

# Segregation analysis for bacterial leaf blight disease resistance genes in rice ‘MR219’ using SSR marker

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## ABSTRACT

Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* is one of the major hindrances in rice (*Oryza sativa* L.) production across the world including Malaysia. Therefore, the development of disease-resistant varieties remains a very economical and effective method of controlling BLB in rice. Based on this background, this study was conducted to analyze segregation pattern of simple-sequence repeat (SSR) markers associated with BLB resistance genes in an F<sub>2</sub> bulk population derived from a resistant variety (IRBB60) and a susceptible variety (MR219). Out of 129 simple sequence repeat (SSR) markers screened, 18 distinct polymorphism markers including R-gene based markers were used to screen 345 F<sub>2</sub> progenies for resistance to BLB. Among the polymorphic markers, the chi-square analyses showed that 15 SSR markers (i.e. RM13 (*xa5*), RM21 (*Xa21*), RM122 (*xa5*), RM153 (*xa5*), RM164 (*Xa13*), RM206 (*Xa10*), RM5509, RM20B, RM25, RM163, RM169, RM218, RM267, RM276, and RM334) had a segregation ratio of 1:2:1 for a single gene model (df = 2.0,  $p \leq 0.05$ ). For phenotypic ratio, the F<sub>2</sub> population segregated in ratio 3:1 (R:S) for resistant and susceptible plants, respectively. This indicated that resistance to BLB caused by pathotype *X. oryzae* pv. *oryzae* (*Xoo*) in the ‘MR219’ × ‘IRBB60’ F<sub>2</sub> population is controlled by single dominant genes. The result presented in this study will help breeders to further breeding research in rice by enabling selection based on the genotype rather than on the phenotype. Similarly, the markers reported in this study will serve as a valuable tool for marker-aided selection for BLB resistance gene.

**Key words:** Disease resistance, F<sub>2</sub> bulk population, *Oryza sativa*, rice bacterial leaf blight, simple sequence repeat.

## INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important staple food that constitutes a large portion of a world standard diet. In spite of its position among the highly rated crops, the geometric growth rate of the global population has called stepping up the current yield for this important crop (Oladosu et al., 2018). In Malaysia, it was estimated that individuals consume 80 kg rice per year which amounts to 26% of the total daily caloric intake. At the moment, the self-sufficient in rice stands at 67% while the remaining 33% shortfall under importation primarily from Pakistan, Vietnam, and Thailand. Despite the high level of government intervention on rice cultivation, the production output is not adding up, which is largely due to the susceptibility of local high yielding varieties to different diseases. The selection and hybridization for yield, yield component, and grain quality traits have led to improvement and release of new rice variety. However, this improvement has led to a gradual loss of genes responsible for biotic and abiotic tolerance. Similarly, the development of high N-receptive new rice varieties has a detrimental effect on pest and disease resistance. Among these diseases, bacterial

leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* is considered one of the most devastating that cause severe yield losses (Chukwu et al., 2019a). Infection of plants at an early stage can cause up to 50% yield while 20%-40% yield reduction was reported at the maximum tillering stage of infection (Chukwu et al., 2019a). In Malaysia, the recent BLB outbreak was reported to cause 50%-70% yield losses (Rafidah et al., 2018). These losses could jeopardize national food security and therefore, it is important to discover the solution to this problem.

This disease occurs at different growth stages and it is characterized by acute wilting of young plants. The pathogen causing leaf blight enters the plant through the wounded part of the plant or water pores. Lesions start as a small spot at the tip of the leaf with wavy margins as the water pores are located at the margins of upper parts of the leaf which later increase in size and gradually turn the leaf to yellow and later death of the plant (Yasmin et al., 2017). Various management strategies were conducted over the years to control this disease. In the past, various disease management strategies have been employed to avoid disease epidemics and reduce yield losses worldwide including Malaysia. Controlling BLB pathogen using chemical application and biological control is not very effective due to variation in sensitivity of pathogenic races. Additionally, due to the toxic residual effect, the use of chemical and antibiotics against BLB has been a major setback plant (Yasmin et al., 2017). Therefore, the development of resistant varieties is the economical, effective and eco-friendly strategy, for controlling this endemic pathogen to minimize disease incidence and yield losses (Chukwu et al., 2019b).

