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RESEARCH ARTICLE

Plant Pathology

Influence of cultivar selection on blast and brown spot diseases in rice: Molecular screening of blast resistance genes

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Abstract: The prevalence of blast and brown spot diseases in rice is a substantial threat to national food security. This study investigated the distribution patterns of blast and brown spot, comparing their occurrence and distribution with respect to cultivar selection and conducting molecular screening for the identification of blast resistance genes. The research was conducted over five cultivating seasons from *Yala* 2017 to *Yala* 2019 in the Northern Province of Sri Lanka. Incidence percentages of the two diseases were calculated in 114 randomly selected fields across the five districts; Jaffna, Mullaitheevu, Kilinochchi, Mannar, and Vavuniya. Molecular markers were used to screen for nine major blast-resistant genes in 25 commonly cultivated rice cultivars. The results showed a significant shift in the disease over the period of study. While blast disease incidence declined after *Maha* 2017, brown spot incidence increased steadily from *Yala* 2017, peaking in *Yala* 2019. Interestingly, farmers' cultivar preferences, often diverging from the Department of Agriculture recommendations, exhibited a strong correlation with disease occurrence. The cultivar *Attakkari* was identified as a highly susceptible cultivar, which had only three R genes and a major contributor to the progression of blast before *Maha* 2017. Despite higher brown spot incidence percentages observed in cultivars At362, Bw367, and Bg450, compared to Bg360, intensive post-*Maha* 2017 cultivation of Bg360 increased the average brown spot incidence to 43%. Cultivars grown after *Maha* 2017, with over five R genes, showed lower blast disease incidence, suggesting a genetic link to susceptibility. This lower incidence of blast was also observed in the disease evaluation test, where we used the same cultivars. Hence, this study highlights rice cultivar selection as a decisive factor influencing disease occurrence. Given Sri Lanka's robust germplasm for blast-resistant genes, strategic cultivar selection has the potential for effective disease management.

Keywords: Blast disease, brown spot disease, cluster analysis, cultivar selection, molecular screening, rice cultivars.

INTRODUCTION

Rice blast is a devastating disease, caused by *Magnaporthe oryzae* (Ascomycota; Pezizomycotina; Dothideomycetes; Pleosporomycetidae; Pleosporales; Pleosporineae; Pleosporaceae) in almost all rice growing areas around the world (Ou, 1985; Rossman *et al*., 1990; Jagadeesh *et al*., 2020). The disease is responsible for 30% of the annual yield loss globally (Nalley *et al*., 2016). Next to blast, brown spot is considered as an important disease in rice fields, which reduces the quantity and quality of rice grains. Brown spot is mainly caused by *Bipolaris oryzae* (Ascomycota; Pezizomycotina; Sordariomycetes; Sordariomycetidae; Magnaporthales; Pyriculariaceae) (Dallagnol *et al*., 2014). The average yield loss owing to brown spot ranges from 4% to 52% (Dhaliwal *et al*., 2018). Both of these diseases together cause severe economic losses in rice. The incidence and severity of the two diseases vary depending on the geographical location and environmental conditions (Hossain *et*

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al., 2017). Blast is a common disease in both *Yala* and *Maha* cultivation seasons which can be controlled by a combination of cultural practices, fungicide application and selection of resistant cultivars. Selection of cultivars based on assessing the genotype for resistance is a promising strategy, which is preferred in highly blast-prone ecosystems since it is environmentally friendly and economically practical.

The resistance of rice plants to blast is controlled by blast resistance genes (R genes). R genes are located within all the 12 chromosomes of rice except chromosome 3 (Liu *et al*., 2010). Rice and *M. oryzae* have been studied as a classical model for gene for gene interaction (Valent, 1997). During a blast infection, elicitor molecules from the pathogen activate the R genes present in rice to create a hypersensitive response, and resistance is acquired through resistance proteins coded by the R genes (Gururani *et al*., 2012). Major blast resistant genes fall under the class of central nucleotide-binding site (NBS) and carboxy-terminal leucine-rich repeats (LRR). These NBS-LRR proteins are classified into two major classes, the N-terminal domain that shares homology with the mammalian Toll-interleukin-1-receptor (TIR) domain, while the other class encodes an amino-terminal coiled-coil motif (CC-NBS-LRR) (Chandrakanth *et al*., 2020).

In the context of blast resistance genes, a noteworthy advancement has been the identification of over 86 dominant R genes out of approximately 350 Quantitative trait loci (QTLs) associated with blast resistance. Among them, 23 were molecularly characterized such as pb1, Pi-a, Pi-b, Pi-d2, Pi-d3, Pi-k, Pik-h/Pi-54, Pik-m, Pik-p, Pish, Pi-t, Pi-ta, Piz-t, Pi-1, Pi-2/Piz-5, Pi5, Pi-9, pi-21, Pi-25, Pi-36, Pi-37, Pi-35, and Pi-64 to date (Fukuoka *et al*, 2014; Ma *et al*, 2015). But resistance can be conferred by major blast resistant genes (Zeigler *et al*., 1994), and several studies have reported that rice plants containing the major blast resistance genes showed a uniform resistant response in nursery experiments (Imam *et al*., 2014; Yan *et al*., 2017). Also, several studies suggested that resistance is substantially associated with the number of R genes, which implies a positive correlation between number of R genes and resistance against *M. oryzae* (Ning *et al*., 2020). On the other hand, regardless of the number, the pattern of R gene combination is a critical factor in determining the level of resistance against *M. oryzae* (Wu *et al*., 2015). It is essential to screen and discover the combination pattern of R genes that exhibit a broad-spectrum and lasting resistance to maximize the practical use of blast resistance breeding in a particular country. Hence, the present study was conducted to determine the distribution pattern of blast and brown spot diseases among the rice cultivars grown in the Northern Province of Sri Lanka over five consecutive rice growing seasons from *Yala* -2017 to *Yala* – 2019, to estimate the level of blast resistance based on the presence of R genes.

MATERIALS AND METHODS

Study site

The study was conducted in the five districts (Jaffna, Mullaitheevu, Kilinochchi, Mannar, and Vavuniya) in the Northern Province of Sri Lanka in randomly selected fields (n = 114). In the study sites, rice is grown in the two monsoonal seasons of *Maha* (September to March) and *Yala* (May to August). The *Maha* season cultivation is mostly dependent on North-East monsoonal rain, whereas the *Yala* season cultivation is dependent on irrigation from tanks and/or ground water. The province had temperatures ranging from 27 ^{o}C to 36 ^{o}C with an average annual rainfall of 1250 mm during the study period.

Disease assessment

The prevalence of rice blast and brown spot diseases was determined during *Yala* – 2017, *Maha* – 2017, *Yala* - 2018, *Maha* – 2018 and *Yala* – 2019. Incidence of blast and brown spot diseases were studied with the assistance of the Rice Research Station, Paranthan, Sri Lanka, and the Pathology division of the Regional Rice Research and Development Centre, Bombuwala, Sri Lanka. The presence of rice blast and brown spot was examined in selected rice fields, where the diseases were recorded historically, by taking 10 points along a diagonal transect in each field by throwing a 1 m × 1 m quadrat. The latitude and longitude of the locations were also recorded. Information on the cultivar grown in a particular location was recorded. Incidence percentage was calculated using the following formula (Nutter, 1997):

$$\text{Incidence\% (IP)} = \frac{\text{Number of samples showing foliar spots}}{\text{Total number of samples}}$$

Average incidence percentage (AIP) was calculated for each cultivar in the studied locations. In addition, severity of the two diseases was assessed based on the 0-9 visual rating scale of the standard disease evaluation system for rice by International Rice Research Institute (IRRI, 2013).

