Mol Breeding (2015) 35:151
DOI 10.1007/s11032-015-0348-8

# Marker-assisted introgression of bacterial blight and blast resistance into DRR17B, an elite, fine-grain type maintainer line of rice

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Received: 7 February 2015 / Accepted: 1 July 2015


**Abstract** DRR17A is a stable wild-abortive cytoplasmic male sterile line with medium-slender grain type. DRR17A and its maintainer line DRR17B are highly susceptible to two of the major rice diseases, bacterial blight (BB) and blast. To improve DRR17B for resistance against BB and blast, we have introgressed a major dominant gene each conferring resistance against BB (*Xa21*) and blast (*Pi54*) into the maintainer line through marker-assisted backcross breeding using RP-Bio-Patho-2 (a near-isogenic line of Samba Mahsuri possessing *Xa21* and *Pi54*) as the donor parent. PCR-based molecular markers tightly linked to *Xa21* and *Pi54* were used for foreground selection of the resistance plants at each backcross generation, while molecular markers tightly linked to the major fertility restorer genes, *Rf3* and *Rf4*, were used for negative selection (i.e. selection of plants possessing non-fertility-restoring alleles at the two loci) at $BC_1$ generation. After foreground selection for the target genes at each backcross generation, the 'positive' plants were screened with parental polymorphic markers for identifying backcross plants possessing maximum recovery of DRR17B genome. Marker-assisted backcrossing was continued till $BC_3$ generation, and a single $BC_3F_1$ plant possessing the target genes with ~94 % recovery of recurrent parent genome was identified and selfed to generate $BC_3F_2$s. A total of six homozygous $BC_3F_2$ plants were identified and advanced. At $BC_3F_5$, six promising, stable, backcross-derived lines possessing high level of resistance against BB and blast, high yield, short plant stature, fine-grain type, have been identified; their maintenance ability and heterotic potential validated through test crosses and these lines are being converted to CMS lines through marker-assisted breeding.

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**Electronic supplementary material** The online version of this article (doi:10.1007/s11032-015-0348-8) contains supplementary material, which is available to authorized users.

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Published online: 08 July 2015

**Keywords** MABB · Bacterial blight · Blast · Pyramiding · Maintainer line improvement · DRR17B

## Introduction

India needs to produce at least 125 million tonnes of rice by year of 2020, to meet the demands of an increasing population. Among the various technological options available for increasing rice production, large-scale adoption of hybrid rice is the most promising one (Ahmed and Siddiq 1998). Hybrids yield 15–20 % over inbred rice varieties (Virmani 1996) and at least 70 public and private bred hybrids are available for commercial cultivation in India. However, most of the hybrids released to date in India and abroad are highly susceptible to biotic stresses like bacterial blight (BB) and blast (Khush and Jena 2009). DRR17A is an elite, highly stable wild-abortive cytoplasmic male sterile (WA-CMS) line, possessing highly desirable medium-slender grain type and developed by the Directorate of Rice Research, Hyderabad, India (AS Hariprasad, Personal Communication). The WA-CMS line is being used for developing promising three-line hybrids at DRR. However, DRR17A and its maintainer line DRR17B are highly susceptible for BB and blast and have a slightly taller plant stature which is not ideal for a good WA-CMS/maintainer line.

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most important diseases of rice worldwide (Mew 1987) and causes yield loss ranging from 74 to 81 % (Srinivasan and Gnanamanickam 2005). Deployment of host plant resistance is the only effective strategy for management of the disease, and till date more than 38 BB resistance genes have been identified (Cheema et al. 2008; Sujatha et al. 2011; Hari et al. 2013). Some of the major resistance genes like *Xa1, Xa4, xa5, Xa7, xa8, xa13*, *Xa21* and *Xa27* have been tagged and mapped by closely linked molecular markers (Sonti 1998; Rao et al. 2002; Sundaram et al. 2014). Some of the BB resistance genes are dominant in nature (e.g. *Xa21*, *Xa33* and *Xa38*). Availability of tightly linked molecular markers in rice has enabled marker-assisted breeding (MAB) for bacterial blight resistance, a reality in India and abroad (Abenes et al. 1993; Yoshimura et al. 1995; Zhang et al. 1996; Huang et al. 1997; Sanchez et al. 2000; Davierwala et al. 2001; Singh et al. 2001; Joseph et al. 2004; Leung et al. 2004; Perez et al. 2008; Sundaram et al. 2008, 2009; Basavaraj et al. 2010; Hari et al. 2011, 2013). The major dominant BB resistance gene *Xa21* was originally introgressed from the wild rice, *Oryza longistaminata* (Ronald et al. 1992; Song et al. 1995), which conferred broad-spectrum of resistance against most of the virulent isolates existing in India. Most importantly, a very closely linked PCR-based marker, pTA248 is available for marker-assisted selection of the gene (Ronald et al. 1992). Many earlier studies have shown that through marker-assisted breeding, *Xa21* can be successfully introgressed into elite rice varieties (Joseph et al. 2004; Gopalakrishnan et al. 2008; Sundaram et al. 2008, 2009; Perumalsamy et al. 2010; Pandey et al. 2013) and into hybrid rice parental lines (Chen et al. 2001; Liyong et al. 2003; Basavaraj et al. 2010; Hari et al. 2011, 2013).

