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Screening of Resistance Genes Linked to Brown Planthopper Using STS Marker in Aromatic Rice Germplasm

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ABSTRACT Brown planthopper (BPH) is a serious insect pest of rice crop throughout rice growing countries, and yield loss due to its infection can be up to 60%. This study aimed to evaluate efficiency of molecular markers for screening BPH resistance accessions among 86 aromatic rice germplasm. Eighty-six accessions of aromatic rice germplasm included two accessions of *Tongil type* (bred in Korea), 28 accessions of *japonica* type and 56 accessions of *indica* type. We applied eight STS markers (pBPH9, pBPH19, pBPH20, pBPH21, AJ09-b, RG457L, RG457B, and 7312.T4A) which were linked to four of BPH resistance genes, *Bph1*, *Bph13(t)*, *Bph10*, and *Bph18(t)* respectively. One *japonica* type accession, 415XIr352, and six indica type accessions possessed one or four positive bands when tested with four STS markers linked to *Bph1* gene. One *indica* type aromatic rice, Basmati9-93, showed the target bands linked to the *Bph10* gene. The other accessions did not show same fragments as the respective resistant lines. *Bph13(t)* is the most widely introduced resistance gene and only one accession showed positive bands implying that this accession might harbor *Bph10* and *Bph18(t)* genes. Three aromatic accessions, Domsiah, Khao Dawk Mali 105 and 415XIr352 showed gene pyramiding of *Bph1* and *Bph13(t)*. Two indica aromatic rice, Ds 20 and Basmati 9-93, possessed at least two BPH resistance genes, *Bph1*, *Bph18(t)* and *Bph13(t)*, *Bph18(t)*, respectively. These results indicates that aromatic rice germplasm have narrow diversities of BPH resistance genes.

Keywords : aromatic rice (*Oryza sativa* L.), brown planthopper (BPH), resistance, STS marker

Aromatic rice (*Oryza sativa* L.) is known for its characteristic of pleasant aroma. One of the aromatic rice varieties, Basmati, is not only in great demand in the domestic markets, and has a premium price in the world market (Arif *et al.*, 2005; Singh *et al.*, 1997).

Brown planthopper (BPH), *Nilaparvata lugens* (Stål) is widely distributed thought out Asia and considered as the most serious pest throughout the rice-growing areas of the world. At high pest density, its feeding damage causes 'Hopperburn' or complete wiling and drying of the rice plant (Dyck and Thomas, 1979; Pathak, 1968; Sexena and Berrion, 1983).

BPH is able to overwinter successfully in low temperature countries such as Japan and colonization occurs annually following long-distance migration from southern China during the rainy season from June to July (Matsumura *et al.*, 2006; Seino *et al.*, 1987; Watanabe *et al.*, 1990).

So far, three Southeast Asian biotypes, *N. lugens* have been collected and identified from 1971 to 1975 in the Philippines (IRRI, 1976; Pathak and Saxena, 1980). The dominant biotype of BPH occurred in the 11 provinces and autonomous region of China has changed from biotype 1 to biotype 2 except in Chengdu and Sichuan. Some individuals of BPH collected from early rice fields at Simao, Yunnam and Nanning, Guangxi were found to be the same as that of Bangladesh and Sri Landka (Saxena and Barrion, 1985).

In Japan, the virulence of the BPH population immigrating into Japanese rice varieties carrying the resistance gene *Bph1* began to increase during 1988-1990 (Sogawa, 1992). The BPH population rapidly became virulent to the rice variety ASD7 (carrying *bph2* gene) in beginning of 1997, and the virulence remained at a high level through 1999 (Tanaka, 1999; Tanaka and Matsumura, 2000)

In Korea, *N. lugens* migrates from the southern part of

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China annually, and is unable to overwinter (Park, 1973; Uhm *et al.*, 1988). Three BPH biotypes have been identified based on their variety reactions: biotype 1 (no gene for resistance), biotype 2 (carrying *Bph1* resistance gene), and biotype 3 (carrying *bph2* resistance gene) (Lee *et al.*, 1982, 1985)

The first released BPH resistant variety, Mudgo, a variety from India, suffered no apparent damage even under heavy insect populations (Pathak *et al.*, 1969). Also, IR26 was highly resistant against the general population (biotype 1) in the Philippines from 1973 to 1975 (Pathak and Khush, 1979) and in Indonesia from 1974 to 1976 (Harahap, 1979). Unfortunately, this resistant variety soon broke down to a new invade (biotype 2) of the insect in the Philippines (Varca and Feuer, 1976), Indonesia (Harahap, 1979), and Vietnam (Huynh, 1977).

Recently, 13 BPH major resistance genes, together with several quantitative trait loci controlling BPH resistance (Alam and Cohem, 1998; Xu *et al.*, 2002). Genetic analysis of BPH resistance has revealed six dominant (*Bph1, 3, 6, 9, 10,* and *13(t)*) and seven recessive (*bph2, 4, 5, 7, 8, 11(t),* and *12(t)*) genes has been reported in *indica* type rice cultivars and two wild relatives. (Cha *et al.*, 2008; Chen *et al.*, 2006; Jena *et al.*, 1992; Jena *et al.*, 2006; Ishii *et al.*, 1994; Hirabayashi and Ogawa, 1996; Lang *et al.*, 1999; Murai *et al.*, 2001).

