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Low-cost high-resolution genotyping assay for detecting multiple biotic stress resistance genes in rice (*Oryza sativa*)

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Abstract

Improving rice, a major crop that feeds more than half of the world's population, has become increasingly important in enhancing food security in a rapidly changing climate. Global warming is known to exacerbate the negative effects of biotic stresses in rice, notably bacterial leaf blight (BLB), sheath blight (SB), and blast caused by the pathogens *Xanthomonas oryzae*, *Rhizoctonia solani*, and *Magnoporthe oryzae*, respectively. Over the last few decades, gel- and capillary-based electrophoresis have been among the most commonly used techniques for screening rice genotypes for biotic stresses. However, contradictory findings from past studies have resulted in an inconclusive comparison of these techniques, including their cost and effectiveness in genotyping rice diseases in the developing world. The present study sought to identify the suitable allele-specific markers for multiplex genotyping of gene variants associated with BLB, SB, and blast, and to compare the cost and feasibility between gel and capillary electrophoresis-based genotyping methods. Using NCBI databases, 32 allele-specific markers were identified and used to screen 14 selected rice genotypes with varying levels of BLB, SB, and blast resistance. Both gel and capillary electrophoresis were used to analyse the amplification products, and the results were validated through sequencing. While capillary electrophoresis provided greater discriminatory power, we found that high-resolution gel agarose electrophoresis was less expensive and more practical for simultaneous screening of BLB, SB, and blast resistance gene variants in rice varieties. The low-cost yet high-resolution genotyping assay developed in this study is particularly useful for large-scale genotyping in laboratories with limited resources.

Keywords: Biotic stress resistance; capillary electrophoresis; genotyping; high-resolution agarose gel electrophoresis; rice; sequencing.
Abbreviations: BLB_bacterial leaf blight; R_resistant; S_susceptible; SB_sheath blight.

Introduction

Rice (*Oryza sativa*) is central to the food security of approximately half of the entire world's population (Hanafiah et al., 2020). The rapid intensification of rice production in recent decades, particularly in Asia, is considered a major agricultural change that often makes the crop more vulnerable to biotic stress (Séré et al., 2013; Bangratz et al., 2020). Prior work estimated that an average of 30% of the global loss in rice yield is due to pathogens and pests (Savary et al., 2019). Major rice diseases across the world include bacterial leaf blight (BLB), sheath blight (SB), and blast caused by the pathogens *Xanthomonas oryzae* (Hajira et al., 2016), *Rhizoctonia solani* (Yu et al., 2017), and *Magnoporthe oryzae* (Wang et al., 2014), respectively. These pathogens pose major obstacles to rice production in Malaysia over the past two decades, although some promising genetic sources of resistance to their strains have been identified (Chukwu et al., 2019; Singh et al., 2019; NurulNahar et al., 2020). For example, the calcineurin-responsive zinc finger (CRaZy) transcription factor involved in *R. solani*-rice interaction during the establishment phase has been reported to regulate expression of pathogenicity-associated genes during host colonisation (Singh et al., 2019).

Polymerase chain reaction (PCR), one of the most common nucleic acid detection/amplification tests, is deemed the gold standard for detecting plant pathogens. It is characterized by high specificity, sensitivity, rapid detection, and high-throughput multiplex detection capability (Lau and Botella, 2017; Tahamtan and Ardebili, 2020). Multiplex PCR can amplify more than one target sequence in a single reaction, saving considerable time and effort, especially when analysing a large number of samples (Elnifro et al., 2000). Since its inception in 1988, multiplex PCR has come a long way and has been successfully employed in many gene-based selections in various crop improvement programs. Several multiplex PCR systems have been developed to detect key genes and markers associated with agronomic traits (Li et al., 2017), grain quality (Cheng et al., 2015), and

disease resistance (Hajira et al., 2016; Yap et al., 2016; Bangratz et al., 2020) in rice. It is important to note that simple sequence repeats (SSRs, or microsatellites) and single nucleotide polymorphisms (SNPs) are the two most commonly used PCR-based markers for genotyping and classifying rice varieties (Cheng et al., 2015; Chandra et al., 2017). Over the past decade, these markers have played an increasingly important role in molecular breeding of rice, with one of the recent central goals being the development of varieties with broad-spectrum resistance to pathogens (Ning and Wang, 2018; Meng et al., 2020).

To date, a handful of methods, including gel agarose electrophoresis and capillary electrophoresis (Zhang et al., 2018; Singh et al., 2020), are commonly used for analysing PCR amplification products. Since the 1970s, agarose gel electrophoresis has been the mainstay method for separating DNA fragments of moderate size of 50-500 bp (Stellwagen, 2009; Mitchenall et al., 2018). While traditional gel electrophoresis using standard agarose is considered a low-throughput technique with several other potential disadvantages (such as the need for DNA staining with ethidium bromide), the technique has been further enhanced with the development of specially formulated high-resolution agarose that can efficiently separate DNA fragments differing in small sizes (Cheng et al., 2015; Mitchenall et al., 2018). For example, the MetaPhor® agarose (Lonza) has demonstrated the capability to separate fragments that differ in size by 20 bp (Cheng et al., 2015). Furthermore, many alternative and comparatively safer DNA-staining reagents, such as SYBR-Green, have been produced to overcome the disadvantage of using traditional ethidium bromide staining of gel (Motohashi, 2019).

