ORIGINAL ARTICLE

Improvement of bacterial blight resistance of the high yielding, fine-grain, rice variety, Gangavati sona through marker-assisted backcross breeding

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Abstract

Gangavati sona (GS) is a high-yielding, fine-grain rice variety widely grown in the Tungabhadra command area in Karnataka, India; however, it is susceptible to bacterial blight (BB). Therefore, the present study was conducted to improve the GS variety for BB resistance. Three BB-resistant genes (*xa5, xa13,* and *Xa21*) were introgressed into the genetic background of susceptible cultivar GS through marker-assisted backcrossing (MABB) by using Improved samba Mahsuri (ISM), a popular, high-yielding, bacterial blight resistant rice variety as a donor parent. Foreground selection was carried out using gene-specific markers, viz., xa5FM (*xa5*), xa13prom (*xa13*), and pTA248 (*Xa21*), while background selection was carried out using well-distributed 64 polymorphic microsatellite markers. The true heterozygote F_1 was used as the male parent for backcrossing with GS to obtain BC_1F_1. The process was repeated in BC_1F_1 generation, and a BC_2F_1 plant (IGS-5-11-5) possessing all three target genes along with maximum recurrent parent genome (RPG) recovery (86.7%) was selfed to obtain BC_2F_2s. At BC_2F_2, a single triple gene homozygote plant (IGS-5-11-5-33) with 92.6% RPG recovery was identified and advanced to BC_2F_5 by a pedigree method. At BC_2F_5, the seven best entries were selected, possessing all three resistance genes with high resistance levels against bacterial blight, yield level, and grain quality features equivalent to better than GS. The improved versions of GS will immensely benefit the farmers whose fields are endemic to BB.

Keywords Rice · Disease · Genomics · Bacterial blight · Markers · Backcrossing

Introduction

Globally, rice is one of the most important staple food crops and is a primary food source for more than half of the world's population. It provides 21% of the energy and 15% of the protein requirements of human beings (Kennedy et al. 2002). Estimates have projected that rice production must be increased by 0.6–0.9% annually by 2050 to meet the food demand of the anticipated 9.8 billion people (Carriger and Vallee 2007). Nevertheless, rice has witnessed yield plateauing in the last two decades due to several production constraints and biotic and abiotic stress factors (Peng et al. 2004). Among the biotic stresses that affect rice crops bacterial blight (BB) disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) (Swings et al. 1990) is the most destructive one, which has a major impact on yield loss ranging from 50 to 90% (Ou 1985; Sere et al. 2005). The disease occurs in the host plant at the seedling, vegetative, and reproductive stages, but infection at the tillering stage causes severe blighting of leaves, resulting in yield loss (Shivalingaiah 2011). Chemical control of this disease is complicated and limited due to the concerns

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over health hazards (Guvvala et al. 2013). Managing the disease using host plant resistance is the most effective, environmentally safe, and economical option (Khush et al. 1989). Therefore, the preferred bacterial blight disease management strategy is developing resistant rice varieties by deploying resistance genes.

Globally, more than 45 BB resistance genes have been identified from diverse sources, which offer resistance to various strains of *Xoo* (Neelam et al. 2020). Many of these resistance genes have been tagged by closely linked molecular markers (Sonti 1998; Rao et al. 2002). Through conventional breeding, a few of these genes, including *Xa4*, have been integrated into numerous high-yielding varieties (Khush et al. 1989). However, the widespread cultivation of these varieties has led to the prevalence of isolates that overcome *Xa4* (Mew et al. 1992). Therefore, deploying multiple BB resistance genes into rice cultivars results in a broad spectrum and more durable level of resistance than varieties with only a single BB resistance gene.

In the Tungabhadra command area of Karnataka state, India, rice is grown extensively in 3.5 lakhs ha. In the northern districts of Karnataka, rice is grown both in *Kharif* and the summer seasons by utilizing the water resources of the Tungabhadra River. Since extensive rice cultivation is carried out in these areas, this region is known as the "Rice Bowl" of Karnataka state. Gangavati sona (GS) is a major rice variety cultivated in these regions. GS is known for its high yield (*Kharif*: 6.5–7.0 t/ha and summer: 7.0–7.5 t/ha), excellent cooking and eating qualities, and suitability to grow in wet and dry seasons without compromising yield levels. Despite its popularity in most rice-growing regions, farmers are facing the problem of moderate to severe BB infection in GS, which leads to significant yield loss, and the Tungabhadra command area is known to be endemic for BB disease.

Previously, Joseph et al. (2004) introduced *xa13* and *Xa21* into the popular basmati rice variety Pusa Basmati1 and released them as Improved Pusa Basmati1 in India for commercial cultivation. Similarly, Sundaram et al. (2008) incorporated three major BB resistance genes (*xa5*, *xa13* and *Xa21*) in the background of a highly popular rice variety, Samba Mahsuri. The "Improved Samba Mahsuri" (ISM) possesses high grain yield and elite grain quality traits of Samba Mahsuri with a high resistance level against BB disease. Therefore, ISM was released for commercial cultivation across India in Samba Mahsuri growing areas in 2008. With this background, in the present study, we employed a marker-assisted backcross breeding (MABB) strategy to incorporate three major BB resistance genes, viz., *xa5, xa13,* and *Xa21,* into the genetic background of Gangavati sona intending to increase the durability and spectrum of resistance of this highly preferred, premium quality rice variety.

Materials and methods

Plant materials

Gangavati sona (GS), a high-yielding, medium, slender, fine grain, BB susceptible, elite rice variety developed and released for commercial cultivation in the year 2012 by Agricultural Research Station, Gangavathi of University of Agricultural Sciences, Raichur, Karnataka, was used as a recurrent parent (RP). Improved Samba Mahsuri (ISM; also known as RPBio-226), a high yielding, medium slender fine grain, low glycemic index (50.99), BB resistant possessing three major BB resistance genes, viz., *xa5*, *xa13* and *Xa21* (Sundaram et al. 2008), developed from the cross SS1113/Samba Mahsuri through marker-assisted backcross breeding (MABB) developed by ICAR-Indian Institute of Rice Research (ICAR-IIRR) Hyderabad in association with CSIR-Centre for Cellular and Molecular Biology (CSIR-CCMB), Hyderabad, India and currently occupied more than 6,00,000 hectares was used as the donor parent (DP) for bacterial blight resistance.