In the present study, we screened eighty-six aromatic rice accessions of different origin for the genetic diversity of resistance genes against BPH for improving aromatic rice breeding efficiency using PCR-based STS (sequence tagged site) markers.

MATERIALS AND METHODS

Rice plant materials and DNA extraction

In the previous studies, among the 260 aromatic introductions preserved in RDA genebank, 86 accessions were evaluated for agronomic traits, physio-chemical characteristics, and aromatic compounds (Kim *et al.*, 2008a, 2008b)

Five seeds of each accession were disinfected with 2% NaOCl solution for 4 hrs and were washed with tap water for overnight and placed on moist filter paper laid on the petri-dish at 30℃ for one-week in growth chamber.

Genomic DNA for PCR analysis were extracted from frozen young leaves of one-week-old seedlings by an improved CTAB (hexadecyl trimethyl ammonium bromide) buffer based method described by Chen and Ronard (1999). The quality of extracted genomic DNA was estimated on 1% agarose gels stained with ethidium bromide. The quantity of DNA was measured by NANO Drop system (Thermo, U.S.A.) and were adjusted to 10 ng/μl with sterilized distill water and stored at 4℃.

Brown planthopper R genes specific STS markers

Eight PCR based allele-specific STS marker set (pBPH9, pBPH19, pBPH20, pBPH21, AJ09-b, RG457L, RG457B and 7312.T4A) of four major BPH resistance (*R*) genes, *Bph1, Bph13(t), Bph10* and *Bph18(t)*, were shown in Table 2 as per previously reported studies (Cha *et al.*, 2008; Lang *et al.*, 1999; Jena *et al.*, 2006; Renganayaki *et al.*, 2002) (Table 1). Three BPH resistance genes, *Bph1, Bph10* and *Bph18(t)* genes, were located on chromosome 12, and *Bph13(t)* gene was located on chromosome 3. We used the eight STS markers for amplification of DNA fragments linked to BPH resistant genes based on previously reported conditions. All of the markers were synthesized by Bioneer Co., Korea.

Estimation of PCR results for the resistance gene was determined by visualization of 536 bp, 610 bp, 535 bp and 885 bp of positive fragments using four SNP primer, pBPH9, pBPH19, pBPH20 and pBPH21, respectively.

Polymerase chain reaction (PCR) analysis

The PCR analysis for the STS allele-specific markers was conducted according to 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 $MgCl_2$, 1X PCR buffer (10 mM Tris-HCl, pH 9.0, and 30 mM KCl), and 1.0 unit of *Taq* DNA polymerase in 20μl PCR reaction volume. PCR amplifications were carried out in a MyGenie96 Thermal cycler (Bioneer Co. Ltd, Korea), and programmed as such a way that template DNA was initially denatured at 95℃ for 4 min, followed by 30 cycles of PCR amplification

steps with the following parameters; 30 sec denaturation at 94℃, 30 sec primer annealing at from 42 to 62℃, and a 60 sec primer extension at 72℃ to facilitate completion of primer extension, with a final extension at 72℃ for 10min. 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

Table 1. Details of sequence tagged site (STS) markers used for screening BPH resistance genes.

Marker	Linked gene	Enzyme	Chro.	Primer	Expected products (bp)		Cited articles
					R	S	
pBPH09	*Bph1*	-	12	F:5'-AGCGCTGGTCGTTGGGGTTGTAGT-3' R:5'-ATTAAAAGTGATCGCAGCCGTTCG-3'	536	773	Cha *et al.*, 2008.
pBPH19		-		F:5'-GGGGTCGCCGAGGTCGTTGTAGA-3' R:5'-TGGCTGAAGCTGCATGGGAGTTGG-3'	610	587	
pBPH20		-		F:5'-GGCTGCCTTATCCCCAACTCC-3' R:5'-GTCGGCGCCGTGCAGGTCGTG-3'	535	676	
pBPH21		-		F:5'-GCTCCGTGTGCATCCCCTCTGTAG-3' R:5'-GACTGGCTTTTCCTTGATTTCTT-3'	885	734	
AJ09-b	*Bph13(t)*	-	3	F:5'-TCGACCTAGAAAGGCCTGTGT-3' R:5'-CACTGGAAATTTGAGCGAGAA-3'	200	179	Renganayaki *et al.*, 2002.
RG457L	*Bph10*	*Hinf I*	12	F:5'-GCAGTGGCAGATGGGATCGT-3' R:5'-GCTCCGAAATCCCAAGCGAT-3'	300, 250, 200	500, 200	Lang *et al.*, 1999.
RG457B				F:5'-CGTTATCCTCAGTTCCTAGG-3' R:5'-TGAGCTGATGGTTTGCATGG-3'	200, 300	550, 200	
7312.T4A	*Bph18(t)*	*Hinf I*	12	F:5'-ACGGCGGTGAGCATTGG-3' R:5'-TACAGCGAAAAGCATAAAGAGTC-3'	566,398	-	Jena *et al.*, 2006.

Table 2. Genotype of 86 aromatic accessions at the BPH resistance genes estimated with eight allele-specific STS markers linked to the resistance genes.