Apart from agarose electrophoresis, another versatile and effective analytical technique is capillary electrophoresis, which is capable of precisely and consistently reporting allele sizes with minimal manual intervention and least size error (Lau and Latif, 2019). Capillary electrophoresis has been commonly used in the past two decades for SSR and SNP detection in numerous rice studies (R. Vemireddy et al., 2007; Kim et al., 2016). A capillary-based SNP genotyping technique called the allele-specific primer (ASP) PCR was developed in the early 2010s with promising results in rice genotyping (Hirotsu et al., 2010; De Wever et al., 2019). Similar to agarose electrophoresis, capillary electrophoresis can be multiplexed, but it requires specialized equipment and fluorescently labelled probes, which are often costly (Aloui et al., 2015; Qi et al., 2019). This is perhaps the main reason why capillary electrophoresis has not been the predominant analytical method in small- or medium-scale laboratories where gel electrophoresis is readily available (Gupta et al., 2010; Mitchenall et al., 2018).

To aid rice breeding programs in resource-limited settings, particularly in developing countries such as Malaysia, it is vital to establish the means to effectively screen the targeted traits genes at the lowest possible cost. Although many resistance genes and linked markers have been reported for major rice diseases, it remains unclear whether these genes and markers are suitable for Malaysian varieties, and if capillary electrophoresis is, in practice, a better method than high-resolution gel electrophoresis for rice genotyping. The present study sought to identify known genes and markers that are suitable for simultaneously screening of BLB, SB, and blast resistance in local rice varieties via multiplex PCR, and to evaluate the efficiency and suitability of gel and capillary electrophoresis-based genotyping for rice improvement programs. The two genotyping methods were compared with simultaneous analysis of markers associated with BLB, SB, and blast resistance. Allele-specific PCR-gel-based markers were determined for the three targeted traits, and low-cost, reliable multiplex assays were developed to assist the rice breeding programs in Malaysia, and possibly elsewhere.

Results

Molecular markers selection and validation

The present study tested 32 sets of published allele-specific markers via uniplex PCR (Supplementary Table 1), in which three of them were used for multiplex analysis (Table 1). These selected primers, including RM6836, RM202, and pTA248, demonstrated functional polymorphisms on local checks (Fig. 1) and the amplified regions were comparable with previous studies (Hossain et al., 2016; Huang et al., 2017; Miah et al., 2017).

High-resolution agarose gel electrophoresis

The multiplex products of 14 selected genotypes (Table 2) were separated using a 4% high-resolution agarose gel for 3 h at 120 V (Fig. 1) (Cheng et al., 2015) and the electrophoresis results are presented in Table 2. Approximately 71%, 36%, and 71% of the varieties showed resistance to BLB, SB, and blast, respectively (Table 2). Based on the multiplex products of the local checks, the ~230 bp (RM6836 primer), ~160 bp (RM202 primer), and ~700 bp (pTA248 primer) amplicon denoted the presence of resistance alleles for blast, SB, and BLB, respectively. On the other hand, the ~250 bp (RM6836 primer), ~180 bp (RM202 primer), and ~600 bp (pTA248 primer) amplicon denoted the susceptible alleles for blast, SB, and BLB, respectively (Table 2).

Capillary electrophoresis and sequencing

Fig. 2 shows the electrophenograms obtained from fluorescence capillary analysis (Nguyen et al., 2013; Petree and Varshney, 2020) of HEX-RM6836 and NED-RM202 for local checks MR219 (Fig. 2A), Mahsuri Mutant (Fig. 2B), and Pulut Hitam 9 (Fig. 2C). The results from capillary electrophoresis were comparable with the analysis from high-resolution gel electrophoresis (Table 3). For example, the product sizes of MR219 labelled with HEX-RM6836 (247.08 bp) and NED-RM202 (185.18 bp) (Fig. 2A) were similar to the products sizes shown on the high-resolution agarose gel. It is important to note that pTA248 was being excluded in capillary electrophoresis genotyping because the method is only feasible for amplicons with size smaller than 500 bp.

Sequencing

To validate the results from electrophoresis-based genotyping, PCR products of local checks were sequenced. The sequencing results confirmed the presence or absence of resistance genes in these varieties demonstrated by both high-resolution agarose gel and capillary analyses (Table 2 and 3; Supplementary Table 2). Two motifs, $(TCT)_7$ and $(TCT)_{15}$, were detected from the amplified products using marker RM6836, which represented resistance and susceptibility alleles for blast disease, respectively (Miah et al., 2017). Similarly, two motifs, $(CT)_{16}$ and $(CT)_{28}$, detected from the amplified products using RM202 were associated

Table 1. Details of molecular markers and local checks employed for multiplex PCR analysis.

