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Genotypic Assessment of Bacterial Leaf Blight Resistance in Indigenous Rice (*Oryza sativa* L.) Germplasm

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A B S T R A C T

One hundred rice genotypes were analyzed to evaluate the genetic polymorphism and identification of resistant lines to bacterial leaf blight (BLB) disease caused by *Xanthomonas oryzae* pv. *oryzae* using simple sequences repeat (SSR) markers. A total of 38 alleles were detected by seven polymorphic markers showing highly polymorphic across all genotypes with an average of 5.42 alleles per polymorphic marker. The marker RM-21, RM-122 and RM-224 produced maximum 6 alleles. The PIC values ranged from 0.345 to 0.688 and marker RM-21 was found to be the most appropriate marker to discriminate BLB resistant rice genotypes owing to the highest PIC value of 0.688. The cluster analysis showed that these genotypes grouped into four clusters, in which cluster IV had maximum forty genotypes followed by cluster III and cluster I. Multiple resistance ® genes (*Xa*21+ *xa*13+*xa*5+*Xa*4) were identified in germplasms such as Dhalakeera, Swarnamasuri, Purple puttu, Veethiruppu showing high level of resistance to BLB, while Navarai black 5571and Kalyani were found to be moderately resistant to BLB disease in both field and controlled condition. These genetically diverse BLB resistant genotypes can be directly utilized in rice BLB resistance breeding programs.

Introduction

The world's most daring problem is to nourish the developing population which is expected to reach 8 billion individuals by 2020, due to expanding population (Kubo and Purevdorj, 2004). As the population builds, the production of food diminishes due to an absence of reasonable land for crop development. The infection caused due to bacterial pathogens becomes a major challenge for the rice breeders. BLB is a vascular disease that causes a white yellow discoloration in rice crop along veins, leaf margins, leaf blades and those lesions may extend to the sheath (Gnanamanickam *et al.*, 1999).

In rice it causes annual yield losses conservatively estimated at 50% (Song and Goodman, 2001). When the rice is infected by *Xanthomonas oryzae* pv. *oryzae*, although the

symptoms of ailment may be determined at tillering stage, the disease can also retain to increase as the plant grows. It is observed that the rice plant at much less than 21 days old are more liable to disease and the bacteria may additionally desire temperature at 28-34˚C for boom.

The improvement of host resistance and utility of chemical and organic measures have been used for the management of BLB (Akhtar *et al.,* 2008). However, for the BLB management, host plant resistance is the most appropriate one to manage the pathogen.

To date, a total of 42 BLB resistance genes (R genes) have been identified in rice including *Xa1, Xa2, Xa3/Xa26, Xa4, xa5, Xa6, Xa7, xa8, xa9, Xa10, Xa11, Xa12, xa13, Xa14, xa15, Xa16, Xa17, Xa18, xa19, xa20, Xa21, Xa22*(t), *Xa23, xa24*(t), *Xa25, xa26*(t), *Xa27,xa28*(t), *Xa29*(t), *Xa29*(t), *Xa31*(t), *Xa33*(t), *xa34(t), Xa35(t), Xa36(t), Xa37, Xa38, Xa39, Xa40, xa41(t), Xa42*. The genes of recessive resistance comprise *xa5, xa8, xa9, xa13, xa15, xa19, xa20, xa24, xa25/Xa25(t), xa26*(t), *xa28*(t), *xa31*(t), and *xa33*(t).Ten of the recessive R genes; *xa5* (Petpisit *et al.*, 1977), *xa8*(Singh *et al.,* 2002), *xa13 (*Ogawa *et al.,* 1987*), xa24* (Khush and Angeles, 1999), *xa26, xa28*(Lee *et al.*, 2003)and *xa32* (Ruan *et al.,* 2008) confer race-specific resistance.

While the other three genes *viz.,xa*15(Ogawa 1996), *xa*19 and *xa*20 (Taura *et al.*, 1992), were created by mutagenesis and each confers a wide spectrum of resistance to *Xoo* (Ogawa, 1996;Lee *et al.,* 2003; Chen *et al.*, 2002).

Determination of genetic diversity can be done by assessing morphological or molecular data. The use of advanced molecular technologies is one of the possible approach to understand their diversity. Evaluation of genetic diversity using DNA marker technology is non-destructive, not affected by environmental factors, requires small number of samples, and does not require large experimental setup and equipment's for measuring physiological parameters (Kanawapee *et al.,* 2011).

Simple sequence repeat (SSR) marker analysis is an important tool for the identification of genetic variation in accessions(Sajib *et al.,* 2012; Ma *et al.,* 2011). SSR markers are highly informative, mostly monolocus, codominant, easily analyzed and cost effective (Garcia *et al.,* 2004) and able to detect high level of allelic diversity (Ni *et al.*, 2002), thus being widely applied in genetic diversity analysis, molecular map construction and gene mapping(Zhang *et al.*, 2007; Ma *et al.,* 2011) and analysis of germplasm diversity (Zhou *et al.,* 2003; Jin *et al.*, 2010); Ma *et al.,* 2011).

SSR markers even in less number can give a better genetic diversity spectrum due to their multi-allelic and highly polymorphic nature(Singh *et al.,* 2016).Therefore the present study was undertaken with the aim to identify BLB resistant rice lines and molecular diversity in rice genotypes using SSR markers.

Materials and Methods

Plant materials

Seeds of one hundred number of rice genotypes (Table 1) were obtained from Department of Plant breeding and Genetics, Agricultural College and Research Institute (AC&RI), Killikulam, Thoothukudi District, together with the comparison line IRBB60 (carrying *Xa21, xa13, xa5, Xa4*) as positive resistant gene check and TN1 as negative gene check for the study. All these lines were grown in field as well as in pots in the glasshouse.