Rice blast, caused by the fungus *Magnaporthe grisea* (anamorph *Pyricularia oryzae*), is a devastating disease, which leads to significant yield loss up to 70–80 % during an epidemic (Khush and Jena 2009). Even though chemicals are available for control of blast, deployment of host plant resistance is one of the best options for managing the disease (Hulbert et al. 2001). At least, 100 genes conferring resistance against blast disease and 347 quantitative trait loci (QTL) associated with blast resistance have been identified (Ballini et al. 2008; Koide et al. 2009), and 19 resistance genes have been cloned and characterized (Sharma et al. 2012). The *Pi54* blast resistance gene was mapped on chromosome 11L from the Vietnamese rice genotype, Tetep that conferred the resistance against predominant races of the pathogen in India (Sharma et al. 2010). Tightly linked (Sharma et al. 2005) and functional markers (Ramkumar et al. 2010) are available for marker-assisted selection of the gene, and a few research groups in India have successfully introgressed *Pi54* into varieties (Narayanan et al. 2002; Joseph et al. 2004; Sundaram et al. 2008, 2009) and parental lines (Basavaraj et al. 2010; Singh et al. 2011; Zhan et al. 2012; Hari et al. 2011, 2013).

As there is an imminent need to improve DRR17B for BB and blast resistance and with the availability of very closely linked/functional markers for both BB

resistance gene, *Xa21*, and the blast resistance gene, *Pi54,* the present study was initiated.

## Materials and methods

### Plant material

RP-Bio-Patho-2 (NIL of Samba Mahsuri, possessing *Xa21* and *Pi54*) was used as the donor parent for bacterial blight and blast resistance genes. DRR17B, the maintainer line of an elite wild-abortive CMS line-DRR17A, was used as the recurrent parent. In addition to these, Taichung Native 1(TN1), HR12 and Tetep were used as susceptible and resistant checks for blast screening, while the recurrent parent and donor parents were used as susceptible and resistant checks, respectively, while screening for BB resistance.

### Marker-assisted selection for BB and blast resistance, fertility restoration

For targeted introgression of *Xa21* and *Pi54* into DRR17B, a marker-assisted backcross breeding programme was adopted. Backcrossing was performed till $BC_3$ generation, after which the plants were advanced through pedigree method. DNA was isolated from the parents and backcross progenies by following the protocol of Zheng et al. (1995). The PCR-based STS marker pTA248 (Ronald et al. 1992), functional marker *Pi54* MAS (Ramkumar et al. 2010) were used to identify the allelic status of *Xa21* and *Pi54* at $BC_1F_1$ and subsequent backcross generations, while the SSR markers DRCG-RF4-14/DRCG-RF4-8 and DRRM-RF3-10/DRRM-RF3-5 (Balaji et al. 2012) closely linked to the major fertility restorer genes, *Rf4* and *Rf3*, were used at $BC_1F_1$ generation to identify plants possessing the non-fertility-restoring allele (i.e. *rf4rf4* and *rf3rf3*) in homozygous condition. Information about the markers used for foreground selection is given in Supplementary Table 1. A set of 79 parental polymorphic SSR markers spread across the 12 chromosomes of rice were utilized for background selection at $BC_2F_4$ generation.

PCR was performed using 1 U of Taq DNA polymerase (Bangalore Genei, Bangalore, India) and 1X PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 1.8 mM MgCl and 0.01 mg/ml gelatin), 5 Pico moles of each primer, 0.05 mM dNTPs and 50 ng template DNA in 25 µl reaction volume with a thermal profile of 94 °C for 5 min (initial denaturation), followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min and a final extension of 7 min at 72 °C. The amplified product of pTA248 (*Xa21*) was electrophoretically resolved on a 1.5 % Seakem LE agarose gel (Lonza, Rockland, ME, USA); the amplicons of *Pi54* MAS (*Pi54*) were dissolved in 2 % agarose, the amplicons of DRCG-RF4-14/DRCG-RF4-8 (*Rf4*) in 2.5 % agarose gel and the amplicons of RRM-RF3-10/DRRM-RF3-5 *(Rf3*), in 3.5 % agarose gel. Those of SSR markers used for background selection were resolved on a 3.5 % agarose gel. Seakem LE agarose gels containing 0.5 mg/ml of ethidium bromide in 0.5X TBE buffer were visualized under UV.

