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ORIGINAL ARTICLE

Improvement of blast resistance of the popular high-yielding, medium slender-grain type, bacterial blight resistant rice variety, Improved Samba Mahsuri by marker-assisted breeding

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Abstract

Improved Samba Mahsuri (ISM) is a popular, high-yielding, bacterial blight resistant rice variety possessing medium-slender grain type. As ISM is highly susceptible to blast disease of rice, through the present study we have transferred two major blast resistance genes, *Pi2* and *Pi54* into the elite variety by marker-assisted backcross breeding. The two blast resistance genes were transferred to ISM through sets of backcrosses. In every backcross generation, PCR-based markers, specific for the blast resistance genes (*Pi2* and *Pi54*) and bacterial blight resistance genes (*Xa21*, *xa13* and *xa5*) were utilized for foreground selection, while a set of 144 parental polymorphic SSR markers were used for background selection and backcrossing was carried out until BC_2 generation. A solitary BC_2F_1 plant possessing *Pi2* or *Pi54* along with *Xa21*, *xa13* and *xa5* and > 90% recovery of ISM genome was selected from the two sets of backcrosses were crossed and the intercross F_1s (ICF_1s) thus obtained were selfed to generate ICF_2s. Homozygous ICF_2 plants carrying all the five resistance genes were identified through markers and advanced through selfing till ICF_5 generation by adopting pedigree method of selection. Three best lines at ICF_5, possessing excellent resistance against bacterial blight and blast and closely resembling or superior to ISM in terms of grain quality: yield and agro-morphological traits have been identified and advanced for multi-location trials.

Keywords Rice · Blast · Bacterial blight · Improved Samba Mahsuri · *Xa21* · *xa13* · *xa5* · *Pi2* · *Pi54*

Abbreviations

MABB	Marker assisted backcross breeding
SSR	Simple sequence repeats
BB	Bacterial blight
ISM	Improved Samba Mahsuri

Introduction

For a majority of the people in India rice is the predominant food and the crop plays a crucial role in the Indian economy (FAO 2015). Among the Indian rice varieties, Samba Mahsuri (also known as BPT 5204), developed by Acharya N.G. Ranga Agricultural University, Guntur, Andhra Pradesh India is grown in more than 3 Mha across the country, mainly in South Indian states of Andhra Pradesh, Telangana, Tamilnadu and Karnataka and occupies an important position among the mega-varieties of rice grown in India. It is a high-yielding variety with excellent cooking and eating qualities and it possess a highly desirable medium slender grain type and thus commands a high market price (Sundaram et al. 2008). Despite these

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features, the yield of Samba Mahsuri is severely limited by several diseases and insect pests. In 2008, ICAR-Indian Institute of Rice Research (ICAR-IIRR), Hyderabad, India and CSIR-Centre for Cellular and Molecular Biology (CSIR-CCMB), Hyderabad, India jointly developed and released a novel bacterial blight disease resistant derivative of Samba Mahsuri, named Improved Samba Mahsuri (ISM; also known as RP Bio 226) through marker-assisted backcross breeding (MABB), possessing three major bacterial blight (BB) resistance genes, viz., *xa1, Xa21* and *xa5* (Sundaram et al. 2008). ISM is presently estimated to grown by rice farmers across the Indian states of Andhra Pradesh, Telangana, Tamilnadu, Karnataka and Chhattisgarh, particularly in bacterial blight endemic regions, in an area of ~ 1, 00,000 ha. However, in the recent past, the spread of the new variety has been observed to be limited in those rice cultivation areas, which are endemic to another major disease of rice, called blast as both the original variety, Samba Mahsuri and its derivative, Improved Samba Mahsuri are highly susceptible to the dreaded disease.

Magnaporthe oryzae, the causative agent of rice blast lead to the significant yield loss in the major rice growing areas in India and as well as in the world. (Khush and Jena 2009). The most appropriate strategy for blast disease management is to deploy host-plant resistance. Till now, at least 100 rice blast resistance genes and several QTLs conferring resistance to blast have been identified (Sharma et al. 2005; Sharma et al. 2012). Among them, two blast resistance genes viz., *Pi2* and *Pi54* are known to be very effective, confer broad-spectrum resistance and hence are considered to be suitable for deployment in India. Further, gene-specific markers are available for both *Pi2* and *Pi54* for deployment in marker-assisted breeding. Considering the imminent need to improve blast disease resistance of ISM in the present study, we attempted to deploy the strategy of marker-assisted backcross breeding (MABB).to transfer *Pi2* and *Pi54* into the genetic background of the bacterial blight resistant version of Samba Mahsuri.

Materials and methods

Plant materials

Two near-isogenic lines of Samba Mahsuri, viz., RPBio Patho-1 and RPBio Patho-2 (Prasad et al. 2011; Madhavi et al. 2011), possessing the blast resistance genes, *Pi2* and *Pi54*y, developed from the crosses, Samba Mahsuri X C101A51 and Samba Mahsuri X Tetep, respectively were used as donor parents. The bacterial blight resistant variety, Improved Samba Mahsuri (ISM) harboring three bacterial blight resistance genes (*Xa21*, *xa13* and *xa5*) was used as the recipient parent. In addition to these, Samba Mahsuri was used as susceptible check while ISM was used as resistant check, respectively in bacterial blight screening experiments. HR12 was used as the susceptible check in blast screening experiments along with C101A51 and Tetep as resistant checks.

Strategy for marker-assisted backcross breeding

Two separate crosses i.e., ISM X RPBio Patho-1 (Cross I) and ISM X RPBio Patho-2 (Cross II) were carried out to combine *Pi54* and *Pi2* into the genetic background of ISM. The F_1s developed from both the crosses were confirmed for their heterozygosity using target resistance gene-specific markers [viz., AP5659-5 (specific for *Pi2*, Fjellstorm et al. 2006) and Pi54MAS (specific for *Pi54*, Ramkumar et al. 2011), pTA248 (specific for *Xa21*; Ronald et al. 1992), xa13prom (specific for *xa13*; Hajira et al. 2016), xa5FM (specific for *xa5*; Hajira et al. 2016)] to identify heterozygous plants in both the crosses and such plants were backcrossed with ISM to generate BC_1F_{1s}. They were subjected for foreground selection using the gene-specific markers mentioned above and positive plants (i.e. heterozygous BC_1F_1 plants) were then analysed for the recurrent parent genome recovery through background selection using a set of 144 SSR parental polymorphic markers evenly distributed across the 12 rice chromosomes to identify those plants with maximum recurrent parent genome recovery in both the crosses. A single such BC_1F_1 plant was then selected from both the crosses (i.e. cross I and II) and backcrossed with ISM to generate BC_2F_1s. Marker-assisted foreground and background selection (as explained above) was repeated among BC_2F_1 plants and a single heterozygous BC_2F_1 plant from both the crosses, possessing the target resistance genes and also with maximum ISM genome recovery were selfed to develop BC_2F_2s. Homozygous positive BC_2F_2 plants with respect to desired target resistance genes (i.e. homozygous for *Xa21, xa13, xa5* and *Pi2* in Cross I and *Xa21, xa13, xa5* and *Pi54* in Cross II) were identified with the help of markers and a single homozygous BC_2F_2 plant each from both the crosses, which closely resembled ISM (based on morphological features) were then crossed to develop intercross F_1s (ICF_1s). The ICF_1s were selfed to generate intercross F_2s (ICF_2s). Among them, homozygous positive plants for the target BB and blast resistance genes (viz., *Xa21, xa13, xa5, Pi2* and *Pi54*) were identified with the help of markers and advanced through pedigree method of selection till ICF_5s. Three promising homozygous ICF_5 lines closely resembling ISM were advanced for further evaluation along with plants of ISM homozygous for either *Pi2* + *Xa21* + *xa13* + *xa5* or *Pi54* + *Xa21* + *xa13* + *xa5* at BC_2F_6 generation.

