



APPLICATION OF MARKER-ASSISTED BREEDING TO IMPROVE BIOTIC STRESS RESISTANCE FOR RAINFED LOWLAND RICE IN NORTHEASTERN THAILAND

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SUMMARY

Northeastern (NE) Thailand has the largest area of rainfed lowland rice, which accounts for more than half of the country's total rice production area. Blast disease and brown planthopper (BPH) are the major constraints for rice production in the NE. Approximately 80% of the area is dominated by KDML105 and RD6 varieties, which are known to be highly susceptible to pests. Germplasm improvement is the main focus to maintain yield stability in the area. Recent advances in rice genomic research have enabled us to identify various rice pest resistance genes/QTLs and provided DNA markers for marker-assisted selection (MAS). MAS has been used to transfer and pyramid BPH and blast resistance genes/QTLs into KDML105, RD6 and other elite varieties. Several promising lines with similar grain quality standards of KDML105 and RD6 have been developed. In this review, we present an overview of the advantages of MAS to improve biotic stress resistance for rainfed lowland rice in NE Thailand.

Key words: Rainfed lowland rice, Northeast Thailand, blast, brown planthopper, marker-assisted selection

Key findings: In this review, we present the advantages of MAS in the transfer and pyramiding of brown planthopper and blast resistance genes into desirable varieties. The developed lines either used as donors or released as new rice varieties for rainfed lowland in NE Thailand.

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INTRODUCTION

Cultivated rice (*Oryza sativa* L.) is the most important staple food crop for Thais and for people from many other Asian countries. Rice production in Thailand can be classified into four ecosystems: rainfed lowland, irrigated, flood prone/deepwater and upland. Rainfed lowland is the most predominant, followed by irrigated, flood prone and upland. Rainfed lowland occupies approximately 5.7 million hectares, which account for more than half of the total rice-growing area (OAE, 2016). The average rice yields in the area are low and fluctuate from year to year, depending on rainfall and ranging from 1.5 and 2.2 t ha⁻¹. Abiotic stresses (i.e. drought, flooding and inherent low soil fertility) and biotic stresses (i.e. blast disease, caused by the fungus, *Pyricularia oryzae* and the brown planthopper, *Nilaparvata lugens*) are the major constraints for rice production in rainfed lowlands in the NE.

The biotic constraints those affect rice production may be due to the effects of changes in farming practices and climate (Ali *et al.*, 2014; Escalada *et al.*, 2015; Heong *et al.*, 2015). Because of labor shortage, farmers are now shifting their rice cultivation patterns from transplanting to dry seed broadcasting for crop establishment, which requires more intensive use of fertilizers, herbicides and pesticides and machine crop harvesting. Although BPH is considered a secondary insect pest in rainfed areas in past decades, at present (from our 2010-2016 observations) the BPH outbreaks are frequently occurring in the provinces bordering Cambodia and Laos such as Ubon Ratchathani, Sisaket, Surin, Buriram, and Nakhon Ratchasima. The most efficient and reliable method of management of blast and BPH is the enhancement of host plant resistance. However, frequent breakdown of resistance is a leading cause of yield instability. Thus, there is a need to develop strategies for providing durable resistance against a broad spectrum of blast pathogens and BPH biotypes across various geographic areas.

Molecular breeding approaches involving DNA markers such as QTL mapping, marker-assisted selection (MAS) and gene pyramiding have been used to develop new

resistant rice varieties (Rao *et al.*, 2014). MAS is a powerful breeding tool to improve biotic stress resistance and shorten the period of varietal improvement. Numerous DNA markers associated with favorable traits have been developed (Chunwongse *et al.*, 1993; Jairin *et al.*, 2005; 2007a; 2007b; 2007c; 2013; Kanjoo *et al.*, 2011; Noenplab *et al.*, 2006; Sakthivel *et al.*, 2009; Siangliw *et al.*, 2003; Toojinda *et al.*, 2005) and applied for MAS in rice breeding programs in Thailand. MAS has been implemented to improve rice traits such as grain quality, abiotic stress tolerance and biotic stress resistance. Much progress (Jairin *et al.*, 2009; Manivong *et al.*, 2014; Rao *et al.*, 2014) has been made by molecular breeders to introgress and pyramid genes and significant QTLs regulating complex quality components and stress tolerance traits into diverse rice genotypes.

