MARKER-ASSISTED BREEDING FOR BACTERIAL BLIGHT RESISTANCE IN PARENTAL LINES OF HYBRID RICE

S. Raghavendra Rao, M. Priyanka, M. Ajay Kumar, C. Ramanaiah, B. Yashwanth, K. Madan Mohan, B. Vidya Chandra, V. Venkateshwarlu, D. Gandhi, N. Jagan Mohan Rao, G.V. Ramana Rao, M. Sai Murali Raj and M. Lalitha Shanti

Ganga Kaveri Seeds Pvt. Ltd, Sunanda Farm, Kandlakoya, Medchal-501401, Medchal District, Hyderabad

SUMMARY

GK5003 and GK5017 are the rice hybrids of Ganga Kaveri which are very popular with Indian farmers. In order to improve the performance of these hybrids under bacterial blight incidence, marker-assisted backcross breeding has been used to introgress *Xa4*, *xa5*, *xa13* and *Xa21* genes into the parental lines of these hybrids. Foreground as well as background selection using molecular markers was done to pick up target plants. Multiplexing of three bacterial blight resistance genes *Xa4*, *xa13* and *Xa21* was standardised and used in this study to screen large populations of maintainers and restorers. Seven isolates from different parts of the country were inoculated under artificial conditions to evaluate disease spectrum. In addition one very virulent isolate from Maruteru was used for field inoculations. The *Xa21* entries in both hybrids showed moderate resistance, whereas the three gene and four gene combinations showed high level of resistance. Under field inoculated conditions at Kodakandla the three and four gene combinations exhibited significant yield advantage over the original hybrids. Replicated yield trials were also conducted in the three states of Uttar Pradesh, Bihar and Chattisgarh which are the market segments of these hybrids. The value added hybrids were on par as compared to original with respect to agronomic and grain quality parameters. This work reports the successful application of marker assisted selection for introgression of disease resistance genes into the parental lines of hybrid rice.

Keywords: bacterial blight, gene pyramiding, marker assisted backcross breeding, multiplex, resistance genes, hybrid rice.

Corresponding author: M. Lalitha Shanti
Fax: + 04044347211
Email: lalitha.shanti@gangakaveri.in

INTRODUCTION

Rice is the staple and most important food crop grown in the Asian subcontinent and the world. It provides food for more than half of the world's population and constitutes a major source of calories for urban and rural inhabitants (Khush, 2005). Hybrid rice is a beneficial option to increase rice productivity. In India, rice hybrids have been released for commercial cultivation and the area given over to them is slowly but steadily increasing. Hybrids yield 15-20% more than inbred rice varieties (Virmani, 1996) and now at least 70 public and private bred hybrids are available for commercial cultivation. However, most of the hybrids released to date in India and abroad are highly susceptible to biotic stresses like bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) (Khush and Jena, 2009). Further, hybrid rice seed production management practices such as leaf cutting and rope pulling in an attempt to increase cross-pollination cause wounds, increasing vulnerability to pests and diseases.

Among the biotic stresses, BB, blast and the brown plant hopper are a major threat to rice production. BB is a major devastating disease that limits rice yields significantly across the world (Ou, 1985; Mew *et al*., 1993) and limits rice production up to 81% in countries like India (Kumar *et al*., 2012).

GK5003, one of the leading hybrids of Ganga Kaveri, was notified to the Central Varietal Release Commitee in 2009 and has a duration of 125 days with long bold grain. GK5017 is another leading 135 days duration hybrid with long bold grain. Both these hybrids are very popular in Eastern Uttar Pradesh, Chattisgarh, Jharkhand, West Bengal and Bihar which constitute the major Rice Bowl of India. The parental lines and the hybrids are not resistant to BB and yield losses of 12-15% are incurred due to this disease in endemic areas.

Chemical control for BB is not effective (Devadath, 1989). Therefore, host plant resistance offers the most effective, economical and environmentally safe option for BB management (Khush *et al*., 1989). Till date 41 rice BB resistance genes have been reported, eight of which have been cloned and characterised (Kim *et al*., 2015; Hutin *et al*., 2015; Ellur *et al*., 2016). DNA fingerprinting studies and pathotype analysis have shown a significant diversity in the

Table 1. List of primers and their amplicon sizes.

Gene	Chromosome number	Primer name	Amplicon sizes	Reference
Xa21	11	pTA248	R- 1000 bp, S-700 bp	Ronald *et al.*, 1992
xa13	8	xa13-Prom	R- 450 bp, S- 270 bp	Sundaram *et al.*, 2011
xa5	5	xa5FM-SF, xa5FM-SR, xa5FM-RF and xa5FM-RR	424 common band, R-134, S-313	Sundaram *et al.*, 2011
Xa4	11	MP1&MP2	R-150 bp, S-140 bp	Ma *et al.*, 1999
Rf3	1	DRRM-RF3-10	R- 200 bp, S-190 bp	Balaji *et al.*, 2012
Rf4	10	RM6100	R-140 bp, S-130 bp	Sheeba *et al.*, 2009

Xoo population in India and other rice-growing countries (Adhikari *et al.*, 1995; Yashitola *et al.*, 1997; Shanti *et al.*, 2001; Gupta *et al.*, 2001; Singh *et al.*, 2002; Debabrata *et al.*, 2008).

Pyramiding multiple genes using conventional breeding alone is cumbersome because of the masking effects of the genes and environmental effects that make it difficult to identify the virulence pattern of each gene precisely, because each gene confers resistance to multiple races of the pathogen. Marker-assisted selection has opened new opportunities in biotechnology and resistance breeding. With molecular markers it has become possible to tag individual genes that confer resistance to different races of the pathogen and identify multiple genes in plants by selecting for markers linked to the genes. On the pathogen side, DNA fingerprinting and pathotype analysis have indicated significant diversity within the population of *Xoo* in India and other rice-growing areas (Adhikari *et al.*, 1995; Yashitola *et al.*, 1997; Shanti *et al.*, 2001; Singh *et al.*, 2003; Lalitha Shanti *et al.*, 2010).