Bacterial isolate and culture media

The virulent isolate of *Xanthomonas oryzae* pv. *oryzae (Xoo)* was collected from Tamil Nadu Agricultural University, Coimbatore. The *Xoo* isolate was multiplied and maintained on *Xoo* specific modified Wakimoto's medium, which contains sucrose 20g, sodium phosphate 0.82g, ferrous sulphate 0.05g, calcium nitrate 0.5g, peptone 5.0g, agar 17.0g and distilled water 1000 ml. The prepared medium was transferred to triangular flasks. The flasks were closed with cotton plugs, covered with kraft paper, and autoclaved at 121ºC for 30 minutes.

Inoculum preparation and plant inoculation

For inoculum preparation, 10 ml of sterile distilled water poured into pathogenic bacterial culture and preserved the inoculum concentration at @10^9 CFU / ml. Before panicle initiation, plants with entirely fresh and expanded leaves were inoculated using the leaf cutting process(Kauffman, 1973). The sterile scissors were dipped into the inoculum and 3 leaves per plant were trimmed from the tip of growing leaf about 2-3 cm apart. BLB lesions were found 15 days after inoculation on the clipped leaves.

Evaluation

During the *Rabi* season of 2019 –2020, the screening was performed in the rice crop at 10, 30, 50 days after planting under field and controlled conditions. The Percent Disease Index (PDI) and scales for evaluating the BLB resistance under field condition was determined based on the method suggested by Nagendran *et al.*, (2013)

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of all Numerical Ratings}}{\text{Total No of leaves graded}} \times \frac{100}{\text{Maximum Grade obtained}}$$

The scoring system used to evaluate breeding lines for BLB resistance in the field (IRRI, 1996; Rafi *et al.*, 2013) as:

Scale	Disease Leaf Area (%)	Description
0	0	Immune
1	1-10	Resistant
3	11-25	Moderate resistant
5	26-50	Moderate susceptible
7	51-75	Susceptible
9	76-100	Highly susceptible

The scoring system used to evaluate breeding lines for BLB resistance in the glasshouse (IRRI, 1996) as:

SI.No	Lesion length (cm)	Description
1.	0-5	Resistant
2.	5-10	Moderately resistant
3.	10-15	Moderately susceptible
4.	>15	Susceptible

Genomic DNA extraction

DNA samples were extracted from young leaves (21 days old seedlings) using the Cetyl-trimethyl ammonium bromide (CTAB) method modified from the protocol of Doyle and Doyle (1990). The quality of genomic DNA was analyzed in 0.8% agarose gel. Total genomic DNA samples were diluted to 100ng/µl using Tris HCL buffer and stored at -20˚C till further use.

SSR markers and PCR amplification

Of the 10 SSR markers screened, 7 markers showed polymorphism (Table 2).

Amplification of DNA fragments was carried out using gene specific markers of *Xa21, xa13, xa5, Xa4*. Each PCR amplification reaction was in a total volume of 10µl containing 5µl of 2X *Taq polymerase* master mix, 0.5µl of DNA, 0.3µl of forward primer, 0.3µl of reverse primer, 3.9µl of water. The polymerase chain reaction was performed in a thermocycler with the following cycles: the initial denaturation at 94˚C for 5 min followed by 35 cycles of denaturation at 94˚C for 30 sec, annealing at 55˚C for 30 sec followed by extension at 72˚C for 1 min and final extension at 72˚C for 10 min. In order to determine polymorphism, PCR products were checked on 3 % agarose in 1X TBE buffer. For pre-staining, ethidium bromide was added to the gel at the concentration of 10µl/ml before the gel was poured. The samples were run on the gel at 140V until the bromophenol blue dye migrated almost to the end of the gel. Thereafter electrophoresis, gel documentation was carried out and identified for the presence (++) and absence (--) of BLB gene linked DNA fragment.

Data analysis

The amplified fragments of all the rice genotypes were scored by comparing with respective resistance (IRBB60) and susceptible (TN1) bands. The data was scored using "++/--" signs for the presence/absence of target gene, respectively.

Cluster analysis based on UPGMA

The binary data matrix generated by polymorphic SSR markers was subjected to further analysis using Darwin software version 6.0. The dissimilarity matrix was used as an input for analysis of clusters. Phylogenetic tree was formed following un-weighted pair group method of arithmetic means (UPGMA) using the tool. In un-weighted pair-group average (UPGMA) clusters are joined based on the average distance between all members in four groups.

Polymorphic information content (PIC)

PIC for SSR markers was calculated as per the formula:

$$PIC = 1 - \sum P_{ij}^2$$

Where, PIC*ij i*s the frequency of the j^{th} allele for the i^{th} marker and summation extends over k alleles.

Results and Discussion

Screening for BLB resistance of rice germplasm under field and controlled conditions

Bacterial leaf blight of rice has been reported in several parts of the world with high incidence and severity (Sonti, 1998). Therefore strategies adapted to particular environments must be developed to avoid possible epidemics. Knowledge of varietal resistance is important for selecting cultivars with durable resistance to the disease (Banito *et al.,* 2010). The results of the present study showed the various levels of resistance to bacterial leaf blight in rice germplasm.

One hundred number of rice germplasm were screened for BLB resistance under field conditions without any artificial inoculation. The percentage of germplasm found to be resistant, moderately resistant, moderately susceptible and susceptible on 50 days after planting under field screening was found to be three per cent, thirty nine per cent, forty seven per cent and twelve per cent, respectively. Among the resistant genotypes Dhalakeera, Purple puttu and Veethiruppu recorded lowest PDI value of 8.90, 9.10 and 9.50 respectively. In this study, three resistant varieties *i.e.* Purpleputtu, Dhalakeera and Veethiruppu

were found to be completely resistant in field conditions. They are recommended for planting in areas where BLB often occurs to cause reduction in yield. However, the responses of these varieties should be further confirmed by artificial screening.