Lane	Varieties[a]	Ecotypes	*Bph1*				*Bph13(t)*	*Bph10*		*Bph18(t)*
			pBPH9	pBPH19	pBPH20	pBPH21	AJ09-b	*RG457L /Hinf* I	*RG457B /Hinf* I	*7312.T4A /Hinf* I
1	Hyangmibyeo1ho	To	SS	SS	SS	SS	RR	SS	SS	SS
2	Hyangmibyeo2ho	To	SS	SS	SS	SS	SS	SS	SS	SS
3	Hyangnambyeo	Ja	SS	SS	SS	SS	RR	SS	SS	SS
4	Aranghyangchalbyeo	Ja	SS	SS	SS	SS	RR	SS	SS	SS
5	Mihayangbyeo	Ja	SS	SS	SS	SS	RR	SS	SS	SS
6	A-2	Ja	SS	SS	SS	SS	RR	SS	SS	SS
7	A-3, Choh Chang	Ja	SS	SS	SS	SS	RR	SS	SS	SS
8	Muhyang99-8	Ja	SS	SS	SS	SS	RR	SS	SS	SS
9	Jahyangna861	Ja	SS	SS	SS	SS	RR	SS	SS	SS
10	Jc149	Ja	SS	SS	SS	SS	SS	SS	SS	SS
11	Jc157	Ja	SS	SS	SS	SS	SS	SS	SS	SS
12	Iari 7447	Ja	SS	SS	SS	SS	RR	SS	SS	SS
13	Padi danat	Ja	SS	SS	SS	SS	RR	SS	SS	SS
14	Kemiri	Ja	SS	SS	SS	SS	RR	SS	SS	SS

Table 2. Genotype of 86 aromatic accessions at the BPH resistance genes estimated with eight allele-specific STS markers linked to the resistance genes. -continued

Lane	Varieties	Ecotypes[a]	*Bph1*				*Bph13(t)*	*Bph10*		*Bph18(t)*
			pBPH9	pBPH19	pBPH20	pBPH21	AJ09-b	*RG457L /Hinf* I	*RG457B /Hinf* I	*7312.T4A /Hinf* I
15	Daebunhyangdo2	Ja	SS	SS	SS	SS	RR	SS	SS	SS
16	Shiyayuuine	Ja	SS	SS	SS	SS	RR	SS	SS	SS
17	Rasomotrafotsy	Ja	SS	SS	SS	SS	RR	SS	SS	SS
18	TALLI	Ja	SS	SS	SS	SS	SS	SS	SS	SS
19	Masino Basmati	Ja	SS	SS	SS	SS	SS	SS	SS	SS
20	Kalomasino Dhan	Ja	SS	SS	SS	SS	SS	SS	SS	SS
21	Gerdeh	Ja	SS	SS	SS	SS	RR	SS	SS	SS
22	KINANDANG PAT	Ja	SS	SS	SS	SS	RR	SS	SS	SS
23	Milagrosa MUTANT	Ja	SS	SS	SS	SS	RR	SS	SS	SS
24	Inaguhu	Ja	SS	SS	SS	SS	RR	SS	SS	SS
25	Flores	Ja	SS	SS	SS	SS	RR	SS	SS	SS
26	Kung-ShanWu-Shen-Ken	Ja	SS	SS	SS	SS	RR	SS	SS	SS
27	Daw Dam	Ja	SS	SS	SS	SS	RR	SS	SS	SS
28	415 X Ir352	Ja	SS	RR	SS	RR	RR	SS	SS	SS
29	Khau Nua Keo	Ja	SS	SS	SS	SS	RR	SS	SS	SS
30	Khau Tan Luong	Ja	SS	SS	SS	SS	RR	SS	SS	SS
31	Goolarath	In	SS	SS	SS	SS	RR	SS	SS	SS
32	Da13	In	SS	SS	SS	SS	SS	SS	SS	SS
33	Basmati 370	In	SS	SS	SS	SS	SS	SS	SS	SS
34	Hyanggaengdo	In	SS	SS	SS	SS	RR	SS	SS	SS
35	Seratus Malam	In	SS	SS	SS	SS	RR	SS	SS	SS
36	Basmati T3	In	SS	SS	SS	SS	SS	SS	SS	SS
37	Jc111	In	SS	SS	SS	SS	SS	SS	SS	SS
38	Arc 6011	In	SS	SS	SS	SS	SS	SS	SS	SS
39	Kaminibhog	In	SS	SS	SS	SS	SS	SS	SS	SS
40	Tarana Deshi	In	SS	SS	SS	SS	RR	SS	SS	SS
41	Basmati	In	SS	SS	SS	SS	SS	SS	SS	SS
42	Iranbyeopssi	In	SS	SS	SS	SS	RR	SS	SS	SS
43	Iranbyeopssi	In	SS	SS	SS	SS	SS	SS	SS	SS
44	Domsiah	In	SS	RR	SS	SS	RR	SS	SS	SS
45	Mulai	In	SS	SS	SS	SS	SS	SS	SS	SS
46	Tareme	In	SS	SS	SS	SS	SS	SS	SS	SS
47	Hyangdo	In	SS	SS	SS	SS	RR	SS	SS	SS
48	Seratus Malam	In	SS	SS	SS	SS	RR	SS	SS	SS
49	Mayataung	In	SS	SS	SS	SS	RR	SS	SS	SS
50	Yekywin Yinkya Hmwe	In	SS	SS	SS	SS	RR	SS	SS	SS
51	Kala Namak	In	SS	SS	SS	SS	SS	SS	SS	SS