For marker-assisted selection at each generation of backcrossing or intercrossing, mini-scale, rapid DNA isolation was done as described in Zheng et al. (1995). PCR protocols as described in Ronald et al. (1992; for *Xa21*), Hajira et al. (2016; for *xa13* and *xa5*), Fjellstorm et al. (2006; for *Pi2*) and Ramkumar et al. (2011; for *Pi54*) were adopted for foreground selection of the target resistance genes. A set of 144 parental polymorphic SSR markers (listed in Supplementary Table 1) were used for background selection as per the protocol explained in Sundaram et al. (2009). The extent of donor parent segment introgression among the selected backcross derived plants was assessed utilizing the software tool, Graphical Genotype V 2.0 (Van Berloo 1999) following the protocol described in Abhilash et al. (2016c).

Assessment of BB and blast resistance in the backcross derived lines of ISM

Screening for blast resistance During Kharif 2016 (i.e. wet season 2016), gene-pyramid lines of ISM, possessing either *Pi2* (at BC_2F_6) or *Pi54* (at BC_2F_6) or *Pi2* + *Pi54* (at ICF_5) were subjected to stress against blast in the UBN beds at ICAR-IIRR (Prasad et al. 2011) along with the susceptible (HR12) and resistant (C101A51 and Tetep) checks using a local isolate, SPI-40 of the blast pathogen, *Magnaporthae oryzae* as per the protocol described in Mohan et al. (2011). Fifteen days after inoculation, the lines were scored for their resistance/susceptibility to the disease based on IRRI-SES scale, 1996 (IRRI 1996).

Screening for bacterial blight resistance: The blast resistant gene-pyramid lines of ISM, possessing either *Pi2* (at BC_2F_6) or *Pi54* (at BC_2F_6) or *Pi2* + *Pi54* (at ICF_5) were screened along with the susceptible (i.e. Samba Mahsuri) and resistant (i.e. ISM) checks for their resistance/susceptibility to bacterial blight during Kharif 2016 (i.e. wet season 2016) using a virulent isolate of the pathogen, *Xanthomonas oryzae* pv. *oryzae*, viz., DX-020 (Laha et al. 2009) through clip-inoculation procedure described by Kauffman et al. (1973). The plants were scored 15 days after inoculation for their resistance/susceptibility as per IRRI-SES scale, 1996 (IRRI 1996).

Evaluation of agro-morphological characters of the improved lines

During wet season 2016 (i.e. June–November), three promising five-gene pyramided lines at ICF_5 (possessing *Xa21, xa13, xa5, Pi2* and *Pi54*) generation, two each of selected lines of ISM possessing *Pi2* (at BC_2F_6) and *Pi54* (at BC_2F_6), the recurrent parent ISM were transplanted (twenty-seven day-old seedlings) into the experimental farm of ICAR-IIRR in 2 m^2 plots in three replications with a 15 cm × 20 cm spacing. The seedlings were raised till maturity by adopting standard package of practices. Data was collected for selected agro-morphological traits, viz., plant height (cm), days to 50% flowering (DFF), panicle length (cm), number of productive tillers per plant, grain yield per 33 plants (i.e. per m^2; gms), 1000-grain weight (g) among 33 plants for each replication (n = 3) as explained in Abhilash et al. (2016c). The data was statistically analyzed following the procedures described in Freeman et al. (1978). Least Significance Difference (LSD) values at 5 to 7 percent level of significance and Coefficient of variation (CV) were calculated using standard errors of mean (S. Em. ±) using Microsoft Excel package. The software package, SAS Version 9.2 (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis. PROC GLM procedure of SAS was utilized for analysis of variance (ANOVA) for determination of significant variation among the improved breeding lines of ISM.

Evaluation for grain physic-chemical quality traits

The selected advanced breeding lines of ISM possessing BB and blast resistance were evaluated for key grain and cooking quality related traits. After harvest, the rice grains were dried directly under sunlight followed by drying to below 14% moisture content, and preserved at room temperature for 90 days. The rice grains were dehusked, polished and evaluated for physical characters like grain length, grain width and L/B ratio. The data was also recorded for the cooking qualities traits, amylose content (%) in which the rice grains were classified into low (9–20%), intermediate (20–25%) and high (25–33%) and Gel consistency which classifies the quality of cooked rice as soft (61–100 mm), medium (41–60 mm) and hard (26–40 mm) following method of Juliano et al. (1973), Cagampang et al. (1973) and Melissa et al. (2009).

Results

Introgression of *Pi2* into ISM

True F_1 plants (i.e. heterozygous ones for the target resistance genes) derived from the cross ISM/RPBio-Patho-1 were backcrossed to the recurrent parent ISM. Among the 270 BC_1F_1s, 130 were found to be heterozygous for *Pi2* and 15 of them also possessed *Xa21, xa13* and *xa5* in homozygous condition. Screening with 75 parental polymorphic SSR markers revealed that a single BC_1F_1 plant (# ISM-9) had the highest recurrent parent genome (RPG) recovery (i.e. 77.3%; Supplementary Table 1) and it was then backcrossed to ISM to generate 126 BC_2F_1s. A total of

60 heterozygous positive plants possessing *Pi2* were identified and subjected for RPG recovery analysis. A solitary BC_2F_1 plant (# ISM-9-18) was identified with maximum RPG ($\sim$ 90.6%) and selfed to develop 496 BC_2F_2s. Out of these, 24 plants were observed to be possess the gene combination *Xa21* + *xa13* + *xa5* + *Pi2* in homozygous condition and a single four-gene homozygous plant (# ISM-9-18-217) was identified using maximum recurrent parent genome recovery (i.e. 94.6%) using background analysis. This plant was further analyzed to assess the extent of linkage drag around the target blast resistance gene, *Pi2*. At the proximal end of the gene, a segment of 0.5 Mb was introgressed from the donor parent, while at the other end, a 0.2 Mb segment was introgressed from the donor parent genome. Thus, in total, a segment of 0.7 Mb (Fig. 1) was introgressed in the vicinity of *Pi2* from RPBio-Patho 1 in the selected BC_2F_2 plant (i.e. plant # ISM-9-18-217), which was later used for intercrossing.