The rice germplasm was evaluated for its BLB resistance under controlled glass house environment using clipping method for the uniform spread of pathogen in plant tissues. The percentage of germplasm found to be resistant, moderately resistant, moderately susceptible and susceptible under artificial screening was two per cent, thirty eight per cent, forty three per cent and sixteen per cent, respectively. Among the resistant genotypes, Swarnamasuri and Purple puttu recorded mean lesion length of 4.38 and 4.96 cm, respectively. As expected, the resistance level among germplasmwas low with more than half of the resources being moderately susceptible or susceptible (Table 3).

Molecular screening for BLB resistance genes using SSR markers

The indigenous lines of 100 rice genotypes were evaluated for the presence and absence of BLB resistance genes *viz., Xa21, xa13, xa5, Xa4* using PCR based gene linked markers (Table 4). Molecular base pairs for total seven SSR markers which corresponds to IRBB60 (positive control) and TN1 (negative control) was given in the Table 2. During the gene survey using RM21 marker, out of 100 genotypes, 30 genotypes along with a positive control amplified 190bp size fragments which indicated the presence of *Xa21* gene. While the remaining 70 genotypes amplified 180 bp DNA fragments which showed the absence of *Xa21* gene. DNA analysis of *xa13* resistance gene in all the selected rice germplasm exhibited the presence of bands with markers RM230 and RM264. With marker RM230, 38 genotypes along with a positive control amplified 280bp size fragments, while with marker RM264, 35 genotypes along with a positive control amplified 180bp indicating the presence of *xa13* gene. DNA analysis of *xa5* resistance gene in all the selected rice germplasm exhibited the presence of bands with SSR markers RM122 and RM164. Out of 100 genotypes, 24 genotypes amplified 240bp corresponding to positive control (IRBB60) which showed the presence of *xa5* specific bands with marker RM122.

While with the marker RM164, 21 genotypes along with positive control amplified 240bp indicating the presence of *xa5* gene. Ramalingam *et al.,* (2001), Lee *et al.,* (2003)and Kihupi *et al.,* (2001)also conducted similar type of polymorphic survey for the presence of *xa5*, *xa13* and *Xa21* genes in rice germplasm. DNA analysis of the genotypes with RM224 and RM167 markers exhibited the presence and absence of *Xa4* gene. With marker RM 224, 46 genotypes showed the amplicon of 160bp corresponding to resistant allele and hence considered as resistant genotypes. While with marker RM 167, out of 100 rice genotypes, 11genotypes along with positive control amplified 140bp size fragments which indicated the presence of *Xa4* gene. Similar type of polymorphic survey was done by Arif *et al.,* (2008)for the presence (++) and absence (--) of *Xa4* gene in rice germplasmin Pakistan.

The resistant genotype Dhalakeera exhibited the presence of positive bands with the markers RM21, RM230, RM224 and RM167. Swarnamasuri exhibited the presence of positive bands for the markers RM21, RM230, RM264 and RM164. Further, Purple puttu and Veethiruppu exhibited the presence of positive bands with the markers RM21, RM264, RM164 and RM224. In the present study, four BLB resistant genes were identified, in which 7 genotypes carrying the

combination of *Xa21+xa13+xa5* genes, while 10genotypes carrying the combination of *Xa21+xa13+Xa4* genes. Further, 7 genotypes were identified with combination of *Xa21+xa5+Xa4*genes and 12 genotypes carrying the combination of *xa13+xa5+Xa4* genes.The lines carrying four R genes i.e., (*Xa21+xa13+xa5+Xa4*) are Kalyani, Navarai black 5571, Purple puttu, Veethiruppu, along with resistant check IRBB60(Table 5). It is worthy of mention that the resistance levels of Dhalakeera, Swarnamasuri and Purple puttu, Veethiruppu were found to be high due to the presence of multi genes (*Xa21+xa13+Xa4*), (*Xa21+xa13+xa5*) and (*Xa21+xa13+ xa5+Xa4*), respectively. Navarai black 5571 and kalyani carrying four R genes showed moderate resistance to BLB under field and artificial screening.

The rice genotypes carrying multi-genes will pave way for the identification of resistant sources for further breeding programmes. Similar results were reported by Sodhi *et al.*, (2003)concluding that combination of *Xa21* with *xa13* and *Xa5* BLB resistance genes are effective against the prevalent strains of *Xanthomonas (Xoo*). Perumalsamy *et al.*, (2010)reported that the genes *Xa4*, *xa5* along with *Xa21* provide wide spectrum of bacterial leaf blight resistance against many *X. oryzae*races.

Polymorphism and marker efficiency

All the 100 germplasm lines were genotyped by using 7 SSR markers. The allele size variation between the smallest and the largest allele at a given SSR was correlated with number of alleles per locus (Table 6). Thus, RM 167 presented the smallest allele size range 100 – 150 bp and RM 122 had the largest allele size range 240 – 300 bp. The informativeness of the markers was revealed by calculating the polymorphism information content (PIC). The PIC value of seven SSR markers ranged from 0.345 (RM 167) to 0.688 (RM 21) with 0.528 average respectively. Above 0.5 values were observed for RM 21 (0.688), RM 230 (0.507), RM 64 (0.664), RM 122 (0.501) and RM 224 (0.603) which showed the high polymorphic nature of primers. Similar studies proved the high informativeness of SSR markers (Kumar and Bhagwat, (2012) and Lang *et al.*, (2014).

