



Marker-assisted Pyramiding of Bacterial Blight Resistance Genes into 'Uma', an Elite Red Rice Variety of Kerala

L.M. Megha¹, Rose Mary Francies², Jiji Joseph³, P. Raji⁴, P.S. Abida⁵

10.18805/BKAP922

ABSTRACT

Background: Uma is a high-yielding, popular red-kernelled rice variety of Kerala; however, the occurrence of BB disease often causes severe yield reductions in this cultivar. Host plant resistance based on multiple genes is the most effective measure to impart durable resistance. Hence, efforts were undertaken to introgress the resistance genes, *xa5*, *xa13* and *Xa21*, from a BB-resistant variety, ISM, into Uma, using the MABB approach.

Methods: Foreground selection was carried out using markers RG 556 and *xa5* SR for *xa5*, RG136 and *xa13* promoter for *xa13* and pTA248 for *Xa21* genes, respectively. The identified R-gene-pyramided plant was subjected to background selection using rice microsatellite markers for recurrent parent genome recovery. The phenotypic evaluation for BB resistance was carried out using the leaf-clipping method following the IRRI standard evaluation system, SES.

Result: Molecular analysis of the recurrent parent Uma revealed the endogenous presence of R gene *xa5*. The evaluation of BC₂F₁ lines identified a single plant pyramided with three resistance genes, with *xa13* and *Xa21* in the heterozygous condition, exhibiting a recurrent parent genome recovery of 70.45% and kernel characteristics similar to those of the Uma. Concurrently, BC₁F₂ lines generated from the cross were phenotypically screened for BB resistance, also identifying resistance to moderate resistance lines to the BB pathogen. Further advancement of these backcross generations was proposed to isolate gene pyramids homozygous for the resistance genes, with near-complete recurrent parent genome recovery.

Key words: BB resistance, Marker-assisted selection, R genes, Rice, Uma.

INTRODUCTION

Kerala, a state in India, has a distinctive food culture, particularly its unique preference for rice. The widespread use of parboiled, red rice grains (matta rice) makes it a healthier dietary choice for the people in the region. Uma (Mo. 16) is one among the popular high-yielding red-kernelled rice varieties grown in the state and is estimated to occupy more than 60% of the paddy cultivation area in the state. It is a semi-dwarf, non-lodging, medium tillering; exhibits resistance against brown plant hoppers and gall midge biotype-5 and produces a yield of about 6 to 6.5 tonnes/ha (Devika *et al.*, 2004; Estelitta *et al.*, 2016). Of late, severe yield reductions are commonly observed in this elite rice cultivar owing to the occurrence of bacterial blight (BB) disease caused by *Xanthomonas oryzae* p.v. *oryzae* (Xoo) (Jerish *et al.*, 2022; Laha *et al.*, 2023). Among the various disease management tactics, pyramiding resistance genes through the marker-assisted backcross breeding (MABB) approach is found to be an effective method to ensure durable resistance. According to Priyadarisini and Gnanamanickam (1999), the rice line NH56 carrying four R genes (*Xa4* + *xa5* + *xa13* + *Xa21*) was found to be resistant to the Kerala isolate of the Xoo pathogen. However, the breakdown of resistance of cultivars with *Xa4* has been reported earlier in the Philippines and India (Pandey *et al.*, 1986; Mew, 1992). Therefore, efforts are currently being undertaken to pyramid the resistance genes *xa5*, *xa13* and *Xa21* into the genetic background of Uma using the MABB approach, with

¹Department of Genetics and Plant Breeding, College of Agriculture, Kerala Agricultural University, Vellayani, Thiruvananthapuram-695 522, Kerala, India.

²Department of Plant Breeding and Genetics, Agricultural Research Station, Kerala Agricultural University, Mannuthy, Thrissur-680 651, Kerala, India.

³Department of Plant Breeding and Genetics, College of Agriculture, Kerala Agricultural University, Vellanikkara, Thrissur-680 656, Kerala, India.

⁴Department of Plant Pathology, Regional Agricultural Research Station, Kerala Agricultural University, Pattambi, Palakkad-679 306, Kerala, India.

⁵Department of Plant Biotechnology and Molecular Biology, College of Agriculture, Kerala Agricultural University, Vellanikkara, Thrissur-680 656, Kerala, India.

Corresponding Author: L.M. Megha, Department of Genetics and Plant Breeding, College of Agriculture, Kerala Agricultural University, Vellayani, Thiruvananthapuram-695 522, Kerala, India. Email: meghuaishu@gmail.com

ORCID: <https://orcid.org/0009-0001-7458-5094>

How to cite this article: Megha, L.M., Francies, R.M., Joseph, J., Raji, P. and Abida, P.S. (2026). Marker-assisted Pyramiding of Bacterial Blight Resistance Genes into 'Uma', an Elite Red Rice Variety of Kerala. *Bhartiya Krishi Anusandhan Patrika*. 41(3): 276-285. doi: 10.18805/BKAP922.

Submitted: 17-02-2026 **Accepted:** 23-07-2026 **Online:** 20-08-2026

Improved samba mahsuri (ISM) serving as the donor parent. Advanced backcross progenies were screened

using marker-assisted selection (MAS) to identify plants carrying all three resistance genes while minimising linkage drag from the donor genome.

MATERIALS AND METHODS

The study was conducted in the Department of Plant Breeding and Genetics, College of Agriculture, Kerala Agricultural University (KAU), Vellanikkara, Thrissur, Kerala. The rice varieties Uma (Mo16) and ISM were used as the parents in the present backcross breeding programme. The rice variety ISM, an Essentially Derived Variety (EDV), served as the donor for bacterial blight resistance genes *xa5*, *xa13* and *Xa21*, while Uma, was used as the recipient parent. The backcross lines (BC_1F_1 s) generated from the cross were evaluated through MAS and the three BC_1F_1 lines (Plant no. 8.3.2, 8.3.3 and 8.3.9) pyramided with *xa5*, *xa13* and *Xa21* genes were identified (Joseph 2016). However, these lines exhibited lower recovery of the recurrent parent genome (21.80 to 23.90%); hence, they were further advanced to develop BC_2F_1 and BC_1F_2 populations, forming the base material for the study (Fig 1).

