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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

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The brown planthopper (BPH) is a major insect pest in rice, and damages these plants by sucking phloem-sap and transmitting viral diseases. Many BPH resistance genes have been identified in *indica* varieties and wild rice accessions, but none has yet been cloned. In the present study we report fine mapping of the region containing the *Bph1* locus, which enabled us to perform marker-aided selection (MAS). We used 273 F8 recombinant inbred lines (RILs) derived from a cross between Cheongcheongbyeo, an *indica* type variety harboring *Bph1* from Mudgo, and Hwayeongbyeo, a BPH susceptible *japonica* variety. By random amplification of polymorphic DNA (RAPD) analysis using 656 random 10-mer primers, three RAPD markers (*OPH09*, *OPA10* and *OPA15*) linked to *Bph1* were identified and converted to SCAR (sequence characterized amplified region) markers. These markers were found to be contained in two BAC clones derived from chromosome 12: *OPH09* on OSJNBa0011B18, and both *OPA10* and *OPA15* on OSJNBa0040E10. By sequence analysis of ten additional BAC clones evenly distributed between OSJNBa0011B18 and OSJNBa0040E10, we developed 15 STS markers. Of these, *pBPH4* and *pBPH14* flanked *Bph1* at distances of 0.2 cM and 0.8 cM, respectively. The STS markers *pBPH9, pBPH19, pBPH20,* and *pBPH21* co-segregated with *Bph1*. These markers were shown to be very useful for marker-assisted selection (MAS) in breeding populations of 32 F6 RILs from a cross between Andabyeo and IR71190, and 32 F5 RILs from a cross between Andabyeo and Suwon452.

INTRODUCTION

The brown planthopper (BPH) is one of the most serious insect pests for rice growers, and exerts its damaging effects by sucking phloem-sap, and transmitting viral diseases such as grassy stunt virus and ragged stunt virus. Heavy infestation by BPH causes extensive death of rice plants by excessive drying, known as "hopper burn", and results in severe yield loss. This insect pest has been controlled in the past mainly by chemical pesticides, but this management strategy causes harmful side effects by also killing rice planthopper predators and severely affecting the balance within this ecosystem (Matteson, 2000).

An alternative to the use of pesticides is the development of cultivars with various BPH resistance genes; this is a key component of the current integrated pest management (IPM) strategy for sustainable agriculture. Many such resistant cultivars have now been developed worldwide since the initial screens of rice germplasm for genetic resistance to brown planthoppers was conducted at the International Rice Research Institute (IRRI) (Pathak et al., 1969). Most of the developed varieties that are resistant to BPH belong to an *indica* subspecies, but have not been cultivated in Korea due to the preference for *japonica* varieties in this country. Hence, there have been many efforts to develop *japonica*-type BPH resistant varieties in Korea. However, only one such variety, 'Hwacheongbyeo', a *japonica* variety harboring the BPH resistance gene *bph2* from IR1154-243 (Yeo et al., 1998), has so far been released, but this strain was found not to be effective in the field. The main cause of the failure to breed *japonica*-type resistant varieties is suspected to be tight linkage between resistance genes and inferior traits, so called 'linkage drag' (Yeo et al., 2002). Isolation of the BPH resistance gene by map-based cloning may help to overcome this impediment by providing precise selection markers and/or genetic material for transformation.

To date, 19 major and many more minor BPH resistance genes have been identified in *indica* varieties and wild rice spe-

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cies. Of the major genes, *Bph1, bph2, Bph9, Bph10* and *Bph18* were mapped on chromosome 12, *Bph3* and *bph4* on chromosome 6, *bph13*, *Bph14, Bph17,* and *bph19(t)* on chromosome 3*, and bph12*, *Bph15* and *Bph16* on chromosome 4 (Alam and Cohen, 1998; Chen et al., 2006; Huang et al., 2001; Jena et al., 2006; Xu et al., 2002; Yang et al., 2004).