Table 2. Genotype of 86 aromatic accessions at the BPH resistance genes estimated with eight allele-specific STS markers linked to the resistance genes. -continued

Lane	Varieties	Ecotypes[a]	*Bph1*				*Bph13(t)*	*Bph10*		*Bph18(t)*
			pBPH9	pBPH19	pBPH20	pBPH21	AJ09-b	*RG457L /Hinf* I	*RG457B /Hinf* I	*7312.T4A /Hinf* I
52	Masino Basmati	In	SS	SS	SS	SS	SS	SS	SS	SS
53	Basmati Dhan	In	SS	SS	SS	SS	SS	SS	SS	SS
54	Basmati	In	SS	SS	SS	RS	SS	SS	SS	SS
55	Basmati 370	In	SS	SS	SS	SS	SS	RS	SS	SS
56	Basmati 9-93	In	SS	SS	SS	SS	RR	RR	RR	SS
57	Basmati 198	In	SS	SS	SS	SS	SS	SS	SS	SS
58	Basmati 370	In	SS	SS	SS	SS	SS	SS	SS	SS
59	Basmati 5836	In	SS	SS	SS	SS	SS	SS	SS	SS
60	Basmati 5854	In	SS	SS	SS	SS	SS	SS	SS	SS
61	Basmati 5875	In	SS	SS	SS	SS	SS	SS	SS	SS
62	Basmati 6113	In	SS	RR	SS	RS	SS	SS	SS	SS
63	Basmati 1	In	SS	SS	SS	SS	SS	SS	SS	SS
64	Basmati 213 C	In	SS	SS	SS	SS	RR	SS	SS	SS
65	Basmati 372	In	SS	SS	SS	SS	SS	SS	SS	SS
66	Chahora 144	In	SS	SS	SS	SS	SS	SS	SS	SS
67	Pakistani Fine	In	SS	SS	SS	SS	SS	SS	SS	SS
68	Ir841-85-1-1-2	In	SS	SS	SS	SS	RR	SS	SS	SS
69	05-Irri-M-46	In	RR	RR	RR	RR	SS	SS	SS	SS
70	Basmati 107	In	SS	SS	SS	SS	SS	SS	SS	SS
71	Basmati 405	In	SS	SS	SS	SS	SS	SS	SS	SS
72	Basmati 5853	In	SS	SS	SS	SS	SS	SS	SS	SS
73	Basmati 5874	In	SS	SS	SS	SS	SS	SS	SS	SS
74	Basmati 6129	In	SS	SS	SS	SS	SS	SS	SS	SS
75	Basmati 6311	In	SS	SS	SS	SS	SS	SS	SS	SS
76	Basmati 6313	In	SS	SS	SS	SS	SS	SS	SS	SS
77	Basmati 6141	In	SS	SS	SS	SS	SS	SS	SS	SS
78	AZUCENA	In	SS	SS	SS	SS	RR	SS	SS	SS
79	Binicol	In	SS	SS	SS	SS	SS	SS	SS	SS
80	Milfor 6	In	SS	SS	SS	SS	RR	SS	SS	SS
81	Dinorado	In	SS	SS	SS	SS	RR	SS	SS	SS
82	Khao Dawk Mali105	In	SS	RR	SS	SS	RR	SS	SS	SS
83	Jasmine 85	In	SS	SS	SS	SS	RR	SS	SS	SS
84	Dellmont	In	SS	SS	RR	SS	SS	SS	SS	SS
85	Aroma	In	SS	SS	SS	SS	SS	SS	SS	SS
86	Ds20	In	RR	RR	RR	RR	SS	SS	SS	RR

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

[a] To, In, and Ja stand for *Tongil Type*, *Indica Type*, and *Japonica type*, respectively.

amplified fragment using SNP markers were scored as presence (1) or absence (0) of amplicons linked to each gene.

RESULTS

Screening of rice accessions using markers linked to the Bph1 gene

Cha *et al.* (2008) developed four *Bph1* specific STS markers, pBPH9, pBPH19, pBPH20 and pBPH21, based on two BAC clones on chromosome 12. To detect the *Bph1* gene, 86 aromatic accessions were analyzed through the presence of amplicons using four STS markers, pBPH9, pBPH19, pBPH20 and pBPH21. One *japonica* accession, 415XIr352, showed both 610 bp and 885 bp bands amplified with pBPH19 and pBPH21, respectively. Six *indica* accessions possessed one or four positive bands linked to the *Bph1* gene. Among six accessions, three, Domsiah, Basmati6113 and Khao Dawk Mali 105 produced a 610 bp positive amplicon with the pBPH19 STS marker. One accession, Dellmont, showed the 535 bp target band detected with pBPH20 STS marker. Especially, two accessions possessed four positive bands amplified with each four candidate STS markers tightly linked to *Bph1* gene (Fig. 1).