Resistance trait	Locus/loci	Chr	Marker name	Marker type	Tm (^{0}C)	Local check (Resistant)	Local check (Susceptible)
Blast	*Piz*, *Pi2*, *Pi9*	6	RM6836	SSR	55	Mahsuri Mutant	MR219
SB	qSBR11-3, QRlh11	11	RM202	SSR	55	Pulut Hitam 9	MR219
BLB	*Xa21*	11	pTA248	STS	55	MR219	Ria

BLB-bacterial leaf blight; Chr-chromosome; SB-sheath blight; SSR-simple sequence repeat; STS-sequence-tagged site, Tm- annealing temperature.

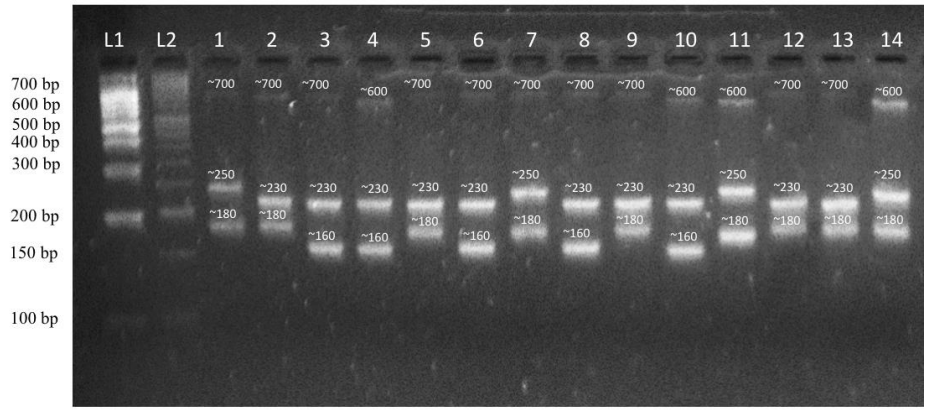

Fig 1. Amplified multiplex products from pTA248, RM6836 and RM202 markers associated with bacterial leaf blight [~600 bp (susceptible); ~700 bp (resistance)], blast [~230 bp (resistance); ~250 bp (susceptible)], and sheath blight [~160 bp (resistance); ~180 bp (susceptible)] resistance genes, respectively. Products separated using 4% high-resolution agarose gel electrophoresis at 120 V for 3 h. L1: 100 bp, L2: 50 bp ladder, 1: MR219, 2: Mahsuri Mutant, 3: Pulut Hitam 9, 4: Ria, 5: MR167, 6: MR185, 7: MR220, 8: MR106, 9: MRQ74, 10: Pulut Malaysia 1, 11: Biris, 12: IR8, 13: Chianung Sen Yu, 14: Tainan 3.

Table 2. Description of uniplex and multiplex results of 14 selected genotypes.

Variety	Uniplex analysis						Multiplex analysis
	Blast		SB		BLB		
	RM6836 (bp)	S/R	RM202 (bp)	S/R	pTA248 (bp)	S/R	RM6836; RM202; pTA248 (bp)
MR219	~250	S	~180	S	~700	R	~250; ~180; ~700
Mahsuri Mutant	~230	R	~180	S	~700	R	~230, ~180; ~700
Pulut Hitam 9	~230	R	~160	R	~700	R	~230; ~160; ~700
Ria	~230	R	~160	R	~600	S	~230; ~160; ~600
MR167	~230	R	~180	S	~700	R	~230; ~180; ~700
MR185	~230	R	~160	R	~700	R	~230; ~160; ~700
MR220	~250	S	~180	S	~700	R	~250; ~180; ~700
MR106	~230	R	~160	R	~700	R	~230; ~160; ~700
MRQ74	~230	R	~180	S	~700	R	~230; ~180; ~700
Pulut Malaysia 1	~230	R	~160	R	~600	S	~230; ~160; ~600
Biris	~250	S	~180	S	~600	S	~250; ~180; ~600
IR8	~230	R	~180	S	~700	R	~230; ~180; ~700
Chianung Sen Yu	~230	R	~180	S	~700	R	~230; ~180; ~700
Tainan 3	~250	S	~180	S	~600	S	~250; ~180; ~600

BLB- bacterial leaf blight; bp- base pair; R- resistant; S- susceptible; SB- sheath blight.

Table 3. Results from fluorescence capillary electrophoresis and sequencing.

Capillary electrophoresis				Sequencing			
HEX-RM6836 (bp)	Blast (S/R)	NED-RM202	SB (S/R)	RM6836 $(TCT)_n$	Blast (S/R)	RM202 $(CT)_n$	SB (S/R)
247.08	S	185.18	S	15	S	28	S
226.64	R	187.06	S	7	R	28	S
226.69	R	164.80	R	7	R	16	R

bp- base pair; R- resistant; S- susceptible; SB- sheath blight.

