



## Development of elite breeding lines conferring *Bph18* gene-derived resistance to brown planthopper (BPH) by marker-assisted selection and genome-wide background analysis in japonica rice (*Oryza sativa* L.)

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### ABSTRACT

Brown planthopper (BPH) is a serious threat to rice production. In this study, we have used the novel resistance gene *Bph18* derived from *Oryza australiensis* and incorporated it into an elite japonica cultivar, Junambyeo, which is highly susceptible to BPH. The *Bph18* gene was introduced by marker-assisted backcross (MAB) breeding into Junambyeo. The backcrossed progenies were evaluated for desirable agronomic and grain quality traits and the selection of improved breeding lines while simultaneously evaluating BPH resistance by bioassays in the greenhouse and foreground selection. Of the 26 advanced backcross breeding lines (ABL), four lines showed agronomic traits similar to those of the recurrent parent, with strong resistance to BPH. Molecular genotyping of the four ABL revealed the conversion of genotypes closely resembling the genotype of Junambyeo. The percentage of donor chromosome segments in ABL decreased from 12.3% in the BC<sub>2</sub> to 9.4%, 8.4% and 5.3% in BC<sub>3</sub>, BC<sub>4</sub> and BC<sub>5</sub> generations, respectively. ABL retained small sizes of the donor chromosome segments on chromosomes 1, 2, 10, 11 and 12 but the genomes of ABL2, ABL3 and ABL4 were homosequential to the recurrent parent on chromosomes 3, 4, 5, 6, 7 and 9 without donor chromosome segment introgression. The ABL1 and ABL2 retained only some small segments of the donor genome on chromosomes 9 and 8, respectively. Fine structure analysis of the *Bph18* flanking region between RM511 and RM1584 markers on chromosome 12 showed a progressive elimination of donor-derived chromosome segments from BC<sub>2</sub> to BC<sub>5</sub> generations. The percentage of *O. australiensis* derived chromosome segment substitution in the recurrent parent background decreased from 28% of the donor parent to 6.7%, 3.9%, 3.4% and 3.4% in BC<sub>2</sub>, BC<sub>3</sub>, BC<sub>4</sub> and BC<sub>5</sub> generations, respectively. However, it was revealed that the *O. australiensis*-derived chromosome segment (1320 kb) in ABL containing the *Bph18* gene was consistently maintained irrespective of advances in backcross generations. BPH resistant elite breeding lines with agronomic and grain quality traits similar to those of the recurrent parent were successfully developed by foreground and background analysis in japonica background without linkage drag.

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### 1. Introduction

Two subspecies of rice (*Oryza sativa* L.), indica and japonica, are the primary sources of food for more than half of the world population. A rapid rise in world population and decreasing agricultural land exert pressure on the production and productivity of rice cultivars (Khush, 2005). However, in recent years, modern

cultivars have become vulnerable to several biotic stresses, mainly brown planthopper (BPH), because of changes in BPH biotypes. BPH (*Nilaparvata lugens* Stal.) is one of the most destructive phloem-sap-feeding insect pests of rice. It damages rice crops every year and the most serious cases of BPH infestations cause hopper burn in rice fields and eventually cause complete yield loss in many Asian countries (Jena et al., 2006; Bui Chi Buu (Viet Nam), personal communication). Host-plant resistance is the most effective breeding strategy to control BPH damage in contrast to the environmentally inimical use of pesticides.

To date, 21 major genes for BPH resistance have been identified and the sources of resistance to BPH come mostly from indica rice cultivars/land races as well as from the wild species *O. australiensis*, *O. officinalis* and *O. minuta* (Ishii et al., 1994; Yang et al.,

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E-mail address: [k.jena@cgiar.org](mailto:k.jena@cgiar.org) (K.K. Jena).

2004; Jena et al., 2006; Rahman et al., 2009). Ten of these genes (*Bph1*, *bph2*, *Bph3*, *Bph9*, *Bph14*, *Bph15*, *Bph18*, *bph19*, *Bph20* and *Bph21*) have been fine-mapped with DNA markers that provide opportunities for marker-assisted selection (MAS) for BPH resistance in rice (Hirabayashi and Ogawa, 1995; Sharma et al., 2003, 2004; Chen et al., 2006; Jena et al., 2006; Zhang, 2007; Jairin et al., 2007; Rahman et al., 2009).

Of the 21 genes for BPH resistance, *Bph1*, *bph2*, *Bph3* and *bph4* have been used in cultivar development by conventional breeding and a number of BPH-resistant indica rice cultivars have been developed (Khush and Brar, 1991). The source of BPH resistance genes in temperate japonica rice germplasm is very limited because of narrow genetic diversity. The transfer of a BPH resistance gene from indica rice cultivars into japonica cultivars by conventional breeding methods often difficult due to high sterility of the progenies, poor plant type, and linkage drag (Jeung et al., 2005).

Molecular markers provide opportunities to map resistance genes and accelerate the application of marker-assisted backcross (MAB) breeding through the precise transfer of target genomic regions into the recurrent parent (Jena and Mackill, 2008; Lewis and Kernodle, 2009). Backcross breeding is often used in a conventional breeding program to transfer specific genes into elite cultivars (Allard, 1960). The basis of MAB breeding is to transfer a specific gene/allele of the donor parent into the recurrent parent genome while selecting against donor introgressions across the rest of the genome. The effectiveness of MAB breeding depends on the availability of closely linked DNA markers for the target locus, the size of the population, the number of backcrosses and the position and number of markers for background selection (Frisch and Melchinger, 2005). MAB breeding provides a new opportunity for the selective transfer of BPH resistance genes into elite indica rice cultivars devoid of linkage drag, eventually restoring the recurrent parent genotype (Jairin et al., 2009). In addition, molecular markers precisely estimate the introgression of chromosome segments from donor parents and can speed up the recipient genome recovery via background selection (Neeraja et al., 2007; Jairin et al., 2009; Suh et al., 2009). Sharma et al. (2004) performed a molecular marker-assisted pyramiding of two BPH-resistance genes, *Bph1* and *bph2*, into a japonica line. Jairin et al. (2009) successfully dissected the linkage drag between the *Bph3* and *Wx<sup>a</sup>* allele and introgressed the BPH resistance gene into a cultivated rice genetic background using marker-assisted selection (MAS).

