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Original Article

Broad resistance of RD6 introgression lines with *xa5* gene from IR62266 rice variety to bacterial leaf blight disease for rice production in Northeastern Thailand

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ABSTRACT

This study focused on the transferred *xa*5 gene from the rice cultivar IR62266 into the genome of the RD6 cultivar for bacterial leaf blight (BLB) resistance using the marker-assisted backcrossing method. Ten RD6 introgression lines were selected from the $BC_2F_{2:3}$ population. The RD6 introgression lines and their parents were evaluated for BLB resistance using 15 isolates of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) collected from northeastern Thailand. The *Xoo* isolates were artificially inoculated onto the leaves of the rice cultivars for validation of BLB resistance at the seedling stage. The results showed that there were statistically significant differences in the BLB lesion length among the test cultivars in most of the *Xoo* isolates. The broad-spectrum resistance values of introgression lines $BC_2F_{2:3}$ 1-13-17-52 and $BC_2F_{2:3}$ 1-28-8-37 were the same (0.6) and close to that of the resistant cultivar IR62266 (0.67). In further application, the new rice lines (RD6 resistance to BLB) can be used for pyramiding with other traits for blast resistance and drought tolerance combined with desirable agronomic attributes, for the development of an RD6 rice variety.

Introduction

RD6 is the most popular glutinous rice variety for people in North and Northeast Thailand and this variety is preferred due to its aroma, starch character and eating quality, which make it very palatable to people in the area (Kharkwal and Shu, 2009). The genetic background of the RD6 variety was derived from the non-glutinous variety KDML105 using gamma radiation, with selection in the M2 population being made for glutinous characters (Khambanonda, 1978). Currently, RD6 is grown in most rainfed, lowland rice-growing areas in northern and northeastern Thailand (Kharkwal and Shu, 2009). Although RD6 has been well accepted by farmers and consumers, its susceptibility to bacterial leaf blight (BLB) disease is a recurring problem for the rainfed areas that grow this variety (International Rice Research Institute, 1994). The disease can reduce grain yield in more than 80 rice-growing countries by up to 50% (Mew, 1989). The seedling period when the plants are aged 21 d is the phase of plant growth which is most susceptible to BLB, with complete loss of the crop sometimes occurring when the disease occurs during the tillering stage (Ou, 1985).

The utilization of host-resistant cultivars carrying major resistance (R) genes is the most economic and efficient strategy to manage BLB disease, and which also has a neutral effect on the environment (Singh et al., 2001; Jena and Mackill, 2008). The IR62266 rice variety, which carries the *xa*5 gene, was released by the International Rice Research Institute and has broad resistance to BLB disease, (Glaszmann, 1987; Ogawa et al., 2004). This variety has been a popular donor parent in rice improvement for BLB resistance and was identified as a good genetic resource for broad spectrum BLB resistance in Thailand (Korinsak et al., 2014). It is also a high yielding variety with good agronomic characteristics (Sriprakorn, 2009).

Due to the recessive character of the *xa*5 gene from IR62266, breeding for RD6 resistance to BLB can be potentially limiting and complicated. Marker-assisted selection (MAS) can overcome the constraints of some conventional breeding methods (Collard and Mackill, 2008). The rice variety IR62266 carries the *xa*5 gene on rice chromosome 5, which is flanked by markers RM122 and RM159 (Pattawatang, 2005). Therefore, these flanking markers can

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be used for MAS for BLB resistance in a rice breeding program (Pinta et al., 2013). Based on consumer preference, RD6 has been well accepted by farmers and consumers in northeastern Thailand, due to its cooking and eating qualities. Therefore, the RD6 rice variety should not only aim at resistance to BLB, but also at maintaining the cooking and eating qualities of the original RD6. Moreover, as this variety is grown throughout northeastern Thailand, it must be able to resist diverse BLB isolates in this production area.

The objective of this study was to evaluate the broad spectrum resistance (BSR) of RD6 introgression lines with the *xa5* gene from the IR62266 rice variety to the BLB pathogen from the rice production area in northeastern Thailand.

