Screening of Restorable WA-CMS Rice Genotypes for Bacterial Blight (BB) Resistance

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Authors' contributions

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ABSTRACT

Aim: To screen and evaluate rice genotypes which were found to be having restorability for WA-CMS cytoplasm for their further use in the hybrid breeding program.
Study Design: Laboratory-experimental design was used in the study.
Place and Duration of the Study: The genotypes used in the study were comprised of promising lines from various experiments, local varieties and landraces, etc. The study was conducted during June 2017 to May 2018.
Methodology: In the present study, 55 rice lines which were found to have restorable capacity for WA-CMS system were screened for 4 Bacterial Blight resistance genes *viz. Xa4, xa5, xa13, Xa21,*

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against most prevalent races of the pathogen throughout the country, using PCR based molecular markers namely, MP1+MP2, xa5 multiplex, xa13 prom and pTA248, respectively.

Results: Bacterial Blight (BB) is considered to be one of the most important diseases of the rice crop. As hybrids are one of the most viable options to increase rice yields, the parental material used for the development of hybrids, must be having genes which show resistance against BB. Out of 55 restorer lines, 43 genotypes amplified for *Xa4,* 7 genotypes namely R. Bhagwati, IRBB5-9, Narendra Usar Dhan 3, Pratikhya, IR 88964-24-2-1-4, IR 94314-20-2-1B, HHZ 5-DT8-DT1-Y1 showed positive bands for *xa5,* 3 genotypes namely PAU 3220, N. Usar Dhan 3 and Pratikhya showed positive bands for *xa13.* However, only one genotype namely Pratikhya amplified for *Xa21.* In combinations, Narendra Usar Dhan 3 amplified for *xa5* and *xa13.* Pratikhya was the only genotype found to have all 4 BB (*Xa4, xa5, xa13, Xa21*) resistance genes under consideration.

Conclusion: Genotypes having different BB resistant genes in combinations along with good capability of restoration for prevalent WA-CMS system can further be used as male parent in the hybrid breeding programme for development of BB resistant hybrids.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food of more than two third of the world population providing approximately 50% of the dietary calorific requirements. Approximately 92% of rice is grown and consumed in Asia, which comprises 55% of the world population [1]. As per estimation, 135 million tonnes of milled rice will be required in India till 2030 which will be increased to 180 million tonnes by 2050 (anonymous). To keep pace with the ever increasing population, there is a need to boost rice production. Among several strategies suggested for increment in the rice production, development of hybrids is one of the most viable and proven technology. Hybrids are feasible and readily adaptable. Success of hybrid rice technology in China has resulted in tremendous enhancement of rice production in the country. In India also very good hybrids have been developed and released from both public and private sectors. Earlier issues related to grain quality have now been resolved and hybrids with very good grain qualities are coming into farming practices [2].

Plant breeders face several problems in developing new varieties. On one side, we are gradually getting higher productions while on the other side, more and more challenges are coming. Few decades ago, there were only few insects pests and diseases which were said to be important in respect of the breeding purposes of rice, but now this number has reached new heights [3] and it has become a necessity that the hybrids which is now being developed, must have resistance against insect pest and diseases prevailing in the region. In Coordinated Research

Improvement Project (AICRP), it is mandatory to test the genotypes for disease and insect pests if the genotypes become advanced in the first year of testing. Therefore, development of rice hybrids resistant to different biotic and abiotic stresses will be effective. In the present time, plant breeders have been equipped with several modern tools and techniques which have made selection and handling of single gene or a group of genes quite simple.