The *Bph18* gene was originally identified in an indica introgression line, IR65482-7-216-1-2, and it expresses strong resistance to

the BPH biotype of Korea. The STS marker 7312.T4A linked to the *Bph18* gene has been developed by using bacterial artificial chromosome (BAC)-clone derived sequence information of a putative BPH resistance gene (Jena et al., 2006). Here, we report on the development of elite breeding lines by introducing the *Bph18* gene into a susceptible japonica cultivar, Junambyeo, using the MAB breeding strategy. The resistance of ABL against the BPH population of Korea was compared with that of the parental lines and background analysis of ABL using genome-wide marker analysis indicated the extent of recurrent parent genome conversion possessing desirable agronomic traits in a short period of time.

## 2. Materials and methods

### 2.1. Plant materials and development of ABL

The introgression line IR65482-7-216-1-2, the source of the *Bph18* gene, was used as the donor parent for BPH resistance and Junambyeo, a BPH-susceptible elite japonica cultivar with good grain quality, was used as the recipient parent. Two Tongil-type cultivars, Taebaekbyeo and Andabyeo, were used as susceptible and resistant checks, respectively, in both seedling and adult stages of BPH screening. Advanced backcross breeding lines (ABL) in a japonica genetic background were developed by the marker-assisted backcross (MAB) breeding strategy (Fig. 1). Four ABL with high yield potential, selected one from each of the BC<sub>2</sub>, BC<sub>3</sub>, BC<sub>4</sub> and BC<sub>5</sub> generations, were used to determine the recurrent parent genetic background and were evaluated for agronomic performance in the field. Pure seeds of IR65482-7-216-1-2 and *O. australiensis* (acc. no. 100882) were obtained from the Plant Breeding, Genetics, and Biotechnology Division of the International Rice Research Institute (IRRI), Los Baños, Philippines. Seeds of Junambyeo, Taebaekbyeo and Andabyeo were obtained from the Rice Research Division of the National Institute of Crop Science (NICS), Rural Development Administration (RDA), Republic of Korea.

### 2.2. Bioassays for BPH resistance in seedlings and adult plants

A BPH bioassay was done using the modified bulk seedling test in the greenhouse following the method of Jena et al. (2006). Seeds of BC progenies along with resistant (R) and susceptible (S) checks were planted into randomly selected rows in seed boxes, and seedlings at the three-leaf stage were infested with 2nd- or 3rd-instar nymphs at a density of 10–12 nymphs per seedling. For adult

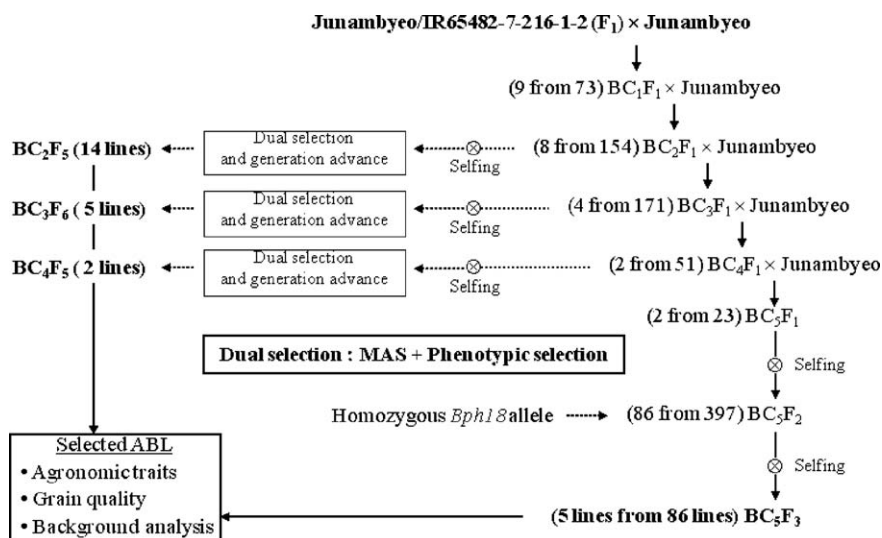


Fig. 1. Scheme for the development of *Bph18* gene-derived advanced backcross breeding lines using marker-assisted foreground and background selection.

plant screening, two seedlings at the three-leaf stage of selected ABL of BC<sub>2</sub>, BC<sub>3</sub>, BC<sub>4</sub> and BC<sub>5</sub> generations and check cultivars were transplanted into plastic pots containing pulverized soil with commercial fertilizer (9.0–4.5–5.7, N–P<sub>2</sub>O<sub>5</sub>–K<sub>2</sub>O) in two replications. Adult rice plants in three sets at different growth stages (maximum tillering, booting and flowering) were infested with 2nd- or 3rd-instar nymphs at a density of 200 nymphs per plant. The progenies were evaluated based on the degree of susceptibility of the susceptible check and the progenies were rated as resistant and susceptible once the S check was dead.

