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# Development of rice introgression lines with brown planthopper resistance and KDML105 grain quality characteristics through marker-assisted selection

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ABSTRACT

'Khao Dawk Mali 105' (KDML105), a Thai aromatic rice cultivar, has been accepted in markets as a prime jasmine rice with premium prices. It has been extensively used as a parental line to develop new cultivars for rainfed lowland areas in Thailand because of its favorable quality and fragrance. However, this cultivar is highly susceptible to brown planthopper (BPH), a phloem sap-feeding insect pest of rice. The main objective of this study was to combine KDML105 essential grain quality traits with BPH resistance from the donor cultivar, 'Rathu Heenati'. The linkage drag between *Bph3* and $Wx^a$ alleles was successfully broken by phenotypic and marker-assisted selections. All introgression lines (ILs) developed in this study showed a broad spectrum resistance against BPH populations in Thailand and had KDML105 grain quality standards. Finally this study was revealed that the ILs can be directly developed into BPH resistant varieties or can be used as genetic resources of BPH resistance to improve rice varieties with the $Wx^b$ allele in rice breeding programs.



## 1. Introduction

Cultivated *indica* rice (*Oryza sativa* L.), a derivation of several Thai cultures, is the most important source of carbohydrate for Thais as well as for Asian population. Increasing rice production from the limited paddy fields for feeding the continuously growing world population is the main challenge in front of all the breeders. Improving yield potential genotypes can increase the rice production, while improving biotic stress genotypes can maintain the stability of rice yield. The *indica* rice cultivar KDML105 (KD) is characterized by its good eating quality with desirable fragrance and has been accepted in markets as premium jasmine rice. Additionally, the cultivar can be widely adapted under rainfed lowland areas in Northeast of Thailand. Thus, KD has been extensively used as a favorable quality parental line to develop new cultivars. However, one limitation of this cultivar is its susceptibility to brown planthopper (BPH), *Nilaparvata lugens* Stål, a major insect pest in rice-producing areas. BPH is causing enormous yield losses every year. Continuous rice culture, extensive use of insecticides and high application rates of nitrogen fertilizer often cause outbreaks of BPH in rice fields. One strategy to minimize the losses due to BPH is the utilization of BPH resistance genes. Consequently, breeding BPH resistant cultivar was an objective of this study in order to stabilize the yield production of KD.

Tagging and mapping of BPH resistance genes in rice have been widely studied. The number of major genes conferring BPH resistance in several cultivated and wild species were identified and mapped with DNA markers, which facilitated marker-assisted selection (MAS) for BPH resistance in rice (Chen et al., 2006; Huang et al., 2001; Ishii et al., 1994; Jena et al., 2003, 2006; Jairin et al., 2007a; Liu et al., 2001; Murai et al., 2001; Murata et al., 2001; Park et al., 2008; Sharma et al., 2003; Su et al., 2002; Sun et al., 2006, 2007; Wang et al., 2001; Yan et al., 2002; Yang et al., 2002, 2004). Molecular markers have been proved very useful in improving backcross breeding through precise transfer of target genomic regions. In addition, it can allow us to estimate the genomic composition and can speed up the recipient genome recovery via background selection (Hospital, 2001). Many studies have been reported the successful introgression of BPH resistance genes from *indica* cultivars and wild species into cultivated rice using MAS (Jena et al., 2006; Sharma et al., 2004; Sun et al., 2006). *Bph3*, one of the major resistance genes derived from the cultivar Rathu Heenati (RH), have been mapped to the short arm of chromosome 6. This

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gene has showed a highly resistant reaction against a broad range of BPH populations in Thailand (Jairin et al., 2007a). RH has been used as a donor of BPH resistance in various conventional breeding programs. However, a poor grain quality such as high amylose content, low gel consistency, chalky endosperm and no fragrance limited the success of breeding lines. Improvement of good cooking and eating cultivars through MAS has been intensively applied in rice breeding programs throughout Asian countries (Liu et al., 2006; Toojinda et al., 2005; Zhang et al., 2005; Zhang, 2007; Zhou et al., 2003). The previous study revealed that, *Bph3* is located very close to $Wx^a$ allele of the *Waxy* locus, approximately 380 kb (Jairin et al., 2007b). In addition, the *Wx* locus and the tightly linked genomic region on the short arm of chromosome 6 are responsible to control cooking and eating qualities determined by the physical and chemical properties of the starch in the endosperm especially the amylose content and gel consistency (Itoh et al., 2003; Lanceras et al., 2000; Zhou et al., 2003). Since the $Wx^a$ allele from RH (*Wx*-RH) is a dominant allele controlling amylose synthesis, resistant progenies carrying the $Wx^a$ have high level of amylose content. To breed the BPH resistance using the *Bph3* allele, linkage drag will be causing a less success in low amylose rice cultivars. It is not only difficult to develop resistant line with low level of amylose content but also it will take longer time via conventional approaches to remove the linkage drag. In this study, phenotypic and marker-assisted selections were performed to break down the linkage drag and we were successful in introgression the *Bph3* allele from RH into KD genetic background. Finally this study was revealed that the MAS is a powerful breeding tool to improve the BPH resistance in low amylose rice cultivars.

## 2. Materials and methods

### 2.1. Development of ILs

RH and KD were used as the parent in this study. RH (acc. no. 11730), a local cultivated rice from Sri Lanka carrying BPH resistance gene *Bph3*, was used as the donor, whereas KD, a landrace cultivar with good cooking and eating quality was used as the recipient. Introgression lines were developed through back-cross breeding using MAS strategy (Fig. 1). The $BC_1$ generation was resulted from the backcrossing of the $F_1$ plants with the recurrent parent. The second and third rounds of backcrossing ($BC_2$ and $BC_3$) were derived from the cross of selected resistant $BC_1$ and $BC_2$ plants based on BPH resistant phenotype and linked markers. A total of 2343 progenies of $BC_3F_2$ were obtained and used to dissect a linkage drag between *Bph3* and *Wx*-RH loci. Two $BC_3F_3$ individual plants were developed from the $BC_3F_2$ resistant plant, which showed heterozygous in the target region on chromosome 6. A total of 330 $BC_3F_4$ individuals derived from an individual $BC_3F_3$ plant that was heterozygous at the *Bph3* locus and homozygous at