Bacterial blight (BB) or bacterial leaf blight (BLB) caused by *Xanthomonas oryzae oryzae* (*Xoo*) is the most destructive disease of rice prevalent mostly in irrigated and rainfed lowland rice growing areas. It affects the rice plant at all stages of growth but maximum damage occurs at tillering stage. In the initial stages, small lesions on leaf tips are observed. But, as the disease progresses, small lesions turn into wavy lesions and cover almost entire leaf portions and thus, affecting the yield to a greater extent. Yield losses in severely infected fields range from 20 to 30%, but this can reach as high as 80% [4,5,6,7]. Employment of host plant resistance has been found to be very reliable in controlling the disease. Till date, 40 BB resistance genes [1] conferring resistance to host against different strains of *Xoo* have been identified, of which, *Xa2, Xa4, Xa7, Xa30, Xa33 and Xa38* have been physically mapped and *Xa1, xa5, xa13, Xa21, Xa26/Xa3* and *Xa27* have been cloned [4,6]. On the basis of studies done on segregating pattern of these 40 BB resistance genes, 14 genes *viz. Xa1, Xa2, Xa3, Xa4, Xa7, Xa10, Xa11, Xa12, Xa14, Xa16, Xa17, Xa18, Xa21* and *Xa22* were found to be dominant while six genes *viz. xa5, xa8, xa13, xa15, xa19* and *xa20* were found to recessive in nature

[8,9]. However, out of these, four genes *viz. Xa4, xa5, xa13, Xa21* were reported to be the most important conferring effective resistance against most prevalent races of the pathogen [10,4,11].

In India, most of the rice hybrids are based on CGMS (Cytoplasmic Genetic Male Sterility) system where three lines namely A-male sterile line, B-maintainer line and R-restorer line are required. During the course of hybrid development, if restorer line having one or more genes conferring resistance against BB is used, the resistance can easily be transferred to the hybrids.

Using conventional breeding tools for screening of resistance is a very time taking and tedious task. Moreover, the accuracy is largely affected by the environments and technical operations. Now, several gene specific and SSR (Simple Sequence Repeats) markers are available for the screening which can be used in Marker Assisted Selection (MAS) of the disease resistance in screening process of progenies. In the present study, genotypes which were found to have restorability for WA-CMS (Wild Abortive-Cytoplasmic Male Sterility) were used for screening for 4 most prevalent BB resistance genes *viz. Xa4, xa5, xa13, Xa21* so that the lines could be further used for the development of better hybrids.

2. MATERIALS AND METHODS

2.1 Plant Material and Molecular Markers

A set of 55 rice genotypes (Table 1) consisting of indigenous and exotic collections and reported to have restorability for WA-CMS system, were used for screening of 4 BB resistance genes *viz. Xa4, xa5, xa13, Xa21* by using different tightly linked gene specific/ PCR-based markers (Table 2).

2.2 DNA Extraction

Genomic DNA was extracted using rapid DNA isolation protocol, reported by Kumar et al. [12]. About 50 mg leaf tissue of 21 days old rice seedlings was cut into small pieces, homogenized and digested using 500 µl of isolated buffer [100 mM Tris-HCl (pH 8.0), 50 mM EDTA, 500 mM NaCl, 1% SDS (w/v) and 0.1 (v/v) of β-mercaptoethanol]. Then 96 µl of 5M potassium acetate were added and the samples were vortexed following centrifugation @ 10000 rpm for one minute. The supernatant was collected and the DNA was precipitated with equal volume of isopropanol. The DNA pellet was then washed in 70% ethanol followed by alcohol was decantation and air drying of DNA pellet. It was further dissolved in 60 µl of distilled water and stored at -20°C freezer.

2.3 Polymerase Chain Reaction (PCR)

Extracted DNA samples were subjected to PCR amplification using the tightly linked/gene specific molecular markers in automated thermal cycler (Applied Biosystems Veriti, USA). The PCR amplification were carried out in 12 µl reaction volume contained 2 µl (100 ng) DNA, 4 µl Premix Taq® Version2.0 (Xcelris genomics, India), 0.25 µl each forward and reverse primer (0.25 µM) and 5.5 µl MilliQ water. Reaction condition was programmed as initial hold at 94°C for 4 minutes followed by 35 cycles of denaturation at 94°C for 30 seconds, 60 seconds annealing at 55°C for linked/ gene specific markers and extension at 72°C for 60 seconds. Further extension was allowed at 72°C for 5 minutes followed by holding the samples at 4°C for 2 minutes. The PCR products were resolved by electrophoresis in 1.5% agarose gels for pTA248, xa5S/xa5SR/R-Multiplex and xa13 prom markers and 2.5% agarose gels for MP1+MP2 markers in 1X TAE buffer. The amplicons were visualized by UV light and documented (UVITEC gel doc system, UK).

