Screening of Rice Blast Resistance Genes from Aromatic Rice Germplasms with SNP Markers

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Rice blast is one of the serious devastating diseases. This study was carried out to determine the genetic diversities of blast resistance (*R*) genes from 86 accessions of aromatic rice with eight SNP markers, z4792, zt4792, z60510, zt6057, k6415, k6411, k39575 and t256, which showed the close-set linkage to 6 major genes, *Piz*, *Piz-t*, *Pik*, *Pik-m*, *Pik-p*, and *Pit*. Four accessions of *indica* type, Mayataung, Yekywin Yinkya Hmwe, Basmati9-93, and Basmati5854, showed the positive amplicons of six major genes. Among 86 accessions, 83 accessions were detected both or one of *Piz* and *Piz-t* genes. Seventy three accessions contained the *Piz* gene with z4792 marker. In addition, 30 and 71 accessions possessed *Piz-t* gene with zt4792 and zt6057 markers, respectively. Ten accessions showed the positive bands for the *Piz-t* gene with both zt4792 and zt6057 markers. Only one accession, Khau Nua Keo, was not amplified for both *Piz* and *Piz-t* gene. But *japonica* type, Gerdeh, possessed only *Piz* gene between *Piz* and *Piz-t*. Fifty two accessions showed the three of *Pik* multiple genes and *Pit* gene. Four accessions, Iari7447, Daebunhyangdo2, Shiyayuuine, and Basmati 6129 possessed a *Pik-p* gene. Especially, *Pit* gene on chromosome 1 was detected with t256 marker in all of 83 accessions, exception of A-2, one accession of *japonica* type.

Keywords : aromatic rice, *Pi* genes, rice blast resistance, SNP marker

Aromatic rices (*Oryza sativa* L.) such as 'Basmati', 'Kelara', and 'Jasmine' are known for their fragrance when cooked. Also, they fetch a premium price in domestic and world markets, often three to four times more than that of the ordinary rice varieties. In Korea, seven varieties of aromatic rice, Hyangmibyeo1ho, Hyangmibyeo2ho, Hyangnambyeo, Aranghyangchalbyeo, Mihyangbyeo, Seolhyangchalbyeo, and Heughyang were bred from 1993 to 2000 (Choi et al., 1995; Ha et al., 2003; Ha et al., 2006;

Moon et al., 1998; Moon et al., 2003).

Rice blast disease, caused by *Magnaporthe grisea*, causes economically significant yield losses annually worldwide and has been one of the most serious diseases of rice because of their pathogenic complexity related pathogen, host, and micro weather (Kwon and Lee, 2002; Lee, 1994; Li et al., 2007; Ou, 1985; Teng et al., 1991).

Especially, the blast fungus is highly variable and numerous; races of blast fungus are present in most field conditions (Ou, 1979; Xia et al., 1993; Valent and Chumley, 1994). The identification and isolation of additional host *R* genes and pathogen avirulence gene are now required to deepen understanding of molecular mechanisms involved in the host-pathogen interaction (Valent, 1990).

So far, about 50 major rice blast *R* genes and 35 avirulence (*Avr*) genes have been reported. A great number of genes with complete or partial resistance to blast in rice have been mapped and developed so far, which included *Pi1(t)*, *Pi2(t)*, *Pi4(t)*, *Pi5(t)*, *Pi6(t)*, *Pi9(t)*, *Pi10(t)* and *Pi11(t)* (Causse et al., 1994), *Pi5(t)* and *Pi7(t)* (Wang et al., 1994), *Pia*, *Pib*, *Pik*, *Pit*, *Pita*, *Pi12(t)*, *Pi17(t)*, *Pi18(t)*, *Pi19(t)*, *Pi20(t)*, *Pi23(t)*, *Pi62(t)*, and *Pi157(t)* (Nagato and Yoshimura, 1998), *Pitq1*, *Pitq5*, *Pi-tq6* and *Pilm2* (Tabien et al., 2000), the recessive gene *pi21* (Fukuoka et al., 2001), the QTLs for partial resistance to leaf blast (Wang et al., 1994) and QTLs for partial resistance to neck blast (Bagali et al., 2000; Sirithunya et al., 2002).