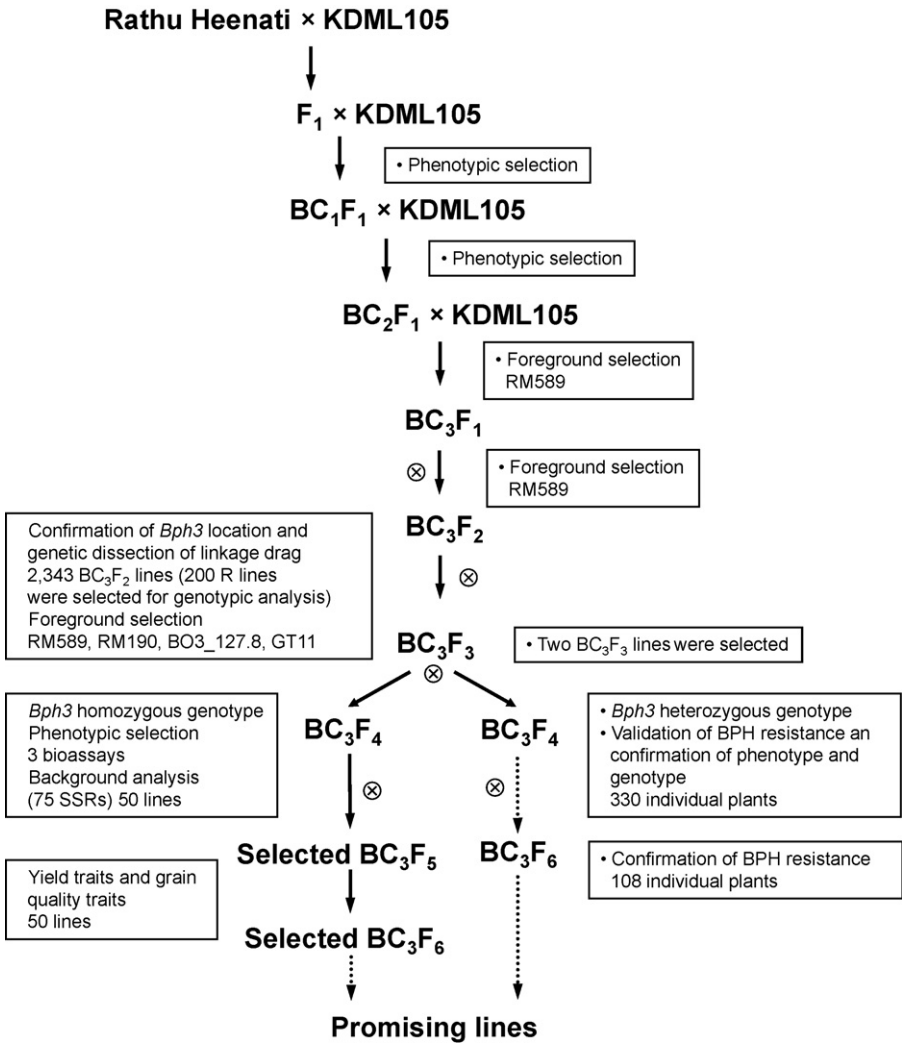


**Fig. 1.** Scheme for the development of BPH resistance introgression lines with details of markers used for foreground and background selection.

**Table 1**
Target molecular markers for MAS.

| Marker | Type | Chr | Trait | Primer sequence | |
|---|---|---|---|---|---|
| | | | | Forward primer | Reverse primer |
| RM190 | SSR | 6 | AC, GC, GT | gctacaaatagccacccacacc | caacacaagcagagaagtgaagc |
| BO3_127.8 | SSR | 8 | FR | cgtggctcgacctttttaat | tcaaaccctggttacagcaa |
| GT11 | STS | 6 | GT | cgagcgagggtttactgttc | ggaggaaacagcagcaactc |
| RM589 | SSR | 6 | BPH | atcatggtcggtggcttaac | caggttccaaccagacactg |

AC = amylose content; FR = fragrance; GC = Gel consistency, GT = Gelatinization temperature; BPH = brown planthopper resistance.

the *Wx*-KD locus ($Wx^b$) were used to confirm the location of *Bph3* on chromosome 6 and validate the BPH resistance. Fifty selected $BC_3F_{4-6}$ ILs derived from the individual plants that were homozygous for *Bph3* and $Wx^b$ were used to determine the recurrent genetic background and evaluate the agronomic trait performance and their grain quality traits.

### 2.2. Bioassays for BPH resistance

Three insect bioassays namely standard seedbox screening (SSBS), modified mass tiller screening (MMTS) and antixenosis on feeding preference (AFP), were used to evaluate BPH resistance. The SSBS was used to evaluate the BPH resistance at the seedling stage of rice under greenhouse condition (Heinrichs et al., 1985). The MMTS (Jairin et al., 2007a) was used to evaluate the BPH resistance of the test lines at the tillering stage. The AFP (Heinrichs et al., 1985) was assessed by monitoring the numbers of BPH nymphs alighting on the test plants. To monitor a broad spectrum resistance against BPH populations in Thailand, a set of 50 selected ILs were screened using SSBS against six BPH populations. Four different biotypes of BPH populations (Jairin et al., 2007a) were collected from Ubon Ratchathani (UBN), Nan (NAN), Kamphaeng Phet (KPP) and Phitsanulok (PSL) provinces in 2004 and two populations were collected from the outbreak fields at Det Udom (DUD), Ubon Ratchathani province and Wang Thong (WTG), Phitsanulok province in 2007.

### 2.3. DNA extraction and PCR amplification

Genomic DNA samples were extracted from young rice leaves following the protocol described by Chen and Ronald (1999). PCR was performed in a 10 μl reaction mixture containing 25 ng of template DNA, 0.5 μM of each primer, 250 μM of each dNTP, 1.5 mM $MgCl_2$, 1 unit *Taq* polymerase and 2 μl of ×10 PCR reaction buffer. Amplification was performed for 35 cycles (1 min at 94 °C, 1 min at 55 °C and 2 min at 72 °C) followed by 5 min at 72 °C. The amplified product was electrophoresed on a 4.5% denaturing silver-stained polyacrylamide gel.