Development and selection of improved BB-resistant lines through the MABB strategy

The recurrent parent GS was crossed with Improved Samba Mahsuri, and the F_1s were analyzed for their heterozygosity (i.e. true F_1s) using the target resistance gene-specific markers, namely, xa5FM specific for *xa5*, xa13prom specific for *xa13* (Hajira et al. 2016) and pTA248 specific for *Xa21* (Ronald et al. 1992) (Fig. 1). True F_1s thus identified were then backcrossed to the recurrent parent, GS, to generate BC_1F_1s. Then, they were subjected to foreground selection using gene-specific markers, and plants that are triple gene heterozygous were subjected to background selection to identify a single plant with maximum recurrent parent genome (RPG) recovery using a set of 64 SSR parental polymorphic markers, which are evenly distributed across the 12 rice chromosomes (Table 1). The extent of recurrent parent genome (RPG) recovery among the selected backcross-derived plants was assessed utilizing the software tool Graphical Genotype V 2.0 (Van Berloo 1999).

A single positive BC_1F_1 plant with maximum RPG recovery was selected and backcrossed with GS to generate BC_2F_1s, and the process of marker-assisted backcrossing was repeated as mentioned above, and a single BC_2F_1 plant with all the three target resistance genes with maximum RPG recovery was selfed to develop BC_2F_2s. Finally, a BC_2F_2 plant possessing *xa5*, *xa13,* and *Xa21* genes in homozygous condition along with maximum
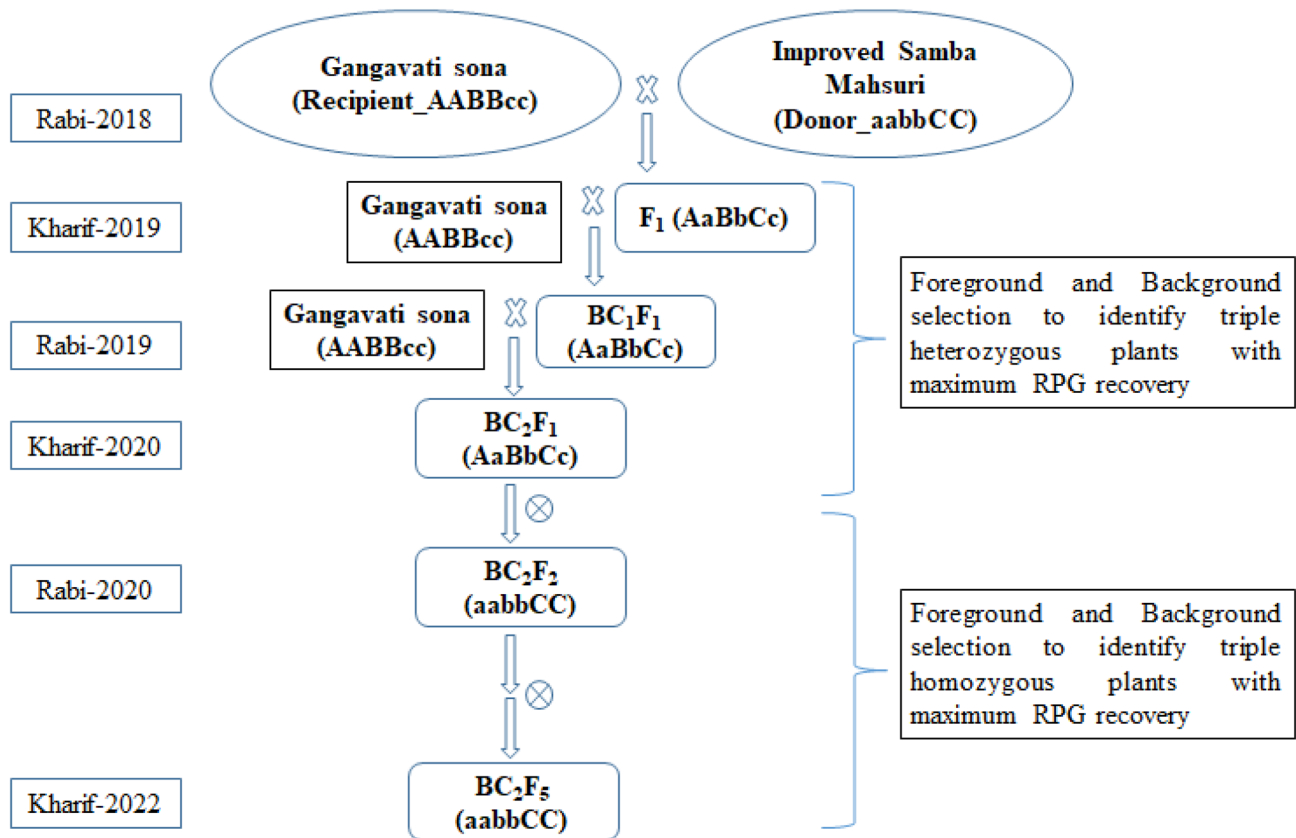
Fig. 1 Outline of the marker-assisted backcross breeding scheme to develop BB resistance in Gangavati sona

Table 1 Microsatellite markers polymorphic between Gangavati sona (recurrent parent) and Improved Samba Mahsuri (donor parent)