Introgression of *Pi54* into ISM

Among the 321 BC_1F_1s generated by backcrossing the true F_1s derived from the cross ISM/RPBio-Patho-2 with ISM, 158 were heterozygous for the target gene *Pi54*. Screening of these plants using gene-specific markers for the three bacterial blight resistance genes revealed that 18 were homozygous. Background selection of these BC_1F_1 plants with SSR markers polymorphic between RPBio-Patho 2 and ISM (i.e. n = 69) helped in identification of a single plant (# ISM-22), possessing maximum RPG of 75.3% (Supplementary Table 1). This BC_1F_1 plant was backcrossed with ISM to produce 108 BC_2F_1s. Foreground selection of these plants resulted in identification of 48 heterozygous plants carrying *Pi54*. A single BC_2F_1 plant (# ISM-22-102) possessing a maximum RPG (i.e. $\sim$ 86.9%) was selected using background analysis and advanced to develop 346 BC_2F_2s. Through analysis with gene-specific markers, 84 four-gene homozygous plants (for *Xa21*, *xa13*, *xa5* and *Pi54*) were identified. Out of these, a solitary plant (# ISM-22-102-179) was identified with recovery of maximum RPG ($\sim$ 93%). This plant was also observed to be introgressed with 0.2 and 0.4 Mb segments from RPBio-Patho 2 at proximal and distal ends of *Pi54*, totaling to $\sim$ 0.6 Mb (Fig. 1). It was then utilized for intercrossing.

Pyramiding of *Pi2* and *Pi54* into ISM

The best BC_2F_2 plants developed from the two sets of backcrosses (i.e. plant # ISM-9-18-217 possessing *Pi2* in homozygous condition from the first cross and # ISM-22-102-179 possessing *Pi54* in homozygous condition from the second cross) were intercrossed to develop 51

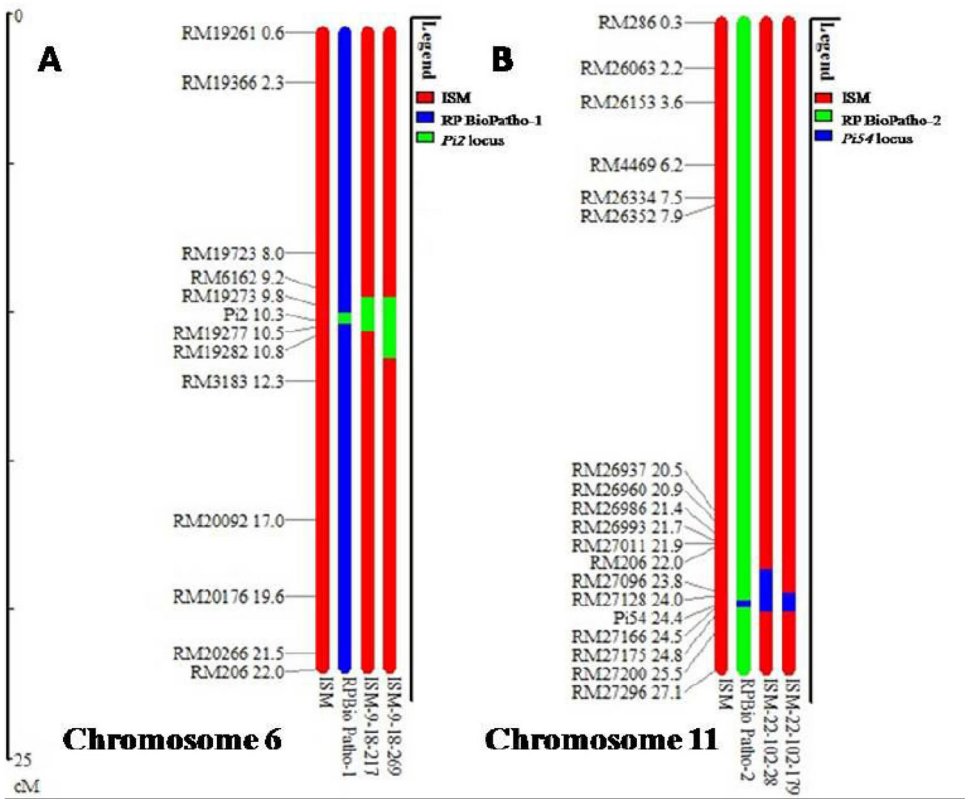

Fig. 1 Analysis of donor genome introgression associated with blast resistance genes in the selected BC_2F_2 plant: **a** At *Pi2* locus, a segment of 0.5 Mb was introgressed at the proximal end, while a 0.2 Mb segment was observed to be introgressed at the distal end from the donor parent genome in the best BC_2F_2 plant (i.e.ISM-9-18-217), Thus, in total, a segment of 0.7 Mb was introgressed from the donor parent with respect to the genomic region in the vicinity of *Pi2*. **b** At *Pi54* locus, a segment of 0.2 and 0.4 Mb was introgressed at proximal and distal ends, respectively from the donor parent genome (totaling to 0.6 Mb) in the best BC_2F_2 plant (i.e.ISM-22-102-28). The position of the polymorphic SSR markers in Mb on Chr. 6 and 11 is given in parenthesis adjacent to each marker

intercross F_1 (i.e. ICF_1) plants. Among them, 46 were identified as true F_1s through analysis with gene-specific markers (Fig. 2), which were selfed to generate an ICF_2 population comprising of 850 plants. Marker-assisted selection, coupled with phenotyping selection resulted in the identified of 50 plants which closely resembled ISM and possessing *Pi2* and *Pi54* in homozygous condition. They were advanced using pedigree-method and three best improved ICF_4 lines (IC-12-9-56, IC-12-199-83, and IC-12-305-106) were identified (Supplementary Fig. 2) to be identical to ISM with respect to morphological traits and they were advanced for evaluation of their disease resistance, agromorphological features and grain quality at ICF_5 generation.

Evaluation of the backcross derived lines of ISM for resistance against blast and BB

HR12 and the recurrent parent ISM (susceptible checks), were found to be highly susceptible to blast (Score 9), while the resistant checks, RPBio Patho-1 (Score 2), RPBio Patho-2 (score 1) were highly resistant to the disease (score; 1–2; Fig. 2a; Table 1). The backcross and intercrossed derived lines displayed excellent resistance against blast similar to the donor parents. With respect to screening for BB resistance (Fig. 2b; Table 1), the recurrent parent (ISM) and donors (RPBio Patho-1 and RPBio Patho-2) were highly resistant to the disease with a lesion length ranging from 0.0 to 1.7 ± 0.3 (Score 1). All the backcross and intercrossed lines displayed high level of resistance as of donor and recurrent parent (Table 1).