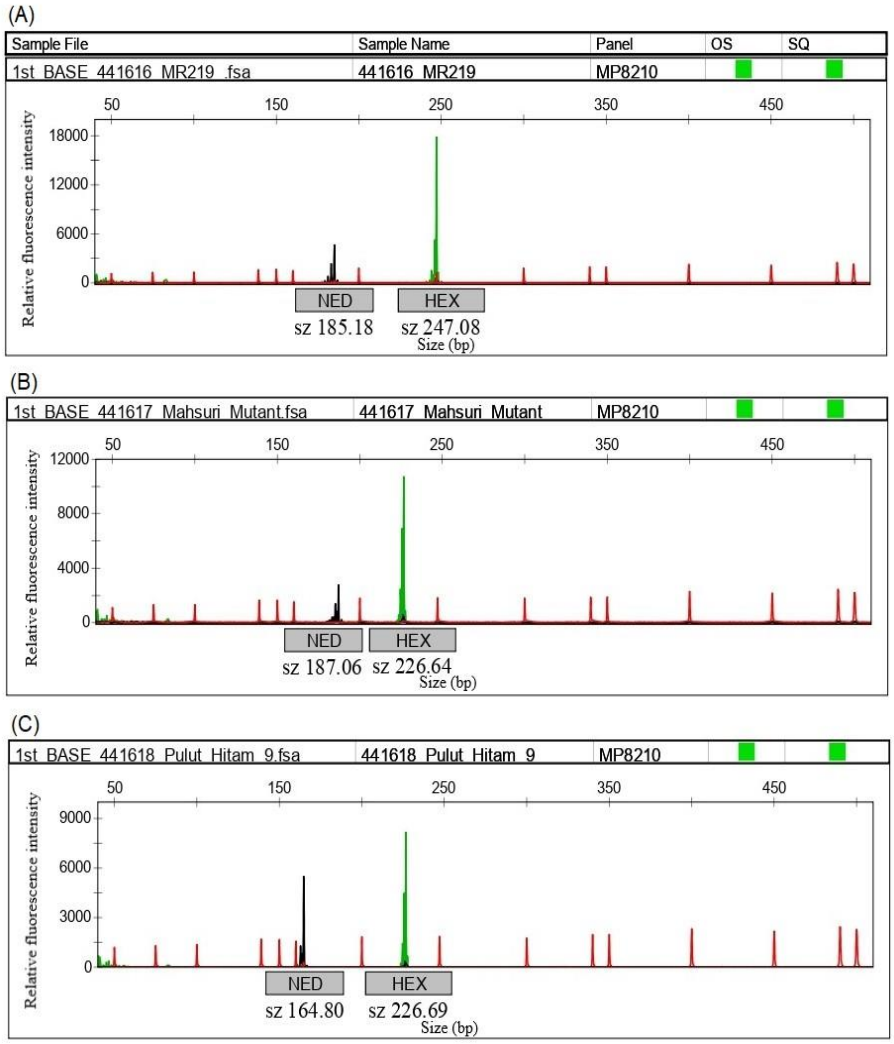

Fig 2. Electropherogram results from capillary electrophoresis for local checks (a) MR219, (b) Mahsuri Mutant and (c) Pulut Hitam 9. Green (HEX) and black (NED) peaks result from the products of RM6836 and RM202 primers, respectively. The red peaks represent internal size standard 500-ROX, while the x- and y-axis represent size of the PCR products in bases and relative fluorescence intensity, respectively.

with resistance and susceptibility to SB disease, respectively (Hossain et al., 2016) (Table 3).

Discussion

During the past decade, crop losses in rice ecosystems of Asia have been largely caused by sheath blight (e.g. 8.75% in China) and bacterial blight (e.g. 8.51% in the Indo-Gangetic Plain) diseases (Savary et al., 2019). Blast is another major rice disease, which is the leading production constraint in Malaysia (NurulNahar et al., 2020). Recent reports suggested that huge grain losses occur mainly due to multiple pests and diseases, which are particularly severe in developing countries (Lau and Botella, 2017; Mesterházy et al., 2020). One of the best means to enhance disease resistance in rice or crops in general is by transferring targeted genes from a resistant variety into an elite variety using functional markers linked to resistance genes in an advanced molecular breeding programme such as gene pyramiding (Ramkumar et al., 2015).

To identify suitable allele-specific markers for BLB, SB, and blast resistance genes for local rice breeding programmes, the present study screened a total of 32 reported markers (Supplementary Table 1) that have been successfully employed in several recent rice breeding programmes in Asia (Hajira et al., 2016; Hossain et al., 2016; Miah et al., 2017; Kadu et al., 2018). Of these, three most suitable markers (RM6836, RM202, and pTA248) linked to genes conferring resistance to each of the targeted rice diseases were selected for multiplex analyses. The main selection criteria include biological (i.e., involvement of resistant and susceptible checks), technical (i.e., functional polymorphisms on local checks) and practical (i.e., effectiveness in breeding programmes) aspects (Platten et al., 2019). These markers also were selected for multiplexing because they produced bands of different sizes (Table 2). According to Miah et al. (2017), RM6836 is tightly linked to the three R-genes (*Pi2*, *Pi9* and *Piz*) located on chromosome 6, which have broad-spectrum resistance to *M. oryzae* that causes rice blast. The marker RM202, associated with SB, was reported to be linked to the *qSBR11-3* and *QRlh11* loci on chromosome 11 (Hossain et al., 2016). Similarly, the marker pTA248 selected for BLB resistance is also located on chromosome 11. It is an STS marker tightly linked to the *Xa21* gene that confers resistance to various races of *X. oryzae pv. oryzae*. A handful of recent breeding programmes

are known to utilize pTA248, (Balachiranjeevi et al., 2018; Nguyen et al., 2018), indicating that this marker is effective and relevant in developing more BLB resistant rice varieties.