S.N.	Chromosome no.	Total no. of markers analysed	No. of polymorphic markers	Polymorphic markers
1	1	65	8	RM 10009, 4554, 10167, 10078, 6324, 4959, 10115, 10695
2	2	39	5	RM 13155, 14140, 13131, 13045, RMES2-1
3	3	38	5	RM 14735, 15630, 15004, 15404, HRM15679
4	4	94	8	RM 16606, 17345, 17377, 16868, 3524, 16373, 16855, 6997
5	5	61	5	RM 169, 18516, 592, 17920, 19147
6	6	75	5	RM 19367, 19410, 1369, 589, 20583
7	7	106	4	RM 6697, 3583, 20923), chr7-1.2
8	8	88	3	RM 6925, 5933, 22565
9	9	50	3	3RM 3769, 3808), RMES9-2
10	10	45	3	RM 5271, chr10-22.1, ESSR 10–22.7
11	11	78	8	RM 21, 26352, 27369, 26558, 26868), chr11-8.9, chr11-25.5, chr11-28.1
12	12	50	7	RM 2877, 28481, 28441, RMS 10060, SSR12-7.4, JGT12-20.24, ESSR12-20.2
Total no. of markers		789	64	

RPG and closely resembling GS based on morphological features along with a high level of resistance against BB was identified and advanced through the pedigree method of breeding to BC_2F_5. The BC_2F_5 lines were also further evaluated for BB resistance under controlled conditions and for grain and cooking quality, key agro morphological traits, including yield parameters under field conditions.

The background genome recovery '*G*' was calculated using the below formula (Sundaram et al. 2008).

$$G = \frac{\left[\left(X + \frac{1}{2}Y\right) * 100\right]}{N},$$

where N = total number of parental polymorphic markers screened, X = number of markers showing homozygosity for recurrent parent allele, Y = number of markers showing heterozygosity for parental alleles.

Phenotypic screening for bacterial blight

The seven pyramided breeding lines of GS at BC_2F_5 generation and parents were challenged with BB pathogen. IXo-20, a virulent isolate of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) (collected from Telangana) during *Kharif* 2022 in the experimental field of ICAR-IIRR farm located at Rajendranagar, Hyderabad, India, to assess their resistance against BB. The pathogen was multiplied on modified Wakimoto's agar media at 28^0C for 72–96 h, harvested after incubation, and diluted with sterile distilled water to get a final concentration of 10^8 cfu/ml. Inoculation was done at the maximum tillering stage following the leaf clip method of Kauffman et al. (1973) by clipping the leaf tip (about 1 to 2 cm) of the uppermost leaves with a sterilized scissor dipped in the bacterial suspension. Disease reactions were recorded 15 days post-inoculation by measuring the lesion length and following the SES scale (standard evaluation system for rice) (IRRI 2013) for resistance/susceptibility.

Evaluation of improved GS lines for agro-morphological traits

Seven improved backcross derived lines of BC_2F_5 generation, possessing BB-resistant genes in the background of GS along with recurrent parent and donor parent, were space planted in the main fields during the *wet season* of 2021–22 of ICAR-IIRR farm, Hyderabad, India, at a spacing of 15×20 cm in four replicates. The field was maintained with the recommended dose of fertilizers (120:60:60 kg NPK per hectare). Phenotypic data of the selected plants for key agro-morphological traits were collected, including days to 50% flowering (DFF), plant height (cm), number of productive tillers per plant, panicle length (cm), number of grains per panicle, grain yield per plant (g), 1000-grain weight (g), *L/B* ratio, grain type and type of panicle exertion among ten plants for each replication ($n = 4$) as explained in Abhilash et al. (2016). The data were statistically analyzed for genetic parameters, and the Coefficient of variation (CV) was calculated using standard errors of the mean (S.Em. $\pm$) as per the procedure described by Freeman (1973). An analysis of variance (ANOVA) was performed using the R Studio version 4.3.1 to determine the variation among the improved breeding lines of GS.

Results

Introduction of *xa5*, *xa13* and *Xa21* genes into Gangavati sona background

The male parent (ISM) and the female parent (Gangavati sona) were sown staggered to match the flowering period for crossing to obtain F_1 seeds. The 46 F_1 plants were inoculated with bacterial culture and scored for their disease reactions. The F_1 plants had disease lesion lengths ranging from 0.8 to 1.2 cm, indicating a resistant nature (Fig. 2). A total of 36 F_1 plants were found to be triple heterozygous (i.e. true F_1s) with respect to the three targets BB-resistant genes (Table 2). Among 142 BC_1F_1s screened, 13 plants were triple heterozygous for target-resistant genes (i.e. *xa5*, *xa13*, and *Xa21*). These plants were analyzed through background selection to assess the RPG recovery using a set of 64 parental polymorphic markers (Table 1). The plant (IGS-5-11) with the highest RPG of 74.2% was identified and used as a pollen donor to produce BC_2F_1s. A total of 137 BC_2F_1s were raised and subjected to foreground selection employing gene-specific markers to identify true heterozygous plants. Eight out of 137 plants were confirmed to possess all three target genes, and they were then subjected to background selection using parental polymorphic markers. A single plant (IGS-5-11-5) with maximum RPG recovery (86.7%) was identified (Fig. 3). This plant was selfed to produce BC_2F_2 plants. A total of 286 BC_2F_2 plants were raised and inoculated with bacterial culture in the field for phenotypic screening with a local isolate of *Xoo*, IX0-20 at the maximum tillering stage. The disease lesion lengths varied from 0.9 to 2.2 cm. The phenotypically resistant plants were subjected to marker analysis with the help of gene-specific markers. Of the 286 plants, a total of 4 plants (1 out of 71) were identified as triple homozygous for all three target genes.

Further, background selection was performed using the parental polymorphic SSR markers to identify a single plant with maximum RPG. The Plant, IGS-5-11-5-33 (Fig. 4), had the highest RPG recovery (92.6%). It was then advanced through the pedigree method till the BC_2F_5 generation. Seven promising BC_2F_5 lines similar to GS, i.e. IGS-5-11-5-33-24-06-06, IGS-5-11-5-33-24-06-24, IGS-5-11-5-33-24-06-30, IGS-5-11-5-33-24-06-91, IGS-5-11-5-33-24-06-93, IGS-5-11-5-33-24-06-184, and IGS-5-11-5-33-24-06-214 were selected and evaluated for BB resistance, agro morphological traits, and yield analysis.