Many earlier studies have shown that through marker-assisted breeding, resistance genes can successfully be 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; Ranjit *et al.*, 2016) and hybrid rice parental lines (Chen *et al.*, 2001; Liyong *et al.*, 2003; Basavaraj *et al.*, 2010; Shanti *et al.*, 2010; Hari *et al.*, 2011). Earlier efforts to generate three-gene pyramids in the backgrounds of rice cvs PR106 (Singh *et al.*, 2001), Pusa Basmati (Joseph *et al.*, 2004) and Samba Mahsuri have been successful (Sundaram *et al.*, 2008; Suh *et al.*, 2013; Hari *et al.*, 2013; Balachiranjeevi *et al.*, 2015).

A number of mapped rice microsatellite markers are available (Mc Couch *et al.*, 2002). Microsatellite markers are highly sequence-specific and co-dominant. These features make them ideal for use in background selection as they permit the detection of alleles coming from both parents and allow accurate determination of the allelic constitution of the offspring. Hence, we used a set of microsatellite markers that are polymorphic between the donor and recurrent parents in selection for the recurrent parent genome at each backcross generation.

The newly developed lines that were obtained in this study exhibited high BB resistance and retained the yield and grain quality traits of the original hybrid. This work represents a successful example of the use of molecular markers in foreground and background selection, for the introgression of genes of interest into the popular parental lines of Ganga Kaveri hybrids GK5003 and GK5017 using marker-assisted selection and conventional breeding.

MATERIALS AND METHODS

Plant materials. IRBB 60, a near isogenic line in the background of IR24, carrying the four resistant genes *Xa4*, *xa5*, *xa13* and *Xa21* served as the donor for all the crosses attempted.

PCR amplification and marker-assisted selection. Miniscale DNA isolation was carried out for PCR analysis of the parents and backcross progenies (Dellaporta *et al.*, 1983). For *Xa4* gene, MP1 and MP2 markers (Ma *et al.*, 1999) were used. For detection of *xa5* gene, *xa5*FM-SF, *xa5*FM-SR, *xa5*FM-RF and *xa5*FM-RR were used (Sundaram *et al.*, 2011). For *xa13*, *xa13* Prom F and Prom R were used (Sundaram *et al.*, 2011). The sequence-tagged-site (STS) marker pTA 248, which is tightly linked to *Xa21* (Ronald *et al.*, 1992) was used to confirm the presence of *Xa21* (Table 1). The PCR mixture contained 50 ng of template DNA, 5 picomoles of each primers, 0.05 mM dNTPs, 1X PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 1.8 mM $MgCl_2$ and 0.01 mg/ml gelatin) and 1 U *Taq* DNA polymerase in a reaction volume of 25 µl. Template DNA was initially denatured at 94°C for 5 min followed by 35 cycles of PCR amplification with the following parameters: 30 s denaturation at 94°C, 30 s annealing at 55°C and 1 min primer extension at 72°C. A final extension was done at 72°C for 5 min. The amplified products were electrophoretically resolved on 2.5% agarose gel and visualized under UV after staining with EZ vision (AMRESCO, USA), a non-carcinogenic dye. With respect to the multiplex PCR assay for simultaneous detection of *Xa21*, *xa13* and *Xa4* the protocol was essentially the same as described above and the amplified products were resolved on 2.5% agarose gels. *xa5* could not be used for multiplex PCR because in resistant lines it shared a common band with *Xa4* and it was difficult to differentiate the two.

Background selection. Microsatellite loci that are polymorphic between donor and recurrent parents were identified by screening 230 microsatellite markers distributed throughout the rice genome. In this screening, different number of markers were identified to be polymorphic between donor and recurrent parents. The parental polymorphic microsatellite markers were used to genotype

foreground-selected plants at each backcross generation to estimate the amount of recurrent parent genome contribution. The data were analysed using Graphical Genotyping Tools (GGT Version 2.0 1999) software programme (Van Berloo, 1999).

Screening for BB resistance. The pyramided lines in the backgrounds of GK5003B, GK5003R, GK5017B and GK5017R and their respective hybrids were evaluated for their reaction to BB under glasshouse conditions at Medchal, Hyderabad, and field conditions at Kodakandla, Siddipet District, Telangana State. Replicated yield trials were conducted at Muzzafarpur (Bihar), Ambikapur (Chattisgarh) and Faizabad (Uttar Pradesh).

Under artificial inoculation in glasshouse conditions, the value-added parental lines and hybrids were evaluated for their reaction to BB using seven isolates of *Xoo* from different parts of India. The cultures were routinely maintained on modified Wakimoto's semi-synthetic medium (per litre: 20 g sucrose, 5 g peptone, 0.5 g calcium nitrate, 1.82 g disodium hydrogen phosphate, 0.05 g ferrous sulphate, 18 g agar, pH 6.8-7; Karaganilla *et al*., 1973). For long-term storage, the cultures were maintained as 40% glycerol stocks at –70°C. The stored cultures were revived and grown on modified Wakimoto's medium for inoculation and DNA experiments. The strains used for inoculation were passed through the susceptible cultivar TN1 and re-isolated before use in inoculation experiments.