The multiplex results obtained from high-resolution agarose gel electrophoresis followed by sequencing analyses and capillary electrophoresis in this study were comparable and consistent with previous studies (Table 2 and 3). The capillary electrophoresis genotyping was proved to be more sensitive than the agarose gel-based electrophoresis because it can separate PCR products that differed by less than 5 bp (Stewart et al., 2011; Aloui et al., 2015). However, the cost of utilizing capillary electrophoresis is much higher than high-resolution agarose gel-based electrophoresis to obtain similar results, especially when conventional capillary electrophoresis involves the use of expensive equipment and fluorescent-labelled primers (Pan et al., 2018). It should be noted that the same high-resolution agarose gel can be easily reused for up to 15 times without compromising the quality of the results.

In this study, each sample analysed by the high-resolution agarose gel electrophoresis costed approximately $0.30 (when the same gel was reused for ten times) as compared to $7.60 for capillary electrophoresis. It is worth noting that the cost of sequencing per sample costed about $6.00. Based on the results, we recommend the local breeding programmes with limited budget but with inexpensive human resources utilize high-resolution agarose gel-based multiplex assay for markers to develop local rice varieties that are resistant to major rice diseases, including BLB, SB, and blast. This practical assay is also useful to researchers who are involved in mass genotyping or work in laboratories with limited resources in terms of modern research infrastructure. Nonetheless, it is important to note that this technique is more tedious and requires more man hours in comparison to capillary electrophoresis.

Materials and Methods

Materials

A total of 10, 13, and 9 allele-specific markers associated with BLB, SB, and blast resistance, respectively, were identified and selected from literature using PubMed and Gene in NCBI databases (https://www.ncbi.nlm.nih.gov). The main selection criteria included their inclusion in previous rice breeding programs and the degree of polymorphism, and the availability of rice genotyping data in Gramene database (https://www.gramene.org). The details of the selected genes and primers are presented in Supplementary Table 1. All primers were synthesized by Integrated DNA Technologies (IDT Inc, Malaysia). Seeds of rice varieties used in this study, including local checks MR219, Mahsuri Mutant, and Pulut Hitam 9 (Table 1), were obtained from the Malaysian Agricultural Research and Development Institute (MARDI).

Nucleic acid extraction

Total genomic DNA was extracted from approximately 25 mg of leaves collected from 4-week-old rice plants using FavorPrep™ Plant Genomic DNA Extraction Mini Kit according to the manufacturer's protocol (Favorgen, Taiwan). The purity of the extracted DNA samples was determined using NanoDrop 2000 UV-Vis spectrophotometer (Thermofisher Scientific, USA), while the quality of the samples was assessed using a 1% agarose gel stained with SYBR® Safe DNA gel stain (Thermofisher Scientific, USA). The samples were electrophoresed in 1x TAE buffer for 30 min and visualized in the AlphaImager Mini Imaging System (ProteinSimple, USA).

Uniplex and multiplex PCR

Uniplex PCR was performed with all allele-specific markers (Supplementary Table 1), while multiplex PCR was performed with the tested markers that demonstrated functional polymorphisms between positive and negative control varieties for each targeted trait (Table 1). Each PCR cocktail for multiplexing was prepared using 10µl premix ready to use 2X Go-Taq Green PCR master mix (Promega, USA), 50 ng template DNA, and 0.4µM each of forward and reverse primer. Amplifications were performed using the Veriti 96-well thermal cycler (Applied Biosystems, USA) following the thermal profile used in McCouch et al. (2002): initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 2 min, and a final extension at 72 °C for 5 min.

Agarose gel electrophoresis

Uniplex PCR products were electrophoresed on a 3% agarose gel, stained SYBR® Safe DNA gel stain (Thermofisher Scientific, USA) and visualized as described for genomic DNA. On the other hand, a 4% high-resolution agarose gel was used to separate multiplex PCR products. The 4% agarose high-resolution gel and 3% agarose gel were prepared by mixing 10 g of high-resolution agarose (Gene Xpress, Malaysia) and 7.5 g of standard agarose (Hydragene, USA) in 250 ml of 1xTAE buffer for resolving multiplex and uniplex PCR products, respectively. PCR products were electrophoresed for 3 h at 120 V. The size of the amplicons was estimated using 50 bp (SMOBIO, Taiwan) and 100 bp DNA size markers (Bioron, Germany).

Capillary electrophoresis

Based on the results of high-resolution gel electrophoresis, the multiplex products of three rice varieties (MR219, Mahsuri Mutant, and Pulut Hitam 9) representing a broad range of genotypes (Table 1) were used to analyse the allelic variation of RM202 (associated with SB) and RM6836 (associated with blast) using fluorescence capillary electrophoresis. For each pair of primers, the forward primer was selected to be labelled with NED or HEX dye at the 5' end. The products were separated using an ABI3100 Genetic Analyzer (Applied Biosystems, USA), and the results were analysed using the GeneMapper 4.0 software (Applied Biosystems, USA). To validate the results obtained from both high-resolution gel and capillary electrophoresis, a set of gel products amplified using primers RM202 and RM6836 were sequenced on an ABI3100 DNA sequencer (Applied Biosystems, USA) in a commercial sequencing facility.