Cluster analysis

A phylogenetic tree (Fig. 1) based on neighbor joining method was constructed using UPGMA. The rice genotypes were grouped into four main clusters (Table 7) *i.e.* cluster I, cluster II, cluster III and cluster IV consisting 25, 10, 25 and 40 genotypes, respectively. Cluster analysis was done to identify the variation between the genotypes and within the genotypes. The resistant genotypes are present in different cluster group having high dissimilarity than other genotypes. It clearly notifies that resistant genotypes had high diversity with BLB resistance.

Genetic diversity is the key determinant of germplasm utilization in crop improvement. Population with high level of genetic variation is the valuable resource for broadening the genetic base in any breeding program. The study of genetic variability was estimated based on the amplification pattern of 7 SSR markers. Brondani *et al.*, (2006) reported the relative efficiency of utilizing the SSR markers for the assessment of genetic diversity. In this study genetic diversity among the accessions were evaluated by model based on clustering approach using the SSR genotypic data. The clusters were developed by using neighbor joining method. This grouping method was further supported by studies of Upadhyay *et al.*, (2012), Nachimuthu *et al.*, (2015) and Singh *et al.*, (2016).

Table.1 List of rice genotypes included in current study

S.No.	Germplasms	S.No.	Germplasms	S.No.	Germplasms	S.No.	Germplasms
1	TN1(Susceptible check)	**26**	Chittini 5520	**51**	Maranellu	**76**	Swarnamasuri
2	IRBB60 (Resistant check)	**27**	Chuvannachittini 7135	**52**	Mallikar	**77**	Thondi
3	Adukan	**28**	Chomala 826	**53**	Mattai	**78**	Thuyamalli
4	Aman	**29**	Company thavalaikannan	**54**	Molikarumbu	**79**	Uma
5	Anjali	**30**	Dhalakeera	**55**	Mapillai samba	**80**	Varakuranellu
6	Annada	**31**	Jai sree ram	**56**	Mulampunchan	**81**	Vanaprabha
7	Aryan 917	**32**	Kunjukunju 7168	**57**	Navarai	**82**	Vattan 5052
8	Aryan 1023	**33**	Kunjukunju 6974	**58**	Navarai Black	**83**	Veethiruppu
9	Aryan 1102	**34**	Kunjukunju 1811	**59**	Navarai Black 5571	**84**	Virendra
10	Aryan 1203	**35**	Karnellu	**60**	Navarai Black 957	**85**	White ponni
11	Aryan 5532	**36**	Karukot	**61**	Navarai Black 6263	**86**	Thamarai
12	Aryan 6333	**37**	Karuvalli	**62**	Noothipattu	**87**	Salem sannam
13	Bharathi	**38**	KaruthaNavara	**63**	Norugan	**88**	Vasanai samba
14	Bommi	**39**	Kalyani	**64**	Ohenellu 6305	**89**	Anna 4
15	Chemban 986	**40**	Kullakar	**65**	Oheruchittini	**90**	Chenellu 6805
16	Chandikar	**41**	Kallondaikar	**66**	Pattani	**91**	Chungamnellu
17	Chembaru 5599	**42**	Kattanoor	**67**	Purple puttu	**92**	Kodikannan
18	Chembaru 4331	**43**	Kalinga	**68**	Poongar	**93**	Varapudha
19	ChittiraiKar	**44**	Koltara samba	**69**	Rajalakshmi	**94**	Krishna hemarathi
20	Chiruchittini 882	**45**	Kothamalli samba	**70**	Sadabahar	**95**	Jaya
21	Chenellu 4735	**46**	Kichali samba	**71**	Shabhagidan	**96**	Abiyan
22	Chenellu 5590	**47**	Kayamma	**72**	Seeraga samba	**97**	Namchenbyeo
23	Chenthodi	**48**	Kuliyadichan	**73**	Surakuruvai	**98**	Gowni
24	Chenkayamma 5523	**49**	Kerala kandasala	**74**	Swarna	**99**	Karsamba
25	Chittini 1123	**50**	Kalakeerai	**75**	Swarnamalli	**100**	White sannam

Table.2 List of gene-specific SSR markers used for screening of BLB resistance genes

Gene	Primer name	Primer Sequence (5'-3')	No of Base pairs	Annealing temp (˚C)	Resistant band (bp)	Susceptible band (bp)
Xa21	RM21	F: ACAGTATTCCGTAGGCACGG	20			
		R: GCTCCATGAGGGTGGTAGAG	20	50	190	180
xa13	RM230	F: GCCAGACCGTGGATGTTC	18			
		R: CACCGCAGTCACTTTTCAAG	20	50	280	270
	RM264	F: GTTGCGTCCTACTGCTACTTC	21			
		R: GATCCGTGTCGATGATTAGC	20	50	180	190
xa5	RM122	F: GAGTCGATGTAATGTCATCAGTGC	24			
		R: GAAGGAGGTATCGCTTTGTTGGAC	24	55	240	270
	RM164	F: TCTTGCCCGTCACTGCAGATATCC	24			
		R: GCAGCCCTAATGCTACAATTCTTC	24	55	240	250
Xa4	RM224	F: ATCGATCGATCTTCACGAGG	20			
		R: TGCTATAAAAGGCATTCGGG	20	55	160	150
	RM167	F: GATCCAGCGTGAGGAACACGT	21			
		R: AGTCCGACCACAAGGTGCGTTGTC	24	55	140	130