Evaluation of resistance. Individual plants were grown in plastic pots under flooded conditions with a mixture of soil and farmyard manure (3:1 ratio). The pots were fertilized with N:P, 100:50 kg/ha with P applied as basal dose and top dressing at 25 days after sowing. Approximately forty-day-old plants were clip-inoculated (Kauffman *et al*., 1973). Top three or four leaves of plants at maximum tillering stage were clip-inoculated with a cell suspension from 48 h old cultures, determined by a spectrophotometer at 620 nm and adjusted to 10^8 CFU ml^{-1}. For each culture-strain combination, five leaves of a plant were inoculated per replication. Each test was replicated three times. Observations were made on the 15th day after inoculation and lesion lengths were measured for classification of disease response. Each plant was classified as resistant (0-4 cm) and susceptible (>4 cm). Statistical analysis was done using the standard statistical procedures to test for significance of the data.

Experimental design and agronomic practices. A total of nine stabilized hybrids in the background GK5003 and eight in the background of GK5017, respectively, and their recurrent parents were evaluated for the agronomic, yield and related characters. The original hybrid was included as control for susceptibility. The experiment was laid in a randomized complete block design with three replications. Twenty-one-day-old seedlings of the original hybrids of GK5003 and GK5017, nine and eight families each of the pyramids of GK5003 and GK5017, were transplanted at one seedling per hill and a spacing of 20 × 20 cm. Each pyramid family, recurrent parent, i.e. the recipient parent and-controls consisted of 40 plants in four rows of 10 plants each. A spacing of 60 cm was maintained between each replication. There were a total of 920 plants per treatment and two treatments in this experiment. Agronomic observations were taken on parent and pyramid families (in all the three locations Muzaffarpur, Ambikapur and Faizabad).

After transplanting, approximately 5 cm of standing water was maintained in the fields. NPK was applied at 120:60:60 kg/ha to the experimental plots in two split applications, half of the nitrogen as a basal dose at the time of transplantation and the rest at mid tillering stage of the crop. Regular plant protection measures were followed to raise a healthy crop, including sprays against the brown plant hopper and blast.

In addition, to assess the effect of bacterial blight on yield, each of the value-added lines were evaluated during the wet seasons of 2015 and 2016. The original hybrids of GK5003 and GK5017, nine and eight families each of the pyramids of GK5003 and GK5017 as well as the controls, resistant checks, i.e. IRBB 60 (near isogenic line in IR24 background containing the four resistant genes *Xa4*, *xa5*, *xa13* and *Xa21*, the donor for the four BB resistance genes) and Taichung Native 1 (TN1), the control for susceptibility, were evaluated.

The lines were evaluated at the Research and Development Farm of Ganga Kaveri Seeds Pvt. Ltd, located at Kodakandla Mandal, Siddipet District, Telangana State, India. This area is located at 17°52' N, 79.50' E at an altitude of 537 m above sea level. The soil of the experimental site was black soil. All plant protection measures were stopped 15 days prior to inoculation to rule out the interactions and masking effects of these chemicals with *Xoo*.

The plants were grown in 10 m^2 plots at the experimental site.

Grain quality parameters. Grain quality parameters were tested using standard methods in the grain testing laboratory at Ganga Kaveri Research and Development Centre (India). The grains of the pyramided and the parent lines were evaluated three months after harvest at 12-14% moisture content for a number of grain physicochemical characteristics. The characters evaluated were milling (%), hulling (%), length-breadth ratio, alkali spreading value, water uptake, volume expansion ratio, kernel length after cooking, elongation ratio and amylose content (%).

RESULTS

Marker-assisted selection for BB resistant genes into different backgrounds. Twenty F_1 plants from each of the crosses between GK5003B/IRBB 60 (donor for the

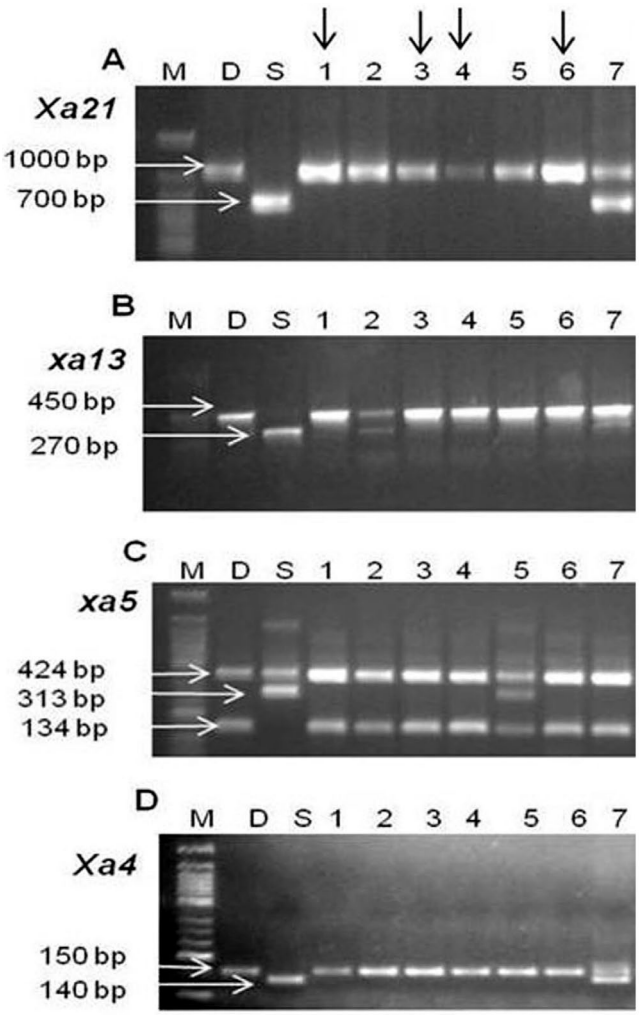

Fig. 1. Foreground selection for *Xa21, xa13, xa5* and *Xa4* in BC_2F_2 generation of GK5017R. M, Molecular weight marker 50 bp ladder; D, donor; S, susceptible control; lanes 1-7, populations of GK5017R. (A) Screening of plants using *Xa21* linked pTA248. (B) Screening of plants positive for *Xa21* with *xa13* linked marker. (C) Screening of plants positive for both *Xa21* and *xa13* using *xa5* linked marker. (D) Screening of the triple positives with *Xa4* linked marker. Arrows indicate selected homozygous plants for all the four genes.