Screening of rice accessions using markers linked to the Bph13(t) gene

Co-dominant STS marker, AJ09-b, was mapped and developed from RAPD markers closely linked to BPH biotype-4 resistance gene, *Bph13(t)* on rice chromosome 3. *Bph13(t)* was scored highest positive bands of four BPH resistance genes amplified with eight PCR based allele-specific STS marker set. Of 86 accessions of tested aromatic rice, forty-two accessions were detected with both 200 bp of positive fragment and 179 bp of susceptible fragment amplified with Aj09-b STS marker. Forty-two positive accessions included one *Tongil*-type accession (Hyangmibyeolho), twenty three *japonica* type accessions and eighteen *indica* type accessions (Fig. 2).

Screening of rice accessions using markers linked to the Bph10 gene

Lang *et al.* (1999) mapped the *Bph10* gene controlling

Fig. 1. Screening of 86 aromatic accessions using four STS markers, pBPH9 (A), pBPH19 (B), pBPH20 (C) and pBPH21 (D), tightly linked to the *Bph1* gene. PCR products were classified into pBPH9 (resistant, 536 bp), pBPH19 (resistant, 610 bp), pBPH20 (resistant, 535 bp) and pBPH21 (resistant, 885 bp). Lane M, 100 bp ladder plus; Lane 1-86, aromatic rice germplasm.

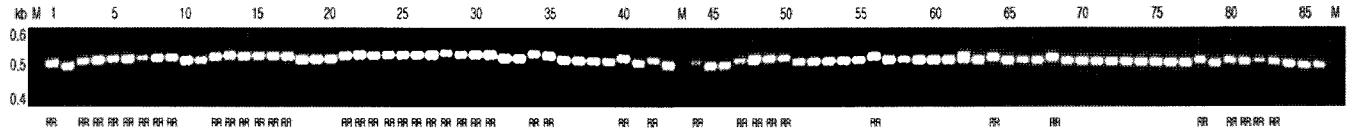

Fig. 2. Screening of the BPH resistance gene (RR), *Bph13(t)* with amplicon amplified with the STS marker, *AJ09-b*. The PCR bands were produced with DNA of 86 accessions. Lane M, 100 bp ladder plus; Lane 1-86, aromatic germplasm.

resistance to BPH biotype 2 and 3, with RFLP and converted one of the linked RFLP markers into a PCR based STS marker, RG457F and RG457R which co-segregated with the *Bph10* gene. None of the *Tongil* and *japonica* accessions showed the positive fragment when the PCR product was cleaved with *Hinf* I. One of fifty-six *indica* accessions, but Basmati9-93 possessed the target band (Fig. 3).

Screening of rice accessions using markers linked to the Bph18(t) gene

Eighty-six accessions were screened with an STS marker, *7312.T4A* linked to the *Bph18(t)* gene and an introgression line (IR65482-7-216-1-2) inherited the gene from the wild rice *Oryz australiensis* (Jena *et al.* 2006). One accession showed the 566 bp and 398 bp of positive double bands when the PCR product was cleaved with the restriction enzyme *Hinf* I (Fig. 4).

DISCUSSION

Nilaparvata lugens (Stål) is present in varying populations on most rice crop, but often its damage is noticed only after the crop suffers hopperburn. Moreover, the insect has been recorded as a symptom of yellowing syndrome and as viral vectors of the grassy stunt virus and ragged stunt virus diseases of rice (Harahap, 1979; Rivera *et al.*, 1966). For rice crops, growing resistant varieties is the most effective and environment-friendly way to control the Brown planthopper (*N. lugens*). Application of molecular markers will enhance the speed and efficiency of the selection process in breeding for BPH resistance program (Alagar *et al.*, 2007; Alam and Cohen, 1998). However, many resistant cultivars have been developed in the world since the initial screens of rice germplasm for genetic resistance to BPH (Pathak *et al.*, 1969).

In this study, we estimated BPH resistance of eighty-six aromatic rice accessions by screening for the presence of 4 major brown plant hopper (BPH) resistance (*R*) genes,

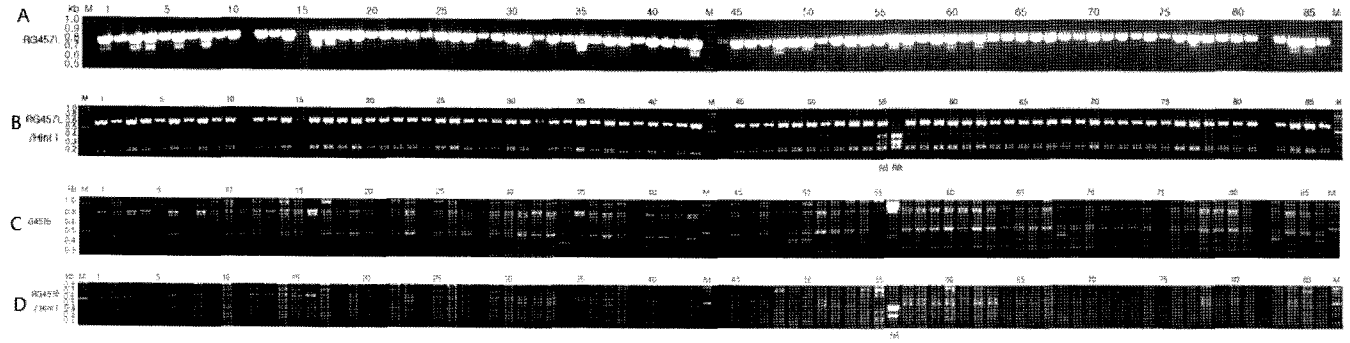

Fig. 3. Banding patterns of the accessions amplified with the STS markers, *RG457L* (A) and *RG457B* (C) that are linked to the *Bph1* gene. The amplified PCR products were digested with *Hinf* I restriction enzyme (B and D). Lane M, 100 bp ladder plus; lane 1-86, aromatic rice germplasm.