3. RESULTS AND DISCUSSION

Bacterial Blight (BB) is one of the most deadly diseases of rice crop. Most of the mega old and new varieties have become susceptible to one or more races of the pathogen. In the present study, 55 rice genotypes which were having restorability capacity for WA-CMS system were screened for genes conferring resistance to major races of the pathogen. For this purpose, molecular markers *viz.* MP1+MP2, xa5 (Multiplex), xa13 prom and pTA248 were used, for screening of *Xa4, xa5, xa13* and *Xa21*, respectively.

The genes under study i.e. *Xa4, xa5, xa13* and *Xa21* produced specific band of 120 bp, 400 bp and 150 bp, 500 bp and 1000 bp indicating their presence while amplification of 150 bp, 400 bp and 300 bp, 250 bp, 600 bp and 800 bp bands indicated the absence of genes, respectively.

Out of 55 genotypes, *Xa4* was found to be present in 43 lines, like wise *xa5, xa13* and *Xa21* was found present in seven, three and one

Table 1. List of genotypes used in the study

S. No.	Name/ Designation	S. No.	Name/ Designation	S. No.	Name/ Designation	S. No.	Name/ Designation	S. No.	Name/ Designation
1.	RAU 1484	12.	HIRANMAYEE	23.	IR 88964-24-2-1-4	34.	HHZ 5-DT8-DT1Y1	45.	IR 07A140SBR-1
2.	PUSA SUGANDH-5	13.	IR06A196	24.	ZHONGHUA 1	35.	PAU 3220	46.	RAJENDRA KASTURI
3.	IR 8896-39-1-4-4	14.	IRK4098SBR-1E	25.	IR 88968-2-1-1-2	36.	PR 35015-5-4	47.	BPT 5204
4.	RAJENDRA BHAGWATI	15.	HUA 565	26.	PRR 78	37.	IR09N142	48.	CR 3825-2-1-2-2-3
5.	IRBB-59	16.	TRC 2013-1	27.	HHZ 9-SAL9-Y3-SUB 1	38.	RYC 674	49.	IR 05A164
6.	NARENDRA USAR DHAN -3	17.	HHZ 25-DT9-Y1-Y1	28.	HHZ 2-Y3-Y1-Y1	39.	IR 04A115	50.	IR 96321-327-300-B-1-1
7.	IRRI-123	18.	HHZ14-SAL10-DT1-DT1	29.	IR 91648-B-89-B-12-1	40.	CR 3622-7-3-1-1-1	51.	PUSA 1638-07-129-2-63
8.	HKR-08-29	19.	HHZ 14-Y7-Y1-DT2	30.	IR 94314-20-2-1-B	41.	IR 06N120	52.	IR 88963-3-7-2-4
9.	AKSHYADHAN	20.	IR 64	31.	IR87761-39-2-3-2	42.	CR 2994-5-3-2-1-1	53.	MGD-1104
10.	PRATIKHYA	21.	SABOUR ARDHJAL	32.	PAU 3207	43.	SWARNA SUB 1	54.	HHZ 17-Y16-Y3-Y1
11.	IR06A177	22.	CRR 547-20-1-1	33.	CN 1646-1-9	44.	IR 1576-1698-2-556-1	55.	R 1530-1546-1418-1-1

Table 2. Chromosome position, primer sequence, annealing temperature of the used tightly linked/gene based molecular markers