Genotyping of the BC_2F_1 population

Of the BC_2F_1 seeds sown, only 21 successfully germinated, attained maturity and were subsequently utilised for screening, whereas the parental lines were raised in replicated plots to facilitate comparison. For cellular DNA isolation, fresh leaf samples were collected from BC_2F_1 s and parents. The modified CTAB method was used for the extraction of DNA from the collected samples (Dellaporta *et al.*, 1983). The quality and quantity ($\mu\text{g/ml}$) of the DNA isolated were analysed using Nanometre (JH BIO Innovations, India). The absorbance ratio (A_{260}/A_{280}) for

the BC_2F_1 plants and the parents ranged from 1.89 to 1.90. Foreground selection was carried out using three STS markers, RG556, RG136 and pTA248, closely linked to the BB resistance genes *xa5*, *xa13* and *Xa21*, respectively (Huang *et al.*, 1997; Sundaram *et al.*, 2008). Further, the presence of resistance genes *xa5* and *xa13* in the BC_2F_1 progenies was also confirmed using functional markers *xa5S/R* and *xa13* promoter, respectively (Chu *et al.*, 2006; Sundaram *et al.*, 2008).

The PCR amplification was performed using the thermal cycler eppendorf master cycler (Eppendorf, Germany, Model: Hamburg 22331) and the reaction mixture of 15 μl was prepared using 2 μl of 10x Taq buffer, 1 μl of dNTP mix, 1.5 μl of MgCl_2 (25 mM), 0.3 μl of Taq DNA polymerase (1 U), 1 μl each of forward and reverse primers, 3 μl of DNA sample and 5.2 μl of distilled water. The PCR reaction profile was as follows: initial denaturation (hot start) at 94°C for 5 min, 35 cycles of denaturation (94°C for 30 sec), annealing (55°C for 30 sec), extension (72°C for 1 min) and final extension at 72°C for 7 min. Restriction digestion was performed for the PCR products of STS markers RG556 and RG136 using enzymes Dra1 and Hind1, respectively, for detecting polymorphism. The amplified PCR products were separated by running them in a 1.5% agarose gel stained with ethidium bromide and the bands obtained were visualised using gel documentation software, UVITEC Fire Reader software (Merck, UK). The location of the amplicon position and its molecular weight were assessed in comparison to the known molecular weight marker. The BC_2F_1 lines having amplicon sizes corresponding to *xa5*, *xa13* and *Xa21* genes were selected for further evaluation. For finding the recurrent parent genome recovery, the selected lines of BC_2F_1 s were

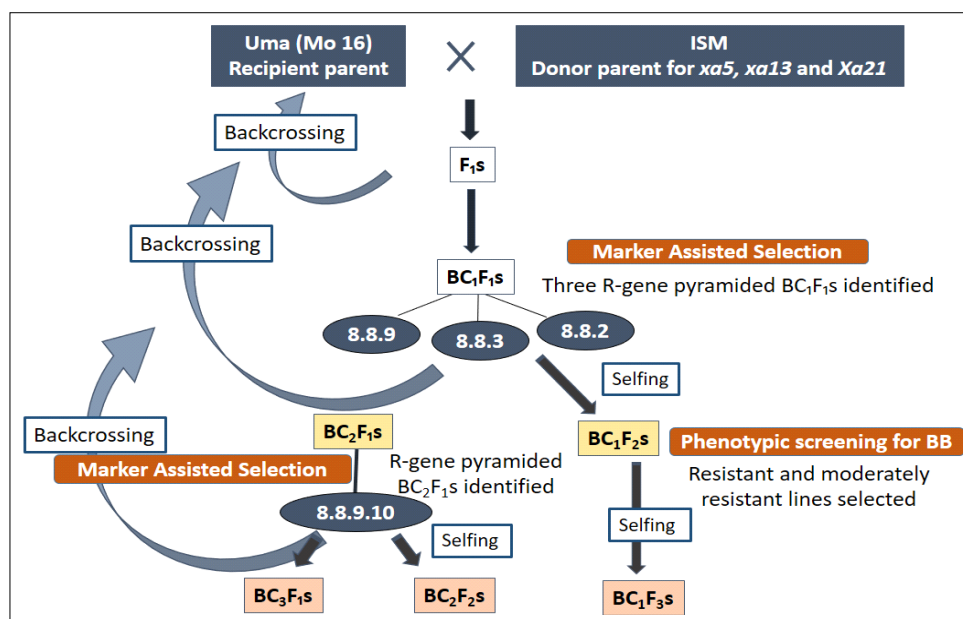


Fig 1: Diagrammatic representation of the marker-assisted breeding programme for bacterial blight resistance in Uma.

further subjected to background selection. A total of 364 rice microsatellite (RM) markers were screened between Uma and ISM, of which only 22 markers were polymorphic. These markers, covering 12 rice linkage groups, were selected for background selection. PCR amplification of samples with background markers, agarose gel separation and documentation were performed as per the protocol described earlier.

Morphological characterisation of introgressed BC₂F₁s and parents

As observations were based on single plants, morphological characterisation of individual BC₂F₁ plants was conducted in a non-replicated experimental block, whereas the parental lines were evaluated in ten replications. All observations were recorded following the standard descriptors for rice developed by the International Rice Research Institute (2002). The traits observed include plant height (cm), days to flowering, leaf blade width (cm), leaf blade length (cm), productive tillers, panicle length (cm), spikelets/panicle, grain length (mm), grain width (mm), decorticated grain length (mm) and decorticated grain width (mm). Mean and standard deviation (SD) values were computed for BC₂F₁s and parental lines.