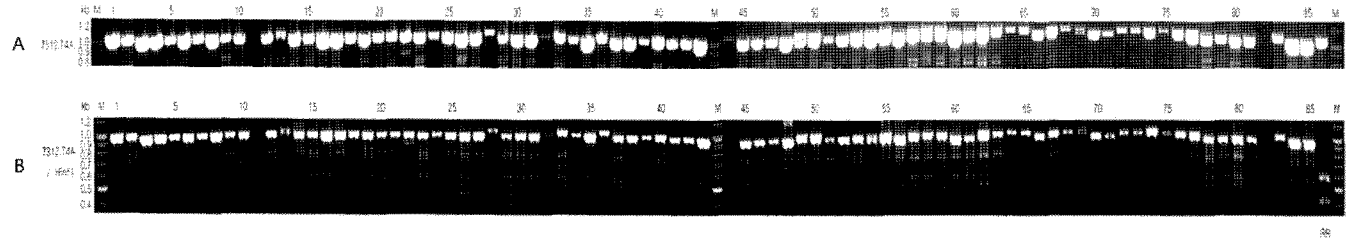

Fig. 4. Agarose gel electrophoresis of PCR products amplified with the STS marker, *7312.T4A*, linked to the *Bph18(t)*. Before (A) and after (B) the amplified PCR products were digested with *Hinf* I restriction enzyme. Lane M, 100 bp ladder plus; Lane 1-86, aromatic rice germplasm.

Bph1, Bph13(t), Bph10 and *Bph18(t)*. Eighty-six accessions included two *Tongil* type (bred in Korea), 28 *japonica* and 56 *indica* accessions.

Thirteen major R genes to BPH resistance have been identified in *indica* type rice cultivars and two wild relatives, *Oryza autraliensis*, and *O. officinalis*. Genetic analysis of BPH resistance genes has revealed 6 dominant (*Bph1, 3, 6, 9, 10,* and *13(t)*) and 7 recessive (*bph2, 4, 5, 7, 8, 11(t),* and *12(t)*) genes controlling BPH resistance (Cha *et al.*, 2008; Chen *et al.*, 2006; Jena *et al.*, 2006; Lang *et al.*, 1999).

Bph1 and *Bph10* were found to be closely linked to RFLP markers, C185 and RG457 on rice chromosome 12 (Jena *et al.*, 1992; Ishii *et al.*, 1994; Hirabayashi and Ogawa, 1996). In this study, one *japonica* accession, 415XIr352, and six *indica* accessions possessed positive bands linked to *Bph1* gene detected with four STS marker, pBPH9 (536 bp), pBPH19 (610 bp), pBPH20 (535 bp) and pBPH21 (885 bp). Many resistance genes to BPH have been identified in *indica* type varieties and wild rice accessions, but have not been cultivated due to the preference for *japonica* type varieties in Korea.

One *indica* accession, Basmati9-93, possessed the target band linked to the *Bph10* gene with the RG457F and RG457R STS marker. But none of eighty-six accessions showed the positive fragment. *Bph13(t)* is the most broadly introduced resistance gene and the *Bph10* and *Bph18(t)* resistance genes showed positive bands in only one accessions in tested eighty-six accessions.

Gene pyramiding has been successfully applied in crop breeding programs, and many varieties and lines possessing multiple attributes have been produced (Huang *et al.*, 1997; Khush, 1981; McCouch *et al.*, 2007; Wang *et al.*, 2001). Three accessions, Domsiah, Khao Dawk Mali 105, 415XIr352, showed the gene pyramiding of *Bph1* and *Bph13(t)*. *Indica typerice*, Ds20 and Basmati9-93 possessed the two BPH resistance genes, *Bph1* and *Bph18(t)*, and *Bph13(t)* and *Bph18(t)*, respectively.

MAS will enhance the speed and efficiency of the selection process in breeding for BPH resistant lines. The genotype information of aromatic accessions at the BPH resistance genes in this study will be informative in the breeding program for combining both BPH resistance and the aroma gene in the breeding program..

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REFERENCES

Arif, M., S. Kousar, M. A. Bajwa, A. Arif and Y. Zapar. 2005. Genetic diversity among rice genotypes of Pakistan through random amplified polymorphic DNA (RAPD) analysis. Pakistan Journal of Botany 37 : 585-592.

Alam, S. N. and M. B. Cohen. 1998. Durability of brown planthopper. *Nilaparvata lugens*, resistance in rice variety IR64 in greenhouse selection studies. Entomol. Exp. Appl. 89: 71-78.

