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RESEARCH ARTICLE

Assessment of marker genotypes associated with the resistant genes in a set of rice cultivars for brown planthopper, blast disease and bacterial leaf blight

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ABSTRACT

Brown planthopper, bacterial leaf blight and rice blast disease are identified as devastating stresses of rice cultivation. Developing host plant resistance is considered an effective and environmentally friendly management strategy. In Sri Lanka, limited studies have been carried out to assess the genetic basis of disease resistance. Bph3, Pikh and Xa21 genes convey significant resistance against biotic stresses. The current study was conducted to identify whether markers linked to Bph3, Pikh and Xa21 genes can facilitate the identification of resistant varieties. A total of 34 varieties were selected for the study and their morphometric and marker allelic data were subjected to descriptive and statistical analyses. Moderate height varieties depicted high yield. Most of the varieties showed significant moderate resistance against stresses. Molecular marker analysis yielded polymorphic banding patterns for all markers. RM 589 marker linked to Bph 3 dominant gene provides overlapping banding patterns for the cultivars with different resistance levels making it impossible to discriminate resistant and susceptible cultivars. RM 206 marker linked to gene pikh provided the expected PCR product size for RM 206 marker is 147 bp. The resistant varieties gave band lengths of 140, 170 bp and susceptible or moderately susceptible varieties produced band lengths of 160 and 155 bp. pTA 248 marker linked to Xa21 locus produced bands length of 670, 740 and 930 bp. The 930 bp band was observed only in IRBB 60 variety. IRBB60 amplified 930 bp fragment confirms the possession of the resistant allele of Xa21 in its background. Presence of 730 bp indicates the susceptible allele. None of the Sri Lankan varieties carries a resistant allele for Xa21 as they have 730 bp allele for pTA 248. The rice varieties such as Bg251, Bg455, Bg450, Bg305, At402, At308 and At354 contain high yielding traits but need to improve for the pest and disease resistance.

Keywords*: Biotic stress, marker assistant breeding in rice, pest and disease resistance in rice, rice breeding in Sri Lanka*

INTRODUCTION

Rice is considered as one of the major food crops. Over three billion people consume rice as their staple food. Global rice demand cannot be fulfilled mainly due to severe influences of abiotic and biotic stresses (Wani and Sah, 2014).

Numerous diseases of rice caused by fungi, bacteria, viruses and nematodes have been recorded in different rice-growing areas of the world. Biotic stress factors influence rice cultivation in many ways; it affects plant growth, seed quality and agricultural practices (Gao *et al*., 2007; Cattivelli *et al*., 2008). Thus, biotic stresses are one of the major areas of concern in fulfilling the required food demand (Gao *et al*., 2007). Biotic stress factors on rice cultivation can be divided into two groups: microbial stresses and insect or pest stresses (Khush, 1977; Smith, 1994).

Rice productivity is significantly affected by different species of stem borers, planthoppers, leafhoppers, gall midge and several other insects in most of the rice-growing areas. Brown planthopper (BPH), *Nilupurvata lugens* is the most destructive insect pest, infesting and multiplying in the basal part of the plant causing significant yield losses (Smith, 1994). Heavily infested plants show symptoms such as chlorosis of stems, wilting of leaves, low productivity, and ultimately death of the entire plant which is named 'hopperburn' (Khush, 1977). The Blast disease (BL) of rice, which is a fungal disease caused by *Pyricularia oryzae*, is the most important disease of rice cultivation and causes devastating yield losses in all rice-growing areas of the world (Sing *et al*., 2011). *P. oryzae* infects the leaves, nodes, panicles and other areal parts of the plant (Mithrasena *et al*., 2012). Bacterial leaf blight (BLB) is another serious disease caused by a bacterium is known as *Xanthornonas oryzae* (Chukwu *et al*., 2019). BLB is predominantly found in countries of Asia and Australia causing yield losses up to 81% (Srinivasan and Gnanamanickam, 2005).

In Sri Lanka, BPH is identified as a major pest in rice cultivation (Nugaliyadde *et al*., 2000). Annually in Sri Lanka, about 10-15% of rice farmlands are infested by BPH and WBHP (White-Backed planthopper), and 5-10% of cultivation ended up partially or completely hopper burned (Nugaliyadde *et al*., 2001). Apart from BHP, BL and BLB are the major devastating diseases that significantly limit the rice yield in Sri Lanka (Weerasinghe *et al*., 2017). Breeding programmes have been conducted to incorporate BL resistance genes into susceptible rice varieties. However, the emergence of virulent races has overcome the effect of some resistance genes and ultimately led to rice varieties with narrow BL resistance. Also, Bg300, Bg358 and Bg352 are some of the popular rice varieties which lack sufficient resistance against BLB disease (Fernando *et al*., 2007). Therefore, BLB, BL and BPH have been recorded as significant and most challenging biotic stresses in rice. Without proper control or management practices, these stress factors can trigger devastating damages to the harvest and vigor of the rice plant. Therefore, these biotic stress factors act as major barriers to accomplishing rice demand, food security and safeguarding the economic status of the country.