### 2.3. Evaluation of agronomic and grain quality traits

The parents and ABL were planted in a four-row plot with 35 plants per row by 30 × 15-cm spacing in a randomized complete block design with three replications and evaluated for agronomic traits in the rice experimental plot of NICS, Suwon, Korea, using the standard evaluation method of rice (RDA, 2003). For each line, five plants in the middle rows were used to determine days to heading (DTH), culm length (CL), panicle number (PN), panicle length (PL), number of grains per panicle (NGP), fertility of spikelets (FER), 1000-grain weight of the brown rice (GW), ratio of seed length/width (L/W) and grain yield (GY; t/ha). Grain yield per plot was evaluated based on a grain harvest of 50 plants in the central row of each plot. Grain quality was estimated for alkali digestion value (ADV), amylose content of milled rice (AC), protein content of brown rice (PC) and chalkiness of brown rice (CK; 0: non-chalkiness, 3: high chalkiness). ADV was evaluated based on the procedure of Little et al. (1958). AC was determined by the relative absorbency of starch-iodine color in a digested solution of 100-mesh rice flour by Juliano's (1971) modified method. PC was calculated by total nitrogen multiplied by 5.95 after determining the nitrogen content of rice material using the Micro-Kjeldahl method (Foss: 2300 Kjeltac Analyzer).

### 2.4. DNA marker analysis for *Bph18* gene validation

The most tightly linked co-dominant STS marker, 7312.T4A with primer sequences F: 5' ACGCGGTGAGCATTGG 3' and R: 5' TACAGCGAAAAGCATAAAGAGTC 3', was used to detect the presence of the *Bph18* gene in backcross-derived BPH-resistant breeding lines (Jena et al., 2006). Polymerase chain reaction (PCR) was performed in a Bio-Rad PTC-200 Thermocycler, Germany, with a total volume of 20 µl containing 20 ng of template DNA, 5 pmole of each primer, 1.5 mM of MgCl<sub>2</sub>, 0.2 mM of dNTP and 0.5 U of *Taq* polymerase. The PCR amplification condition was with one cycle of denaturation at 95 °C for 5 min, followed by 35 cycles at 95 °C for 30 s, 58 °C for 30 s and 72 °C for 1 min, with a final extension at 72 °C for 10 min. After determining the amplification success, the PCR product was used for restriction digestion. A total reaction volume of 15 µl with 1 µl restriction buffer, 0.5 µl (10 U/µl) of *Hinf*I restriction enzyme and 5 µl of PCR product were incubated for 3 h at 37 °C. The restriction digestion of PCR products was detected using 1.5% agarose gel electrophoresis with ethidium bromide staining.

### 2.5. Molecular analysis of genetic background of ABL

Some 260 SSR markers of known chromosomal positions distributed evenly on the 12 chromosomes with an average interval of 5.9 cM were used in a genome-wide survey to identify the chromosome segment substitution locations in the four ABL compared with the donor line. The SSR markers polymorphic between the two parents were used for background genotyping to recover the recipient parent genome. The four ABL and the donor parent (IR65482-7-216-1-2) were also analyzed to detect the introgression

of chromosome segments from the wild species *O. australiensis* (acc. no. 100882) by using the polymorphism information between the progenitors (IR31917-45-3-2 and *O. australiensis*). The lengths of substituted chromosome segments in ABL were estimated based on the graphical genotyping procedure of Xi et al. (2006). A chromosome segment of ABL flanked by homozygous marker alleles of the donor parent was considered as a 100% donor type, but a chromosome segment of ABL flanked by homozygous marker alleles of the recipient parent was considered as a 0% donor type, and a chromosome segment flanked by one marker allele of the donor parent and another marker allele of the recipient parent was considered as a 50% donor type. SSR analysis was conducted following the procedure of McCouch et al. (2002). The PCR products were detected using 4% denaturing polyacrylamide gel electrophoresis with silver staining.

### 2.6. Data analysis

The linkage and orientation of SSR markers on chromosomes were assigned following the SSR map constructed by McCouch et al. (2002) and as depicted in Gramene ([www.gramene.org/](http://www.gramene.org/)). MapChart 2.0 was used for the graphical presentation of the genetic linkage map between SSR markers (Voorrips, 2002). The least significant difference (LSD) and Duncan's multiple range test (DMRT) were used for multiple mean comparisons using the SAS statistical software package (version 8.2; SAS Institute, Cary, NC).

## 3. Results

### 3.1. Development of advanced backcross breeding lines with the *Bph18* gene

F<sub>1</sub> progenies were produced from a cross between the BPH-susceptible cultivar Junambyeo and IR65482-7-216-1-2 (donor parent of BPH resistance gene *Bph18*) using MAB breeding scheme (Fig. 1). BC<sub>1</sub> progenies were obtained by backcrossing the F<sub>1</sub> plants with Junambyeo as the recurrent parent. In the BC<sub>1</sub> generation, individual plants heterozygous at the *Bph18* locus were identified and used for further backcrossing with the recurrent parent. The advanced backcross progenies of BC<sub>2</sub>, BC<sub>3</sub>, BC<sub>4</sub> and BC<sub>5</sub> were obtained from the crosses of selected resistant BC<sub>1</sub>F<sub>1</sub> (9 plants from 73 plants), BC<sub>2</sub>F<sub>1</sub> (8 plants from 154 plants), BC<sub>3</sub>F<sub>1</sub> (4 plants from 171 plants), BC<sub>4</sub>F<sub>1</sub> (2 plants from 51 plants) and BC<sub>5</sub>F<sub>1</sub> (2 plants from 23 plants) plants based on the dual-selection procedure of BPH-resistant phenotype and foreground selection using the *Bph18*-gene-specific DNA marker (Fig. 1). Progenies of each BC generation were advanced by selection and selfing, and promising BPH-resistant breeding lines were developed. Finally, ABL of BC<sub>2</sub>F<sub>5</sub> (14 lines), BC<sub>3</sub>F<sub>6</sub> (5 lines), BC<sub>4</sub>F<sub>5</sub> (2 lines) and BC<sub>5</sub>F<sub>3</sub> (5 lines) were selected as promising lines having BPH resistance with homozygous marker alleles of the *Bph18* gene and desirable agronomic traits (Fig. 1 and Table 1).

