Original Research

Journal Home page : www.jeb.co.in ★ E-mail : editor@jeb.co.in

Journal of Environmental Biology

JEB™ p-ISSN: 0254-8704
e-ISSN: 2394-0379
CODEN: JEBIDP

DOI : http://doi.org/10.22438/jeb/41/1/MRN-1093

Identification of BLB resistant genes in some rice varieties for development of high yielding bacterial leaf blight tolerant types

K. Majumder[1]*, S.I. Mondal[1], R. Mallick[1] and T. Dasgupta[2]

[1]Department of Genetics and Plant Breeding, Institute of Agricultural Science, University of Calcutta, Kolkata-700 019, India
[2]IRDM Faculty Centre, School of Agriculture and Rural Development, Ramakrishna Mission Vivekananda Educational and Research Institute, Kolkata-700103, India

***Corresponding Author Email :** kasturi.majumder525@gmail.com

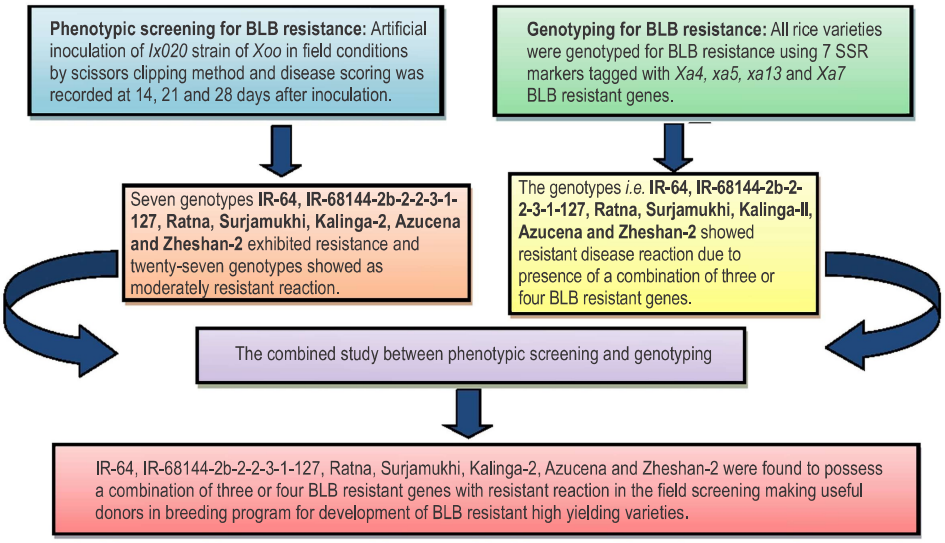
Paper received: 21.01.2019 *Revised received*: 02.05.2019 *Accepted*: 20.08.2019

Abstract

Aim : To identify bacterial leaf blight resistant genes in some rice varieties so that these resistant varieties can be used as a good source of donor for BLB resistant genes in genetic enhancement program.

Methodology : A total of sixty-one rice genotypes including resistant and susceptible checks were screened in field condition by artificial inoculation using *IX020* strain of *Xoo* for two years (*Kharif* 2016 and 2017). These varieties were also genotyped for seven SSR markers tagged with major BLB resistant genes, *i.e., Xa4, xa5, xa13* and *Xa7.*

Results : In artificial screening, significant disease development was recorded and the varieties were categorized using disease scoring scale of IRRI, 1996 where seven cultivars exhibited resistance, while twenty-seven were found to be moderately resistant. In genotyping, there was distinct difference in banding position for resistant and susceptible genotypes. Genotypes having resistant disease reaction, carrying BLB resistant genes were identified.



Interpretation : Genotypes IR-64, IR-68144-2b-2-2-3-1-127, Ratna, Surjamukhi, Kalinga-2, Azucena and Zheshan-2 expressed bands of RM markers closely linked to *Xa4, xa5, xa13* and *Xa7* BLB resistant genes and field testing also confirmed resistant host reaction against pathogens.

Key words: Artificial screening, BLB resistant genes, High yield, *Oryza sativa*

How to cite : Majumder, K., S.I. Mondal, R. Mallick and T. Dasgupta: Identification of BLB resistant genes in some rice varieties for development of high yielding bacterial leaf blight tolerant types. *J. Environ. Biol.*, **41**, 85-91 (2020).

