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Pyramiding of blast and bacterial leaf blight resistance genes into rice cultivar RD6 using marker assisted selection

W. Pinta[1], T. Toojinda[2], P. Thummabenjapone[1] and J. Sanitchon[3,4]*

[1]Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University Khon Kaen, 40002, Thailand.
[2]Rice Gene Discovery Unit, Kasetsart University, Kanphaeng Saen, Nakhon Pathom, 73140, Thailand.
[3]Center of Excellence on Agricultural Biotechnology: (AG-BIO PERDO-CHE), Bangkok 10900, Thailand and Agricultural Biotechnology Research Center for Sustainable Economy, Khon Kaen University, Khon Kaen 40002.
[4]Plant Breeding Research Center for Sustainable Agriculture, Khon Kaen University, Khon Kaen, 40002, Thailand.

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Blast caused by the fungus *Magnaporthe oryzae* (Hebert) Barr. and bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. oryzae (*Xoo*) are two major diseases of rice (*Oryza sativa*). The use of varietal resistance is the most appropriate strategy for controlling the diseases, and molecular assisted selection can potentially accelerate breeding programs. The objective of this study was to pyramid genes conferring resistance to blast and bacterial leaf blight diseases to rice cultivar RD6, using molecular assisted selection. Near-isogenic lines (NIL) derived from two blast resistant crosses (RD6 × P0489 and RD6 × Jao Hom Nin) were pyramided with IR62266 (*xa5*), to transfer bacterial leaf blight resistance to RD6 introgression lines. Five flanking sets of simple sequence repeat (SSR) markers (RM319/RM212, RM48/RM207, RM224/RM144, RM313/RM277 and RM122/RM159: four for blast and one for BLB resistance) were used for screening of introgression lines carrying five quantitative trait loci (QTLs) from the BC_1F_2 generation through to $BC_2F_{2:3}$ generation, and 12 pyramiding lines were identified. Gene validation for blast and bacterial leaf blight diseases was accomplished using artificial inoculation under greenhouse conditions. $BC_2F_{2:3}$ 2-8-2-24 and $BC_2F_{2:3}$ 2-8-2-25 showed greater levels of blast broad spectrum resistance (BSR) whereas $BC_2F_{2:3}$ 2-8-2-36 expressed the highest of bacterial leaf blight resistance with a high blast BSR.

Key words: Gene pyramiding, introgression lines, molecular marker, Near-isogenic lines, SSR.

INTRODUCTION

Blast caused by the fungus *Magnaporthe oryzae* (Hebert) Barr. and bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. oryzae (*Xoo*) are the most serious diseases of rice, that cause severe yield losses throughout the world (Ou, 1985). These two diseases occur in more than 80 rice growing countries resulting yield losses estimated at more than 50% (Ou, 1985; Mew, 1989). Both pathogens have tremendous genetic diversity under natural environment conditions, and a high genetic diversity of the pathogens has been observed in most planting areas. *M. oryzae* has a wide range of alternative hosts, especially grass species that persist throughout the year (Mackill et al., 1986).

The fragrant glutinous rice cultivar, RD6 is a high quality and popular rice cultivar among rice growers in North and Northeast Thailand. However, this cultivar is suscep-

*Corresponding author. E-mail: jirawat@kku.ac.th.

tible to both blast (Wongsaprom et al., 2010) and BLB diseases (June, 1994).

The improvement of rice varieties for resistance to the diseases that are prevalent and destructive is necessary for the sustainability of rice grain yields. Past attempts to achieve varietal resistance to blast and BLB disease have been disappointing, largely due to high levels of variability in the disease populations in growing areas (Sreewongchai et al., 2010). Breeding for broad spectrum resistance is necessary to improve blast resistance in rice. Resistance genes can be specific for different causal pathogens. Pyramiding disease resistant genes into a single genetic background might be expected to give more durable disease resistance, as more resistant genes are incorporated into single genotypes (Koide et al., 2010). Marker assisted backcrossing (MAB) is one of the most anticipated and frequently cited benefits of molecular markers as indirect selection tools in breeding programs (Semagn et al., 2006). The BLB resistance genes have as many as 24 major genes of host plant resistance which have been identified and used in rice breeding programs (Rao et al., 2002). In addition, Naveed et al. (2010) detected the BLB resistance gene *xa5* in Pakistani rice germplasm by using linked markers.