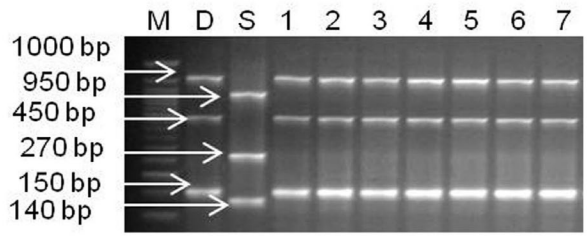

Fig. 2. Multiplex PCR of *Xa21, xa13* and *Xa4* in BC_2F_3 generation of 5017R. M, Molecular weight marker 50 bp ladder; D, donor; S, susceptible control; lanes 1-7, 5017R population.

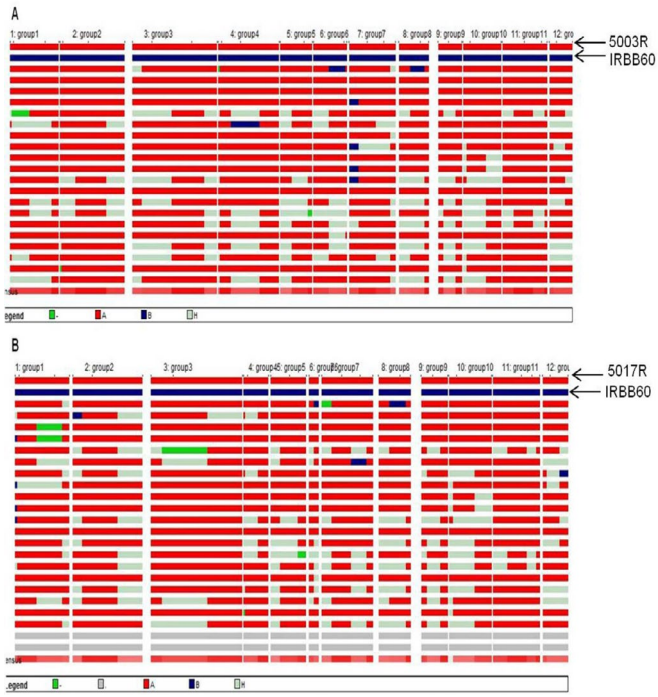

Fig. 3. GGT image of GK5003R (A), GK5017R (B) in BC_2F_1.

four BB resistant genes), GK5003R/IRBB 60, GK5017B/IRBB 60, GK5017R/IRBB 60, were screened for the R gene-linked markers and were backcrossed using the female parent. The resulting BC_1F_1 lines were first checked for presence of the *Xa21* resistance allele in heterozygous condition. All plants carrying the *Xa21* resistant allele in heterozygous state were checked for the presence of the *xa5* allele in heterozygous condition. Plants containing resistant alleles for both genes were further screened for the *Xa4*. Finally, the triple positives in heterozygous condition were screened for the presence of *xa13* as described in Materials and Methods (Fig. 1).

From BC_2F_1 all the populations were screened using multiplex PCR for the genes *Xa4*, *Xa21* and *xa13*. This multiplex PCR saved both resources and time and was a very efficient system for screening multiple genes simultaneously, accurately and rapidly. One single tube could detect the presence of three genes in homozygous and heterozygous allele conditions (Fig. 2).

A total of 17/345 (GK5003R), 14/450 (GK5003B), 11/250 (GK5017R) and 16/450 (GK5017B) BC_1F_1 plants were found containing the four bacterial blight resistance genes in heterozygous condition. These plants were screened with markers for background selection. A total of 230 rice microsatellite markers were used in this study. Table 2 shows the polymorphic markers for each parental combination. These markers were used to genotype the target plants and identify plants having maximum recurrent parental genome (Fig. 3). These plants were further backcrossed with the recurrent parent. In case of the B lines in addition to these markers the lines were also screened for the presence/absence of *Rf3* and *Rf4* to eliminate the fertility restoration factor (Fig. 4). This was continued up to BC_3F_1.

Table 2. Polymorphic microsatellite markers spanning the 12 chromosomes for each genotype.

CHR. Loc	GK5003R	GK5003B	GK5017R	GK5017B
1	RM (1, 151, 246, 212)	RM (246, 81, 212)	RM (1, 151, 246, 212)	RM (151, 246)
2	RM (485, 110, 324, 240)	RM (154, 174, 324, 475)	RM (154,174, 240)	RM (154, 110, 279, 324)
3	RM (231,7, 442)	RM (7,132, 55)	RM (514, 231, 7)	RM (231, 7, 503)
4	RM (307, 401, 273, 349)	RM (280, 307)	RM (401, 307, 273)	RM (307, 401, 273)
5	RM (153, 169, 473)	RM 13	RM (159, 437, 163)	RM (13, 169)
6	RM (508, 3)	RM (589, 276)	RM (204, 589)	RM (204, 589)
7	RM (481, 11, 234, 248)	RM (234, 320)	RM (248, 481, 11, 234)	RM (481, 11)
8	RM (407, 547, 342)	RM (152, 447)	RM (310, 152, 223)	RM (152, 447)
9	RM (296, 460, 583)	RM (242, 460, 278, 296)	RM (296, 460, 583)	RM (219, 278)
10	RM (474, 216, 117)	RM (484, 216)	RM (474, 216, 117)	RM (271, 216)
11	RM (286, 202, 473, 254)	RM (21, 254)	RM (181,167, 21, 206)	RM (21, 206, 286)
12	RM (20, 19, 511)	RM (19, 277, 511)	RM (19, 20, 511)	RM (20, 11, 17, 19)
Total	40	30	38	31