Molecular screening of rice blast resistant genes

This study was designed to check the presence of blast resistant genes in 25 selected cultivars (Bg 94-1, Bg 360, Bg 352, Suwandal, At 308, Moddaikaruppan, Bg 366, Pachchaperumal, Bg 300, At 362, Bw 351, Bg 250, At 402, Bg 251, Karuthaheenati, At 353, Bw 367, Bg 358, Ld 365, Bg 450, Attakari, Bw 372, Bg 369, Bg 406, and Co 10) grown during the study period. Genomic DNA was isolated from leaves using PhytoSpin D™ Plant Genomic DNA extraction kit. Eight PCR markers were used to detect nine different blast resistant genes; Pita/ Pita2, Pik, Pikp, Piz, Pikh, Pizt, Pi9, and Pib; details of the markers are listed in Table 1. The PCR reaction mixture was prepared as follows: 1X PCR buffer, 2.5 mM $MgCl_2$, 0.4 mM dNTP, 0.4 µM of each primer 1.5 U of GoTaq DNA polymerase (Promega, USA). The PCR amplification was performed with the following thermal profile: an initial denaturation of 94 ˚C for 5min, 35 cycles at 94 ˚C for 30 s, primer annealing at different temperatures for 45 s (Table 1), 72 ˚C for 2 min, and a final extension at 72 ˚C for 8 min. The PCR products were separated by gel electrophoresis on 1% agarose gel in 1X TAE buffer at 60 V for 2 hours. A 100 bp DNA ladder was used as a molecular weight size marker.

Disease evaluation

The disease assessment was conducted at the Regional Rice Research Station, Bombuwala, Sri Lanka. Seeds of the 25 cultivars were collected from the Rice Research Station, Paranthan, Sri Lanka. Seeds were surface sterilized with 70% ethanol and germinated in plastic pots containing sterile soil under greenhouse conditions. Each pot contained 25 seeds per cultivar. Urea (0.2 g/pot) was applied to increase vegetative growth and plants were watered daily. The concentration of conidial suspension of *M. oryzae* was adjusted to 1×10^5 per ml, and 10 ml of the suspension was inoculated into each pot using an atomizer when the plants were at the 4th or 5th leaf stage. After inoculation, pots were kept in a moist chamber for 48 h and then transferred to the green house. Disease scoring was carried out based on the Standard Evaluation System (SES) of the International Rice Research Institute (IRRI, 2013) after 7, 14, and 21 d. Scores of 0-3 were considered as resistant (R), 4-5 as moderately resistant (MR), and 6-9 as susceptible (S) as reported by Imam *et al*. (2014).

Table 1: List of PCR markers used to detect blast resistant genes

Gene	Primer name	Forward primer sequence 5'- 3'	Primer name	Reverse primer sequence 5' – 3'	AT (°C)	AL (bp)	References
Pita/Pita2	Pita3 F	AGTCGTGCGATGCGAGGACAGAAAC	Pita3 R	GCATTCTCCAACCCTTTTGCATGCAT	59	861	Hayashi *et al*., 2006
Piz	Z56592 F	GGACCCGCGTTTTCCACGTGTAA	Z56592 R	AGGAATCTATTGCTAAGCATGAC	60	292	Hayashi *et al*., 2004
Piz-t	Zt56591 F	TTGCTGAGCCATTGTTAAACA	Zt56591 R	ATCTCTTCATATATATGAAGGCCAC	60	257	Hayashi *et al*., 2006
Pik	RGA4_F3	GGAAAGCTGATATGTTGTCG	RGA4_R3	ACTCGGAGTCGGAGAGTCAG	55	1200	Ariya-anandech *et al*., 2018
Pikp	K3957 F	ATAGTTGAATGTATGGAATGGAAT	K3957 R	CTGCGCCAAGCAATAAAGTC	60	148	Hayashi *et al*., 2006
Pikh	Candidate GM F	CATGAGTTCCATTTACTATTCCTC	Candidate GM R	ACATTGGTAGTAGTGCAATGTCA	55	1500	Sharma *et al*., 2005
Pib	Pi28 F	GACTCGGTCGACCAATTCGCC	Pi28 R	ATCAGGCCAGGCCAGATTTG	60	388	Hayashi *et al*., 2006
Pi9	195R-1 F	ATGGTCCTTTATCTTTATTG	195R-1 R	TTGCTCCATCTCCTCTGTT	56	2000	Qu *et al*., 2006

AT: Annealing temperature; AL: Amplicon length

Cluster analysis

Cluster analysis was performed individually utilizing disease reaction scores obtained at 7, 14, and 21 d, and the number of blast resistant genes present in cultivars. For the analysis, the following programs were used in the following order in the Phylip V3.698 : Clique, Neighbor, and Draw tree. Finally, the result file was viewed in Mega X Software Version 10.1.8. The generated dendrograms were compared to identify similar clustering patterns.

RESULTS AND DISCUSSION

Distribution pattern of blast and brown spot diseases

Rice blast and brown spot have been reported as major rice diseases and are prevalent in all the rice growing seasons and districts in Sri Lanka, causing varying degrees of damage (Seneviratne & Jeyanandarajah, 2004). Both diseases infect an economically essential component, the seed, which decreases yield, degrades seed quality, and renders food unsafe for human consumption (Figure 1). In terms of disease intensity at the study sites, the IP of blast ranged from 10% - 81%. The highest incidence of 81% was observed in the Paranthan area of the Kilinochchi district, which was severely affected by blast (Supplementary Table S1) during the *Yala* - 2017 and *Maha* - 2017.

After *Maha* – 2017, the blast incidence was not observed in any of the studied fields. However, the incidence of brown spot increased steadily from *Yala* – 2017 to *Yala* – 2019 and the IP ranged from 0% - 88% in the studied fields. *Yala* – 2019 cultivation had the highest brown spot disease (range of IP = 15.24% - 88.75%) and farmers experienced severe losses due to this (Supplementary Table S1). Since both are fungal diseases and present in the same host, epidemiology of one disease can influence the other disease as reported in previous studies (Bahous *et al*., 2003; Terensan *et al*., 2022). Also, *M. oryzae* is reported as a weak competitor in fields (Jia, 2009).