### Screening for BB resistance

Four virulent isolates of the bacterial blight pathogen, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) collected from BB hot-spot locations in India, viz. DX-020 (Hyderabad, Telangana), DX-002 (Faizabad, Uttar Pradesh), DX-066 (Raipur, Chhattisgarh) and DX-049 (Maruteru, Andhra Pradesh), were used to screen the donor and recurrent parents along with backcross-derived lines of DRR17B for BB resistance under both glasshouse and field conditions. The *Xoo* strains were cultured and stored as described by Laha et al. (2009). The rice plants were clip-inoculated with a bacterial suspension of $10^{8-9}$ cfu/ml at maximum tillering stage (45–55 days after transplanting) through the methodology of Kauffman et al. (1973). Approximately 5–10 leaves were inoculated per plant, and disease reaction was scored 14 days after inoculation. Individual plants of the segregating backcross generations were screened under field condition following the same procedure. In addition to measurement of BB lesion length, the disease score was also calculated as per IRRI standard evaluation system (IRRI-SES) scale, which is based on per cent diseased leaf area (IRRI 1996).

### Screening for blast resistance

A local isolate of *Magnaporthe oryzae* (SPI-40) from Directorate of Rice Research (DRR), Hyderabad,

Andhra Pradesh, India (Madhan Mohan 2011), was used to screen the donor and recurrent parents along with backcross-derived lines of DRR17B for blast resistance under in vivo conditions following uniform blast nursery (UBN) method at Directorate of Rice Research (DRR), Hyderabad, India. The pathogen strains were cultured and stored as described by Srinivas Prasad et al. (2011). The young seedlings at four-leaf stage were inoculated with the fungal conidial suspension at a concentration of $1 \times 10^5$ conidia/ml, and high relative humidity was maintained for disease development. Inoculated seedlings were monitored for the development of blast lesions 1 week after inoculation. The plants were scored and evaluated on a 0–9 scale as per IRRI-SES scale (IRRI 1996).

### Screening for agro-morphological characteristics

Thirty-day-old seedlings of the selected $BC_3F_5$ lines along with parents were transplanted in the Rajendranagar Experimental farm of Indian Institute of Rice Research, Hyderabad, India, at a spacing of $15 \times 20$ cm in 12-row plots in three replications. Standard agronomic practices were followed to raise a healthy crop, which were evaluated during the wet season (June–November) in 2013. Data were recorded for the agronomic traits, viz. plant height (cm), spikelet fertility (%), productive tillers (number), panicle length (cm), grains per panicle (number) and days to 50 % flowering.

### Evaluation of heterosis of the experimental hybrids developed from improved DRR17B

Four selected backcross-derived lines (viz., GP17 B112-3-98-118-12-204-8, GP17B112-3-98-118-12-204-15, GP17B112-3-98-118-12-204-90 and GP17B 112-3-98-118-12-204-234) and DRR17B were crossed with the elite restorer line RPHR1005 to evaluate grain yield standard heterosis of the derived hybrids during wet season 2014. The popular, high-yielding variety, BPT5204 (Samba Mahsuri), possessing medium-slender grain type served as the varietal check, while the elite hybrid, DRRH3, possessing medium-slender grain type served as the hybrid check.

## Results

### Introgression of BB and blast resistance genes into DRR17B background

The heterozygosity of the $F_1$s developed by crossing RP-Bio-Patho-2 (NIL of BPT5204, possessing *Xa21* and *Pi54*) and the recurrent parent DRR17B was confirmed using pTA248, the co-dominant marker specific for *Xa21*, and *Pi54* MAS, functional marker for *Pi54*. The positive $F_1$ plants were then backcrossed with DRR17B to obtain $BC_1F_1$. A total of 261 $BC_1F_1$ plants were genotyped, of which 43 plants were double positives (i.e. *Xa21* + *Pi54*; Fig. 1). These were then screened with *Rf3*- and *Rf4*-specific markers to identify plants devoid of the two genes (i.e. *rf3rf3, rf4rf4*), and a total of nine such plants were identified (Supplementary Figure 1). These were then subjected to background selection using 79 parental polymorphic SSRs to identify plants possessing maximum recurrent parent genome recovery. The best $BC_1F_1$ plant GP17B112, possessing a recovery of 73.41 % was identified and used for the next backcross generation. Backcrossing was continued up to $BC_3$ generation, wherein a $BC_3F_1$ plant possessing *Xa21* + *Pi54* and 93.4 % recovery of DRR17B genome was identified and selfed to generate $BC_3F_2$s, which were then screened with gene-specific markers to identify homozygous plants. From a total of 544 $BC_3F_2$ plants, a total of 52 plants homozygous for both *Xa21* and *Pi54* were identified and advanced. At $BC_3F_5$, a total of six promising lines possessing grain type and agro-morphological traits similar to DRR17B were identified (viz. GP17B112-3-98-118-12-204-8, GP17B112-3-98-118-12-204-15, GP17B 112-3-98-118-12-204-90, GP17B 112-3-98-118-12-204-168, GP17B112-3-98-118-12-204-234, GP17B 112-3-98-118-12-204-276), and these were then screened for BB and blast resistance. The details of number of plants screened, number of plants identified to possess non-restoring alleles at *Rf3* and *Rf4* and percentage recurrent parent genome recovery of the best selected plant at each backcross generation are given in Supplementary Table 2.