### 3.2. Validation of *Bph18* and BPH resistance in ABL

BPH resistance conferred by the *Bph18* gene in ABL was validated by foreground selection using the STS marker 7312.T4A. Some 26 selected ABL showed a homozygous-resistant marker allele of IR65482-7-216-1-2, whereas ABL susceptible to BPH showed a homozygous-susceptible marker allele of Junambyeo (Fig. 2). A BPH bioassay of cultivar Junambyeo, IR65482-7-216-1-2 and ABL at the seedling and adult stages showed strong BPH resistance in IR65482-7-216-1-2 and the 26 selected ABL, but Junambyeo expressed high susceptibility to BPH similar to cultivar Taebaekbyeo (Table 2 and Fig. 3a and b).

**Table 1**

List of four advanced backcross breeding lines, check varieties and recurrent and donor parents used in this study.

Cultivar/breeding line	Description (generaton) <sup>a</sup>	Gene	Remarks
Junambyeo	Recurrent parent	None	Korean elite japonica variety
IR65482-7-216-1-2	Donor parent	<i>Bph18</i>	<i>O. australiensis</i> introgression line with <i>Bph18</i> gene
IR83261-3-7-15-4-3-1	ABL1 (BC <sub>2</sub> F <sub>5</sub> )	<i>Bph18</i>	Junambyeo genetic background
IR83261-1-1-18-3-3-3-1-2	ABL2 (BC <sub>3</sub> F <sub>6</sub> )	<i>Bph18</i>	Junambyeo genetic background
IR83261-3-7-15-7-11-2-10-2	ABL3 (BC <sub>4</sub> F <sub>5</sub> )	<i>Bph18</i>	Junambyeo genetic background
IR83261-1-1-18-3-3-11-1-5-1	ABL4 (BC <sub>5</sub> F <sub>3</sub> )	<i>Bph18</i>	Junambyeo genetic background
Andabyeo	Check	<i>Bph1</i>	Tongil-type variety for resistant check
Taebaekbyeo	Check	None	Tongil-type variety for susceptible check

<sup>a</sup> ABL: advanced backcross breeding lines having *Bph18* gene.**Table 2**

Reaction patterns of selected ABL to brown planthopper (BPH) at seedling and tillering stages.

Cultivar/line	Reaction patterns at different growth stages <sup>a</sup>				Gene
	Seedling 9 DAI	Tillering 14 DAI	Booting 14 DAI	Flowering 14 DAI	
Junambyeo	S	S	S	S	None
IR65482-7-216-1-2	R	R	R	R	<i>Bph18</i>
ABL 1	R	R	R	R	<i>Bph18</i>
ABL 2	R	R	R	R	<i>Bph18</i>
ABL 3	R	R	R	R	<i>Bph18</i>
ABL 4	R	R	R	R	<i>Bph18</i>
Andabyeo	MR	R	R	MR	<i>Bph1</i>
Taebaekbyeo	S	S	S	S	None

<sup>a</sup> DAI: days after infestation, R: resistant, MR: moderately resistant, S: susceptible.

### 3.3. SSR-based genetic background analysis of ABL

Four ABL along with the BPH-resistant donor line IR65482-7-216-1-2 and recurrent parent Junambyeo were used for background analysis. Some 260 SSR markers were used and a genetic linkage map covering a 1512.40-cM region of the *O. sativa* genome was constructed (Fig. 4). The marker polymorphism between Junambyeo and IR65482-7-216-1-2 was 84.4%. Each ABL contains some SSR marker-defined chromosome segments from the donor in the genetic background of the recurrent parent, Junambyeo. The percentage of donor parent chromosome substitution in ABL1 (BC<sub>2</sub>F<sub>5</sub>), ABL2 (BC<sub>3</sub>F<sub>6</sub>), ABL3 (BC<sub>4</sub>F<sub>5</sub>) and ABL4 (BC<sub>5</sub>F<sub>3</sub>) was 12.3, 9.4, 8.4 and 5.3, respectively (Table 3). Chromosomes 3 and 5 did not inherit any substituted chromosome segment from the donor parent in the four ABL. Interestingly, ABL2, ABL3 and ABL4 in BC<sub>3</sub>-BC<sub>5</sub> generations exhibited no introgression of donor chromosome segments on chromosomes 3, 4, 5, 6, 7 and 9. However, these lines commonly share the substituted chromosome segments of the donor parent not only on chromosome 12 around the *Bph18* gene locus but also on chromosome 10 in the Junambyeo genetic background (Table 3 and Fig. 4). The percentage of *O. australiensis* chromosome segment substitution in ABL1, ABL2, ABL3, ABL4 and the donor parent, IR65482-7-216-1-2, was 6.7, 3.9, 3.4, 3.4 and 28.3, respectively (Table 3). All four selected ABL inherited the chromo-

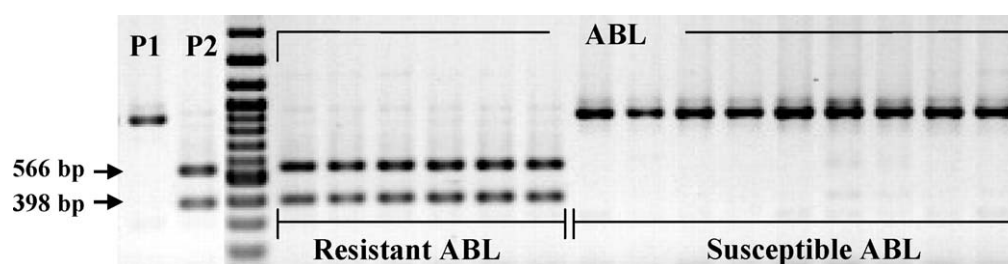
some segments of *O. australiensis* on some regions of chromosomes 10 and 12.