More than 32 bacterial blight resistance genes in a series from *Xa1* to *Xa34* has been discovered, identified and designated as reported by Ram et al. (2010). Marker-assisted selection (MAS) using simple sequence repeat (SSR) marker was selected as molecular markers for the present study due to its co-dominant nature which can detect both hetero- and homozygous alleles. SSR markers can be efficiently applied for developing unique DNA profiles of rice genotypes because have a high level of polymorphism and greater information (Chukwu et al., 2019b).

Because MR219 is the most cultivated rice variety in Malaysia, covering almost 70% to 90% of the cultivated areas as at the time of release in 2002 (Oladosu et al., 2014), efforts are constantly being made to increase its yield potentials. 'MR219' have good characteristics including high yield (7 to 10 t ha<sup>-1</sup>), average maturation period (105 to 111 d), intermediate amylose content (21.4%), long and slender grains and moderate resistance to blast, BLB and brown planthopper (BPH) (Bashar et al., 2014). Unfortunately, after years of intensive cultivation, this variety began to show more susceptibility to bacterial blight disease. While the donor parent 'IRBB60', carrying four BLB resistance genes namely *Xa4*, *xa5*, *xa13*, and *Xa21*, is widely grown rice variety developed by International Rice Research Institute, Philippines (IRRI, 2002). According to Shanti et al. (2010), 'IRBB60' was successful in the parental lines of hybrid rice KMR3 and PRR78. At the moment, BLB is a major problem in rice production in Malaysia, and no resistant variety is available for commercial cultivation. Hence, the objective of this study was to analyze the segregation pattern of SSR markers associated with BLB resistance genes in an F<sub>2</sub> bulk population derived from IRBB60 (resistant variety) and MR219 (susceptible variety).

## MATERIALS AND METHODS

### Plant materials

Three hundred and forty-five F<sub>2</sub> hybrid rice population derived from a cross between a local rice variety MR219 (susceptible variety) and IRBB60 (resistant variety) were genotyped with selected SSR markers associated with BLB resistance gene. 'MR219' was sourced from the gene bank at the Malaysian Agricultural Research and Development Institute (MARDI), while 'IRBB60' was collected from the International Rice Research Institute (IRRI), Philippines.

### Genomic DNA segregation

Three-week-old seedlings were used for the genomic DNA extraction, from where 0.5 g young leaf were excised from the growing plant. Modified CTAB method by Oladosu et al. (2015) was adopted in this experiment for the DNA extraction. Young leaves were cut into small pieces before grinding into fine powder using mortar and pestle in 0.3 to 0.4 mL liquid nitrogen and transferred into micro-centrifuge 2.0 mL Eppendorf tube. After that, 500 µL 2x CTAB buffer (40.91 g NaCl; 1 M Tris-HCl, pH 8.0; 0.5 M EDTA, and 100 µL β-mercaptoethanol) were added and mixed thoroughly. The samples were then incubated at 65 °C for 1 h in a water bath to breakdown the cell such as carbohydrate and protein. Then, the supernatant was transferred into a new tube and 500 µL chloroform-isoamyl alcohol (24:1) were added. The samples

were then centrifuged at 14 000 rpm for 5 min at 4 °C using centrifuge (5430 R, Eppendorf, Hamburg, Germany). The supernatant aqueous layer was then transferred to a new 2.0 mL Eppendorf tube and 500 µL isopropanol were added. The samples were again incubated at -20 °C overnight. After that, the samples were centrifuged at 14 000 rpm for 5 min at 4 °C and the isopropanol discarded and DNA pellet remain was washed with 500 µL 70% ethanol. Then, the pellets were air-dried at room temperature. The DNA pellet was dissolved in 100 µL TE buffer (1 M Tris-HCl, pH 8.0; and 0.5 M EDTA). Then, 1.0 µL RNase (10 mg mL<sup>-1</sup>) was added to remove the enzyme. The samples were incubated at 37 °C for 30 min and 10 µL (1/10) sodium acetate and 200 µL, 2 volume of absolute ethanol was added and stand overnight. Then, the samples were centrifuged at 14 000 rpm for 5 min. After that, the supernatant was discarded and the DNA pellets were again rinsed with 500 µL 70% ethanol and the pellets were air-dried for 1 h. The DNA pellet was then dissolved in 100 µL TE buffer before final centrifuged for 5 min at 5000 rpm. The DNA quality was checked using nano-drop spectrophotometer machine (NanoDrop 1000, Thermo Fisher Scientific, Waltham, Massachusetts, USA) and analyzed purity with 1% agarose gel by electrophoresis. After adjusting final concentrations to 50 ng µL<sup>-1</sup>, the stock solution was stored at -20 °C and the working solution at 4 °C.