Introduction

Rice (*Oryza sativa* L.) is one of the oldest domesticated and most important food crops grown worldwide. Rice is the staple food for half of the world's population, particularly in China, India, Indonesia, Japan and Southeast Asia. More than half of the world population depends on rice for their daily required calories (Jiang et al., 2013). Unfortunately, rice production is impeded by several diseases of fungal, bacterial and viral origin. Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *Oryzae* (*Xoo*) is one of the most devastating disease leading to severe yield loss in rice production throughout the world and it is the most serious disease of rice in South East Asia, particularly in Japan, Philippines, Indonesia and India (Khan *et al.,* 2009). In some areas of Asia, it can reduce crop yield by 50-80% (Yadav *et al.,* 2013). To circumvent the problem, attempts were made earlier by researchers to identify and characterize BLB resistance genes. The total number of 40 genes conferring resistance to BLB has been identified in rice (Kumar *et al.,* 2012; Kim *et al.,* 2015). Among them, nine genes namely *Xa1, Xa3/Xa26, xa5, Xa10, xa13, Xa21, Xa23, xa25* and *Xa27* have been cloned(Nino-Liu *et al.,* 2006; Liu *et al.,* 2011; Tian *et al.,* 2014; Wang *et al.,* 2015) and five genes namely *Xa4, xa5, Xa7, xa13* and *Xa21* have been reported as major resistance genes (Singh *et al.,* 2015).

Rice varieties carrying number of resistant genes express a higher level of resistance than a variety containing a single resistance gene (Dilla-Ermita *et al.,* 2017). Consequently, pyramiding of more than one major resistance gene was reported to deliver durable resistance against *Xoo* (Rajpurohit *et al.,* 2011). In case of recessively inherited resistance genes such as xa5 and *xa13,* conventional breeding tools suffer in gene pyramiding program. The conventional methods of plant selection for BLB resistance are not easy due to large environmental effects and low narrow sense heritability of BLB resistance. This hinders the development of an accurate, rapid and reliable screening technique. Individuals with target gene in a segregating population can be identified with the assistance of DNA markers (Khan *et al.,* 2014). All these limitations can be overcome by using marker-assisted selection (MAS) which enables the evaluation and expression of recessive resistance genes and allows for pyramiding of multiple resistance genes in a desirable genetic background. Marker-assisted selection having a very promising future prospects in rice breeding area where adoption of different markers is expected to increase in future. However, developing effective strategies for using markers would facilitate a greater level of the adoption. Microsatellite marker analysis is very useful to identify major gene locus for BLB resistance that can be helpful for plant breeders to develop new cultivar (Bhuiyan *et al.,* 2005).

Hence, the available resources for developing new markers from DNA sequence data as a result of rice genome sequencing and research in functional genomics would be helpful (Chukwu et al.,2019).Recent progress and technical advances in marker-assisted selection permit the rapid and accurate identification of individuals that contain genes for BLB resistance. In the present study, sixty-one rice genotypes were screened using seven Simple Sequence Repeats (SSRs) RM markers linked to *Xa4, xa5, xa13* and *Xa7* genes in view to identify and tag the BLB resistance/susceptible rice varieties. The main objective was to identify desirable donors against bacterial leaf blight infection.

Materials and Methods

Plant materials and experimental design: The experimental materials comprising fifty eight rice genotypes (Table 2) along with three checks, namely IRBB 60 (*Xa4+xa5+xa13+Xa21*), IRBB 7 (*Xa7*) and IR 24 (no resistant gene) were collected from International Rice Research Institute (IRRI), Philippines ; Cornell University, USA and the Department of Genetics and Plant Breeding, Institute of Agricultural Science, University of Calcutta, India. Twenty-one day old seedlings were transplanted during *Kharif* season or rainy season 2016 and 2017, in a randomized block design with three replications at the Agricultural Experimental Farm, University of Calcutta.

Strain revival and pathogenicity test: The strain IX020, a culture of Xoo was obtained from the Indian Institute of Rice Research, (IIRR), Hyderabad, India and sub cultured on nutrient agar and potato sucrose agar (PSA) medium at 27°C. Inoculum suspensions (10^8 cfu ml^{-1}) were prepared by using sterile distilled water from 48 hr old cultures on potato sucrose agar medium.

Phenotypic screening by artificial inoculation for BLB resistance: Fifty-eight rice genotypes along with three checks were screened against *Xoo* strains under epiphytotic condition during *Kharif* season in 2016 and 2017. For pathogenicity test, the rice plants were inoculated at booting stage with IX020 strain by scissors clipping method. The top (2.5–6 cm) portion of completely developed leaves was clipped off one by one, with sterilized scissors dipped in a bacterial suspension containing 10^8cfu ml^{-1}. Appearance of disease symptoms in plants was noted at 24 hr time interval after inoculation. The disease scoring was recorded at 14, 21 and 28 days after inoculation from 10 randomly selected plants and 5 leaves per plant from each replication in each rice germplasms. Observations were recorded by measuring the lesions and percent of diseased leaf area using a disease scoring scale of IRRI (1996).