The genetic improvement of KDML105 and RD6 (non-glutinous and glutinous jasmine rice, respectively) for resistance to blast (*qBL1*, *qBL11*, *qBL12*), bacterial leaf blight (BLB) (*Xa21*, *xa5*, *xa33*), BPH (*Bph3*, *qBPH4*, *qBPH12*) and gall midge (*Gm1*) has been performed through MAS in both foreground and background selections (Jairin *et al.* 2009; Jantaboon *et al.*, 2011; Kanjoo *et al.*, 2011; Korinsak *et al.*, 2016; Kotcharerk *et al.*, 2015; Punyawaew *et al.*, 2016; Siangliw *et al.*, 2003; Toojinda *et al.*, 2005; Win *et al.*, 2012; Wongsaprom *et al.*, 2010). Advances associated with next-generation sequencing technologies, coupled with the completion of high-quality reference genome sequences, provide opportunities for more efficient genetic mapping and genomic analyses in rice. These techniques have been applied to identify and develop high-throughput DNA markers that are closely linked to the desired genes/QTLs from traditional Thai germplasm. Through collaborations with several government organizations and universities in Thailand, new and improved rice varieties those have excellent grain quality with yield potential and multiple durable resistance against rice pests for rainfed lowland have been bred and distributed to farmers.

Rice production in NE Thailand

The NE region is known as the Khorat plateau, a

saucer-shaped tableland situated 90-200 meters above sea level. Precipitation is the only source of water for most rice-growing areas in the wet season. The average annual rainfall is from 1,200 to 1,400 mm, with 107 to 132 rainy days. Neither the total nor the average annual rainfall is a limiting factor for rice production. However, erratic rainfall patterns and poor distribution create additional problems for farmers in the region. Low rainfall in the southwest and high rainfall in the northeast is a general pattern in NE Thailand. Annual rainfall varied markedly with year and site (Haefele *et al.*, 2006, Polthanee and Promkhambut, 2014).

The total area of rice cultivation in the wet season of 2015 is approximately 5.7 million ha⁻¹, accounting for 64% of the total rice growing area in Thailand. Total production is approximately 11 million tons of paddy rice or approximately 40% of the total production of the country in the wet season (OAE, 2016). The irrigated area covers approximately 0.2 million ha, around 2.8% of the total rice growing area in 2015. Half of the dry season growing area depends on water resources from rivers, swamps or ground water using electric or gas/diesel pumps. It is difficult to increase irrigation area and facilities due to undulating topography and limited water resources. The photoperiod-sensitive varieties KDML105, RD6 and RD15 (RD6 and RD15 are mutants of KDML105 obtained by irradiation with gamma rays) are the main varieties, which grow approximately 90% of the rice production in the rainfed lowland area in the NE during the wet season.

Rainfed lowland rice improvement in NE

Breeding rice for the rainfed lowland ecosystem of NE is a major challenge for breeders. Climate and heterogeneous rainfed lowland rice ecosystems are the most important factors for rice production in the NE. Photoperiod-sensitive, good quality rice varieties for cooking and eating rice are required in these areas, making genetic improvement in the NE more difficult and slower than breeding in irrigated areas. Thus, the primary breeding objectives for rainfed lowland include the incorporation of grain appearance, aroma and cooking characteristics relative to consumer preference,

insect pest and disease resistance into future rice varieties, while high yield potential is the main breeding objective in irrigated areas.

The molecular breeding strategy has become a powerful tool to deploy the identified genes of interest and transfer them into a desired background. Since the initial period of genetic development in NE Thailand, there has been little success in developing new rice varieties to replace KDML105 in pest outbreak risk areas. KDML105 is premium Thai aromatic rice for the world market, and farmers grow these varieties for their income. Unlike KDML105, farmers grow RD6 for household consumption. Therefore, replacing RD6 with new, improved rice varieties may be easier than replacing KDML105.