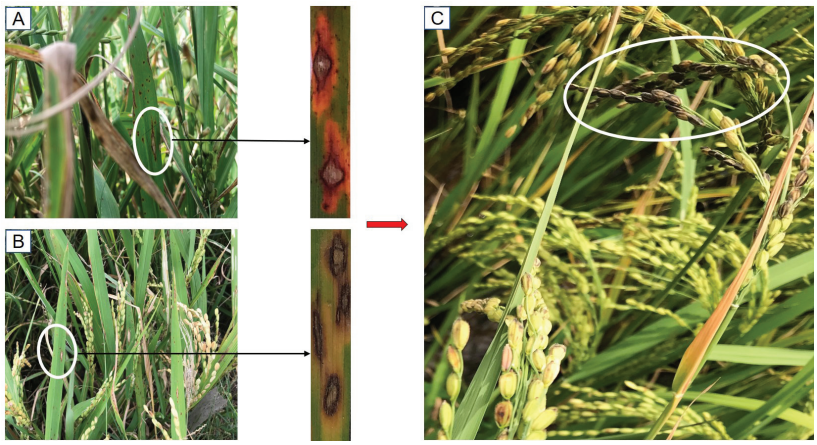

Figure 1: Symptoms of blast and brown spot diseases. A) Lesions of rice blast disease. B) Lesions of brown spot disease. C) Panicle infected with both diseases and turned black in colour.

The distribution of blast disease was observed to be higher in *Maha* – 2017 (AIP = 32.31) than *Yala* – 2017 (AIP = 28.22). Generally, the *Maha* season cultivation progress by a heavy rainfall leading to higher humidity that is conducive to blast fungus growth (Rice Research and Development Institute, Bathalagoda). Although a severe blast incidence was expected, as recorded in previous years (Food and Agriculture Organization/ World Food Programme, 2017; Northern Provincial Council of Sri Lanka 2019), it was not observed in *Maha* or *Yala,* 2017 except in few areas in the Kilinochchi (Kandavalai, Tharmapuram, Paranthan) and Mullaitheevu (Udayarkaddu) districts where Attakkari was the predominant cultivar (Supplementary Table S1), which was identified as a blast susceptible cultivar.

In contrast, the incidence of brown spots was low at the beginning of the study, but then increased to a level where farmers abandoned the fields without harvesting. There may be multiple factors contributing to this change in disease occurrence patterns. A few factors, such as the selection of cultivars by farmers and the presence of blast resistance genes in the cultivars grown in the region were analysed to check their influence on disease occurrence patterns. The *in - vitro* disease evaluation by analysing the presence of R genes in the major cultivars was also carried out to validate field observations on the distribution and severity of blast disease.

Cultivar selection and the occurrence of diseases

The study focussed on the selection of cultivars and their correlation with the distribution patterns with blast and brown spot diseases. It was assumed that this correlation was one of the determining factors for the occurrence pattern of the diseases. Blast infection was observed in 17 different cultivars while the distribution of brown spots was observed in 27 cultivars (Figure 2). It was found that the selection of the cultivar Attakkari during *Maha* – 2017 and *Yala* – 2017 was the significant determining factor for blast occurrence during these two seasons. The earlier field trial conducted by the rice research stations failed due to the severe incidence of blast disease. It was identified as a highly blast - susceptible cultivar and this led to a higher incidence percentage (IP) in locations where it was cultivated, particularly in the districts of Kilinochchi, and Mullaitheevu where farmers have actively grown this cultivar. Similarly, several pockets in the Mannar district were severely infected by blast due to the selection of the same cultivar (Supplementary Table S1).

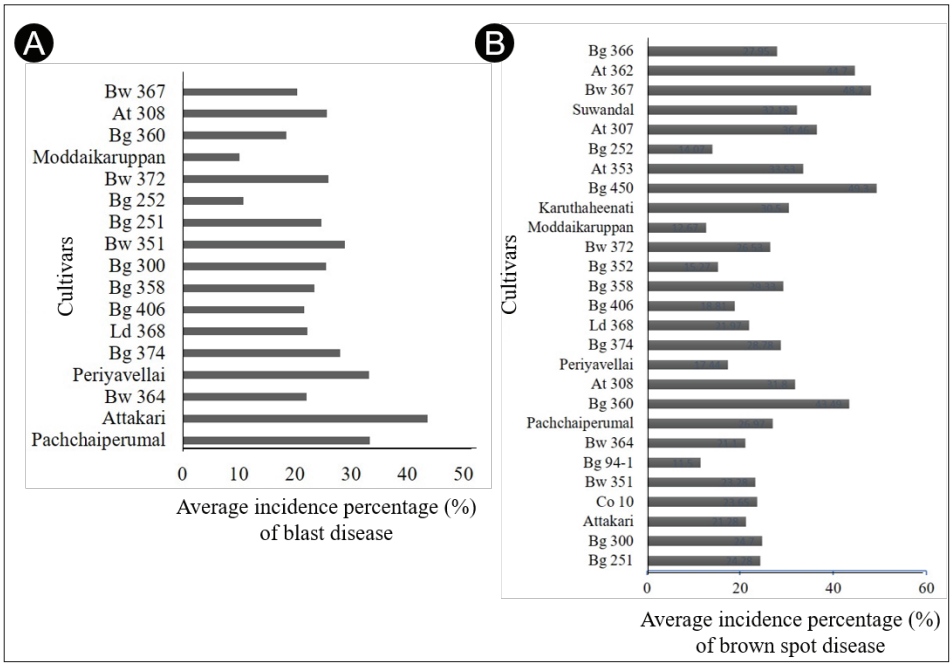

Figure 2: Distribution of A) blast and B) brown spot diseases among different rice cultivars grown in the study area with respect to average incidence percentage (AIP).

Despite the fact that the Department of Agriculture has advised farmers to avoid planting the cultivar Attakkari due to its blast susceptibility, based on their field trials, farmers have cultivated this cultivar extensively until *Maha* – 2017 because it produces a high yield, round bold type of seeds, and has a strong market demand.

On the other side, significantly low blast IP (10.18%) was noticed in the Jaffna district during *Maha* – 2017, and infections were not observed in *Yala* – 2017 where farmers cultivated the red rice cultivar Moddaikaruppan to a higher extent, which is comparably resistant to blast. Therefore, selection of cultivars could be a reason for the absence of blast disease after *Maha* – 2017 to *Yala* – 2019 in the study sites. In addition to this cultivar Attakkari, Pachchaiperumal also had a higher blast IP, but the cultivation extent of this particular cultivar was limited to a few locations in Kilinochchi and Jaffna districts. Notably, Moddaikkaruppan and Bg 252 were identified as significantly resistant cultivars to blast and recorded with symptoms below the severity scale of 2.

Based on the field survey and interviews conducted with the farmers and the Department of Agriculture, after *Maha* – 2017, farmers have grown a wide range of cultivars based on the availability of seed material, consumer preference and seeds stored from the previous cultivation. Briefly, higher brown spot infection was noticed in Bg 450, Bw 367, At 362, and Bg 360 while Bg 94-1, Periyavellai, Bg 352, Moddaikaruppan and Bw 364 showed lower infection. Farmers have cultivated Bg 360 in 50% of the studied locations during *Yala* – 2019 (Supplementary Table S1). Interestingly, Bg 360 was grown in the locations where severe brown spot infections were recorded. The highest average incidence percentage (AIP) was noticed in Shiruneelaceni (88.75%) and second highest was in Andankulam (85.4%) from the Mannar district. A similar yield reduction was observed in Vallipunam area from the Mullaitheevu district, where the same cultivar was grown and recorded with an AIP of 58.6%. Cultivar Bg 360 was chosen for intensive cultivation because of the market demand after *Yala* – 2018. Five cultivars, namely Periyavellai (2.6%), At 353 (1.8%), Bg 366 (1.8%), Bw 364 (2.6%), and Moddaikaruppan (2.6%) showed significant resistance to brown spot. However, they were grown only in a few locations.