Genotyping for BLB resistance: The genomic DNA of all rice genotypes were isolated from three week seedlings following standard Cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1990). PCR amplification was done using seven SSR markers, *i.e., RM 224, MP 1, RM 13, RM 153,RM 264, RM 230* and *RM 251* (Table 1) which are closely linked to BLB resistant genes *viz. Xa4, xa5, xa13* and *Xa7,* respectively, and were used to detect the presence or absence of these BLB resistant genes. Each PCR reaction tube contained 14 µl of H_2O,5 µl template DNA, 0.5 µl dNTPs (Bioline, UK), 0.5 µl each of forward and reverse primers (Eurofins Genomics; Bangalore, India), 2.5 µl 10x reaction buffer (Bioline, UK), 1 µl 50 mM $MgCl_2$ (Bioline, UK) and 0.1 µl Taq DNA polymerase (Bioline, UK) using

a thermal cycler (Eppendorf AG 6321,Germany). Amplification was carried by preheating at 95°C for 5 min, followed by 37 cycles of denaturation at 95°C for 30 sec, 30 sec for primer specific annealing, 1 min elongation at 72°C and final extension at 72 °C for 5 min. The SSR products were separated in agarose gel (2%), through a horizontal gel electrophoresis (Bio-Rad,USA). DNA fragments were expressed using ethidium bromide staining procedure. The gels were documented using gel documentation unit (UVP,USA). A 100 bp DNA ladder (Fermentas Life Sciences, USA) was used to calculate the fragment size.

Results and Discussion

Screening with artificial inoculation was done consecutively for two years in 2016 and 2017 (Table 2). It was done during *Kharif* season or rainy season under epiphytotic condition because artificial inoculation was more effective for development of disease, if inoculation was done after rainy days. Similar finding was reported by Tasleem-uz-Zaman *et al.* (2000) and Thimmegowda *et al.* (2011) because this disease is prevalent in rainfed conditions, particularly during humid (83-93)% condition. In phenotypic screening not a single variety was found completely free off disease. The initial symptoms of BLB were like either water soaking to yellowish stripes on leaf blades or starting at leaf tips then gradually increase in length and width with wavy margin. Generally, symptoms were observed on both edges of the leaf, and rarely symptoms were recorded on one edge with variable intensities. Lesions turned yellow to white as the disease intensified and tended to dry quickly. All these symptoms were observed within 10 to 12 days after inoculation of bacterial culture in resistant control checks, IRBB 7 and IRBB 60, respectively whereas susceptible control, IR 24 (no resistant gene) showed symptoms only after 5 days. Seven cultivars like IR-64, IR-68144-2b-2-2-3-1-127, Ratna, Surjamukhi, Kalinga-2, Azucena and Zheshan-2 exhibited resistance, twenty-seven moderately resistant and the rest were either moderately susceptible or susceptible. Similar variable expression of disease among genotypes were earlier reported by Akhtar *et al.* (2011); Madhavi *et al.* (2011); Ram *et al.* (2011); Thimmegowda *et al.* (2011); Sharma *et al.* (2012) and Acharya *et al.* (2018). Among them, Acharya *et al.* (2018) found twenty-eight genotypes as resistant, three moderately resistant, two moderately susceptible, two susceptible and twenty-two genotypes highly susceptible Thimmegowda *et al.*(2011) reported three genotypes as resistant, six moderately resistant, twenty-three moderately susceptible, twenty-four susceptible and fifteen highly susceptible.

The same sixty-one rice genotypes, including three checks, were screened for four bacterial leaf blight resistance genes *viz. Xa4, xa5, xa13 and Xa7* using 7 markers namely RM 224, MP 1, RM 13, RM 153, RM 264, RM 230 and RM 251, respectively, linked to these genes. The resistant and susceptible genes showed a distinguishable difference in banding position. The susceptible variety IR 24 exhibited different bp fragment than *Xa4, xa5, xa13* and *Xa7* resistant lines IRBB 60 (*Xa4*+ *xa5*+ *xa13*+ *Xa21*) and IRBB 7 (*Xa7*). The *xa5* resistant gene was linked with RM 13 and RM 153 markers. The presence of 161 bp and 213 bp fragments in the genotypes confirmed that the resistant gene was present in the genotype. While, in susceptible line, IR 24, the expressed bp fragment was 131 bp and 192 bp, respectively, for these markers. In other words, if the fragment size of the genotypes was either 131 bp or 192 bp then the expression was susceptible. Similarly, the bp fragments of resistant and susceptible lines were compared for all resistant genes. Finally, the expression of bp fragments among all genotypes was checked (Table 2, Fig. 1). Genotypes IR-64, Ratna, Surjamukhi, Kalinga-II and Zheshan-2 showed resistant reaction in phenotypic screening were also characterized by the presence of bands for four resistant genes, namely *Xa4, xa5, xa13* and *Xa7* revealed from PCR data (Table 2). Interestingly, these five genotypes expressed their resistant reaction all along their growth stage with score of 1-2 disease scoring scale. Among