### 2.4. DNA markers for MAS

The SSR marker RM589 co-segregated with the *Bph3* locus were used to determine the presence of *Bph3* gene (Jairin et al., 2007a). One SSR marker, RM190, representing the *Wx* gene was used to select the presence of *Wx* allele of KD ($Wx^b$). The SSR marker BO3_127.8 co-segregated with rice grain aroma (Wanchana et al., 2005) was used to select the fragrance allele of KD. The STS marker GT11 was used to identify the allele corresponding to low gelatinization temperature (Table 1).

### 2.5. Breaking down the linkage between Bph3 and $Wx^a$

To break down a linkage between the *Bph3* and *Wx*-RH loci, a total of 2343 $BC_3F_2$ progenies were screened for BPH resistance using MMTS in the greenhouse. A total of 200 resistant and moderately resistant plants were selected for genotyping with DNA markers. Two SSR markers, RM589 and RM190, closely linked to *Bph3* and *Wx*-RH loci, respectively were used to identify the genotype of $BC_3F_2$ individuals. Only progenies those carrying heterozygous genotypes at the *Wx*-RH and *Bph3* region were selected to generate $BC_3F_3$.

### 2.6. Determination of genetic background

Based on the high-resolution rice linkage map with SSR markers (McCouch et al., 2002), about 120 SSR primer sets were selected and tested on the parental cultivars RH and KD. A total of 75 polymorphic SSR markers distributed throughout rice genome (approximately 5–7 markers spanning each chromosome) were then used to determine the recurrent genetic background of the 50 selected ILs from the $BC_3F_4$ generation.

### 2.7. Field evaluation of ILs

The parents and IL progenies were evaluated for essential agronomic traits in the rice field. They were grown in rainfed lowland condition during the wet season (June to October, 2007) at the Ubon Ratchathani Rice Research Center. For each line, five plants were sampled at heading (when >50% plants showed panicles). Individual plants were evaluated for plant height (cm) and tiller number per plant. Only forty-eight plants in the middle rows were used to determine the grain yield (g/plant) and its components. Grain weight was calculated at 14% grain moisture content.

### 2.8. Determination of the grain quality traits of ILs

Procedures given by Lanceras et al. (2000) were used to determine the amylose content (AC), gel consistency (GC) and gelatinization temperature (GT). The percentage of grains with chalky endosperm was measured as the number of grains with opacity (counted by visual assessment) in 100 milled rice grains. The determination of aroma was carried out by sensory method according to Wanchana et al. (2005).

## 3. Results

### 3.1. Evaluation of parents for BPH resistance

Three bioassays, SSBS, MMTS and AFP, were used to evaluate the BPH resistance of parental cultivars. At the seedling and tillering stage of rice plants, RH expressed strong resistance to the BPH, while KD was completely susceptible to the BPH in SSBS and MMTS bioassays (Table 2). Under free choice conditions in AFP test, BPH avoided settling on seedlings of RH. The number of BPH nymphs settled on KD remained significantly higher and increased further after 72 h (Fig. 2).

**Table 2**
Average damage score of the parents to brown planthopper at vegetative stage (seedling and tillering stages) of rice plants.

| Cultivar | Seedling stage by SSBS | | | Tillering stage by MMTS | | |
|---|---|---|---|---|---|---|
| | 7 DAI | 10 DAI | 14 DAI | 7 DAI | 15 DAI | 23 DAI |
| Rathu Heenati | 1.0 | 2.2 | 2.4 | 1.0 | 1.0 | 1.0 |
| KDML105 | 6.5 | 8.9 | 9.0 | 5.0 | 9.0 | 9.0 |
| TN1 | 7.0 | 9.0 | 9.0 | 5.0 | 9.0 | 9.0 |

DAI = Days after infestation.
Damage score: 1 = very slight damage, 9 = all plants dead.

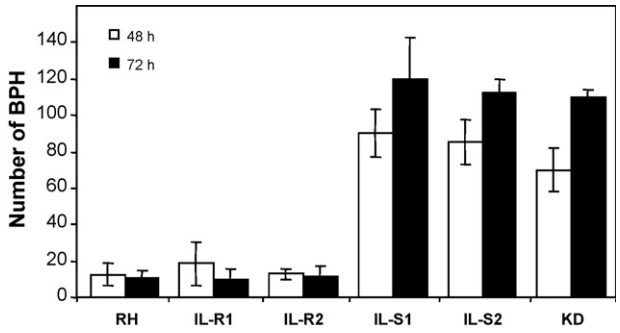


**Fig. 2.** Number of BPH nymphs (means ± SE) settled on rice cultivars (RH = Rathu Heenati; KD = KDML105) and introgression lines (IL-R = resistant introgression lines; IL-S = susceptible introgression lines) in a choice test at 48 and 72 h after infestation.

### 3.2. Genetic dissection of the Bph3 and unfavorable $Wx^a$ allele

*Bph3* and $Wx^a$ were linked in a coupling phase in the rice cultivars RH (Jairin et al., 2007a). The physical distance between the two loci was approximately 380 kb (Fig. 3). A total of 2343 $BC_3F_2$ progenies were used to dissect the linkage using MMTS and MAS. A variation was observed for BPH resistance in $BC_3F_2$ plants based on the MMTS screening. The $BC_3F_2$ plants were classified into three resistant patterns as resistant, moderately resistant and susceptible. Two hundred $BC_3F_2$ resistant and moderately resistant plants were selected for MAS. Ten $BC_3F_2$ progenies those carrying fragrance and GT alleles and showing heterozygous or homozygous on the *Bph3* and/or *Wx* region were selected. Two SSR markers, RM589 and RM190, closely linked to *Bph3* and *Wx* loci, respectively were used to analyze $BC_3F_3$ progenies derived from the selected resistant $BC_3F_2$ lines (#101), which showed heterozygous at the *Bph3* region and KD homozygous at the *Wx* region (Fig. 3). Only two $BC_3F_3$ progenies with slender grains that carry RH homozygous and heterozygous genotypes at *Bph3* region and KD homozygous genotype at *Wx* region were selected to generate $BC_3F_4$.