According to observations, brown spot disease became the major rice disease following the introduction of Bg 360 (‘*Keeri samba’*), not only in the Northern Province but also in other regions of Sri Lanka (Nalaka *et al*., 2021; Senuka, 2023). Bg 360 is a white ‘*samba’* type cultivar with a higher market price. Most farmers save seeds from previous harvest to cultivate in the next planting season, without realizing that infected seeds can be a significant source of the pathogen’s inoculum. This practice can result in the disease persisting continuously, especially when weather conditions are favorable. This may have promoted the existence of brown spot throughout the study period. Moreover, a study by Chakrabarti (2001) reported that brown spot is generally severe in soils with low pH, low availability of potassium (K_2O), and deficient in essential and trace elements. The dominant group of soil in the Northern Province is Reddish Brown Earth (RBE). The association of Low HumicGley (LHG) with RBE soil (92%) enhances paddy cultivation. However, RBE has low organic matter content and the nitrogen and phosphorus status are usually poor, while the potassium status varies from medium to low (Northern Provincial Council of Sri Lanka, 2014). Improper soil amendments has led to subsequent degradation in soil quality because farmers have failed to implement significant improvements in their agronomic practices. This could possibly be one of the reasons for the emergence of brown spot as a major rice disease.

Detection of blast resistant genes by PCR and pathogenicity assay to assess resistance

This study was conducted to investigate the influence of R genes on blast disease in cultivars grown at the study sites. Twenty-five cultivars were genotyped for the presence of nine blast resistant genes (R genes) in this study. Table 2 summarizes the presence of R genes in the cultivars, which ranged from 3 to 9 based on the results obtained from gel electrophoresis (Supplementary Figures S1–S8). The cultivar Attakari, which was identified as susceptible to blast in the field, contained the fewest R genes (n=3). Cultivars Bg 360, Bg 352, Bg 250, At 353, Bg 369, Bg 300 and At 362 were found to contain more than five genes out of the nine tested R genes, with less impact from blast. These cultivars were grown to a larger extent after *Maha* – 2017.

Furthermore, the results revealed that cultivars grown after *Maha* – 2017 possessed more than four blast resistant genes. As the number of R genes increased, resistance also switched from moderately resistant (MR) to resistant (R) response in the disease severity assessment. In fact, during the disease assessment, MR to R responses were recorded for all the 24 cultivars with the exception of cv. Attakkari. This finding clearly indicates that several cultivars possess resistance towards the blast disease.

Based on disease severity assessment, cultivars were categorized into three groups, where 17 cultivars were highly resistant (score 0-3), seven were moderately resistant (score 4-5), and one (cv. Attakari) showed susceptible reaction (score 6-9). Data on the presence of R genes and the response to disease screening of all the cultivars were consistent.

Table 2: Status of blast resistant genes in cultivars grown in the study sites and the response to susceptibility/resistance levels

		Gene									Total number	DR	DR
Code	Cultivar	Pita	Pita2	Pikh	Piz	Pizt	Pik	Pikp	Pib	Pi9	of R genes	Score	Status
N1	Bg 94-1	1	1	0	1	0	1	1	1	0	6	3	R
N2	Bg 360	1	1	1	0	0	1	1	1	0	6	1	R
N3	Bg 352	1	1	1	1	0	1	1	1	0	7	2	R
N4	Suwandal	0	0	1	1	0	0	1	1	1	5	1	R
N5	At 308	1	1	0	0	0	1	1	1	0	5	2	R
N6	Moddaikaruppan	0	0	1	1	1	1	1	1	0	6	0	R
N7	Bg 366	0	0	1	1	0	0	1	1	0	4	3	R
N8	Pachchaperumal	1	1	1	1	1	1	1	1	1	9	0	R
N9	Bg 300	1	1	1	1	0	1	1	1	0	7	4	MR
N10	At 362	1	1	1	1	1	1	1	1	0	8	0	R
N11	Bw 351	1	1	1	0	0	0	1	1	0	5	5	MR
N12	Bg 250	1	1	1	1	0	1	1	1	1	8	4	MR
N13	At 402	0	0	0	1	0	0	1	1	0	3	3	R
N14	Bg 251	1	1	1	1	0	0	1	1	0	6	4	MR
N15	Karuthaheenati	0	0	1	1	0	1	1	1	0	5	1	R
N16	At 353	1	1	1	1	1	1	1	1	0	8	1	R
N17	Bg 450	0	0	1	0	1	1	1	1	0	5	0	R
N18	Bw 367	1	1	1	0	1	0	1	1	0	6	1	R
N19	Bg 358	1	1	1	1	0	0	1	1	0	6	1	R
N20	Ld 365	1	1	1	1	0	1	1	1	0	7	1	R
N21	Attakari	0	0	1	0	0	0	1	1	0	3	6	S
N22	Bw 372	0	0	1	0	0	1	1	1	0	4	5	MR
N23	Bg 369	1	1	1	1	0	1	1	1	0	7	0	R
N24	Bg 406	1	1	1	1	0	0	1	1	0	6	5	MR
N25	Co 10	1	1	1	1	0	1	1	1	0	7	4	MR

'1' represents presence of amplicon and '0' represents absence of amplicon. DR: disease resistance, R: resistant, MR: moderately resistant, S: susceptible.

The cluster analysis revealed that various cultivars correspond to similar groupings in the dendrograms (Figure 3) constructed using disease screening results and PCR assay findings. In both dendrograms, cv. Attakkari and cv. At 402 (clusters C and D) were grouped separately, whereas certain cultivars were shown to be in similar groups (clusters A and B). Cultivars Co10, Bg 300, Bg 250 for example, were grouped together (Cluster A). Bg 450 and Moddaikkaruppan were also grouped together (Cluster B). However, a definite correlation was not observed in cultivars that were in the moderately resistant category, which were grouped in different clusters. This is further illustrated as a scatter plot derived using the data generated (Figure 4). The plot clearly separates the susceptible cv. Attakari from the rest, and the resistant varieties are grouped together as illustrated by the cluster diagram.

The findings highlight a significant influence of cultivar selection on the severity of these two diseases. Furthermore, other factors such as agronomic practices and climate change may have also significantly contributed to the dynamics of disease incidence.