In Sri Lanka, current rice breeding and germplasm assembling programmes have been implemented to breed resistant rice cultivars for biotic and abiotic stresses (Heinrichs and Pathak, 1980; Kumari *et al*., 2007; Samarasinghe *et al*., 2008). Even though numerous agronomical practices and agrochemicals are available to control most of the pests and diseases, they do not provide cost-effective,

durable and environmentally friendly results (Jayawardana *et al.*, 2014). For some diseases like BLB, there is no chemical control method available to practice, and for BL disease, fungicides will cost more and result in serious environmental pollution. Development of improved disease-resistant rice varieties via breeding approaches is considered the most economical and environmentally friendly strategy in disease management. For that, suitable resistant genes with a broad-spectrum resistance must be incorporated into local rice varieties. To date, many genes resistant to biotic stresses have been identified in the rice genome. *Bph3*, *Pikh* and *Xa21* were identified as widely available resistant genes in Sri Lankan rice germplasm showing a high degree of resistance against different biotypes of BPH, BL and BLB, respectively (Jayawardana *et al.*, 2014; Weerasinghe *et al.*, 2017). In conventional breeding approaches, breeders use phenotypic screenings to identify desired resistant genes which is very time-consuming and laborious (Jayawardana *et al.*, 2013). However, identification of resistant genes using tightly linked polymorphic DNA markers provides more efficient and accurate results (Joshi *et al.*, 2009). DNA markers can be used as an identification tool when desired genes are transferred from one varietal background to another in a breeding programme (Fjellstorm *et al.*, 2002). In Sri Lanka, sufficient DNA marker analyses have not been conducted to assess the genetic basis of disease resistance in local rice varieties. Therefore, this study was conducted to identify whether molecular markers reported to be linked to *Bph3*, *Pikh*, and *Xa21* genes in previously published studies can facilitate the identification of resistant varieties and their resistant levels in marker-assisted breeding programs in Sri Lanka and assist breeders to have a better understanding of resistant genes and resistant levels in the desired lines.

MATERIALS AND METHODS

Plant material

The morphometric details of 34 rice cultivars (Table 1) with different resistant levels (R=Resistant, R/MR=Resistant/Moderate resistant, MR=moderate resistant, MR/MS=Moderate resistant/Moderate susceptible, MS=Moderate susceptible, S=Susceptible) against BL, BHP and BLB were selected using recommended rice variety book released by Rice Research and Development Institute (RRDI), Bathalagoda, Sri Lanka. Ptb33, Tetep, and IRBB60 rice varieties were used as reference resistant varieties for BPH, BL and BLB, respectively. Seeds samples were obtained from RRDI. After that, seeds were soaked in a beaker to germinate, and germinated seeds were planted and maintained for 14 d under greenhouse conditions at University of Peradeniya.

Table 1: Rice cultivars used in the analysis.

No.	Cultivar	Maturity time/months (*Yala* & *Maha*)	Average yield (t ha^{-1})	Recommendation	Disease resistant level			
					BL	BLB	BPH	GM
1	At303	3.0	6.2	General cultivation	MR/MS	S	MS	MR
2	At306	3.0	4.7	General cultivation	MR	MR	R/MR	R/MR
3	At307	3.0	7.0	General cultivation	R	MS	R	R
4	At308	3.0	6.5	General cultivation	R	MS	MS	MR
5	At309	3.0	6.0	General cultivation	MR	MR/MS	MR	MR
6	At311	3.0	5.2	General cultivation	MR	MS	MR	R/MR
7	At353	3.5	5.2	Acid saline condition in Nilwala region	MS	S	MS	MR
8	At354	3.5	6.5	Saline area	MS	S	MR/MS	MR/MS
9	At362	3.5	7.0	General cultivation	MR/MS	MR/MS	R/MR	R/MR
10	At373	3.5	4.9	General cultivation	S	MR/MS	MR	R
11	At401	4-4.5	5.0	Costal saline area	MS	MS	MR/MS	R
12	At402	4-4.5	6.7	Southern province	S	MS	R/MS	R
13	Bg250	2.5	4.5	Drought & flood-prone area	MR	MR	R	MR
14	Bg251	2.5	5.5	Rain fed and drought-prone area	R/MR	MS	MR	R/MR
15	Bg252	2.5	4.2	Rain fed	R	MR/MS	MR	R/MR
16	Bg300	3.0	5.0	General cultivation	MR	MR	MR	R
17	Bg304	3.0	4.7	General cultivation	S	MR	MR	R
18	Bg305	3.0	6.9	General cultivation	S	MR/MS	MR	R
19	Bg310	3.0	5.6	Saline prone areas	R/MR	MR/MS	R/MR	S
20	Bg357	3.5	5.8	General cultivation	MR/MS	MR	MR	R
21	Bg359	3.5	5.9	Wet zone	MR	MR	MR	R
22	Bg360	3.5	4.2	General cultivation	S	MR	MR	R
23	Bg374	3.5	5.9	General cultivation	MR	MS	MR	MR
24	Bg450	4-4.5	5.3	General cultivation	S	MR/MS	MR/MS	R
25	Bg455	4-4.5	6.0	Flood prone areas	R	MS	MR/MS	R/MR
26	Bg94_1	3.5	4.1	General cultivation	MR	MS	S	S
27	Bw100	4-4.5	4.5	LCWZ	R	MR	MS	S
28	Bw367	3.5	5.2	General cultivation with iron toxicity	R/MR	MR	R/MR	MR/MS
29	Bw372	3.5	4.2	Wet tolerance to iron toxicity	MR	MR/MS	MR	R/MR
30	Bw302	3.0	3.2	Wet zone	R	MS	S	MS
31	Ld355	3.5	4.5	Southern province	MR	MR	MR	R
32	Ld356	3.5	5.8	Kaluthara & Galle districts	S	MR	MR	MR
33	Ld365	3.5	4.5	Wet zone	MR	MS	MR	MR
34	Ld368	3.5	4.6	Wet zone	MS	MR/MS	MR	R/MR