Table 1: List of markers linked to bacterial leaf blight resistance genes and their primer sequences

Gene	Character	Chromosome	Markers		Sequences of markers (5'->3')	References
Xa 4	Dominant	11	RM 224	F	ATCGATCGATCTTCACGAGG	Sun *et al.* (2003)
				R	TGCTATAAAAGGCATTCGGG	
			MP 1	F	ATCGATCGATCTTCACGAGG	Ma *et al.* (1999)
				R	TCGTATAAAAGGCATTCGGG	
xa 5	Recessive	5	RM 13	F	TCCAACATGGCAAGAGAGAG	Mc-Couch *et al.* (1996)
				R	GGTGGCATTCGATTCCAG	
			RM 153	F	GCCTCGAGCATCATCATCAG	Blair *et al.* (2003)
				R	ATCAACCTGCACTTGCCTGG	
xa 13	Recessive	8	RM 264	F	GTTGCGTCCTACTGCTACTTC	Basharat *et al.* (2006)
				R	GATCCGTGTCGATGATTAGC	
			RM 230	F	GCCAGACCGTGGATGTTC	Chen *et al.* (1997)
				R	CACCGCAGTCACTTTTCAAG	
Xa 7	Dominant	6	RM 251	F	GAATGGCAATGGCGCTAG	Chen *et al.* (1997)
				R	ATGCGGTTCAAGATTCGATC	

Table 2 : Reaction of rice varieties to bacterial leaf blight genes and their response against BLB isolate

Variety No	Name of genotypes	Resistance genes genotyped by flanking markers							BLB reactions against Xoo isolates (IX020)	
		Xa4 RM 224	MP 1	*xa5* RM 13	RM 153	*xa13* RM 264	RM 230	*Xa7* RM 251		
	Banding position of resistant control (IRBB 60 and IRBB 7)	196 bp	159 bp	161 bp	213 bp	203 bp	181 bp	173 bp		
	Banding position of susceptible control (IR 24)	171 bp	136 bp	131 bp	192 bp	179 bp	169 bp	162 bp		
1	SURJAMUKHI	R	R	R	R	R	R	R	1.71	R
2	IR 68144-2B-2-2-3-1-127	R	R	R	R	R	R	-	2.01	R
6	IR 64	R	R	R	R	R	R	R	1.98	R
10	RATNA	R	R	R	R	R	R	R	1.68	R
43	KALINGA-2	R	R	R	R	R	R	R	1.01	R
44	AZUCENA	R	R	R	R	R	R	-	1.97	R
49	ZHESHAN-2	R	R	R	R	R	R	R	1.58	R
3	IR 5882-23-1-3-1	S	R	R	S	R	-	S	3.06	MR
4	PATHAREA	R	S	R	S	R	R	S	3.01	MR
7	CN 1646-2	R	R	S	S	R	R	R	3.31	MR
9	IR 50	R	S	R	S	R	R	R	3.65	MR
11	ARC-10086	R	S	R	R	S	R	R	4	MR
13	PSRBC-68	R	-	R	S	S	S	R	4.23	MR
18	DEOK JEOK JODO	R	R	-	S	S	R	R	4.18	MR
19	JALDI-6	R	R	R	S	S	R	R	3.81	MR
24	NAVEEN	R	R	R	R	-	R	R	1.94	MR
25	ORYZICA-L-LONOS-5	R	R	R	S	R	S	-	3.17	MR
29	KALAPAHAR	R	-	S	R	R	S	S	3.01	MR
30	DOUBLE CAROLINA	R	S	S	R	S	S	R	3.89	MR
31	KHARBELA	R	-	R	S	R	S	S	3.99	MR
33	TREMBESE	S	R	S	S	-	R	R	3.75	MR
39	1-ZEO-TZE	R	R	R	S	R	R	S	3.45	MR
40	PAROMA-AHU	R	S	R	S	R	R	S	3.03	MR
41	1ET-20144	R	S	R	S	R	S	S	3.63	MR
42	PHUDUGEY	S	R	R	S	S	R	S	3.92	MR
45	ARC-10372	S	R	R	S	S	S	R	3.95	MR
46	TCHIBANGA	R	S	S	R	R	S	S	3.18	MR
47	PRAVAT	R	R	R	S	R	R	-	3.39	MR
48	CENIT	R	R	S	S	R	S	R	4.11	MR
50	M-202	R	R	R	S	R	R	S	2.9	MR
51	NORTAI	R	S	S	R	S	R	S	3.52	MR
52	D-J-123	R	S	S	S	S	R	R	4.07	MR
53	N-12	R	S	R	S	S	R	S	3.83	MR
58	IR-64 QTL	-	S	R	S	R	-	S	3.52	MR
5	NIPPON BARE	-	R	S	S	R	-	S	5.91	MS
8	IR 6844-120	S	S	R	S	R	R	S	5.25	MS
14	PNR-519	R	S	S	S	S	R	S	5.75	MS
16	KASALATH	S	R	S	R	-	S	S	5.97	MS
17	CN 1646	-	S	R	S	-	S	R	5.84	MS
20	RATHUWEE	S	-	S	S	S	R	R	5.75	MS
21	JALDI-13	R	S	S	S	-	R	S	5.85	MS
22	KRISHNA HAMSA	R	-	S	S	S	R	S	5.75	MS
23	IA-1	S	R	S	S	R	-	S	5.3	MS
26	IA-2	S	R	R	S	-	S	S	5.86	MS
27	ASSAMLAYA	-	S	R	S	R	-	-	5.56	MS
28	CAROLINA- GOLD-SEL	-	-	R	S	S	R	-	5.61	MS
32	IR-1552	R	S	S	S	S	-	R	5.09	MS
34	MTU-1010	R	S	S	S	R	R	S	5.07	MS

