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Studies on interaction of rice and bacterial leaf blight causing *Xanthomonas oryzae* pv. *oryzae*

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ABSTRACT

Bacterial leaf blight pathogen, *Xanthomonas oryzae* pv. *oryzae* is a major biotic constraint in rice production which eventually make the global food grain sustainability under stress. Host resistance being the only strategy of management of the disease, the present study focused on screening of 28 near isogenic lines and/or varieties under field condition which revealed resistant reaction of the following lines *viz*., IRBB 21, IRBB 57, IRBB 58 and IRBB 61 against *Xanthomonas oryzae* pv. *oryzae* (NCBI Accession No. MH464904). Besides, R genes *viz*., *Xa21*, *xa5* and *xa13* were identified in these near isogenic lines where different combinations of genes have been identified. With the intention of understanding the kind of avr genes present in X. o. pv. oryzae isolate and thereby elucidating the kind of interaction persists among rice and bacterial leaf blight pathogen, gene specific amplification and identification of avr genes were performed. The results revealed that *avr* genes *viz*., *PthXo1*, *avrxa3*, *AvrXa10*, *Avr/pthC8b* and *Avr/pth56B* were positively amplified whereas *Avr/pth3* was absent in the isolate even at repeated attempts. Interaction of *X.* o. *pv.* oryzae against ADT 38 rice variety through SDS PAGE analysis of the pathogen and abiotic agents (Biotin (0.1mM), Riboflavin (0.5mM), Chloramphenicol (0.1mM), Ethrel (1µl/ml)) applied samples showed that defense related proteins were induced alike in all the samples treated irrespective of the treatment when compared to untreated control.

KEY WORDS: *AVR* GENE BACTERIAL LEAF BLIGHT, R GENE, RICE

INTRODUCTION

Plants are confronted consistently with pathogens and based on the molecular determinants in plants (Pathogen recognition receptor (PRRs), Resistance genes) and in pathogen (Pathogen associated molecular patterns (PAMPs), *Avr* genes) both interacts to build defense responses where PRRs and PAMPs interacts to provide broad spectrum resistance (horizontal resistance) whereas R genes and *Avr* genes interaction may result in gene

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specific resistance (vertical resistance) (Vander Plank, 1984). Rice is one of the most significant cultivated food crops which feeds half of the population worldwide where the demand for rice is progressively increasing in developing countries (Khush and Jena, 2009) whereas bacterial leaf blight pathogen, *Xanthomonas oryxae* pv. *oryzae* (*Xoo*) becoming one among the important delimiting biotic factor that reduces rice production up to 81 per cent (Kumar *et al.* 2012). Host resistance is the only management strategy that could strongly dependent to contain the disease as the practice of application of chemicals in management of disease fronting adverse backlashes (Rajpurohit *et al.* 2011, Dokku *et al.* 2013, Suh *et al.* 2013; Sundaram *et al.* 2014; Arunakumari *et al.* 2016; Hajira *et al.* 2016; Gao *et al.* 2018).

Hence, most favorable strategies for crop improvement program for the disease resistance is either selection of donor source of resistance against *Xoo* and thereby using for resistance breeding or by introduction of resistance genes into desired variety or cultivars. Since understanding of interaction between rice and bacterial leaf blight pathogen is still remain ambiguous, researches on elucidating the mechanism of interaction and defense response has significant role. Interaction between rice and bacterial leaf blight pathogen is tend to follow the classical gene-for-gene relationship (Flor 1971), however, there exists distinctive differences for the interaction of R genes of bacterial leaf blight disease from characterized R genes of other crops where researchers also identified closely linked molecular markers (Yoshimura *et al.* 1995, Sonti 1998; Rao *et al.* 2002, Gu *et al.* 2008; Khan *et al.* 2015; Arunakumari *et al.* 2016; Hajira *et al.* 2016; Chukwu *et al.* 2019).

Exploration, identification, and utilization of new resistant germplasms in rice breeding are the strategical steps to control the bacterial blight disease of rice. The *Xa21* gene has been successfully introgressed into several elite rice varieties and hybrid rice parental lines all over the world either singly or in combination with other major resistance genes such as *Xa4*, *xa5*, and *xa13* (Singh et al. 2001; Joseph et al. 2004; Sundaram et al. 2008; Sundaram et al. 2009; Perumalsamy et al. 2010; Pandey et al. 2013).