Evaluation of pyramided lines for BB resistance

The pyramided lines were evaluated for BB reaction under field conditions using IXo-20 strain (Table 3). The donor
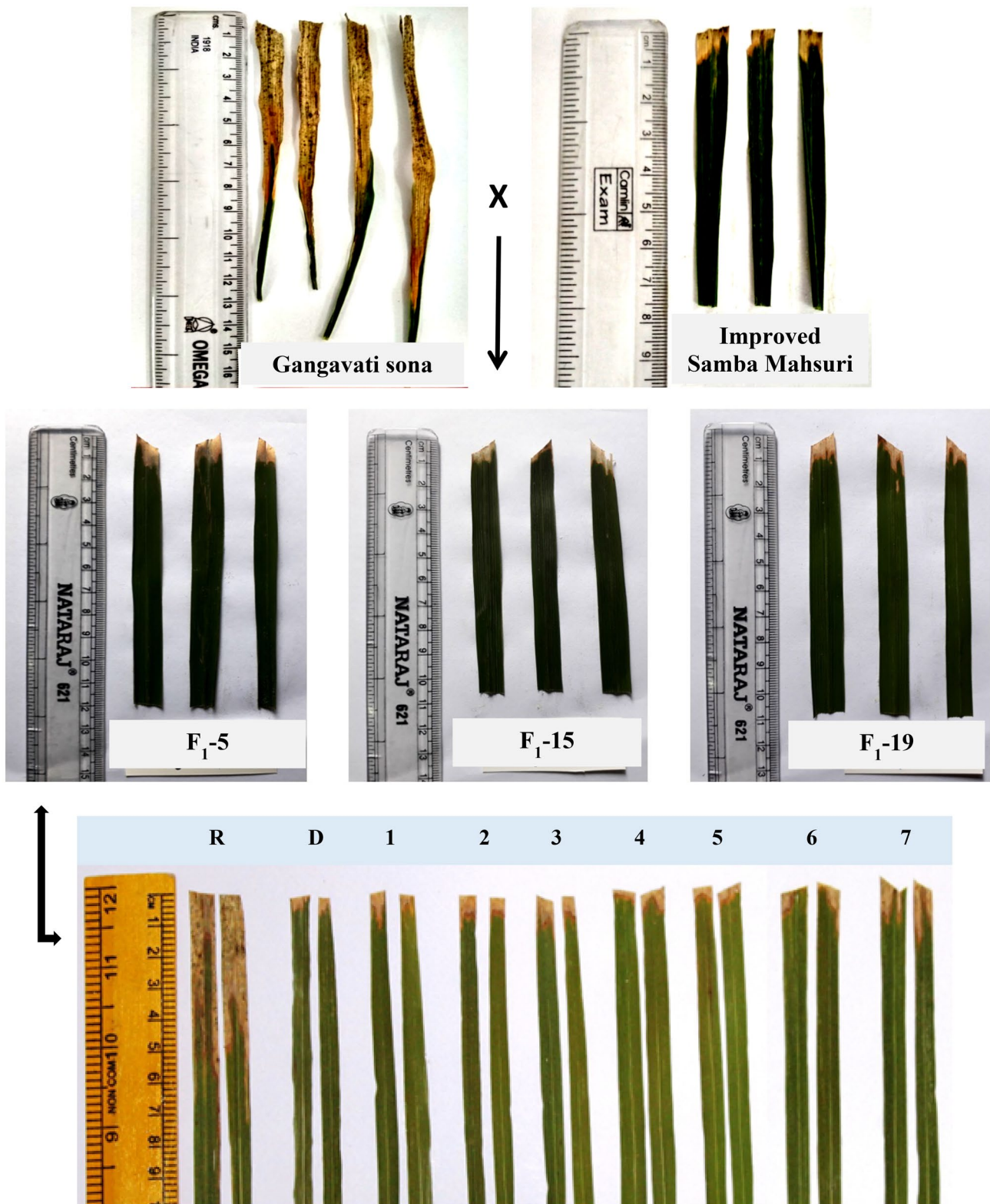
Fig. 2 Disease reaction of parents, F_1's and derived BC_2F_5 for *Xoo* strain. R: Recurrent Parent; D: Donor Parent; 1–7: Derived BC_2F_5 plants (1: IGS-5-11-5-33-24-06-06, 2: IGS-5-11-5-33-24-06-24, 3: IGS-5-11-5-33-24-06-30, 4: IGS-5-11-5-33-24-06-30, 5: IGS-5-11-5-33-24-06-93, 6: IGS-5-11-5-33-24-06-184, **7**-IGS-5-11-5-33-24-06-214)

Table 2 Number of plants confirmed for foreground and background selection in each backcross population