It will take more than 11-13 years from crossing to the release of new varieties by conventional breeding. The new breeding strategy that suggested conducting an early generation interstation testing based on F₄ bulks to replace an intra-station testing has been proposed to reduce the time of improvement to 9-10 years (Cooper *et al.*, 1999). However, the period for variety development can be shortened using MAS combined with rapid generation advancement and early generation testing (Toojinda *et al.*, 2005). Marker-assisted backcrossing (MAB) has been extensively used to transfer genes/QTLs for abiotic and biotic stress resistance into KDML105 and RD6 (Jairin *et al.*, 2009; Korinsak *et al.*, 2016; Kotcharerk *et al.*, 2015; Kotchasatit *et al.*, 2016; Sinsaitai *et al.*, 2015; Toojinda *et al.*, 2005; Wongsaprom *et al.*, 2010). New resistant/tolerant varieties were developed using MAS and released to farmers in rainfed lowland areas of NE, such as RD18, RD20, RD51 and Thanya Sirin (TYS).

Identification of DNA markers linked to biotic stress resistance

Blast is the most destructive disease in rainfed lowland rice in the NE and causes economically significant annual crop losses. With changing agricultural practices and climate, BPH has become a serious insect pest in the region. Severe field outbreaks were observed from 2010 through the current year (Figure 1). Several % QTLs for blast and BPH resistance have been

identified and used in rice breeding programs (Jairin *et al.*, 2005; 2007a; 2007c; 2013; Noenplab *et al.*, 2006). Simple sequence repeat

(SSR) marker system in rice is commonly used for identification of markers linked to resistance genes in the early stages of MAS.

Figure 1

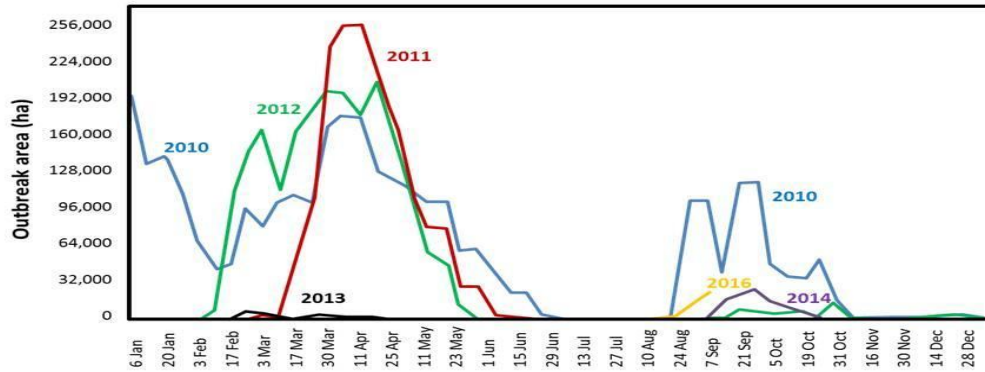


Figure 1. Current brown planthopper outbreak areas in Thailand. The outbreaks occurred in irrigated areas in the central and lower north from January to June, while outbreaks in rainfed lowland occurred from August to October in northeast Thailand. Data from the outbreak areas were recorded by Department of Agricultural Extension, Ministry of Agriculture, Thailand.

Several donors for biotic stress resistance have been studied and selected for crossing to identify the position of resistance genes on rice chromosomes (Table 1 and Figure 2). Bulk segregant analysis (BSA) and QTL mapping techniques have been intensively applied for identification of DNA markers linked to the biotic stress resistance. Hundreds of rice blast fungus isolates and BPH populations collected from farmers' fields in Thailand were used to study and screen rice germplasm. Resistant genotypes were selected and used as genetic materials for the discovery of resistance genes/QTLs for breeding programs.