Alagar, M., S. Suresh, R. Samiyappan and D. Saravanakumar. 2007. Reaction of resistant and susceptible rice genotypes against brown planthopper (*Nilaparvata lugens*). Phytoparasitica 35 : 346-356.

Cha,Y. C., H. S. Ji, D. W. Yun, B. O. Ahn, M. C. Lee, S. C. Suh, C. S. Lee, E. K. Ahn, Y. H. Jeon, I. D. Jin, J. K. Sohn, H. J. Koh and M. Y. Eun. 2008. Fine mapping of the rice *Bph1* gene, which confers resistance to the brown planthopper (*Nilaparvata lugens* Stal), and development of STS markers for marker- assisted selection. Mol. Cells. 26 : 146-151.

Chau, L. M. 2007. State of insecticide resistance of brown planthopper in Mekong Delta, Vietnam. Omonrice 15:185-190.

Chen, D. H. and P. C. ROnard. 1999. A rapid DNA Minipreparation Method Suitable for AFLP and other PCR Applications. Plant Molecular Biology Reporter 17 : 53-57.

Dyck, V. A. and B. Thomas. 1979. The brown planthopper problem. In: Brown Planthopper: Threat to Rice Production in Asia IRRI, Los Baños, Philippines. pp. 3-17.

Harahap, Z. 1979. Breeding for resistance to brown plnthopper and grassy stunt virus in Indonesia. In: Brown Planthopper: Threat to Rice Production in Asia.IRRI, Los Baños, Philippiness. pp. 201-208.

Hirabayashi, H. and T. Ogawa. 1996. RFLP mapping of brown plant hopper resistance gene *Bph1* in rice, Int. Rice Reas. Newsl. 21 : 54-55.

Huang, N., E. R. Angeles, J. Domingo, G. Magpantay, S. Singh, G. Zhang, N. Kumaravadivel, J. Bennett and G. S. Khush. 1997. Pyramiding of bacterial blight resistance genes in rice; marker-assisted selection using RFLP and PCR. Theor. Appl. Genet. 95 : 313-320.

Hutnh, N. V. 1977. New biotype of brown planthopper in the Mekong Delta of Vietnam. IRRN 2 : 10

Int. Rice Rer. Inst. 1976. Selection and studies on sources of resistance. Biotypes. IRRI Ann. Rep. 1975 : 107-108.

Int. Rice Rer. Inst. 1978. Biotype studies. Genetis of BPH biotypes. IRRI Ann. Rep. 1977 : 68.

Ishii, T., D. S. Brar, D. S. Multani and G. S. Khush. 1994. Molecular tagging of genes for brown planthopper resistance and earliness introgressed from *Oryza australiennsis* into cultivated rice, *O. sativa*. Genome 37 : 217-221.

Jena, K. K., J. U. Jeung, J. H. Lee, H. C. Choi and Brar. D. S. 2006. High-resolution mapping of a new brown planthopper (BPH) resistance gene, *Bph18(t)*, and marker-assisted selection for BPH resistance in rice (*Oryza sativa* L.). Theor Appl Genet 112 : 288-297.

Jena, K. K., G. S. Khush and G. Kochert. 1992. RFLP analysis on rice (*Oryza sativa* L.) introgression lines. Theor Appl. Genet. 84 : 608-616.

Jonson, V. A., J. W. Gibbons, K. A. K. Moldenhauer, Z. Wang, and Y. Jia. 2002. In: Breeding, Genetics, and Physiology: Rice variety improvement using marker-assisted selection. B. R. Wells. Rice Research Series. pp. 66-72.

Khush, G. S. 1981. Breeding rice for multiple disease and insect resistance. In: Rice improvement in China and other Asian countries. International Rice Research Institute, Los Banos, Philippines. pp. 220-237.

Kim, J. S., S. N. Ahn, Y. H. Cho, J. G. Gwag, T. S. Kim, J. R. Lee and S. Y. Lee. 2008b. Estimation of agronomic characteristics of domestic aromatic rice germplasm and foreign aromatic rice germplasm in RDA Genebank, Korea. Korean J. Crop Sci. 53 : 261-272.

Kim, J. S., S. N. Ahn, H. K. Kang, Y. H. Cho, J. G. Gwag and S. Y. Lee. 2008a. Estimation of Physico-chemical Characteristics of domestic aroma rice and foreign aroma rice. Korean J. Crop Sci. 53 : 203-216.

Lang, N. T., D. Brar, S. Khush, G. S., N. Huang and B. C. Buu. 1999. Development of STS markers to identify Brown Planthopper resistance in a segregating population. OMONRICE 7 : 27-38.

Lee, J. O., H. G. Goh and Y. H. Kim. 1985. Study on brown planthopper biotypes. 2. Resistance mechanisms of different rice varieties to Korean brown planthopper biotypes. Res. Rep. ORD, (S.P.M.U.) 27 : 73-78.

Lee, J. O., H. G. Goh, Y. H. Kim, C. C. Kim and J. S. Park. 1982. Reactions of some Korean rice varieties to brown planthopper biotype 2. Int. Rice Res. Newsl. 7 : 10.