DNA extraction and PCR

Immature leaf samples were collected from the 37 rice varieties (including three reference varieties). DNA was extracted from each leaf sample using CTAB (Cetyl Trimethylammonium Bromide) method (Oard and Dronavalli, 1992). Extracted DNA samples were PCR amplified with markers linked to resistant genes *Pikh* for rice BL, *Bph3* for BPH and *Xa21* for BLB (Table 2) in a 10 µL PCR mixture containing 1.0 µM of forward and reverse primers (0.5 µL from each), 0.5 µL of DNA template, 3.5 µL of nuclease-free water with 5.0 µL of Go Taq® Green Master Mix 2X. The PCR profile used in the Thermal cycler (Takara, Japan) for three primers as follows; initial denaturation step is performed at 94 ℃ for 5 min, after those 35 cycles of 94 ℃ for 30 s for the denaturation process; then, primer annealing temperature (Table 2) for 90 s, and 72 ℃ for 2 min for finally extension step for 10 min. After that, amplified PCR products were separated using ethidium bromide-stained 2.0% agarose gel electrophoresis.

Table 2: DNA markers used in PCR amplification.

Marker	Gene	Forward and reverse primer sequence (5'- 3')	T_a/ ℃	Reference
RM 206	*Pikh*	ATCGATCGATCTTCACGAGG GGGCTTACGGAAAATATCGT	55	Jiang *et al*. (2012)
RM 589	*Bph3*	ATCATGGTCGGTGGCTTAAC CAGGTTCCAACCAGACACTG	55	Liu *et al*. (2009)
pTA 248	*Xa21*	AGACGCGGAAGGGTGGTTCCCGGA AGACCGGTAATCGAAAGATGAAA	55	Williams *et al*. (1996)

Data analysis

No. of days to maturity (*Yala* season), No. of days to maturity days in (*Maha* season), plant height, basal leaf sheath color, BL resistance, BLB resistance, BPH resistance, 1000 grain weight, average yield and bushel weight were categorized under the morphometric data (Table 3). All quantitative morphometric data were analyzed in Minitab 18 (Minitab Inc, USA). Morphometric parameters were subjected to distribution analysis and histograms were constructed for each parameter. Correlation analyses were conducted among yield parameters, including 1000 grains weight, average yield, bushel weight and plant height. The principal component analysis (PCA) was conducted using disease-resistance data of 34 varieties. The dendrograms were constructed for each disease based on allelic data using the Single Linkage and Euclidean Distance method in Minitab 18. Combining all allelic data another dendrogram was constructed using the Average Linkage and Euclidean Distance method in the statistical package Minitab 18 to depict the overall clustering of cultivars based on the allelic polymorphisms.

Table 3: The phenotypic data of studied rice varieties (RRDI, Sri Lanka).

Variety	Average yield (t ha^{-1})	Maturity days (*Yala*)	Maturity days (*Maha*)	Height (cm)	Basal Leaf sheath color	Recommendation	BL	BLB	BPH	GM	1000 grain weight	Bushel weight
Bg94_1	4.1	105	105	55.0	Green	General cultivation	MR	MS	S	S	28.3	20.8
Bw100	4.5	135	135	108.0	Dark green	LCWZ and iron toxic soil	R	MR	MS	S	19.3	21.8
Bg450	5.3	125	125	64.0	Purple	General cultivation	S	MR/MS	MR/MS	R	14.2	21.2
Bg300	5.0	93	93	72.2	Green	General cultivation	MR	MR	MR	R	26.8	21.9
Bw302	3.2	90	90	98.0	Green	WZ	R	MS	S	MS	21.3	20.9
At303	6.2	90	90	74.0	Green	General cultivation	MR/MS	S	MS	MR	26.0	21.5
At353	5.2	90	90	80.0	Green	Acid saline condition in Nilawala region	MS	S	MS	MR	24.1	19.2
At354	6.5	95	95	67.0	Green	Saline area	MS	S	MR/MS	MR/MS	26.1	20.5
At401	5.0	115	115	85.0	Green	Costal saline area	MS	MS	MR/MS	R	25.0	20.0
At402	6.7	115	115	85.0	Green	Southern province	S	MS	R/MR	R	23.7	21.4
Bg304	4.7	87	87	68.0	Green	General cultivation	S	MR	MR	R	23.5	20.1
Ld355	4.5	105	105	70.0	Green	Southern province	MR	MR	MR	MR	16.4	21.0
Ld356	4.5	105	105	66.0	Green	Kaluthara and Galle districts	S	MR	MR	MR	17.6	22.6
Bg357	5.8	104	104	56.0	Green	General cultivation	MR/MS	MR	MR	R	22.6	20.2
Bg359	5.9	104	104	64.0	Green	WZ	MR	MR	MR	R	23.6	21.6
Bg360	4.2	105	105	52.0	Green	General cultivation	S	MR	MR	R	13.6	20.6
Bg305	6.9	94	94	70.0	Green	General cultivation	S	MR/MS	MR	R	23.7	21.0
At362	7.0	110	110	70.0	Green	General cultivation	MR/MS	MR/MS	R/MR	R/MR	25.7	20.6
At306	4.7	102	102	63.0	Green	General cultivation	MR	MR	R	MR	21.9	19.0
Bg250	4.5	85	85	75.0	Green	Drought and flood prone areas	MR	MR	R	MR	24.0	19.9
At307	7.0	97	97	59.0	Green	General cultivation	R	MS	R	R	23.3	22.5
Ld365	4.5	102	102	40.0	Green	WZ	MR	MS	MR	MR	16.0	22.6
At308	6.5	95	95	77.0	Green	General cultivation	R	MS	MS	MR	17.5	21.8
Bw367	5.2	105	105	97.0	Green	General cultivation tolerance to iron toxicity	R/MR	MR	R/MR	MR/MS	15.0	22.0
Ld368	4.6	102	102	23.0	Green	WZ	MS	MR/MS	MR	R/MR	16.0	22.6
Bw372	4.2	104	104	98.0	Green	WZ tolerance to iron toxicity	MR	MR/MS	MR/MS	R	21.4	22.5
At309	6.0	95	95	52.8	Green	General cultivation	MR	MR/MS	MR	MR	22.6	18.5
Bg310	5.6	97	97	65.0	Green	Saline prone areas	R/MR	MR/MS	R/MR	S	27.5	21.3
Bg251	5.5	80	80	87.0	Green	Rain fed and drought prone areas	R/MR	MS	MR	R/MR	21.5	20.3
At373	4.9	103	103	56.0	Green	General cultivation	S	MR/MS	MR	R	10.5	19.8
Bg455	6.0	130	130	77.0	Green	Flood prone areas	R	MS	MR/MS	R/MS	24.8	21.3
At311	5.2	93	93	67.0	Green	General cultivation	MR	MS	MR	R/MR	20.7	17.3
Bg252	4.2	85	85	81.0	Green	Rain fed	R	MR/MS	MR	R/MR	17.8	21.5
Bg374	5.9	107	107	68.0	Green	General cultivation	MR	MS	MR	MR	22.2	22.2