SSR analysis was performed to identify and localize the *Bph3* gene derived from Rathu Heenati (RH) and PTB33. For mapping the *Bph3* locus, backcross populations from crosses of PTB33×RD6 and RH×KDML105 were developed and evaluated for BPH resistance. From the linkage analysis, the *Bph3* locus was mapped between two flanking SSR markers, RM589 and RM588 on chromosome 6 (Jairin *et al.*, 2007a). Furthermore, *Bph3* was physically mapped to an approximately 190-kb interval flanked by the markers RM19291 and RM8072

(Jairin *et al.*, 2007b). Recently, the resistance gene in PTB33 was identified to encode a unique short consensus repeat domain protein that confers antibiotic resistance to BPH (Ren *et al.*, 2016).

To detect the *bph4* locus, BSA with SSR markers on chromosome 6 was used to analyze F₂ populations derived from crosses of TN1×Babawee and Babawee×KDML105. From the genetic linkage map and QTL analysis, the *bph4* locus was assigned to the same chromosomal region of *Bph3* between the two flanking markers RM589 and RM586 (Jairin *et al.*, 2010). The results confirm previous studies using a classical genetic approach in which *Bph3* was closely linked to *bph4* (Angeles *et al.*, 1986; Ikeda and Kaneda 1981; Sidhu and Khush 1979). In addition, we found that the recessive gene *bph4* could behave as a dominant gene under certain genetic backgrounds (Jairin *et al.*, 2010).

BPH resistance in Abhaya, an Indian rice gall midge-resistant variety, was studied using backcross introgressed lines from a cross between Abhaya and KDML105. Through BSA,

Table 1. Donors for biotic stress resistance and DNA markers used in rice breeding programs for rainfed lowland in NE Thailand.

Donor	Trait	Gene/QTL	DNA marker	Reference
Rathu Heenati	BPH, WBPH	<i>Bph3</i> , <i>qBPH4.1</i>	RM589, RM586, RM8213, RM5953	Jairin <i>et al.</i> , 2007a; Sun <i>et al.</i> , 2005
Abhaya, RGD9905	BPH	<i>qBPH12</i>	RM277, RM260	Jairin <i>et al.</i> , 2005
IR71033-121-15	BPH, BL	<i>qBPH6</i> , <i>qLB11</i>	RM589, RM586, RM224, RM2191	Jairin <i>et al.</i> , 2007c; 2013
Jao Hom Nin	BL	<i>qBL1</i> , <i>qBL11</i> , <i>qBL12</i>	RM319, RM212, RM144, RM224, RM227, RM463	Noenplab <i>et al.</i> , 2006; Wongsaprom <i>et al.</i> , 2010
IRRIC-BPHWRI-67-111	BPH	<i>qBPH4.2</i>	RM317, RM5709	Jairin <i>et al.</i> , 2014
Babawee	BPH	<i>bph4</i>	RM589, RM586	Jairin <i>et al.</i> , 2010
W1263	GM	<i>Gm1</i>	RM5526, RM23899, RM23946	Biradar <i>et al.</i> , 2004
KDML105, Basmati 370	Eating quality	<i>Wx^b</i> , <i>badh2</i>	RM190, BADEX7-5	Jairin <i>et al.</i> , 2009; Sakthivel <i>et al.</i> , 2009
MNTK-BB aroma	BLB, aroma, amylose	<i>Xa5</i> , <i>Xa21</i> , <i>xa33</i> , <i>badh2</i> , <i>Wx^b</i>	RM122, RM507, RM30, RM7243, RM5509, RM400, PAXa5, PB7-8, BADEX7-5	Chunwongse <i>et al.</i> , 1993; Korinsak <i>et al.</i> , 2009; Korinsak <i>et al.</i> , 2014; Sakthivel <i>et al.</i> , 2009
SL-Sub1/Xa21	SUB, BLB, aroma, amylose	<i>Sub1</i> , <i>Xa21</i> , <i>badh2</i> , <i>Wx^b</i>	R10783indel, RM190, RM204, RM224, PB7-8, BADEX7-5	Chunwongse <i>et al.</i> , 1993; Sakthivel <i>et al.</i> , 2009; Siangliw <i>et al.</i> , 2003; Toojinda <i>et al.</i> , 2005
IR57514-PMI-5-B-1-2		<i>qBL12</i> , <i>Sub1</i>	RM227, RM463, R10783indel	Sinsaitthai <i>et al.</i> , 2015