### 3.4. Fine structure analysis of the chromosome 12 region surrounding the *Bph18* locus

Twenty-one additional SSR markers were used to fill the gaps on chromosome 12 surrounding the *Bph18* gene locus in the four ABL (Fig. 5). Donor chromosome segments were comparatively reduced in BC<sub>5</sub>-derived ABL surrounding the *Bph18* gene. However, the ratio of percentage of introgressed donor segments did not show a significant difference among the ABL as evident from the result of 16 polymorphic SSR markers analyzed on chromosome 12. The four ABL showed a common substituted chromosomal segment size of 1320 kb from *O. australiensis* around the *Bph18* gene region (Fig. 5). We could not find any donor-derived segment outside the region between RM511 and RM1584 on chromosome 12.

### 3.5. Agronomic traits and grain quality performance of ABL

The results of agronomic traits of ABL evaluated in the field and laboratory showed that most of the morphological traits of ABL, including plant type and grain quality were similar to those of the recurrent parent, Junambyeo (Table 4). Traits such as days to head-



**Fig. 2.** PCR amplification of resistant and susceptible ABL from a cross between Junambyeo and IR65482-7-216-1-2 using the primer set of 7312.T4A as a complete R or S marker associated with the *Bph18* gene. PCR products were cleaved by *Hinf*I and gels were stained by ethidium bromide. P1: Junambyeo (recurrent parent), P2: IR65482-7-216-1-2 (donor parent), ABL: advanced backcross breeding lines.

ing, panicle number, grain yield, 1000-grain weight of brown rice, chalkiness, amylose content of milled rice, protein content of brown rice and alkali digestion value of the selected four ABL were almost the same as those of Junambyeo. However, the culm length of ABL1 and ABL4 was 4–5 cm more than that of Junambyeo. The panicle length of ABL4 was 2.6 cm longer than that of Junambyeo. The grain yield of ABL did not show a significant difference from Junambyeo even though the number of grains per panicle of some selected ABL was more than that of the recurrent parent. This may be due to a reduction in fertility of spikelets per se and gain in number of grains per panicle. All of the selected ABL were recovered with japonica grain characteristics of the recurrent parent with a non-chalky appearance and similar values for AC, PC, ADV and grain shape (short grain type) having homozygous alleles of the *Bph18* gene (Table 4).

**4. Discussion**

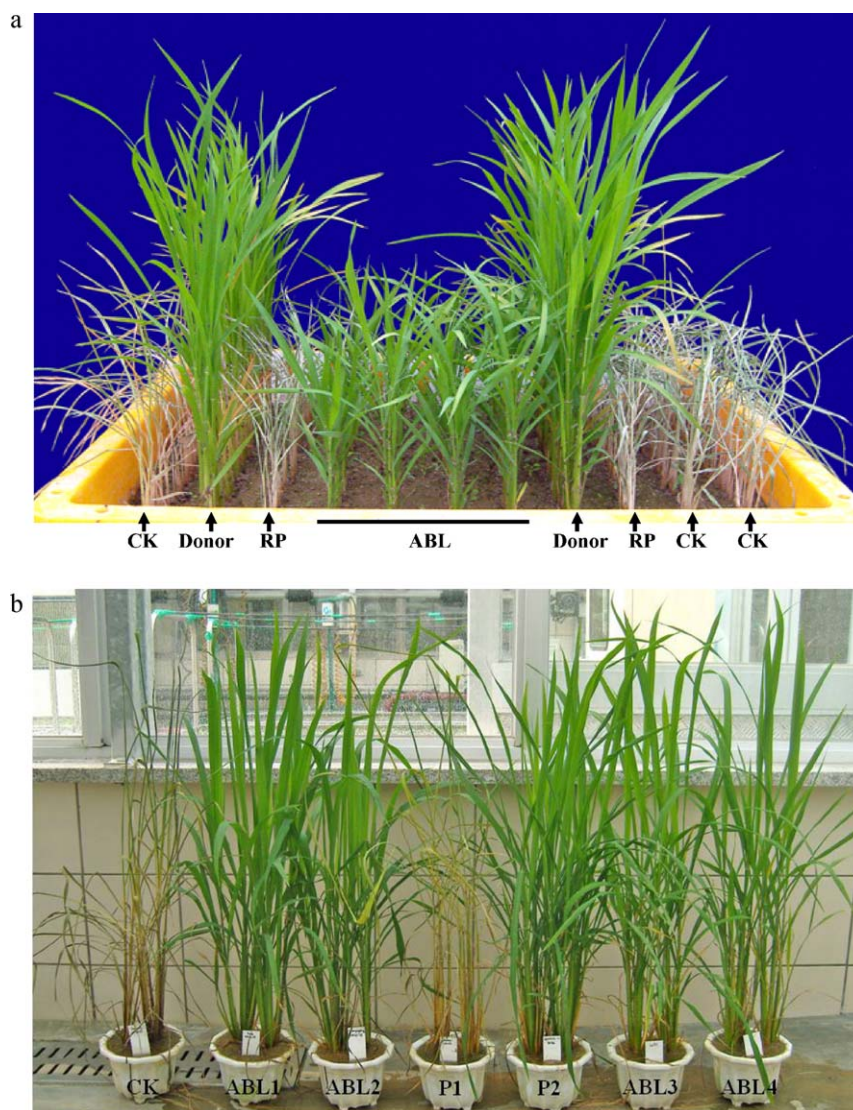
Most of the japonica cultivars grown in the temperate region are susceptible to BPH. Although BPH was considered as a minor insect pest in the region for decades, since 2005, BPH outbreaks have been occurring frequently (Jena et al., 2006). It is imperative to develop new BPH-resistant rice cultivars with high yield potential and grain quality using modern tools of biotechnology. However, it is often difficult to incorporate BPH resistance genes into a japonica genetic background from indica or exotic germplasm sources by conventional breeding methods due to the unexpected linkage drag encountered in the progenies, which affects yield and grain quality characteristics of rice cultivars (Jeung et al., 2005; Yeo and Shon, 2001). It is also challenging to achieve a definite goal of BPH resistance using conventional breeding strategies when the target gene is linked with an unfavorable dominant gene (Jairin et al., 2009). Nevertheless, using the tools of biotechnology, it is plausible to transfer valuable genes of resistance to biotic and abiotic stresses in rice without linkage drag (Mackill, 2007). Our study focuses on combining the useful agronomic traits of Junambyeo with *Bph18*-derived resistance in backcross breeding lines by conversion to the recurrent parent genotype using molecular genotyping with SSR markers. We successfully transferred the *Bph18* gene from donor indica line IR65482-7-216-1-2 into Junambyeo, an elite japonica cultivar, by MAS and several generations of backcrossing.