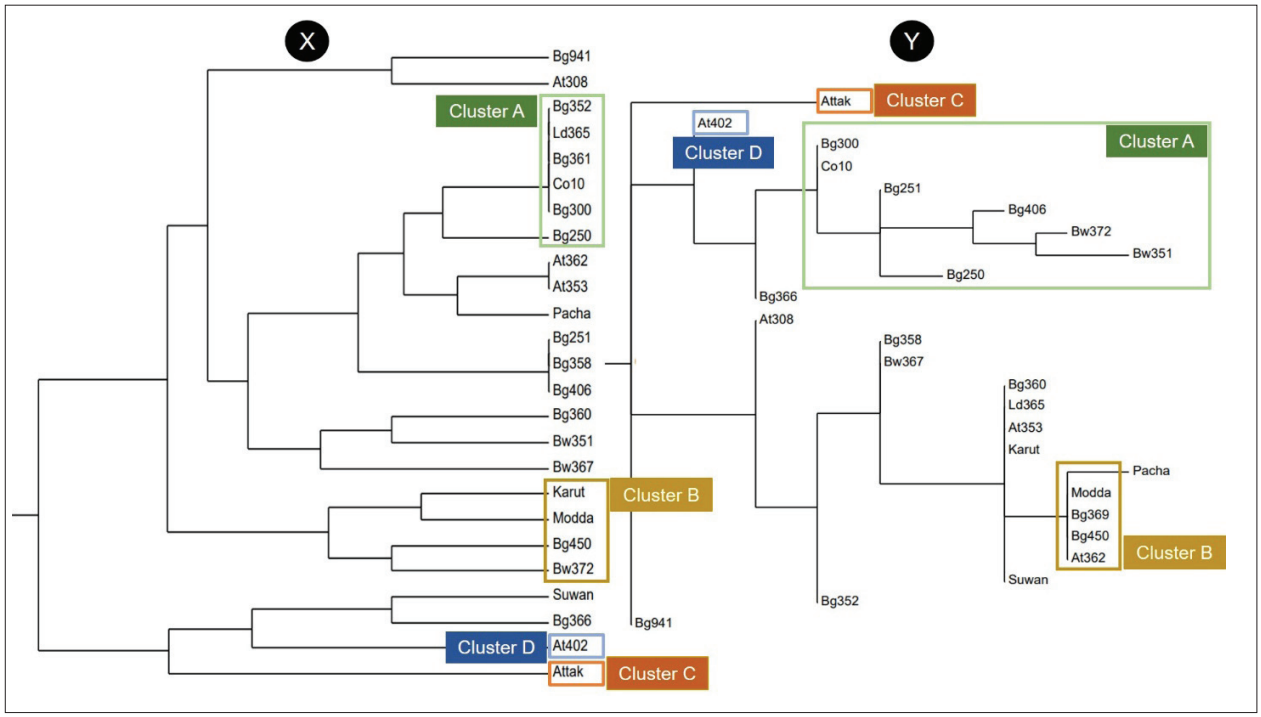

Figure 3: Dendrograms constructed based on the cluster analysis to compare the results of disease screening assay and the presence of resistance genes in 25 cultivars grown in the Northern Province. X: Dendrogram produced based on the number of blast resistant genes present in 25 cultivars. Y: Dendrogram based on resistance/susceptible levels in the blast disease screening. The similar clustering patterns of the cultivars are indicated by the use of the same color boxes.

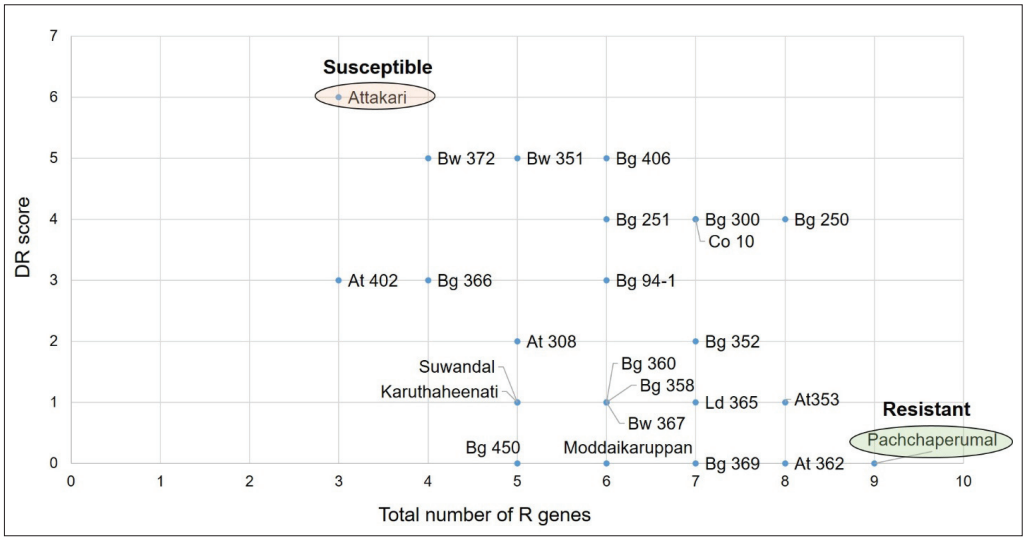

Figure 4: Scatter plot drawn for the total number of blast R genes against disease resistance score (DR score)

CONCLUSION

The study reveals that choosing the susceptible cultivar Attakkari before *Maha* - 2017 has led to a severe incidence of blast. This is further supported by *in-vitro* disease evaluation where the number of R genes present in the cultivars was assessed. Therefore, the absence of blast disease after the *Maha* - 2017 may be attributed to a change in cultivar selection by the farmers. They are now growing cultivars with more blast-resistant genes than before *Maha*-2017. Similarly, the brown spot incidence was higher in locations where cultivar Bg 360 was cultivated. Additionally, when farmers grew disease-resistant cultivars of both diseases, the IP of both diseases decreased. Furthermore, it was observed that the accumulation of blast resistance genes enhances blast resistance in the tested cultivars. Therefore the selection of cultivars has a significant impact on the occurrence of the disease.

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Supplementary data

Detection of blast resistant genes in 25 cultivars grown in study sites.

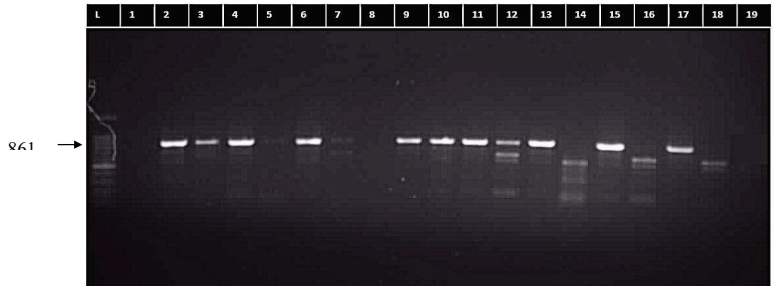

Figure S1a: Gel image showing the presence of *Pi-ta/Pi-ta2* gene using the marker of Pita3-F/Pita3-R (861 bp). L: 100 bp ladder, Lane 1: Negative control, Lane 2: *Bg 94-1*, Lane 3: *Bg 360*, Lane 4: *Bg 352*, Lane 5: *At 308*, Lane 6: S*uwandal*, Lane 7: *Moddaikaruppan*, Lane 8: *Bw 372*, Lane 9: *Bg 406*, Lane 10: *Pachchaiperumal*, Lane 11: *Bg 358*, Lane 12: *Bg 300*, Lane 13: *Ld 365*, Lane 14: *Karuthaheenati*, Lane 15: *At 362*, Lane 16: *Bg 366*, Lane 17: *Bg 251*, Lane 18: *At 402*, Lane 19: *Bg 450*