Evaluation of agromorphological traits for improved lines

Among the improved lines, BC_2F_5 plant, # ISM-9-18-217-15 and ICF_5 plant # IC-12-9-56 displayed grain yield per plant (17. 1 ± 0.2 and 17.0 ± 1.2 g, respectively) equivalent to ISM (i.e., 17.1 ± 1.7 g), while other backcross and intercross derived lines displayed grain yield higher than that of the recurrent parent (21.6 ± 1.0–26.0 ± 0.8; Table 2). All the introgressed lines were observed to be equivalent to or slightly taller than the recurrent parent ISM (83.0 ± 4.0–93.0 ± 0.5), except a single line, ISM 22-102-179-8 (80.6 ± 4.7) (Fig. 3; Table 2), which was found to be considerably shorter than ISM. The gene pyramid lines were found to perform equivalent to or better than ISM in terms of number of productive tillers per plant (12.3 ± 0.3–17.7 ± 1.3), panicle length (19.7 ± 0.9–22.0 ± 0.3 cm) and 1000 grain weight (14.5 ± 0.4–18.5 ± 2.0 g; Table 2). Four improved lines were observed to flower 9–11 days earlier than ISM along with better panicle exsertion (Table 2). The five-gene pyramid line, # IC-12-305-106 was significantly better in terms of number of tillers, plant height, panicle length, yield per plant, 1000 grain weight and also better exsertion of panicles with respect to the recurrent parent, ISM (Table 2).

Quality traits of the improved lines

All the improved lines were observed to be of medium-slender grain type, possessed intermediate values with respect to amylose content (20–25%) and had hard gel

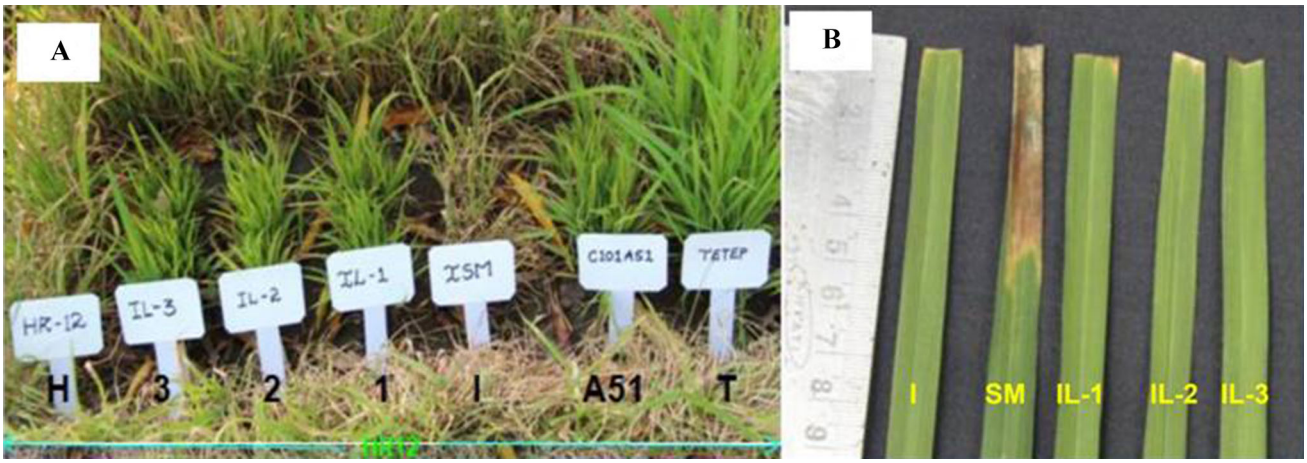

Fig. 2 Phenotypic screening of the selected five-gene pyramid ICF_4 lines against rice blast and bacterial blight diseases. **a** Screening of the selected five-gene pyramid ICF_4 plants, viz., plant # IC-12-9-56 (IL-1), IC-12-199-83 (IL-2), IC-12-305-106 (IL-3) against blast disease under uniform blast nursery. T- Tetep (donor parent and resistant check); A51-C101A51 (donor parent and resistant check); I-Improved Samba Mahsuri (recurrent parent and susceptible check); H- HR12 (susceptible check). **b** Screening of the selected five-gene pyramid ICF_4 plants, viz., plant # IC-12-9-56 (IL-1), IC-12-199-83 (IL-2), IC-12-305-106 (IL-3) against bacterial blight disease through clip-inoculation method. I- Improved Samba Mahsuri (recurrent parent and resistant check); SM- Samba Mahsuri (susceptible check). All the three selected five-gene homozygous ICF_4 lines were resistant to both bacterial blight and blast diseases

Table 1 Reaction of selected pyramided lines of ISM after inoculation with bacterial blight and blast pathogen

Improved lines	Reaction against bacterial blight[a]		Reaction against blast[b]	
	DX020		SPI40	
	Score	R/S	Score	R/MR/S
ISM	0. 0 ± 0.0	R	9	S
RPBio- Patho 1	1.3 ± 0.3	R	2	R
RPBio- Patho 2	1.7 ± 0.3	R	1	R
TN1	8.7 ± 0.3	S	–	–
HR12	–	–	9	S
ISM-9-18-217-15	1.3 ± 0.3	R	1	R
ISM-9-18-217-18	0.7 ± 0.3	R	2	R
ISM-22-102-179-8	0.3 ± 0.3	R	2	R
ISM-22-102-179-13	0.7 ± 0.3	R	1	R
IC-12-9-56	1.3 ± 0.3	R	1	R
IC-12-199-34	1.7 ± 0.3	R	2	R
IC-12-305-106	0.7 ± 0.3	R	1	R

[a]The backcross derived lines at BC_2F_5 (*xa5* + *xa13* + *Xa21* + *Pi2* and *xa5* + *xa13* + *Xa21* + *Pi54*) and five-gene pyramid lines at ICF_3 generation (*xa5* + *xa13* + *Xa21* + *Pi2* + *Pi54*), was screened with a single *Xoo* isolate, DX-020 (DRR isolate-used to screen in glass house conditions)

[b]The backcross derived lines at BC_2F_5 (*xa5* + *xa13* + *Xa21* + *Pi2* and *xa5* + *xa13* + *Xa21* + *Pi54*) and five-gene pyramid lines at ICF_3 generation (*xa5* + *xa13* + *Xa21* + *Pi2* + *Pi54*), were screened with a single blast isolate SPI-40 under controlled conditions by UBN method

R resistant, *S* susceptible

consistency (Table 3), equivalent to the values noted for ISM.