Most of the reported R genes of other crop-pathogen systems are dominant in nature, unlike in rice where one-third of the R-genes conferring resistance to bacterial leaf blight disease have been reported as recessive (Verdier *et al.* 2012). Forty resistant genes, *Xa1* to *Xa39* have been identified in rice system which were against bacterial leaf blight pathogen (Kim *et al.* 2015).

Such R genes correspondingly recognize *Avr* gene products of the bacterial leaf blight pathogen directly or indirectly which could mediate defense response. However, such responses to contain the disease never ensue often in a host-pathogen system as the pathogen could evolve and evoke the virulence to cause the disease with distinct avirulence and virulence factor. Hence, screening of rice lines against various strains or pathotypes of bacterial leaf blight pathogen has prominent role to play. TAL effector dependent R genes induces downstream expression of R genes, for example, *Xa10* contains a binding element for the TAL effector, *AvrXa10* (*EBEAvrXa10*) in its promoter, and which induces *Xa10* expression (Tian *et al.* 2014). Hence, *Avr* and R gene plays imperative changes in host system to combat bacterial leaf blight disease in rice. Therefore, identification of the presence of *Avr* genes in the pathotypes prevailing in each location and its virulence nature against the cultivars and Near Isogenic Lines (NILs) could help in developing resistant donor parent for the crop improvement program through resistant breeding. Moreover, for successful deployment of stable resistance genes, their characterization and availability of tightly linked markers will greatly facilitate the development of new versions of cultivars (Vikal and Bhattia, 2017).

The present study is focused on identification of R genes present in NILs which confer downstream defense response after recognition (directly or indirectly) of *Avr* genes and also the marker assisted genotyping of major R genes of bacterial leaf blight of rice.

MATERIAL AND METHODS

Bacterial leaf blight symptom of rice was isolated from various locations *viz*., Coimbatore, Aduthurai, Gudalur, Krishnagiri, Vellur, Theni, Wayanad, Hyderabad and New Delhi during the survey and were brought into laboratory for isolation of the pathogen under *in vitro* condition. Briefly, under *in vitro,* the diseased samples collected were sliced off into leaf bits containing both the healthy and infected portions were transferred into an Eppendorf tube containing sterile water. Later, the leaf bits were crushed using sterile rod to release bacterial colonies as ooze and the loopful of suspension was streaked onto the autoclaved, solidified Peptone sucrose agar media ($CaNO_3$-0.5g, $FeSO_4$. $7H_2O$- 0.5g, sucrose-15g, peptone-5g, Na_2HPO_4. $7H_2O$-2g, agar-15g, distilled water-1000ml) poured into the Petri dish. Triplicates of samples were maintained for isolation and were incubated at 25°C for the formation of yellow, mucoid, doom shaped colonies with entire margins were developed and were sub cultured for further studies. Pathogenicity of the isolate was proved in bacterial leaf blight susceptible varieties *viz.,* ADT 38 and TN1. Seeds of the susceptible varieties were collected in a jute bag and immersed overnight in water and on next day, the soaked seeds in jute bag were kept overnight, air tightened inside the hay to sprout. Sprouted seeds were collected on the

next day for sowing in pots filled with clay loamy soil. Seedlings emerged from the pots were transplanted to another one after 15 days of sowing which were maintained in a growth chamber of temperature of 25^{0}C and 85-90% relative humidity. Forty eight hours old bacterial suspension prepared in nutrient broth conditioned to 0.1 value in spectrophotometer at 600nm were employed for testing pathogenicity nature of the isolate through clip inoculation method (Kauffman *et al.* 1973).

Concisely, clip inoculation method was performed into the seedlings with maximum tillering stage where surface sterilized scissors were used to nick out the top 5cm of leaves of the seedling after dipping into the bacterial suspension prepared earlier in the nutrient broth. Remaining bacterial suspension was sprayed onto the cut ends and margins of leaves to permeate the entry of bacterial colonies into the leaves and cause the infection. Observation for symptom appearance from the day of inoculation was recorded and the organism was re-isolated from the lesion and compared with that of the original isolate and hence proved the pathogenicity. Characterization of bacterial leaf blight pathogen was instigated by isolation of total genomic DNA from the bacteria which was performed using lysis buffer method (Chen and Kuo, 1993) where the bacterial culture was multiplied in 100 ml nutrient broth kept for 48hr in rotary shaker at 180rpm. Saturated culture was harvested in 1.5 ml of Eppendorf tube and allowed for centrifugation for 3 min at 12,000 rpm. 200 µl of lysis buffer (40 mM Tris-acetate pH 7.8, 20 mM sodium-acetate, 1 mM EDTA, 1% SDS) was suspended in the cell pellet and lysed by vigorous pipetting. 66 µl of 5M NaCl was added and mixed well which remove most proteins and cell debris and then the viscous mixture was centrifuged at 12,000 rpm for 10 min at 4°C.