Recently, many rice varieties with complete resistance to *M. grisea* have been developed, but in many cases this resistance has been breakdown within a few years of the initial cultivation owing to the emergence of stronger virulent isolates of rice blast fungus (Bonman et al., 1986; Han et al., 2001; Kiyosawa, 1981; Mackill and Bonman, 1992; Yaegashi, 1994). Partial or field resistance of rice blast has received much attention as a means of effective control of a parasite under natural field condition and conferring durable blast resistance when exposed to new races of that parasite (Hittalmani et al., 2000; Liu et al., 2005; Wang et al., 1994).

This study was carried out to acquire information for genetic diversities of resistance genes against rice blast

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disease in aromatic rice germplasms for improving aromatic rice breeding efficiency using PCR-based markers including several candidate SNP (single nucleotide polymorphisms) markers.

Materials and Methods

Rice plant materials and DNA extraction. In our previous studies, we described the origin and characteristics of 260 accessions of aromatic rice germplasms introduced from 20 origins and preserved in Korean RDA genebank. Eighty six of 260 aromatic rice accessions were evaluated with agronomic traits, phytochemical characteristics, and analysis of aromatic compounds in 2007 (Kim et al., 2008a and Kim et al., 2008b). The eighty six aromatic rice accessions were offered from National Agrobiodiversity Center of RDA, Korea, in Table 2. Five rice seeds of each accession were disinfected with 2% NaOCl solution at 4 hrs and were washed with tap water at overnight and placed on moist filter paper laid on the petri-dish at 30°C for one-week in growth chamber.

Genomic DNA was extracted from frozen young leaves of one-week-old seedlings by an improved CTAB (hexadecyl trimethyl ammonium bromide) method based on the procedure described by Murray and Thompson (1980). The extracted genomic DNA was estimated on 1% agarose gels staining of ethidium bromide for quality test. The quantity of extracted DNA was measured by Nano Drop system (Thermo, U.S.A.) and they were diluted to 10 ng/μl with the sterilized distilled water and stored at 4°C.

SNP markers specific for rice blast R gene. Table 1, eight PCR based allele-specific SNP marker set (z4792, zt4792, z60510, zt6057, k6415, k6411, k39575 and t256) of 6 major rice blast resistance (*R*) genes, *Piz*, *Piz-t*, *Pik*, *Pik-m*, *Pik-p* and *Pit*, were previously reported another studies (Cho et al., 2007; Hayashi et al., 2004; Hayashi et al., 2006). Two of blast resistance genes, *Piz* and *Piz-t* genes,

were reported that closed to same position of on chromosome 6, and related to four SNP marker, z4792, zt4792, z60510, and zt6057 (Hayashi et al., 2004).

We used the eight SNP markers for amplification of DNA fragments linked to rice blast resistant genes based on each previous research papers. All of the rice blast resistance markers were synthesized at the oligo synthesis facility of Bioneer Co., in Korea.

Polymerase chain reaction (PCR) analysis. The PCR analysis for the SNP allele-specific markers were conducted based on procedures described by Bioneer PCR Pre-mix kit manual (Bioneer Co. Ltd, Korea). The PCR reaction mixture contained 50 ng of genomic DNA, 5 pmol of each primer set, 2.5 mM of each dNTPs, 1.5 mM MgCl₂, 1X PCR buffer (10 mM Tris-HCl, pH 9.0, and 30 mM KCl), and 1 unit of Taq DNA polymerase in 20 μl PCR reaction volume. PCR amplifications were carried out in a My-Genie96 Thermal cycler (Bioneer Co. Ltd, Korea), and programmed that template DNA was initially denatured at 95°C for 4 min, followed by 30 cycles of PCR amplification steps with the following parameters; a 30 sec denaturation at 94°C, 30 sec primer annealing at from 42 to 62°C, and a 60 sec primer extension at 72°C allowed for completion of primer extension, with a final extension at 72°C for 10 min. Initially 4 μl of the amplified products were electrophoretically resolved on a 1.5% agarose gel in 0.5X TAE (Tris-acetate-EDTA) buffer (pH 8.0) and visualized under UV light after staining with 0.1 μg/ml of ethidium bromide (Et-Br). The amplified fragment using SNP markers were scored as presence (1) or absence (0) of amplicon linked each gene DNA fragment.