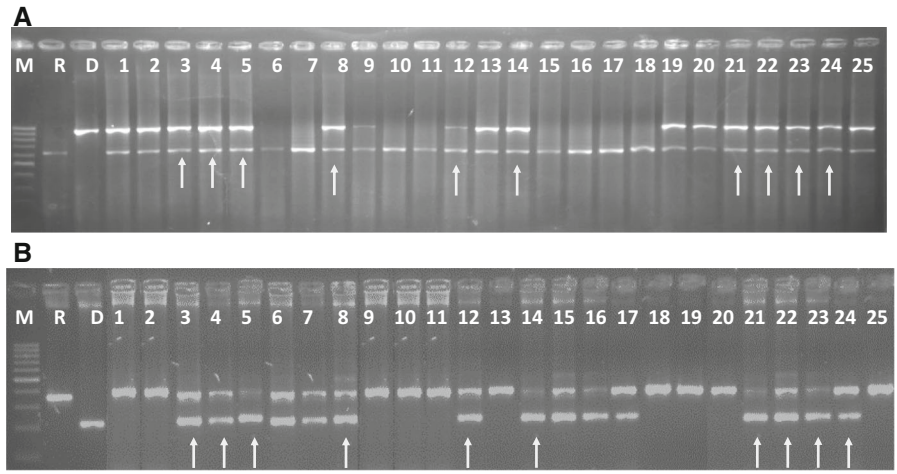


**Fig. 1** Foreground selection for *Xa21* and *Pi54* among BC1F1 plants using gene-specific markers. The BC1F1 plants were screened through PCR for *Xa21* gene using the marker pTA 248 (**a**) and for *Pi54* gene using the marker *Pi54* MAS (**b**). *M* marker, *R* recurrent parent (i.e. DRR17B) and *D* donor parent (i.e. RP-Bio-Patho-2). *Arrows* indicates plants which were heterozygous (i.e. positive) for both the genes

### Evaluation of BB and blast resistance of the selected backcross-derived lines

The selected backcross-derived lines (mentioned above) were evaluated for their resistance to BB and blast under glass house condition. The resistance check Tetep harbouring the blast gene *Pi54* showed a blast disease score of 1, whereas the susceptible checks, DRR 17B and HR12, showed a score of 9. The selected six backcross-derived lines showed a very high level of resistance with score of 0 (Fig. 2a). With respect to screening for bacterial blight, the donor RP-Bio-Patho-2 displayed a score of 3, whereas the recurrent parent, DRR17B, showed score of 9. The selected backcross-derived lines showed a very high level of resistance against BB with a score of 3 (Fig. 2b). The details of results of screening of the selected lines against BB and blast are given in Table 1.

### Selection for agro-morphological and yield characters in backcross-derived lines

The selected six $BC_3F_5$ backcross-derived lines showing good level of resistance against BB and blast disease were evaluated for agro-morphological traits. Significant difference was observed in plant height, whereas no such difference was observed in number of productive tillers, panicle length and number of grains per panicle for these lines when compared with the recurrent parent (Table 2). Further except two lines viz. GP17B112-3-98-118-12-204-168 and GP17B112-3-98-118-12-204-276 remaining lines displayed good maintenance ability when test-crossed with IR58025A and DRR17B (Table 2).

### Evaluation of heterosis of hybrids derived by crossing improved versions of DRR17B lines with the elite restorer line, RPHR1005

The four elite, improved versions of DRR17B along with DRR17B were crossed with popular restorer line RPHR1005 to assess their heterotic potential. Two improved lines, viz. GP17B112-3-98-118-12-204-15 and GP17B112-3-98-118-12-204-234, showed a higher level of heterosis as compared to the crosses between DRR17B and the restorer line (Table 3). Further, all the hybrids developed from the improved DRR17B lines were resistant to BB and blast and possessed MS grain type (data not shown), indicating that the improved versions have good potential for development of high-yielding, disease-resistant rice hybrids after their conversion to CMS lines.

## Discussion

DRR17B is a recently developed maintainer line of rice, possessing stable maintenance ability, with medium duration and having semi-tall plant type, and medium-slender (MS) grain type. As DRR17A and its maintainer parent, DRR17B, are highly susceptible to two important diseases, bacterial blight and blast, the present study was aimed at targeted improvement of the maintainer parent for disease resistance by introgressing a major dominant gene