Phenotypic screening of the BC₁F₂ population for BB resistance

The BC₁F₂ lines (106 nos) produced by selfing of R gene pyramided BC₁F₁ lines (8.3.2, 8.3.3 and 8.3.9) were phenotypically screened for bacterial blight resistance following the leaf clip method (Kauffman *et al.*, 1973; Jalil *et al.*, 2023; Kanipriya *et al.*, 2024) for identification of resistant and susceptible genotypes. The isolate of *Xanthomonas oryzae* pv. *oryzae* (Xoo) collected from the Regional Agricultural Research Station, Pattambi, Kerala, was used to screen the BC₁F₂ population and parents to evaluate their reactions to pathogen infection. The BB susceptible recurrent parent, Uma, was used as the susceptible check in this study. The local strains of the bacterial blight pathogen were isolated from infected rice leaves. After 4-5 days of incubation, typical round, yellowish colonies of Xoo were selected, subcultured to obtain pure cultures and multiplied on sucrose agar medium. The cultures were then preserved as glycerol stocks at 4°C for further use. The identity of the pathogen was also confirmed using species specific primers. For inoculation, bacterial suspension was prepared in sterile distilled water to get a final concentration of 10⁸ CFU/ml and was immediately used for inoculation following the leaf clipping technique at the crop's maximum tillering stage (Kauffman *et al.*, 1973). In each plant, a minimum of three leaves were inoculated early in the morning by cutting away 1-2 cm of the leaf tip with scissors that were dipped in bacterial suspension on the cutting edge. Individual plants of the BC₁F₂ population and parents were evaluated for field infection of bacterial blight and the disease incidence was scored 15 days after inoculation. Lesion length was

measured and the disease severity was scored 1-9 as per the IRRI standard evaluation system, SES (IRRI, 2002).

Production of advanced generations of BB-resistant lines

For the production of BC₂F₂s and BC₃F₁s, half the total number of panicles in the R-gene-pyramided BC₂F₁ line was self-pollinated and the other half was backcrossed to the recurrent parent, Uma, respectively. Concurrently, all the panicles of the BC₁F₂s exhibiting resistance or moderate resistance to BB pathogen infection during phenotypic screening were self-pollinated to obtain BC₁F₃s. At maturity, the seeds were harvested and dried to 13 per cent moisture to aid prolonged storage.

Statistical analysis

In the above study, the recurrent parent genome contribution (G) in the R gene pyramided lines of BC₂F₁ based on SSR data of background selection was estimated by using the formula:

$$G = [(X + 1/2Y) * 100]/N$$

Where,

N= The total number of parental polymorphic markers screened.

X= The number of markers showing homozygosity for the recurrent parent allele.

Y= The number of markers showing heterozygosity for parental alleles (Sundaram *et al.*, 2008).

The graphical representation of the parental genome contribution using molecular marker data was carried out using Graphical Geno Types (GGT) software version 2.0 (van Berloo, 1999). The software generates a similarity matrix following the method of Sneath and Sokal (1973) and performs clustering based on the default similarity coefficient. A dendrogram is then generated to visualise the relationships among the samples. The variability in morphological characters of the backcross progenies (BC₂F₁s) was assessed through statistical measures, viz., mean and SD. Clustering of BC₂F₁s and parents was also carried out based on morphological traits.

RESULTS AND DISCUSSION

Presently, our study evaluated BC₂F₁ and BC₁F₂, the advanced segregating generations of backcross Uma × ISM, for BB resistance through molecular marker-based genotypic and phenotypic screening approaches, respectively.

Foreground selection in BC₂F₁s

The presence of the R-gene *xa5* in BC₂F₁s was confirmed through PCR amplifications of STS marker RG556 and functional marker *xa5SR*. The analysis revealed a monomorphic banding pattern in all BC₂F₁ plants as well as the parents for both the markers assayed (Fig 2a and 2b). These results indicated the endogenous presence of the recessive resistant allele of the *xa5* gene in homozygous condition (*xa5/xa5*) in both parents and all BC₂F₁s screened. Such an endogenous presence of the *xa5* gene in several genotypes has been reported by earlier workers;

i.e., Singh *et al.* (2015) reported the presence of the *xa5* gene in 15 cultivars out of 35 wild rice accessions evaluated for the presence of BB-resistant genes. Another widely cultivated high-yielding variety in Kerala, Jyothi, has been reported to naturally possess the *xa5* gene, conferring BB resistance (Gorakhanath *et al.*, 2017). It is important to note that, despite carrying the resistant allele of the R-gene *xa5*, Uma remains susceptible to the BB pathogen. This suggests that *xa5*, when deployed singly, does not provide resistance against the BB pathogen isolates prevalent in Kerala. It was reported that *xa5* provides only moderate resistance to the BB pathogen and possesses only a relatively small but consistent residual effect (Li *et al.*, 2001; Sundaram *et al.*, 2008) and also tends to break down over time (Khan *et al.*, 2014; Carpenter *et al.*, 2020). Hence, pyramiding multiple R-genes was recommended to achieve durable resistance against the BB pathogen (Bharathkumar *et al.*, 2008; Yugander *et al.*, 2017).

The introgression of the recessive *xa13* R-gene in BC_2F_1 plants was confirmed using the STS marker RG136 and the functional marker *xa13* promoter. Similar to *xa5*,

xa13 is also a recessive R-gene, but in contrast, *xa13* had no residual effects and showed prominent race specificity (Li *et al.*, 2001). MAS is particularly useful for the selection of recessively inherited R-genes like *xa5* and *xa13* (Sundaram *et al.* 2008; Singh *et al.* 2015). The STS marker pTA248 was used to detect the introgression of the dominant *Xa21* R-gene. Amplification results for both R-genes (*xa13* and *Xa21*) showed that all BC_2F_1 plants, except plant no. 8.3.9.10, exhibited a monomorphic banding pattern identical to the recurrent parent, Uma, for their respective markers screened. However, plant No. 8.3.9.10 displayed bands corresponding to both Uma and ISM, indicating its heterozygous allelic status for both *xa13* (Fig 2c and 2d) and *Xa21* (Fig 2e). Hence, the results of the foreground selection concluded that among the 21 BC_2F_1 plants screened, only plant no. 8.3.9.10 was introgressed with BB resistance genes *xa13* and *Xa21* from the donor parent, ISM. The *xa5* gene was found endogenously present in both parents and was inherited by all BC_2F_1 s. The resistant alleles of the *xa13* and *Xa21* genes were detected only in plant no. 8.3.9.10, where they were introgressed in a heterozygous state (*xa13/Xa13* +