Table.3 Screening of rice germplasm under field and controlled conditions

S.no	Germplasms	Field screening			Artificial screening	
		PDI	Scale	Description	LL (cm)	Description
1.	TN 1	45.70	5	MS	18.75	S
2.	IRBB60	12.30	3	MR	6.25	MR
3.	Adukan	20.80	3	MR	10.00	MR
4.	Aman	30.20	5	MS	14.50	MS
5.	Anjali	30.30	5	MS	9.50	MR
6.	Annada	30.60	5	MS	7.50	MR
7.	Aryan 917	54.10	7	S	16.25	S
8.	Aryan 1023	55.00	7	S	11.25	MS
9.	Aryan 1102	63.50	5	MS	9.50	MR
10.	Aryan 1203	23.70	3	MR	7.50	MR
11.	Aryan 5532	24.40	3	MR	8.25	MR
12.	Aryan 6333	30.40	5	MS	13.50	MS
13.	Bharathi	50.00	5	MS	14.50	MS
14.	Bommi	30.80	5	MS	11.75	MS
15.	Chemban 986	15.20	3	MR	7.25	MR
16.	Chandikar	25.60	3	MR	11.25	MS
17.	Chembaru 5599	30.90	5	MS	9.50	MR
18.	Chembaru 4331	69.70	7	S	17.25	S
19.	ChittiraiKar	43.20	5	MS	10.00	MR
20.	Chiruchittini 882	40.10	5	MS	8.25	MR
21.	Chenellu 4735	21.20	3	MR	14.75	MS

22.	Chenellu 5590	20.50	3	MR	6.25	MR
23.	Chenthodi	60.60	7	S	14.25	MS
24.	Chenkayamma 5523	17.80	3	MR	12.14	MS
25.	Chittini 1123	40.70	5	MS	16.25	S
26.	Chittini 5520	20.50	3	MR	12.50	MS
27.	Chuvannachittini 7135	47.50	5	MS	14.25	MS
28.	Chomala 826	35.60	5	MS	14.60	MS
29.	Company thavalaikannan	65.80	7	S	18.25	S
30.	Dhalakeera	8.90	1	R	5.47	MR
31.	Jai sree ram	71.80	7	S	9.50	MR
32.	Kunjukunju 7168	48.10	5	MS	13.50	MS
33.	Kunjukunju 6974	30.50	5	MS	14.75	MS
34.	Kunjukunju 1811	20.80	3	MR	9.75	MR
35.	Karnellu	40.90	5	MS	17.75	S
36.	Karukot	20.50	3	MR	11.75	MS
37.	Karuvalli	39.70	5	MS	13.75	MS
38.	KaruthaNavara	34.50	5	MS	12.75	MS
39.	Kalyani	20.70	3	MR	7.75	MR
40.	Kullakar	30.20	5	MS	13.25	MS
41.	Kallondaikar	43.10	5	MS	11.25	MS
42.	Kattanoor	34.90	5	MS	19.25	S
43.	Kalinga	21.60	3	MR	14.50	MS
44.	Koltara samba	19.75	3	MR	13.50	MS
45.	Kothamalli samba	22.60	3	MR	9.80	MR
46.	Kichali samba	22.10	3	MR	16.25	S
47.	Kayamma	40.20	5	MS	11.75	MS
48.	Kuliyadichan	30.90	5	MS	13.75	MS
49.	Kerala kandasala	20.80	3	MR	8.25	MR
50.	Kalakeerai	35.50	3	MR	9.25	MR
51.	Maranellu	60.50	7	S	12.25	MS
52.	Mallikar	30.50	5	MS	11.75	MS
53.	Mattai	19.60	3	MR	11.75	MS
54.	Molikarumbu	22.50	3	MR	20.25	S
55.	Mapillai samba	40.60	5	MS	9.75	MR
56.	Mulampunchan	20.50	3	MR	7.75	MR
57.	Navarai	35.60	5	MS	13.25	MS
58.	Navarai Black	30.80	5	MS	11.75	MS
59.	Navarai Black 5571	22.50	3	MR	8.25	MR
60.	Navarai Black 957	49.50	5	MS	14.75	MS
61.	Navarai Black 6263	70.50	7	S	19.75	S
62.	Noothipattu	45.50	5	MS	14.00	MS

63.	Norugan	22.20	3	MR	9.25	MR
64.	Ohenellu 6305	30.40	5	MS	9.25	MR
65.	Oheruchittini	30.60	5	MS	8.75	MR
66.	Pattani	43.50	5	MS	13.75	MS
67.	Purple puttu	9.10	1	R	4.96	R
68.	Poongar	20.10	3	MR	9.50	MR
69.	Rajalakshmi	69.50	7	S	18.50	S
70.	Sadabahar	30.50	5	MS	12.75	MS
71.	Shabhagidan	31.90	5	MS	8.50	MR
72.	Seeraga samba	24.00	3	MR	9.62	MR
73.	Surakuruvai	42.50	5	MS	13.10	MS
74.	Swarna	20.40	3	MR	9.50	MR
75.	Swarnamalli	32.30	5	MS	14.25	MS
76.	Swarnamasuri	21.60	3	MR	4.38	R
77.	Thondi	22.50	3	MR	9.75	MR
78.	Thuyamalli	21.50	3	MR	12.75	MS
79.	Uma	20.50	3	MR	9.50	MR
80.	Varakuranellu	35.10	5	MS	16.75	S
81.	Vanaprabha	22.10	3	MR	13.75	MS
82.	Vattan 5052	60.50	7	S	16.25	S
83.	Veethiruppu	9.50	1	R	9.75	MR
84.	Virendra	42.50	5	MS	9.25	MR
85.	White ponni	41.20	5	MS	11.75	MS
86.	Thamarai	18.00	3	MR	9.75	MR
87.	Salem sannam	30.80	5	MS	12.75	MS
88.	Vasanai samba	41.30	5	MS	13.20	MS
89.	Anna 4	20.90	3	MR	7.51	MR
90.	Chenellu 6805	73.90	7	S	12.50	MS
91.	Chungamnellu	43.70	5	MS	14.50	MS
92.	Kodikannan	47.60	5	MS	16.25	S
93.	Varapudha	19.60	3	MR	8.25	MR
94.	Krishna hemarathi	31.50	5	MS	16.95	S
95.	Jaya	20.90	3	MR	13.25	MS
96.	Abiyan	40.70	5	MS	7.50	MR
97.	Namchenbyeo	24.50	3	MR	6.15	MR
98.	Gowni	21.50	3	MR	8.75	MR
99.	Karsamba	46.60	5	MS	12.22	MS
100.	White sannam	68.90	7	S	19.25	S