Sl. No.	Gene/QTL	Primers		Chr. No.	Annealing temp. (°C)	Amplicon size	References
1.	*Xa* 4	MP1+MP2	F- ATCGATCGATCTTCACGAGG	11	55	150, 120	Suh et al, (2013)
			R- TGCTATAAAAGGCATTCGG				
2.	*xa* 5	Xa5s (Multiplex)	F- GTCTGGAATTTGCTCGCGTTCG	5	55	400, 300, 150	Sundaram et al,
		Xa5s(Multiplex)	R- TGGTAAAGTAGATACCTTATCAAACTGGA				(2011)
		Xa5sr/R(Multiplex)	F- AGCTCGCCATTCAAGTTCTTGAG				
		Xa5sr/R(Multiplex)	R- TGACTTGGTTCTCCAAGGCTT				
3.	*xa* 13	Xa13 Prom	F- GGCCATGGCTCAGTGTTTAT	8	55	500, 250	Chu et al, (2006)
			R- GAGCTCCAGCTCTCCAAATG				
4.	*Xa* 21	pTA248	F- AGACGCGGAAGGGTGGTTCCCGGA	11	55	1000,800, 600	Ronald et al (1992)
			R- AGACGCGGTAATCGAAGATGAAA				

genotypes, respectively {Fig. 2(a), 2(b), 2(c), 2(d)}. Ma et al. [13], Suh et al. [6], Suh et al. [14], using PCR based molecular marker MP1+MP2 and found 150 bp band size positive for *Xa4* resistance gene. Sundaram et al. [15], Pradhan et al. [11], Sinha et al. [16] also used xa5 multiplex marker and found the similar results with 400 bp and 150 bp positive for *xa5* resistance gene. Swathi et al. [17]; Singh et al. [18], Arunakumari et al. [19], Dash et al. [20], and Hajira et al. [21], also used *xa13* prom marker and found the similar results with 500 bp positive for *xa13* resistance gene. Bharania et al. [22], Kottapalli et al. [23], Deng et al. [24], Luo et al. [25], Xu et al. [26], Magar et al. [27], Singh et al. [18]; Swathi et al, [17]; Arunakumari et al. [19], Dash et al. [20], Pradhan et al. [28] and Sinha et al. [16] also used pTA248 marker and found the similar results with 1000 bp positive for *Xa21* resistance gene.
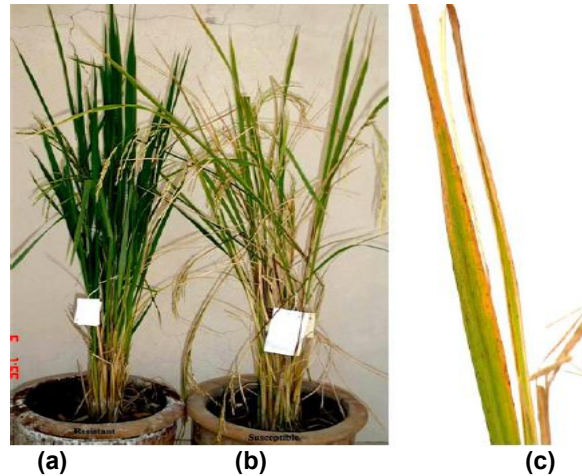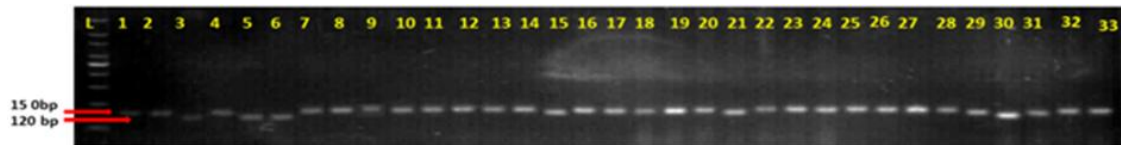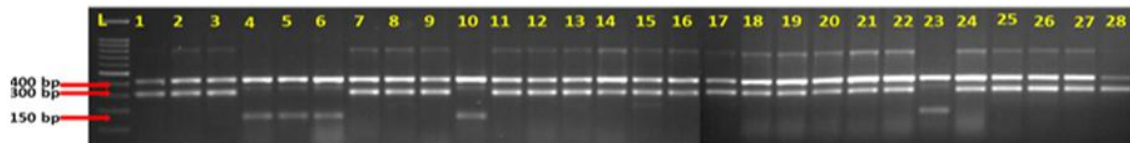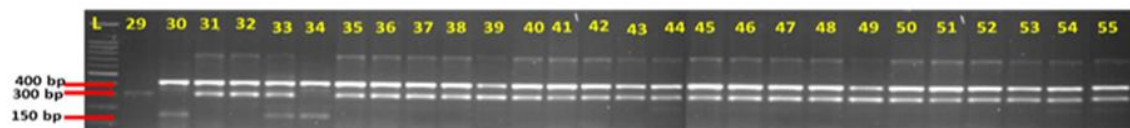