| S.N.	Generation	Total no. of plants analysed	Total No. of plants with all three genes	% Recurrent parent genome (RPG) recovery	Plant with maximum RPG%
1	F_1	46	36	–	
2	BC_1F_1	142	13	74.2%	IGS-5-11
3	BC_2F_1	137	8	86.7%	IGS-5-11-5
4	BC_2F_2	286	4	92.6%	IGS-5-11-5-33
5	BC_2F_5			98.3%	IGS-5-11-5-33-24-06-06
					IGS-5-11-5-33-24-06-24
					IGS-5-11-5-33-24-06-30
					IGS-5-11-5-33-24-06-91
					IGS-5-11-5-33-24-06-93
					IGS-5-11-5-33-24-06-184
					IGS-5-11-5-33-24-06-214
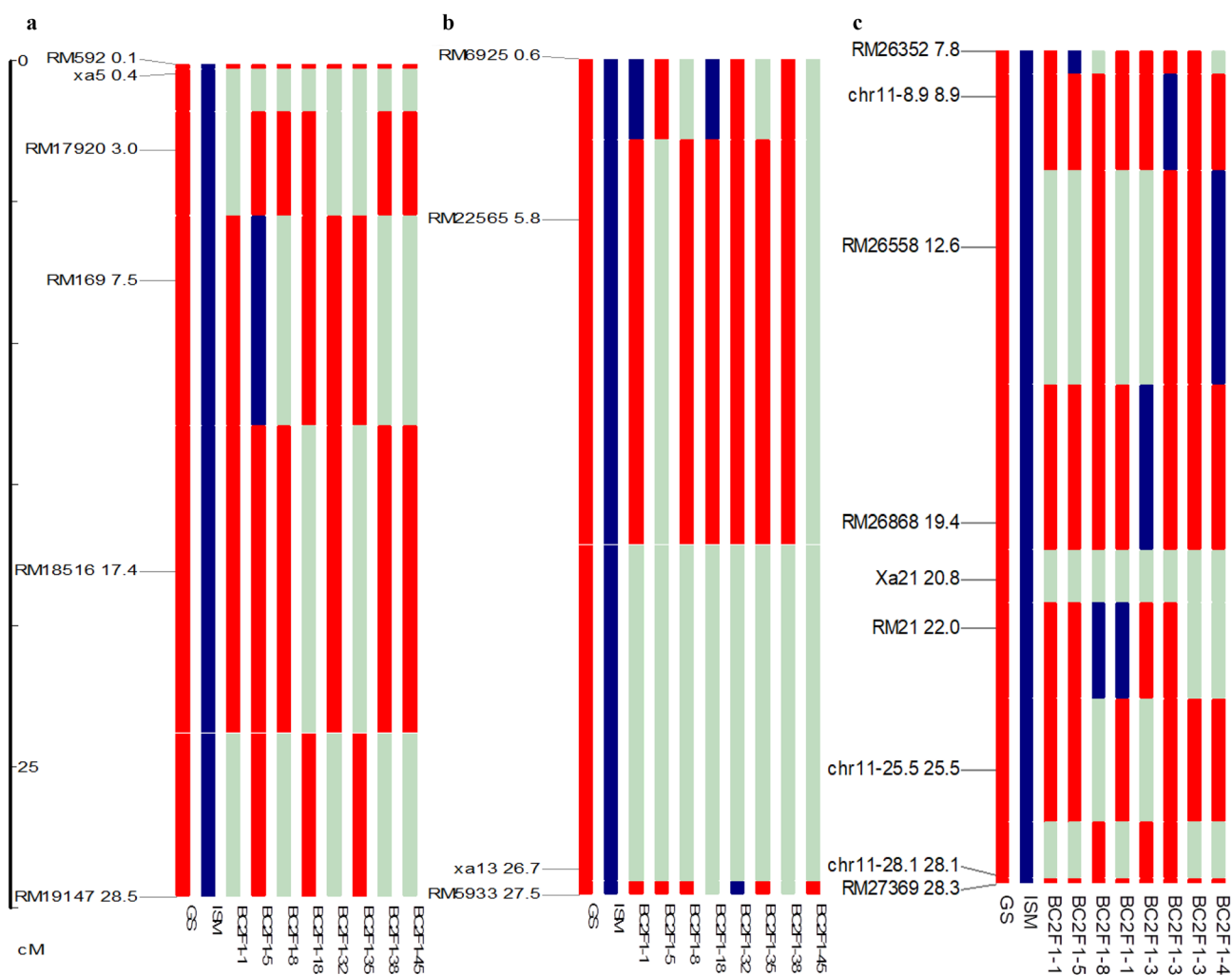
Fig. 3 Graphical representation of genome recovery in selected BC_2F_1 population through graphical genotype analysis. **a** Extent of background genome recovery in the genomic regions in the vicinity of *xa5* on chromosome 5. **b** Extent of background genome recovery in the genomic regions in the vicinity of *xa13* on chromosome 8. **c** Extent of background genome recovery in the genomic region in the vicinity of *Xa21* on Chromosome 11
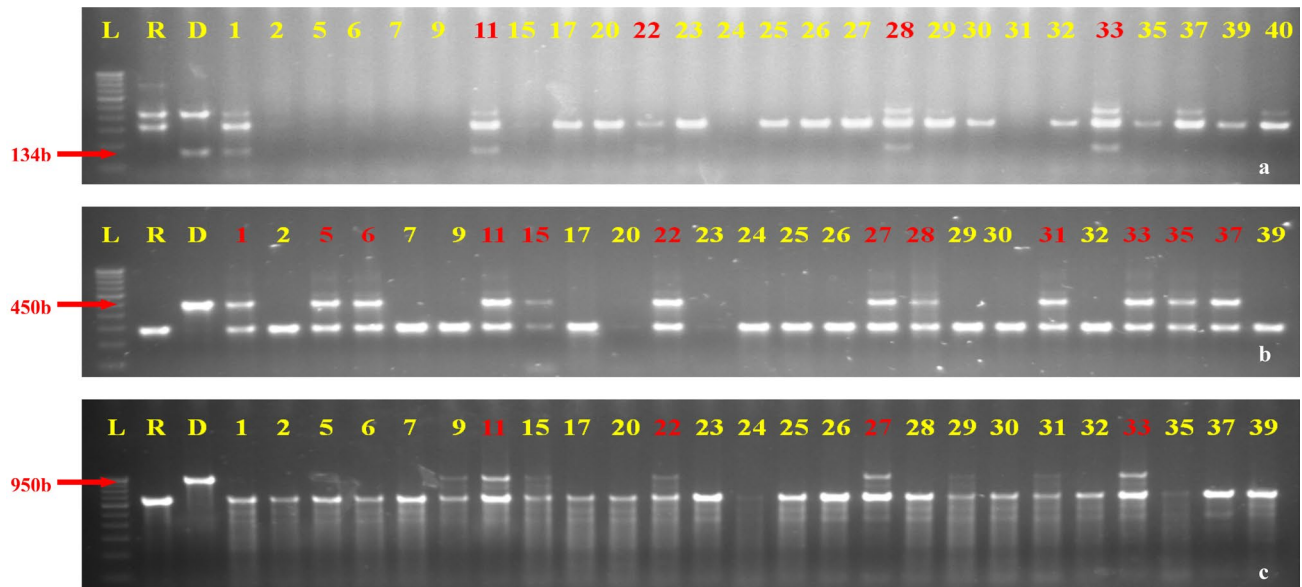
Fig. 4 Identification of homozygous BC$_2$F$_2$ (IGS-5-11-5-33) plants possessing *xa5* + *xa13* + *Xa21*. **a** Analysis of *xa5* gene using xa5FM marker **b** Analysis of *xa13* gene using xa13prom marker. **c** Analysis of *Xa21* gene using pTA248 marker. *L* Ladder, *R* recurrent parent GS; *D*, donor parent ISM; 1-39, 40 test samples