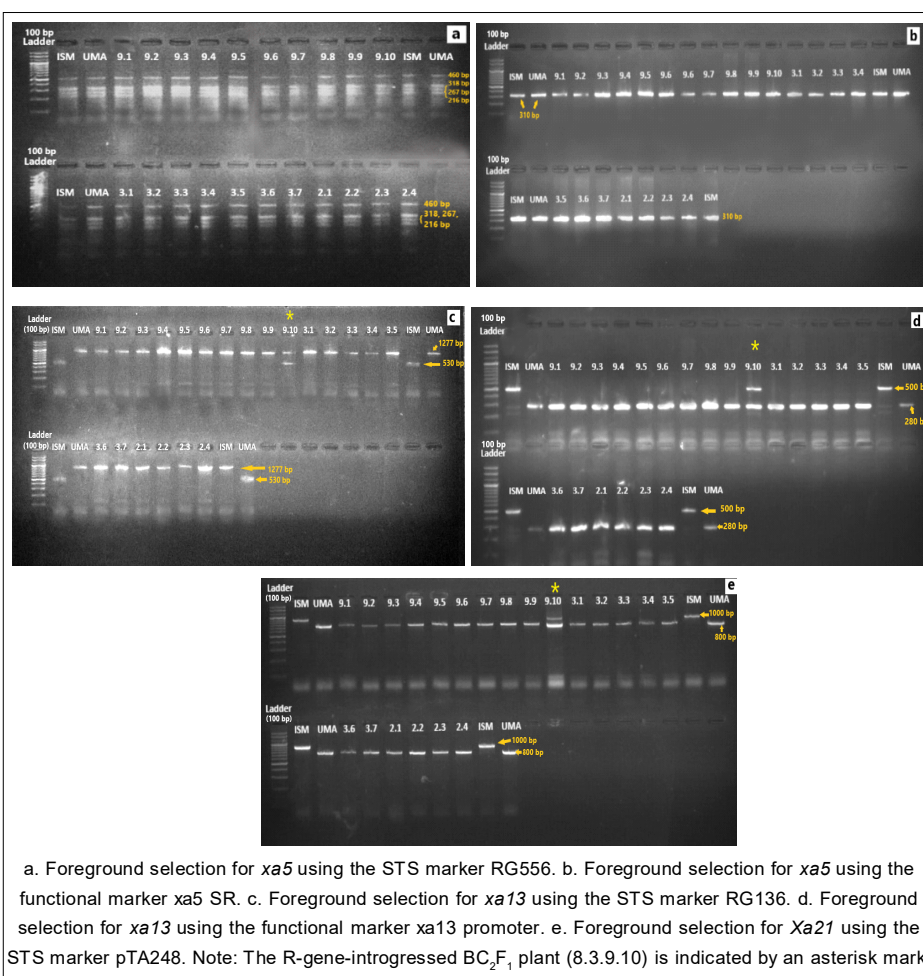


Fig 2: Foreground selection of BC_2F_1 s using molecular markers linked to BB-resistant genes.

xa21/Xa21). The effectiveness of the *Xa21* R-gene in providing broad-spectrum resistance to BB in rice has been well established through numerous studies (Sundaram *et al.*, 2008; Shanti *et al.*, 2010). Lines carrying *Xa21* in combination with *xa5*, *xa13*, or both have confirmed strong resistance, highlighting the effectiveness of *Xa21* in enhancing BB resistance levels in rice (Huang *et al.*, 1997; Sanchez *et al.*, 2000; Singh *et al.*, 2001; Pradhan *et al.*, 2016; Sagar *et al.*, 2018). The broad-spectrum resistance conferred by *Xa21* and *xa13* is attributed to their synergistic effect in combating the BB pathogen. However, in the present study, the alleles of *xa13* and *Xa21* being in a heterozygous condition will hinder the complete resistance expression of the R-gene-pyramided plant. Hence, further advancing the generation (BC_2F_2) was recommended for attaining homozygosity for the alleles concerned.

Background selection for recurrent parent genome recovery

The efficiency of MABB can be greatly enhanced through background selection, facilitating the earlier development and release of BB-resistant cultivars (Joseph *et al.*, 2004). The R-gene introgressed BC_2F_1 plant (8.3.9.10) showed similarity to the recurrent parent Uma at 13 out of 22 microsatellite markers, while it matched the donor parent ISM at four loci and remained heterozygous at five loci. The recurrent parent genome recovery, computed following Sundaram *et al.* (2008), was 70.45%, as against the expected recovery of 87.5%. This lower magnitude of

recurrent parent genome recovery may be attributed to the relatively low recovery (21.80%) observed in the corresponding R-gene introgressed BC_1F_1 (8.3.9) parent, as previously reported by Joseph (2016). Furthermore, given the size of the rice genome (approximately 400–450 Mbp), the use of a relatively small number of markers (only 22) for background selection may have influenced the accuracy of the estimated recurrent parent genome recovery in the R-gene introgressed plant. The genome recovery in the R-gene introgressed BC_2F_1 plant was also represented through Graphical Genotyping Software (GGT) version 2.0 (Fig 3a). Additionally, the dendrogram generated grouped the parents and Plant No. 8.3.9.10 into two clusters, where cluster 1 contained only the donor parent ISM (monogenic), while cluster 2 included the recurrent parent Uma and plant no. 8.3.9.10, reaffirming their similarity (Fig 3b). Therefore, advancing the backcross generation is expected to enhance the recurrent parent genome contribution.

Morphological characterisation of R-gene-pyramided BC_2F_1 and parents

To assess the phenotypic resemblance of the R-gene-pyramided BC_2F_1 to its parents, morphological characterisation was conducted (Table 1). Based on morphological traits, clustering of the BC_2F_1 plants and parents was performed. At an 80% similarity coefficient, they were grouped into 10 major clusters, with the donor parent ISM forming a separate monogenic cluster (Fig 4). The R-gene introgressed BC_2F_1 plant (8.3.9.10) exhibited 45.39%