RESULTS AND DISCUSSION

Statistical analysis

Histograms were constructed for each parameter to identify the distribution of frequency. Constructed histograms were categorized into two groups: pest and diseases (Figure 1) and yield parameters (Figure 2). In disease-resistant distribution analysis, all diseases demonstrated high frequency under the moderate resistant category (3=Moderate resistant). Sixteen varieties exhibited moderate resistance against BPH. BL disease showed the highest number of susceptible cultivars (7 varieties). Considering the overall resistant levels, BPH demonstrated the highest total no. of varieties under the moderate resistant level. None of the rice varieties demonstrated resistance or resistant/moderate resistant to BLB disease. Rice cultivars such as Bg94_1, Bw302, Bw100, At303 and At353 did not show a significant (P>0.05) overall resistance against selected stress factors. For some diseases, such as BLB, studied set of rice varieties did not contain enough overall resistance (Fernando *et al*., 2007; Samarasinghe *et al.*, 2008). In yield parameter analysis, except for four varieties (At311, At309, At306, At353), all other varieties demonstrated relatively high bushel weight (<20 kg). Maturity days in *Yala* and *Maha* seasons did not exhibit any particular relationship with rice varieties (Bambaradeniya *et al*., 2004; Munasinghe *et al*., 2017). It remains unchanged for two seasons. The average yield of most of the cultivars varied from 4.25 to 4.65 t ha^{-1}. Most of the cultivars produce an average yield greater than 4 t ha^{-1} (Figure 2). In Sri Lanka, the hybrid cultivar Bg407H accounts for the highest yield, 8 t ha^{-1} (Abeysekara and Jayawardena, 2012). The bushel weight is used as an indicator of good yield gain, and it is associated with grain filling (Seyoum *et al*., 2012; Ranawake *et al*., 2014). Rice varieties with low bushel weight account for poor grain quality. Poor grain quality causes many problems in milling processes such as low head rice recovery, low milling yield and broken grains ultimately resulting in a low market price. Rice varieties like At311, At309, At306 and At353 demonstrated a bushel weight lower than 20 kg.