The *Bph18* gene was identified in an introgression line, IR65482-7-216-1-2, which inherited the gene from the wild species *O. australiensis* (Jena et al., 2006). IR65482-7-216-1-2 confers a broad spectrum of resistance against the BPH biotype of Korea, Japan, China and Viet Nam (data not shown). Nonetheless, it is difficult to transfer the *Bph18* gene into susceptible japonica cultivars from an indica genetic background without linkage drag using conventional breeding methods. Here, we used the MAB breeding method to integrate the *Bph18* gene into a japonica cultivar by phenotype and genotype selection. Using STS marker 7312.T4A derived from a putative resistance gene and the flanking SSR markers (RM463, RM3813) for the *Bph18* gene ensured efficient foreground selection of ABL (Jena et al., 2006). The co-dominant nature of these STS and SSR markers could be very useful in addition to gene-based markers for the introgression of the *Bph18* locus into a wide range of recipient elite cultivars. The selfed progenies or recombinant homozygote plants in the target region were selected from 353 to 586 BC<sub>2.5</sub>F<sub>2</sub> plants with phenotypic and foreground selection. The sizes of the segregating progenies were reduced by preselecting plants for resistance by BPH bioassays of the progenies before applying MAS, which is an efficient breeding approach that recovered the desirable phenotype of the recurrent parent and introduced the resistance gene.

**Table 3**  
Simple sequence repeat markers with polymorphism between recurrent parent and donor parent and substituted chromosome segments from donor parent in advanced backcross breeding lines of rice.

Chr. no. <sup>a</sup>	No. of markers	No. of PM <sup>b</sup>	% of P <sup>c</sup>	Interval (cM) <sup>d</sup>	Chr. length (cM) <sup>e</sup>	Chromosome segments of donor (%) <sup>f</sup>				Chromosome segments of <i>O. australiensis</i> (%) <sup>g</sup>				Donor
						ABL1 (BC <sub>2</sub> F <sub>5</sub> )	ABL2 (BC <sub>3</sub> F <sub>6</sub> )	ABL3 (BC <sub>4</sub> F <sub>5</sub> )	ABL4 (BC <sub>5</sub> F <sub>3</sub> )	ABL1 (BC <sub>2</sub> F <sub>5</sub> )	ABL2 (BC <sub>3</sub> F <sub>6</sub> )	ABL3 (BC <sub>4</sub> F <sub>5</sub> )	ABL4 (BC <sub>5</sub> F <sub>3</sub> )	
1	34	31	88.6	5.3	181.8	15.3	0	10.8	0	2.5	0	0	0	34.7
2	29	24	82.8	5.4	157.9	5.6	0	5.6	0	5.6	0	5.6	0	36.1
3	26	23	88.5	6.3	164.4	0	0	0	0	0	0	0	0	27.3
4	21	15	71.4	6.2	129.6	1.9	0	0	0	0	0	0	0	4.7
5	22	20	87.0	5.5	122.0	0	0	0	0	0	0	0	0	29.1
6	22	17	77.3	5.7	124.4	3.4	0	0	0	0	0	0	0	30.7
7	21	18	85.7	5.6	118.3	3.4	0	0	0	0	0	0	0	19.4
8	19	16	84.2	6.3	119.9	0	21.4	0	0	0	3.5	0	0	15.4
9	15	14	93.3	6.1	92.1	37.2	0	0	0	37.2	0.0	0	0	44.1
10	15	13	86.7	5.6	83.8	34.5	46.1	29.7	29.7	6.6	19.7	6.6	6.6	19.7
11	20	16	80.0	5.8	115.1	4.9	3.5	23.8	4.9	4.9	0	4.9	4.9	45.4
12	16	15	88.2	6.4	103.1	41.3	41.3	30.4	23.8	23.8	23.8	23.8	23.8	32.4
Total	260	222	84.4	5.9	1512.4	12.3	9.4	8.4	5.3	6.7	3.9	3.4	3.4	28.3

<sup>a</sup> Chromosome number. <sup>b</sup> Number of polymorphic markers between Junambyeo and IR65482-7-216-1-2. <sup>c</sup> Percentage of polymorphism between Junambyeo and IR65482-7-216-1-2. <sup>d</sup> Average marker interval. <sup>e</sup> Chromosome length in centiMorgans (cM). <sup>f,g</sup> ABL1, ABL2, ABL3 and ABL4: advanced backcross breeding lines in Junambyeo genetic background, Donor: IR65482-7-216-1-2 (IR31917-45-3-2/*O. australiensis*).