Results

MAS of rice blast resistance accessions of aromatic rice germplasm. Application of molecular markers will enhance the efficiency of rice evaluation and improvement

Table 1. Details of single nucleotide polymorphisms (SNP) markers tightly linked to the major rice blast resistant genes

Genes	Number of Chromosome	Markers	Primer sequence		Expected size (bp)
			Forward (5'-3')	Reverse (5'-3')	
<i>Piz</i>	6	z4792	cgcacgcttccgaactactcccgt	agtgttggtcggcacgtgcttg	340
<i>Piz</i>		zt4792	tatatgttgggaggagtaggac	agtgttggtcggcacgtgcttg	340
<i>(Piz-t)</i>		z60510	ggagttgtgcgacggtgccgttac	gcgcggaccggccagctagttgac	360
		zt6057	gaagctcaactgaacgtgacgc	actggaagtcctctatatgcc	360
<i>Pik</i>	11	k6415	gcattagatagtgatgaaagccgg	tgttcacccaggcaattggc	218
<i>Pik</i>		k6441	tgtaaaatacttctatgcgcaggc	gtttatggagagtagtcgctg	404
		k39575	ggtgttgggaacctgaacccta	ttctgttcgtcgatgctc	158
<i>Pit</i>	1	t256	ggatagcagaagaacttgagacta	catgtcttaacataagaagttctc	322

Table 2. Genotyping of 86 accessions of aromatic rice germplasm estimated with eight of allele-specific SNP markers linked to the major of rice blast resistant gene

Lanes	Varieties	Ecotypes	<i>Piz</i>	<i>Piz-t</i>	<i>Piz,Piz-t</i>	<i>Piz-t</i>	<i>Pik</i>	<i>Pik-m</i>	<i>Pik-p</i>	<i>Pit</i>
			z4792	zt4792	z60510	zt6057	K6415	Pk6441	K39575	t256
1	Hyangmibyeo1ho	Tongil	1 ^a	1	1	0	1	0	1	1
2	Hyangmibyeo2ho	Tongil	1	1	1	0	1	1	1	1
3	Hyangnambyeo	Japonica	1	0	1	1	0	1	1	1
4	Aranghyangchalbyeo	Japonica	1	0	1	1	0	1	1	1
5	Mihayangbyeo	Japonica	1	0	1	1	0	1	1	1
6	A-2	Japonica	1	1	1	1	1	1	1	0
7	A-3, Choh Chang	Japonica	1	0	1	1	1	1	1	1
8	Muhyang99-8	Japonica	0	1	1	1	1	1	1	1
9	Jahyangna861	Japonica	1	1	1	1	0	1	1	1
10	Jc149	Japonica	1	0	1	1	1	1	1	1
11	Jc157	Japonica	1	0	1	1	1	1	1	1
12	Iari 7447	Japonica	1	0	1	1	0	0	1	1
13	Daebunhyangdo2	Japonica	1	0	1	1	0	0	1	1
14	Shiyayuuine	Japonica	1	0	1	1	0	0	1	1
15	Rasomotrafotsy	Japonica	1	0	1	1	1	1	1	1
16	TALLI	Japonica	1	0	1	1	1	1	1	1
17	Masino Basmati	Japonica	1	0	1	1	1	1	1	1
18	Kalomasino Dhan	Japonica	1	0	1	1	1	1	1	1
19	Gerdeh	Japonica	1	0	1	0	0	1	1	1
20	KINANDANG PAT	Japonica	1	0	1	1	1	1	1	1
21	Milagrosa Mutant	Japonica	1	1	1	1	0	1	0	1
22	Inaguhu	Japonica	1	0	1	1	1	0	1	1
23	Flores	Japonica	1	1	1	1	0	1	1	1
24	Kung-ShanWu-Shen-Ken	Japonica	1	1	1	1	0	1	1	1
25	Daw Dam	Japonica	1	0	1	1	1	1	1	1
26	415 X Ir352	Japonica	1	1	1	0	1	0	1	1
27	Khau Nua Keo	Japonica	0	0	0	0	0	1	0	1
28	Khau Tan Luong	Japonica	1	0	1	1	0	1	0	1
29	Goolarath	Indica	1	1	1	1	0	1	1	1
30	Da13	Indica	1	0	1	1	1	1	1	1
31	Basmati 370	Indica	1	0	1	1	1	1	1	1
32	Hyanggaengdo	Indica	1	1	1	0	1	1	1	1
33	Seratus Malam	Indica	1	0	1	1	1	1	1	1
34	Basmati T3	Indica	1	0	1	1	1	1	1	1
35	Jc111	Indica	1	0	1	1	1	1	1	1
36	Arc 6011	Indica	1	0	1	1	0	1	1	1
37	Kaminibhog	Indica	0	1	1	1	1	1	1	1
38	Tarana Deshi	Indica	1	1	1	1	1	0	1	1
39	Basmati	Indica	0	1	1	1	1	1	1	1
40	Iranbyeopssi	Indica	1	0	1	1	1	1	1	1
41	Iranbyeopssi	Indica	1	0	1	1	1	0	1	1
42	Domsiah	Indica	1	0	1	1	1	1	1	1
43	Mulai	Indica	1	0	1	1	1	1	1	1
44	Tareme	Indica	1	0	1	1	1	1	1	1
45	Hyangdo	Indica	1	0	1	1	1	1	1	1
46	Seratus Malam	Indica	1	0	1	1	1	1	1	1
47	Mayataung	Indica	1	1	1	1	1	1	1	1
48	Yekywin Yinky Hmwe	Indica	1	1	1	1	1	1	1	1