Clear supernatant obtained after centrifugation was transferred into new vial and an equal volume of chloroform was added followed by vortex until the solution turned milky. Subsequently, centrifuged the solution at 12,000 rpm for 3 minutes, and the supernatant extracted was transferred to another vial and the DNA was precipitated with 100% ethanol, washed twice with 70% ethanol, dried, and redissolved in 50 µl of 1 x TE buffer. Later, the quality of the DNA was assessed by 1.2% agarose gel electrophoresis. To assess the genetic identity of the pathogen, PCR was performed using 16s rRNA primers (27F: 5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') which shows the amplicon size of 1537bp. The PCR product was outsourced for sequencing to identify upto the species level of bacteria. Hence, the bacteria identified as *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) through molecular tools were used for screening of bacterial leaf blight pathogen against rice cultures in field. Thirty one rice lines were sown in rice beds and were transplanted to fields after 20 days of sowing such that each lines had a minimum of ten hills in a row. Integrated nutrient management practices were followed as per the package of practices for rice crop. Maximum tillering was obtained at 45 days old crop and in which clip inoculation was carried out following the method of Kauffman *et al.* (1973) described above. As the bacterial infection spread from the cut ends of the leaves, the lesion length was recorded at 7 and 14 days after inoculation of bacterial suspension and the lesion length was compared with that of the resistance reaction to bacterial leaf blight mentioned in Standard Evaluation System of Rice.

Screening of these culture lines were carried out twice during the period of 2017-18 and 2018-19 and confirmed the resistant reaction nature of the culture lines in comparison with the existing susceptible cultivars. To understand the interaction between host and pathogen, presence of resistance genes and *avr* genes in rice cultures and *X. o.* pv. *oryzae* respectively were identified using polymerase chain reaction amplification through the gene specific primers. Genomic DNA of thirty one rice lines were isolated using conventional CTAB method in which 0.5g of leaf samples were ground with 500µl of CTAB buffer using pestle and mortar. The contents were transferred to a vial and kept in water bath at 65^{o}C for 15 minutes followed by cooled at room temperature. Chloroform and Isoamyl alcohol mixture at 24:1 ratio were prepared and equal volume of the solution was added into vial and centrifuged at 12,000 rpm for 15 minutes. Aqueous phase thus formed was transferred into a new vial and poured equal volume of ice cold isopropanol and incubated overnight at -20^{o}C. Pellet was separated from the solution by centrifugation at 12,000rpm for 15 minutes and added 200µl of 70% of ethanol. Air dried the pellet and kept the pellet dissolved in sterile water at -20^{o}C for further studies.

Genomic DNA in each rice lines were checked at 0.8% of agarose gel electrophoresis. A total of 25µl of reaction mixture for polymerase chain reaction amplification of genomic DNA of 31 rice lines were performed. Primers *viz., xa5* (F: 5' GCACTGCAACCATCAATGAATC 3'; R: 5' CCTAGGAGAAACTAGCCGTCCA 3'), *xa13* (F: 5' CCTGATATGTGAGGTAGT 3'); R: 5' GAGAAAGGCTTAAGTGC 3') and *PTA 248* (F: 5' AGACGCGGGAAGGGTGGTTCCCGGA 3'); (R: 5' AGACGCGGGTAATCGAAAGATGAAA 3') (Robert *et al.* 1992) each at 100pm/µl were used to identify the presence of R genes (*xa5, xa13* and *Xa21*).