Disease resistance. The gene pyramids in the backgrounds of GK5003 and GK5017 showed a very high degree of resistance as compared to their parents to all the seven *Xoo* isolates inoculated (Fig. 5A, B). There were varying degrees of resistance to each of the isolates, but no isolate could break the resistance of any of the three gene and four-gene pyramids. In case of *Xa21* the plants showed moderate resistance. This is in line with our earlier studies (Shanti and Shenoy, 2005; Lalitha Shanti *et al.*, 2010).

Amongst the lines tested, in case of GK5003, entry No. 130 (four gene combination) showed the lowest mean lesion length across the isolates at 1.7 cm indicating that it was highly resistant to the *Xoo* isolate and 102 (*Xa21*) showed a susceptible reaction with the lesion length measuring 4 cm. The rest of the entries were intermediate between the two ranges.

In GK5017, entry 202 (four gene combination) showed the lowest mean lesion length with respect to disease across the seven isolates at 2.2 cm and 213 (*Xa21*) showed the highest of 4 cm. The others were intermediate between these two ranges.

Yield and agro morphological traits of pyramided lines. The entries along with the recurrent parent were evaluated at multilocation trials in three test locations. Initially, 20 lines of each value-added hybrid containing different combinations were evaluated (data not shown). From these the best lines having superior yield and grain quality nearer to parent were selected and finally these best entries were promoted.

Among the entries of GK5003 the single gene introgressed entries outperformed the other gene combinations: 118 showed the highest average yield across the three locations, 102 showed the least among the entries evaluated (Table 3a).

GK5017 also showed a similar trend with *Xa21* entry 202, recording the highest average yield across locations while entry 211 had the lowest average yield among the selected entries (Table 3b).

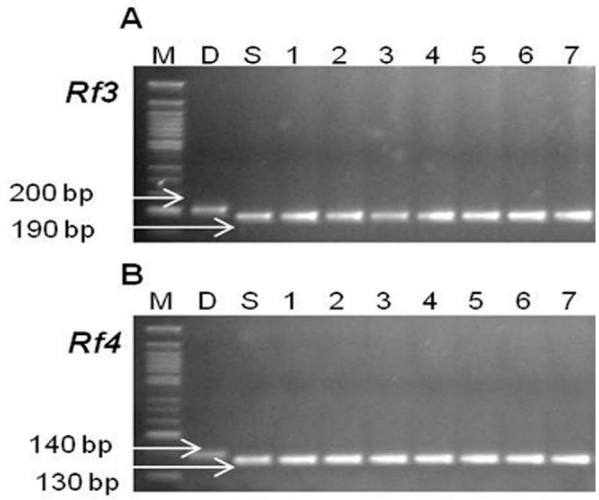

Fig. 4. *Rf3* and *Rf4* screening in maintainers of GK5017. Restoration genes are absent in maintainers and hence show the lower band. M, Molecular weight marker 50 bp ladder; D, restorer; S, maintainer; 1-7, selected maintainer lines.

Under BB artificial inoculated conditions at Kodakandla, the control GK5003 showed a yield reduction of 18.8% (Table 4a). The four gene combination entry 130 showed the minimum yield reduction of 1.5% under diseased conditions. Entry 106 showed a yield reduction of 13%, 102 showed 7.9% and 118 showed 8.8% under BB conditions. Hence, these entries were not considered for further evaluation. The percentage of yield insulation against the disease thereby mitigating the yield loss,ranged from 5.9 to 17.3% in the value-added hybrids.

GK5017 showed a yield reduction of 13.6% under disease pressure whereas the four gene combination 209 showed 0.9% reduction in yield followed by *Xa21* introgressed 204 (Table 4b). The percentage of yield insulation ranged from 7.6 to as high as 12.7% in the value-added hybrids.

In GK5003, *Xa21* showed moderate resistance to a very virulent BB isolate from Maruteru which was used for field inoculation studies. Entries No. 118, 102 and 106 showed a high percentage of yield reduction under inoculated