**Fig. 3.** (a) Seedling reaction of the parents and four advanced backcross breeding lines (ABL) with *Bph18* gene to BPH. CK: check cultivar (Taebaekbyeo), Donor: *Bph18* donor parent (IR65482-7-216-1-2), RP: recurrent parent (Junambyeo). (b) BPH reaction of parents and ABL at adult plant stage. CK: susceptible check, P1: recurrent parent Junambyeo, P2: donor parent IR65482-7-216-1-2. ABL1, ABL2, ABL3 and ABL4: advanced backcross breeding lines with *Bph18* gene. Note the resistance reaction of *Bph18* donor parent and ABL1, ABL2, ABL3 and ABL4 at adult plant stage.

Our results demonstrate that a major broad-spectrum resistance gene, *Bph18*, from the donor parent IR65482-7-216-1-2 was transferred into the Junambyeo background. A small chromosome segment (1320 kb in size from *O. australiensis*) containing the *Bph18* gene from the donor parent was introduced and the improved ABL of BC<sub>2</sub>, BC<sub>3</sub>, BC<sub>4</sub> and BC<sub>5</sub> generations contained donor-derived chromosome segments of 12.3%, 9.4%, 8.4% and 5.3%, respectively, in the

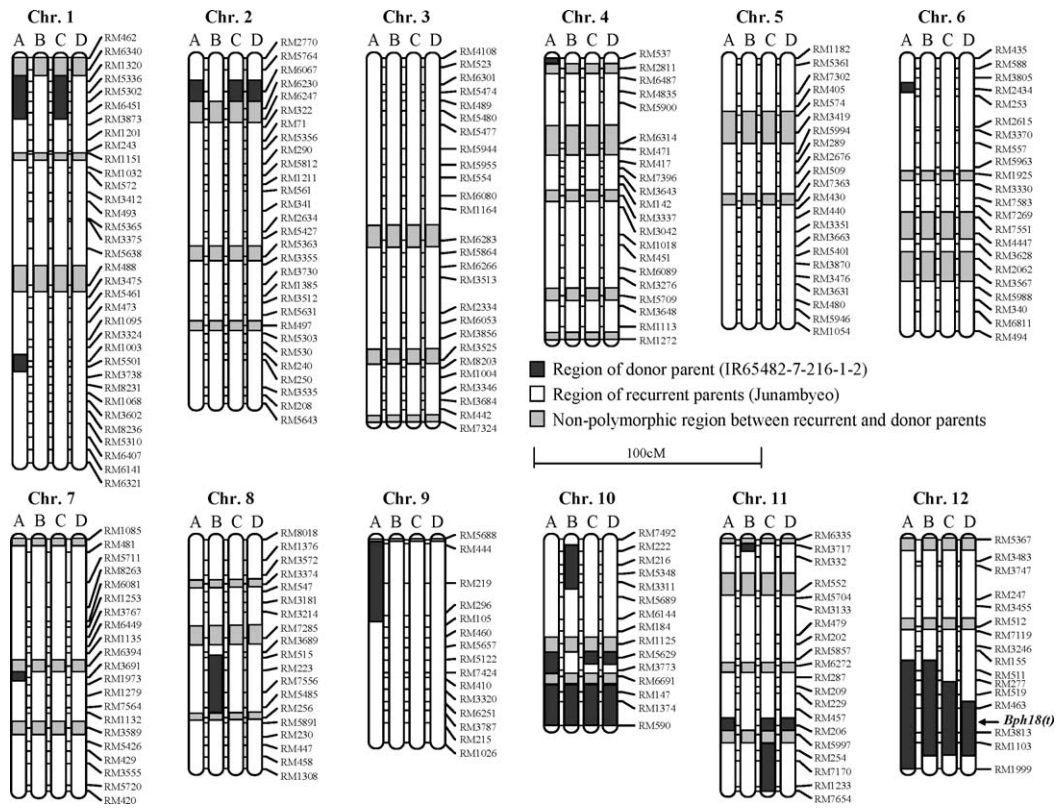
background of recurrent parent Junambyeo. The chromosome segments of *O. australiensis* in the ABL could be reduced up to 3.4% using the MAB strategy compared with the segments present in the donor parent, which was 28.3%. Although the donor parent's chromosome segments decreased when advancing the backcrosses, the selected BC plants expressed a similar phenotypic effect when compared with Junambyeo. This result may be attributed to the phenotype

**Table 4**

Performance of principal agronomic traits and grain quality of four ABL, which were selected as the most promising lines from 11 selected ABL.

Cultivar/breeding line	Agronomic traits <sup>a</sup>									Traits related to grain quality <sup>b</sup>			
	DTH (d)	CL (cm)	PL (cm)	PN	NGP	FER (%)	GY (t/ha)	GW (g)	L/W (cm)	CK	AC (%)	PC	ADV
Junambyeo	120 a	73 c	20.1 b	13 a	109 c	89 a	8.17 a	22.7 a	1.74 b	0a	18.2 ab	6.7 ab	6.7 a
ABL1	121 a	77 ab	22.3 ab	13 a	136 a	81 c	7.94 a	22.8 a	1.76 ab	0a	18.1 ab	7.0 a	6.6 a
ABL2	120 a	76 abc	20.7 bc	15 a	114 bc	83 bc	8.31 a	22.3 a	1.74 b	0a	18.4 a	6.8 ab	6.6 a
ABL3	120 a	74 bc	21.3 abc	14 a	130 ab	82 bc	8.06 a	21.7 a	1.79 a	0a	17.8 b	6.5 b	6.5 a
ABL4	121 a	78 a	22.7 a	15 a	124abc	85 ab	8.25 a	21.9 a	1.75 ab	0a	17.9 ab	6.8 ab	6.7 a