Fig. 1. Symptoms of bacterial leaf blight- (a) Resistant, (b) Susceptible, and (c) Leaf of a susceptible plant (Khan et al. 2014) [7]
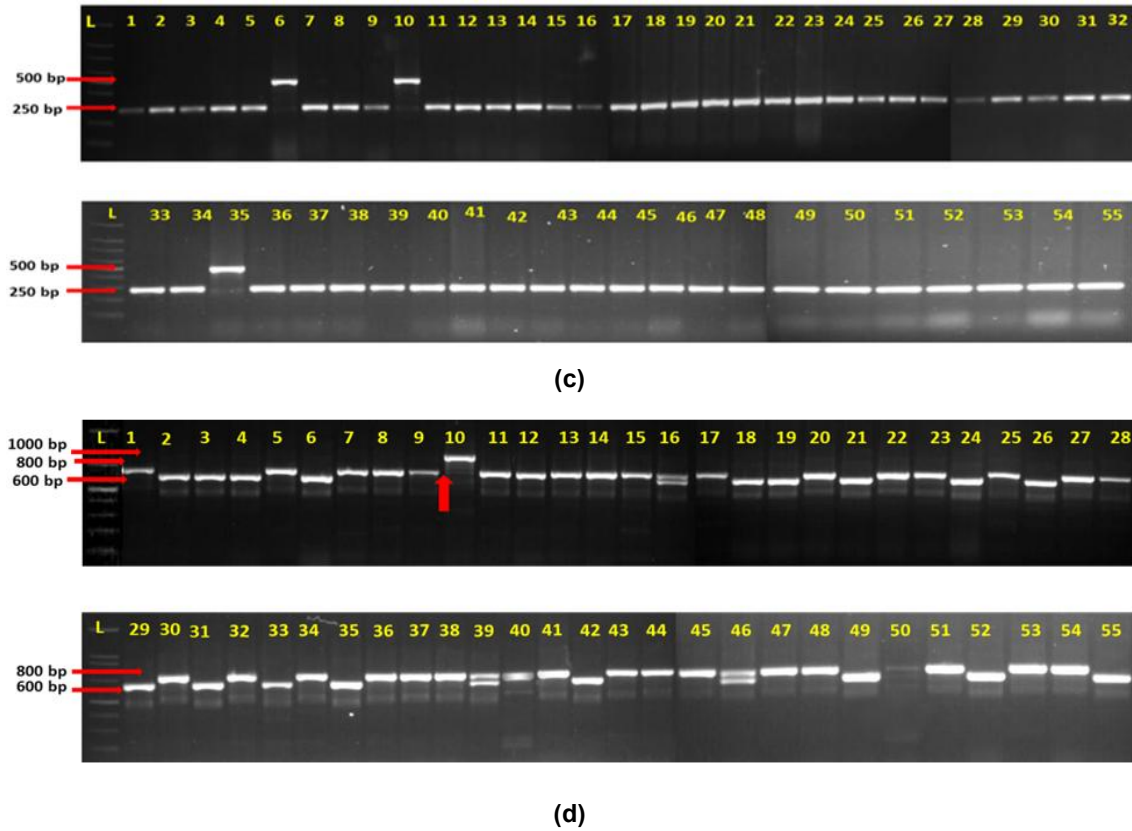