BPH=brown planthopper; BL=blast; SUB=submergence; GM=gall midge; BLB=bacterial leaf blight

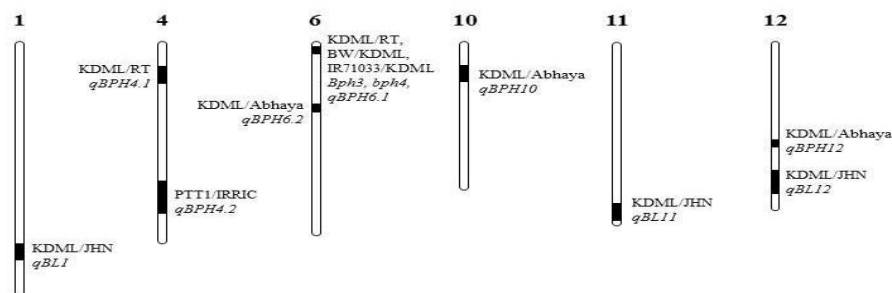


Figure 2. Putative locations of genes and major QTLs for blast and brown planthopper resistance reported on chromosomes 1 (Noenplab *et al.*, 2006), 4 (Jairin *et al.*, 2007a; 2014; Sun *et al.*, 2005), 6 (Jairin *et al.*, 2005; 2007a; 2007c; 2010; 2013), 10 (Jairin *et al.*, 2005), 11 (Noenplab *et al.*, 2006) and 12 (Jairin *et al.*, 2005; Noenplab *et al.*, 2006).

four AFLP fragments were co-segregated with BPH resistance. Linkage analysis revealed that these fragments were localized on rice chromosomes 6, 10 and 12. These results indicated that multiple BPH resistance QTLs play a major role in BPH resistance in Abhaya (Jairin *et al.*, 2005).

Two major QTLs for BPH and blast resistance were mapped using RILs from a cross between KDML105 and IR71033-121-15, an introgression line derived from a wild species of *Oryza minuta*. SSR mapping showed that two major QTLs for BL and BPH resistance from IR71033-121-15, designated *qBPH6* and *qBL11*, were present on chromosomes 6 and 11, respectively. The resistance loci were located in the same genomic regions as in previous reports (Jairin *et al.*, 2013; Noenplab *et al.*, 2006).

The RILs from a cross between KDML105 and Jao Hom Nin (JHN) were used as rice materials to identify QTLs associated with leaf and neck blast resistance. Six QTLs were identified and mapped onto rice chromosomes 1, 11 and 12 with contribution of JHN for all the resistance QTL alleles (Noenplab

et al., 2006). All markers linked to blast resistance genes/QTLs have been used for MAS to develop new rice varieties for NE.

MAS for biotic stress resistance

MAS greatly increases the efficiency of artificial selections in rice breeding. MAS can assist plant breeders to pyramid several desired target genes and significant QTLs into an individual background. The method can be useful to select for traits those are difficult to measure and are expressed late in development. MAS for the traits of the cooking and eating qualities (aroma, texture, and taste) along with biotic/abiotic stresses has been intensively applied in rice breeding programs for rainfed lowland rice (Jairin *et al.*, 2009; Kotcharerk *et al.*, 2015; Korinsak *et al.*, 2016; Toojinda *et al.*, 2005; Wongsaprom *et al.*, 2010). Using MAS, we can simply, quickly and simultaneously combine multiple genes/QTLs into a single genotype to develop broad-spectrum and durable resistance against blast fungus and BPH (Table 2).

Table 2. Promising rice lines and varieties developed by MAS for biotic stress resistance and good cooking and eating quality for wet and dry seasons of rainfed lowland in NE Thailand.