PDI – Per cent Disease Index MR - Moderately Resistant S - Susceptible
I -Immune HS - Highly Susceptible LL - Lesion length
R - Resistant MS - Moderately Susceptible

Table.4 Screening of rice germplasm for *Xa21, xa13, xa5 and Xa4* genes using SSR markers

S.no	Germplasms	Gene status						
		RM21 (*Xa21*)	RM230 (*xa13*)	RM264 (*xa13*)	RM122 (*xa5*)	RM164 (*xa5*)	RM224 (*Xa4*)	RM167 (*Xa4*)
1	TN 1 (Susceptible check)	--	--	--	--	--	--	--
2	IRBB60 (Resistant check)	++	++	++	++	++	++	++
3	Adukan	--	--	++	++	++	++	++
4	Aman	--	--	++	--	--	++	++
5	Anjali	--	--	++	--	--	++	--
6	Annada	--	++	--	--	--	++	--
7	Aryan 917	--	--	--	--	--	--	--
8	Aryan 1023	--	--	--	--	--	++	--
9	Aryan 1102	--	++	--	--	--	++	--
10	Aryan 1203	++	--	--	++	--	++	--
11	Aryan 5532	--	++	--	++	--	++	--
12	Aryan 6333	--	--	--	++	--	--	--
13	Bharathi	--	--	--	++	++	--	--
14	Bommi	++	++	--	--	--	--	--
15	Chemban 986	--	++	--	--	--	--	--
16	Chandikar	--	++	--	--	--	--	--
17	Chembaru 5599	--	++	++	--	--	--	--
18	Chembaru 4331	--	--	--	--	--	--	--
19	ChittiraiKar	--	++	--	--	--	++	--
20	Chiruchittini 882	--	--	--	++	--	++	--
21	Chenellu 4735	--	++	--	++	++	++	--
22	Chenellu 5590	--	++	++	++	--	++	--
23	Chenthodi	--	--	++	--	--	--	--
24	Chenkayamma 5523	--	--	++	--	++	++	--
25	Chittini 1123	--	--	++	--	--	--	--
26	Chittini 5520	--	++	--	--	++	--	--
27	Chuvannachittini 7135	--	--	--	--	++	--	++
28	Chomala 826	--	--	--	--	--	--	++
29	Company thavalaikannan	--	--	--	--	--	--	--
30	Dhalakeera	++	++	--	--	--	++	++
31	Jai sree ram	--	--	--	--	++	--	--
32	Kunjukunju 7168	--	--	++	--	--	++	--
33	Kunjukunju 6974	--	--	++	--	--	++	--
34	Kunjukunju 1811	++	--	++	--	--	++	--

35	Karnellu	--	--	++	--	--	--	--
36	Karukot	--	--	++	--	++	--	--
37	Karuvalli	--	--	++	--	--	--	++
38	KaruthaNavara	--	--	++	--	--	--	--
39	Kalyani	++	--	++	++	++	++	--
40	Kullakar	--	--	++	++	--	--	--
41	Kallondaikar	++	--	--	++	--	--	--
42	Kattanoor	--	--	--	++	++	--	--
43	Kalinga	++	++	--	++	--	--	--
44	Koltara samba	++	++	--	--	--	++	++
45	Kothamalli samba	++	--	--	++	--	++	--
46	Kichali samba	--	++	--	--	--	++	--
47	Kayamma	--	--	--	++	--	--	--
48	Kuliyadichan	--	++	--	--	--	--	--
49	Kerala kandasala	--	++	--	--	--	++	--
50	Kalakeerai	++	++	--	--	--	++	--
51	Maranellu	--	--	--	--	--	--	--
52	Mallikar	++	--	--	--	--	--	--
53	Mattai	--	--	--	--	++	--	--
54	Molikarumbu	--	--	--	--	--	--	--
55	Mapillai samba	--	--	++	--	--	++	--
56	Mulampunchan	--	--	--	++	--	--	--
57	Navarai	--	--	--	++	--	++	--
58	Navarai Black	++	--	--	--	--	--	--
59	Navarai Black 5571	++	++	++	++	++	--	++
60	Navarai Black 957	--	--	--	--	--	++	--
61	Navarai Black 6263	--	--	--	--	--	--	--
62	Noothipattu	--	++	++	--	--	--	--
63	Norugan	--	++	++	--	--	--	++
64	Ohenellu 6305	--	++	--	--	--	--	--
65	Oheruchittini	--	--	--	--	--	++	--
66	Pattani	--	--	--	--	++	--	++
67	Purple puttu	++	--	++	--	++	++	--
68	Poongar	++	--	--	++	--	++	--
69	Rajalakshmi	--	--	--	--	--	--	--
70	Sadabahar	++	--	--	++	--	--	--
71	Shabhagidan	++	--	--	--	--	--	--
72	Seeraga samba	++	--	--	--	--	++	--
73	Surakuruvai	++	--	--	--	--	--	--
74	Swarna	++	--	--	--	--	++	--
75	Swarnamalli	--	--	--	--	--	++	--