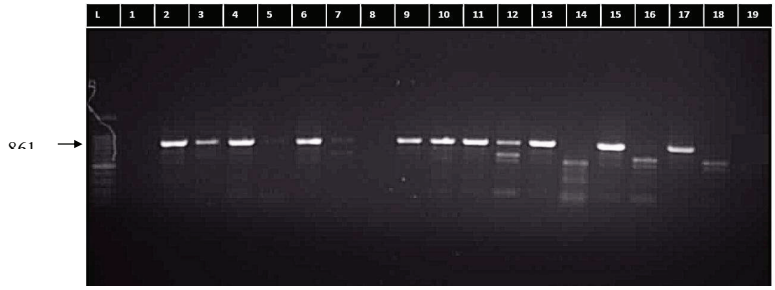

Figure S1b: Gel image showing the presence of *Pi-ta/Pi-ta2* gene using the marker of Pita3-F/ Pita3-R (861 bp). L: 100 bp ladder Lane 1: *Co10*, Lane 2: *Bg 369*, Lane 3: *Bw 351*, Lane 4: *Attakkari*, Lane 5: *Bg 250*, Lane 6: *Bw 367*, Lane 7: *At 353*

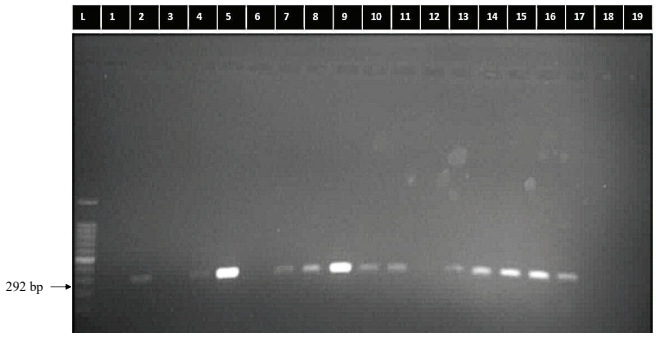

Figure S2a: Gel image showing the presence of *Piz* gene using the marker of Z56592 – F/ Z56592 – R (292 bp). L: 100 bp ladder, Lane 1: Negative control, Lane 2: *Bg 360*, Lane 3: *Bg 94-1*, Lane 4: *Bg 352*, Lane 5: *Suwandal*, Lane 6: *At 308*, Lane 7: *Moddaikaruppan*, Lane 8: *At 402*, Lane 9: *Bg 406*, Lane 10: *Pachchaiperumal*, Lane 11: *Bg 358*, Lane 12: *Bw 367*, Lane 13: *Ld 365*, Lane 14: *Karuthaheenati*, Lane 15: *At 362*, Lane 16: *Bg 366*, Lane 17: *Bg 251*, Lane 18: *Bw 372*, Lane 19: *Bg 450*

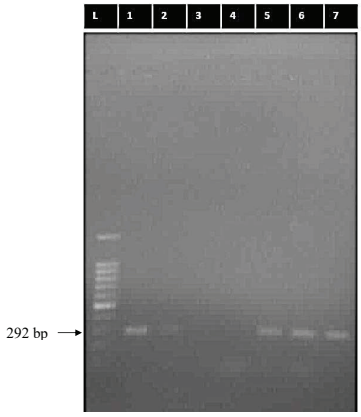

Figure S2b: Gel image showing the presence of *Piz* gene using the marker of Z56592 – F/ Z56592 – R (292 bp). L: 100 bp ladder, Lane 1: *Co10*, Lane 2: *Bg 369*, Lane 3: *Bw 351*, Lane 4: *Attakkari*, Lane 5: *Bg 250*, Lane 6: *Bg 300*, Lane 7: *At 353*

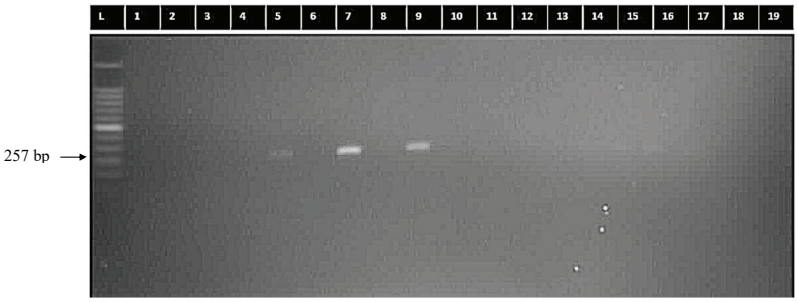

Figure S3a: Gel image showing the presence of *Pizt* gene using the marker of Z56591 – F/ Z56591 – R (257 bp). L: 100 bp ladder, Lane 1: Negative control, Lane 2: *Bg 94-1*, Lane 3: *Bg 360*, Lane 4: *Bg 352*, Lane 5: *Bw 367*, Lane 6: *At 308*, Lane 7: *Moddaikaruppan*, Lane 8: *Bg 366*, Lane 9: *Bg 450*, Lane 10: *At 402*, Lane 11: *Bg 358*, Lane 12: *Suwandal*, Lane 13: *Ld 365*, Lane 14: *Karuthaheenati*, Lane 15: *Pachchaiperumal*,, Lane 16: *At 362*, Lane 17: *At 353*, Lane 18: *Bw 372*, Lane 19: *Bg 406*

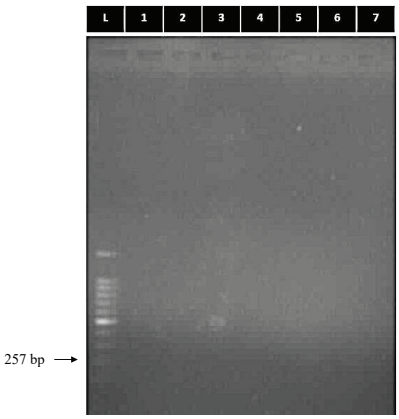

Figure S3b: Gel image showing the presence of *Pizt* gene using the marker of Z56591 – F/ Z56591 – R (257 bp). L: 100 bp ladder, Lane 1: Co10, Lane 2: Bg 369, Lane 3: Bw 351, Lane 4: *Attakkari*, Lane 5: *Bg 250*, Lane 6: *Bg 300*, Lane 7: *Bg 251*