Marker-assisted selection (MAS) allows the identification of multiple resistance genes in plants (Akhtar et al., 2010). The introgression of two quantitative trait loci (QTLs) conferring resistance to blast disease from Jao Hom Nin (JHN) into RD6 has been successful through MAS, with two introgression lines being released for cultivation in North and Northeast Thailand in 2008 (Wongsaprom et al., 2010). The improved cultivar of RD6 has become extensively grown because of its resistance to blast. The resistance to BLB disease is quantitative when using NILs with four resistance genes (R gene) (*Xa4*, *xa5*, *xa13*, and *Xa21*) that expressed a higher level and more durable resistance after pyramiding of the R gene (Li et al., 2001). Phuc et al. (2005) reported that marker assisted selection was accurate for improving the resistance of rice varieties to BLB. The resistant genes to BLB, *Xa4*, and *xa13*, links to microsatellites markers RM144 and RM122, respectively, and *xa5* links to STS marker (RG136). The objective of this study was to pyramid blast and bacterial leaf blight resistance genes into RD6 using marker assisted backcrossing, in order to achieve durable disease resistance.

MATERIALS AND METHODS

Population development

A near inbred line (NIL) derived from pyramiding between (1) the cross of RD6 (susceptible) × JHN (lowland *indica* cultivar with broad-spectrum resistance to blast disease) and (2) RD6 × P0489 (recombinant derived from Azucena × IR64 with blast resistance gene) was used as a recurrent parent, while IR62266 was used as a BLB donor parent. JHN is a blast resistant line carrying QTLs conferring blast resistance in chromosomes 1 and 11 (Noenplab et al., 2006), while P0489 is also a blast resistant line carrying resistant QTLs in chromosomes 2 and 12 (Suwannual et al., 2009). IR 62266 carries the gene *xa5,* conferring bacterial leaf blight resistance in chromosome 5 (Pattawatang, 2005).

A schematic diagram of breeding program is presented in Figure 1. Population development was started with the crossing between NIL and IR62266. F_1 plants were back crossed to the recurrent parent to achieve BC_1F_1. MAS were used to select individual BC_1F_1 plant with resistance alleles. Resistant plants were identified and consequently backcrossed. BC_2F_1 plants were derived by the same method. Resistant BC_2F_1 plants were identified and subsequently allowed to self-pollinate to produce BC_2F_2. MAS were performed to classify the genotype group of the BC_2F_2 plants. The homozygous BC_2F_2 plants were identified and grown to produce $BC_2F_{2:3}$ seed for validation.

Marker assisted selection

To select desirable BC_1F_1, BC_2F_1 and BC_2F_2 lines, 8 SSR flanking markers (RM319/RM212, RM48/RM207, RM224/RM144 and RM313/RM277) associated with blast resistance and 2 markers associated to BLB (RM122/RM159) (Table 1), were used for marker assisted selection (MAS). The DNA of individual plants was extracted using the method (with slight modifications) of Dellaporta et al. (1983). Aliquots of the extracted DNA were run on 1% agarose gel electrophoresis to check the quality and quantity when compared to λ-DNA standard.

Polymerase chain reaction (PCR) was carried out in 10 µl reaction containing 50 ng of DNA template, 1 X PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTP, 0.2 mM reverse and forward primer, and 0.5 unit of *Taq* DNA polymerase. The standardized amplification was initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s; primer annealing at 55°C for 30 s; primer extension at 72°C for 2 min and final extension at 72°C for 7 min. The PCR products were separated on 4.5% polyacrylamide denaturing gel (SequiGen, BioRad Laboratory) at 70 watt for 1 h 30 min. DNA bands were resolved using silver staining.

In addition, three markers BADH, SNP3 and GLU23 associated with quality traits for aroma, gelatinization temperature and glutinous, respectively were used to select homozygous genotypes.