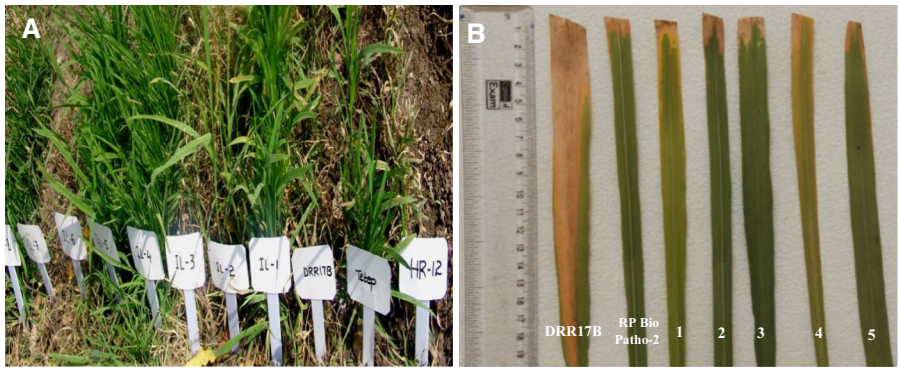


**Fig. 2** Screening of selected BC3F5 plants for blast and bacterial blight resistance. **a** Screening against rice blast disease through uniform blast nursery (UBN) method revealed that the susceptible check HR12 and recurrent parent DRR17B were highly susceptible to blast disease, while the resistant donor (i.e. check), Tetep, along with the selected backcross-derived lines at BC3F5 generation (viz., IL-1: GP17B112-3-98-118-12-204-8, IL-2: GP17B112-3-98-118-12-204-15, IL-3: GP17B112-3-98-118-12-204-90, IL-4: GP17B 112-3-98-118-12-204-168, IL-5: GP17B112-3-98-118-12-204-234, IL-8: GP17B112-3-98-118-12-204-276) showed high level of resistance. **b** Screening against bacterial blight disease under glasshouse conditions revealed that the recurrent parent, DRR17B, was highly susceptible to the disease, while the donor parent, RP-Bio-Patho-2, and the selected backcross-derived lines at BC3F5 generation (viz. 1: GP17B112-3-98-118-12-204-8, 2: GP17B 112-3-98-118-12-204-15, 3: GP17B112-3-98-118-12-204-90, 4: GP17B 112-3-98-118-12-204-168, 5: GP17B112-3-98-118-12-204-234) were highly resistant to bacterial blight

**Table 1** Phenotypic screening of selected $BC_3F_5$ lines against BB and blast diseases

| S. No | Line/genotype | Phenotypic screening for resistance against BB and Blast | |
|---|---|---|---|
| | $BC_3F_5$ lines | BB# | Blast* |
| 1 | GP17B112-3-98-118-12-204-8 | 1.97 ± 0.23 | 1 |
| 2 | GP17B112-3-98-118-12-204-15 | 2.30 ± 0.20 | 1 |
| 3 | GP17B112-3-98-118-12-204-90 | 2.07 ± 0.14 | 1 |
| 4 | GP17B112-3-98-118-12-204-168 | 2.23 ± 0.08 | 1 |
| 5 | GP17B112-3-98-118-12-204-234 | 2.23 ± 0.28 | 1 |
| 6 | GP17B112-3-98-118-12-204-276 | 2.20 ± 0.26 | 1 |
| | Parents/checks | | |
| 7 | DRR 17B (recurrent parent) | 21.6 ± 0.70 | 9 |
| 8 | RP-Bio-Patho-2 (donor parent) | 2.13 ± 0.23 | 1 |
| 9 | Tetep | – | 1 |

# A total of five plants from each of the backcross-derived lines, the donor and recurrent parents were screened for BB resistance under glass house conditions, and lesion length (cm) was calculated as an average of five leaves per plant

* A total of twenty seedlings from each of the backcross-derived lines, the donor and recurrent parents were screened in the Uniform Blast Nursery (UBN), and disease score was calculated as per IRRI-SES (IRRI 1996)

each conferring resistance against BB (i.e. *Xa21*) and blast (*Pi54*) through MABB, so that the hybrids developed from the improved version of the maintainer line would also be resistant.

Backcross breeding is one of the most commonly deployed methods for improvement of one or few target traits which are lacking in elite crop varieties (Stoskopf et al. 1993). This is particularly true with respect to parental lines of rice hybrids wherein targeted and precise improvement of one or few traits is usually desired, as gross changes in the genetic background of an elite maintainer or restorer line will make it unfit for use in heterosis breeding (Zhou et al. 2011). Molecular markers can aid backcross breeding, reducing the duration and the cost involved (Collard and Mackill 2008). There are many successful