### 3.3. Validation of BPH resistance in the ILs

A rice population consisted of 330 individuals derived from a heterozygous (at the *Bph3* region) line of $BC_3F_4$ was screened for BPH resistance using MMTS method to investigate the accuracy of the marker for MAS. The BPH resistance scores of the 330 lines were showed a continuous low to high distribution ranging from 1 to 9, respectively. The segregation of BPH resistance in the populations by directly assaying the phenotype was found that resistant, moderately resistant and susceptible plants segregated in a 1:2:1 segregation ratio ($\chi^2 = 1.09$, $P = 0.58$). The corresponding $BC_3F_4$ plants were genotyped as homozygous resistance, segregating heterozygous and homozygous susceptibility. The segregation of $BC_3F_4$ population showed a good fit to the expected ratio of 1:2:1 ($\chi^2 = 1.04$, $P = 0.59$). Investigation of the marker selection efficiency based on the results of the genotypic and phenotypic analysis by BPH resistance was revealed that RM589 marker had high selection accuracy of 80.6%. Further investigation to confirm the phenotype of $BC_3F_6$ individuals derived from a heterozygous line of $BC_3F_5$ for BPH resistance revealed that among the 108 $BC_3F_6$ plants, 25 individuals were resistant, 57 were moderately resistant and the remaining 26 were susceptible, which fit a 1:2:1 segregation based on the $\chi^2$ test ($\chi^2 = 0.35$, $P = 0.84$). Finally results revealed the fact that the BPH resistance in the ILs can be conferred by a single dominant gene.

### 3.4. Evaluation of ILs for BPH resistance

RH, KD and the mixture seeds of selected ILs were evaluated to confirm the resistance against BPH at the seedling stage. Only the donor and the selections have showed high resistance to the BPH,

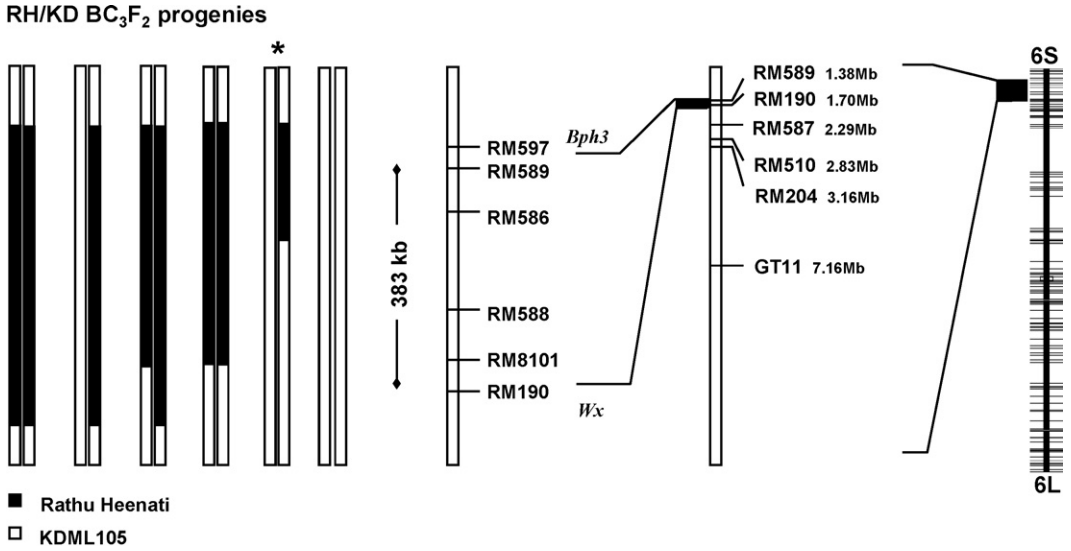


**Fig. 3.** Fine-scale mapping of two loci, *Bph3* and *Wx*-RH, controlling BPH resistance and amylose content, respectively. The locations of two genes, *Bph3* and *Wx* are shown in the linkage map on chromosome 6. Names of SSR markers and genes are shown on the right. Graphical genotypes of the region in six $BC_3F_2$ plants are shown on the left; white blocks regions derived from KDML105 and black blocks regions derived from Rathu Heenati. The progeny with an asterisk (#101) was selected to develop the $BC_3F_3$.

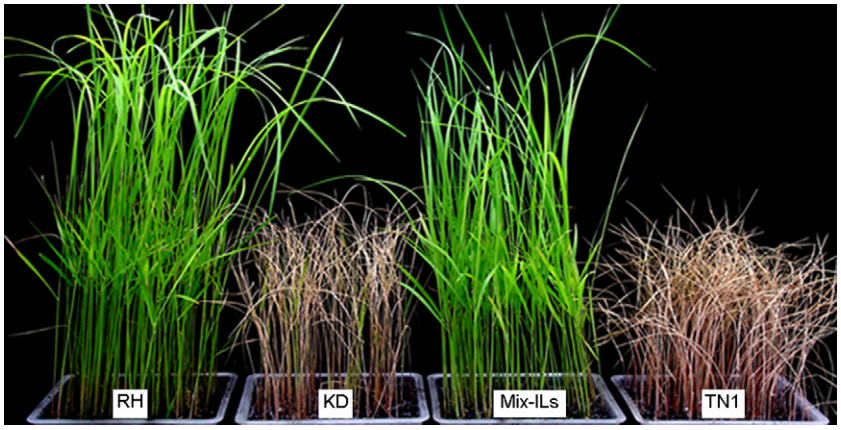


**Fig. 4.** The levels of resistance to BPH at seedling stage of the parents and the mixture seeds of some selected introgression lines. RH = Rathu Heenati; KD = KDML105; Mix-ILs = mixture seeds of introgression lines; TN1 = susceptible cultivar TN1.