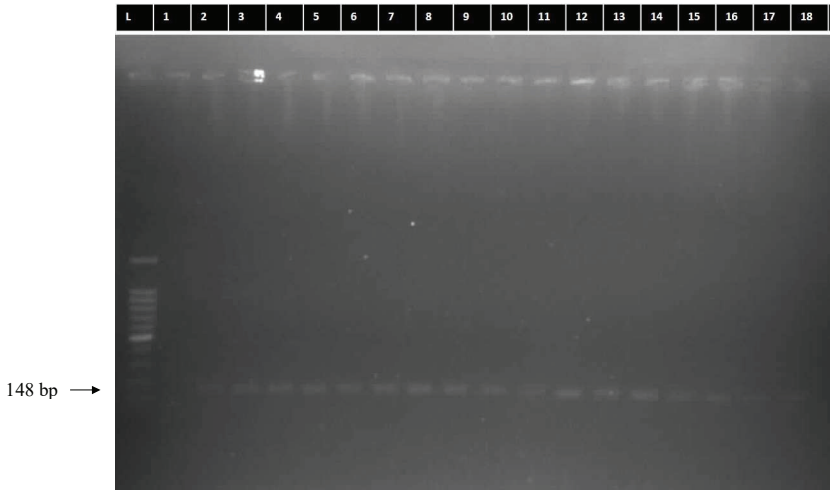

Figure S4a: Gel image showing the presence of *Pikp* gene using the marker of K3957 - F / K3957- R (148 bp). L: 100 bp ladder, Lane 1: Negative control, Lane 2: Bg 94-1, Lane 3: Bg 360, Lane 4: Bg 352, Lane 5: Bw 367, Lane 6: Co 10, Lane 7: *Moddaikaruppan*, Lane 8: Bg 251, Lane 9: Bg 450, Lane 10: Bg 300 Lane 11: At 308, Lane 12: *Suwandal*, Lane 13: Ld 365, Lane 14: Bg 366, Lane 15: *Pachchaiperumal*,, Lane 16: *Attakkari*, Lane 17: At 353, Lane 18: Bw 372

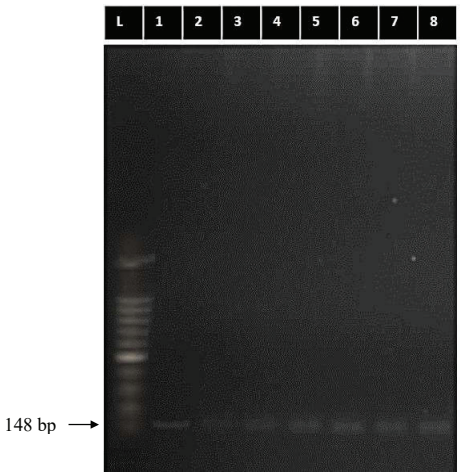

Figure S4b: Gel image showing the presence of Pikp gene using the marker of K3957 - F / K3957- R (148 bp). L: 100 bp ladder, Lane 1: *Bg 406*, Lane 2: *Bg 358*, Lane 3: *Bg 369*, Lane 4: *Bw 351*, Lane 5: *At 362*, Lane 6: *Bg 250*, Lane 7: *At 402*, Lane 8: *Karuthaheenati*

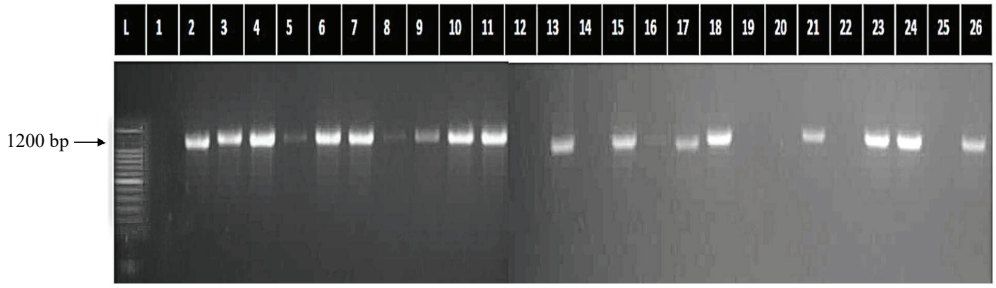

Figure S5: Gel image showing the presence of *Pik* gene using the marker of RGA4_F3/ Z56591 RGA4_ R3 (1200 bp). L: 100 bp ladder, Lane 1: Negative control, Lane 2: *Bg 94-1*, Lane 3: *Bg 360*, Lane 4: *Bg 352*, Lane 5: *Bw 367*, Lane 6: *Co 10*, Lane 7: *Moddaikaruppan*, Lane 8: *Bg 251*, Lane 9: *Bg 450*, Lane 10: *Bg 300*, Lane 11: *At 308*, Lane 12: *Suwandal*, Lane 13: *Ld 365*, Lane 14: *Bg 366*, Lane 15: *Pachchaiperumal*,, Lane 16: *Attakkari*, Lane 17: *At 353*, Lane 18: *Bw 372*, Lane 19: *Bg 406*, Lane 20: *Bg 358*, Lane 21: *Bg 369*, Lane 22: *Bw 351*, Lane 23: *At 362*, Lane 24: *Bg 250*, Lane 25: *At 402*, Lane 26: *Karuthaheenati*

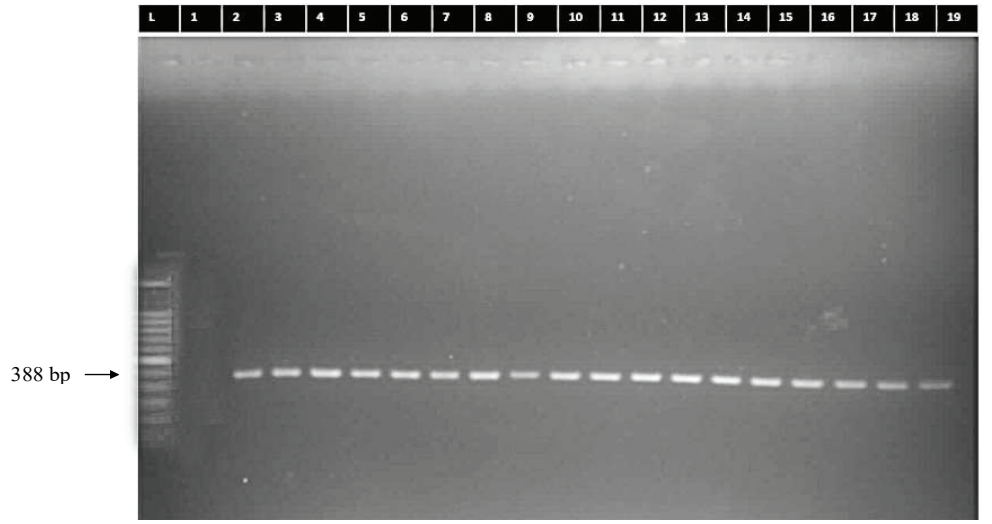

Figure S6a: Gel image showing the presence of *Pib* gene using the marker of Candidate Pi28 - F/ Pi28- R (388 bp). L: 100 bp ladder, Lane 1: Negative control, Lane 2: *Bg 94-1*, Lane 3: *Bg 360*, Lane 4: *Bg 352*, Lane 5: *Bw 367*, Lane 6: *At 402*, Lane 7: *Moddaikaruppan*, Lane 8: *Bg 251*, Lane 9: *Bg 450*, Lane 10: *Bg 300*, Lane 11: *Ld 365*, Lane 12: Suwandal, Lane 13: *At 308*, Lane 14: *Bg 366*, Lane 15: *Pachchaiperumal*,, Lane 16: *Attakkari*, Lane 17: *At 353*, Lane 18: *Bw 372*, Lane 19: *Bg 406*, Lane 20: *Bg 358*, Lane 21: *Bg 369*, Lane 22: *Bw 351*, Lane 23: *At 362*, Lane 24: *Bg 250*, Lane 25: *Co 10*, Lane 26: *Karuthaheenati*