76	Swarnamasuri	++	++	++	--	++	--	--
77	Thondi	--	++	++	--	++	++	--
78	Thuyamalli	--	++	--	--	--	--	--
79	Uma	++	++	++	--	--	++	--
80	Varakuranellu	--	++	--	--	--	--	--
81	Vanaprabha	--	++	++	--	--	++	--
82	Vattan 5052	--	--	++	--	--	++	--
83	Veethiruppu	++	--	++	--	++	++	--
84	Virendra	++	--	++	--	++	--	--
85	White ponni	++	--	--	--	--	++	--
86	Thamarai	--	--	--	--	--	++	--
87	Salem sannam	++	++	--	--	--	--	--
88	Vasanai samba	++	++	++	--	--	--	--
89	Anna 4	--	++	++	++	++	++	--
90	Chenellu 6805	--	--	--	--	--	++	--
91	Chungamnellu	--	--	--	++	--	--	--
92	Kodikannan	--	--	++	++	--	--	--
93	Varapudha	--	--	++	--	++	++	--
94	Krishna hemarathi	--	++	--	--	--	--	--
95	Jaya	++	++	--	--	--	--	--
96	Abiyan	--	++	--	--	--	++	--
97	Namchenbyeo	--	++	--	--	--	++	--
98	Gowni	++	++	++	--	--	++	--
99	Karsamba	--	++	--	--	--	--	--
100	White sannam	--	--	--	--	--	--	--

Table.5 Multi-genic lines carrying different combinations of BLB resistant genes

S.no	Combination of BLB resistant genes	No.ofgermplasms	Name of the germplasms
1	*Xa21+xa13+xa5*	7	Kalyani, Kalinga, Navarai black 5571, Purple puttu, Swarnamasuri, Veethiruppu, Virendra
2	*Xa21+xa13+Xa4*	10	Dhalakeera, Kunjukunju1811, Kalyani, Koltara samba, Kalakeerai, Navarai black 5571, Purple puttu, Uma, Veethiruppu, Gowni
3	*Xa21+xa5+Xa4*	7	Aryan 1203, Kalyani, Kothamalli samba, Navarai black 5571, Purple puttu, Poongar, Veethiruppu
4	*xa13+xa5+Xa4*	12	Adukan, Aryan 5532, Chenellu 4735, Chenellu 5590, Chenkayamma 5523, Kalyani, Navarai black 5571, Purple puttu, Thondi, Veethiruppu, Anna 4, Varapudha
5	*Xa21+xa13+xa5+Xa4*	5	IRBB60, Kalyani, Navarai black 5571, Purple puttu, Veethiruppu

Table.6 Details of the SSR primers used in present study, allele size (bp) and polymorphism information content (PIC)

SSR Markers	Total alleles	Allele size (bp)		PIC
		Minimum	Maximum	
RM21	6	100	210	0.688
RM230	5	240	280	0.507
RM264	5	150	200	0.664
RM122	6	240	300	0.501
RM164	5	240	280	0.392
RM224	6	100	160	0.603
RM167	5	100	150	0.345
Total	38	1170	1580	3.700
Mean	5.42	167.14	225.71	5.528

Table.7 Composition of clusters formed in phylogenetic tree diagram for rice genotypes

Cluster number	No.of genotypes	Name of the germplasms
I	25	Kattanoor, Bharathi, Mattai, Kullakar, Kayamma, Kodikannan, Maranellu, Navarai black 6263, Poongar, Aryan 1203, Sadabahar, Chungamnellu, Mulampunchan, Navarai, Surakuruvai, Shabhagidan, Rajalakshmi, Molikarumbu, Navarai black, White ponni, Oheruchitteni, Chenellu 6805, Swarnamalli, TN 1, Uma
II	10	Pattani, Chuvannachitteni 7135, Jai sree ram, Karuvalli, Chomala 826, White sannam, Company thavalaikannan, Chembaru 4331, Chemban 986, Aryan 917
III	25	Navarai black 5571, Adukan, Kalyani, Anna 4, Thondi, Chenkayamma 5523, Varapudha, Veethiruppu, Virendra, Swarnamasuri, Karukot, Chittini 5520, Chenellu 4735, Vattan 5052, Kunjukunju 6974, Mapillai samba, Karuthanavara, Karnellu, Kunjukunju 1811, Aman, Anjali, Chittini 1123, Chenthodi, Ohenellu 6305, IRBB 60
IV	40	Chittiraikar, Kunjukunju 7168, Annada, Chandikar, Thamarai, Chiruchitteni 882, Aryan 6333, Norugan, Aryan 1023, Abiyan, Kerala kandasala, Kichali samba, Namchenbyeo, Chenellu 5590, Aryan 5532, Varakuranellu, Kuliyadichan, Karsamba, Chembaru 5599, Noothipathu, Krishna hemarathi, Thuyamalli, Koltara samba, Dhalakeera, Kalakeerai, Bommi, Gowni, Vanaprabha, Vasanai samba, Salem sannam, Kalinga, Jaya, Purple puttu, Swarna, Seeraga samba, Navarai black 957, Kothamalli samba, Mallikar, Kallondaikar, Aryan 1102.