Table continue

35	BON-AHU	S	S	R	S	R	S	S	6.02	MS
36	PADMINI	S	-	R	R	S	R	-	5.42	MS
37	TRIGUNA	R	S	-	S	R	S	S	5.12	MS
38	CO-39	S	R	-	S	-	-	R	5.2	MS
54	DD-62	S	R	S	S	-	R	S	5.23	MS
55	ARC-10352	R	S	S	R	S	S	S	5.98	MS
57	HEERA	S	-	R	S	S	R	S	6	MS
12	PEH-KUH	S	R	S	S	S	S	S	7.62	S
15	DULAR	-	R	S	S	-	-	S	7.36	S
56	DANAGURI	-	R	-	S	-	-	-	7.56	S

Note: Rice genotypes were scored as the presence (R) and absence (S) of resistant genes linked to these markers and (-) denotes no results

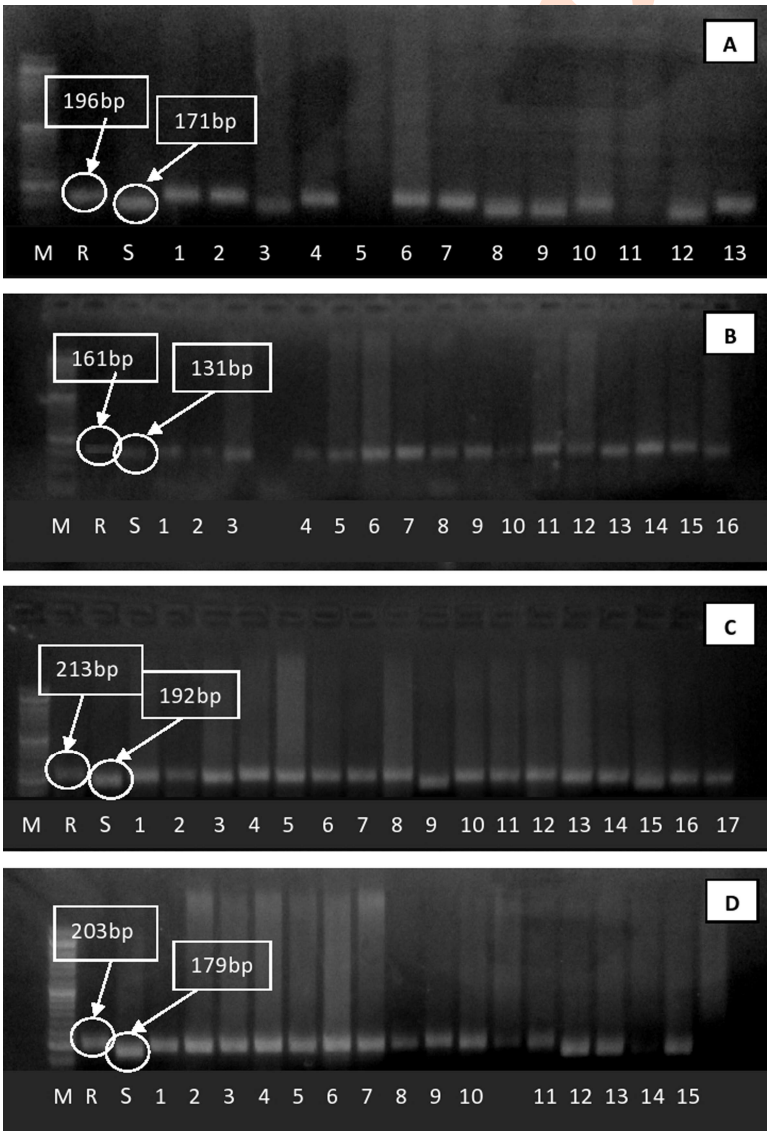


Fig. 1 : Electropherogram of some rice cultivars along with resistant and susceptible cheaks generated by using different genic markers- A. RM 224, B. RM 13, C.RM 153 and D. RM 264 (where M represents 100 bp DNA marker, R and S represent resistant and susceptible controls and number 1-17 represent rice cultivars).