Recently, high-resolution maps have been constructed to identify genes such as *Bph1*, *bph2*, *Bph3*, *Bph13(t)*, *Bph18(t)*, and *bph19(t),* but none have been isolated thus far (Chen et al., 2006; Jena et al., 2006; Murai et al., 2001; Renganayaki et al., 2002). The genomic region containing the *Bph1* locus has been narrowed down to 2.7cM since Hirabayashi and Ogawa (1995) demonstrated its linkage with the RFLP marker *XNpb248* at a 10.7% recombination value (Cha et al., 1999; Huang et al., 1997; Jeon et al., 1999; Kim and Sohn, 2005; Sharma et al., 2002). Previously in our laboratory we mapped *Bph1* between the *RG413* and *RG901* markers on chromosome 12 at distances of 7.5cM and 8.4cM, respectively, using F4 lines from a cross between Nakdongbyeo and Mudgo (Cha et al., 1999). We report here the results of a fine mapping analysis of *Bph1* using F8 recombinant inbred lines (RILs) from a cross between Hwayeongbyeo and Cheongcheongbyeo together with newly developed STS markers that are more tightly linked to *Bph1*. We also demonstrate the utility of these markers for marker-assisted selection (MAS).

MATERIALS AND METHODS

Plant materials

An F_8 recombinant inbred (RI) population of rice plants was derived from a cross between Cheongcheongbyeo and Hwayeongbyeo. Cheongcheongbyeo is an *indica*-type resistant cultivar harboring *Bph1* from Mudgo, whereas Hwayeongbyeo is a *japonica*-type susceptible cultivar (Lee et al., 1981). Using 273 lines from this RI population, we constructed a linkage map and evaluated the responses of the plants to BPH. Two more populations, comprising 32 F6 RILs from a cross between Andabyeo (with *Bph1*) and IR71190 (without *Bph1*), and 32 F5 RILs from a cross between Andabyeo and Suwon452 (without *Bph1*), were used to confirm the linkage of the STS markers with *Bph1*. To validate the applicability of the markers linked to *Bph1* to MAS, correlations between the linked markers and the BPH response were evaluated in 44 varieties (Table 1).

Evaluation of the response to BPH in rice plants

The bulked seedling test was used to evaluate the response to BPH in rice plants as described previously (Athwal et al., 1971). Briefly, the rice seeds were presoaked and sown in rows in plastic seed boxes of (40 × 60 × 10) cm. Forty progeny lines were subsequently placed in a box, along with the parental cultivars Cheongcheongbyeo and Hwayeongbyeo as resistant and susceptible controls, respectively. When the seedlings reached the three or four leaf stage, they were infected with 2nd- to 3rd-instar nymphs of BPH biotype I, which had been reared and maintained at the National Institute of Crop Science RDA, at a density of 10 nymphs per seedling. The responses of the seedlings to BPH were recorded when seedlings of the susceptible controls had nearly wilted and died out. The responses of the progeny lines were classified as resistant, segregating or susceptible. This bioassay was conducted three times.

Fine mapping, and development of STS markers for MAS

To develop new markers that are tightly linked to the *Bph1* locus, RAPD analysis was performed using 656 random 10-mers obtained from Operon Technologies Inc. (Alameda, USA). A bulked segregation analysis (BSA) was then performed using two genomic DNA bulks from seven susceptible and seven resistant F8 progeny, respectively. Selected RAPD markers that showed clear segregation between resistant and susceptible DNA bulks were converted to sequence characterized amplified region (SCAR) markers (Paran and Michelmore, 1993) by sequencing the products of these markers.

We then searched for BAC clones containing these SCAR markers and the previously selected *Bph1*-linked RFLP markers, *RG413* and *RG901* (Cha et al., 1999), by hybridization to Nipponbare BAC library filters. Using the BAC contig information from TIGR Rice Genome Annotation-release 5, 10 BAC

Table 1. The 44 rice varieties used to test the efficacy of the *Bph1*-linked STS markers developed in this study for marker-aided selection (MAS).