Discussion

Samba Mahsuri (BPT5204), a very popular high yielding Indian rice variety fetches premium price for farmers due to its excellent grain and cooking quality attribues. However, it is highly susceptible to many biotic and abiotic stresses. We had earlier developed a near isogenic line (NIL) of Samba Mahsuri, possessing resistance against a dreaded disease of rice called bacterial blight (BB; conferred by the genes, *Xa21*, *xa13* and *xa5*) in collaboration with CSIR-CCMB. The NIL was released as a new variety in 2008 for cultivation with the name Improved Samba Mahsuri (ISM; Sundaram et al. 2008). The newly released bacterial blight resistant variety was observed to give 15–30% more yield than Samba Mahsuri and other bacterial blight susceptible varieties in areas endemic to bacterial blight and hence it is being enthusiastically cultivated in Indian states of Telangana, Andhra Pradesh, Tamilnadu, Karnataka and Chhattisgarh in an estimated area of ~ 1, 00,000 ha. This is one of the first examples of a rice variety developed in India through molecular marker-assisted selection and successfully released and commercialized. As ISM was observed to recently show low to moderate blast susceptibility at a few locations across India, we planned and executed the current study, aimed to improve ISM for durable blast resistance by utilising an adoptable strategy, marker assisted incorporation of two, broad spectrum blast resistance genes, *Pi54* and *Pi2*.

MABB is a fastidious strategy, which involves the introgression of one or more target genes into elite crop variety/hybrid to improve one or few traits lacking in the original variety/hybrid (Wen and Gao 2012). The purpose of backcross breeding is to achieve a progressive reduction in the donor genome content to retain the characteristics of the recurrent parent in the improved varieties apart from introgression of one or more target genes (for e.g. genes conferring resistance against diseases). As single gene conferred resistance is known to be short lived (Victoria and Zeigler 1993; Shanti et al. 2010; Hua et al. 2015), rice breeders are striving to breed durable resistant varieties by pyramiding two or more genes into elite varieties and hybrids. There are a few earlier reports, where rice breeders have successfully adopted marker-assisted breeding strategy for improvement of rice varieties for resistance against blast (Das and Rao 2015; Tanweer et al. 2015; Usatov et al. 2016), bacterial blight (BB) (Pradhan et al. 2015; Sundaram et al. 2008, 2009), BB and blast (Singh et al. 2011; Hari et al. 2013; Balachiranjeevi et al. 2015; Ellur et al.

Table 2 Analysis of agro-morphological characters of improved ISM lines along with parents under field conditions

S. no.	Plant identity	Days to 50% flowering (DFF)	Mean plant height (cm)	No. of productive panicles/plant	Panicle length (cm)	Grain yield per plant (g)	1000 seed weight (g)	Panicle exerstion
1	RPBio-226	103.0 ± 1.2	82.0 ± 0.6	12.3 ± 0.9	19.2 ± 0.6	17.1 ± 1.7	12.2 ± 0.7	PE
2	RPBio Patho-1	96.0 ± 1.2	68.8 ± 0.2	15.6 ± 0.3	19.2 ± 0.4	15.3 ± 0.4	17.1 ± 0.1	PE
3	RPBio Patho-2	104.7 ± 0.3	69.8 ± 0.7	16.3 ± 0.7	19.5 ± 0.5	16.2 ± 0.1	17.9 ± 0.2	PE
Cross (C-I)—*xa5* + *xa13* + *Xa21* + *Pi2*								
4	ISM-9-18-217-15	94.0 ± 0.6	85.6 ± 1.9	15.7 ± 1.2	21.0 ± 1.2	17.1 ± 0.2	14.5 ± 0.4	FE
5	ISM-9-18-217-18	102.0 ± 1.2	83.0 ± 4.0	17.3 ± 1.7	21.8 ± 0.2	21.6 ± 1.0	15.4 ± 1.4	FE
Cross(CII)—*xa5* + *xa13* + *Xa21* + *Pi54*								
6	ISM-22-102-179-8	93.0 ± 1.2	80.6 ± 4.7	13.3 ± 0.9	20.6 ± 0.9	25.3 ± 2.6	18.0 ± 1.2	FE
7	ISM-22-102-179-13	103.0 ± 1.2	83.6 ± 2.3	12.3 ± 0.3	19.7 ± 0.9	20.1 ± 0.6	17.3 ± 1.0	FE
Intercross (IC)—*xa5* + *xa13* + *Xa21* + *Pi2* + *Pi54*								
8	IC-12-9-56	92.0 ± 1.2	87.1 ± 1.9	16.0 ± 2.5	19.8 ± 1.3	17.0 ± 1.2	15.3 ± 0.8	FE
9	IC-12-199-83	94.7 ± 2.6	84.8 ± 4.0	12.3 ± 1.3	20.7 ± 0.7	19.3 ± 0.8	15.9 ± 1.2	FE
10	IC-12-305-106	103.3 ± 0.9	93.0 ± 0.5	17.7 ± 1.3	22.0 ± 0.3	26.0 ± 0.8	18.5 ± 2.0	FE
	CV%	2.22	5.52	14.11	2.11	8.46	6.56	
	LSD (p = 0.05)	6.33	13.07	6.20	3.75	4.16	4.01	
	F	15.82	8.08	2.39	2.11	6.15	21.55	
	P value (<0.05)	< 0.0001	< 0.0001	0.0505	0.079	0.0004	< 0.0001	

ISM reccurent parent, *RPBio Patho-1*, *RPBio Patho-2* donor parents, *MS* medium slender, *CV* coefficient variance, *CD* critical differtial at 5% probability level, ± standard error and values given are mean of three replications, *PE* partially exserted, *FE* fully exserted

2016; Abhilash et al. 2016a, b, c). Taking cues from these reports, the present study was formulated and executed.

Pi54, a major blast resistance gene derived from Tetep (a Vietnamese rice line) is known to be very efficient under Indian circumstances (Rai et al. 2011; Sharma et al. 2010). NILs of Swarna and Samba Mahsuri possessing *Pi54* displayed excellent resistance in multi-location trials of All India Coordinated Rice Improvement Project (AICRIP) trials (DRR Progress report, Vol. 2, 2008–2013). Along with *Pi54*, we also considered another major blast rsistance gene, *Pi2*, a gene known to confer broad-spectrum resistance (originally derived from the *indica* rice line, 5173; Mackill and Bonman 1992) for introgression into ISM. The efficacy of *Pi2* gene in conferring excellent resistance against blast in the several parts of India has been reported (Variar et al. 2009; Imam et al. 2014; Khanna et al. 2015; Indian Council of Agricultural Report 2009–2013). Further, a recent study (Ellur et al. 2016), revealed that the gene pyramid combination, *Pi2* + *Pi54* is very effective in the southern states of India such as Kerala, Tamilnadu apart from northern and eastern parts of India. Hence based on these reports, we narrowed down on the two genes, *Pi2* and *Pi54* for introgression into ISM.