the seven resistant genotypes revealed from phenotypic screening, two genotypes namely IR-68144-2b-2-2-3-1-127 and Azucena though were found to possess three resistant genes (*Xa4, xa5* and *xa13*) but expressed similar resistant reaction like that of previous five resistant genotypes. Thus, the presence of gene *Xa7* was not found critical for resistant reaction. On the contrary, presence of one resistant gene namely *Xa4* in Peh-kuh, Dular and Danaguri showed susceptible reaction in field condition.

Earlier, Rajpurohit *et al.* (2011), similar to present study, found that the cultivars containing a single major resistant gene exhibited susceptible reaction. It was also noticed that the presence of two genes either *xa13* and *Xa7* in Rathuwee or *xa13* and *Xa4* in Jaldi-13, Nipponbare, PNR- 519 and Krishna-Hamsa etc or *xa13* and *Xa5* in Heera and IR-6844-120 did not produce resistant or moderately resistant reaction in phenotypic screening (Table 2). Similar to the results of the present study, Acharya *et al.* (2018) reported that genotypes showed the presence of *Xa7* gene only, they were found susceptible in greenhouse condition. They also noticed that the rice genotypes having a combination of two genes, *Xa4* and *Xa7* gene showed moderately susceptible reaction for both inoculums. Similar to the findings, they recorded that the genotypes having more than two BLB resistance genes were found resistant or moderately resistant in greenhouse conditions against both inoculums. The present findings corroborates with the reports of Huang *et al.* (1997) who carried out DNA marker-assisted selection to pyramid four bacterial blight resistance genes, *i.e., Xa-4, xa-5, xa-13* and *Xa-21*.

They reported that the pyramid lines with more number of resistant genes showed a wider spectrum and a higher level of resistance than lines with only a single gene. Similar findings were reported by Jamal *et al.* (2018) where plants bearing more number of resistance genes demonstrated BLB resistance in phenotypic screening. Joseph *et al.* (2004) pyramided two major BLB-resistant genes, *i.e. xa13 and Xa21* into the genetic background of highly popular basmati rice variety Pusa Basmati 1, which was released for commercial cultivation as 'Improved Pusa Basmati 1' during 2007 (Rani, 2008). But, some *Xoo* strains are virulent on varieties possessing a combination of *Xa21* and *xa13* (Yugander *et al.,* 2017). Sundaram *et al.* (2008) pyramided three BB-resistant genes, *Xa21, xa13* and *xa5* into the genetic background of elite mega-variety of rice, Samba Mahsuri, which is a highly popular medium-slender and fine-grain type rice variety. The improved BB-resistant version of Samba Mahsuri possess high yield and a high level of resistance against BB disease. Thus, more than two BLB resistant genes through gene pyramiding will provide BLB resistance.

In general, the knowledge of effective resistance genes and pathogen population structure would be helpful in deploying suitable resistance genes in different rice growing areas. The plant breeders are eager to develop high yielding varieties having tolerance or resistance to specific biotic stresses as challenging situation for a particular agro-climatic zone. Looking into high yield *vis-a-vis* presence of BLB resistant genes among all genotypes, Zheshan 2 and IR-68144-2b-2-2-3-1-127, an indica type from China and IRRI, were combination of both traits. Several high yielding varieties, like, MTU 1010, ARC 10352 and IA 2 exhibited high yield, but failed against disease due to absence of desirable resistant genes. Identification of genotypes showing presence of gene as amplified by specific markers and their response in phenotypic screening would be more reliable than only phenotypic screening to identify BLB resistance (Acharya *et al.,* 2018). The present study identifies good genetic resources carrying BLB resistance genes in different rice genotypes which will facilitate the use of diverse donor base in breeding for BLB resistance in rice helping to mitigate the vulnerability of rice lines to severe disease. Marker assisted selection expedites and makes easy the plant breeding program in screening and incorporation of BLB resistant genes into high yielding types (Pradhan *et al.,* 2015). Hence, these seven varieties can be used as a good source of donor for BLB resistant genes in genetic enhancement program.The donors may be used in future hybridization program for incorporation of resistant genes in high yielding genetic background.

Acknowledgments

We are thankful to the Department of Science and Technology, Government of West Bengal for providing financial support. We are grateful to Prof. Adam H. Price, University of Aberdeen, UK for providing different rice genotypes. We acknowledge with appreciation for receiving the strain of Bacterial Leaf Blight from Indian Institute of Rice Research (IIRR), Hyderabad, India.