Table 3 Disease reaction of improved pyramided lines of GS with bacterial blight pathogen

S.N.	Entry	Reactions against BB (IX0-20)	
		Score (cm)	Reaction
1	GS	8.0 ± 0.39	S
2	ISM	0.5 ± 0.13	R
3	IGS-5-11-5-33-24-06-06	1.1 ± 0.21	R
4	IGS-5-11-5-33-24-06-24	1.7 ± 0.79	R
5	IGS-5-11-5-33-24-06-30	1.2 ± 0.08	R
6	IGS-5-11-5-33-24-06-91	1.4 ± 0.19	R
7	IGS-5-11-5-33-24-06-93	1.2 ± 0.11	R
8	IGS-5-11-5-33-24-06-184	1.3 ± 0.18	R
9	IGS-5-11-5-33-24-06-214	1.3 ± 0.22	R

S susceptible, *R* resistant

parent ISM was highly resistant to BB with a disease lesion length of 0.5 ± 0.13 cm, and the recurrent parent, GS, was highly susceptible, with a disease lesion length of 8.0 ± 0.39 cm. All seven pyramided BC$_2$F$_5$ lines were witnessed to be resistant to BB, with average lesion lengths ranging from 1.1 to 1.7 cm.

Assessment of pyramid lines for yield and agro-morphological characters

Among the seven BC$_2$F$_5$ pyramided lines, the IGS-5-11-5-33-24-06-30 exhibited significantly better performance over the RP, and GS concerning yield and other agro-morphological traits (Table 4). IGS-5-11-5-33-24-06-06 was early (93.4 days to 50% flowering) compared to others, and GS and also exhibited the highest value for plant height (101.2 cm). IGS-5-11-5-33-24-06-214 also produced more productive tillers per plant (12.1). All the pyramided lines were found on par or better than both parents in terms of panicle length, number of grains per panicle, grain yield per plant, and 1000-grain weight. Further, all the improved lines had an LB ratio equivalent to GS (~ 3.8), and all the lines had full exerted panicles similar to the recurrent parent.

Estimation of genetic variability parameters

Genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) estimates (Table 4) were found to be lower (1.04–8.02%) for traits days to 50% flowering, plant height, panicle length, number of grains per panicle, grain yield per plant, 1000 seed weight, L/B ratio and were moderate (18.27–20.63%) for number of productive tillers per plant revealed the presence of variability for the traits under study. Similarly, estimates for heritability in a broad sense were high (78.41–94.59%) for all the traits except for the trait L/B ratio (moderate-60%). The genetic advance as a per cent of mean (GAM) was recorded to be lower (1.66–9.78%) for days to 50% flowering, plant height, panicle length, number of grains per panicle, 1000 seed weight, L/B ratio; moderate (14.55%) for grain yield per plant and was high (33.35%) for number of productive tillers per plant.

Table 4 Performance of parents and improved pyramided lines for agro-morphological traits

S.N.	Plant identity	Days to 50% flowering (DFF)	Plant height (cm)	No. of productive tillers/plant	Panicle length (cm)	Number of grains per panicle	Grain yield per plant (g)	1000 seed weight (g)	L/B ratio	Grain type	Panicle exertion
1	GS	95.3±0.50	98.4±0.62	8.2±0.11	22.6±0.13	254.8±6.65	22.0±0.39	14.2±0.15	3.8±0.05	MS	FE
2	ISM	104.8±1.26	92.9±0.50	8.2±0.11	21.7±0.34	224.5±5.32	18.0±0.29	12.4±0.30	3.7±0.07	MS	PE
3	IGS-5-11-5-33-24-06-06	93.9±0.75	98.1±0.88	8.2±0.28	22.5±0.08	260.9±2.81	22.3±0.55	14.0±0.27	3.7±0.01	MS	FE
4	IGS-5-11-5-33-24-06-24	94.6±0.25	97.0±2.37	8.5±0.59	22.8±0.16	264.8±3.52	22.7±0.61	14.1±0.18	3.7±0.02	MS	FE
5	IGS-5-11-5-33-24-06-30	97.4±0.25	97.8±1.87	7.8±0.41	22.7±0.14	253.5±0.82	23.3±0.78	14.0±0.04	3.8±0.01	MS	FE
6	IGS-5-11-5-33-24-06-91	95.3±0.65	93.1±0.80	11.6±0.99	22.6±0.21	266.8±2.18	23.1±1.30	13.9±0.21	3.7±0.04	MS	FE
7	IGS-5-11-5-33-24-06-93	95.5±1.22	95.4±0.87	11.6±1.71	22.6±0.16	252.9±1.60	22.5±0.34	14.0±0.05	3.7±0.01	MS	FE
8	IGS-5-11-5-33-24-06-184	99.8±1.04	101.2±1.28	11.0±1.51	22.8±0.14	264.9±3.68	23.1±0.57	14.1±0.10	3.7±0.01	MS	FE
9	IGS-5-11-5-33-24-06-214	102.0±0.71	98.9±1.27	12.1±0.52	22.7±0.00	260.0±5.60	23.8±0.33	14.0±0.06	3.7±0.02	MS	FE
	Mean	98	96.97	9.69	22.57	256	22.31	13.86	3.71		
	CV (%)	3.76	2.93	19.75	1.60	5.00	7.73	3.96	1.11		
	CD (5%)	1.28	1.87	1.35	0.26	5.94	0.90	0.27	0.05		
	Mean SS	70.94**	18.04**	15.59**	15.53**	40.36**	30.62**	33.83**	6.81**		
	GCV	3.75	2.73	18.27	1.48	4.98	7.53	3.88	1.04		
	PCV	3.86	3.03	20.63	1.68	5.23	8.02	4.11	1.35		
	h^2b	94.59	80.99	78.48	78.41	90.78	88.11	89.14	60.00		
	GAM@5%	7.52	5.06	33.35	2.71	9.78	14.55	7.54	1.66		