Initial denaturation of 5 min at 94°C followed by 40 cycles of 1 min of denaturation at 94°C, 1 min of annealing at 55°C for *xa5 and xa13* gene primers and 59^{o}C for *Xa21* gene primer, and 1 min of extension at 72°C and final extension was 10 min at 72°C. PCR amplification of genomic DNA of *X. o.* pv. *oryzae* was performed using

Avr gene primers specific to *avrxa3 gene, Avr/pth3 gene, Avr/pth56B, Avr/pthC8b* and *AvrXa10, Pth/Xo1* (Table 4.). PCR conditions followed were initial denaturation of 5 minutes at 94°C followed by 40 cycles of 1 minute of denaturation at 94°C, 1 minute of annealing at 55°C and 1 minute of extension at 72°C and final extension was 10 minutes at 72°C. Resistance genes in host and *avr* genes in the pathogen are the important molecular determinants that downstream the defense mechanism in host system by induction of secretion of several proteins.

An experiment was laid out to detect the presence of proteins secreted after bacterial leaf blight susceptible rice variety, ADT 38 was confronted with the pathogen and abiotic agents *viz.,* Biotin (0.1mM), Riboflavin (0.5mM), Chloramphenicol (0.1mM), Ethrel (1µl/ml). Virulent bacterial colonies were inoculated into nutrient broth and incubated for 48hr in BOD incubator with 180rpm till the spectrophotometer read 0.1 at 600nm. Bacterial suspension was clip inoculated into the 40 days old plant followed by abiotic agents were applied 24 hr after the inoculation. Leaves were collected 24hr after application of abiotic agents separately from each and were subjected to protein extraction for SDS PAGE analysis to detect the presence of protein fractions secreted.

RESULTS AND DISCUSSION

Organism causing bacterial leaf blight disease in rice was isolated from the leaf samples collected during the survey in Coimbatore, Aduthurai, Gudalur, Krishnagiri, Vellur, Theni, Wayanad, Hyderabad and New Delhi under *in vitro* condition. The virulent strains of the bacterial leaf blight isolates were demarcated with that of the non-virulent one as the former appeared yellow, round, mucoid, convex colonies after 72 hr of incubation whereas the latter remained white, slimy, mucoid and convex as detailed by Webster and Gunnell (1992). Pathogenicity tests for 9 isolates were proved for all the 9 isolates in ADT 38 and TN1 which showed initial symptom of the disease after 7 days of inoculation. Virulence spectrum of *X. o.* pv. *oryzae* isolates were compared after inoculating in ADT 38 and TN1 varieties revealed that Coimbatore isolate imparted highest virulence than any other. While comparing the lesion size formed by each isolate, highest lesion size was observed in the variety inoculated with Coimbatore isolate whereas the least was observed with New Delhi isolate.

Genomic DNA of nine bacterial leaf blight pathogens were extracted and the molecular characterization of the nucleic acids using 16S rRNA primers showed amplification at 1200 bp and partial sequencing of the isolates revealed the identity of the organism as *Xanthomonas oryzae* pv. *oryzae*. Screening of 29 rice lines along with improved samba mahsuri (resistant check) and TN1 (Susceptible check) against bacterial leaf blight pathogen (Coimbatore) showed formation of typical bacterial leaf blight symptom after 7 days of inoculation. Lesion size were recorded during 7th and 14th day of inoculation, revealed the resistant reaction offered against the pathogen by the rice cultures. When compared to the resistant check, improved samba mahsuri, rice cultures showed better resistive potential. Three rice cultures *viz.,* IRBB 57, IRBB 58, IRBB 61 were categorized as resistant among the 29 culture lines according to the Standard Rice Evaluation System of IRRI, Philippines whereas 11 were moderately resistant (IRBB 7, IRBB 8, IRBB 11, IRBB 13, IRBB 14, IRBB 21, IRBB 50, IRBB 56, IRBB 63, IRBB 64 and IRBB 66) and 13 culture lines (IRBB 1, IRBB 3, IRBB 4, IRBB 5, IRBB 10, IRBB 51, IRBB 52, IRBB 53, IRBB 54, IRBB 55, IRBB 59, IRBB 60 and IRBB 62) were moderately resistant (Table 1, Table 2).