References

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

Akhtar, M. A., F. M. Abbasi, H. Ahmad, M. Shahzad, M.A. Shah and A. H. Shah: Evaluation of rice germplasm against *Xanthomonas oryzae* causing bacterial leaf blight. *Pak. J. Bot.,* **43**, 3021-3023 (2011).

Basharat, H. S., X. H. Ding, L.X. Zeng, T. Akshay, Z. M. Zhang, R. Z. Zeng and G. Q. Zhang: Pyramiding four bacterial blight resistance genes into rice cultivars in South China. *Mol. Plant Breed.,* **4**, 493–499 (2006).

Bhuiyan, M.A.: Efficiency in evaluating salt tolerance in rice using phenotypic and marker assisted selection. Mymensingh, Bangladesh, **96** (2005).

Blair, M.W., A.J.Garris, A.S. Iyer, B. Chapman, S. Kresovich and S.R. McCouch : High resolution genetic mapping and candidate gene identification at the xa5 locus for bacterial blight resistance in rice (*Oryza sativa* L.). *Theor. Appl. Genet.,* **107**, 62-73 (2003).

Chen, X., S.Temnykh, Y. Xu, Y. G. Cho and S. R. McCouch: Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). *Theor. Appl. Genet.,* **95**, 553-567 (1997).

Chukwu, S. C.,M. Y. Rafii, S. I. Ramlee, S. I. Ismail,Y. Oladosu, E. Okporie and M. Jalloh: Marker-assisted selection and gene pyramiding for resistance to bacterial leaf blight disease of rice (*Oryza sativa* L.). *Biotechnol. Equip.,* **33**, 1-16 (2019). https://doi.org/ 10.1080/ 1310 28 18. 2019.1584054

Dilla-Ermita, C. J., E. Tandayu,V. M. Juanillas, J. Detras, D.N. Lozada, M.S. Dwiyanti and M. Mendioro: Genome-wide association analysis tracks bacterial leaf blight resistance loci in rice diverse germplasm. *Rice,* **10**, (2017). https://doi.org/10.1186/s12284-017-0147-4

Doyle, J.J. and J.L. Doyle: Isolation of plant DNA from fresh tissue. *Focus,* **12**, 39-40(1990).

Huang, N., E.R. Angeles, J. Domingo, G. Magpantay, S. Singh, G. Zhang and G.S. Khush: Pyramiding of bacterial blight resistance genes in rice: Marker-assisted selection using RFLP and PCR. *Theor. Appl. Genet.,* **95**, 313-320 (1997).

IRRI.: International Rice Research Institute: Standard Evaluation System (SES) for Rice. 4th Edn., p.52 (1996).

Jamal, G. and S. Rahman: Marker-analysis of leaf resistance bacterial in rice. *Med. Bio. Tech. J.,* **2**, 199-206 (2018).

Jiang, S.H., H. Zhou, D.Z. Lin, Y.J. Dong, S.H. Ye and X.M. Zhang: Identification and gene mapping of a thermo-sensitive leaf-color mutant at seedling stage in rice. *Chin. J. Rice Sci.,* **27**, 359-364 (2013).

Joseph, M., S. Gopalakrishnan, R.K. Sharma, V.P. Singh, A.K. Singh, N.K. Singh and T. Mohapatra: Combining bacterial blight resistance and Basmati quality characteristics by phenotypic and molecular marker-assisted selection in rice. *Mol. Breed.,* **13**, 377-387(2004).

Khan, J.A., H.M.I. Arshad, F.F. Jamil and S. Hasnain: Evaluation of rice genotypes against bacterial leaf blight (BLB) disease. *Pak. J. Phytopathol.,* **21**, 26-30(2009).

Khan, M.A., M. Naeem and M. Iqbal: Breeding approaches for bacterial leaf blight resistance in rice (*Oryza sativa* L.), current status and future directions. *Eur. J. Plant Pathol.,* **139**, 27-37 (2014).

Kim, S.M., J.P. Suh, Y. Qin, T.H. Noh, R.F. Reinke and K.K. Jena: Identification and fine-mapping of a new resistance gene, Xa40, conferring resistance to bacterial blight races in rice (*Oryza sativa* L.). *Theor. Appl. Genet.,* **128**, 1933-1943(2015).

Kumar, P.N., K. Sujatha, G.S. Laha, K.S. Rao, B. Mishra, B.C. Viraktamath and M.S. Madhav: Identification and fine-mapping of Xa33, a novel gene for resistance to *Xanthomonas oryzae* pv. oryzae. *Phytopathology,* **102**, 222-228 (2012).