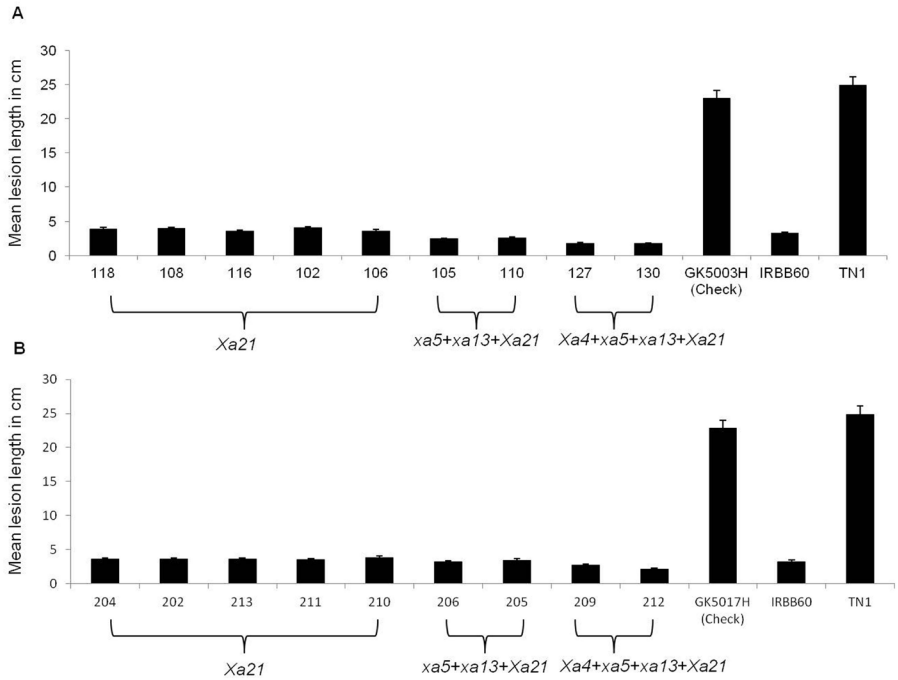

Fig. 5. Varying degrees of bacterial blight reaction of the value added hybrids of GK5003H (A) and GK5017H (B).

Table 3a. Grain yield of GK5003 value added hybrids at three locations as compared to the control*.

S. No.	Gene combination	Entry	Muzaffarpur	Ambikapur	Faizabad	Mean
1	*Xa21*	118	3722 ± 55	3696 ± 102	2726 ± 51	3381
2	*Xa21*	108	3560 ± 87	3559 ± 36	2693 ± 3	3270
3	*Xa21*	116	3645 ± 109	3167 ± 88	2626 ± 51	3146
4	*Xa21*	102	3408 ± 116	3399 ± 58	2367 ± 33	3058
5	*xa13+Xa21*	106	3788 ± 165	3150 ± 29	2499 ± 47	3146
6	*xa5 + xa13+Xa21*	105	3669 ± 86	3408 ± 51	2717 ± 101	3265
7	*xa5 + xa13+Xa21*	110	3682 ± 131	3608 ± 51	2727 ± 79	3339
8	*Xa4+xa5+xa13+Xa21*	127	3651 ± 131	3510 ± 84	2789 ± 113	3317
9	*Xa4+xa5+xa13+Xa21*	130	3459 ± 22	3408 ± 51	2618 ± 43	3162
10	GK5003 (Check)	104	3142 ± 30	3059 ± 46	2377 ± 67	2859
	Location average[a]		3573	3397	2614	3195
	SD		191	211	150	
	CV		5.3%	6.2%	5.7%	

*Grain yield of entries given in Kg/ha ± Standard error, values given are mean of three replications.
[a]The location average includes the yield data of the lines mentioned in the table as well as the check line which varied depending on the location.

Table 3b. Grain yield of GK5017 value added hybrids at three locations as compared to the control*.

S. No	Gene combination	Entry	Muzaffarpur	Ambikapur	Faizabad	Mean
1	*Xa21*	204	4134 ± 52	3786 ± 86	3941 ± 83	3954
2	*Xa21*	202	4051 ± 132	3823 ± 150	3834 ± 234	3903
3	*Xa21*	213	4190 ± 106	3502 ± 117	3983 ± 188	3892
4	*Xa21*	211	4000 ± 29	3784 ± 109	3861 ± 39	3882
5	*Xa21*	210	4005 ± 98	3862 ± 90	4050 ± 76	3972
6	*xa5+xa13+Xa21*	206	4290 ± 66	4082 ± 18	4167 ± 120	4180
7	*xa5+xa13+Xa21*	205	4352 ± 75	3908 ± 51	3978 ± 135	4079
8	*Xa4+xa5+xa13+Xa21*	209	4790 ± 208	4084 ± 72	4300 ± 208	4391
9	GK5017H (Check)	203	3850 ± 29	3367 ± 89	3601 ± 58	3606
	Location average[a]		4184.7	3822	3968	3991
	SD		274.9	274.4	200.5	
	CV		6.6%	7.2%	5.1%	

*Grain yield of entries given in Kg/ha ± Standard error, values given are mean of three replications.
[a] The location average includes the yield data of the lines mentioned in the table as well as the check line which varied depending on the location.

Table 4a. Effect on yield of GK5003 and the value-added pyramids under disease free and artificial disease conditions.

S. No	Gene combination	Entry No.	Natural	Inoculated	Lesion length	% of yield reduction	% yield saved due to resistance
1	*Xa21*	118	3290 ± 38	3000 ± 33	3.8 ± 0.19	8.8	10
2	*Xa21*	108	3295 ± 102	3250 ± 126	3.9 ± 0.12	1.4	17.4
3	*Xa21*	116	3227 ± 37	3050 ± 58	3.5 ± 0.14	5.5	13.3
4	*Xa21*	102	2569 ± 101	2367 ± 88	4.0 ± 0.06	7.86	10.9
5	*Xa21*	106	3139 ± 141	2733 ± 33	3.6 ± 0.16	12.93	5.9
6	*xa5+xa13+Xa21*	105	3253 ± 130	3175 ± 120	2.4 ± 0.07	2.4	16.4
7	*xa5+xa13+Xa21*	110	3202 ± 114	3133 ± 88	2.6 ± 0.04	2.2	16.6
8	*Xa4+xa5+xa13+Xa21*	127	3351 ± 103	3283 ± 117	1.8 ± 0.16	2	16.8
9	*Xa4+xa5+xa13+Xa21*	130	3248 ± 100	3200 ± 88	1.7 ± 0.10	1.47	17.3
10	GK5003H (Check)	104	2996 ± 119	2433 ± 233	23 ± 1.1	18.79	

*Experiments were conducted in 10 m² plots in three replications as described in Materials and Methods. Yield of 10 m² plots extrapolated to per hectare yield (1ha = 10,000 m²).