**Table 2** Agro-morphological characteristics of six selected backcross-derived lines (Wet season 2013)

| S. No. | Selected $BC_3F_5$ line | Plant height (cm) | Spikelet fertility (%) (IR 58025A × selected $BC_3F_5$) | Productive tillers (number) | Panicle length (cm) | Grains per panicle (number) | Days to 50 % flowering |
|---|---|---|---|---|---|---|---|
| 1 | GP17B112-3-98-118-12-204-8 | 80.33 ± 0.88 | 0 | 10.30 ± 0.88 | 22.67 ± 0.33 | 308.00 ± 4.36 | 105 |
| 2 | GP17B112-3-98-118-12-204-15 | 77.00 ± 1.00 | 0 | 11.30 ± 0.33 | 24.67 ± 0.67 | 343.33 ± 5.55 | 97 |
| 3 | GP17B112-3-98-118-12-204-90 | 75.67 ± 0.67 | 0 | 12.70 ± 0.33 | 21.33 ± 0.88 | 281.33 ± 2.03 | 105 |
| 4 | GP17B112-3-98-118-12-204-168 | 79.33 ± 0.67 | 24 | 9.30 ± 0.33 | 24.67 ± 0.33 | 335.33 ± 4.33 | 100 |
| 5 | GP17B112-3-98-118-12-204-234 | 75.67 ± 0.67 | 0 | 10.30 ± 0.33 | 22.33 ± 0.33 | 300.67 ± 3.76 | 97 |
| 6 | GP17B112-3-98-118-12-204-276 | 77.67 ± 0.33 | 18 | 10.70 ± 0.33 | 24.33 ± 0.88 | 330.33 ± 8.25 | 100 |
| 7 | DRR 17B | 95 ± 0.66 | 0 | 10.70 ± 0.43 | 23.33 ± 0.63 | 284.00 ± 6.45 | 105 |
| 8 | RP-Bio-Patho-2 | 73.33 ± 0.86 | 0 | 11.30 ± 0.65 | 21.67 ± 0.33 | 190.33 ± 2.45 | 105 |

**Table 3** Grain yield and heterosis exhibited by the experimental hybrids derived by crossing the improved lines of DRR17B with RPHR1005

| S. No | Cross/genotype | Days to 50 % flowering | Single plant yield (g) | Grain yield heterosis over standard check for medium-slender (MS) grain type, BPT 5204 (%) | Grain yield heterosis over hybrid check DRRH3 (%) |
|---|---|---|---|---|---|
| 1 | DRR17B X RPHR 1005 | 103 | 27.2 ± 0.70 | 73.58 | 22.68 |
| 2 | GP17B112-3-98-118-12-204-8 X RPHR1005 | 97 | 21.5 ± 0.09 | 37.20 | −3.02 |
| 3 | GP17B112-3-98-118-12-204-15 X RPHR 1005 | 97 | 27.6 ± 0.61 | 76.13 | 24.49 |
| 4 | GP17B112-3-98-118-12-204-90 X RPHR 1005 | 103 | 25.8 ± 0.61 | 64.64 | 16.37 |
| 5 | GP17B112-3-98-118-12-204-234 X RPHR 1005 | 95 | 29.2 ± 0.47 | 86.34 | 31.70 |
| 6 | BPT 5204 (standard check for MS grain type) | 115 | 15.67 ± 0.44 | – | – |
| 7 | DRRH3 (hybrid check for MS grain type) | 100 | 22.17 ± 0.33 | – | – |

instances wherein marker-assisted backcross breeding has been deployed for targeted improvement of elite varieties and parental lines of rice hybrids. Marker-assisted selection (MAS) has been successfully applied for improving resistance against biotic stresses like bacterial blight, blast and BPH in rice (Narayanan et al. 2002; Joseph et al. 2004; Sundaram et al. 2008, 2009; Basavaraj et al. 2010; Sreewongchai et al. 2010; Hari et al. 2011, 2013; Singh et al. 2012; Zhan et al. 2012; Khanna et al. 2015). Breeders can easily transfer beneficial alleles into other genetic backgrounds, whenever DNA markers tightly linked to the resistance genes have been identified. There are many successful instances wherein marker-assisted backcross breeding has been deployed for targeted improvement of elite varieties and parental lines of rice hybrids. For example, Sundaram et al. (2008, 2009) improved two elite rice varieties, Samba Mahsuri and Triguna, for BB resistance by deploying *Xa21, xa13* and *xa5* through marker-assisted backcross breeding (MABB). Similarly, Singh et al. (2012) improved an elite basmati restorer line, PRR78, through MABB by introgressing two blast genes *Piz-5* and *Pi54*. Later, Hari et al. (2011, 2013) improved an elite restorer line,

KMR3R, and a popular maintainer line, IR58025B, for BB and blast resistance through MAS by combining two dominant resistance genes (*Xa21* and *Pi54*). Recently, Khanna et al. (2015) developed near-isogenic lines (NILs) of Pusa Basmati-1 possessing single major blast resistance genes, *Pi54*, *Pi1*, *Pita*, *Pi2*, *Pib*, *Pi5* and *Pi9,* through MABB. In the present study, we used previously reported DNA markers for bacterial blight and blast resistance along with stringent phenotype-based selection for agro-morphological characters to improve an elite maintainer line, i.e. DRR17B. This approach of combining MAS with phenotype-based selection can improve precision in breeding in the initial stages and can reduce costs significantly in the later stages of backcross breeding.