Similarly, Bharathkumar *et al.* (2014) also tested the resistance reaction of rice and bacterial leaf blight pathogen and categorized pathotypes of *X. o.* pv. *oryzae* isolates prevailing in different states of India. Incongruence with the present study, Rajappan and Ravi (2015) evaluated twenty two gene pyramided rice cultures against bacterial leaf blight disease to estimate the resistant reactions against the disease during 2012-2015. These rice cultures were gene pyramided with resistance genes (*Xa1* to *Xa21*) to bacterial leaf blight and with the combinations of the same which in turn give resistive nature to the lines. Identification of such combinations of R genes in the lines are advantageous for the identification of donor parent intended to development of a resistant varieties. Gene specific primers pertaining to *xa5, xa13* and a functional marker of *Xa21* gene (*PTA 248*) were amplified using rice genomes of twenty eight rice cultures and three cultivars where the results showed that polymorphic genotypes attained after gel electrophoresis were directly related to the resistant nature of the lines (Table 3).

Gene specific amplification of *Xa21* marker in 31 rice lines/varieties, 8 lines showed (IRBB 11, IRBB 55, IRBB 56, IRBB 57, IRBB 58, IRBB 59, IRBB 60, IRBB 61, IRBB 64, IRBB 65 and IRBB 66) amplification at 900 bp thereby confirmed the presence of *Xa21* gene (Figure 1). Similarly, presence of *xa5* gene amplification established after the comparison of gene amplification pattern similar to that of IRBB 5. The results revealed that IRBB 50, IRBB 59, IRBB 60, IRBB 61, IRBB 63, IRBB 64, IRBB 66 showed similar *xa5* gene marker amplification at 270 bp (Figure 2). In the case of *xa13* gene, rice lines *viz.,* IRBB 51, IRBB 53, IRBB 54, IRBB 56, IRBB 58, IRBB 60, IRBB 61, IRBB 63, IRBB 65 were showed similar banding pattern of that of IRBB 13 which remarked the presence of *xa13* gene (Figure 3).

Table 1. Field screening of rice cultures against bacterial leaf blight pathogen during 2017-18

Sl. No	Rice lines	Average lesion size (cm) 7th day	Average lesion size (cm) 14th day	Resistant reaction
1.	IRBB 1	7.5^{l}	8.2^{ijk}	MS
2.	IRBB 3	5.6^{f}	6.6^{efgh}	MS
3.	IRBB 4	7.8^{l}	8.2^{ijk}	MS
4.	IRBB 5	6.8^{ijk}	7.0^{ghi}	MS
5.	IRBB 7	3.9^{c}	4.2^{bc}	MR
6.	IRBB 8	4.8^{d}	5.0^{cde}	MR
7.	IRBB 10	5.1^{e}	5.5^{def}	MR
8.	IRBB 11	6.1^{hi}	6.6^{efgh}	MS
9.	IRBB 13	3.6^{c}	4.2^{bc}	MR
10.	IRBB 14	3.6^{c}	4.3^{bc}	MR
11.	IRBB 21	1.8^{a}	2.2^{a}	R
12.	IRBB 50	4.6^{d}	5.0^{cde}	MR
13.	IRBB 51	6^{gh}	6.6^{efgh}	MS
14.	IRBB 52	6.7^{ij}	7.0^{ghi}	MS
15.	IRBB 53	6.7^{ij}	7.0^{ghi}	MS
16.	IRBB 54	5.7^{fg}	6.6^{fghi}	MS
17.	IRBB 55	6.7^{ij}	7.0^{ghi}	MS
18.	IRBB 56	6.8^{k}	7.2^{ghif}	MS
19.	IRBB 57	2.6^{b}	2.9^{a}	R
20.	IRBB 58	2.5^{b}	2.8^{a}	R
21.	IRBB 59	6^{fg}	6.5^{efg}	MS
22.	IRBB 60	7^{k}	7.3^{hij}	MS
23.	IRBB 61	2.7^{b}	3.2^{ab}	MR
24.	IRBB 62	6.7^{ij}	7.5^{hij}	MS
25.	IRBB 63	3.7^{c}	4.3^{bcd}	MR
26.	IRBB 64	5.8^{fg}	6.6^{fghi}	MS
27.	IRBB 65	6^{fg}	6.7^{fghi}	MS
28.	IRBB 66	4.6^{d}	5.2^{cdef}	MR
29.	DV-85	2.8^{b}	3^{ab}	MS
30	ISM	5.9^{fg}	5.6^{def}	MR
31.	TN1	8^{m}	8.8^{jk}	MS
	CD (0.05)	0.311	1.37	