Table 2. Continued

Lanes	Varieties	Ecotypes	<i>Piz</i>	<i>Piz-t</i>	<i>Piz,Piz-t</i>	<i>Piz-t</i>	<i>Pik</i>	<i>Pik-m</i>	<i>Pik-p</i>	<i>Pit</i>
			z4792	zt4792	z60510	zt6057	k6415	k6441	k39575	t256
49	Kala Namak	Indica	1	0	1	1	1	1	1	1
50	Masino Basmati	Indica	0	1	1	1	1	1	1	1
51	Basmati Dhan	Indica	1	0	1	1	1	0	1	1
52	Basmati	Indica	1	1	1	1	1	0	1	1
53	Basmati 370	Indica	0	0	1	1	1	1	1	1
54	Basmati 9-93	Indica	1	1	1	1	1	1	1	1
55	Basmati 198	Indica	1	0	1	1	1	1	1	1
56	Basmati 370	Indica	1	0	1	1	1	1	1	1
57	Basmati 5836	Indica	1	1	1	0	1	1	1	1
58	Basmati 5854	Indica	1	1	1	1	1	1	1	1
59	Basmati 5875	Indica	1	0	1	1	1	1	1	1
60	Basmati 6113	Indica	1	1	1	0	0	1	1	1
61	Basmati 1	Indica	1	0	1	1	1	1	1	1
62	Basmati 213 C	Indica	1	0	1	1	1	1	1	1
63	Basmati 372	Indica	1	0	1	1	1	1	1	1
64	Chahora 144	Indica	1	0	1	1	1	1	1	1
65	Pakistani Fine	Indica	0	0	1	1	1	1	1	1
66	Ir841-85-1-1-2	Indica	1	1	1	0	1	1	1	1
67	05-Irri-M-46	Indica	1	0	1	1	1	1	1	1
68	Basmati 107	Indica	1	0	1	1	1	1	1	1
69	Basmati 405	Indica	1	0	1	1	1	1	1	1
70	Basmati 5853	Indica	1	1	1	0	1	1	1	1
71	Basmati 5874	Indica	1	0	1	1	1	1	1	1
72	Basmati 6129	Indica	0	0	1	1	0	0	1	1
73	Basmati 6311	Indica	1	0	1	1	0	1	0	1
74	Basmati 6313	Indica	1	1	1	0	1	1	1	1
75	Basmati 6141	Indica	1	1	1	0	1	1	1	1
76	AZUCENA	Indica	1	0	1	1	0	1	1	1
77	Binicol	Indica	0	0	1	1	1	1	1	1
78	Milfor 6	Indica	1	1	1	1	0	1	1	1
79	Dinorado	Indica	1	0	1	1	0	1	0	1
80	Khao Dawk Mali105	Indica	1	1	1	1	0	1	1	1
81	Jasmine 85	Indica	0	1	1	0	1	1	1	1
82	Dellmont	Indica	1	0	1	1	0	1	1	1
83	Aroma	Indica	1	0	1	1	0	1	1	1
84	Ds20	Indica	0	1	1	0	1	1	1	1