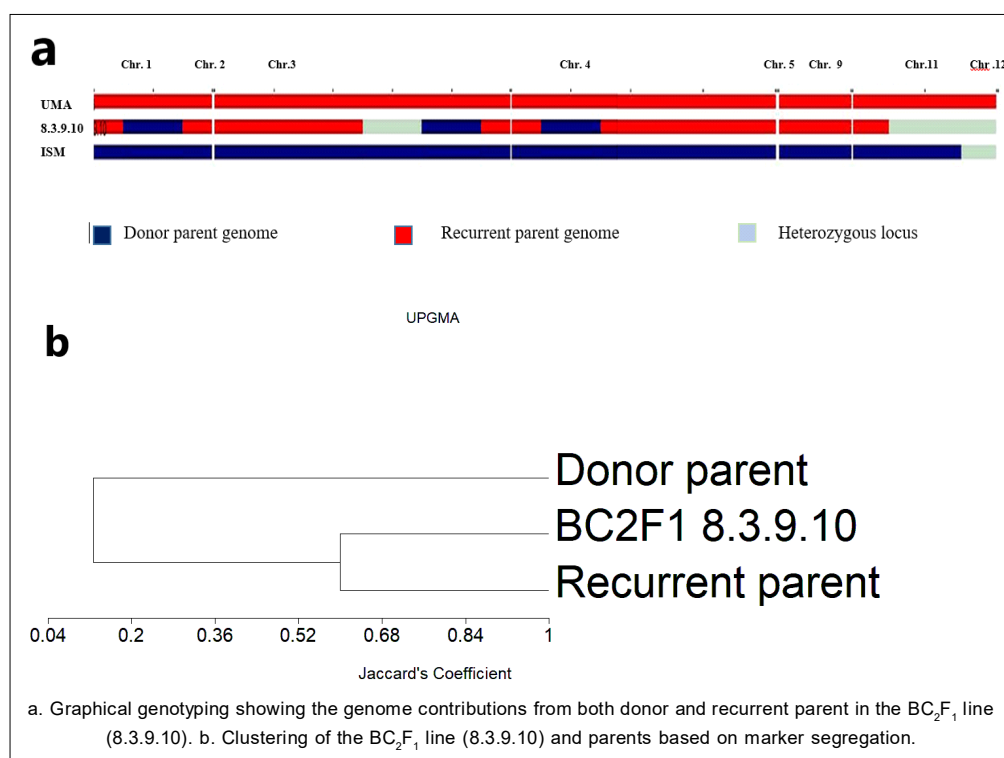


Fig 3: Recovery of the recurrent parent genome in R-gene introgressed BC_2F_1 (8.3.9.10).

Table 1: Morphological characteristics of BC₂F₁s and parents (Uma and ISM).

Plant no.	Days to flowering	Plant height (cm)	Leaf width (cm)	Leaf blade length (cm)	Productive tillers/plant	Panicle length (cm)	Spikelets/panicle	Grain length (mm)	Grain width (mm)	Decorticated grain length (mm)	Decorticated grain width (mm)
8.3.2.1	132	48.9	0.87	30.4	8	13.9	68	7.5	2	5	2
8.3.2.2	132	37.5	0.83	28.7	6	16.73	66	7.5	2	5	1.5
8.3.2.3	124	38	0.7	21.8	6	18.56	69.67	6	2	5	2
8.3.2.4	115	43.6	0.7	22.6	10	16.67	73.67	7	2	5.5	2
8.3.3.1	128	36	0.7	21.3	3	9.6	20	7	2	4	1.5
8.3.3.2	125	37.9	0.7	23.3	4	10.4	33	6	2	4	2
8.3.3.3	128	39.2	0.7	20.5	10	14.37	59.33	7	2	5	2
8.3.3.4	135	43.5	0.7	24.9	7	17.27	81.33	7.5	2	5	2
8.3.3.5	123	50.5	0.9	34.1	8	11.4	69	7	2	4	1.5
8.3.3.6	115	62	0.9	36.9	9	13.8	59	7	2	5	1.5
8.3.3.7	119	51	0.77	32.5	9	18.13	66.33	7	2	5	2
8.3.9.1	155	33	0.77	19.3	5	14.2	22.33	6.5	2	5	2
8.3.9.2	119	43.6	0.7	17.9	5	13.5	47.67	6.5	2	4	1.5
8.3.9.3	138	34.5	0.8	22.2	4	11.97	71.33	6	2	5	2
8.3.9.4	117	54	0.93	29.5	10	16.8	80	7	2	5	2
8.3.9.5	115	42	0.83	24.9	17	15.33	67	6	1.5	5	1.5
8.3.9.6	124	41	0.9	28.3	9	13.53	46.33	6	2	5	2
8.3.9.7	136	35	0.77	20	9	15.2	75.67	6.5	1.5	5	1.5
8.3.9.8	120	59	0.97	31.3	10	17.3	74	6.5	2	5	1.5
8.3.9.9	119	54	1	33	5	17.53	71.33	7	2	4	1.5
8.3.9.10	115	56.1	0.9	28.5	6	13.46	47.33	7	2	6	1.5
BC ₂ F ₁ s Mean±SD	125.4±10.01	44.78±8.68	0.81±0.1	26.28±5.5	7.62±3.12	14.75±2.55	60.4±17.82	6.74±0.52	1.95±0.15	4.83±0.53	1.76±0.26
Uma Mean±SD	120.1±3.9	57.17±3.33	0.94±0.09	31.1±5.2	13.8±1.69	16.37±1.22	85.86±7.82	6.74±0.28	2.81±0.07	5.8±0.19	2.34±0.08
ISM Mean±SD	147.8±2.49	48.62±2.41	0.91±0.05	36.97±3.36	8.3±0.82	21.94±0.76	93.61±4.12	6.49±0.18	1.74±0.13	5.47±0.36	1.45±0.11

similarity to the recurrent parent Uma and 26.86% similarity to the donor parent ISM. Consistent with the molecular clustering pattern, morphological clustering also placed the R-gene introgressed plant closer to the recurrent parent Uma than to the donor parent ISM. The R-gene-introgressed BC₂F₁ plant (8.3.9.10) was early flowering (115 days), showing greater similarity to Uma (120 days) than to ISM (148 days). However, the BC₂F₁ plant (8.3.9.10) exhibited a significant reduction in the number of tillers per plant and spikelets per panicle compared to rice variety Uma, possibly owing to poor adaptation of the backcross progenies arising from linkage drag. Notwithstanding these reductions, the grain length, decorticated grain length and kernel colour of the BC₂F₁ plant (8.3.9.10) closely resembled those of Uma, indicating successful retention of the Matta (Red kernelled rice) rice characteristics preferred by the consumers of Kerala (Fig 5a and 5b). In contrast the grain width and decorticated grain width in the R-gene introgressed line were lower than those of rice variety Uma resulting in comparatively slender grains resembling those of the donor parent ISM. These observations suggest incomplete recovery of the recurrent parent phenotype, thereby necessitating further backcrossing to enhance recurrent parent traits recovery in the R-gene introgressed line.

Integrating agro-morphological evaluation with molecular genotyping is more effective for the rapid recovery of desirable genotypes possessing a higher proportion of the recurrent parent genome (Olalekan *et al.*, 2019; Chukwu *et al.*, 2020).