**Table 3**
The reaction to BPH populations, collected in Thailand in 2004 and 2007, of some selected introgression lines. The SSBS was used to evaluate the resistance.

| Designation | Reaction to BPH populations* | | | | | |
|---|---|---|---|---|---|---|
| | UBN | DUD | NAN | KPP | WTG | PSL |
| UBN03078-101-342-9 | R | R | R | R | R | R |
| UBN03078-101-342-11 | R | R | R | R | R | R |
| UBN03078-101-342-14 | R | MR | R | R | R | R |
| UBN03078-101-342-4-24 | R | R | R | MR | R | MR |
| UBN03078-101-342-4-32 | R | MR | R | MR | R | MR |
| UBN03078-101-342-4-114 | R | R | R | MR | R | MR |
| UBN03078-101-342-4-126 | R | R | R | R | R | R |
| UBN03078-101-342-4-143 | R | R | R | R | R | R |
| UBN03078-101-342-4-144 | R | MR | MR | R | R | MR |
| UBN03078-101-342-4-148 | R | R | R | R | R | R |
| UBN03078-101-342-6-49 | R | R | R | R | R | MR |
| UBN03078-101-342-6-56 | R | R | R | R | R | R |
| UBN03078-101-342-6-58 | R | R | R | R | R | R |
| KDML105 | S | S | S | S | S | S |
| Rathu Heenati | R | R | R | R | R | R |

R = resistant; MR = moderately resistant; S = susceptible.
* Four different biotypes of BPH populations (Jairin et al., 2007a) were collected from four provinces, Ubon Ratchathani (UBN), Nan (NAN), Kamphaeng Phet (KPP) and Phitsanulok (PSL), in 2004. Two BPH populations were collected from the outbreak fields from Det Udom (DUD), Ubon Ratchathani province and Wang Thong (WTG), Phitsanulok province in 2007.

whereas the recipient parent and the susceptible cultivar TN1 were completely susceptible to the BPH (Fig. 4).

Under free choice conditions in the AFP test, the settling response of BPH initially was not significantly different on all cultivated rice. The number of BPH on RH and resistant IL plants were significantly lower than TN1, susceptible IL and KD plants after 24 h of infestation. The BPH nymphs were first randomly landed on all rice plants and started to move to the susceptible plants increasingly after 24 h of infestation. The number of BPH nymphs settled on the susceptible plants remained significantly higher and was found increasing after 72 h (Fig. 2).

To validate the level and broad spectrum of resistance against BPH populations collected in Thailand, the selected lines were evaluated at the seedling stage using SSBS. Six BPH populations were selected based on its variations from the previous study (Jairin et al., 2007a). All selected lines carrying *Bph3* showed resistance to all BPH populations (Table 3). The result indicated that a broad spectrum BPH resistance gene which has been introgressed from RH to KD was effectively against the variation of BPH populations found in Thailand.

### 3.5. Agronomic performance of ILs in the field trial

The agronomic performance of the selected ILs was evaluated in the rainfed lowland field in 2007. The results showed almost all of

**Table 4**
Performance of principal agronomic and grain quality traits of some selected introgression lines, which were randomly selected from fifty selected introgression lines.

| Designation | DH | PN | GP | NP | NU | PH | GY | ML | MB | ML/MB | GW | CK |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| UBN03078-101-342-9 | 130 | 9.8 | 2.51 | 79.8 | 8.4 | 122.8 | 24.5 | 7.8 | 2.3 | 3.4 | 34.1 | 1.13 |
| UBN03078-101-342-11 | 131 | 9.6 | 2.83 | 84.0 | 10.2 | 130.8 | 31.2 | 7.4 | 2.5 | 3.0 | 36.0 | 1.05 |
| UBN03078-101-342-14 | 130 | 8.8 | 2.31 | 69.0 | 6.4 | 133.0 | 24.8 | 7.4 | 2.1 | 3.5 | 31.2 | 1.01 |
| UBN03078-101-342-4-24 | 128 | 11.2 | 2.72 | 96.2 | 13.4 | 152.2 | 32.0 | 7.3 | 2.2 | 3.3 | 29.7 | 1.40 |
| UBN03078-101-342-4-32 | 127 | 11.6 | 3.53 | 122.8 | 20.6 | 159.4 | 29.3 | 7.7 | 2.2 | 3.5 | 25.5 | 1.37 |
| UBN03078-101-342-4-114 | 127 | 15.4 | 3.43 | 132.6 | 19.4 | 132.0 | 34.2 | 7.6 | 2.2 | 3.4 | 30.4 | 0.47 |
| UBN03078-101-342-4-126 | 128 | 12.0 | 2.26 | 68.6 | 13.8 | 153.2 | 26.1 | 7.6 | 2.4 | 3.1 | 32.1 | 1.02 |
| UBN03078-101-342-4-143 | 127 | 19.2 | 3.72 | 132.4 | 16.6 | 143.8 | 23.3 | 7.7 | 2.1 | 3.7 | 27.1 | 1.35 |
| UBN03078-101-342-4-144 | 127 | 15.4 | 2.68 | 96.0 | 10.8 | 154.6 | 25.9 | 7.8 | 2.0 | 3.9 | 29.4 | 0.61 |
| UBN03078-101-342-4-148 | 127 | 19.4 | 3.62 | 123.2 | 17.0 | 152.2 | 35.8 | 7.9 | 2.0 | 4.0 | 28.1 | 0.90 |
| UBN03078-101-342-6-49 | 127 | 14.8 | 3.01 | 92.4 | 16.2 | 137.6 | 26.1 | 8.0 | 2.4 | 3.3 | 31.6 | 1.28 |
| UBN03078-101-342-6-56 | 127 | 13.0 | 4.07 | 122.4 | 13.8 | 139.6 | 24.5 | 7.8 | 2.4 | 3.3 | 32.7 | 1.87 |
| UBN03078-101-342-6-58 | 127 | 13.2 | 3.03 | 93.4 | 38.0 | 143.4 | 25.4 | 7.8 | 2.5 | 3.1 | 33.4 | 1.29 |
| KDML105 | 127 | 13.8 | 2.91 | 102.6 | 11.8 | 139.0 | 22.0 | 7.8 | 2.2 | 3.6 | 29.4 | 0.83 |
| Mean* | 128.1 | 12.1 | 3.2 | 122.0 | 19.4 | 144.3 | 26.0 | 7.6 | 2.3 | 3.3 | 31.5 | 1.14 |

DH = days to heading (d); PN = panicle number; GP = grain weight per panicle (g); NP = number of grain per panicle; NU = number of unfilled-grain; PH = plant height (cm); GY = grain yield per plant (g/plant); ML = milled rice kernel length (mm); MB = milled rice kernel breadth (mm); ML/MB = milled rice kernel breadth/milled rice kernel length; GW = 1000-grain weight (g); CK = chalkiness of endosperm (%).
* Mean value from all fifty selected introgression lines.