### **Microsatellite DNA markers for SSR amplification**

A total of 129 primers sets from Gramene database ([www.gramene.org](http://www.gramene.org)) and previous studies associated with bacterial leaf blight resistance gene (Chu et al., 2006; Iyer-Pascuzzi and McCouch, 2007) were used for screening both parents to determine the efficient and polymorphic primers.

### **Polymerase chain reaction (PCR) and genotypic screening for marker segregation**

PCR reactions were performed in a total volume of 15 µL containing 2.0 µL genomic DNA, 1.0 µL (5 mM) of each forward and reverse primer, 3.0 µL (1 mM) dNTPs, 1.5 µL (5x) buffer (green go tag and colorless go taq), 0.8 µL (25 mM) MgCl<sub>2</sub>, 0.1 µL DNA Taq polymerase, and 4.1 µL nuclease-free water. The PCR products were amplified using Mastercycler gradient machine (Eppendorf) with an initial denaturing for 94 °C for 5 min followed by 35 cycles of the polymerization reaction with 30 s at 94 °C denaturing, 30 s primer annealing at either 54, 55 or 60 °C (depending on the primer pair), and 1 min at 72 °C for primer extension, followed by a final extension step at 72 °C for 1 min by rapid cooling to 4 °C prior to analysis. The PCR products were loaded on the gel electrophoresis machine, 5 µL of each PCR products were loaded on a 2.0% agarose gel stained with ethidium bromide in 1x TBE buffer. The gel was run at a constant voltage of 80 V for 1 h and gel picture was visualized under UV light and documented using the Bio-Rad molecular imager (Molecular Imager Gel Doc XR System, Bio-Rad Laboratories, Hercules, California, USA). The 100 bp DNA ladder was used for scoring of bands in each gel. The size bands scoring was done according to the size of the DNA ladder already defined by ChemiDoc with Image Lab Software (Bio-Rad). Based on the band obtained from the Gel Doc image analyzer, the progenies were scored with respect to similarity with the two-parent. The resistant parent (IRBB60) was scored (R) while the susceptible parent (MR219) was scored as (S), however, heterozygote progenies are scored as (H) which indicated genotypic resemble with both 'IRBB60' and 'MR219'.

### **Phenotypic screening in the F<sub>2</sub> population**

Field evaluation and screening of F<sub>2</sub> were carried out after 60 d of transplanting. The leaf blades were inoculated with the BLB pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) following the protocol described by Kauffman et al. (1973), where 3 cm below the leaf tips were cut using scissors. For inoculation, the inoculum of high virulence pathogen (pathotype *Xoo* P<sub>7.0</sub>) was used in this study provided by Pest and Diseases Laboratory in MARDI Seberang Perai, Penang. The pathotype was cultured 48 h before inoculation. Scoring and recording were done 30 d after inoculation. The plants were evaluated for BLB resistance by measuring the length of lesions and the leaf length based on the Standard Evaluation System (SES) recommended by IRRI. Data were recorded as resistant if lesion length was less than 5 cm and described as susceptible if lesion length was more than 5 cm.

### **Statistical analysis**

Data for the segregation pattern were tested using chi-square ( $\chi^2$ ) analysis. This analysis for the phenotypic and genotypic ratio was calculated using the chi-square formula,  $\chi^2 = (O - E)^2/E$ , where  $E$  is expected value and  $O$  is the observed value

(Ashkani et al., 2011). For the single-gene model, it was considered as significant ( $p \leq 0.05$ ) if chi-square value was greater than 5.99 (df = 2) for genotypic, and 3.84 (df = 1) for phenotypic ratio. The genotypic ratio by chi-square analysis was tested against the expected Mendelian ratio (1:2:1), while the phenotypic ratio for the goodness fit of 3:1.