Line/variety	Photoperiodism	Donor	Trait	Approach	Background
UBN03078	Photoperiod sensitive	Rathu Heenati	BPH	MAB	KDML105
UBN07007	Photoperiod sensitive	Rathu Heenati, Jao Hom Nin	BPH, BL	MAP	KDML105
Hom Mali 821	Photoperiod sensitive	IR49830, Abhaya	SUB, BPH	MAP	KDML105
UBN13073	Photoperiod sensitive	W1263, Jao Hom Nin	GM, BL	MAP	RD6
RD18, TYS	Photoperiod sensitive	Jao Hom Nin	BL	MAB	RD6
RGDU06337	Photoperiod sensitive	KDML105	Fragrance	MAB	IR57514
PSL13279	Photoperiod insensitive	Rathu Heenati	BPH	MAB	PTT1
UBN13066	Photoperiod insensitive	IRRIC-BPHWRI-67-111	BPH	MAB	PTT1
UBN14001	Photoperiod insensitive	MNTK-BB, SL- <i>Sub1/Xa21</i> , RD49	SUB, BLB, BL, BPH	MAP	RD49, MNTK-BB

DNA markers have not only been used for foreground selection but have also been utilized for background selection to recover recurrent parent genomes (Jairin *et al.*, 2009; Korinsak *et al.*, 2016; Wongsaprom *et al.*, 2010). Examples of the successful breeding MAS programs to improve biotic stress resistance are given below:

1) We successfully introduced the *Bph3* allele from RH into the KDML105 background via three generations of backcrossing. *Bph3* is located very close to a *Wx^a* allele of the *waxy* locus (Jairin *et al.*, 2009). The *waxy* locus and the tightly linked genomic region on the short arm of chromosome 6 are responsible for controlling cooking and eating quality, as determined by the physical and chemical properties of starch in the endosperm, especially

the amylose content and gel consistency. To reduce the linkage drag between the *Bph3* and *Wx^a* alleles, MAS integrated with phenotypic selection was used. The SSR markers, RM190, RM589 and B03_127.8 were used to select the *Wx^b*, *Bph3* and fragrance alleles. This is a successful example of an integrated approach to plant breeding (Jairin *et al.*, 2009). The improved lines (UBN03078) not only showed excellent cooking and eating quality in the milled rice, but also expressed a broad spectrum resistance against BPH populations in Thailand (Jairin *et al.*, 2009). Kotcharerk *et al.*, (2015) and Kotchasatit *et al.*, (2016) used the promising line as a donor to improve PTT1 and KDML105, respectively, for resistance to BPH and blast (Figure 3).