#Average lesion length for 25 leaves per plot measured 15 days after inoculation.

Table 4b. Effect on yield of GK 5017 and the value-added hybrids under disease free and artificial disease conditions.

S. No	Gene combination	Entry No.	Yield (kg/ha) under disease free condition*	Yield (kg/ha) under disease pressure*	Lesion length in cm#	% of yield reduction	% yield saved due to resistance
1	*Xa21*	204	3734 ± 88	3650 ± 126	3.6 ± 0.23	2.3	11.3
2	*Xa21*	202	3617 ± 44	3450 ± 29	3.8 ± 0.08	4.6	9
3	*Xa21*	213	3688 ± 69	3467 ± 88	4.0 ± 0.1	5.99	7.6
4	*Xa21*	211	3593 ± 103	3433 ± 120	3.5 ± 0.14	4.45	9.2
5	*Xa21*	210	3742 ± 68	3592 ± 51	3.9 ± 0.19	4	9.6
6	*xa5+xa13+Xa21*	206	3716 ± 109	3567 ± 153	3.2 ± 0.13	3.1	10.5
7	*xa5+xa13+Xa21*	205	3643 ± 81	3500 ± 58	3.5 ± 0.19	3.9	9.7
8	*Xa4+xa5+xa13+Xa21*	209	3633 ± 67	3600 ± 153	2.8 ± 0.11	0.9	12.7
9	GK5017H (Check)	203	3549 ± 116	3067 ± 67	22.8 ± 1.04	13.6	

*Experiments were conducted in 10 m² plots in three replications as described in Materials and Methods. Yield of 10 m² plots extrapolated to per hectare yield (1ha = 10,000 m²).

#Average lesion length for 25 leaves per plot measured 15 days after inoculation.

conditions hence these entries were dropped and only the entries 108 and 116 were considered. The three gene and four gene combinations showed high degrees of resistance and the yield loss was significantly reduced under inoculated conditions (Table 3). In case of *Xa21* introgressed 108 entry yield insulated under disease conditions was 17.4% as compared to the control. The next best entry was the four gene combination 130 which showed 17.3% insulation. The three gene combinations showed an average of 16.5% insulation in yield under disease pressure.

GK5017 also showed a similar trend with the single gene combination showing moderate resistance and the four gene combination showing high levels of resistance. Single gene entry 213 showed a yield loss of 6% under diseased conditions. Hence this entry was dropped. Entry 204 (*Xa21* introgressed) showed a yield insulation of 11.3%; the four gene combination 209 showed the highest insulation of 12.7% and the three gene combinations showed an average yield insulation of 10%.

Grain quality characteristics of the pyramided lines. GK5003 has long bold grain type. The different combinations tested also showed similar grain characteristics as that of the parent (Table 5a, Fig. 6). The single gene, two genes, three gene and four gene combinations were all comparable to the parent in their grain characters. GK5017 also feel under the long bold grain type. The grain quality parameters of the single, two and three gene combinations were similar to those of the parent (Table 5b, Fig. 7).

DISCUSSION

In the present study, four BB resistance genes *Xa4*, *xa5*, *xa13* and *Xa21* have been introgressed into GK5003 and GK5017 using marker-assisted backcross breeding in conjunction with phenotypic selection. The objective of this work was the development of broad spectrum resistance to BB in the above two hybrids. Earlier reports of marker-assisted introgression (Gopalkrishnan *et al.*, 2008; Sundaram *et al.*, 2008; Basavaraj *et al.*, 2010; Shanti *et al.*, 2010; Hari *et al.*, 2011) have been successful.

Multiplexing of three genes *Xa4*, *xa13* and *Xa21* was of great advantage as it accelerated the screening process. Three genes could be tested in one tube at one shot, saving time and resources (cost effectiveness per data point).

Our work is in line with earlier reports of successful background selection (Liu *et al.*, 2003; Gopalakrishnan *et al.*, 2008; Sundaram *et al.*, 2008; Hari *et al.*, 2013). The

Table 5a. Grain quality parameters of GK5003 and its value added hybrids*#.

Designation	Physical characteristics									Cooking characteristics			
	Paddy Length (mm)	Paddy Breadth (mm)	Paddy L/B Ratio (mm)	Grain Length (mm)	Grain Breadth (mm)	L/B Ratio (mm)	Grain Type	1000 Seed Wt/g	Chalkiness	KLAC (mm)	Elongation Ratio	Volume Expansion Ratio	Water Uptake (ml)
105	9.1	2.5	3.6	6.09	2.31	2.64	LB	22.51	0 Absent	9.8	1.6	3.6	300
106	8.5	2.6	3.3	6.04	2.39	2.53	LB	23.22	4 VOC	9.0	1.5	3.5	270
108	9.3	2.6	3.6	6.53	2.43	2.69	LB	26.54	3 VOC	10.2	1.6	3.5	300
110	9.1	2.5	3.6	6.05	2.28	2.65	LB	22.65	0 Absent	9.8	1.6	3.7	330
116	8.1	2.6	3.1	6.50	2.56	2.54	LB	26.41	12 OC	10.2	1.6	3.7	340
118	8.9	2.5	3.6	6.09	2.35	2.59	LB	23.21	9 VOC	10.2	1.7	3.4	350
127	9.0	2.6	3.5	6.01	2.43	2.47	LB	23.63	1 VOC	9.4	1.6	3.7	340
130	8.2	2.5	3.3	6.27	2.31	2.71	LB	23.64	8 VOC	10.0	1.6	3.5	330
GK5003 CHECK	8.8	2.5	3.5	6.22	2.35	2.65	LB	22.97	3 VOC	10.6	1.7	3.7	250

*The grain quality parameters determined as described in Materials and Methods.