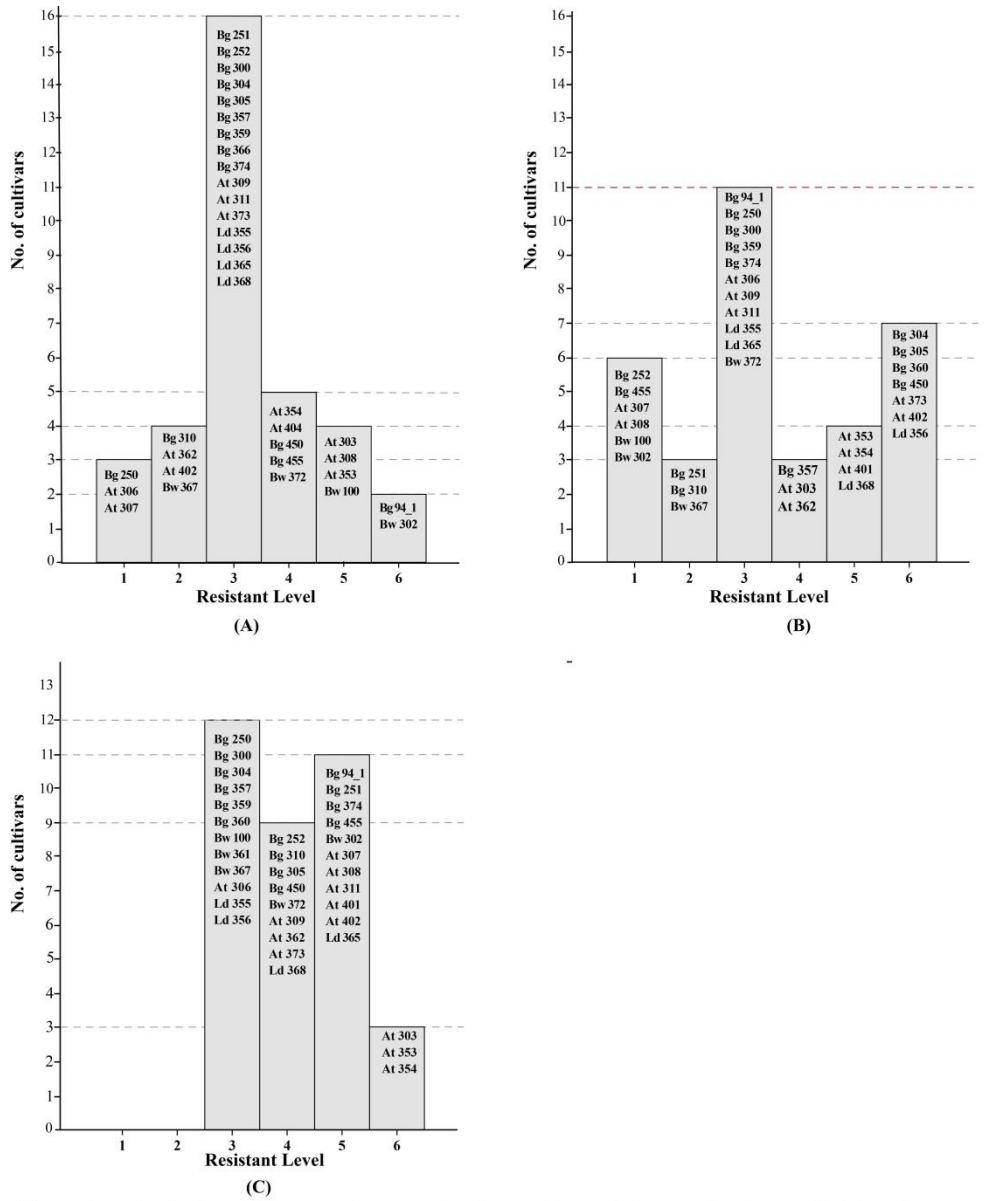

Figure 1: Distribution of disease-resistant levels of rice cultivars, (A): Brown planthoper; (B): Blast disease; (C): Bacterial leaf blight. X axis demonstrates the resistance level 1: R; 2: R/MR; 3: MR; 4: MR/M.S; 5: MS; 6: S. Y axis demonstrates the number of rice cultivars in each resistant level. Relevant rice cultivars under each resistant level showed inside bars.