## RESULTS AND DISCUSSION

### Microsatellite DNA marker screening of parental lines and F<sub>2</sub> population

Out of 129 SSR primers screened only 42 (33%) markers showed clear polymorphism between BLB resistant and susceptible to parental lines. Among 42 primers, 18 best polymorphic SSR markers including gene-based markers were evaluated on the 345 F<sub>2</sub> progenies derived from MR219 × IRBB60 (Table 1). The patterns of all the markers were similar to segregation ratio of 1:2:1 except for 3 markers (RM400, RM264, and RM281). Figure 1 showed the banding pattern of polymorphic SSR markers related to BLB genes, RM206 with 22 samples and two parents. Band photo was taken after running the gel electrophoresis on 2% agarose gel stained with ethidium bromide.

### Marker segregation data analysis

The observed segregation ratio for BLB resistance and susceptibility in F<sub>2</sub> progenies for 18 polymorphic microsatellite DNA markers is presented in Table 2. The chi-square analysis ( $\chi^2$ ) showed a good fit to expected Mendelian segregation ratio (1:2:1) for 15 SSR markers, RM13, RM21, RM122, RM153, RM164, RM206, RM5509, RM20B, RM25, RM163,

**Table 1. Information on the 18 best polymorphic SSR markers used for segregation study in rice.**

| Nr | SSR primers            | Chr | Primer sequence (forward)   | Primer sequence (Reverse)   | Expected size (bp) |
|----|------------------------|-----|-----------------------------|-----------------------------|--------------------|
| 1  | RM164 ( <i>Xa13</i> )  | 5   | F: TCTTGCCCGTCACTGCAGATATCC | R: GCAGCCCTAATGCTACAATTCTTC | 246                |
| 2  | RM206 ( <i>Xa10</i> )  | 11  | F: CCCATGCGTTTAACTATTCT     | R: CGTCCATCGATCCGATATGG     | 147                |
| 3  | RM21 ( <i>Xa21</i> )   | 11  | F: ACAGTATTCCGTAGGCACGG     | R: GCTCCATGAGGGTGGTAGAG     | 157                |
| 4  | RM153( <i>xa5</i> )    | 5   | F: GCCTCGAGCATCATCATCAG     | R: ATCAACCTGCACTTGCTGG      | 201                |
| 5  | RM400 ( <i>xa33</i> )  | 6   | F: ACACCAGGCTACCCAACTC      | R: CGGAGAGATCTGACATGTGG     | 321                |
| 6  | RM13 ( <i>xa5</i> )    | 5   | F: TCCAACATGGCAAGAGACAG     | R: GGTGGCATTCCGATCCAG       | 141                |
| 7  | RM5509 ( <i>xa33</i> ) | 6   | F: GATGATCCATGCTTTGGCC      | R: TTCCAGCAGAAAGAAGACGC     | 255                |
| 8  | RM122 ( <i>xa5</i> )   | 5   | F: GAGTCGATGTAATGTCATCAGTGC | R: GAAGGAGGTATCGCTTTGTTGGAC | 227                |
| 9  | RM20B                  | 11  | F: ATCTTGTCCTGCAGGTCAT      | R: GAAACAGAGGCACATTTCATG    | 140                |
| 10 | RM25                   | 8   | F: GGAAAGAATGATCTTTTCATGG   | R: CTACCATCAAAACCAATGTTC    | 146                |
| 11 | RM163                  | 5   | F: ATCCATGTGCGCCTTTATGAGGA  | R: CGCTACCTCCTTCACTTACTAGT  | 124                |
| 12 | RM169                  | 5   | F: TGGCTGGCTCCGTGGGTAGCTG   | R: TCCCGTTGCCGTTTATCCCTCC   | 167                |
| 13 | RM218                  | 3   | F: TGGTCAAACCAAGGTCCTTC     | R: GACATACATTCTACCCCGG      | 148                |
| 14 | RM264                  | 8   | F: GTTGCGTCTACTGCTACTTC     | R: GATCCGTGTCGATGATTAGC     | 178                |
| 15 | RM267                  | 5   | F: TGCAGACATAGAGAAGGAAGTG   | R: AGCAACAGCACAACTTGATG     | 156                |
| 16 | RM276                  | 6   | F: CTCAACGTTGACACCTCGTG     | R: TCCTCCATCGAGCAGTATCA     | 149                |
| 17 | RM281                  | 8   | F: ACCAAGCATCCAGTGACCAG     | R: GTTCTTCATACAGTCCACATG    | 138                |
| 18 | RM334                  | 5   | F: GTTCAGTGTTGAGTCCACC      | R: GACTTTGATCTTTGGTGGACG    | 182                |

**Figure 1. SSR primer of RM206 showing the banding patterns in F<sub>2</sub> progenies derived from cross MR219 × IRBB60 linked to bacterial leaf blight (BLB) resistance genes.**



L: 100 bp ladder, S: susceptible to BLB, R: resistant to BLB, H: heterozygous, 1-22 lanes: F<sub>2</sub> progenies.