Evaluation for resistance to blast and bacterial leaf blight

Twelve (12) lines of selected $BC_2F_{2:3}$ and the parental lines were grown in plastic trays (seed seeds per hill, two replications) at the Rice Gene Discovery Unit (RGDU), Kasetsart University, Thailand, in 2011. The susceptible check cultivar KDML105, resistant check varieties IR64, P0489 and Jao Hom Nin, and the two donors, were used as the controls for blast resistance. For BLB validation, 13 of $BC_2F_{2:3}$ (12 lines with blast and BLB resistance and one line with blast resistance but not having BLB resistance), were grown together with KDML105 and IRBB5, as susceptible and resistance checks, respectively. Urea fertilizer (46-0-0) at the rate of 312.5 kg/ha was applied three days before inoculation.

Blast inoculation

A factorial experiment in Completely Randomized Design (CRD) was laid out. Eight isolates of *M. oryzae* (Hebert) Barr. (THL185, THL653, THL658, THL142, THL119, THL191, THL949 and B1-2) which represented geographical locations in rice growing areas in North and Northeast Thailand, were used for blast evaluation. The

Table 1. SSR markers used for MAS in F_1, BC_1F_1, BC_2F_1 and BC_2F_2 populations.

Marker	Disease resistance	Chro.	Direction	Sequence
RM 319	Blast	1	Forward	5'ATCAAGGTACCTAGACCACCAC 3'
			Reverse	3'TCCTGGTGCAGCTATGTCTG 5'
RM 212	Blast	1	Forward	5'CCACTTTCAGCTACTACCAG3'
			Reverse	3'CACCCATTTGTCTCTCATTATG5'
RM 48	Blast	2	Forward	5'TGTCCCACTGCTTTCAAGC3'
			Reverse	3'CGAGAATGAGGGACAAATAACC5'
RM207	Blast	2	Forward	5'CCATTCGTGAGAAGATCTGA3'
			Reverse	3'CACCTCATCCTCGTAACGCC5'
RM224	Blast	11	Forward	5'ATCGATCGATCTTCACGAGG3'
			Reverse	3'TGCTATAAAAGGCATTCGGG5'
RM144	Blast	11	Forward	5'TGCCCTGGCGCAAATTTGATCC3'
			Reverse	5'GCTAGAGGAGATCAGATGGTAGTGCATG3'
RM313	Blast	12	Forward	5'TGCTACAAGTGTTCTTCAGGAC3'
			Reverse	3'GCTCACCTTTTGTGTTCCAC5'
RM277	Blast	12	Forward	5'CGGTCAAATCATCACCTGAC3'
			Reverse	3'CAAGGCTTGCAAGGGAAG5'
RM122	BLB	5	Forward	5'GAGTCGATGTAATGTCATCAGTGCC3'
			Reverse	3'GAAGGAGGTATCGCTTTGTTGGAC5'
RM159	BLB	5	Forward	5'GGGGCACTGGCAAGGGTGAAGG3'
			Reverse	3'GCTTGTGCTTCTCTCTCTCTCTCTCTC5'

methodology of Mackill and Bonman (1986) was used for the preparation of fungus conidia. Inoculation was done with an airbrush spray, using 21 days old seedlings, followed by incubation at 24 to 28°C. Disease scoring was done 7 days after inoculation, using the standard of Roumen et al. (1997).

Bacterial inoculation

Ten (10) isolates of *Xanthomonas oryzae* pv. oryzae were used, representing the virulent isolates of North Thailand; the isolates comprised BB2009-1377, BB2009-1369, BB2009-1361, BB2009-1204, BB2009-1120, BB2009-928, BB2009-922, BB2009-786, BB2009-758 and BB2009-348. Inoculum with a concentration of 10^8 cfu/ml was prepared. The 30 days seedlings were inoculated using the method described by Ou et al. (1971). Disease response was observed by measuring the mean lesion length of two inoculated leaves at 14 days after inoculation and scored as follows:

HR = highly resistant: < 1 cm.
R = resistant: 1 to 3 cm.
MR = moderately resistant: 3 to 6 cm.
MS = moderately susceptible: 6 to 10 cm.
S = susceptible: >10 cm (IRRI, 1996).

Data analysis

Data recorded for blast and BLB severity scored were converted to a percentage severity index (SI) using the following formula:

$$SI = \{\Sigma(N_i \times V_i) / V \times N)\} \times 100$$

Where, N_i is the number of plant in each level; V_i is the disease score with differences among individual for plant number; V is the maximum disease score and N is the total plant number.