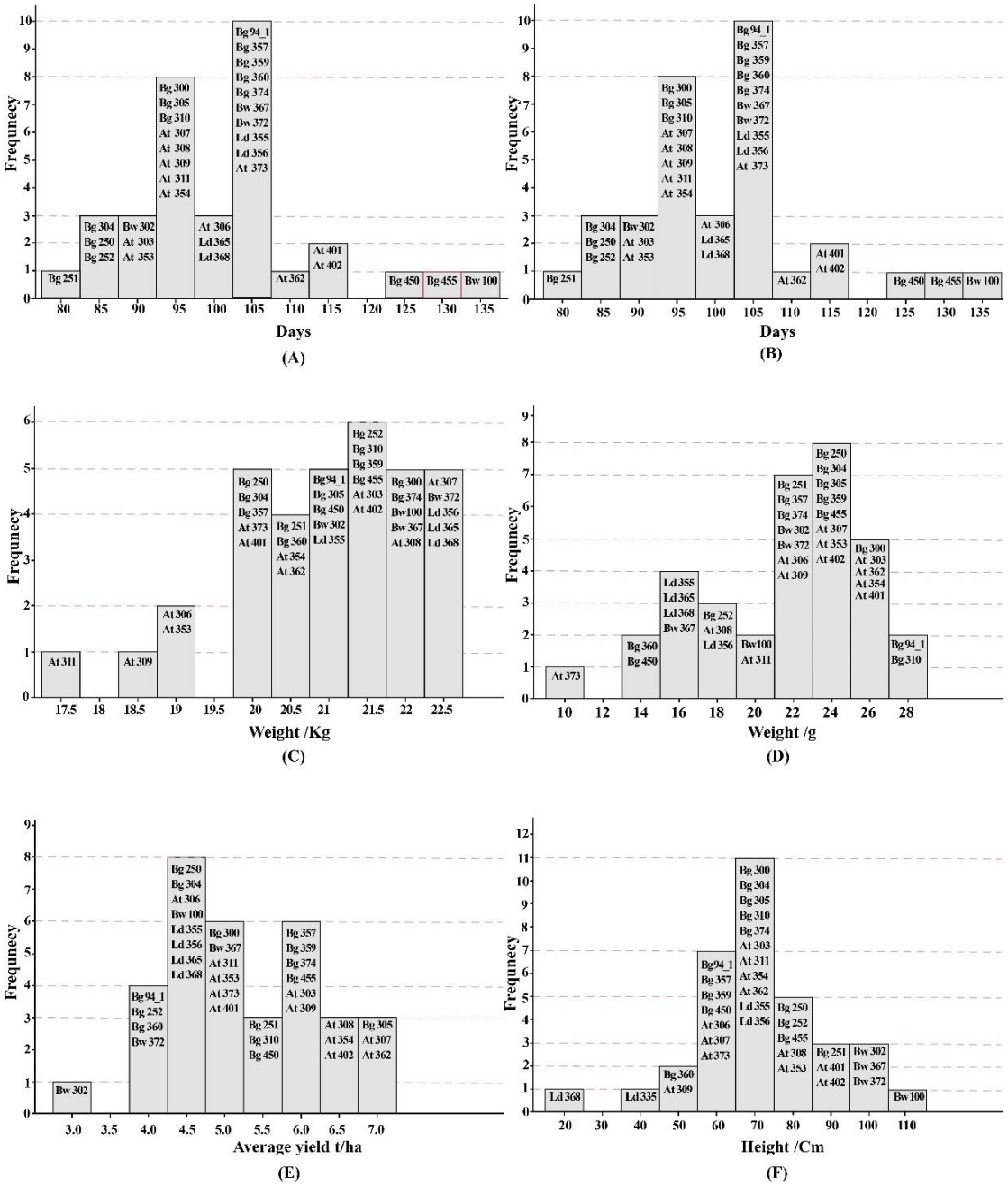

Figure 2: Distribution of morphometric data of rice cultivars, (A): Maturity days in *Yala* season; (B): Maturity days in *Maha* season; (C): Bushel weight; (D): 1000 grains; (E): Average yield; (F): Plant height. Y axis denotes the number of rice cultivars in each category. Relevant rice cultivars under each category are mentioned inside the bars.

Principal component analysis (PCA)

The PCA yielded three components corresponding to three disease-resistant levels of 34 rice varieties. The first two components described a total variance of 82%. The cumulative score for PC1 and PC2 was obtained as 82% (Table 4).

Table 4: Eigenanalysis of the correlation matrix.

Eigenvalue	1.4686	0.9956	0.5359
Proportion	0.490	0.332	0.179
Cumulative	0.490	0.821	1.000

PC1 and PC2 obtained Proportion values of 0.490 and 0.332, respectively. PC1 was significantly (P<0.05) positively associated with BPH and BLB. However, PC2 was largely positively associated with BL and BLB (Table 5).

Table 5: Eigenvectors of principal components.

Variable	PC1	PC2	PC3
BL	-0.099	0.995	0.022
BLB	0.703	0.086	-0.706
BPH	0.704	0.055	0.708

Correlation analysis

Correlation analysis was conducted to understand the association of the yield with the agronomic characters of the studied rice cultivars. The average yield was significantly (P<0.05) negatively correlated with plant height. When the plants become taller it tends to lodge and thereby limit yield. The 1000 grain weight significantly (P<0.05) positively correlated with average yield (Table 6). The results obtained from the present study are supported by the similar observations reported for plant height (Qmar *et al.*, 2005) and 1000 grains weight (Ullah *et al.*, 2011; Ranawake *et al.*, 2014). Moreover, Ranawake *et al.* (2014) show a correlation between filled grain percentage and 1000 grains weight. Therefore, plant height and 1000 grains weight can be considered as good criteria for the selection of high yielding rice cultivars in breeding programmes.

Table 6: Correlation analysis between yield parameters.

Parameter	Average yield (t ha^{-1})	Height (cm)	1000 Grains weight
Height (cm)	-0.078		
	0.660		
1000 grains weight	0.356	0.163	
	0.039*	0.357	
Bushel weight	0.006	0.036	-0.148
	0.972	0.842	0.405

* *P*-value lowers than 0.05

Molecular marker analysis

The polymorphic band patterns were resultant for all three makers; *RM 206*, *RM 589*, and *pTA 248*. The polymorphic banding patterns of three makers were used to assure the presence of a particular gene and to assess the resistance level of a variety. Ptb33, IRBB60 and Tetep were used as reference varieties that contain desired genes that are evaluated in this study. Resistance of all varieties has been successfully used in the varietal improvement programmes in Sri Lanka (Kudagamage and Nugaliyadde, 1995; Nugaliyadde, 2000; Weerasinghe *et al*., 2017) (Figure 3).

RM 589 marker linked *Bph 3* locus depicted length polymorphism with bands length of 180, 185, 190 and 200 bp. Rice varieties with *Bph 3* gene have shown a high degree of resistance to a broad spectrum of BHP biotypes (Jairin *et al*., 2007). Ptb33, the reference cultivar used in the *Bph 3* marker analysis found to contain two BHP resistant genes *Bph3* (dominant) and *bph2* (recessive) (Khush 1979). The BPH resistance in Ptb33 is mainly generated by the monogenic dominant *Bph 3* gene (Nugaliyadde, 2001). Ptb 33 produced a band length of 185 bp indicating the presence of *Bph 3* gene. BPH resistant cultivars produced a band length of 185 bp. Most of the moderate resistant cultivars (R/MR, and MR) depicted band lengths of 185 and 190 bp. Most of the susceptible cultivars also gave a band length of 185 bp, and it overlaped with the resistant cultivar differentiation process. The polymorphic banding pattern of *Bph 3* gene indicates that the resistant levels of the cultivars are associated with the *Bph 3* gene. Previous genetic analyses have shown that Ptb33 derived lines Bg300, Bg379-2 have gained moderate against BPH due to *Bph 3* dominant gene (Kudagamage and Nugaliyadde, 1995; Nugaliyadde, 2001). Since, in the present marker analysis, most of the cultivars gave overlapping banding patterns for different resistance levels. Thus, the banding patterns did not provide a distinct polymorphism to differentiate resistant and susceptible cultivars.