(a)

(b)

(c)

(d)

Fig. 2. PCR amplification of markers linked to bacterial leaf blight resistance genes, *Xa4, xa5, xa13* and *Xa21* using primers (a) MP1+MP2, (b) xa5 (Multiplex), (c) pAT248, and (d) xa13 prom, respectively

(L-ladder, 1-RAU 1484, 2- P. SUGANDH 5, 3-IR88966-39-1-4-4, 4-R. BHAGWATI, 5-IRBB5-9, 6- N.USAR DHAN3, 7-IRRI123, 8- HKR-08029, 9-AKSHYADHAN, 10-PRATIKHYA, 11-IR 06A177, 12- HIRANMAYEE, 13-IR06A196, 14- IRK4098SBR-1E, 15- HUA 565, 16- TRC 2013-1, 17-HHZ 25- DT9-Y1-Y1, 18- HHZ 14-SAL10-DT1-DT1, 19- HHZ 14-Y7-Y1-DT2, 20- IR64, 21-SABOUR ARDHJAL, 22- CRR 547-20-1-1, 23- IR 88964-24-2-1-4, 24- ZHONGHUA1, 25- IR 88968-2-1-1-2, 26- PPR78, 27- HHZ 9-SAL9-Y3-SUB1, 28- HHZ 2-Y3-Y1-Y1, 29- IR 91648-B-89-B-12-1, 30-IR 94314-20-2-1-B, 31- IR 87761-39-2-3-2, 32- PAU 3207, 33- CN 1646-1-9, 34- HHZ 5-DT8-DT1-Y1, 35- PAU 3220, 36- PR 35015-5-4, 37- IRO9N 142, 38- RYC 674, 39- IR 04A115, 40- CR 3622-7-3-1-1-1, 41- IR 06N120, 42- CR 2994-5-3-2-1-1, 43- SWARNA SUB1, 44- IR 1576-1698-2-556-1, 45- IR 07A140SBR-1, 46- R. KASTURI, 47- BPT 5204, 48- CR 3825-2-1-2-2-3, 49- IR 05A164, 50- IR96321-327-300-B-1-1, 51-PUSA 1638-07-129-2-63, 52-IR 88963-3-7-2-4, 53-MGD 1104, 54-HHZ 17-Y16-Y3-Y1, 55-R 1530-1546-1-418-1-1)

The present study also revealed that the genotype N. Usar Dhan 3 was having a combination of *xa5* and *xa13* gene and, that the only single genotype *viz.* Pratikhya carried all the 4 BB gene i.e, *Xa4*, *xa5*, *xa13* and *Xa21*. Kottapalli et al. [23] also reported *xa5*, *xa13* and *Xa21* as the best gene combination for the control of BB disease in rice.

4. CONCLUSION

For sustainable management of the rice bacterial blight disease, stacking of genes conferring resistance to the disease is necessary. In the hybrid rice technology, if the stacking could be done in parental genotypes, it could be easy to develop multiple good hybrids having resistance to BB. In the present study, 55 genotypes having restorable capacity to WA-CMS germplasm were screened for four most prevalent strains (Xa4, xa5, xa13, Xa21) of bacterial blight. Out of 55 genotypes, several were having one or more genes showing great value for breeders. The genotypes like N. Usar Dhan 3 and Pratikhya could further be utilized for transfer and stacking of two or more (even up to four) most important genes for resistance to bacterial blight through marker assisted breeding programme.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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