Bioassay of BC₁F₂s

The BC₁F₂ plants developed in parallel (Joseph 2016) were evaluated for resistance to the BB pathogen using the leaf-clipping method described by Kauffman *et al.* (1973). The BC₂F₁ plants were not subjected to phenotypic screening, as they may be heterozygous at the bacterial blight resistance loci; in particular, since *xa13* confers resistance only in the homozygous recessive state. Phenotyping in this generation may thereby result in loss of plants bearing the desirable allele in the heterozygous state. The recurrent parent, Uma, was used as the susceptible check and the disease severity was scored 1-9 according to the IRRI standard evaluation system (Fig 6a, b and c). The donor parent revealed a score of 1, indicating resistance, while Uma had a score of 9, indicating high susceptibility. The BC₁F₂ plants displayed a higher percentage of resistant (54.72%) and moderately resistant (20.75%) genotypes than of susceptible (3.77%) and highly susceptible (10.38%) genotypes (Table 2). These results indirectly suggest the presence of the appropriate R-gene

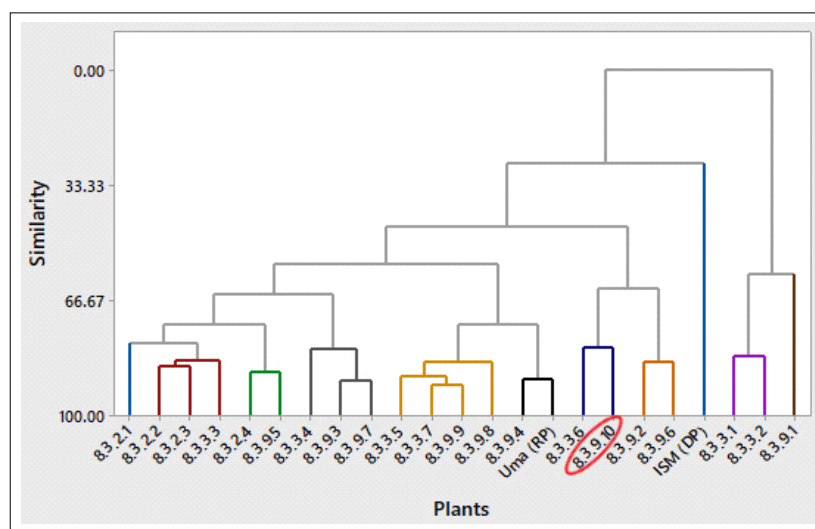


Fig 4: Clustering of BC₂F₁s and parents based on morphological traits.

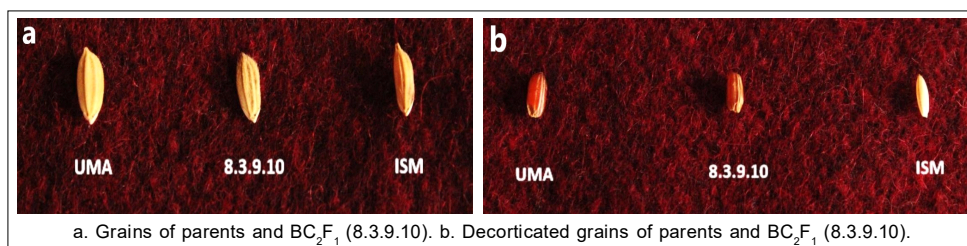


Fig 5: Grain and kernel characteristics of R-gene pyramided BC₂F₁ (8.3.9.10) and parents.

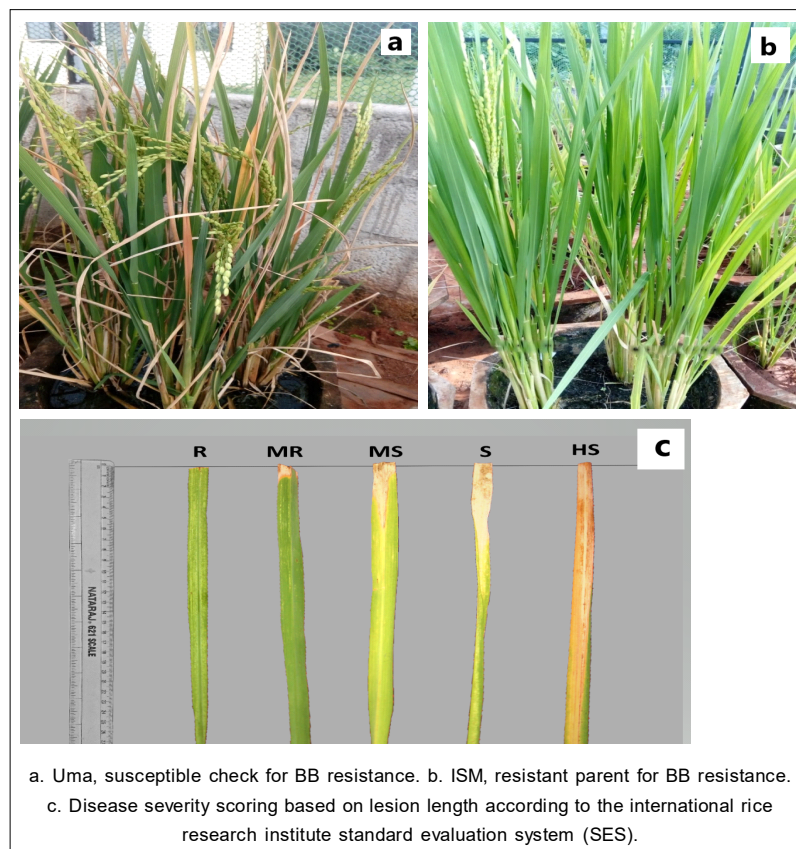


Fig 6: Phenotypic screening for bacterial blight (BB) resistance.

Table 2: Phenotyping screening of BC_1F_2 s and parents for BB infection according to SES (IRRI, 2002).