Disease reaction for blast was classified by SI using the method described by Sirithanya (1998) whereas for BLB was done by SI, based on the method of IRRI (1996). Broad spectrum resistance (BSR) for both diseases was calculated using the method reported of Ahn (1994). Analysis of variance and mean comparisons were undertaken (Gomez and Gomez, 1984).

We measure photoperiod sensitivity of promising lines by growing the line in late of February (Start point of long day season in Thailand) and then records the days to flowering.

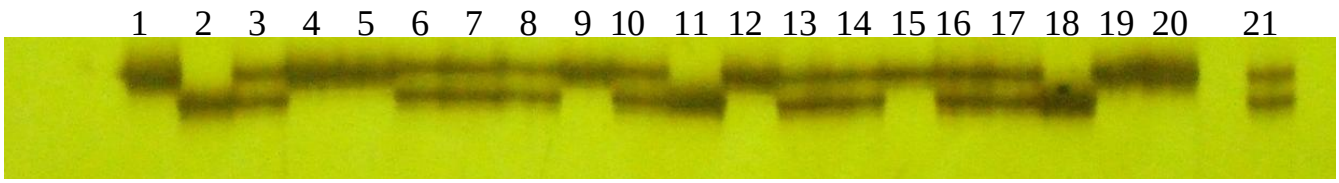

Figure 2. Banding patterns amplified by RM122 marker, showing segregation of xa5 gene in the BC2F2 population, Lane 1 = IR62266 (resistance parent), Lane 2 = RD6 (susceptible parent), Lane 3-21 = BC2F2 population.

Table 2. Severity index (SI) and blast disease reactions.

SI	Reaction to blast disease
0	Very resistant: VR
< 20 %	Resistant: R
21 - 40 %	Moderately resistant: MR
41 - 60 %	Moderately susceptible: MS
61 - 80 %	Susceptible: S
81 - 100 %	Very susceptible: VS

Source: Sirithanya (1998)

Table 3. Analysis of variance of $BC_2F_{2:3}$ selected lines and check varieties against 8 isolates of *P. oryzae* and 10 isolates of *X. oryzae.*

SOV	Blast		BLB	
	d.f.	MS	d.f.	MS
Isolates (I)	7	14.81**	9	42.27**
Genotypes (G)	17	4.564**	16	77.27**
I × G	119	0.87**	144	1.822**
Error	144	0.387	170	1.034
C.V. (%)	31.89		26.69	

RESULTS

Population development was started with the crossing between NIL and IR62266. F_1 plants were back crossed to the recurrent parent, resulting in 156 BC_1F_1. MAS were used to select individual plant with resistance alleles. Two plants were identified and consequently backcrossed. BC_2F_1 plants were selected for the same purpose and 5 BC_2F_1 plants were selected and subsequently allowed to self-pollinate to produce BC_2F_2. MAS were performed to classify the genotype group of the BC_2F_2 plants (Figure 2). Twelve homozygous BC_2F_2 plants were identified and grown to produce $BC_3F_{2:3}$ seed for validation.

Validation of blast resistance

Significance of mean squares was found in relation to genotype (G), blast isolate (I) and the G × I interaction (Table 3). The $BC_2F_{2:3}$ lines had significantly different levels of resistance. IR64 (the resistant check) and P0489 (the donor parent), showed resistance to most blast isolates (BSR = 0.75), while KDML105 (the susceptible check) and RD6, (the recurrent parent) were susceptible and moderately susceptible, respectively, to most isolates (BSR = 0.63, 0.50) (Table 4). All of the 12 $BC_2F_{2:3}$ lines had high levels of resistance, with a BSR of 0.75. Among these lines, $BC_2F_{2:3}$-2-8-24 and $BC_2F_{2:3}$-2-8-25 showed 0.88 of BSR, which was greater than IR64 and P0489. Although the remainder of the lines showed similar BSR (0.75), $BC_2F_{2:3}$-2-8-36 was resistant to most isolates, and very resistant (VR) for the two isolates, THL658 and THL653 (Table 4). Interestingly, the susceptible check cultivar, KDML105, was resistant to THL658 and THL142, whereas RD6 (the susceptible parent cultivar) was also resistant to THL142 and THL949

Table 4. Reaction of $BC_2F_{2:3}$ population two parental lines and 2 check varieties to 8 blast isolates, and their photo-period sensitivity.