Materials and methods

Plant materials and Xoo isolates

The experimental materials for this backcross breeding program consisted primarily of the popular Thai rice cultivar RD6 as the recurrent parent, and the BLB resistant line, IR62266, as the donor parent. RD6 was crossed with IR62266, and the F_1 was crossed back to RD6 through $BC_2F_{2:3}$ using MAS with the flanking simple sequence repeat (SSR) markers for BLB resistance, aromatic, waxy and cooking quality (Fig. 1 and Table 1). Selected lines containing the flanking SSR markers were classified. Subsequently, assessment for BLB resistance was performed using artificial inoculation with 15 *Xoo* isolates representing the virulent isolates of northeastern Thailand and also virulence in the RD6 variety (Fig. 2).

Marker-assisted selection for bacterial leaf blight resistance quantitative trait loci

Genomic DNA samples from young leaves from each generation and their parents were extracted according to the method described by Dellaporta et al. (1983) with slight modifications. Individual plants of each generation were selected based on the *xa*5 gene using two franking SSR markers (RM122 and RM159), following the procedure of Pattawatang (2005), together with aromatic (BADH), waxy (Glu) characteristics (Wanchana et al., 2003) and cooking quality (RM190) (Tabkhkar et al., 2012) (Table 1 and Fig. 1).

Polymerase chain reaction (PCR) for SSR markers was carried out in a volume of 10 μL containing 25 ng of genomic DNA, 0.2 μM of each of the forward and reverse primers, 0.2 mM of each of dNTP, 1.8 mM $MgCl_2$, 0.1 unit *Taq*DNA polymerase and 1 × PCR buffer. DNA amplification was performed in a DNA thermal cycler for 5 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 55 °C and 2 min at 72 °C, with a final extension of 7 min at 72 °C. The amplification products were separated on 4.5% polyacrylamide gel electrophoresis, with detection by silver staining.

Phenotypic evaluation of resistance to bacterial leaf blight

Greenhouse conditions

Fifteen virulent *Xoo* isolates from a diverse outbreak area (Fig. 2) were used for evaluation of the 10 RD6 introgression lines together with RD6 non-introgression line, parents and check varieties (IRBB5 as resistant and KDML105 as susceptible checks). The *Xoo* isolates used in the pathogenicity test were grown on nutrient agar media, and the concentration of bacterial inoculation was measurement using a spectrophotometer (optical density = 0.3 at 600 nm). For the $BC_2F_{2:3}$ population, the parents and check varieties were planted in a randomized complete block design with two replications (four plants per replication), in the 2012 wet season (August–October) at Khon Kaen University, Khon Kaen, Thailand. BLB assay was performed at the seedling stage. At 21 d after planting, artificial inoculation was done using the leaf-clipping method (Kauffman et al., 1973). Seven days after inoculation, the plant reaction to BLB was measured in terms of lesion length on individual plants. Plants with a BLB lesion length of less than 6 cm were classified as being resistant, while those with a BLB lesion length of more than 6 cm were classified as being susceptible (International Rice Research Institute, 1996).

Data analysis

Analysis of variance of BLB scores data was done. Mean and SD values of the BLB lesion length for each line and variety were calculated, and the means of all lines were compared using Tukey's Honest Significant Difference test with the R program v 2.10 (R Development Core Team, 2010). Then the broad-spectrum resistance (BSR) was calculated using Equation (1) (Ahn, 1994):

$$BSR = S/T \tag{1}$$

where S is the number of isolates to which plants showed resistance and T is the total number of isolates.

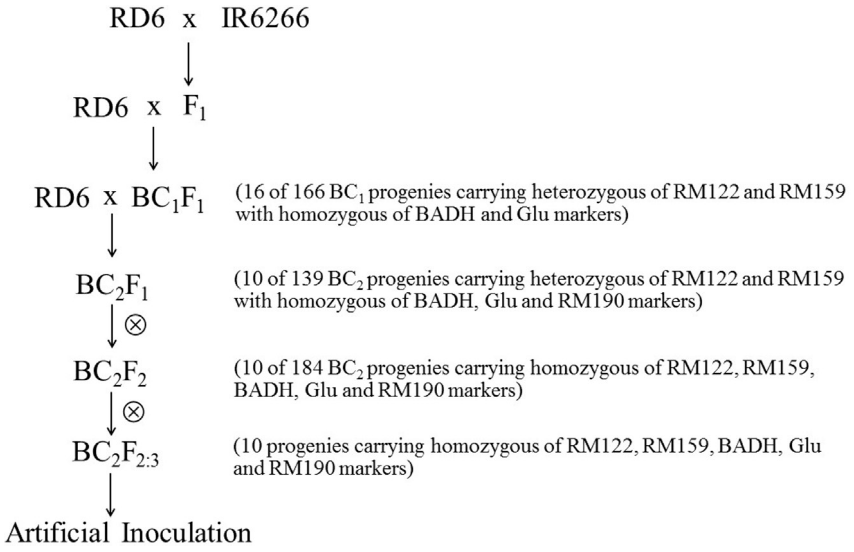

Fig. 1. Breeding scheme for introgression of *xa5* gene from IR62266 to RD6 rice variety using marker-assisted selection.