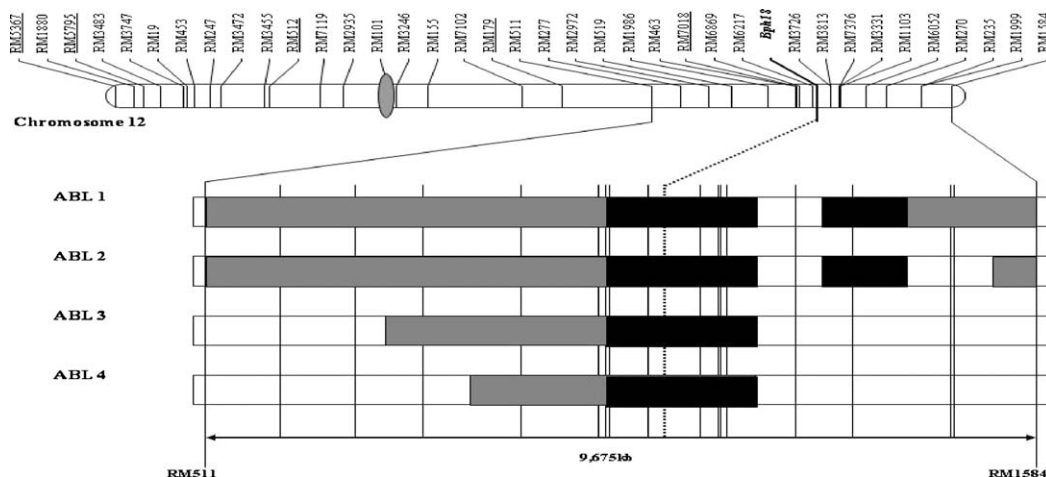
<sup>a</sup> DTH: days to heading, CL: culm length (cm), PL: panicle length (cm), PN: panicle number, NGP: number of grains per panicle, FER: fertility of spikelets (%), GY: grain yield (t/ha), GW: 1000-grain weight of brown rice (g), L/W: ratio of seed length/width. <sup>b</sup> CK: chalkiness of endosperm (0: non-chalky, 3: highly chalky), AC: amylose content of milled rice (%), PC: protein content of brown rice (%), ADV: alkali digestion value (1–7). <sup>a,b</sup> Means followed by the same letter are not significant at the 5% significance level by the least significant difference test (LSD = 0.05).



**Fig. 4.** Background selection of four advanced backcross breeding lines (ABL) in Junambyeo genetic background. Letters A, B, C and D on top are ABL1, ABL2, ABL3 and ABL4, respectively. The black box indicates substituted chromosome segments of the donor parent in ABL.

selection of desirable traits combined with the genotypic selection conducted in every backcross generation and foreground selection for the BPH resistance gene. Additionally, larger chromosome segments flanking the *Bph18* locus from *O. australiensis* had no negative effect on the expression of a desirable phenotype. Of the four ABL from different backcross generations analyzed in this study to decide on appropriate backcrossing time for selection of elite lines, our results suggested that two or three backcrosses and five generations of selfing could recover the recurrent parent genotype without linkage drag. This result is also consistent with results from simulation studies showing that two or three BC generations could be saved by using markers compared with conventional back-

crossing (Frisch et al., 1999). A substantial reduction in generation time, confirmation of BPH resistance and agronomic trait expression point to the possible utility of major gene traits in rice that could significantly lessen the time required to complete backcross trait conversion in plant breeding applications. In a theoretical perspective, a plant breeder requires at least five backcross generations to achieve 99.99% homozygosity of the recurrent parent genome using a conventional breeding approach (Allard, 1960). Hence, the MAB strategy using a dual-selection approach is an effective pathway to introduce the major genes in rice breeding programs as has been achieved in the introduction of the submergence-tolerance gene (*SUB1*) in rice (Neeraja et al., 2007; Septiningsih et al., 2009).



**Fig. 5.** Fine structure of introgressed chromosome segment containing the *Bph18* gene on chromosome 12 in selected advanced backcross breeding lines (ABL). Underlined markers were non-polymorphic between Junambyeo and IR65482-7-216-1-2. The region containing the *Bph18* gene introgressed from IR65482-7-216-1-2 and *O. australiensis* is shown in light black and deep black color, respectively.

The main agronomic traits of the ABL were similar to those of Junambyeo. The increasing grain yield of ABL2 and ABL4 may be attributed to the increasing number of grains per panicle and panicle length. However, the spikelet fertility of those ABL was lower than that of Junambyeo, and grain yield did not increase significantly compared with that of Junambyeo. The grain quality related traits of ABL showed no difference with recurrent parent because the backcross progenies possessing undesirable grain quality traits were eliminated in early generation by dual selection. It is noteworthy to mention that one of the ABL2 progenies possessing the *Bph18* gene and high yield potential with good grain quality was selected as an elite breeding line (Suweon 523) for the development of a new cultivar and the line is now under a local adaptability test in Korea.

## 5. Conclusions

We have successfully developed *Bph18* version of the commercially cultivated japonica elite cultivar Junambyeo by using marker-assisted backcross breeding and incorporating the resistance gene *Bph18* that conferred enhanced resistance to BPH. The recovery of the recurrent parent genome by molecular genotyping and selection could increase the efficiency of the MAB strategy, and this was achievable in a short span of time. This study could have an impact in rice breeding and it is applicable for the introduction of important agronomic traits into the genomes of japonica and indica rice.

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