RM169, RM218, RM267, RM276, and RM334, for a single gene model ( $df = 2.0, p \leq 0.05: 5.99$ ). Other markers accept the hypothesis with significant difference ( $p \leq 0.05$  and  $p \leq 0.01$ ) and did not fit the expected segregation ratios. From previous studies, McCouch et al. (1996) revealed two microsatellites markers RM122 and RM13 related to *xa5* resistance gene. Other study on blast also supported with finding whereas genetic analyses on polymorphic simple sequence repeat (SSR) markers on F<sub>2</sub> population derived from the cross of Pongsu Seribu 2 (resistant) and Mahsuri (susceptible) with good fit of 1:2:1 ratio for single-gene model (Ashkani et al., 2012).

### Phenotypic screening for BLB resistance in F<sub>2</sub> population

Scoring data for the length of lesion (LL) for BLB of F<sub>2</sub> plants were scored according to Kauffman et al. (1973) while for percentage diseased leaf area (%DLA) were categorized according to Standard Evaluation System (SES) from IRRI (2002). Pathogenicity assay in Table 3 showed the expected and observed segregation ratios of resistant and susceptible plants in the F<sub>2</sub> population inoculated with pathotype *Xoo* P<sub>7.0</sub>. The results revealed that F<sub>2</sub> population with  $\chi^2$  1.18 and 0.3055 probability segregated in a 3:1 (R:S) ratio for resistant and susceptible plants followed a single gene model ( $df = 1.0, p \leq 0.05$ ), respectively. This indicates that resistance to BLB caused by pathotype *Xoo* P<sub>7.0</sub> in F<sub>2</sub> population was most likely controlled by single dominant genes. This result agreed with the previous study in blast disease resistance genes in a segregating F<sub>2</sub> population of rice (Ashkani et al., 2011). In previous studies, two or more BLB R-gene were found very effective method against BLB isolates from the rice-growing region of the world (Chukwu et al., 2019a). Similarly, studies revealed that pyramiding lines of 'IRBB60' are resistant to all the Philippines races and the Punjab isolates of *Xoo* (Singh et al., 2001).

**Table 2. Marker analysis in F<sub>2</sub> progenies derived from crossing between MR219 × IRBB60.**

| Markers               | Chromosome | Marker analyzed |        |        | $\chi^2(1:2:1)$ | Probability |
|-----------------------|------------|-----------------|--------|--------|-----------------|-------------|
|                       |            | RR = R          | Rr = H | rr = S |                 |             |
| RM13 ( <i>xa5</i> )   | 5          | 93              | 175    | 77     | 1.56            | 0.4584      |
| RM21 ( <i>Xa21</i> )  | 11         | 90              | 176    | 79     | 0.84            | 0.6570      |
| RM122 ( <i>xa5</i> )  | 5          | 92              | 155    | 98     | 3.76            | 0.1526      |
| RM153 ( <i>xa5</i> )  | 5          | 75              | 181    | 89     | 1.97            | 0.3734      |
| RM164 ( <i>Xa13</i> ) | 5          | 100             | 172    | 73     | 4.23            | 0.1206      |
| RM206 ( <i>Xa10</i> ) | 11         | 90              | 176    | 79     | 0.84            | 0.6570      |
| RM400                 | 6          | 69              | 193    | 83     | 6.01*           | 0.0495      |
| RM5509                | 6          | 74              | 189    | 82     | 3.53            | 0.1712      |
| RM20B                 | 11         | 95              | 174    | 76     | 2.12            | 0.3465      |
| RM25                  | 8          | 80              | 179    | 86     | 0.69            | 0.7047      |
| RM163                 | 5          | 96              | 174    | 75     | 2.58            | 0.2753      |
| RM169                 | 5          | 90              | 172    | 83     | 0.29            | 0.8653      |
| RM218                 | 3          | 77              | 183    | 85     | 1.65            | 0.4382      |
| RM264                 | 8          | 90              | 192    | 63     | 8.63**          | 0.0134      |
| RM267                 | 5          | 90              | 160    | 95     | 1.96            | 0.3753      |
| RM276                 | 6          | 97              | 157    | 91     | 2.99            | 0.2242      |
| RM281                 | 8          | 92              | 190    | 63     | 8.43**          | 0.0148      |
| RM334                 | 5          | 84              | 178    | 83     | 0.36            | 0.8353      |

\*, \*\*Significant at the 0.05 and 0.01 probability levels, respectively ( $df = 2.0; \chi^2 = 5.99$ ).