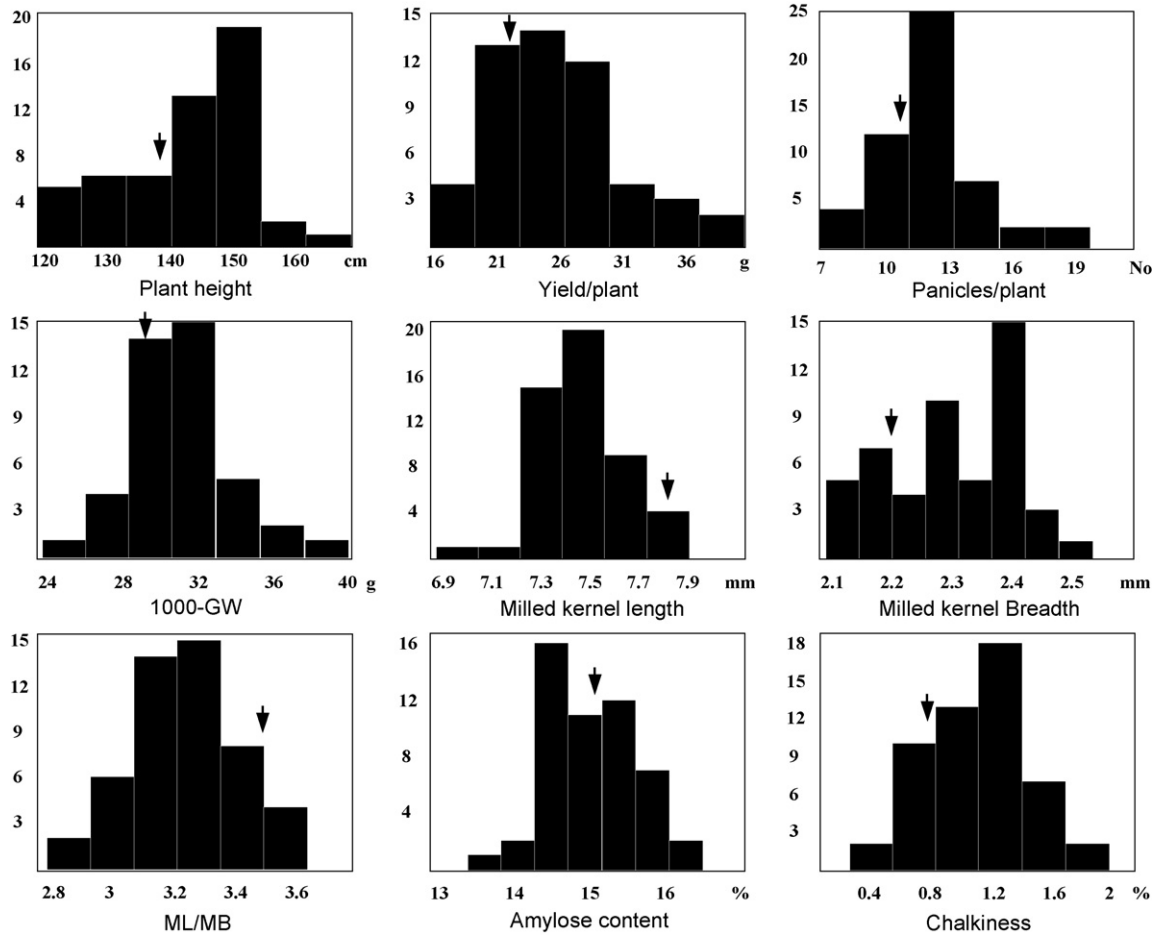


**Fig. 5.** Frequency distribution of plant height (cm), grain yield per plant (g/plant), panicle per plant, 1000-grain weight (g), milled rice kernel length (mm), milled rice kernel breadth (mm), milled rice kernel length/breadth, amylose content (%) and chalkiness of rice grain (%) in $BC_3F_{5-6}$ progenies. Arrows show the mean of KDML105.

the morphological traits of ILs, including plant type, flowering date and appearance grain quality were as same as those of KD (Table 4). The distribution of agronomic traits based on the phenotypes in the ILs is shown in Fig. 5. The average plant height of ILs varied from 122.2 to 164.6 cm. Thirteen of the ILs had shorter plant height than KD (139.0 cm). The average plant height of the ILs was found 3.8% higher than that of KD. The numbers of days to flowering of the selected lines were almost same as those of KD (127 days). However, some selected ILs had 3–7 days delayed flowering than KD. The average number of panicles and grain yield per plant of the selections were 10.0 and 18.4% higher than those of KD, respectively. The average number of filled-grains and 1000-grain weight of the ILs were 18.9 and 6.5% higher than those of KD, respectively. The grain yield per plant of ILs was ranged from 17.6 to 39.0 g. All of the selected lines were awnless and white pericarp, unlike the donor RH, which has prominent awning and red pericarp.

The correlation of the phenotypic performance is shown in Table 5. Panicle number per plant was correlated positively with plant height ($r = 0.54$, $P < 0.001$). Grain yield per plant showed significant positive correlations with panicle number per plant, number of grain per panicle as well as with plant height ($r = 0.37$, $P < 0.01$; $r = 0.40$, $P < 0.01$; $r = 0.42$, $P < 0.01$, respectively) and was not significant with 1000-grain weight. However, 1000-grain weight was correlated positively with grain width ($r = 0.57$, $P < 0.001$). Flowering date was correlated negatively with grain yield and number of grain per panicle ($r = -0.37$, $P < 0.01$; $r = -0.39$, $P < 0.01$, respectively).