Liu, Q., M. Yuan, Y. A. N. Zhou, X. Li, J. Xiao and S. Wang : A paralog of the MtN3/saliva family recessively confers race-specific resistance to *Xanthomonas oryzae* in rice. *Plant Cell Environ.,* **34**, 1958-1969 (2011).

Ma, B., W. Wang, B. Zhao, Y. Zhou, L. Zhu and W. Zhai: Studies of PCR marker for the rice bacterial blight resistance gene Xa 4. *HEREDITAS,* **21**, 9-12 (1999).

Madhavi, K.R., M.S. Prasad, G.S. Laha, K.M. Mohan, M.S. Madhav and B.C. Viraktamath: Combining blast and bacterial blight resistance in rice cultivar, improved Samba Mahsuri. *Indian J. Plant Prot.,* **39**, 124-129(2011).

McCouch, S.R., L.S. Sebastian, R. Ikeda, N. Huang, T. Imbe and W.R. Coffman: Molecular mapping of resistance to rice tungrospherical virus and green leafhopper. *Phytopathol.*,86, 25–30 (1996).

Nino-Liu, D.O., P.C. Ronald and A.J. Bogdanove: *Xanthomonas oryzae* pathovars: Model pathogens of a model crop. *Mol. Plant Pathol.,* **7**, 303-324 (2006).

Pradhan, S.K., D.K. Nayak, S. Mohanty, L. Behera, S.R. Barik, E. Pandit and A. Anandan: Pyramiding of three bacterial blight resistance genes for broad-spectrum resistance in deepwater rice variety. *Jalmagna Rice,* **8**, 19 (2015) doi: 10.1186/s12284-015-0051-8

Rajpurohit, D., R. Kumar, M. Kumar, P. Paul, A. Awasthi, P.O. Basha and H.S. Dhaliwal: Pyramiding of two bacterial blight resistance and a semi dwarfing gene in Type 3 Basmati using marker-assisted selection. *Euphytica,* **178**, 111-126 (2011).

Ram, T., G.S. Laha, R. Deen, J.M. Ramos, C.M. Veracruz and D.S. Brar: *Oryza rufipogon*, a valuable source for resistance to bacterial blight of rice. *Plant Breed,* **130**, 715–718 (2011).

Rani, S.: High yielding rice varieties of India. Directorate of Rice Research, ICAR. (2008).

Sharma, B. and M.P. Pandey: Identification of rice germplasm with resistance to bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*). *Bangladesh J. Agric. Res.,* **37**, 349-353 (2012).

Singh, A.K., E. Dharmraj, R. Nayak, P.K. Singh and N.K. Singh: Identification of bacterial leaf blight resistance genes in wild rice of eastern India. *TURK. J.,* **39**, 1060-1066 (2015).

Sun, X., Z. Yang, S. Wang and Q. Zhang: Identification of a 47-kb DNA fragment containing *Xa4*, a locus for bacterial blight resistance in rice. *Theor. Appl. Genet.,* **106**, 683–687 (2003).

Sundaram, R.M., M.R. Vishnupriya, S.K. Biradar, G.S. Laha, G.A. Reddy, N.S. Rani and R.V. Sonti : Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite indica rice variety *Euphytica,* **160**, 411-422 (2008).

Tasleem-uz-Zaman, K., M.A. Gilla and M.G. Khan: Screening of rice varieties/lines for resistance to bacterial leaf blight. Pak. *J. Phytopathol.,* **12**, 71-72 (2000).

Thimmegowda, P.R., D.S. Ambika, A.R. Manjunatha, P.P. Sataraddi and M. Chandrashekar: Screening germplasm for resistance to bacterial blight of rice caused by *Xanthomonas oryzae* pv. *oryzae. Int. J. Sci. Nat.,* **2**, 659-661 (2011).

Tian, D., J. Wang, X. Zeng, K. Gu, C. Qiu, X. Yang and F.F. White: The rice TAL effector–dependent resistance protein *XA 10* triggers cell death and calcium depletion in the endoplasmic reticulum. *Plant Cell,* **26**, 497-515 (2014).

Wang, C., X. Zhang, Y. Fan, Y. Gao, Q. Zhu, C. Zheng and B. Yang: XA23 is an executor R protein and confers broad-spectrum disease resistance in rice. *Molecu. Plant,* **8**, 290-302 (2015).

Yadav, S., A. Singh, N. Goel and A.K. Singh: Identification of Indian rice germplasm lines with bacterial leaf blight (BLB) resistance genes. *Indian J. Genet. PL. BR.,* **73**, 310-31(2013).

Yugander, A., R.M. Sundaram, D. Ladhalakshmi, S.K. Hajira, V. Prakasam, M.S. Prasad and G.S. Laha: Virulence profiling of *Xanthomonas oryzae* pv. *oryzae* isolates, causing bacterial blight of rice in India. *Eur. J. Plant Pathol.,* **149**, 171-191 (2017).