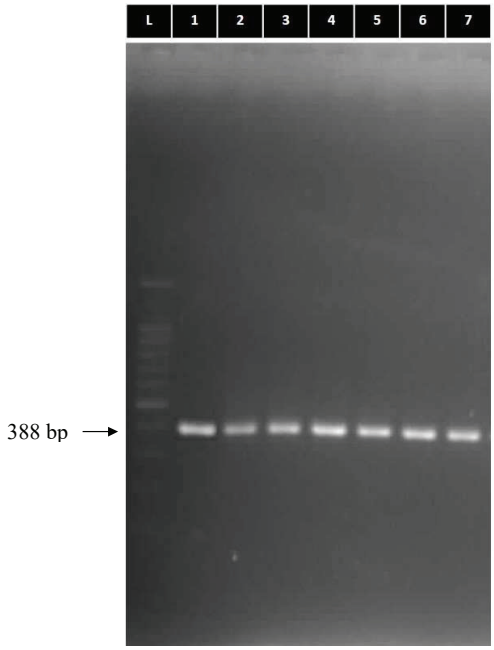

Figure S6b: Gel image showing the presence of *Pib* gene using the marker of Candidate Pi28 - F/ Pi28- R (388 bp). L: 100 bp ladder, Lane 1: *Bg 358*, Lane 2: *Bg 369*, Lane 3: *Bw 351*, Lane 4: *At 362*, Lane 5: *Bg 250*, Lane 6: *Co 10*, Lane 7: *Karuthaheenati*

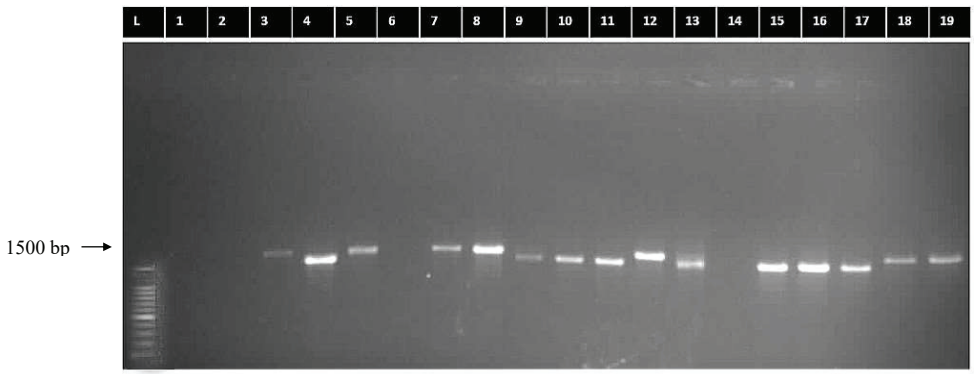

Figure S7a: Gel image showing the presence of *Pikh* gene using the marker of Candidate GM - F/ Candidate GM - R (1500 bp). L: 100 bp ladder, Lane 1: Negative control, Lane 2: *Bg 94-1*, Lane 3: *Bg 360*, Lane 4: *Bg 352*, Lane 5: *Bw 367*, Lane 6: *At 402*, Lane 7: *Moddaikaruppan*, Lane 8: *Bg 251*, Lane 9: *Bg 450*, Lane 10: *Bg 300*, Lane 11: *Ld 365*, Lane 12: *Suwandal*, Lane 13: *At 308*, Lane 14: *Bg 366*, Lane 15: *Pachchaiperumal*, Lane 16: *Attakkari*, Lane 17: *At 353*, Lane 18: *Bw 372*, Lane 19: *Bg 406*

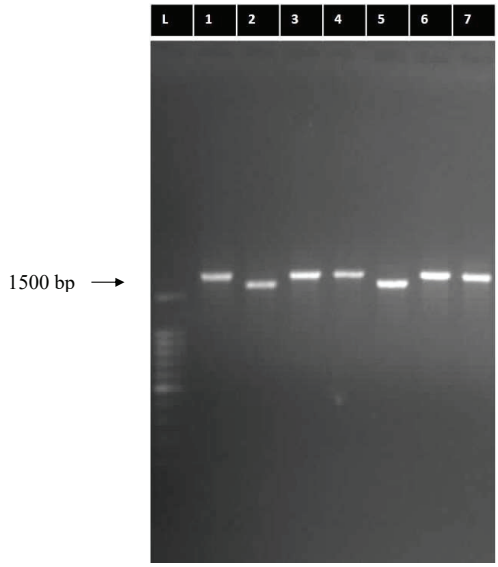

Figure S7b: Gel image showing the presence of *Pikh* gene using the marker of Candidate GM - F/ Candidate GM - R (1500 bp). L: 100 bp ladder, Lane 1: *Bg 358*, Lane 2: *Bg 369*, Lane 3: *Bw 351*, Lane 4: *At 362*, Lane 5: *Bg 250*, Lane 6: *Co 10*, Lane 7: *Karuthaheenati*

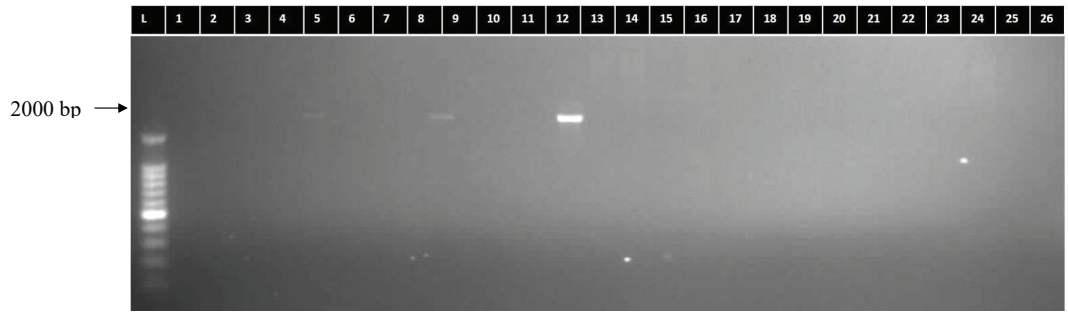

Figure S8: Gel image showing the presence of *Pi9* gene using the marker of Candidate 195R-1 F/ 195R-1 R (2000 bp). L: 100 bp ladder, Lane 1: Negative control, Lane 2: *Bg 94-1*, Lane 3: *Bg 360*, Lane 4: *Bg 352*, Lane 5: *Bw 367*, Lane 6: *At 402*, Lane 7: *Moddaikaruppan*, Lane 8: *Bg 251*, Lane 9: *Bg 450*, Lane 10: *Bg 300*, Lane 11: *Ld 365*, Lane 12: *Suwandal*, Lane 13: *At 308*, Lane 14: *Bg 366*, Lane 15: *Pachchaiperumal*, Lane 16: *Attakkari*, Lane 17: *At 353*, Lane 18: *Bw 372*, Lane 19: *Bg 406*, Lane 20: *Bg 358*, Lane 21: *Bg 369*, Lane 22: *Bw 351*, Lane 23: *At 362*, Lane 24: *Bg 250*, Lane 25: *Co 10*, Lane 26: *Karuthaheenati*