Variety[a]	BPH resistance gene[b]	Donors	Response to BPH[c]
Mudgo	*Bph1*	Original donor	R
TKM6	*Bph1*	Original donor	S
CO22 (IT 000588)	*Bph1*	Original donor	R
ASD7	*bph2*	Original donor	R
Milyang30	*Bph1*	Mudgo	R
Milyang34 (IT 006216)	*Bph1*	Mudgo	R
Nampungbyeo	*Bph1*	Mudgo	R
Chilseongbyeo	*Bph1*	Mudgo	R
Andabyeo	*Bph1*	Mudgo	R
Kanto PL4 (IT173362)	*Bph1*	Mudgo	R
Cheongcheongbyeo	*Bph1**	Mudgo	R
Changsongbyeo	*Bph1**	Mudgo	R
Baekunchalbyeo	*Bph1*	TKM6	R
IR26 (IT001886)	*Bph1*	TKM6	R
IR28 (IT001892)	*Bph1*	TKM6	R
IR29 (IT001893)	*Bph1*	TKM6	R
IR30 (IT001899)	*Bph1*	TKM6	R
Hangangchalbyeo	*Bph1**	TKM6	R
Yeongpungbyeo	*Bph1**	TKM6	R
Namyeongbyeo	*Bph1*	Mudgo & TKM6	R
Gayabyeo	*Bph1**	Mudgo & TKM6	R
Samgangbyeo	*Bph1**	Mudgo & TKM6	R
Namcheonbyeo	*Bph1*	Mudgo & TKM6	S
Hwacheongbyeo	*bph2*	IR1154-243	S
Susceptible varieties	Hwayeongbyeo, Nagdongbyeo, Keumho2, Dasanbyeo, Milyang23, Yongmunbyeo, Yongjubyeo, Jungwonbyeo, Taebeakbyeo, Pungsanbyeo, IR71190, Suwon452, Nipponbare, IR8, IR20, IR24, Tongil, Milyang23, Ilpumbyeo, Dongjinbyeo.		

[a]Figures in parentheses are the accession numbers of the rice varieties distributed by the National Genebank of Rural Development Administration, Korea.
[b]Varieties highlighted by an asterisk contain part of the genetic background of the wild rice *O. nivara*.
[c]The responses to BPH were surveyed using brown planthopper biotype I. 'R' and 'S' indicate resistance and susceptibility, respectively.

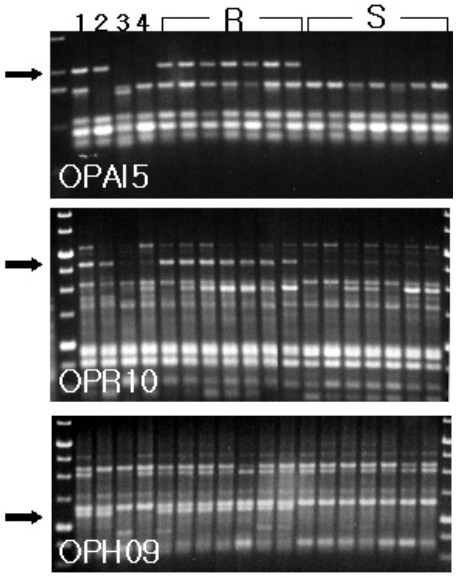

Fig. 1. RAPD analysis and the development of *Bph1*-linked markers (*OPH09, OPH10, OPH15*). Arrows indicate specific bands in resistant plants. Lane 1, DNA pooled from seven resistant lines; lane 2, Mudgo (resistant var.); lane 3, Nagdongbyeo (susceptible var.); lane 4, DNA pooled from seven susceptible lines. R, resistant lines; S, susceptible lines.

clones evenly distributed over the region containing the SCAR markers along with *RG413* and *RG901* were eventually selected. The end fragments of these selected BAC clones were isolated using the vectorette-PCR method and used in subsequent RFLP analysis. For RFLP analysis, genomic DNA was prepared from leaf tissue, and digested with a number of restriction enzymes (*Xba*I, *Sca*I, *Dra*I, *Hin*dIII, *Eco*RI, *Bam*HI, and *Eco*RV) followed by electrophoresis and hybridization according to the method of Causse et al. (1994). Based upon the sequences of the BAC clones that were found to harbor the *Bph1* gene in the above-mentioned fine mapping experiment, STS markers were designed to develop PCR-based selection markers for MAS. The primer pairs for the STS markers consisted of more than 20 nucleotides, and the PCR annealing step was performed above 60°C.

Linkage analysis was carried out using Mapmaker V2.0 for the Macintosh, with a greater than 2.0 LOD score and a recombination fraction θ equal to 0.4 (Lander et al., 1987). Map distances were calculated using the Kosambi function (Kosambi, 1944).

RESULTS

Fine mapping of *Bph1*

By RAPD analysis using 656 random 10-mer primers, we identified three markers (*OPH09*, *OPA10* and *OPA15*) linked to *Bph1* and converted them to SCAR markers (Fig. 1). Based upon information from TIGR Rice Annotation release 5, *OPH09* was found to be in BAC clone OSJNBa0011B18, and both *OPA10* and *OPA15* in BAC clone OSJNBa0040E10 on chromosome 12. Based upon the BAC clone contig information also in TIGR Rice Annotation release 5, we selected 10 BAC clones that were evenly distributed over the region containing the SCAR markers, along with *RG413* and *RG901*. These were OSJNBa0025J01, OSJNBa0022K16, OSJNBb0092G12, OSJNBa0027H05, OSJNBa0028L05, OSJNBb0076G11, OSJNBa0043F23, OSJNBa0011B18, and OSJNBa0022B07.