HR: Highly Resistant (<1cm); R: Resistant (1-3cm); MR: Moderately Resistant (3-6cm); Moderately Susceptible (6-10cm); Susceptible (>10cm)

Genotyping of *Xa21, xa5* and *xa13* genes showed that all the three genes were present in IRBB 60 and IRBB 61. *Xa21* and *xa5* genes were present in IRBB 59 alone whereas *xa5* and *xa13* genes were amplified in IRBB 63 and IRBB 65 lines. To contain the bacterial leaf blight of rice, researchers paved major attention in introgression of multiple resistance genes through breeding program. Guvvala *et al.* (2013) have experimented gene pyramiding of R genes *viz., Xa4, xa5, xa13* and *Xa21* into bacterial leaf blight susceptible mahsuri variety and various such related works are in progress (Pinta *et al.* 2013, Pradhan *et al.* 2015, Chukwu *et al.* 2019).

Hence, the present study to identify the donor parents carrying R genes for resistance breeding program

Table 2. Field screening of rice cultures against bacterial leaf blight pathogen during 2018-19

Sl. No	Rice lines	Average lesion size (cm) 7th day	Average lesion size (cm) 14th day	Resistant reaction
1.	IRBB 1	6^{def}	8^{f}	MS
2.	IRBB 3	6^{def}	8^{f}	MS
3.	IRBB 4	6^{def}	8^{f}	MS
4.	IRBB 5	7^{f}	7^{f}	MS
5.	IRBB 7	2^{a}	4^{abc}	MR
6.	IRBB 8	5^{bcd}	5^{bcd}	MR
7.	IRBB 10	6^{def}	8^{f}	MS
8.	IRBB 11	4^{abc}	6^{def}	MR
9.	IRBB 13	4^{abc}	6^{def}	MR
10.	IRBB 14	4^{abc}	6^{def}	MR
11.	IRBB 21	2^{a}	4^{abc}	MR
12.	IRBB 50	5^{bcd}	6^{def}	MR
13.	IRBB 51	6^{def}	8^{f}	MS
14.	IRBB 52	7^{ef}	7^{f}	MS
15.	IRBB 53	7^{ef}	7^{f}	MS
16.	IRBB 54	6^{def}	8^{f}	MS
17.	IRBB 55	7^{ef}	7^{f}	MS
18.	IRBB 56	4^{abc}	6^{def}	MR
19.	IRBB 57	3^{ab}	3^{ab}	R
20.	IRBB 58	3^{ab}	3^{ab}	R
21.	IRBB 59	5^{bcd}	5^{bcd}	MS
22.	IRBB 60	7^{ef}	7^{ef}	MS
23.	IRBB 61	3^{ab}	3^{ab}	R
24.	IRBB 62	7.66^{ef}	7.66^{ef}	MS
25.	IRBB 63	4^{abc}	6^{def}	MR
26.	IRBB 64	4^{abc}	6^{def}	MR
27.	IRBB 65	6^{def}	6^{def}	MR
28.	IRBB 66	5^{bcd}	5^{bcd}	MR
29.	DV-85	3^{ab}	3^{ab}	R
30	ISM	5^{bcd}	5^{bcd}	MR
31.	TN1	8.8^{f}	7.66^{f}	MS
	CD (0.05)		1.78	1.85

HR: Highly Resistant (<1cm); R: Resistant (1-3cm); MR: Moderately Resistant (3-6cm); Moderately Susceptible (6-10cm); Susceptible (>10cm)

has pursued its importance. In the bacterial leaf blight pathogen, *Xanthomonas oryzae* pv. *oryzae* (NCBI Accession No. MH464904) after gene specific amplification of *avr* genes *viz.*, *pthXo1* (433 bp), *avrxa3* (623 bp), *AvrXa10* (562), *Avr/pthC8b* (420 bp) and *Avr/pth56B* (623) were obtained whereas *Avr/pth3* gene was absent in the strain (Figure 4). As a testimonial to the above results, Wu *et al.* (2007) came into conclusion that *avrXa3* gene containing three nuclear localization signal (NLS) motifs which is consistently present in all members of the *avrBs3/pthA* family was identified in JXOIII strain of *X. o.* pv. *oryzae*. Numbers of the *avr/pth* genes vary among different strains of *X. o.* pv. *oryzae*.