MABB strategy is known to be very helpful in limiting the number of backcrosses required for near-complete recovery of the recurrent parent genome (RPG). There are some earlier reports wherein the recurrent parent genome (RPG) has been successfully recovered by using just two or three back crosses. (Abhilash et al. 2016c; Balachiranjeevi et al. 2015; Singh et al. 2011). A similar approach was followed in the current study, wherein the number of backcrosses was limited to just two. In addition to the deployment of marker-assisted selection for accelerate recovery of the RPG at each stage of backcrossing, the donor parents used in the present study are actually pre-breeding lines with Samba Mahsuri genetic background possessing medium-slender grain type (Prasad et al. 2011). These factors helped to restrict to two rounds of

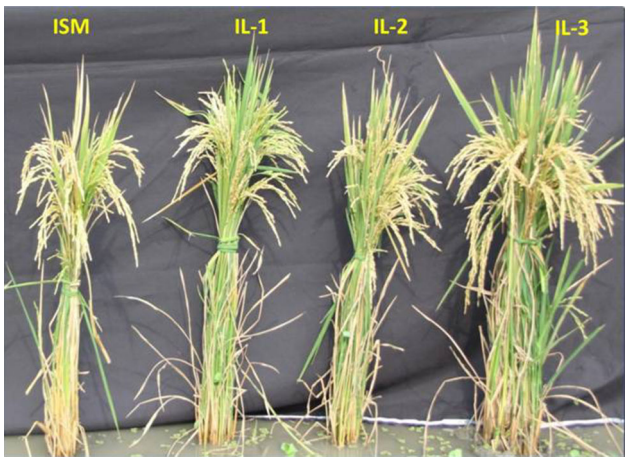

Fig. 3 Single plants of selected five-gene pyramid ICF_4: ISM-Improved Samba Mahsuri (recurrent parent), selected five-gene pyramid ICF_4 plants, viz., plant # IC-12-9-56 (IL-1), IC-12-199-83 (IL-2), IC-12-305-106 (IL-3). While IL-1 and IL-2 was observed to be equivalent to Improved Samba Mahsuri, IL-3 was observed to be superior to the recurrent parent

backcrossing. A single plant (# ISM-9-18-217) at BC_2F_2 (derived from Cross I), possessing *Pi2* gene with maximum recurrent parent genome recovery (94.6%) was observed to have very limited linkage drag of only 0.7 Mb around *Pi2*. Similarly, another BC_2F_2 plant (# ISM-22-102-179 l; derived from cross II) possessing *Pi54* gene with maximum recurrent parent genome recovery ($\sim$ 93%) had a linkage drag of only $\sim$ 0.6 Mb in the vicinity of *Pi54*. Thus when these plants were intercrossed, as expected, the derived lines closely resembled Samba Mahsuri and ISM in terms of most of the agro-morphological characters and grain type, while possessing excellent resistance against BB and blast.

Phenotypic screening of the improved versions of ISM possessing either *Pi2* or *Pi54* or both the genes revealed that all the lines are highly resistant to both blast and BB (Table 1; Fig. 3a, b). Among these lines, ICF_4 lines possessing the complete complement of BB and blast resistance genes i.e. combinations of *Pi2*, *Pi54* and *xa5*, *xa13*, *Xa21* were observed to possess excellent resistance against blast as compared to be backcross derived lines incorporated with single blast resistant genes (*Pi2* or *Pi54*). As the recurrent parent used in the present study is a BB resistant variety having three BB resistant genes (*xa*5, *xa*13 and *Xa*21), all the improved lines of ISM at BC_2F_5 and ICF_4 generation were observed to be highly resistant to BB (0.3 ± 0.3–1.7 ± 0.3), similar to ISM (Table 1; Fig. 2b). The results obtained in the present study with respect to resistance against BB and blast are in congruence with the results obtained in our previous reports (Abhilash et al. 2016a, b, c; Hari et al. 2013, Balachiranjeevi et al. 2015), wherein no negative interaction was observed between blast and BB resistance genes.

Interestingly, most of the ISM derived lines in the present study were found to be equivalent to ISM in terms of grain yield, some of them were observed to be superior with respect to yield (Table 2) and panicle exsertion. Further all the selected lines, possessing five resistance genes were observed to be retaining the fine-grain type and other grain quality attributes of the ISM (Table 2; Supplementary Fig. 2). It can be expected that the blast resistant lines of ISM which have been developed through the study may command the same premium price for farmers in the market similar to Samba Mahsuri (BPT5204) or ISM as they are identical in most of the key attributes related to grain and cooking quality. The approach adopted in the present study, i.e. combining phenotype-based selection with MAS was helpful in not only recovering good features of the recurrent parent, but also helpful in selection of superior lines (with better yield and panicle exsertion) as

Table 3 Analysis of grain and cooking qualities of ISM improved lines

Lines	Grain qualities				cooking qualities	
	KL	KB	L/B ratio	Grain type	AC (%)	GC (mm)
ISM	4.92	1.84	2.66	MS	21.47	22
RPBio Patho-1	5.09	1.80	2.84	MS	22.57	22
RPBio Patho-2	5.20	1.81	2.88	MS	23.93	22
ISM-9-18-217-15	4.92	1.88	2.62	MS	22.58	22
ISM-9-18-217-18	5.19	1.85	2.81	MS	23.40	22
ISM-22-102-179-8	5.16	1.76	2.93	MS	22.79	22
ISM-22-102-179-13	5.28	1.82	2.89	MS	23.84	22
IC-12-9	5.08	1.80	2.83	MS	21.64	22
IC-12-199	4.73	1.84	2.57	MS	22.02	22
IC-12-305	5.18	1.95	2.66	MS	24.28	24

ISM reccurent parent, *RPBio Patho-1*, *RPBio Patho-2* donor parents, *KL* kernel length, *KB* kernel breadth, *L/B* kernel length/kernel breadth, *AC* amylose content, *GC* gel consistency

compared to ISM in the later generations of backcrossing and intercrossing.

The significant contribution of this study is the achievement of complete recovery of yield, yield related traits along with grain yield and cooking quality related characters of ISM in the derived lines of ISM possesing, *Pi2* and *Pi54* along with achievement of a marginal improvement in its panicle exsertion and yield in some of the improved lines (Tables 2, 3). Even though in a recent study, improved breeding lines of Samba Mahsuri possessing blast and BB resistance have been developed (Madhavi et al. 2016), the improved breeding lines developed in the reported study were confirmed to be possessing only *Xa21* and *xa13* and not *xa5*. However, in our study, we have ensured transfer of the complete complement of the bacterial blight resistance genes, viz., xa5, *xa13* and *Xa21* into Samba Mahsuri along with transfer of the two blast resistance genes and hence it can be considered as an improvement over the report of Madhavi et al. (2016). In addition, we have also analyzed the key grain and cooking quality attributes of the pyramided lines of ISM possessing *Pi2* and *Pi54* and demonstrated their equivalence to ISM.