We isolated the end fragments of these BAC clones by vectorette PCR, and sequenced the resulting PCR products. These BAC end sequences were then used to develop 11 STS markers. Using these STS markers in conjunction with the previously identified *Bph1*-linked RFLP markers in a linkage analysis of an F8 RIL population from a cross between Hwayeongbyeo and Cheongcheongbyeo, a fine map of the *Bph1* region was constructed (Fig. 2). The *Bph1* gene was found to be located between *b92G12P1* and *a28L05p2* at distances of 1.1 cM and 0.8 cM, respectively. *Bph1* was thus most likely to be on BAC clone OSJNBa0040P10 or OSJNba0028L05, which are located between these flanking markers. Based on the sequences of these two BAC clones obtained from TIGR Rice Annotation release 5, we subsequently designed and mapped five markers (*pBPH5*, *pBPH 4*, *pBPH 9*, *pBPH 14* and *pBPH 7*). The *Bph1* region was then narrowed down to a 1.0 cM region (120 kB) between the *pBPH4* and *pBPH14* markers, and cosegregated with *pBPH9* (Fig. 2).

Applicability of the *Bph1* STS markers to MAS

We tested the applicability of the *pBPH9* maker to MAS in two other rice populations. These were 32 F6 progeny from a cross between Andabyeo (a resistant variety harboring *Bph1*) and IR71190 (susceptible), and 32 F5 progeny from a cross between Andabyeo and Suwon452 (susceptible). Our results showed that *pBPH9* could indeed discriminate between resistant and susceptible lines (Fig. 3). In the vicinity of *pBPH9* on BAC clone OSJNBa0040P10, we designed and tested three more markers (*pBPH19-21*) in MAS with the same Andabyeo/IR71190 and Andabyeo/Suwon452 populations (Table 2). These markers also showed the same ability to discriminate between BPH resistant and susceptible groups (Fig. 5).

The *pBPH9* marker was then further tested on 44 varieties. It amplified two distinct PCR products: a 536 bp fragment in varieties harboring *Bph1* such as Mudgo, TKM6, Samgangbyeo, and a 773 bp fragment in varieties without *Bph1* (Fig. 4). Only one rice variety, Namyeongbyeo, gave results that differed from expectation. Namyeongbyeo has been shown previously to have strong resistance to all of the BPH biotypes (I, II, and III) and to be derived from resistant ancestors Mudgo and TKM6 with *Bph1*, and IR4-93-2 with *bph2* (Sohn et al., 1987). This variety also showed strong resistance in our current bioassay, but a susceptible band pattern was observed with the *pBPH9* marker. It is noteworthy, however, that the genetics of the BPH resistance in Namyeongbyeo had not yet been described, and the gene underlying this resistance remains unclear. Hence, given that the response spectrum to the BPH biotypes of Namyeongbyeo differs from that of Mudgo, Namyeongbyeo may contain the *bph2* gene, not the *Bph1* gene.

Segregation distortion of markers in the *Bph1* gene region

In our F8 RIL lines generated from a cross between Cheongcheongbyeo and Hwayeongbyeo, the response to BPH segregated as 235:107 resistant:susceptible. This ratio deviates from the 1 RR: 1 SS ratio expected for a single dominant gene model ($x^2 = 47.91$) (Table 3). In fact all the markers used in our current fine mapping study showed similar deviations, with segregation ratios close to 2 RR: 1 SS (Table 3). This indicates that segregation distortion occurs over the *Bph1* gene region. The frequency of the *indica* type allele was also observed to be about two-fold higher than its *japonica* type counterpart.