Conclusion

The successful development of a low-cost yet high-resolution genotyping assay using gel-based electrophoresis from the current study allows functional polymorphisms BLB, SB, and blast resistance genes to be analysed simultaneously. This would play an important role in fuelling the development of rice varieties with improved resistance, especially for laboratories with limited resources in developing or underdeveloped regions.

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References

Acharya A, Adhikari N, Amgain RB, Poudel A, Yadav R, Poudyal K (2018) Identification of rice genotypes resistant to bacterial leaf blight disease using SSR markers. J Inst Agr Anim Sci. 35(1):113-120.

Aloui C, Sut C, Cognasse F, Granados V, Hassine M, Chakroun T, Garraud O, Laradi S (2015) Development of a highly resolutive method, using a double quadruplex tetra-primer-ARMS-PCR coupled with capillary electrophoresis to study CD40LG polymorphisms. Mol Cell Probes. 29(6):335-342.

Ashkani S, Rafii MY, Sariah M, Siti NAA, Rusli I, Harun AR, Latif MA (2011) Analysis of simple sequence repeat markers linked with blast disease resistance genes in a segregating population of rice (*Oryza sativa*). Genet Mol Res. 10(3):1345-1355.

Balachiranjeevi CH, Bhaskar Naik S, Abhilash Kumar V, Harika G, Mahadev Swamy HK, Hajira SK, Dilip Kumar T, Anila M, Kale RR, Yugender A, Pranathi K, Koushik MBVN, Suneetha K, Bhadana VP, Hariprasad AS, Laha GS, Rekha G, Balachandran SM, Madhav MS, Senguttuvel P, Fiyaz AR, Viraktamath BC, Giri A, Swamy BPM, Jauhari Ali, Sundaram RM (2018) Marker-assisted pyramiding of two major, broad-spectrum bacterial blight resistance genes, *Xa21* and *Xa33* into an elite maintainer line of rice, DRR17B. PLoS one. 13(10):e0201271.

Bangratz M, Wonni I, Kini K, Sondo M, Brugidou C, Béna G, Gnacko F, Barro M, Koebnik R, Silué D, Tollenaere C (2020) Design of a new multiplex PCR assay for rice pathogenic bacteria detection and its application to infer disease incidence and detect co-infection in rice fields in Burkina Faso. PLoS one. 15(4):e0232115.

Chandra H, Yadava YK, Bishnoi P, Kumar P (2017) Genetic diversity analysis using microsatellite based simple sequence repeats (SSR) markers in Basmati *indica* rice. *Oryza sativa*. 3.

Cheng A, Massawe F, Ismail I, Osman M, Hashim H (2015) High resolution agarose-based system for single-tube genotyping of *fgr* and *Waxy* genes in rice: MAGE to displace PAGE? Plant Omics. 8(4):348-352.

Chukwu SC, Rafii MY, Ramlee SI, Ismail SI, Hasan MM, Oladosu YA, Magaji UG, Akos I, Olalekan KK (2019) Bacterial leaf blight resistance in rice: a review of conventional breeding to molecular approach. Mol Biol Rep. 46(1):1519-1532.

De Wever J, Everaert H, Coppieters F, Rottiers H, Dewettinck K, Lefever S, Messens K (2019) The development of a novel SNP genotyping assay to differentiate cacao clones. Sci Rep. 9(1):1-10.

Elnifro EM, Ashshi AM, Cooper RJ, Klapper PE (2000) Multiplex PCR: optimization and application in diagnostic virology. Clin Microbiol Rev. 13(4):559-570.

Fu C, Wu T, Liu W, Wang F, Li J, Zhu X, Huang H, Liu ZR, Liao Y, Zhu M, Chen J, Huang Y (2012) Genetic improvement of resistance to blast and bacterial blight of the elite maintainer line Rongfeng B in hybrid rice (*Oryza sativa* L.) by using marker-assisted selection. Afr J Biotechnol. 11(67):13104-13114.

Gupta V, Dorsey G, Hubbard AE, Rosenthal PJ, Greenhouse B (2010) Gel versus capillary electrophoresis genotyping for categorizing treatment outcomes in two anti-malarial trials in Uganda. Malar J. 9(1):1-8.

Hajira SK, Sundaram RM, Laha GS, Yugander A, Balachandran SM, Viraktamath BC, Sujatha K, Balachiranjeevi CH, Pranathi K, Anila M, Bhaskar S, Abhilash V, Mahadevaswamy HK, Kousik M, Dilip Kumar T, Harika G, Rekha G (2016) A single-tube, functional marker-based multiplex PCR assay for simultaneous detection of major bacterial blight resistance genes *Xa2*1, *xa13* and *xa5* in rice. Rice Sci. 23(3):144-151.