a: The rice blast resistant genes scored as the presence (1) or absence (0) of amplicons linked to eight of allele specific SNP markers.

program. Eighty four accessions of aromatic rice germplasm were screened for the presence of 6 major rice blast resistance (*R*) genes, *Piz*, *Piz-t*, *Pik*, *Pik-m*, *Pik-p*, and *Pit*, using the eight of SNP markers listed in Table 1. An allele specific PCR marker assays the genotype of SNP marker by estimating the presence or absence of PCR amplicon from a pair of PCR reactions.

All of the eighty four accessions of aromatic rice germplasms possessed at least more than three positive bands of the eight rice blast resistance markers in Table 2. *Indica*

type of four accessions, Mayataung, Yekywin Yinkya Hmwe, Basmati9-93, and Basmati5854, showed the positive amplicon of 6 major rice blast resistance genes (Table 2).

Genetic diversity of *Piz* and *Piz-t* gene. Estimation of PCR results for the *Piz* and *Piz-t* rice blast resistance genes were determined by visualization of amplicons on 340 bp, 240 bp, and 360 bp of positive fragments using two set of SNP primer, set 1 (z4792 and zt4792) and set2 (z60510 and zt6057) (Fig. 1). *Piz* gene on chromosome 6 was scored to

Fig. 1. Electrophoretical band patterns of PCR products for 84 aromatic rice germplasms. The amplified fragments using SNP markers, z4792 (A), zt4792 (B), z60510 (C), and zt6057 (D), were scored as presence (+) or absence (-) of amplicons linked to *Piz* gene. M, molecular marker (Bioneer 100 bp ladder plus); lanes 1-84, eighty four accessions of aromatic rice.

73 accessions of aromatic rice germplasm, two accessions of two accessions of *tongil* type, twenty four accessions of *japonica* type, and forty seven accessions of *indica* type (Fig. 1).

Piz-t gene on chromosome 6 was detected in eighty three accessions of aromatic rice germplasm with together or any one of zt4792 and zt6057 SNP marker. Among the eighty three, thirty accessions and seventy one accessions were amplified with each one of allele specific SNP marker, zt4792 and zt6057, respectively. Eighteen accessions including six of *japonica* type and twelve of *indica* type showed

the positive bands with both zt4792 and zt6057 SNP markers (Fig. 1).

Eighty three accessions possessed at least one genes of *Piz* and *Piz-t* genes identified with SNP marker z60510. And only one accession, Khau Nua Keo, was not amplified any one of *Piz* and *Piz-t* gene with four SNP markers. But *japonica* type aromatic rice, Gerdeh, possessed only *Piz* gene (Fig. 1).

Genetic diversity of *Pik multi* gene. Fifty two accessions show the positive reactions of all of three *Pik* multiple

Fig. 2. PCR analysis of 84 aromatic rice germplasms to evaluate rice blast resistance genes. *Pik* multi genes, *Pik* (A), *Pik-m* (B), and *Pik-p* (C), were scored as presence (+) or absence (-) of target amplicon. Each gene of *Pik* multi genes amplified with allele specific SNP markers, k6441 (*Pik*), k39575 (*Pik-m*), and k6415 (*Pik-p*) M, molecular marker (Bioneer 100 bp ladder plus); lanes 1-84, eighty four accessions of aromatic rice.