Table 2. Primer sequences, and sizes of the PCR product of the STS markers that co-segregate with *Bph1*

Marker	Primer sequence	No of bases (bp)	Size of PCR product[a]	
			Resistant	Susceptible
pBPH9F	5′-AGCGCTGGTCGTTGGGGTTGTAGT	24	536	773
pBPH9R	5′-ATTAAAAGTGATCGCAGCCGTTCG	24		
pBPH19F	5′-GGGGTCGCCGAGGTCGTTGTAGA	23	610	587
pBPH19R	5′-TGGCTGAAGCTGCATGGGAGTTGG	24		
pBPH20F	5′-GGCTGCCTTATCCCCAACTCC	21	535	676
pBPH20R	5′-GTCGGCGCCGTGCAGGTCGTG	21		
pBPH21F	5′-GCTCCGTGTGCATCCCCTCTGTAG	24	885	734
pBPH21R	5′-GACTGGCTTTTCCTTGATTTCTT	23		

[a]PCR product sizes were measured in Mudgo as the resistant variety and Nagdongbyeo as the susceptible variety.

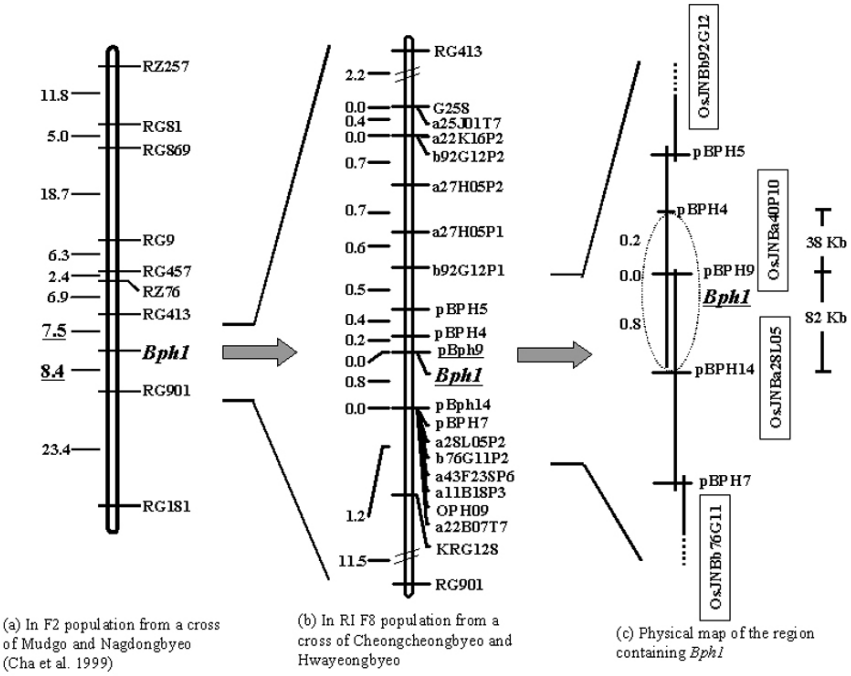

Fig. 2. A comparison between linkage maps and a physical map of *Bph1* on rice chromosome 12. The maps shown were generated using an F2 population from a cross between Nagdongbyeo and Mudgo (Cha et al., 1999) and an F8 RIL population from a cross between Hwayeongbyeo and Cheongcheongbyeo. In the physical map, the names inside the boxes represent BAC clones consisting of a BAC contigs that includes *Bph1*. The numbers indicate the genetic distances (cM, left) and physical distances (Kbp, right) between the markers. The marker order in map (b) was decided from linkage analysis using Mapmaker V2.0, with a greater than 2.0 LOD score. The dotted circle indicates the predicted region containing the actual *Bph1* gene.

DISCUSSION

We employed an F8 RIL population generated from a cross between Cheongcheongbyeo and Hwayeongbyeo for fine mapping of the *Bph1* gene. To our knowledge, previous examples of the mapping of BPH resistance genes using highly advanced inbred lines such as these are rare. Because the lines in our mapping populations were almost fixed, we evaluated the response of each line in three individual experiments, with 10 individual plants tested in each. By performing repetitive evaluations in this way, phenotyping errors are likely to have been greatly reduced.

It has been reported that rice cultivars known to be resistant to BPH show a susceptibility ratio of 1-2% (Athwal et al., 1971). In addition, the response of rice plants to BPH is often variable, being affected by such factors as insect density, age or status of the plants infected, and the resistant variety type (Kaneda et al., 1981; Kim et al., 1989; Nemoto et al., 1989; Yeo and Sohn, 1995). In addition, responses to BPH infection may be affected by other biotic or abiotic stress conditions and incomplete penetrance of the resistance gene. However, the use of a RIL mapping population had the distinct advantage of increasing the precision with which we could evaluate the response of rice plants to BPH in spite of the plasticity of this trait.