The improved versions of Samba Mahsuri possessing BB and blast resistance, developed in the present study may confer a distinct advantage to Samba Mahsuri farmers whose fields are affected by both BB and blast. Further, the improved lines of ISM developed in this study can also be used as donors to transfer BB and blast resistance into other genetic backgrounds as they possess high yield and medium-slender grain type. Among the improved lines of ISM, the intercross derived line # IC-12-305-106 (possessing *Xa21* + *xa13* + *xa5* +*Pi2* + *Pi54*) possessed very good phenotype and better panicle exerstion has been identified as one of the best line (Table 2) and this line will shortly be nominated for multi-location trials across India.

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Compliance with ethical standards

Conflict of interest All author declares that they have no conflict of interest.

References

Abhilash KV, Balachiranjeevi CH, Bhaskar NS, Rambabu R, Rekha G, Madhav KR, Vijay S, Pranathi K, Harika G, Mahadevaswamy HK, Anila M, Hajira SK, Yugander A, Hariprasad AS, Madhav M, Laha GS, Balachandran SM, Sundaram RM, Prasad MS (2016a) Marker-assisted introgression of bacterial blight and blast resistance genes into RPHR 1005, restorer line of the popular rice hybrid, DRRH-3. J Plant Biochem Biotechnol 25(4):400–409

Abhilash KV, Balachiranjeevi CH, Bhaskar NS, Rambabu R, Rekha G, Vijay S, Pranathi K, Harika G, Aruna J, Hajira SK, Mahadevaswamy HK, Anila M, Yugander A, Hariprasad AS, Madhav MS, Laha GS, Balachandran SM, Prasad MS, Sundaram RM (2016b) Marker-assisted improvement of the elite restorer line of rice, RPHR-1005 for resistance against Bacterial blight and Blast. J Genet 95(4):895–903

Abhilash KV, Balachiranjeevi CH, Bhaskar NS, Rambabu R, Rekha G, Madhavi KR, Vijay S, Pranathi K, Harika G, Mahadeva SHK, Anila M, Hajira SK, Yugander A, Hariprasad AS, Madhav MS, Laha GS, Balachandran SM, Sundaram RM, Prasad MS (2016c) Development of gene-pyramid lines of the elite restorer line, RPHR-1005 possessing durable bacterial blight and blast resistance. Front Plant Sci 7:1195

Balachiranjeevi CH, Bhaskar NS, Abhilash V, Akanksha S, Viraktamath BC, Madhav MS, Hariprasad AS, Laha GS, Prasad MS, Balachandran SM, Neeraja CN, Satendra Kumar M, Senguttuvel P, Kemparaju KB, Bhadana VP, Ram T, Harika G, Mahadeva Swamy HK, Hajira SK, Yugander A, Pranathi K, Anila M, Rekha G, Kousik MBVN, Dilip Kumar T, Kulkarni SR, Giri A, Sundaram RM (2015) Marker-assisted introgression of bacterial blight and blast resistance into DRR17B, an elite, fine-grain type maintainer line of rice. Mol Breed 35:151

Cagampang GB, Perez CM, Juliano BO (1973) A gel consistency test for eating quality of rice. J Sci Food Agric 24:1589–1594

Das J, Rao JN (2015) Molecular marker assisted gene stacking for biotic and abiotic stress resistance genes in an elite rice cultivar. Front Plant Sci 6:698

Ellur RK, Khanna A, Yadav A, Pathania S, Rajashekara H, Singh VK, Gopalakrishnan S, Bhowmick PK, Nagarajan M, Vinod KK, Prakash G, Mondal KK, Singh NK, Prabhu KV, Singh AK (2016) Improvement of Basmati rice varieties for resistance to blast and bacterial blight diseases using marker assisted backcross breeding. Plant Sci 242:330–334

FAO (2015) The State of Food and Agriculture. Rome. http://www.fao.org

Fjellstrom R, McClung AM, Shank AR (2006) SSR markers closely linked to the Pi-z locus are useful for selection of blast resistance in a broad array of rice germplasm. Mol Breed 17:149–157

Freeman GH, Gomez KA, Gomez AA (1978) Statistical procedures for agricultural research with emphasis on rice. Biometrics 34(4):721

Hajira SK, Sundaram RM, Laha GS, Yugander A, Balachandran SM, Viraktamath BC, Sujatha K, Balachiranjeevi CH, Pranathi K, Anila M, Bhaskar S, Abhilash KV, Mahadevaswamy HK, Kousik M, Dilipkumar T, Harika G, Rekha G (2016) A Single-Tube, functional marker-based multiplex PCR assay for simultaneous detection of major bacterial blight resistance genes in rice, *Xa21*, *xa13* and *xa5*. Rice Sci 23:1224–1227

Hari Y, Srinivasarao K, Basavraj C, Viraktamath A, Hari Prasad S, Laha GS, Ahmed IM, Natrajkumar P, Sujatha K, Prasad MS, Pandey M, Ramesha MS, Neeraja CN, Balachandran SM, Rani NS, Balachandra K, Madan Mohan K, Venkata S, Arun Sama K, Shaik Hajira, Balachiranjeevi C, Pranathi K, Reddy Ashok, Seshumadhav M, Sundaram RM (2013) Marker assisted

introgression of bacterial blight and blast resistance into IR58025B, an elite maintainer line of rice. Plant Breed. https://doi.org/10.1111/pbr.12056

Hua LX, Liang LQ, He XY, Wang L, Zhang WS, Liu W, Liu XQ, Lin F (2015) Development of a marker specific for the rice blast resistance gene *Pi39* in the Chinese cultivar Q15 and its use in genetic improvement. Biotechnol Biotechnol Equip 29(3):448–456

Imam J, Alam S, Mandal NP, Variar M, Shukla P (2014) Molecular screening for identification of blast resistance genes in North East and Eastern Indian rice germplasm (*Oryza sativa* L.) with PCR based makers. Euphytica 196:199–211

Juliano BO, Antonio AA, Esmama BV (1973) Effect of protein content on distribution and properties of rice protein. J Sci Food Agric 24:295–306

Kauffman HE, Reddy APK, Hsieh SPY, Merca SD (1973) An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. Plant Dis Rep 56:537–540

Khanna A, Sharma V, Ellur RK, Shikari AB, Gopala Krishnan S, Singh UD, Prakash G, Sharma TR, Rathour R, Variar M, Prashanthi SK, Nagarajan M, Vinod KK, Bhowmick PK, Singh NK, Prabhu KV, Singh BD, Singh AK (2015) Development and evaluation of near-isogenic lines for major blast resistance gene(s) in Basmati rice. Theor Appl Genet 128:1243–1259

Khush GS, Jena KK (2009) Current status and future prospects for research on blast resistance in rice (*Oryzasativa L.*) Adv Genet Genom Control Rice Blast Dis 1–10