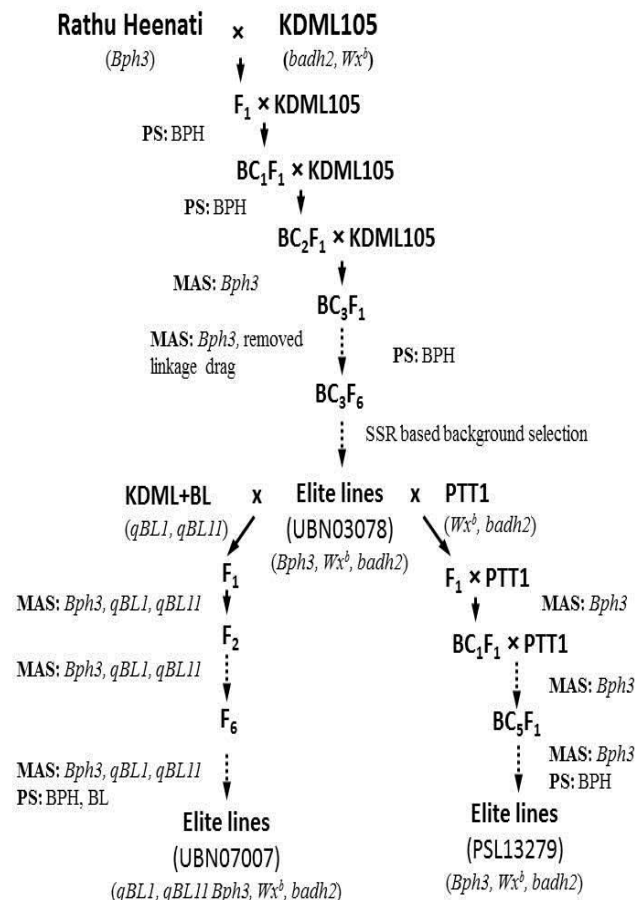


Figure 3. An example of marker-assisted backcross scheme showing development of the introgression line UBN03078. The introgression line was chosen as a donor for brown planthopper resistance to improve rice varieties with the *Wx^b* allele in rice breeding programs.

2) The Rice Department collaborated with RGDU of the National Centre for Genetic Engineering and Biotechnology (BIOTEC) to develop a promising rice line, RGDU06337-MAS-122-B-B, by introgressed fragrance alleles from KDML105 into IR57514-PMI-5-B-1-2, another promising rice line carrying submergence tolerance (*Sub1*) and blast resistance (*qBL1*, *qBL11*). Four DNA markers including R10783indel, Aromarker, Waxy and GT11 were used in the selection of submergence tolerance and cooking quality (Jantaboon *et al.*, 2011). Four SSR markers, RM319, RM212, RM144 and RM224, flanking *qBL1* and *qBL11*, respectively, were used to select rice plants carrying blast resistance (Sinsaithai *et al.*, 2015). The cooked rice from the promising line RGDU06337-MAS-122-B-B is intermediately sticky and moderately soft in texture with the aroma. The promising line gave approximately 80% higher yield than KDML105 under heavy blast infestation conditions, while under blast-free conditions, it gave approximately 11% higher yield than KDML105 (Sinsaithai *et al.*, 2015).

3) MAB was successfully utilized in transferring blast resistance QTLs into RD6. Two QTLs (*qBL1*, *qBL11*) conferring broad-spectrum resistance to leaf and neck blast in JHN (Noenplab *et al.*, 2006) were transferred to RD6 using the flanking markers RM319, RM212, RM144 and RM224. The developed lines showed lower disease scores than RD6, whereas their agronomic performance was not significantly different from RD6. MAB accelerated the development of broad-spectrum blast resistance in the genetic background of RD6 within four years (Wongsaprom *et al.*, 2010). The developed lines were released as new rice varieties (RD18, TYS), and farmers in the NE could benefit from growing the new varieties. In addition, the dominant gall midge resistance gene *Gml* was also introgressed into the promising line to develop blast and gall midge resistance.

4) MAS has been used to pyramid disease and insect resistance genes and major QTLs into elite rice cultivars. Rice lines, MNTK-BB aroma and SL-*Sub1/Xa21*, developed by RGDU, Kasetsart University, were used as parents for BLB and submergence

tolerance. The aim of this breeding program is to improve aromatic rice lines with intermediate amylose, submergence tolerance and resistance to blast, BLB and BPH. In the background of rice cultivar RD49 and MNTK-BB aroma, we pyramided seven genes/QTLs for grain quality traits (aroma and intermediate amylose content), blast, BLB and BPH resistance into RD49 and MNTK-BB aroma. Two markers, BADEX7-5 (Sakthivel *et al.*, 2009) and RM190, were used to select lines carrying aroma and intermediate amylose. Six SSR markers on rice chromosomes 1, 11 and 12 were used to select lines to confer blast resistance (*qBL1*, *qBL11*, *qBL12*). Two functional markers (Chunwongse *et al.*, 1993; Korinsak *et al.*, 2014) were used to select the *xa5* and *Xa21* alleles. Since the locations of BPH and blast resistance genes in RD49 have not yet been identified, BPH and blast resistance lines were chosen based on phenotypic data (Figure 4). All introgression lines significantly improved disease and insect pest resistance. The results of our research suggest that MAS is an efficient and rapid approach to develop biotic resistance and shorten the period of varietal improvement in rice.