Lines and varieties	Blast isolate								BSR	Photo-period sensitivity
	THL 658	B1-2	THL 119	THL 653	THL 191	THL 142	THL 949	THL 185		
$BC_2F_{2:3}$ 2-7-5-2	VR	MR	R	R	MR	MR	R	R	0.75	sensitive
$BC_2F_{2:3}$ 2-7-5-43	VR	MR	R	VR	R	R	R	R	0.75	sensitive
$BC_2F_{2:3}$ 2-7-5-67	R	MS	R	VR	R	R	R	R	0.75	sensitive
$BC_2F_{2:3}$ 2-8-2-19	VR	MS	R	VR	R	MR	VR	R	0.75	sensitive
$BC_2F_{2:3}$ 2-8-2-24	R	MR	MR	VR	VR	R	R	R	0.88	NS
$BC_2F_{2:3}$ 2-8-2-25	VR	MR	R	R	R	R	R	R	0.88	NS
$BC_2F_{2:3}$ 2-8-2-26	VR	MR	R	VR	R	R	R	R	0.75	sensitive
$BC_2F_{2:3}$ 2-8-2-27	VR	MS	MR	VR	R	MR	R	R	0.75	sensitive
$BC_2F_{2:3}$ 2-8-2-36	VR	MR	R	VR	R	R	R	R	0.75	NS
$BC_2F_{2:3}$ 2-8-2-38	R	S	R	VR	R	R	R	R	0.75	sensitive
$BC_2F_{2:3}$ 2-8-2-45	VR	MR	MR	R	R	R	R	R	0.75	sensitive
$BC_2F_{2:3}$ 2-8-2-52	R	MR	R	R	R	R	R	R	0.75	sensitive
RD6	VS	MS	MR	S	MR	R	R	S	0.50	sensitive
P0489	R	MS	R	R	MR	R	R	MR	0.75	NS
Jao Hom Nin	VR	MS	MR	R	R	R	R	R	0.75	NS
KDML105 (check; S)	R	MS	MR	MR	MS	R	MR	MS	0.63	sensitive
IR 64 (check; R)	R	MR	R	R	R	MR	S	R	0.75	-

Reactions; VR: Very resistant, R: Resistant, MR: Moderately resistant, MS: Moderately susceptible, S: Susceptible and VS: Very susceptible. BSR: 0 = susceptible; 1= resistant; NS = not photoperiod sensitive.

revealing that susceptible lines also possess resistant alleles which can be transferred. Most of the pyramided lines were photo-period sensitive (like RD6); however, by the visual selection, three of the promising lines including $BC_2F_{2:3}$-2-8-24, $BC_2F_{2:3}$-2-8-25 and $BC_2F_{2:3}$-2-8-36 was not photo-period sensitive (Table 4).

Validation of BLB resistance

The significance of genotype, isolate and their interaction were similar in their responses to blast (Table 5). IRBB5 (the resistant check) and IR62266 (the donor parent) had 1.00 of BSR, whereas KDML105 (the susceptible check) and RD6 (the susceptible parent) showed susceptibility and moderately susceptibility to most of *X. oryzae* isolates with 0.00 of BSR. The twelve introgression lines containing the resistance gene *xa05* gave a range of 0.4 - 0.8 for BSR. $BC_2F_{2:3}$-2-8-2-36 was the most resistant line with 0.8 of BSR, followed by $BC_2F_{2:3}$-2-7-5-2, $BC_2F_{2:3}$-2-7-5-43, $BC_2F_{2:3}$-2-7-67 and $BC_2F_{2:3}$-2-8-2-45 with the same BSR of 0.7. $BC_2F_{2:3}$-2-7-5-25 the line carrying 4 QTLs of blast resistance, but not carrying the gene *xa05,* gave a susceptible level of 0.00 BSR.

The markers related to aroma, high grain quality, were also used in selection program. Moreover, visualized and sensory test were used as routine for typical RD6 types.