In the present study, we deployed two PCR-based functional markers, viz. pTA248 and *Pi54* MAS, for foreground selection of the BB and blast resistance genes, *Xa21* and *Pi54*, respectively. Precise foreground selection is vital for success of MABB (Hospital et al. 1997). As the two markers are functional markers, no recombination can be expected between the marker and the gene, thus ensuring 100 % selection accuracy. The $F_1$ plants and backcross plants were again validated with another marker, RM206, which is reported to be very closely linked to *Pi54* (Sharma et al. 2005). No recombination was noticed between *Pi54* MAS and RM206, indicating that both the markers are highly useful for tracking the introgression of *Pi54* in breeding populations. In addition to the target resistance genes, we deployed molecular markers closely linked to the major fertility restorer genes, *Rf3* and *Rf4,* at $BC_1F_1$ generation. This permitted us to identify resistance gene(s)-positive plants which also possessed non-restoring alleles in homozygous condition at $BC_1F_1$ generation. This was necessary as the donor parent has been characterized to possess *Rf4* through marker analysis (data not shown). In addition to foreground selection for the target genes and non-restoration trait, we also carried out background selection using large number (#12) of parental polymorphic SSR markers located on carrier chromosome (i.e. Chr. 11) and flanking the target resistance genes and a reasonable number of markers (# 4–6) located on non-carrier chromosomes to minimize the linkage drag, thus limiting the number of backcrosses. Earlier, Sundaram et al. (2008) used same strategy while improving the Samba Mahsuri for BB resistance through MABB. Even though the extent of recovery of the recurrent parent genome was considerably lower (Supplementary Table 2) than the expected value (i.e. 75 %) in the first backcross generation, by the third backcross generation, we were able to identify a resistance gene(s)-positive plant possessing ~93.4 % recovery of DRR17B genome, which is nearly equal to the expected value of 93.75 %. Further, only a small region of <2 Mb has been introgressed from the donor parent in the vicinity of *Xa21* and *Pi54* on chromosome 11 (i.e. target chromosome), and most of the non-target chromosomes have only negligible segments from the donor parent (i.e. RP-Bio-Patho-2). Further, the selected backcross plants also closely resembled DRR17B in terms of most agro-morphological traits and grain quality (Table 2) with introgression line # 2 (GP17B112-3-98-118-12-204-15) possessing maximum recovery of the recurrent parent genome on the target chromosome (Supplementary Figure 2), thus demonstrating the utility of background selection as reported earlier by Sundaram et al. (2008, 2009).

In the early generations of backcrossing, genotypic background analysis was carried out and the plants with maximum recurrent parent genome recovery were used for backcrossing up to $BC_3F_1$ generation. From $BC_3F_2$ to $BC_3F_4$ generations, strict phenotypic selections were followed and those plants which were similar to recurrent parent DRR17B in almost of the key agro-morphological traits (viz., number of days to flowering, number of tillers, grain type and gain number) were selected and advanced further. In $BC_3F_4$ generation, six stable lines which were similar to or better than DRR17B were identified. They were then subjected to genotypic background selection with parental polymorphic SSR markers spread all over the 12 chromosomes including markers flanking the gene of interest (Supplementary Figure 2). The process of intensive marker-assisted selection coupled in the initial stages of backcrossing coupled with a stringent phenotypic selection in the later stages resulted in near-complete recovery of the recurrent parent genome in all the six elite backcross-derived improved lines of DRR17B, with only small segments flanking the target genes possessing donor chromosomal introgression.

The advanced backcross-derived improved lines (ABILs) developed in the present study were screened for BB and blast resistance under controlled conditions by artificially inoculating with virulent isolates

of respective pathogens. All the six improved ABILs screened showed significantly higher level of resistance to both the diseases compared to the recurrent parent, DRR17B (Table 1). Bacterial blight resistance gene, *Xa21,* provides a broad-spectrum resistance to majority of the pathotypes in India barring a few reports (Shanti et al. 2010; Sundaram et al. 2008). *Xa21* has tremendous potential in BB resistance breeding programme and can be used either singly or in combination with other BB resistance genes (Song et al. 1995; Khush et al. 1990; Wang et al. 1996; Chen et al. 2000; Sundaram et al. 2008). This is evident from several recent reports (Joseph et al. 2004; Hari et al. 2011, 2013) where the gene had been deployed singly in hybrid rice parental lines. As no additional broad-spectrum, dominant BB resistance gene was available, when the study was initiated in 2008, we introgressed only *Xa21*. However, there are recent reports about identification and fine mapping of at least two broad-spectrum, wild-rice-derived BB resistance genes, *Xa33* (Natrajkumar et al. 2012; Shaik et al. 2014) and *Xa38* (Bhasin et al. 2012). We are in the process of introgressing *Xa33* in the genetic background of improved lines of DRR17B possessing *Xa21* and *Pi54*. Similar to the BB resistance *Xa21* gene, the blast resistance gene, *Pi54,* is known to be a dominant and broad-spectrum and is effective throughout India (Sharma et al. 2005; Rai et al. 2011; Singh et al. 2011; Hari et al. 2013), having deployed in many Indian hybrid rice parental lines and varieties. Even though, owing to the very dynamic nature of these pathogen, it is very difficult to predict the durability of resistance conferred by a single gene (i.e. *Pi54*), data from All India Coordinated Rice Improvement Project (DRR Progress report, Vol. 2, 2008–2013) clearly indicate that NILs of Samba Mahsuri and Swarna possessing *Pi54* are highly effective across the country. In these trials, which were carried out in >25 test centres, the *Pi54*-containing lines showed resistance reaction against multiple isolates with very low severity index values (2.6–2.7), which was significantly different when compared with the susceptible checks (SI values of 6.8–6.9). This clearly indicates that *Pi54* gene is very effective against most of the isolates across the country. However, in order to enhance the spectrum and durability of blast resistance, we have started introgressing an additional broad-spectrum blast resistance gene, *Pi2,* into DRR17B.