Future outlook and challenges

The future challenge for breeders in the NE is to develop new cultivars with higher yield, good grain quality, multiple and durable resistance against major pests and wide adaptation to rainfed lowland ecosystems in the NE to replace KDML105 and RD6 in the pest outbreak risk areas. Genotyping by sequencing (GBS) is the current application of NGS protocols that have been applied for discovery of novel alleles for important rice phenotypes from hundreds of germplasm accessions in our gene bank. A Thai rice diversity panel from a collection of 600 purified homozygous rice accessions representing a broad range of genetic variation has been selected and is being analysed for genome-wide association analysis. The GBS is also being applied to traditional bi-parental mapping populations (F₂, RILs, BILs, NILs) to find genes underlying the phenotypic variation through saturation mapping with a high density of SNP markers. High throughput MAS using SNPs for high yield potential and multi-

Figure 4

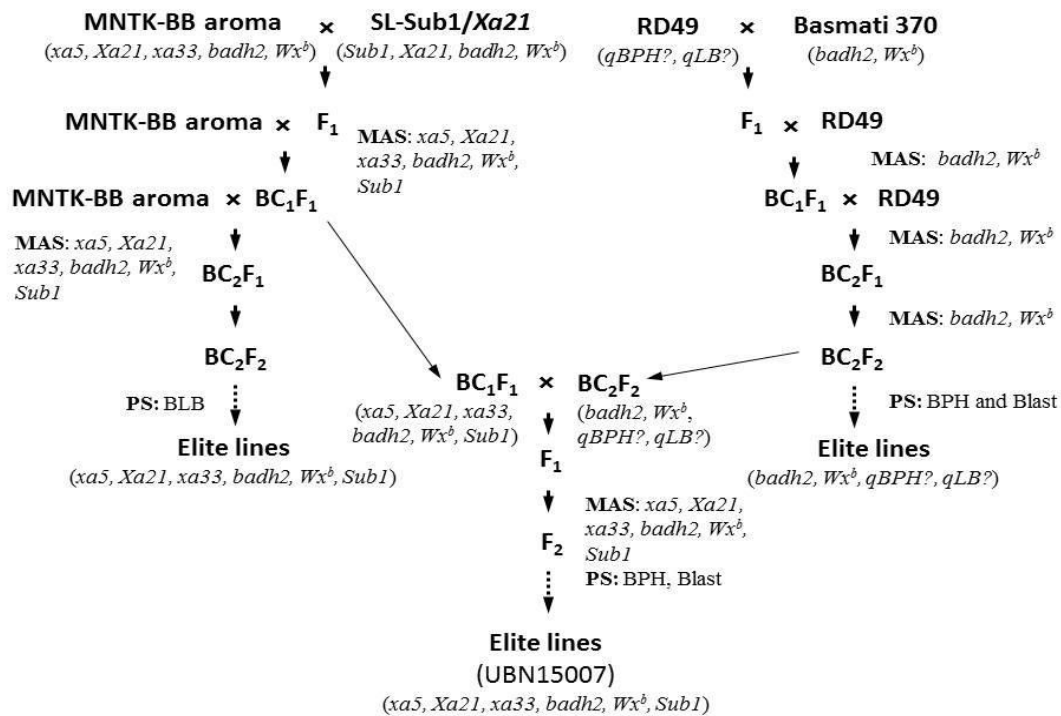


Figure 4. An example of a gene-pyramiding scheme to develop the aromatic rice lines with intermediate amylose, submergence tolerance and resistance to blast, bacterial leaf blight and brown planthopper. Six target chromosome segments were introgressed into the elite rice lines.

resistance to biotic stresses with desired grain quality to shorten our breeding programs is in progress. This may eventually lead to improved rice varieties those maintain productivity or increase yield potential for rainfed lowland rice in NE Thailand. We are not only developing durable biotic stress resistant rice varieties but also seek to understand the genome and genetics of rice pests. Information on adaptation of rice pests to host plants at the molecular level will provide strategies for sustainable deployment of our limited germplasm resources.

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