Fig. 3. Agarose gel photograph of 84 aromatic rice germplasms for the SNP marker t256 sized 322 bp. The presence of the amplicon was scored as presence (1) or absence (0) of amplicon linked *Pit* gene. M, molecular marker (Bioneer 100 bp ladder plus); lanes 1-84, eighty four accessions of aromatic rice.

genes, *Pik*, *pik-p*, and *Pik-m*, located on chromosome 11 with tightly linked three SNP markers k6415, k6441, and k39575. Among the eighty four accessions, four accessions, Iari7447, Daebunhyangdo2, Shiyayuuine, and Basmati 6129 possessed only *Pik-p* gene of three *Pik* multi genes (Fig. 2). Three accessions of *japonica* type (Milagrosa mutant, Khau Nua Keo, and Khau Tan Luong) and two accessions of *indica* type (Basmati6311 and Dinorado) were showed the positive amplicon for *Pik-m* of three *Pik* multi genes with k6441 SNP marker. Each of the seven accessions of *japonica* type (Hyangnambyeo, Arnahyangchalbyeo, Mihayangbyeo, Jahyangna86-1, Gerdeh, Flores,

and Kung-Shan Wu-Shen-Ken) and *indica* type (Goolarath, Arc6011, Azucena, Milfor6, Khao Dawk Mali 105, Aroma, and Ds20) aromatic rice germplasms was possessed the positive bands of *Pik-m* and *Pik-p* genes. Allele specific resistance genes of *Pik* and *Pik-p* gene were detected in three accessions of *japonica* type (Hyangmibyeo1ho, Inaguhu, and 415xIr352) and four accessions of *indica* type (Tarana Deshi, Iranbyeopssi, Basmati Dhan, and Basmati), respectively (Fig. 2).

Genetic diversity of *Pit* gene. Especially, one of the NBS-LRR family genes, *Pit* gene founded on chromosome 1 of

indica rice variety K59, was detected as 322 bp of positive bands in all of 83 accessions of aromatic rice germplasm with t256 SNP marker, exception A-2, one of *japonica* type (Fig. 3).

Discussion

In Asian cultivated rice, aroma is one of the most valuable traits in grain quality. The rice world market ranks aromatic rice at the top and for the 4–5 million tons of aromatic rice worth 2–2.5 billion US Dollars traded in the world and the greatest market will become more competitive in the near future (McCouch et al., 2007; Singh et al., 1997).

Recently, more than 50 major blast resistance genes have been identified (Chauhan et al., 2002; Chen et al., 2002; Berruyer et al., 2003; Liu et al., 2004; Liu et al., 2005; Pan et al., 1999; Sallaud et al., 2003; Wu et al., 2003; Zhu et al., 2004). However, a number of blast resistance genes below have been mapped, which should be regarded as an essential starting point to isolate these important genes through map-based cloning approach.

Plant breeders often use cultivars developed in other countries to broaden the genetic background of the improved cultivars being developed such as the major fungal diseases of last and sheath blight. But most breeding programs of aromatic rice have a narrow genetic diversity of breeding resources (Khush, 1981). In Pakistan, out of 7 basmati varieties were currently under cultivation, five had 'Basmati 370' as one of the parent cultivar (Arif et al., 2005). Also, in Korea, only seven varieties of aromatic rice were bred and distributed as cultivars. Most of these varieties have a parent originated from Japan (Dohoku144 and Miyagaori) and IRRI (IR841-76-1), exception of Heughyangbyeon that was mutated Sanghaehyanghyeolla with 300Gy of γ -ray (Choi et al., 1995; Ha et al., 2003; Ha et al., 2006; Moon et al., 2003).

Our results, the genetic frequency of the six major rice blast resistance genes, *Piz*, *Piz-t*, *Pik*, *Pik-m*, *Pik-p*, and *Pit*, ranged from 30% to 99%. Significantly, eighty three accessions (99%) were detected with both or one of *Piz* and *Piz-t* genes. Ten accessions (12%) showed the positive bands for the *Piz-t* gene with both zt4792 and zt6057 SNP markers. Only one accession, Khau Nua Keo, was not amplified with both *Piz* and *Piz-t* gene with four SNP markers. Especially, *japonica* type aromatic rice, Gerdeh, possessed only *Piz* gene between *Piz* and *Piz-t*.