Several recent studies have been conducted in an attempt to narrow down the location of *Bph1*. Recently, this gene was reported to be located at a distance of 2.7 cM from two AFLP markers, *em5814N* and *em2802N* (Sharma et al., 2002). However, this distance is too great to enable the isolation *Bph1* or to screen accurately for plants harboring this gene using these markers. In the current experiments we found that *Bph1* was 0.2 cM and 0.8 cM from the STS markers *pBPH4* and *pBPH14*, respectively, and was completely linked to *pBPH9*. Each of these STS markers is contained in BAC clone OSJNBa0040P10 within a genomic region 78.9 cM from the top of chromosome 12. The interval between *pBPH4* and *pBPH14* was found to be about 120 kb, and contains 15 genes according to TIGR Rice Genome Annotation-release 5. Expression

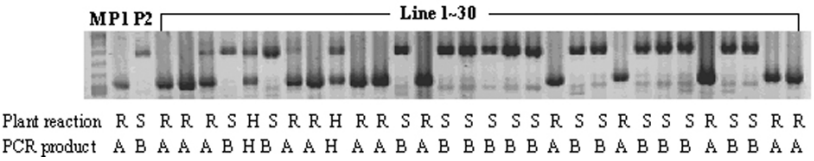

Fig. 3. Segregation pattern of *pBPH9* in F6 RIL lines derived from a cross between Andabyeo and IR71190. R, resistant to BPH; S, susceptible to BPH; H, segregating.

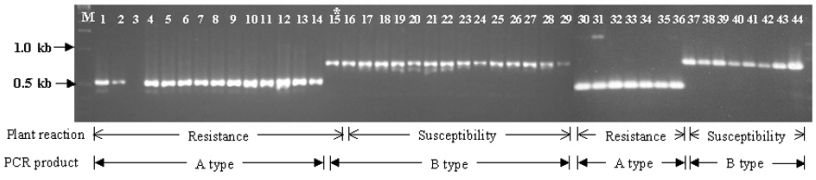

Fig. 4. PCR products amplified using the *pBPH9* marker that is specific for *Bph1*. The numbers above each lane denote 22 BPH resistant varieties of rice as follows: 1, Mudgo; 2, TKM6; 3, ASD7; 4, Cheongcheongbyeo; 5, Nampungbyeo; 6, Yeongpungbyeo; 7, Milyang30; 8, Hangangchalbyeo; 9, Chilseongbyeo; 10, Samgangbyeo; 11, Changsongbyeo; 12, Gayabyeo; 13, Baekunchalbyeo; 14, Andabyeo; 15, Namyeongbyeo; 30, IR26; 31, IR28; 32, IR29; 33, IR30; 34, CO22; 35, Kanto PL4; and 36, Milyang34; and 22 susceptible varieties 16, Hwacheongbyeo; 17, Hwayeongbyeo; 18, Nagdongbyeo; 19, Keumho2; 20, Namcheonbyeo; 21, Dasanbyeo; 22, Milyang23; 23, Yongmunbyeo; 24, Yongjubyeo; 25, Jungwonbyeo; 26, Taebeakbyeo; 27, Pungsanbyeo; 28, IR71190; 29, Suwon452; 37, Nipponbare; 38, IR8; 39, IR20; 40, IR24; 41, Tongil; 42, Milyang23; 43, Ilpumbyeo; and 44, Dongjinbyeo. M, DNA size marker. PCR products were classified into 'A' type (resistant, 536 bp) and 'B' type (susceptible, 773 bp). (*) The Namyeongbyeo variety shows a discrepancy between its BPH response and PCR pattern.

Table 3. Segregation of *Bph1* and the indicated linked markers in the Hwayeongbyeo/Cheongcheongbyeo F8 RIL population

Marker	Number of genotypes[a]			x^2 value[b]	p[c]
	RR	SS	Total		
RG413	109	51	160	21.03	$p < 0.01$
G258	110	50	160	22.50	$p < 0.01$
a27H05p1	110	52	162	51.20	$p < 0.01$
b92G12p1	225	111	336	44.23	$p < 0.01$
PBPH5	229	113	342	39.35	$p < 0.01$
PBPH9	219	102	321	42.64	$p < 0.01$
Bph1	235	107	342	47.91	$p < 0.01$
pBPH14	233	104	337	49.38	$p < 0.01$
OPH09	110	47	157	25.28	$p < 0.01$
a22B07T7	111	47	158	25.92	$p < 0.01$
KRG128	109	52	161	20.18	$p < 0.01$
RG901	127	42	169	42.75	$p < 0.01$