In blast disease resistance, *RM 206* marker linked to gene *pikh* was used to evaluate the polymorphic band patterns. Tetep exhibits a high degree of resistance against blast disease and contains several blast-resistant genes including *pikh* (Sharma *et al*., 2012). The expected PCR product size for *RM 206* marker is 147 bp (Sharma *et al*., 2005). *RM 206* marker analysis provided bands length of ~140, 155, 160 and 170 bp. Most of the resistant varieties gave band lengths of 140, 170 bp and susceptible or moderately susceptible varieties produced band lengths of 160 and 155 bp (Figure 3). *pTA 248* marker linked to *Xa21* locus produced bands length of 670, 740 and 930 bp. 930 bp band was observed only in IRBB 60 variety. IRBB60 carries resistant genes *Xa21*, *Xa4*, *Xa5*, and *Xa13* against BLB (Huang *et al*., 1997). In IRBB60 amplified 930 bp fragment confirms the presence of the resistant allele of *Xa21* and 730 bp indicates the susceptible allele. Among many resistant genes, *Xa21* confers a broad spectrum of resistance against all most Asian BLB biotypes (Khush *et al*., 1990). Among the analyzed rice varieties, none of the varieties carries a resistant allele for *Xa21*

(Figure 3). It could be a major reason for the absence of BLB resistant varieties in the studied set of rice varieties. Susceptible cultivars produced two types of banding pattern lengths of 740 and 670 bp. At 303 and At353 depicted band length of 670 bp and the rest of the susceptible and moderate susceptible cultivars gave a band length of 740 bp. The cultivars produced bands length of 740 or 670 bp exhibited moderate resistance/moderate susceptibility to BLB attacks.

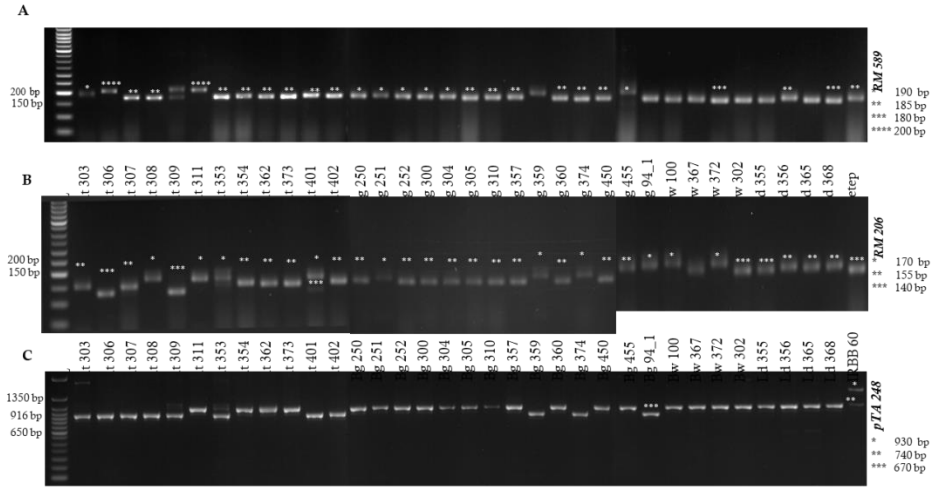

Figure 3: The polymorphism of three tightly linked markers, A: *RM 589* for brown planthopper resistance gene *Bph3*; B: *RM 206* for Rice blast resistance gene *Pi54*; C: *pTA 248* for Bacterial leaf blight resistance gene *Xa-21* observed in 2% agarose gel. The band sizes are indicated at the left side of the figure and the respective rice cultivars are indicated at the top of the image. The markers used in the analysis given at the right side of the image. Ptb 33, Tetep and IRBB 60 rice cultivars were used as reference cultivars for resistance genes *Bph 3*, *Pi54* and *Xa-21*.

The dendrograms A, B and C shown in Figure 4 were developed using the Single Linkage and Euclidean Distance method. The dendrogram D was developed using the Average Linkage, and Euclidean Distance method considering all three markers together. Dendrogram A was constructed using BL marker information and it showed four clusters at 83.33% of similarity level. Dendrogram B was obtained by analyzing allelic data of BLB disease and it produced three clusters at 80.12% similarity level. Dendrogram C developed based on BPH allelic data showed four clusters at 70.1% similarity coefficient. Within all clusters of all dendrograms (A, B, and C), mixing of rice cultivars with resistant levels were observed. Hence, a significant (P<0.05) clustering was not observed in the cultivars with similar resistant levels. To obtain an overall better separation of resistant cultivars dendrogram D was constructed based on the allelic information of all three markers. Each cluster comprised cultivars with different resistance levels. None of the clusters was biased towards a particular resistant level.