DISCUSSION

Selection for quantitative trait loci (QTL) of disease resistant lines, in which numerous loci are accumulated, is referred to as gene pyramiding. This is difficult to achieve using conventional breeding approaches due to a low accuracy in the identification of desirable genotypes, and because of the laborious and time consuming process. This study demonstrated that MAS can deliver genotypes of interest in a limited time and a small population (Qi-ming et al., 2006). $BC_2F_{2:3}$ pyramided lines were achieved in 2.5 years, while gene validation and the objectives of the breeding program were accomplished in three years.

The success of the breeding program was defined by BSR to blast and BLB. The recurrent parent obtained blast resistance on chromosomes 2 and 12 from P0489, the recombinant line of Azucena × IR64 (the donor, containing strongly resistant genes) (Sallaud et al., 2003) as well as the resistant genes on chromosomes 1 and 11 from RD6 × Jao Hom Nin (Wongsaprom et al., 2010). This study demonstrated the availability of recombinants for gene introgression.

For blast validation, P0489 and IR64 had the same level of resistance. However, the two varieties were not resistant to B1-2 and THL 185, respectively. Nonetheless, most of the resistant lines were moderately resis-

Table 5. Reaction of $BC_2F_{2:3}$ populations and four varieties to 10 BLB isolates.

Lines and varieties	Bacterial leaf blight isolate										BSR*
	1377	1369	1361	1204	1120	928	922	786	758	348	
$BC_2F_{2:3}$ 2-7-5-2	MR	R	R	R	R	MR	R	R	MR	R	0.7
$BC_2F_{2:3}$ 2-7-5-43	MR	R	R	R	R	MR	R	R	MR	R	0.7
$BC_2F_{2:3}$ 2-7-5-67	MR	R	R	R	R	MR	R	R	MR	R	0.7
$BC_2F_{2:3}$ 2-8-2-19	R	MR	R	R	R	MR	R	MR	MS	R	0.6
$BC_2F_{2:3}$ 2-8-2-24	MR	MR	R	R	R	MR	R	R	MS	R	0.6
$BC_2F_{2:3}$ 2-8-2-25	MR	R	R	R	R	MS	R	MR	MR	R	0.6
$BC_2F_{2:3}$ 2-8-2-26	R	R	R	R	R	MR	MR	R	MR	R	0.7
$BC_2F_{2:3}$ 2-8-2-27	MR	R	R	R	R	MS	MR	MR	MS	MR	0.4
$BC_2F_{2:3}$ 2-8-2-36	MR	R	R	R	R	R	R	R	MS	R	0.8
$BC_2F_{2:3}$ 2-8-2-38	MR	R	R	R	R	MR	R	MR	MS	R	0.6
$BC_2F_{2:3}$ 2-8-2-45	R	MR	R	R	R	MR	R	R	MS	R	0.7
$BC_2F_{2:3}$ 2-8-2-52	MR	R	R	R	R	MS	R	MR	MS	R	0.6
$BC_2F_{2:3}$ 2-7-5-25*	MS	MS	MS	MS	MR	MS	MR	MS	MR	MR	0
RD6	S	MS	MS	MS	MS	S	MS	MS	MS	MS	0
IR 62266	R	R	R	R	R	R	R	R		R	1
KDML 105 (check; S)	S	MS	MS	MS	MS	S	MS	MS	MS	MR	0
IRBB 5 (check; R)	R	R	R	R	R	R	R	R	R	R	1

Reactions; VR: Very resistant, R: Resistant, MR: Moderately resistant, MS: Moderately susceptible, S: Susceptible, VS: Very susceptible. *BSR: 0= susceptible, 1= resistance, ** No BLB resistance gene

tant to the isolate B1-2, the isolate with the most virulence. Due to only eight markers associated with two major and two minor QTLs from resistant lines were used in the program. The possibility that change the high resistant to moderate resistant performance might be due to: 1) Several minors QTL have not been selected, therefore incomplete additive gene effects from resistant line could not be selected, 2) there are interaction between genes from different parental lines (non-additive gene effects). Further pyramiding might be needed to incorporate more resistance genes, to overcome B1-2. The BSR of the two lines $BC_2F_{2:3}$-2-8-2-24 and $BC_2F_{2:3}$-2-8-2-25 was greater than that for P0489 and IR64. This may reflect the non-additive effects of other resistant loci contributed from susceptible parent. Korinsak et al. (2009) reported the blast resistance locus of KDML105 located on chromosome 8.