Table 1
Quantitative trait locus-specific polymerase chain reaction (PCR) primers used for the identification of *xa5* gene, aromatic, waxy and cooking qualities.

Marker name	Chromosomes	Trait	Sequences	PCR product (bp)
RM122	5	*xa5*	5′GTGTCGATGTAATGTCATCAGTGC 3′ 3′GAAGGAGGTATCGCTTTGTTGGAC 5′	229
RM159	5	*xa5*	5′GGGGCACTGGCAAGGGTGAAGG3′ 3′GCTTGTGCTTCTCTCTCTCTCTCTCTCTC5′	252
RM190	6	Cooking quality	5′CTTTGTCTATCTCAAGAACAC3′ 3′TTGCAGATGTTCTTCCTGATG5′	120
Glu	6	Waxy	5′TGCAGAGATCTTCCACAGCA3′ 3′GCTGGTCGTCACGCTGAG5′	209
BADH	8	Aroma	5′AGAATGGCGTTCCCAATTCCTGCTC3′ 3′TTCAAGGAGACTTGTACCATCCCCA5′	422

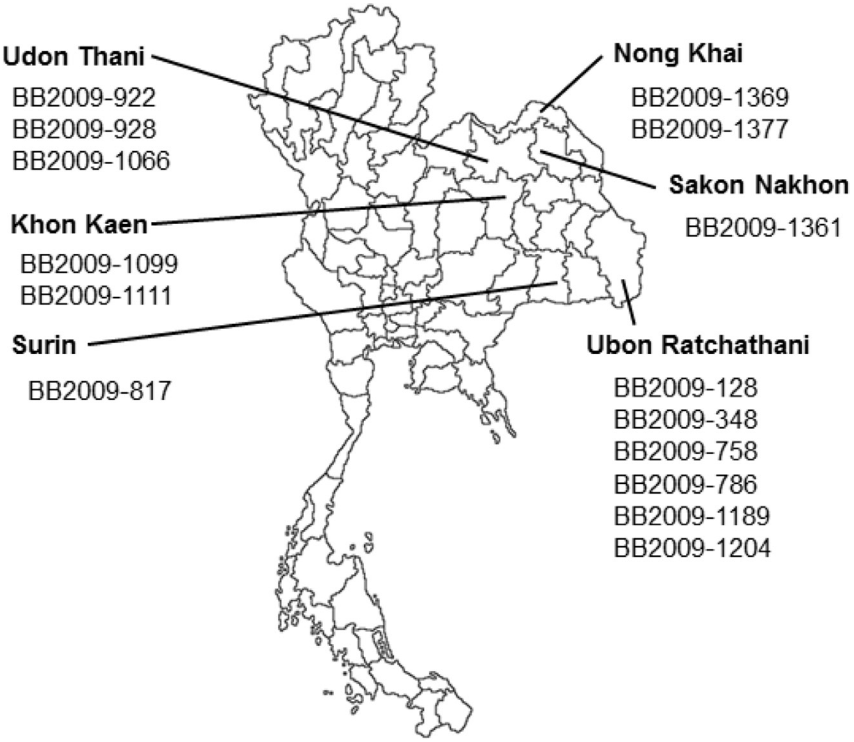

Fig. 2. Distribution of *Xanthomonas oryzae* pv. *oryzae* isolates from northeast Thailand used for bacterial leaf blight evaluation in this study.