CV coefficient of variation, *CD* critical difference, *Mean SS* Mean sum of squares, *GCV* genotypic coefficient of variance, *PCV* phenotypic coefficient of variance, *h^2b* broad sense of heritability, *GAM* genetic advance in % of mean, *MS* medium slender, *FE* full exerted, *PE* partially exerted

Discussion

Recently, there has been a dramatic shift in rice production due to several biotic and abiotic stress factors, which not only reduces yield and quality but also potentially negatively impact the livelihood of millions of rice farmers. Many biotic and abiotic stress factors constantly challenge Rice cultivation. Among the biotic stresses, bacterial blight (BB) is caused by the Gram-negative bacteria *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) causes significant yield loss in irrigated and rainfed lowland areas, accounting for 60% of rice-growing areas in India (Ismail et al. 2013). BB disease caused by the *Xoo* pathogen is highly dynamic, leading to effective resistance overcoming after a few years of cultivation. This has necessitated breeders to search for novel resistance (R) genes. Extensive cultivation of single *R* gene-containing cultivars leads to increased selection pressure on the pathogen, which results in the breakdown of resistance (Chukwu et al. 2019). Thus, the pyramiding of multiple R genes over time and space is an effective strategy to enhance the durability and spectrum of resistance. Pyramiding multiple *R* genes with conventional breeding is challenging, owing to potential linkage drag with other undesirable traits that are arduous to break even after several generations of backcrossing (Tanksley et al. 1989). In addition, when more than two genes are incorporated, it is difficult to differentiate the effect of each gene precisely through phenotype-based selection, which is adopted in conventional breeding.

Further, in the presence of a dominant and a recessive allele, the effect of the recessive gene is masked. The rapid advances and low cost of molecular markers, which are tightly linked to gene(s) of interest, have precisely made it possible to identify plants with multiple *R* genes. The past efforts to incorporate multiple genes with the help of molecular markers in the elite but susceptible rice cultivars PR106 (Singh et al. 2001), Pusa Basmati (Joseph et al. 2004), and Samba Mahsuri (Sundaram et al. 2008) have been very successful.

Resistance breeding for developing improved lines by pyramiding multiple resistance genes with the help of molecular markers is effective for BB disease (Joseph et al. 2004; Sundaram et al. 2008, 2009). At least 47 genes (Sundaram et al. 2014; Neelam et al. 2020) conferring resistance to BB have been identified in rice germplasm. Among them, a combination of three major genes was found to be more effective against the disease, viz., major recessive genes *xa5* and *xa13* located on chromosome 5 and 8, respectively, and a major dominant, broad-spectrum resistant gene *Xa21* located on Chr. 11 derived from *O. longistaminata* are known to confer durable resistance against BB disease (Sundaram et al. 2008; Lalitha et al. 2013; Pradhan et al. 2015; Ramalingam et al. 2017; Rekha et al. 2018).

Gangavati sona is a premium fine grain medium slender rice variety known for its high yield and year-round cultivation in high-productivity regions of the Northern Karnataka region of India. It is highly preferred by farmers of the Gangavati area, popularly known as the "rice bowl of Karnataka" due to its medium slender (MS) grain type, where MS grain type is the dominant market segment in south India. However, the premium quality rice variety is highly susceptible to BB disease. In this context, we have introgressed three major BB resistance genes i.e., *xa5*, *xa13*, and *Xa21*, into GS to develop durable BB-resistant lines using marker-assisted backcross breeding (MABB) strategy without comprising GS yield and quality characteristics.

In modern breeding programs, molecular markers linked tightly to target genes are utilized to improve the selection efficiency of target traits or genes in a backcross breeding program (Jena and Mackill 2008). This involves foreground selection, where gene-specific markers are used to track target trait introgression (Hospital and Charcosset 1997), and background selection to identify the backcross-derived plant possessing maximum recovery of the recurrent parent genome (RPG) using co-dominant markers, which are polymorphic among the parents. This study used three co-dominant markers: xa5FM for the gene *xa5*, xa13prom for *xa13* (Hajira et al. 2016), and pTA248 for *Xa21* (Ronald et al. 1992) for foreground selection. These markers were also used successfully by Ramalingam et al. (2017); Rekha et al. (2018) and Dasari et al. (2022). Further, 64 parental polymorphic SSR markers were used for background selection.