Diseased leaf area (%)	Scale	Description	Plants screened for BB resistance (%)		
			BC_1F_2 s	ISM	Uma
1-5	1	Resistant (R)	54.72	100.00	0.00
6-12	3	Moderately resistant (MR)	20.75	0.00	0.00
13-25	5	Moderately susceptible (MS)	10.38	0.00	0.00
26-50	7	Susceptible (S)	3.77	0.00	0.00
>50 %	9	Highly susceptible (HS)	10.38	0.00	100.00

combinations (*xa5* + *xa13* + *Xa21*) in the BC_1F_2 individuals that exhibited resistant and moderately resistant reactions to the BB pathogen, in contrast to the recurrent parent Uma. However, further confirmation through molecular assays and phenotypic evaluation in the BC_1F_3 generation is necessary to conclusively determine the specific order and nature of gene combinations responsible for the observed resistance to BB infection.

CONCLUSION

The present study successfully evaluated the backcross generations derived from the cross between rice varieties Uma and ISM through integrated molecular marker-based genotypic screening and phenotypic assessment approaches. Genotypic screening of BC_2F_1 plants for

bacterial blight resistance identified only a single plant (8.3.9.10) carrying the resistance genes *xa13* and *Xa21*. This limited recovery may be attributed to the reduced population size arising from poor germination of the F_1 s, thereby underscoring the need for further evaluation of cross-compatibility between the parental lines. Phenotypic screening for bacterial blight resistance could not be performed in the gene-introgressed BC_2F_1 plant (8.3.9.10) due to the heterozygous status of the resistance loci (*xa13/Xa13* and *xa21/Xa21*), particularly considering the recessive nature of *xa13*, which may impede phenotypic expression of resistance. Furthermore, the identified plant (BC_2F_1 8.3.9.10) exhibited a recurrent parent genome recovery of 70.45%, which was substantially lower than the theoretically expected 87.5% at the BC_2 stage. Therefore, in view of the

heterozygous status of the resistance genes and the comparatively low recovery of the recurrent parent genome, the breeding programme was further advanced to develop BC₂F₂ and BC₃F₁ generations. In addition, the lower marker density employed in the present study may also have contributed to the reduced estimation of recurrent parent genome recovery, highlighting the necessity for screening with a larger set of polymorphic markers for more precise background selection.

In parallel, the BC₁F₂ lines were phenotypically screened for BB resistance using the leaf-clipping method, which revealed a higher proportion of resistant to moderately resistant plants against the BB pathogen. These lines were subsequently advanced to the BC₁F₃ generation. The advanced backcross populations will undergo further evaluation through marker-assisted selection (MAS) and phenotypic screening to identify superior genotypes pyramided with BB resistance genes and exhibiting near-complete recovery of the recurrent parent genome, 'Uma'.

ACKNOWLEDGEMENT

We are grateful for the financial assistance and contributions of the DBT (Department of Biotechnology) project 'Rice-Gene Pyramiding to develop cultivars with durable resistance to Bacterial Leaf Blight through Marker Assisted-Selection' for the commencement of this programme and Tintumol Joseph for providing the experimental material for the conduct of the present study.

Author contributions

Experimental conception and design were done by Rose Mary Francies. Study material preparation, execution, data collection, analysis and result interpretation were performed by L.M. Megha. The draft manuscript was prepared by L.M. Megha and statistical analysis was done by both Rose Mary Francies and L.M. Megha. The manuscript was reviewed and improved by Rose Mary Francies, Jiji Joseph, P.S. Abida and P. Raji. All authors read and approved the final manuscript. Rose Mary Francies, Jiji Joseph, P.S. Abida and P. Raji provided the necessary facilities for the study.

Conflict of interest

The authors declare that they have no relevant financial or non-financial interests to disclose.

REFERENCES

- Bharathkumar, S., Paulraj, R.S.D., Brindha, P.V., Kavitha, S. and Gnanamanickam, S.S. (2008). Improvement of bacterial blight resistance in rice cultivars Jyothi and IR50 via marker-assisted backcross breeding. *Journal of Crop Improvement*. **21**(1): 101-116.
- Carpenter, S.C.D., Mishra, P., Ghoshal, C., Dash, P.K., Wang, L., Midha, S., Laha, G.S., Lore, J.S., Kositratana, W., et al. (2020). An *xa5* resistance gene-breaking indian strain of the rice bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae* is nearly identical to a thai strain. *Frontiers in Microbiology*. **11**: 1-8.
- Chu, Z., Yuan, M., Yao, J., Ge, X., Yuan, B., Xu, C., Li, X., Fu, B., Li, Z., Bennetzen, J.L., Zhang, Q. and Wang, S. (2006). Promoter mutations of an essential gene for pollen development result in disease resistance in rice. *Genes and Development*. **20**(10): 1250.
- Chukwu, S.C., Rafii, M.Y., Ramlee, S.I., Ismail, S.I., Oladosu, Y., Muhammad, I., Musa, I., Ahmed, M., Jatto, M.I. and Yusuf, B.R. (2020). Recovery of recurrent parent genome in a marker assisted backcrossing against rice blast and blight infections using functional markers and SSRs. *Plants*. **9**(11): 1-15.
- Dellaporta, S.L., Wood, J. and Hicks, J.B. (1983). A plant DNA miniprep: Version II. *Plant Molecular Biology Reporter*. **1**(4): 19-21.
- Devika, R., Bai, N.R. and Kumary, S.L. (2004). 'Uma' (MO 16) and 'Revathy' (MO 17): Two promising rice varieties with seed dormancy. *Journal of Tropical Agriculture*. **42**(1-2): 13-16.
- Estelitta, S., Bonny, B.P., Helen, S., Suma, A. (2016). Package of Practices Recommendations/ Crops 2016. 15th ed. Kerala Agricultural University, Thrissur.
- Gorakhanath, K.P., Francies, R.M. and Devidas, P.N. (2017). Pyramiding of bacterial blight resistance genes in rice variety Jyothi (Ptb 39) through marker assisted selection. *Oryza-An International Journal on Rice*. **54**(4): 367.
- Huang, N., Angeles, E.R., Domingo, J., Magpantay, G., Singh, S., Zhang, G., Kumaravadiel, N., Bennett, J. and Khush, G.S. (1997). Pyramiding of bacterial blight resistance genes in rice: Marker-assisted selection using RFLP and PCR. *Theoretical and Applied Genetics*. **95**(3): 313-320.
- IRRI, (International Rice Research Institute). (2002). Standard Evaluation System for Rice. 4th ed. International Rice Research Institute, Manila.
- Jalil, M., Bakhtiar, B., Efendi, E. and Zakaria, S. (2023). Marker-assisted breeding and F₃ progenies characterization for improving local rice variety "Tinggong." *Agricultural Science Digest*. **43**(2): 143-149. doi: 10.18805/ag.DF-496.
- Jerish, J.R., Narayanan, R. and Murugan, S. (2022). Genetic diversity analysis for bacterial leaf blight disease resistance in rice (*Oryza sativa* L.). *Agricultural Science Digest*. **42**(4): 444-448. doi: 10.18805/ag.D-5365.
- Joseph, M., Gopalakrishnan, S., Sharma, R.K., Singh, V.P., Singh, A.K.K., Singh, N. and Mohapatra, T. (2004). Combining bacterial blight resistance and Basmati quality characteristics by phenotypic and molecular marker-assisted selection in rice. *Molecular Breeding*. **13**(4): 377-387.
- Joseph, T. (2016). Gene Pyramiding for Bacterial Blight Resistance in Rice Variety Uma (Mo 16). M.Sc. Thesis, Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara.
- Kanipriya, R., Ramanathan, A., Gopalakrishnan, C., Ramalingam, J. and Saraswathi, R. (2024). Pathotyping and virulence analysis of *Xanthomonas oryzae* pv. *oryzae* causing bacterial blight of rice in Tamil Nadu. *Agricultural Science Digest*. **44**(2): 282-288. doi: 10.18805/ag.D-5828.
- Kauffman, H.E., Reddy, A.P.K., Hsieh, S.P.Y. and Merca, S.D. (1973). An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. *Plant Disease Reporter*. **57**(6): 537-541.