Hanafiah NM, Mispan MS, Lim PE, Baisakh N, Cheng A (2020) The 21st century agriculture: When rice research draws attention to climate variability and how weedy rice and underutilized grains come in handy. Plants. 9(3):365.

Hirotsu N, Murakami N, Kashiwagi T, Ujiie K, Ishimaru K (2010) Protocol: a simple gel-free method for SNP genotyping using allele-specific primers in rice and other plant species. Plant Methods. 6(1):1-10.

Hossain MK, Jena KK, Bhuiyan MAR, Wickneswari R (2016) Association between QTLs and morphological traits toward sheath blight resistance in rice (*Oryza sativa* L.). Breed Sci.15154.

Huang Z, Young ND, Reagon M, Hyma KE, Olsen KM, Jia Y, Caicedo AL (2017) All roads lead to weediness: Patterns of genomic divergence reveal extensive recurrent weedy rice origins from South Asian *Oryza*. Mol Ecol. 26(12):3151-3167.

Kadu TP, Kale SS, Chavan NR, Agrawal T, Verulkar SB (2018) Pyramiding of three bacterial blight resistance genes in rice cultivar using marker assisted selection. Int J Bio-resource and Stress Manag. 9(2):243-248.

Kim S-R, Ramos J, Ashikari M, Virk PS, Torres EA, Nissila E, Hechanova SL, Mauleon R, Jena KK (2016) Development and validation of allele-specific SNP/indel markers for eight yield-enhancing genes using whole-genome sequencing strategy to increase yield potential of rice, *Oryza sativa* L. Rice. 9(1):1-17.

Lau HY, Botella JR (2017) Advanced DNA-based point-of-care diagnostic methods for plant diseases detection. Front Plant Sci. 8:2016.

Lau WCP, Latif MA (2019) Current breeding approaches for developing rice with improved grain and nutritional qualities. In: Qureshi A, Dar Z, Wani S, (eds) Quality breeding in field crops, pp. 199-216. Springer,Cham

Li JZ, Cao YR, Li MQ, Zhao WL, Sun HZ, Zhao QZ (2017) A multiplex PCR system for detection of wide compatibility allele *S5-n* and erect panicle allele *dep1* in rice. Crop Breed Appl Biotechnol. 17(3):250-258.

McCouch S, Teytelman L, Xu Y, Lobos KB, Clare K, Walton M, Fu B, Maghirang R, Li Z, Xing Y, Zhang Q (2002). Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Res. 9(6): 199-207.

Meng X, Xiao G, Telebanco-Yanoria MJ, Siazon PM, Padilla J, Opulencia R, Bigirimana J, Habarugira G, Wu J, Li M, Wang B, Lu G-D, Zhou B (2020) The broad-spectrum rice blast resistance (R) gene *Pita2* encodes a novel R protein unique from *Pita*. Rice. 13(1):1-15.

Mesterházy Á, Oláh J, Popp J (2020) Losses in the grain supply chain: Causes and solutions. Sustainability. 12(6):2342.

Miah G, Rafii MY, Ismail MR, Puteh AB, Rahim HA, Latif MA (2017) Marker-assisted introgression of broad-spectrum blast resistance genes into the cultivated MR219 rice variety. J Sci Food Agr. 97(9):2810-2818.

Mitchenall LA, Hipkin RE, Piperakis MM, Burton NP, Maxwell A (2018) A rapid high-resolution method for resolving DNA topoisomers. BMC Res Notes. 11(1):1-7.

Motohashi K (2019) Development of highly sensitive and low-cost DNA agarose gel electrophoresis detection systems, and evaluation of non-mutagenic and loading dye-type DNA-staining reagents. PLoS one. 14(9):e0222209.

Nadarajah K, Omar NS, Rosli MM, Shin Tze O (2014) Molecular characterization and screening for sheath blight resistance using Malaysian isolates of *Rhizoctonia solani*. BioMed Res Int. 2014.

Nguyen TT, Lakhan SE, Finette BA (2013) Development of a cost-effective high-throughput process of microsatellite analysis involving miniaturized multiplexed PCR amplification and automated allele identification. Hum Genomics. 7(6).

Nguyen HT, Vu QH, Van Mai T, Nguyen TT, Vu LD, Nguyen TT, Vu LD, Nguyen TT, Nguyen LV, Vu HTT, Nong HT, Dinh TN, Toshitsugu N, Vu LV (2018) Marker-assisted selection of *Xa21* conferring resistance to bacterial leaf blight in indica rice cultivar LT2. Rice Sci. 25(1):52-56.

Ning Y, Wang GL (2018) Breeding plant broad-spectrum resistance without yield penalties. Proc Natl Acad Sci USA. 115(12):2859-2861.

NurulNahar E, Adam P, Mazidah M, Roslan I, Rafii YM (2020) Rice blast disease in Malaysia: Options for its control. J Trop Agr Food Sci. 48(1):11-23.

Pan J-Z, Fang P, Fang X-X, Hu T-T, Fang J, Fang Q (2018) A low-cost palmtop high-speed capillary electrophoresis bioanalyzer with laser induced fluorescence detection. Sci Rep. 8(1):1-11.