The improved lines containing a combination of three resistant genes revealed a high level of resistance nature upon inoculation with the *Xoo* strain. The lines with only the *xa5* gene introgressed exhibited a little to partial resistance due to the presence of only one resistant gene, whereas lines with more resistant gene combinations (*xa13* or *Xa21*) exhibited a good resistance level. Even though the lines with single *xa13* or *Xa21* and/or other lines with two gene combinations are resistant, we have characterized only the three gene pyramided lines, considering the resistance they provided would be more durable and long-lasting. The data on disease lesion lengths of improved lines (Table 3) were recorded post-15 days after inoculation (DAI). The inoculated leaves were also examined at 21 DAI, wherein the lesion lengths had expanded in single- and two-gene introgressed lines but not in three-gene pyramided lines, indicating that there is a kind of quantitative complementation and additive nature of the resistance genes deployed. A higher level of resistance was also observed in improved introgression lines with multiple BB resistance genes, compared to those with fewer resistance genes in studies by Sundaram et al. (2008) and Dasari et al. (2022).

The genes *xa5*, *xa13*, and *Xa21* introduced into the GS background are well characterized. *xa5*, a recessive

resistance gene, encodes a variant form of transcription factor γIIa (Iyer and McCouch 2004) and the *xa13* gene is also recessive which has shown a mutation in the promoter region of a gene that is a homolog of the nodulin MtN3 (Chu et al. 2006), while *Xa21*, a dominant gene that encodes NBS-LRR domains of a receptor kinase (Song et al. 1995).

Microsatellite markers (SSR) that are found to be polymorphic among the parents, ISM, and GS were utilized for background selection in our study. Marker-assisted background selection is beneficial in recovering RPG by reducing the number of backcrosses (Hospital and Charcosset 1997), and background selection in early backcross generations was found to help recover RPG quickly (Chen et al. 2001; Joseph et al. 2004). Many studies (Bai et al. 2006; Hasan et al. 2015; Sundaram et al. 2008; 2009) suggest that a minimum of 3–4 backcrosses are required to recover RPG completely, while recent studies indicate that with stringent MABB, it is possible to get maximum RPG recovery even with two rounds of backcrossing (Singh et al. 2001; Basavaraj et al. 2010; Miah et al. 2015; Abhilash Kumar et al. 2017; Rekha et al. 2018; Swathi et al. 2019).

In the present study, the backcrossing was restricted to two generations since both parents used to share the same Samba Mahsuri lineage. The plant with the highest RPG recovery in BC_1F_1 was 74.2% in IGS-5-11 and 86.7% in BC_2F_1 (IGS-5-11-5). In BC_2F_2 generation, the line IGS-5-11-5-33 exhibited 92.6% RPG recovery and 98.3% in the selected best improved BC_2F_5 (IGS-5-11-5-33-24-06-30) line. The high RPG recovery obtained in this study can be attributed to the fact that the stringent phenotypic selection was carried out at every backcross generation and selfing. This helps in the reduction of time and resources used as well. Earlier, Singh et al. (2012) followed a similar strategy and reported increased RPG recovery. The results obtained in the present study are in accordance with the earlier reports (Sundaram et al. 2008; Pradhan et al. 2015; Dasari et al. 2022).

When challenged with the *Xoo* pathogen under field conditions, GS's improved, bacterial blight-resistant lines revealed that all the pyramided lines were resistant to bacterial strain IXo-20. Similar results were also observed in earlier studies (Yoshimura et al. 1995; Huang et al. 1997; Sanchez et al. 2000; Singh et al. 2001; Sundaram et al. 2008; Dokku et al. 2013; Gitishree and Rao 2015; Dasari et al. 2022) wherein *xa5*, *xa13,* and *Xa21* were introgressed in combinations or singly.

The main objective of this study was to develop improved lines of GS with durable resistance to BB. Many studies (Sanchez et al. 2000; Sundaram et al. 2008) have reported the quantitative complementation of resistance genes when they are introgressed in different combinations. Deploying three resistant genes into the GS background may help the farmers to reap the yield advantages in disease-affected areas by providing durable resistance. Parallel results were reported by Sundaram et al. 2009 and Dasari et al. 2022 in other genetic backgrounds of Indica rice cultivars. The improved lines using GS as a recurrent parent are expected to show high resistance against BB. This disease is widely prevalent, limiting rice production in Karnataka (Raichur, Koppal, and Ballari) and other Indian states (Aruna Kumari et al. 2016).

From the agro morphological data, the improved lines of GS possessing resistance against BB disease performed better when compared to GS without yield penalty. We observed that some lines were superior to Recurrent parent GS. Further, the selected lines possessing BB resistance were found to retain medium slender grain types and other grain quality traits as of GS. All the improved lines exhibited full panicle exertion and plant height similar to GS. It can be anticipated that the improved GS lines for BB resistance developed through marker-assisted backcrossing will benefit the farmers who regularly grow GS as they are similar in quality and yield, with an added advantage of three resistance genes against BB disease.

Conclusions

Through this MABB strategy, we have successfully introgressed three major BB resistance genes, i.e. *xa5, xa13,* and *Xa21,* into the background of the susceptible variety Gangavati sona (GS), without yield penalty and retaining better grain quality and other related traits of the elite variety. The identified lines possessing resistance genes have very good phenotypes and high yield levels. Hence, a few of these lines have been nominated for All India Co-ordinated Crop Improvement (AICRIP) trails for varietal release to farmers. These introgressed lines may help the farmers of GS, whose fields are affected by BB disease. In addition, these improved lines can be used in future disease-resistance breeding programs as donors. Our work demonstrated the importance of marker-assisted backcrossing for improving the popular varieties to BB resistance while retaining all the other desirable attributes for which farmers have been growing for many years with enhanced durable broad-spectrum resistance to BB.

Author contributions Experimental idea and design: MSA, CAM, CG, PMS, RMS; execution and performing experiment: MSA, CAM, CG, BM, PSB, RMS; infrastructural facilities: LVSR, KB, GSL, RMS; data analysis: SR, CAM; manuscript preparation: CAM, MSA; editing

manuscript: PS, KBK, RMK, RL, JD, JMN, MG; field selections, data collection and genotyping: CAM, MH, VA, DA, VGI, GU.

Declarations

Conflict of interest The authors declare that they have no competing interests.

Ethical approval Not applicable.

Consent to participate Not applicable.

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