IR62266 (the donor parent) and IRBB5 (the BLB resistant check) had the same reaction to *X. oryzae* with BSR of 1.00, reflecting the fact that two rice varieties possess the gene *xa05* for BLB resistance (Blair and McCouch, 1997). Despite $BC_2F_{2:3}$ introgression lines being selected on markers, crossing over due to the genetic distance of 1.8 cM between the flanking markers RM122 and RM159, might have occurred. Consequently, none of the $BC_2F_{2:3}$ lines had the same BSR as donor. However, the line $BC_2F_{2:3}$-2-8-2-36 was most likely resistant to the 10 isolates of *X. oryzae*. Disease reaction also showed that the 758 was the most virulent isolate. We selected introgression line by only marker associated with major and some minor QTL effects. Therefore, it could not get the complete resistance. The photoperiod non-sensitivity occurred by donor transmission, which actually was an advantage in introgression line. This makes this line grown all years round. In the same year, field experiment was also conducted to assess resistance as well as grain yield. Unfortunately, the field experiment was flooded, resulting to failure for data collection.

Conclusions

Twelve (12) $B_2F_{2:3}$ pyramiding lines were successfully enhanced with resistance to blast and BLB. $BC_2F_{2:3}$-2-8-2-24, $BC_2F_{2:3}$-2-8-2-25 and $BC_2F_{2:3}$-2-8-2-36 are lines with resistance to blast. After BLB evaluation, the line $BC_2F_{2:3}$-2-8-2-36 showed high potential as a promising line for both blast and BLB resistance, delivered and selected through MAS.

Despite the pyramiding of lines containing four QTLs for blast and one gene for BLB, none of them were resistant to all isolates of the two disease pathogens. This indicates that further backcrossing and pyramiding are required to broaden the spectrum of resistance. Farmers in the North and Northeast regions of Thailand will benefit from growing the resistance RD6 cultivar. The

promising lines derived from the blast and BLB evaluation studies carry the trait of non-sensitivity to photoperiod. This characteristic is an additional benefit in that it allows rice farmer to grow them at any time during the year.

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REFERENCES

Ahn SW (1994). International collaboration on breeding for resistance to rice blast. *In* Zeigler, R.S., Leong, S.A. and P.S. Teng (ed.). Rice blast disease. CAB International, Wallingford, Oxon, U.K. 137-153.

Akhtar S, Bhat MA, Wani SA, Bhat KA, Chalkoo S, Mir MR, Wani SA (2010). Marker assisted selection in rice. Phytology 2:66-81.

Blair MW, McCouch SR (1997). Microsatellite and sequence-tagged site markers diagnostic for the rice bacterial leaf blight resistance gene xa-5. Theor. Appl. Genet. 95:174-184

Dellaporta SL, Wood J, Hicks JB (1983). A plant DNA minipreparation: Version II. Plant Mol. Biol. Report. 1:19-21.

Gomez KA, Gomez AA (1984). Statistical procedures for agricultural research. 2nded. New York: John Wiley and Sons.

IRRI (1996). Standard evaluation system for rice. The International Rice Research Institute, Manila, Philippines.

June (1994). International rice research notes. International Rice Research Institute. 19:1-42.

Koide Y, Kawasaki A, Yanoria MJ, Hairmansis T, Nguyet A, Bigirimana NTM, Fujita J, D., Kobayashi N, Fukuta Y (2010). Development of pyramided lines with two resistance genes, Pish and Pib, for blast disease (*Magnaporthe oryzae* B. Couch) in rice (*Oryza sativa* L.). Plant Breed. 129:670-675.

Korinsak S, Toojinda T, Jantasuriyarat C (2009). Identification of a new blast resistance gene in Thai jasmine rice 'KDML 105' by using microsatellite markers. The 35th Congress on Science and Technology of Thailand (STT35); 2009 October 15-17; Chonburi, Thailand.