### 3.6. Grain quality traits of ILs

The quality traits of fifty selected lines were measured using seeds harvested from Ubon Ratchathani in the wet season of 2007. Almost all of the selected ILs was found to meet the KD grain quality standards (Table 6). The distribution of grain and eating quality of the ILs are shown in Fig. 5. The appearance character of milled rice and grain shape were measured and compared among

**Table 5**
Correlation coefficients between panicle number (PN), plant height (PH), grain yield per plant (GY), grain weight per panicle (GP), number of grain per panicle (NP), number of unfilled-grain (NU), flowering date (FD), 1000-grain weight (GW), milled rice kernel breadth (MB), milled rice kernel length (ML) and milled rice kernel breadth/milled rice kernel length (ML/MB) of fifty selected introgression lines.

| Traits | PN | PH | GY | GP | NP | NU | FD | GW | MB | ML |
|---|---|---|---|---|---|---|---|---|---|---|
| PH | 0.54*** | | | | | | | | | |
| GY | 0.37** | 0.42** | | | | | | | | |
| GP | 0.40** | 0.41** | 0.31* | | | | | | | |
| NP | 0.42** | 0.40** | 0.40** | 0.88*** | | | | | | |
| NU | 0.20 | 0.13 | 0.05 | 0.13 | 0.48*** | | | | | |
| FD | −0.31* | −0.34* | −0.37** | −0.33* | −0.39** | −0.04 | | | | |
| GW | 0.07 | 0.04 | −0.02 | 0.13 | −0.05 | 0.09 | 0.13 | | | |
| MB | 0.22 | 0.13 | −0.05 | 0.25 | 0.04 | 0.29* | 0.21 | 0.63*** | | |
| ML | −0.01 | −0.12 | 0.08 | −0.02 | 0.03 | −0.11 | −0.27 | −0.25 | −0.29* | |
| ML/MB | −0.19 | −0.16 | 0.07 | −0.21 | −0.02 | −0.27 | −0.26 | −0.61*** | −0.92*** | 0.63*** |

*** $P < 0.001$.
** $P < 0.01$.
* $P < 0.05$, respectively.

**Table 6**
The genotype and grain quality traits of some selected introgression lines detected by the DNA marker profile as described in Table 1 and the percentage of parental genome recovery of the selections using 75 SSR markers. RH and KD stand for Rathu Heenati and KDML105 alleles, respectively.

| Designation | *Bph3* allele | AC allele | GT allele | FR allele | AC | GT | FR | GC | KD genome (%) | RH genome (%) | Residual heterozygosity (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| UBN03078-101-342-9 | RH | KD | KD | KD | 16.06 | 7.0 | 2 | 65.0 | 86.6 | 10.4 | 3.0 |
| UBN03078-101-342-11 | RH | KD | KD | KD | 15.39 | 6.9 | 1 | 60.0 | 86.6 | 9.0 | 4.5 |
| UBN03078-101-342-14 | RH | KD | KD | KD | 14.95 | 7.0 | 1 | 115.0 | 84.8 | 12.1 | 3.0 |
| UBN03078-101-342-4-24 | RH | KD | KD | KD | 15.61 | 6.9 | 2 | 100.0 | 85.3 | 13.2 | 1.5 |
| UBN03078-101-342-4-32 | RH | KD | KD | KD | 14.58 | 7.0 | 1 | 105.0 | 88.2 | 8.8 | 2.9 |
| UBN03078-101-342-4-114 | RH | KD | KD | KD | 14.39 | 7.0 | 2 | 80.0 | 88.2 | 8.8 | 2.9 |
| UBN03078-101-342-4-126 | RH | KD | KD | KD | 15.25 | 7.0 | 1 | 67.5 | 86.8 | 8.8 | 4.4 |
| UBN03078-101-342-4-143 | RH | KD | KD | KD | 14.19 | 7.0 | 2 | 77.5 | 86.8 | 10.3 | 2.9 |
| UBN03078-101-342-4-144 | RH | KD | KD | KD | 14.56 | 7.0 | 2 | 65.0 | 91.2 | 8.8 | 0.0 |
| UBN03078-101-342-4-148 | RH | KD | KD | KD | 15.73 | 7.0 | 1 | 72.5 | 89.7 | 7.4 | 2.9 |
| UBN03078-101-342-6-49 | RH | KD | KD | KD | 14.43 | 7.0 | 1 | 46.5 | 83.8 | 13.2 | 2.9 |
| UBN03078-101-342-6-56 | RH | KD | KD | KD | 15.39 | 7.0 | 1 | 60.0 | 83.8 | 13.2 | 2.9 |
| UBN03078-101-342-6-58 | RH | KD | KD | KD | 15.22 | 7.0 | 1 | 70.0 | 85.1 | 10.4 | 4.5 |
| KDML105 | KD | KD | KD | KD | 15.28 | 7.0 | 2 | 75.0 | 100.0 | – | – |
| Mean* | | | | | 15.03 | 7.0 | | 81.2 | 86.9 | 9.9 | 3.2 |

AC = amylose content (%); GC = gel consistency (mm); GT = gelatinization temperature (1–2 = high and 6–7 = low); FR = fragrance (1 = mild; 2 = strong).
* Mean value from all fifty selected introgression lines.

the recipient parent and ILs. The milled rice kernel length of the selections was ranged from 6.9 to 7.9 mm. The average milled kernel of the ILs was found 4.2% shorter than that of KD (7.8 mm). The average ML/MB ratio of the selections was 8.0% lower than that of KD (3.55). Only three selected lines were higher than KD. The chalkiness of the ILs was dramatically less than that of the donor RH (46.88%). The percentage of chalky occurrence in rice grains of selected lines was ranged from 0.35 to 1.87%. Percentage chalkiness of five selected lines was observed lower than KD.

Grains of the fifty selected $BC_3F_{5-6}$ lines were subjected to cooking and eating quality analysis including AC, GT, GC, and fragrance (FR). The cooking and eating quality of some selected lines are summarized in Table 6. Almost all of the selections were found to meet the KD grain quality standards and had a desirable intermediate AC of 14.19–16.06% similar to those of KD (15.28%). The average AC of the selected lines was 15.01%, which was approximately 1.6% lower than in KD. The gelatinization temperature of a grain was measured by the alkaline spreading value (ASV). The selections had the similar ASV score of 6.9–7.0 as that of KD (0.7). GC was measured by the length of the gel. The average length of the gels of the selected lines was 81.2 mm, slightly higher than KD (75 mm), while the average length of the gel of the donor was only 20 mm. Compared to donor, all the selections showed a decreased AC accompanied with an increase in GT and GC. Aroma is one of the most important characters of KD. All selected ILs were aromatic with score of 1–2. Despite of few variations of aromatic scent observed among the selections, some of ILs had similar aromatic scent as the original aroma of KD (Table 6).