Results

Development of RD6 introgression lines with xa5 *resistance genes through marker-assisted selection*

The results of the MAS in this study are shown in Fig. 1 and Table 2. In the F_1 generation, 38 F_1 plants from the crosses between RD6 and IR62266 were tested for true hybrids using the RM122–RM159 markers. Nineteen positive F_1 plants were backcrossed to generate 166 BC_1F_1 plants which were screened with RM12–RM159, BADH, and Glu markers. Sixteen out of those 166 plants were identified as carrying the positive alleles (heterozygous of RM122–RM159 and homozygous of BADH and Glu) at selected markers. Based on the marker profile as well as agro-morphological similarity to the RD6 parent, 16 BC_1F_1 plants were selected and used to generate139 BC_2F_1 plants. Subsequently, 10 individuals were identified as carrying the positive alleles [heterozygous of RM122–RM159 and homozygous of BADH, Glu and cooking quality (RM190)] at selected markers, which were sown for selfing to produce BC_2F_2 progenies. Finally, 10 BC_2F_2 plants were identified as being homozygous for RM122, RM159, BADH, Glu and RM190 linked markers. These 10 plants were then selfed to produce $BC_2F_{2:3}$ progenies for the evaluation of their reactions to BLB disease (Fig. 1).

Reaction to bacterial leaf blight disease

The 10 $BC_2F_{2:3}$ progenies of each RD6 introgression, homozygous for *xa*5, aroma, waxy and cooking quality, together with the non-introgression line, parents and check varieties, were evaluated for their reaction to the 15 *Xoo* isolates which are prevalent in Thailand (Fig. 2). The analysis of variances showed significant differences among the introgression lines, their parents and check varieties (Table 2). IR62266, the donor resistant parent for *xa*5, showed greatest resistance to the *Xoo* isolates (with an average BLB lesion length of 4.60 cm) while, RD6, the recurrent parent, was very susceptible (with an average BLB lesion length of 11.41 cm). The RD6 introgression lines had a shorter lesion length than the non-introgression lines, with the lesion length in the range 5.17–6.99 cm compared with 11.69 cm for the non-introgression line ($BC_2F_{2:3}$ 1-28-8-33*). The introgression lines, $BC_2F_{2:3}$ 1-28-8-37 and $BC_2F_{2:3}$ 1-13-17-52, had shorter mean lesion lengths across

Table 2
Lesion length of RD6 introgression lines carrying *xa5* compared with parents and check varieties.

Lines	*xa5*	Lesion length of each *Xanthomonas oryzae* pv. *oryzae* isolate (cm)														
		128	348	758	786	817	922	928	1066	1099	1111	1089	1204	1361	1367	1377
$BC_2F_{2:3}$ 1-13-9-6	1	7.30	2.74	8.77	9.09	10.54	7.00	2.45	7.35	7.23	2.83	8.46	6.40	2.30	4.13	2.48
$BC_2F_{2:3}$ 1-13-9-13	1	7.92	1.84	7.70	9.57	12.28	8.90	1.48	9.24	7.87	2.14	8.65	7.34	7.40	5.50	5.60
$BC_2F_{2:3}$ 1-13-9-18	1	7.07	2.61	8.00	10.41	9.32	8.40	1.24	6.56	8.61	2.19	8.01	7.19	2.51	5.04	2.48
$BC_2F_{2:3}$ 1-13-17-52	1	5.18	2.01	8.47	7.80	10.76	5.40	1.86	9.25	7.67	1.54	8.16	5.12	2.46	3.74	1.98
$BC_2F_{2:3}$ 1-28-8-1	1	9.97	2.18	9.49	8.62	10.14	10.00	2.92	10.66	7.90	3.61	11.79	7.25	6.25	2.53	1.56
$BC_2F_{2:3}$ 1-28-8-16	1	7.81	1.36	8.79	9.94	10.96	9.22	3.55	7.39	8.12	1.69	9.06	6.71	4.74	5.35	3.60
$BC_2F_{2:3}$ 1-28-8-18	1	5.68	2.08	8.35	8.28	8.19	8.05	1.60	5.28	8.81	1.23	8.34	7.07	2.00	5.05	2.73
$BC_2F_{2:3}$ 1-28-8-21	1	5.76	2.73	9.77	7.32	8.75	6.90	1.94	6.63	8.59	2.39	8.88	6.25	1.06	5.25	2.96
$BC_2F_{2:3}$ 1-28-8-34	1	5.53	3.36	7.64	8.71	10.49	8.20	2.38	6.39	10.35	2.50	8.56	4.56	1.16	3.99	2.16
$BC_2F_{2:3}$ 1-28-8-37	1	5.47	1.90	7.70	8.25	10.85	6.30	1.95	5.42	7.62	1.54	8.19	5.50	1.19	3.88	1.76
$BC_2F_{2:3}$ 1-28-8-33[a]	0	12.45	6.65	13.37	12.80	13.23	12.30	8.07	11.77	13.38	10.30	13.82	12.69	11.80	11.53	11.13
RD6	0	11.49	9.20	12.53	13.19	11.66	12.10	9.79	12.93	12.12	8.50	11.57	14.19	9.10	11.70	11.09
IR62266	1	4.97	0.74	8.16	5.56	6.59	4.60	1.17	6.11	7.06	1.24	8.47	4.94	2.56	4.61	2.19
KDML105	S Check	11.21	6.83	12.96	15.74	12.51	12.60	8.28	11.60	12.53	6.28	13.06	13.09	10.89	10.72	10.61
IRBB5	R Check	3.81	0.90	4.05	4.69	5.06	5.20	0.98	3.86	4.19	1.55	3.60	1.50	0.73	3.63	2.00
p value		<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
$LSD_{.05}$		1.54	0.77	1.23	2.11	2.20	2.03	1.24	1.60	1.57	0.65	1.70	1.47	1.19	0.87	0.65
CV (%)		9.7	11.9	6.4	10.6	10.2	11.4	17.6	9.3	8.4	9.2	8.6	9.4	12.6	7.1	7.2