Laha GS, Reddy CS, Krishnaveni D, Sundaram RM, Prasad MS, Ram T, Muralidharan K and Viraktamath BC (2009) Bacterial blight of rice and its management DRR Technical Bulletin No 41. Directorate of Rice Research (ICAR), Rajendra nagar, Hyderabad 1–37

Mackill DJ, Bonman JB (1992) Inheritance of blast resistance genes in near isogenic lines of rice. Phytopathology 82:746–749

Madhavi KR, Prasad MS, Laha GS, Mohan MK, Madhav MS, Viraktamath BC (2011) Combining blast and bacterial blight resistance in rice cultivar, Improved Samba Mahsuri. Indian J Plant Prot 39:124–129

Madhavi KR, Rambabu R, Abhilash Kumar V, Vijay Kumar S, Aruna J, Ramesh S, Sundaram RM, Laha GS, Madhav MS, Ravindra Babu V, Prasad MS (2016) Marker assisted introgression of blast (*Pi-2* and *Pi-54*) genes into the genetic background of elite, bacterial blight resistant indica rice variety, Improved Samba Mahsuri. Euphytica 212:331–342

Melissa A, Fitzgerald SR, McCouch Robert D H (2009) Not just a grain of rice: the quest for quality. Trends Plant Sci 14(3):1360–1385

Mohan K, Madhavi KR, Kumar V, Virakatamath BC (2011) Rice blast disease and its management technical bulletin No. 57. Directorate of Rice Research (ICAR) Hyderabad 52

Pradhan SK, Nayak DK, Mohanty S, Behera L, Barik SR, Pandit E, Lenka S, Anandan A (2015) Pyramiding of three bacterial blight resistance genes for broad-spectrum resistance in deepwater rice variety, Jalmagna. Rice 8:19

Prasad MS, Madhav MS, Laha GS, Lakshmi DL, Krishnaveni D, Mangrauthia SK, Balachandran SM, Sundaram RM, Arunakanthi B, Mohan KM, Madhavi KR, Kumar V, Viraktamath BC (2011) Rice blast disease and its management. Directorate of rice research (ICAR). Tech Bullet 57:52

Rai AK, Kumar SK, Gupta SK, Gautam N, Singh NK, Sharma TR (2011) Functional complementation of rice blast resistance gene *Pi-k^h* (*Pi54*) conferring resistance to diverse strains of *Magnaporthe oryzae*. J Plant Biochem Biotechnol 20(1):55–65

Ramkumar G, Srinivasa Rao K, Mohan MK, Sudarshan I, Sivaranjani AKP, Gopalakrishna K, Neeraja CN, Balachandran SM, Sundaram RM, Prasad MS, Shobha Rani N, Ram Prasad AM, Viraktamath BC, Madhav MS (2011) Development and validation of functional marker targeting an In Del in the major rice blast disease resistance gene *Pi54 (Pikh)*. Mol Breed 27:129–135

Ronald PC, Albano B, Tabien R, Abenes MLP, Wu KS, McCouch SR, Tanksley SD (1992) Genetic and physical analysis of the rice bacterial blight disease resistance locus *Xa21*. Mol Gen Genet 236:113–120

Shanti ML, Shenoy VV, Lalithadevi G, Mohan Kumar V, Premalatha Naveen P, Kumar G, Shashidhar HE, Zehr UB, Freeman WH (2010) Marker-assisted breeding for resistance to bacterial leaf blight in popular cultivar and parental lines of hybrid rice. J Plant Pathol 92:495–501

Sharma TR, Madhav MS, Singh BK, Shanker P, Jana TK, Dalal V, Pandit A, Singh A, Gaikwad K, Upreti HC, Singh NK (2005) High resolution mapping, cloning and molecular characterization of the *Pik^h* gene of rice, which confers resistance to *M. grisea*. Mol Genet Genom 274:569–578

Sharma TR, Rai AK, Gupta GK, Singh NK (2010) Broad spectrum blast resistance gene *Pikh* cloned from the rice line tetep designated as *Pi54*. J Plant Biochem Biotechnol 19:1

Sharma TR, Rai AK, Gupta SK, Vijayan J, Devanna BN, Ray S (2012) Rice blast management through host-plant resistance: retrospect and prospects. Agric Res 1:37–52

Singh AK, Gopala Krishnan S, Singh VP, Prabhu KV, Mohapatra T, Singh NK, Sharma T, Nagarajan M, Vinod KK, Singh D, Singh UD, Chander S, Atwal SS, Seth R, Singh VK, Ellur RK, Singh A, Anand D, Khanna A, Yadav S, Goel N, Singh A, Shikari AB, Singh A, Marathi B (2011) Marker assisted selection: a paradigm shift in Basmati breeding. Indian J Genet Plant Breed 71(2):120–128

Sundaram RM, Vishnupriya MR, Biradar SK, Laha GS, Reddy AG, Rani NS, Sarma NP, Sonti RV (2008) Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite indica rice variety. Euphytica 160:411–422

Sundaram RM, Priya MRV, Laha GS, Shobha Rani N, Srinivasa Rao P, Balachandran SM, Ashok Reddy G, Sarma NP, Sonti RV (2009) Introduction of bacterial blight resistance into Triguna, a high yielding, mid-early duration rice variety by molecular marker assisted breeding. Biotechnol J 4:400–407

Tanweer FA, Rafii MY, Sijam K, Rahim HA, Ahmed F, Latif MA (2015) Current advance methods for the identification of blast resistance genes in rice. C. R. Biol. 338:321–334. https://doi.org/10.1016/j.crvi.2015.03.001

Usatov AV, Koatylev PI, Azarin KV, Markin NV, Makarenko MS, Khachumov VA, Bibov M, Yu (2016) Introgression of the rice blast resistance genes *Pi1*, *Pi2* and *Pi33* into Russian rice varieties by marker-assisted selection. Indian J Genet Plant Breed 76(1):18–23

Van Berloo R (1999) GGT Software for display of graphical genotypes. J Hered 90:328–329

Variar M, Vera CCM, Carrillo MG, Bhatt JC, Sangar RBS (2009) Rice blast in India and strategies to develop durably resistant cultivars. Adv Genet Genom Control Rice Blast Dis 359–374

Victoria CJ, Zeigler RS (1993) Field breeding for durable rice blast resistance in the presence of diverse pathogen populations. Curr Plant Sci Biotechnol Agric 18:215–218

Wen S, Gao B (2012) Introgressing blast resistant gene *Pi-9(t)* into Elite rice restorer Luhui17 by marker-assisted selection. Rice Genom Genet 2(4):31–36

Zheng K, Huang N, Bennett J, Khush GS (1995) PCR-based marker assisted selection in rice breeding. IRRI Discussion Paper Series No 12. International Rice Research Institute, Manila, Philippines