R: Resistant; S: susceptible; H: segregant; RR: plants with a banding pattern alike to the resistant parent alleles; Rr: heterozygous plants; rr: plants with a banding pattern alike to the susceptible parent alleles;  $\chi^2$ : actual value of the chi-square test for resistant/susceptible ratio.

**Table 3. Expected and observed segregation ratios of resistant and susceptible plants in F<sub>2</sub> population inoculated with pathotype *Xoo* P7.0 of *Xanthomonas oryzae* pv. *oryzae* for the genetic cross between MR219 × IRBB60.**

| Generation     | Reaction        | Pathogenicity test |                | $\chi^2(3:1)$ | P      |
|----------------|-----------------|--------------------|----------------|---------------|--------|
|                |                 | Expected Nr        | Observation Nr |               |        |
| F <sub>2</sub> | Resistance (R)  | 259                | 250            | 0.29          |        |
|                | Susceptible (S) | 86                 | 95             | 0.89          |        |
|                | Total plant     | 345                | 345            | 1.18          | 0.3055 |

$df = 1.0; \chi^2 = 3.84$ .

To have a comprehensive understanding of the molecular mechanism for broad-spectrum resistance to rice BLB and for further confirmation report result, additional genetics studies are needed to investigate whether quantitative and qualitative genes affect the level of the resistance in rice 'IRBB60'. As reported by Ashkani et al. (2011), application of MAS in rice have shown that the use of tightly linked DNA markers to targeted genes can be identified more efficiently in a segregating population at any plant growth stage. Molecular MAS background analysis of segregates is convenient in determining the relation contribution of the progenitor parents. In addition, the finding of Sanchez et al. (2000) revealed the effectiveness of MAS in gene pyramiding for BLB R-gene, mostly for recessive genes, such as *xa13* and *xa5* that are challenging to select as compared with the conventional background in the existence of a dominant gene such as *Xa21*. Hence, pyramiding multiple resistance genes in a single rice cultivar is recommended as a strategy to delay or prevent the breakdown of resistance. The disposition of rice varieties that have multiple BLB R-genes is predictable to lead to more durable resistance (Salgotra et al., 2012). As reported by Chukwu et al. (2019b), the possibility of coincident pathogen transformations for virulence to overcome the resistance conferred by two or more effective genes is much lower than for a single gene.

## CONCLUSIONS

In this study, genotypic analyses on 18 polymorphic SSR markers were identified. Among the polymorphic markers, the chi-square analyses showed that 15 SSR markers had a segregation ratio of 1:2:1. Phenotypic data based on disease reaction of resistance and susceptibility to bacterial leaf blight (BLB) *Xanthomonas oryzae* pv. *oryzae* pathotype *Xoo* P<sub>7.0</sub> also segregated in a 3:1 (R:S) ratio in the F<sub>2</sub> population. Therefore, resistance to BLB pathotype *Xoo* P<sub>7.0</sub> in 'IRBB60' is more likely controlled by a single dominant gene. These markers RM13 (*xa5*), RM21 (*Xa21*), RM122 (*xa5*), RM153 (*xa5*), RM164 (*Xa13*), RM206 (*Xa10*), RM5509, RM20B, RM25, RM163, RM169, RM218, RM267, RM276, and RM334 offer a potential, fast, easy and consistent method in MAS for screening BLB in future breeding programs. Molecular marker analysis with SSR markers gives a quick evaluation of the genetic background of the recombinant. This is a successful example of applying an integrated approach to plant breeding. MAS was used for identifying plants with multiple BLB R-genes and conventional phenotypic selection for recovering 'MR219' quality traits. In addition, SSR based background analysis helped in determining the amount of 'MR219' alleles in the combination that influenced desirable traits from 'IRBB60', such as BLB resistance and increase the grain yield. These studies are expected to have a high impact on the yield steadiness and sustainability of Malaysia rice production.

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