Li ZK, Sanchez A, Angeles E, Singh S, Domingo J, Huang N, Khush GS (2001). Are the dominant and recessive plant disease resistance genes similar? A case study of rice R genes and *Xanthomonas oryzae* pv. oryzae races. Genetics 159:757-765.

Mackill DJ, Bonman JM (1986). New hosts of *Pyricularia oryzae*. Plant Disease 70:125-127.

Mew TW (1989). An overview of the world bacterial blight situation. *In* Proceedings of the International Workshop on Bacterial Blight of Rice, March 14 - 18, 1988. The International Rice Research Institute. pp. 7-12. Manila, Philippines.

Naveed SA, Babar M, Arif A, Zafar Y, Sabar M, Ali I, Chragh M, Arif M (2010). Detection of bacterial blight resistant gene xa5 using linked marker approaches, Afr. J. Biotechnol. 9(24):3549-3554.

Noenplab A, Vanavichit A, Toojinda T, Sirithunya P, Tragoonrung S, Sriprakhon S, Vongsaprom C (2006). QTL mapping for leaf and neck blast resistance in Khao DawkMaIIL105 and Jao Hom Nin recombinant inbred lines. Science Asia. 32:133-142.

Ou SH, Nuque FL, Silva JP (1971). Varietal resistance to bacterial blight of rice. Plant Disease. 55:17-21.

Pattawatang P (2005). Mapping of quantitative trait loci controlling bacterial blight resistance in rice. Master of Science (Agricultural Biotechnology), Major Field: Agricultural Biotechnology, Interdisciplinary Graduate Program, Kasetsart University, Thailand.

Phuc NV, Lang NT, Buu BC (2005). STS and microsatellite marker-assisted selection for bacterial blight resistance in rice, *Oryza sativa* L. OmonRice. 13:18-25.

Qi-ming D, Shi-quan W, Ai-ping Z, Hong-yu Z, Ping L (2006). Breeding rice restorer lines with high resistance to bacterial blight by using molecular marker-assisted selection. Rice Sci. 13:22-28

Rao KK, Lakshminarasu M, Jena KK (2002). DNA markers and marker-assisted breeding for durable resistance to bacterial blight disease in rice. Biotechnology Advance. 20:33-47.

Roumen E, Levy M, Nottegham JL (1997). Characterization of the European pathogen population of *Magnapothe grisea* by DNA fingerprinting and pathotype analysis. Eur. J. Plant Pathol. 103:363-371.

Sallaud C, Lorieux M, Roumen E, Tharreau D, Berruyer R, Svestasrani P, Garsmeur O, Ghesquiere A, and Notteghem J. L (2003). Identification of five new blast resistance genes in the highly blast-resistant rice variety IR64 using a QTL mapping strategy. Theor. Appl. Genet. 106:794-803.

Semagn K, Bjornstad A, Ndjiondjop MN (2006). Progress and prospects of marker assisted backcrossing as a tool in crop breeding programs. Afr. J. Biotechnol. 5:2588-2603.

Sirithanya P (1998). Mapping gene controlling blast resistance in rice (*Oryzae sativa* L.). Ph.D. Thesis. Kasetsart University.

Sreewongchai T, Toojinda T, Thanintorn N, Kosawang C, Vanavichit A, harreau D, Sirithunya P (2010). Development of elite indica rice lines with wide spectrum of resistance to Thai blast isolates by pyramiding multiple resistance QTLs. Plant Breeding. 129:176-180.

Suwannual T, Saksirirat W, Toojinda T, Sirithunya P, Sanitchon J (2009). Gene pyramiding through marker assisted selection for increasing blast resistance in the rice cultivar RD6. In: 35th Congress on Science and Technology of Thailand. 15-17 October 2009. Burapha University, Chonburi, Thailand. pp. 1-6.

Wongsaprom C, Sirithunya P, Vanavichit A, Pantuwan G, Jongdee B, Sidhiwong N, Lanceras-Siangliw J, Toojinda T (2010). Two introgressed quantitative trait loci confer a broad-spectrum resistance to blast disease in the genetic background of the cultivar RD6, a Thai glutinous jasmine rice. Field Crop Res. 119:245-251.