In molecular breeding programmes, breeders generally deploy MAS for improvement of one or few target traits and carry out phenotype-based selection for improvement or retaining certainly key agro-morphological and grain quality characters, for which reliable markers are not available. This strategy was earlier adopted by Joseph et al. (2004), Gopalakrishnan et al. (2008), Sundaram et al. (2008), Singh et al.(2011) and Hari et al. (2011, 2013). Based on the experience gathered from these studies, we adopted MAS for selection of lines possessing *Xa21, Pi54, rf3* and *rf4* and morphology-based selection for certain key agro-morphological characters like plant height, grain type, number of productive tillers and days to flower among the backcross-derived lines. At $BC_3F_5$ generation, we identified six lines (based on visual selection carried out from $BC_1$ generation onwards), viz. GP17B112-3-98-118-12-204-8, GP17B112-3-98-118-12-204-15, GP17B112-3-98-118-12-204-90, GP17B 112-3-98-118-12-204-168, GP17B112-3-98-118-12-204-234 and GP17B112-3-98-118-12-204-276, which were significantly shorter (75–80 cm) than the recurrent parent DRR17B (95 cm). For a CMS (or its maintainer line), possessing a short plant stature is an important morphological character (Virmani and Kumar 2004) as it assists in better seed set, provided that the restorer line is taller than the CMS lines. In addition, two ABILs (GP17B112-3-98-118-12-204-8 and GP17B112-3-98-118-12-204-90) were similar in duration to DRR17B 105 days, while some of the ABILs were significantly early in duration as compared to DRR17B (Table 2). This could be an important trait for development of hybrids in the early or mid-early category.

The ABILs were test-crossed with the WA-CMS line IR58025A to study their stability of sterility maintenance (Table 2). Except two lines, all the other ABILs showed complete sterility, and these are being crossed with DRR17A for line conversion through MAS, a strategy adopted by Hari et al. (2013). When the selected ABILs possessing stable maintenance and medium-slender grain type were crossed with a medium-slender (MS) grain type restorer, RPHR1005 (Ramesha et al. 2010), some of the newly developed hybrids were observed to possess not only medium-slender grain type, but also a higher level of heterosis as compared to the popular hybrid, DRRH3 (Table 3). Further, the experimental hybrids were resistant to BB and blast disease, indicating that high-yielding hybrids

possessing MS grain type along with BB and blast resistance could be developed from the improved lines of DRR17B after their line conversion.

All the improved lines showed good level of BB and blast resistance, while the recurrent parent DRR17B was observed to be completely susceptible for both the diseases (Table 1). Among the backcross-derived lines, we made rigorous phenotype-based selections for characters which were equivalent to or better than DRR17B (i.e. more number of grains per panicle, dwarf plant type, and early or late flowering). In this process, we could recover some of the backcross-derived lines possessing lesser plant height as compared to DRR17B (Table 2) more number of gains per panicle and those possessing early flowering plant type as compared to the recurrent parent (Table 2), while retaining the premium medium-slender (i.e. fine) grain type exactly similar to DRR17B, whereas selections made for shorter height than recurrent parent ideal for maintainer line (Table 2). The improved lines also showed good maintainer ability when crossed with WA-CMS line, IR58025A, indicating their equivalence to DRR17B with respect to maintenance ability (Table 2). Interestingly, hybrids derived from two of the improved lines viz., GP17B112-3-98-118-12-204-15 and GP17B112-3-98-118-12-204-234, exhibited more grain yield heterosis when compared to the hybrids derived from crosses with DRR17B (Table 3).

In conclusion, through the present study, we have developed improved versions of the stable maintainer line DRR17B, possessing resistance against BB and blast along with MS grain type, complete sterility maintenance ability and demonstrated the heterotic potential of the improved lines.

**Acknowledgments** The authors would like to acknowledge the funding support provided by the Department of Biotechnology (DBT), Government of India, for execution of the research study through the Grant # BT/PR11705/AGR/02/646/2008. The authors also thank Project Director, ICAR-Indian Institute of Rice Research, for providing all the necessary facilities.

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