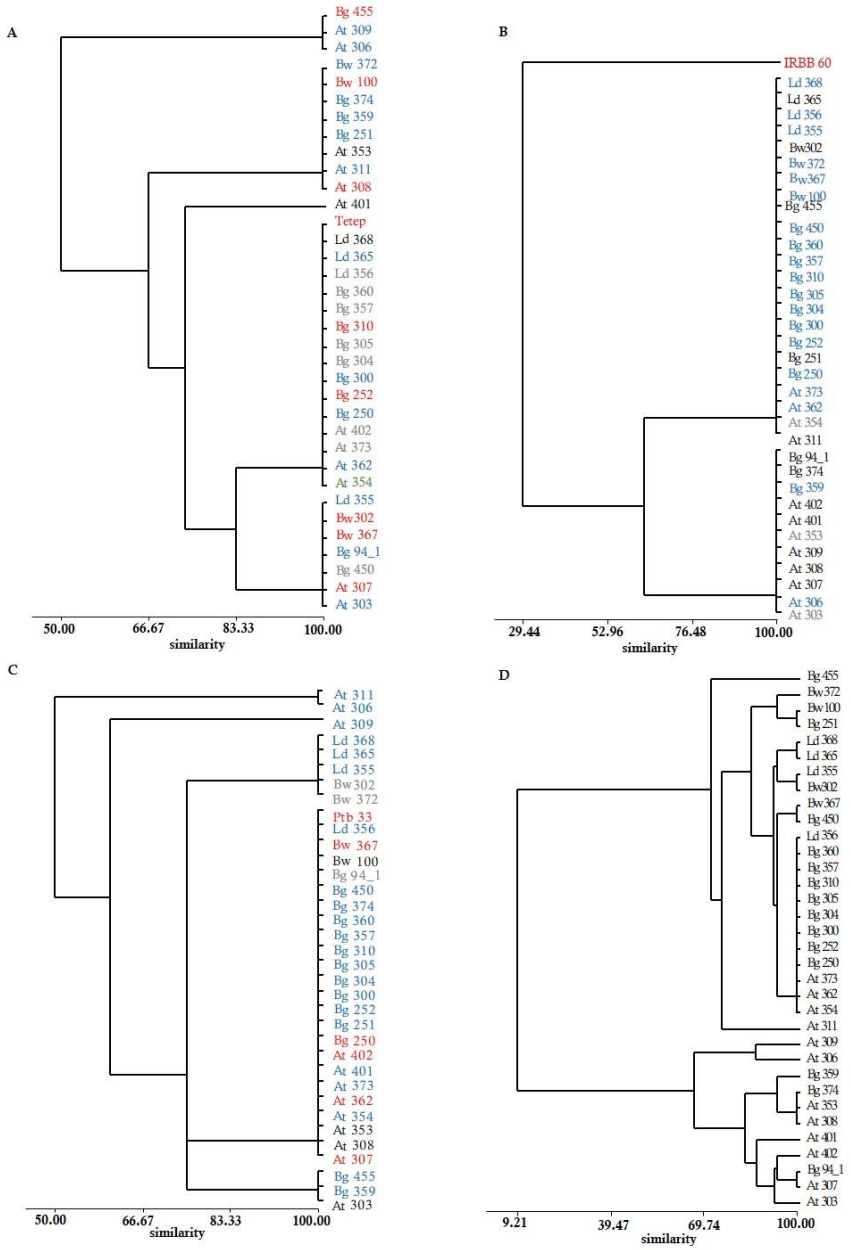

Figure 4: Dendrograms constructed for 34 rice cultivars based on alleic patterns of resistant alleles. Dendrograms A: Blast disease; B: Bacterial leaf blight; and C: Brown planthopper constructed using Single Linkage and Euclidean Distance method. Red – Resistant cultivars; Blue – Moderate resistant cultivars; Black – Moderate susceptible cultivars; Grey – Susceptible cultivars. Dendrogram D was constructed using average Linkage and Euclidean distance method considering all three markers together.

The genetic basis of a resistance variety does not generate from a single gene; it requires the overall effect of more than 20 genes and QTLs to create gene-based resistance (Ronald *et al*., 1992; Fjellstrom *et al*., 2004; Jairin *et al*., 2007). Therefore, the presence of a single resistant gene cannot generate enough resistance against pathogens. In this study, three resistant genes were genotyped using DNA markers to evaluate the applicability of resistance genes in MAS. In Sri Lanka, similar studies have not been conducted in recent years focusing on several diseases at once. In this study, results were compared with statically analyzed phenotypic data to evaluate the validity of the results of molecular markers analysis. Together with phenotypically analyzed data, the marker-assisted selection process can be used to identify the presence of a particular resistant gene with other promising traits.

CONCLUSIONS

Major resistant genes *Bph3*, *Xa21*, and *Pikh* are associated with BPH, BLB and BL, and these genes involving in providing a gene-based resistance against different biotypes/pathotypes. *RM 206*, *pTA 248*, and *RM 589* markers produced polymorphic banding patterns highlighting their importance of them in variety selection. Markers analysis and dendrograms created based on allelic information did not create a clear separation according to resistant levels. The polymorphic marker analysis of *Bph3*, *Xa21* and *Pikh* cannot be used to distinguish resistant cultivars from susceptible ones, for the studied set of rice cultivars. The statistical analysis of phenotypic data suggests that agronomical traits are important yield contributing traits and these traits would be most effective in future rice breeding programmes. The present study suggests that rice varieties such as Bg251, Bg455, Bg450, Bg305, At402, At308 and At354 are equipped with high yielding traits.

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