Both *Piz* and *Piz-t* genes have been used for conferring blast resistance to Japanese cultivars, because their importance was emphasized by Hayashi et al. (2004) in rice breeding in Japan. The previous studies described that Niigatawase, a resistant donor cultivar of *Piz*, and Toride 1, a resistant donor cultivar of *Piz-t*. The *Piz* gene was mapped

close to *Piz-t* on chromosome 6 of Japanese *indica* rice variety K59.

The *Piz-t* and *Piz* genes, co-segregated with zt4792 and zt6057 markers, was flanked on one side by z4794 with one recombination and on the other side by z60510 and z5765 markers with two combinations. Also, the *Piz* gene co-segregated with three markers, z4792, z60510, and z5765. The gene was flanked on one side by z6050 and z4794 markers with one combination and on the other side by marker z3943 with two combinations (Hayashi et al., 2004).

Hayashi et al. (2006) revealed that three alleles of the *Pik* locus, *Pik*, *Pik-p* and *Pik-m* are mapped on the same chromosomal region by linkage analysis using 300 to 2,100 F₂ segregation populations.

The genes *Pik* and *Pik-m* originated in the Chinese *japonica* cultivars To-To (Kiyosawa, 1968) and Hokushi Tami (Kiyosawa, 1978), respectively, whereas *Pik-p* originated in the *indica* cultivar Pusur (Kiyosawa, 1969a). With exception of *Pik-s*, the *Pik* multi resistance genes to distinct rice blast isolates are similar. The resistance specificities of *Pik* and *Pik-m* mostly overlap each other and show a similar resistance response to distinct blast isolates. But *Pik-m* has a broader resistance spectrum than *Pik* and is composed of two, or more than two, genetic factors (Ashikawa et al., 2008; Kiyosawa and Nomura, 1988).

In this study, fifty two accessions showed the three of *Pik* multiple genes and *Pit* gene. Four accessions, Iari7447, Daebunhyangdo2, Shiyayuuine, and Basmati 6129 possessed only a *Pik-p* gene of *Pik* multi genes.

Pit gene was reported as a major resistance gene on chromosome 1 which is inherited from an *indica* rice variety K59 and confers essential race-specific resistance against the fungal pathogen, *Magnaporthe grisea*, and is a member of the nucleotide-binding site-leucine-rich repeat (NBS-LRR) family of evolution R genes (Hayashi et al., 2006).

Cho et al. (2007) described that none of the Korean rice varieties was found to be have positive bands of the *Pit* gene, with t256 SNP marker. But the tested Korean rice varieties were non-aromatic rice varieties. *Pit* gene was founded in an *indica* rice variety K59. Fortunately in our results, *Pit* gene was detected all of 83 accessions of aromatic rice germplasms, two *tongil* types, twenty five *japonica* types, and fifty six *indica* types, and expected one accession of *japonica* type, A-2, and originated from Bhutan.

The host R genes and pathogen avirulence genes of rice blast fungus and rice plant are required to deepen understanding of molecular mechanisms involved in the host-pathogen interaction. But, the molecular marker is a useful tool for assessing genetic variations and resolving cultivar identities.

In our study, four accessions of *indica* type, Mayataung, Yekywin Yinkya Hmwe, Basmati9-93, and Basmati5854, showed the positive bands to six major rice blast resistance genes, *Piz*, *Piz-t*, *Pik*, *Pik-m*, *Pik-p*, and *Pit*. The origins of four candidate accessions possessed to China, Myanmar, and Pakistan.

Transferring blast resistance genes to different genetic backgrounds is very cumbersome and tedious. Since it would be difficult to identify under field conditions using conventional approaches in order for marker-assisted selection to facilitate at early selection phase with greater accuracy (Gu et al., 2005; Melchinger et al., 1990; Mohan et al., 1997).

In this study, the marker-assisted selection of rice blast resistance genes will help in the breeding program in multi diseases resistant aromatic rice varieties in genetic resources of aromatic rice. Some of these germplasma may have special properties that are important to breeding program whereas others may possess proprietary technology.

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