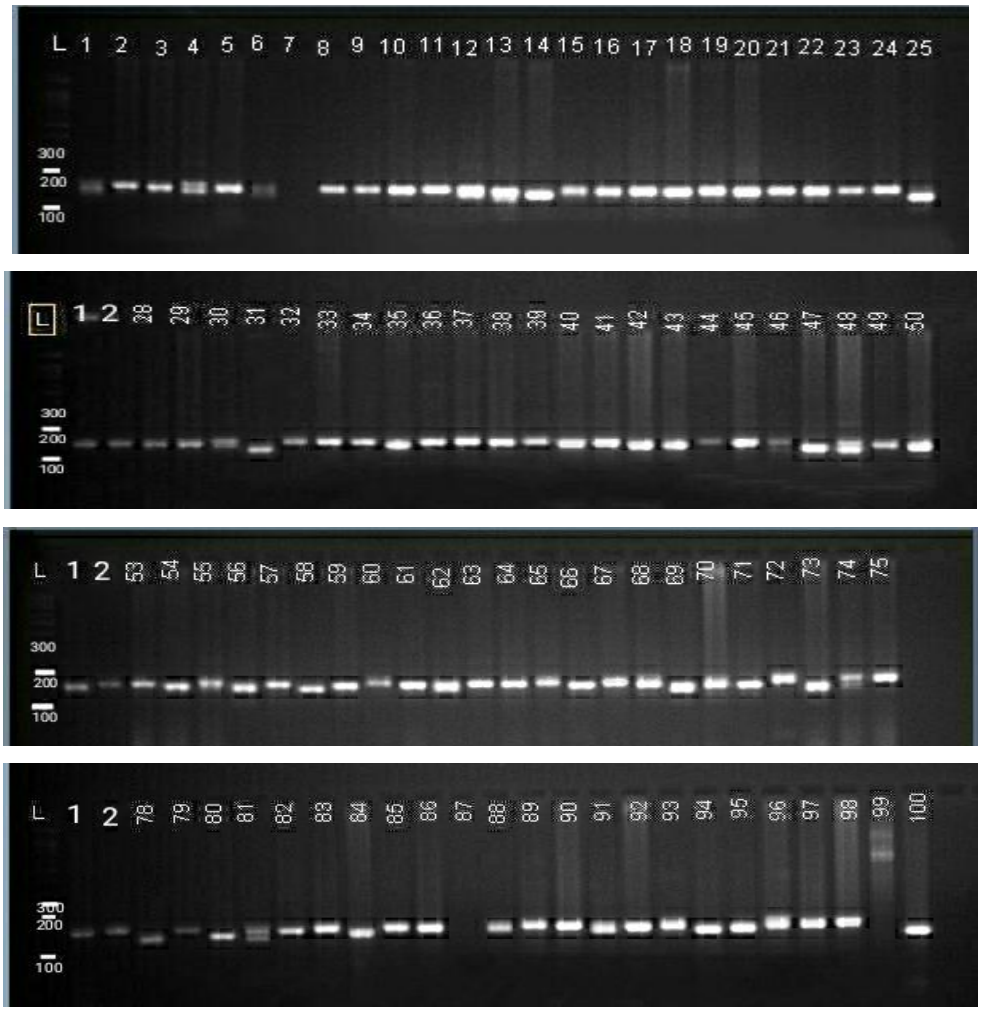

Fig.1 Molecular profile of SSR marker RM224 -*Xa4*

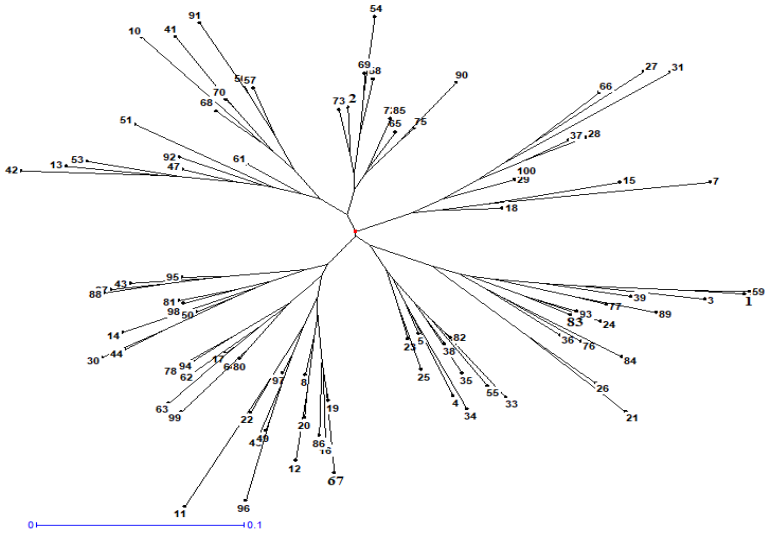

Fig.2 Phylogenetic tree constructed based on molecular marker (SSR) data

The genotypes consists uprooted tree grouped 100 germplasm into four major clusters. Clusters I, II III and IV were consisting number of genotypes *viz*., 25, 10, 25 and 40. The UPMGA cluster tree analysis showed that the genotypes present in same cluster are genetically similar and different cluster are genetically dissimilar (Mubassir *et al.*, 2016).

In the present study, bacterial leaf blight resistant genotypes were identified through polymorphic SSR markers. The BLB resistant genotypes were Veethiruppu, Purple puttu, Dhalakeera and Swarnamasuri. IRBB-60 (Positive check), Veethiruppu and Swarnamasuri genotypes were present in cluster III, Purple puttu and Dhalakeera were present in cluster IV. This showed genetic similarity and dissimilarity between the genotypes. The study result indicated that genetically diverse BLB resistant genotypes can be used for elite molecular breeding program and also used as a baseline for improvement of rice varieties.

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