[a]RR and SS represent the genotypes of Cheongcheongbyeo and Hwayeongbyeo, respectively.
[b]x^2 values based on a ratio of 1 (RR) :1 (SS)
[c]With one degree of freedom (*df*), the x^2 value at the 1% significance level is 6.635

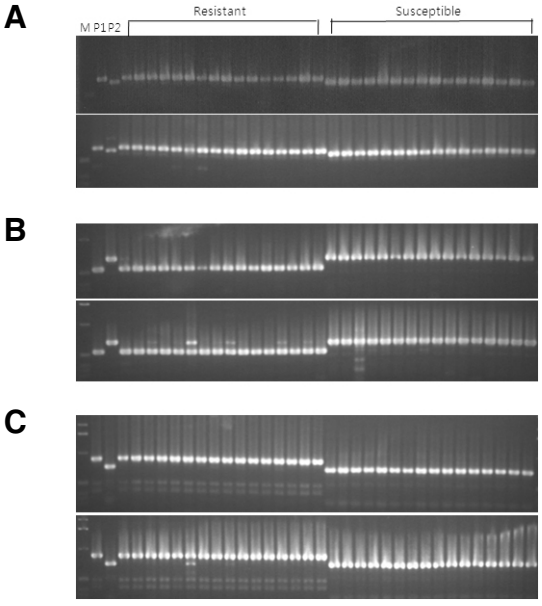

Fig. 5. PCR profiles of resistant and susceptible plants in 32 F6 RILs from a cross between Andabyeo and IR71190 (upper photo), and 32 F5 RILs from a cross between Andabyeo and Suwon452 (lower photo) generated using *Bph1* selection markers, *pBPH19*(A), *pBPH20*(B), and *pBPH21*(C). Lane M, 1 kb ladder; lane P1, parent 1 (Andabyeo); lane P2, parent 2 (IR71190 in upper photo and Suwon452 in lower photo).

and transformation experiments to precisely identify *Bph1* are now underway in our laboratory using this information.

The four STS markers that we developed, *pBPH9*, *pBPH19*, *pBPH20*, and *pBPH21*, were found to be completely linked to *Bph1* in our mapping population derived from a Cheongcheongbyeo/Hwayeongbyeo cross, and could discriminate between resistant and susceptible plants from two further rice populations with precision. In addition, *pBPH9* was successfully used to accurately classify 44 rice varieties according to the presence of *Bph1* with only one exception, Namyeongbyeo. However, whether BPH resistance in Namyeongbyeo is in fact due to *Bph1* remains unclear. These markers thus proved to be very precise, convenient and reliable tools for marker-aided selection. Selection by bioassay during the breeding of BPH resistant rice cultivars is both tedious and time-consuming as it needs requires continuous rearing of insects, and the BPH resistancet trait is highly variable under different environment conditions. The use of MAS with our STS markers could greatly reduce both the cost and time required to breed varieties of rice harboring *Bph1*.

We found in our present analysies of a RIL population generated from a cross between Cheongcheongbyeo and Hwayeongbyeo that segregation distortion occurs in the *Bph1* gene region.

All of the markers tested in this region showed segregation distortion from *RG413* to *RG901* (19.2 cM), and the frequency of the Cheongcheongbyeo (*indica*) allele was about two-fold higher than that of the Hwayeongbyeo (*japonica*) allele. This had been also been observed previously in a Nakdongbyeo/Mudgo F2 population (Cha et al., 1999). Moreover, segregation distortion has also been reported in a similar genomic region in rice in studies of a Zhai-Ye-Qing 8 (*indica*)/Jing-Xi 17 (*japonica*) F2 population (Xu et al., 1997). A gametophyte gene, *ga-13*, which is the likely cause of this effect, was found to be located in this region. It has been postulated that *japonica* type alleles that are desirable for rice quality and plant stature are tightly linked to *Bph1*. This is known as 'linkage drag' and has hindered the breeding of *japonica* type BPH resistant varieties. Considering the segregation distortion toward *indica* type alleles in this region, the frequency of recombinants harboring the *Bph1* gene from *indica* with a *japonica* background would be very low. The use of MAS, however, should enable the selection of desirable recombinants at an earlier generation, and thus overcome this obstacle.

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