Petree C, Varshney GK (2020) MultiFRAGing: Rapid and simultaneous genotyping of multiple alleles in a single reaction. Sci Rep. 10:3172.

Platten JD, Cobb JN, Zantua RE (2019) Criteria for evaluating molecular markers: comprehensive quality metrics to improve marker-assisted selection. PLoS one. 14(1):e0210529.

Qi S, Zhu L, Wang X, Du J, Yang Q, Li Y (2019) Near-infrared turn-on fluorescent probe for discriminative detection of Cys and application in in vivo imaging. RSC Adv. 9(71):41431-41437.

R. Vemireddy L, Archak S, Nagaraju J (2007) Capillary electrophoresis is essential for microsatellite marker based detection and quantification of adulteration of Basmati rice (*Oryza sativa*). J Agr Food Chem. 55(20):8112-8117.

Ramkumar G, Prahalada G, Hechanova SL, Vinarao R, Jena KK (2015) Development and validation of SNP-based functional codominant markers for two major disease resistance genes in rice (*O. sativa* L.). Mol Breed. 35(6):1-11.

Savary S, Willocquet L, Pethybridge SJ, Esker P, McRoberts N, Nelson A (2019) The global burden of pathogens and pests on major food crops. Nat Ecol Evol. 3(3):430-439.

Séré Y, Fargette D, Abo ME, Wydra K, Bimerew M, Onasanya A, Akator SK (2013) 17 managing the major diseases of rice in Africa. In: Wopereis MCS, Johnson DE, Ahmadi N, Tollens E, Jalloh A (eds) Realizing Africa's Rice Promise, pp 213. Centre for Agriculture and Biosciences International, England.

Sharma TR, Madhav MS, Singh BK, Shanker P, Jana TK, Dalal V, Pandit A, Singh A, Gaikwad K, Upreti HC, Singh NK (2005) High-resolution mapping, cloning and molecular characterization of the *Pi-k h* gene of rice, which confers resistance to *Magnaporthe grisea*. Mol Genet Genom. 274(6):569-578.

Singh AK, Nayak R. Singh PK (2015) Identification of bacterial leaf blight resistance genes in rice (*Oryza sativa* L.). Int J Sci Nat. 6(2):283-287.

Singh N, Huang L, Wang DB, Shao N, Zhang XE (2020) Simultaneous detection of a cluster of differentiation markers on leukemia-derived exosomes by multiplex immuno-polymerase chain reaction via capillary electrophoresis analysis. Anal Chem. 92(15):10569-10577.

Singh P, Mazumdar P, Harikrishna JA, Babu S (2019) Sheath blight of rice: a review and identification of priorities for future research. Planta.250(5):1387-1407.

Stellwagen NC (2009) Electrophoresis of DNA in agarose gels, polyacrylamide gels and in free solution. Electrophoresis. 30(S1):S188-S195.

Stewart S, Wickramasinghe D, Dorrance AE, Robertson AE (2011) Comparison of three microsatellite analysis methods for detecting genetic diversity in *Phytophthora sojae* (Stramenopila: Oomycete). Biotechnol Lett. 33(11):2217-2223.

Tahamtan A, Ardebili A (2020) Real-time RT-PCR in COVID-19 detection: issues affecting the results. Expert Rev Mol Diagn. 20(5):453-454.

Tanweer FA, Rafii MY, Sijam K, Rahim HA, Ahmed F, Ashkani S, Latif MA (2015) Introgression of blast resistance genes (putative *Pi-b* and *Pi-kh*) into elite rice cultivar MR219 through marker-assisted selection. Front Plant Sci. 6:1002.

Wang X, Lee S, Wang J, Ma J, Bianco T, Jia Y (2014) Current advances on genetic resistance to rice blast disease. Rice-Germplasm, Genetics and Improvement.195-217.

Wang Y, Pinson SRM, Fjellstrom R, Tabien R (2012) Phenotypic gain from introgression of two QTL, qSB9-2 and qSB12-1, for rice sheath blight resistance. Mol Breed. 30(1):293-303.

Wang ZX, Yano M, Yamanouchi U, Iwamoto M, Monna L, Hayasaka H, Katayose Y, Sasaki T (1999) The *Pib* gene for rice blast resistance belongs to the nucleotide binding and leucine-rich repeat class of plant disease resistance genes. Plant J. 19(1):55-64.

Yap R, Hsu YC, Wu YP, Lin YR & Kuo CW (2016) Multiplex PCR genotyping for five bacterial blight resistance genes applied to marker-assisted selection in rice (*Oryza sativa*). Plant Breed. 135(3):309-317.

Yu YY, Jiang CH, Wang C, Chen LJ, Li HY, Xu Q, Guo JH (2017) An improved strategy for stable biocontrol agents selecting to control rice sheath blight caused by *Rhizoctonia solani*. Microbiol Res. 203:1-9.

Zhang Y, Zhu L, Zhang Y, He P, Wang Q (2018) Simultaneous detection of three foodborne pathogenic bacteria in food samples by microchip capillary electrophoresis in combination with polymerase chain reaction. J Chromatogr A. 1555:100-105.