cho et al. (2005) reported that IRBL5-M, a monogenic line of *Pi5* gene, showed the stable resistance to blast isolates inoculation and to the evaluation at the field. However, the *Pi5* gene was not identified in Korean *japonica* rice varieties but in only *Tongil-type* (Korean *indica*) Taebaeg (Cho et al. 2007). Two amplicons, 138 bp and 157 bp by RM296 and RM105 at *Pi5* locus, respectively, would be useful to introduce the alleles of *Pi5* gene originated from *Tongil-type (indica)* Taebaeg.

Acknowledgement

This work was supported in part by Basic Fund of NICS, and Biogreen21 program (code no. 2007-0301-034-034), RDA, Republic of Korea.

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