LSD = least significant difference; CV = coefficient of variation.

[a] RD6 non-introgression line.

the *Xoo* isolates (less than the other lines), with averages of 5.17 cm and 5.43 cm, respectively. In addition, the mean lesion lengths of IRBB5 and KDML105, as resistance and susceptible check varieties, respectively, were 4.60 cm and 11.26 cm, respectively (Table 2). The results indicated that the RD6 introgression lines with the *xa*5 gene provided effective resistance against the predominant *Xoo* isolates in Thailand.

Based on the BSR values, IR62266, the donor parents with *xa*5 gene on chromosome 5, had a high average BSR value (0.67). In contrast, RD6 had a low BSR value (0.00). The RD6 introgression lines had BSR values in the range 0.33–0.60 with the non-introgression line, while the BSR value with the non-introgression was 0.00. Both $BC_2F_{2:3}$ 1-28-8-37 and $BC_2F_{2:3}$ 1-13-17-52 had the same BSR value (0.60). These results indicated that the two RD6 introgression lines with the *xa5* gene provided effective resistance against the predominant *Xoo* isolates in Thailand at almost the same level as the resistant parent (IR62266). In addition, IRBB5 and KDML105 (the resistant and susceptible check varieties, respectively) had BSR values of 1.00 and 0.00, respectively (Table 3), indicating that RD6 and KDML105 had the same reaction to the *Xoo* isolates but were different to IRBB5 and IR62266.

Discussion

BLB disease caused by the *Xoo* is a serious constraint to RD6 rice production in Thailand. The wide cultivation of this variety in northeastern Thailand raises the risk of extensive crop losses due to this pathogen. The main objective of this study was to determine the RD6 introgression lines with the *xa5* gene from IR62265, for use in developing a broad resistance variety to BLB disease in northeastern Thailand.

IR62266 and IRBB5 are well known as sources of resistance to BLB in several rice-breeding programs (Glaszmann, 1987; Singh et al., 2001; Ogawa et al., 2004; Sriprakorn, 2009; Naveed et al., 2010; Korinsak et al., 2014). Both the IR62266 and IRBB5 rice varieties possess the *xa5* gene for BLB resistance (Blair and McCouch, 1997). In the current study, IR62266 and IRBB5 had different reactions to *Xoo* isolates in terms of the mean lesion length and BSR value regarding resistance (Table 3), though the results of this study suggested that IRBB5 had broader resistance than IR62266 to *Xoo* isolates. However, IR62266 was used as the donor parent since the agronomic attributes of IR62266 are greater than for IRBB5 (Sriprakorn, 2009).

Table 3
Disease reaction and broad-spectrum resistance (BSR) of RD6 introgression lines carrying *xa5* compared with parents and check varieties.