Table 3. List of primers

S. No.	Genes	Nucleotides	Annealing temperature
1.	16S rRNA	27F: AGAGTTTGATCCTGGCTCAG 1492R: GGTTACCTTGTTACGACTT	55
2.	PTA248	F: AGACGCGGGAAGGGTGGTTCCCGGA R: AGACGCGGGTAATCGAAAGATGAAA	59
3.	xa13	F: CCTGATATGTGAGGTAGT R: GAGAAAGGCTTAAGTGC	55
4.	xa5	F: GCACTGCAACCATCAATGAATC R: CCTAGGAGAAACTAGCCGTCCA	55
5.	Avrxa3	F: CATCTTGTTCCCACATCACG F: GCCGGAATTGATCAGAAGAG	55
6.	Avr/pth3	F: AGGACATAATCAGGGCGTTG R: CCAATACGGCGATTGACTCT	55
7.	Avr/pth56b	F: GCCGGAATTGATCAGAAGAA R: CATCTTGTTCCCACATCACG	55
8.	Avr/pthC8b	F: GCCGGAATTGATCAGAAGAA R: CATCTTGTTCCCACATCACG	55
9.	AvrXa10	F: ATCTGCTCCGTCAGTTCGAT R: TGGCCTGTGTCCAACTGTAA	55
10.	PthXo1	F: GAGAGCATTGTTGCCCAGTT R: CTGAAGTAGGGGACGGTTTG	55

Similar to the identification of *avr* genes in individual strain, researchers have identified presence of 15 *avr3/pth* genes in Korean race 1 (KACC10331) (Lee *et al.* 2005) whereas Yang and White (2004) demonstrated 25–32 *avr/pth* genes in different Philippine strains. After inoculation of *X. o.* pv. *oryzae* suspension into rice lines carrying R genes, *avr* gene products interact correspondingly with that of R gene product and based on the downstream action of the corresponding gene interaction compatibility or incompatibility were established

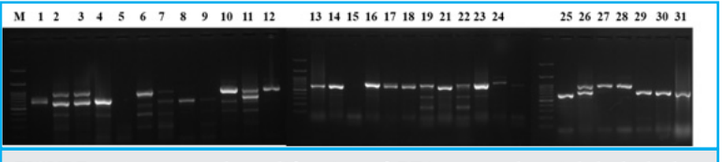

FIGURE 1. Agarose gel amplification of *PTA248* marker tightly linked to *Xa21* gene in 31 rice lines

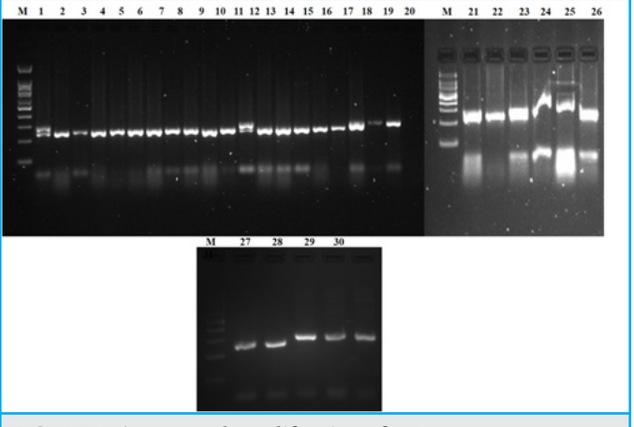

FIGURE 2. Agarose gel amplification of *xa5* gene

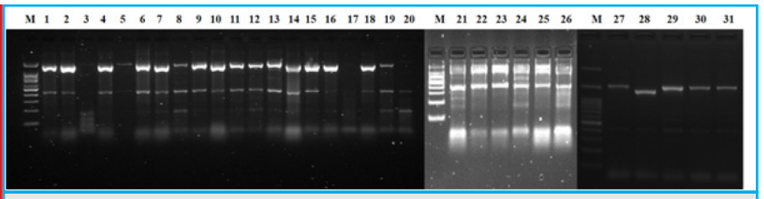

FIGURE 3. Agarose gel amplification of *xa13* genes

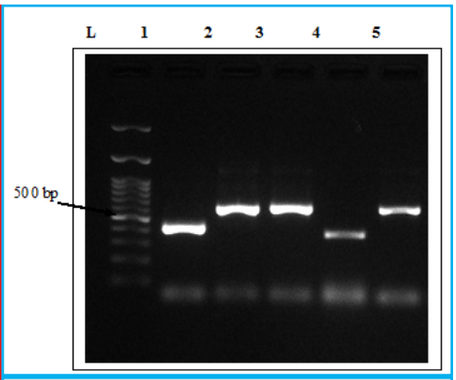

FIGURE 4. Agarose gel amplification of Avr genes (1. *PthXo1*, 2. *avrxa3*, 3. *AvrXa10*, 4. *Avr/pthC8b*,5. *Avr/pth56B*,) in *X.* o. pv. *Oryzae*