### 3.7. SSR based background analysis

A total of 75 polymorphic SSR markers distributed throughout 12 rice chromosomes were used for background analysis in the fifty selected ILs from the $BC_3F_4$ population. The average distance between adjacent markers was ranged from 11.4 cM (chromosome 5) to 30.2 cM (chromosome 3). Among these the percent of markers homozygous for the recipient allele was ranged from 60 to 100%. The background analysis in the ILs revealed the recovery up to 91.2% of the recurrent parent alleles after three generations of backcrossing. The average parental genome recovery of the ILs was 86.9% of the KD genome while that of RH genome was 9.9% with residual heterozygosity of 3.2% (Table 6).

## 4. Discussion

When the unexpected linkage drag occurred, it will endeavor to achieve a breeding goal using conventional approaches in particular when the target gene is linked with an unfavorable dominant gene. However, the goal can be obtained using molecular markers as a tool for selection. The main objective of this study was to combine KD grain quality traits with BPH resistance, and it was successfully introgressed the BPH resistance gene from RH into KD by MAS in three generations of backcrossing and dissected the linkage drag between the introgressed *Bph3* and $Wx^a$ allele, which mainly responsible for an unfavorable characteristic of the grain quality traits, from the donor cultivar.

The quality of the rice grain is one of the primary breeding objectives of rice improvement programs. There is a strong emphasis in Thailand on increasing the quality of rice cultivars with biotic and/or abiotic tolerance. Consequently, KD has been widely used as a base for the grain quality traits nationwide. KD is mostly growing under rainfed lowland areas in the Northeast, the largest area for producing the best quality rice in Thailand. Almost all cultivated rice growing in these areas are susceptible to BPH. Although BPH has been considered as a minor insect pest in the rainfed areas for decades, in the recent time the BPH outbreaks have been frequently occurred in the areas. Breeding new BPH resistant cultivars with high grain quality and wide adaptability under rainfed lowland areas are, therefore, becoming necessary.

RH was found very effective against BPH populations in Thailand and in South East Asia. This cultivar has been considered to confer a broad spectrum and durability of resistance against BPH. However, RH having major disadvantages of poor grain quality as well as its appearance because of a high AC, a hard GC, a low GT, a chalky endosperm, no fragrance together with a prominent awning and a red pericarp. It had been determined that AC, GC and GT, are controlled by the *Waxy* region on chromosome 6 (He et al., 2006; Wang et al., 2007). Unfortunately, we have found that the major BPH resistance gene in RH was linked to the $Wx^a$ allele. According to the previous study, a co-segregated SSR marker with major BPH resistance gene in RH was located near the $Wx^a$ approximately 380 kb based on the genome sequence of Nipponbare (http://www.gramene.org/). This is a case of linkage drag that usually occurs in breeding programs. This might be one of the reasons that we could not develop BPH resistant promising line with good cooking and eating quality using *Bph3* by conventional

approaches. For example, BPH resistant cultivars or promising lines carrying *Bph3* (i.e., IR72, IR56, IR60, IR13540-56-3-2-1 and PSL2), which have been developed in various institutes, are having high amylose content in the endosperm.

To reduce the linkage drag, MAS integrate with phenotypic selection was used to select rice lines carrying recombinants heterozygous in the target region from a total of 2343 $BC_3F_2$ derived from the cross between RH and KD. The number of the progenies was reduced by screening for BPH resistance before applying MAS. This is a successful example of an integrated approach to plant breeding. A small chromosome segment containing a favorable gene from the donor cultivar was introduced into elite lines. The improved lines contained a fragment less than 190 kb of the *Bph3* region from the donor parent (Jairin et al., 2007b). The ILs showed the same broad spectrum resistance against BPH populations in Thailand as the donor cultivar RH. The results have confirmed that the major broad spectrum resistance gene from RH has transferred to the elite lines. However, the levels of resistance of some ILs were not as high as that of RH. Some unidentified minor resistance genes might be lost during backcrossing. Further investigations are required for identification of the locations and effects of the other minor resistance genes.

The essential agronomic characteristics of the ILs developed in this study were almost same as those of KD. No significant alteration was observed in agronomic characters of the improved ILs compared to KD except for the grain yield. The increase in grain yield of the ILs was probably because of increase in number of panicles per plant, number of grains per panicle and plant height but not because of 1000-grain weight.

Molecular marker-assisted selection is proved as an effective approach to improve good cooking and eating quality of the milled rice. Effectiveness of MAS for quality traits was successful in the previous studies and several advanced breeding lines/varieties have been developed (Joseph et al., 2004; Liu et al., 2005, 2006; Toojinda et al., 2005; Zhang et al., 2005; Zhang, 2007; Zhou et al., 2003). In this study, BPH resistant lines were successfully improved with maintaining high grain quality using MAS approach. The results revealed that the *Wx* region on chromosome 6 have major effects on the rice grain quality. The improved ILs lines can be directly developed into varieties, which will have an impact on the yield stability in KD-producing areas. In addition, the ILs can be served either as an immediate sources of broad spectrum and durable BPH resistance to improve good grain quality in breeding programs or as a material to combine several target genes by crossing and MAS.

## 5. Conclusion

ILs with brown planthopper resistance and KD grain quality characteristics were successfully developed by the integration of phenotypic- and marker-assisted selections in three generations of backcrossing. The linkage drag between the *Bph3* and $Wx^a$ allele was successfully dissected and the BPH resistance gene was introgressed into the KD genetic background. The improved lines were not only showed the excellent cooking and eating quality of the milled rice but they also expressed a broad spectrum resistance against BPH populations in Thailand. The ILs developed in this study will have an impact on the yield stability and sustainability in KD-producing areas.

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