Li Q., S. Y. Luo, A. X. Sbi, S. M. Wei and F. K. Huang. 1997. The biotypes of Brown planthopper (*Nilaparvata lugens* Stål.) with a view to its control. Acta Entomol. Sin., 40 : 139-146.

Matsumura, M., A. Otuka and T. Watanabe. 2006. Migration prediction and monitoring of rice Planthoppers in Japan. Proceedings of the International Symposium on Area-Wide Management of Insect Pests. Food and Ferertilizer Technology Center. Okinawa, Japan.

McCouch, S. R., X. Chen, O. Panaud, S. Temnykh, Y. Xu, Y. G. Cho, N. Huang, T. Ishii and M. Blair. 1997. Microsatellite marker development, mapping and application in rice genetics and breeding. Plant Mol. Bio. 35 : 89-99.

McCouch, S. R., M. Sweney, J. Li, H. Jiang, M. Thoson, E. Septiningish, J. Edwards, P. Moncada, J. Xiao, A. Garris, T. Tai, C. Martinez, J. Thome, M. Sugiono, A. McClung, L. P. Yuan and S. N. Ahn. 2007. Through the genetic bottleneck: *O. rufipogon* as a source of trait enhancing alleles for *O. sativa*. Euphytica 154 : 317-339.

Melchinger, A. E. 1990. Use of molecular markers in breeding for oligogenic disease resistance. Plant Breed. 104 : 1-19.

Park, J. S. 1973. Studies on the recent occurrence tendency of major insect pests on rice plant. Symposium on Plant Environmental Research. In Commemoration of Dr. Kim's 60th birthday. pp. 91-102.

Pathak, M. D., C. H. Cheng and M. E. Fortuno. 1969 Resistance to *Nephotettix impicticeps* and *Nilaparvata lugens* in varieties of rice. Nature 22 : 502-504.

Patak, M. D. and R. C. Saxena. 1980. Breeding approaches in rice. In: Maxwell, F., and Jennings, P. R., eds. Plants Resistance to Insect. John Wiley and Sons, Inc., New York. pp. 422-456.

Pathak, M. D. 1968. Ecology of common insect pest of rice. Ann. Rev. Ent. 13 : 257-294.

Pathak, M. D. and G. S. Khush. 1979. Studies of varietal resistance in rice to the brown planthopper at IRRI. In: Brown Planthopper: Threat to Rice Production in Asia. IRRI. Los Baños, Philippines. pp. 287-301.

Renganayaki, K., A. K. Fritz, S. Sadasivam, S. Pammi, S. E. Harrington, S. R. McCouch, S. M. Kumar and A. S. Reddy. 2002. Mapping and progress toward map-based cloning of brown planthopper biotype-4 resistance gene introgressed from *Oryza officinalis* into cultivated rice, *O. sativa*. Crop Sci. 42 : 2112-2117.

Rievra, C. T., S. H. Ou and T. T. Iida. 1966. Grassy stunt disease of rice and its transmissions by the planthopper *Niaparvata lugens* Stål. Plant Disease Reporter 50 : 453-456.

Saxena, R. C. and A. A. Barrion. 1985. Biotypes of brown planthopper, *Nilaparvata lugens* (Stål) and strategies in deployment of host plant resistance. Insect Sci. Applic. 6 : 271-289.

Saxena, R. C. and A. A. Berrion. 1983. Biotypes of the Brown planthopper, *Nilaparvata lugens* (Stål). Korean J. Plant Prot. 22 : 52-66.

Seino, H., Y. Shiotsuki, S. Oya and Y. Hirai. 1987. Prediction of long didtance migration of rice planthoppers to northern Kyushu considering low-level jet stream. J. of Agricultural

Meteorology 43 : 203-208.

Singh, G.S., K. S. Rao and K. G. Saxena. 1997. Energy and economic efficiency of the mountain farming system: A case study in the north-western Himalaya. J. Sust. Agric. 9 : 25-49.

Sogawa, K. 1992. A change in biotype property of brown planthopper populations immigrating into Japan and their probable source areas. Proceedings of the Agricultural Meteorology 43 : 203-208.

Tanaka, K. 1999. Quatitative genetic analysis of biotypes of the brown planthopper, *N. lugens*. Entomol. Exp. Et Appl.90 : 279-287.

Tanaka, K. and M. Matsumura. 2000. Development of virulence to resistant rice varieties in the brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae), immigrating into Japan. App. Entomology and Zoology 3 : 529-533.

Uhm, K. B., J. S. Park, Y. I. Lee, K. M. Choi, M H. Lee and J. O. Lee. 1988. Relationship between some weather conditions and immigration of the brown planthopper, *Nilaparvata lugens* Stål. Korean J. App. Entomol. 27 : 200-210. (in Korean with English abstract).

Wang. X. Y., P. D. Chen and S. Z. Zhang. 2001. Pyramiding and marker-assisted selection for powdery mildew resistance genes in common wheat. Acta. Genetic Sinica. 28 : 640-646.

Watanabe, T., H. Seino, C. Kitamura and Y. Hirai. 1990. A computer program, LLJET, utilizing an 850 mb weather chart to forecast long-distance rice planthopper migration. The Bulletin of the Kyushu National Agricultural Experiment Station 26 : 233-260.