- Khan, M.A., Naeem, M. and Iqbal, M. (2014). Breeding approaches for bacterial leaf blight resistance in rice (*Oryza sativa* L.), current status and future directions. *European Journal of Plant Pathology*. **139**(1): 27-37.
- Laha, G.S., Prasad, M.S., Krishnaveni, D., Kannan, C., Ladhakshmi, D., Prakasam, V., Basavaraj, K. and Jasudasu, G.S. (2023). Production Oriented Survey 2023, All India Coordinated Research Project on Rice, ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad.
- Li, Z.K., Sanchez, A., Angeles, E., Singh, S., Domingo, J., Huang, N. and Khush, G.S. (2001). Are the dominant and recessive plant disease resistance genes similar?: A case study of rice R genes and *Xanthomonas oryzae* pv. *oryzae* races. *Genetics*. **159**(2): 757-765.
- Mew, T.W. (1992). Changes in race frequency of *Xanthomonas oryzae* pv. *oryzae* in response to rice cultivars planted in the philippines. *Plant Disease*. **76**(10): 1029-1032.
- Olalekan, K.K., Rafii, M.Y., Salleh, A.M., Mohamed, M.T.M., Ahmad, K., Misran, A., Abro, T.F., Oladosu, Y., Arolu, I.W., Samuel, C. and Usman, M. (2019). Analysis of recurrent parent genome recovery in marker-assisted backcross breeding program in watermelon. *International Journal of Scientific and Technology Research*. **8**(8): 945-955.
- Pandey, M.P., Singh, H. and Mani, S.C. (1986). Breakdown of *Xa4* gene for resistance to bacterial blight (BB) at Pantnagar, India. *International Rice Research Newsletter*. **11**: 19-20.
- Pradhan, S.K., Nayak, D.K., Pandit, E., Behera, L., Anandan, A., Mukherjee, A.K., Lenka, S. and Barik, D.P. (2016). Incorporation of bacterial blight resistance genes into lowland rice cultivar through marker-assisted backcross breeding. *Phytopathology*. **106**(7): 710-718.
- Priyadarisini, V.B. and Gnanamanickam, S.S. (1999). Occurrence of a subpopulation of *Xanthomonas oryzae* pv. *oryzae* with virulence to rice Cv. IRBB21 (*Xa21*) in Southern India. *Plant Disease*. **83**(8): 781-781.
- Sagar, V., Krishnan, S.G., Mondal, K.K., Prakash, G., Nagarajan, M. and Singh, A.K. (2018). Development of basmati rice genotypes with resistance to both bacterial blight and blast diseases using marker assisted restricted backcross breeding. *Indian Journal of Genetics and Plant Breeding*. **78**(1): 36-47.
- Sanchez, A.C., Brar, D.S., Huang, N., Li, Z. and Khush, G.S. (2000). Sequence tagged site marker-assisted selection for three bacterial blight resistance genes in rice. *Crop Science*. **40**(3): 792-797.
- Shanti, M.L., Kumar Varm, C.M., Premalatha, P., Devi, G.L., Zehr, U. and Freeman, W. (2010). Understanding the bacterial blight pathogen-combining pathotyping and molecular marker studies. *International Journal of Plant Pathology*. **1**(2): 58-68.
- Singh, A.K., Dharmraj, E., Nayak, R., Singh, P.K. and Singh, N.K. (2015). Identification of bacterial leaf blight resistance genes in wild rice of eastern India. *Turkish Journal of Botany*. **39**(6): 1060-1066.
- Singh, S., Sidhu, J.S., Huang, N., Vikal, Y., Li, Z., Brar, D.S., Dhaliwal, H.S. and Khush, G.S. (2001). Pyramiding three bacterial blight resistance genes (*xa5*, *xa13* and *Xa21*) using marker-assisted selection into indica rice cultivar PR106. *Theoretical and Applied Genetics*. **102**(6-7): 1011-1015.
- Sneath, P.H.A. and Sokal, R.R. (1973). Numerical Taxonomy: The Principles and Practice of Numerical Classification. WF Freeman and Co., San Francisco.
- Sundaram, R.M., Vishnupriya, M.R., Biradar, S.K., Laha, G.S., Reddy, G.A., Rani, N.S., Sarma, N.P. and Sonti, R.V. (2008). Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite indica rice variety. *Euphytica*. **160**(3): 411-422.
- van Berloo, R. (1999). Computer note. GGT: software for the display of graphical genotypes. *Journal of Heredity*. **90**(2): 328-329.
- Yugander, A., Sundaram, R.M., Ladhakshmi, D., Hajira, S.K., Prakasam, V., Prasad, M.S., Sheshu, M.M., Ravindra, B.V. and Laha, G.S. (2017). Virulence profiling of *Xanthomonas oryzae* pv. *oryzae* isolates, causing bacterial blight of rice in India. *European Journal of Plant Pathology*. **149**(1): 171-191.