Lines	*xa5*	Reaction to *Xanthomonas oryzae* pv. *oryzae* isolates[†]															BSR
		128	348	758	786	817	922	928	1066	1099	1111	1089	1204	1361	1367	1377	
$BC_2F_{2:3}$ 1-13-9-6	1	MS	R	MS	MS	S	MS	R	MS	MS	R	MS	MS	R	MR	R	0.40
$BC_2F_{2:3}$ 1-13-9-13	1	MS	R	MS	MS	S	MS	R	MS	MS	R	MS	MS	MS	MR	MR	0.33
$BC_2F_{2:3}$ 1-13-9-18	1	MS	R	MS	S	MS	MS	R	MS	MS	R	MS	MS	R	MR	R	0.40
$BC_2F_{2:3}$ 1-13-17-52	1	MR	R	MS	MS	S	MR	R	MS	MS	R	MS	MR	R	MR	R	0.60
$BC_2F_{2:3}$ 1-28-8-1	1	MS	R	MS	MS	S	MS	R	S	MS	MR	S	MS	MS	MR	R	0.33
$BC_2F_{2:3}$ 1-28-8-16	1	MS	R	MS	MS	S	MS	MR	MS	MS	R	MS	MS	MR	MR	MR	0.40
$BC_2F_{2:3}$ 1-28-8-18	1	MS	R	MS	MS	MS	MS	R	MR	MS	R	MS	MS	R	MR	R	0.53
$BC_2F_{2:3}$ 1-28-8-21	1	MR	R	MS	MS	MS	MS	R	MS	MS	R	MS	MS	R	MR	R	0.47
$BC_2F_{2:3}$ 1-28-8-34	1	MR	MR	MS	MS	S	MS	R	MS	S	R	MS	MR	R	MR	R	0.53
$BC_2F_{2:3}$ 1-28-8-37	1	MR	R	MS	MS	S	MS	R	MR	MS	R	MS	MR	R	MR	R	0.60
$BC_2F_{2:3}$ 1-28-8-33[a]	0	S	MS	S	S	S	S	MS	S	S	S	S	S	S	S	S	0.00
RD6	0	S	MS	S	S	S	S	MS	S	S	MS	S	S	MS	S	S	0.00
IR62266	1	MR	HR	MS	MR	MS	MR	R	MS	MS	R	MS	MR	R	MR	R	0.67
KDML105	S Check	S	MS	S	S	S	S	MS	S	S	MS	S	S	S	S	S	0.00
IRBB5	R Check	MR	HR	MR	MR	MR	MR	R	MR	MR	R	MR	R	HR	MR	R	1.00

[a] RD6 non-introgression line; [†] HR = highly resistant (lesion length < 1 cm); R = resistant (1 cm < lesion length < 3 cm); MR = moderately resistant (3 cm < lesion length < 6 cm); MS = moderately susceptible (6 cm < lesion length < 10 cm); S = susceptible (10 cm < lesion length).

The resistance of RD6 introgression lines as shown by lower BSR values than for IR62266 might be reflected in major and some minor quantitative trait locus (QTL) effects. Despite the $BC_2F_{2:3}$ introgression lines being selected by only the flanking markers associated with the major gene (*xa*5), the $BC_2F_{2:3}$ lines did not have the same BSR as IR62266. This might have been due to crossing over between the flanking markers RM122 and RM159. However, the lines $BC_2F_{2:3}$ 1-28-8-37 and $BC_2F_{2:3}$ 1-13-17-52 were most likely to have the same level of resistance to *Xoo* isolates as IR62266.

MAB with SSR flanking markers reduced the time needed for the evaluation of the genetic background of the recombinants by overcoming the need for year-round selection. There have been several reports on the successful utilization of MAB for the transfer of BLB resistance genes into elite rice varieties (Shanti et al., 2010; Rajpurohit et al., 2011; Deng et al., 2012; Pinta et al., 2013; Suh et al., 2013). In the present study, the introgression of the BLB resistance gene (*xa5*) from IR62266 into RD6 to obtain RD6 introgression lines using the MAB method was able to overcome potential obstacles when breeding for BLB resistance using conventional breeding methods, due to the recessive allele of the resistance gene from IR62266 (*xa5*). Selection using conventional strategies is limited in its output and requires an extra step of selfing to discard phenotypes associated with recessive genes. Therefore, breeders need to identify heterozygous genotypes that carry a recessive allele which conventional practice cannot identify. This study demonstrated that generally, the MAB approach is an effective strategy for the selection of recessive genes. However, to select only single flanking markers linked to major resistance genes can result in the loss of some minor genes that can result in the $BC_2F_{2:3}$ lines having less resistance than the resistant parent. Moreover, in this study all RD6 introgression lines produced slightly different lesion lengths to the *Xoo* isolates (Table 2) due to their differences in minor modifier genes for BLB resistance. Therefore, the MAB approach should be selected based on both major and minor genes, to overcome some major and minor gene/QTL effects.

Conflict of interest

The authors declare that there are no conflicts of interest.

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