to cause disease susceptibility or resistance. Disease susceptibility gene, *Os8N3* or susceptible allele of recessive R gene *xa13* were found to be specifically induced by TAL effectors, *PthXo1* (Romer *et al.* 2010, Yin *et al.* 2017).

Hence, as *PthXo1* gene was present in the Coimbatore strain of *X. o.* pv. *oryzae,* it could induce the expression of disease susceptibility allele, *Xa 13* gene which thereby results in susceptibility of the line. *Avr/pth56B* and *Avr/pthC8b* were also identified in *X. o.* pv. *oryzae* isolate of Coimbatore, whereas there are ambiguity over the detailed evidence regarding the interaction with the R genes and those *avr* genes. In an extension to the identification type of R genes and *avr* genes present in the host and pathogen, to analyze the PR protein or other defense protein induction, SDS PAGE analysis was performed in rice variety, ADT 38, after inoculation of the pathogen followed by abiotic chemical agents (Biotin (0.1mM), Riboflavin (0.5mM), Chloramphenicol (0.1mM), Ethrel (1µl/ml)). The results revealed that comparing to the untreated control, all the treatments has induced the defense proteins consistently at 66.4 kDa thereby demonstrated that the susceptible rice variety could induce defense proteins with the application of abiotic agents (Figure 5). SERK2 protein is a 69 kDa proteins which is secreted as immune response mediated by the LRR receptor kinases, *Xa21* and the bacterial pathogen interaction. Hence, the apparent molecular weight of the protein observed in SDS-PAGE are consistent with that of SERK2 protein. In congruence to the above study, Wu et al. (2011) has conducted an experiment to compare the expression of PR proteins in compatible and incompatible interactions and results showed that in both the case the induction was noticed. Six out of ten PR proteins (PR1, PR2, PR3, PR4b, PR8, and PR-pha) showed enhanced expression in Xa21-mediated resist-

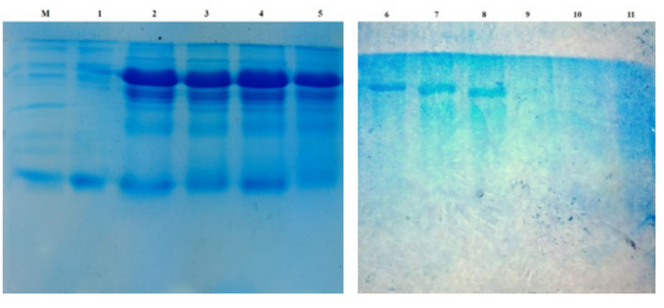

FIGURE 5. Defense protein induction in rice-*X.o.pv.oryzae* interaction by SDS PAGE analysis M- Protein ladder (14-96 kDa),1-control, 2-Biotin (0.1mM), 3-Riboflavin (0.5mM), 4-Chloramphenicol (0.1mM), 5-Ethrel (1µl/ml), 6-8- *Xoo* treated ADT 38, 9-10-Sterile water (control) treated ADT 38

ance responses at late stages after inoculation with *X. o.* pv. *oryzae* (Hou *et al.* 2011).

CONCLUSION

Interaction between rice and bacterial leaf blight pathogen, *Xanthomonas oryzae* pv. *oryzae* resulted in induction of defense related proteins which were observed through SDS PAGE analysis. The defense related proteins synthesized were mediated by the direct and/or indirect interaction of resistance genes and corresponding avirulence genes of rice cultures and *X. o.* pv. *oryzae* respectively. *PthXo1, avrxa3, AvrXa10, Avr/pthC8b* and *Avr/pth56B* were the A*vr* genes identified from *X. o.* pv. *oryzae,* Coimbatore isolate.

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CONFLICT OF INTEREST

All authors have declared no conflicts of interest in this communication

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