#The experiments were replicated with similar results. LB = Long bold, (< than 10%); VOC = Very occasionally present, (11-20%); OC = Occasionally present, > 20% present.

Table 5b. Grain quality parameters of GK5017 and its value added hybrids*#.

Designation	Paddy Length (mm)	Paddy Breadth (mm)	Paddy L/B Ratio (mm)	Grain Length (mm)	Grain Breadth (mm)	L/B Ratio (mm)	Grain Type	1000 Seed Wt/g	Chalkiness (%)	KLAC (mm)	Elongation Ratio	Volume Expansion Ratio	Water Uptake (ml)
201	9	2.6	3.4	6.5	2.4	2.7	LB	22.87	0 Absent	9	1.4	3.6	230
202	9.2	2.5	3.7	6.3	2.4	2.6	LB	25.01	13 OC	9.8	1.5	3.6	270
203	9.1	2.7	3.3	6.1	2.5	2.5	LB	25.8	11 OC	10	1.6	3.5	380
204	9	2.7	3.3	6.3	2.4	2.6	LB	25.61	4 VOC	9.8	1.6	3.6	340
205	9.1	2.7	3.4	5.9	2.5	2.4	LB	25.23	22 Present	10	1.7	3.7	320
206	9.1	2.8	3.2	5.9	2.4	2.5	LB	25.96	16 OC	9	1.5	3.6	350
207	9.2	2.5	3.7	5.8	2.4	2.5	LB	25.9	10 VOC	10	1.7	3.7	300
208	8.8	2.7	3.3	6	2.5	2.4	LB	25.97	14 OC	10	1.7	3.6	300
209	9.2	2.5	3.7	5.9	2.4	2.5	LB	25.65	13 OC	9.8	1.7	3.7	350
210	9.3	2.6	3.6	5.8	2.3	2.5	LB	25.75	10 VOC	10	1.7	3.5	350
211	9.1	2.6	3.5	5.7	2.4	2.4	SB	26	9 VOC	9.8	1.7	3.7	350
212	9.1	2.5	3.7	5.8	2.4	2.4	SB	27.01	11 OC	10	1.7	3.7	350
213	10	2.5	4	6.2	2.4	2.6	LB	26.36	8 VOC	10	1.6	3.7	350
214	9.2	2.6	3.5	6	2.4	2.5	LB	25.93	14 OC	10	1.7	3.7	350

*The grain quality parameters determined as described in Materials and Methods.

#The experiments were replicated with similar results.

LB = Long bold, (< than 10%); VOC = Very occasionally present, (11-20%); OC = Occasionally present, > 20% present.

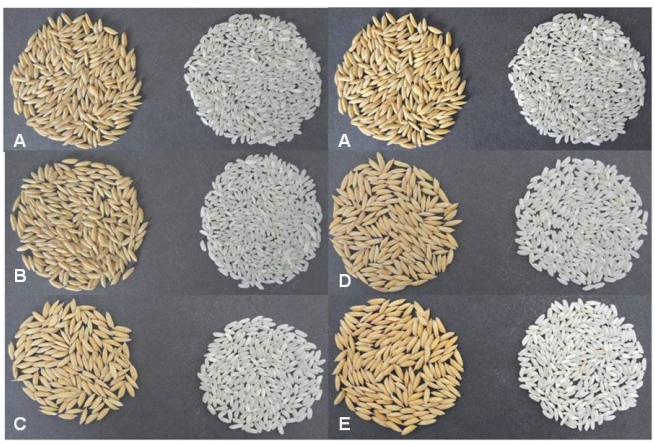

Fig. 6. Hulled and de hulled seed of control and value added hybrids of GK5003. A, GK5003 Check (104); B, Entry 105; C, Entry 130; D, Entry 108; E, Entry 116.

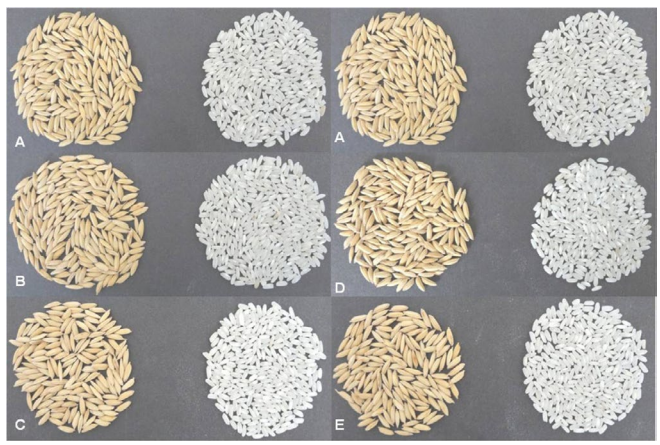

Fig. 7. Hulled and de hulled seed of control and value added hybrids of GK5017. A, GK5017 Check (203); B, Entry 204; C, Entry 211; D, Entry 206; E, Entry 209.

polymorphic markers for each cross ranged from 31 to 40 on the average. The desired plant type could be obtained through stringent phenotypic evaluation, in addition to background selection from BC_1F_1

The pyramided lines containing various gene combinations (single, two or three gene) exhibited various degrees of resistance to BB. All the combinations were resistant against the disease to all isolates inoculated under both artificial as well as field conditions.

The better performance of the three gene and four gene combinations is in agreement with earlier results (Shanti and Shenoy, 2005; Sundaram *et al.*, 2008; Shanti *et al.*, 2010; Lalitha *et al.*, 2013a, 2013b).

The donor IRBB 60 contains fertility restoration genes and is a partial restorer. Hence while improving the maintainer lines for BB resistance it is imperative that the backcross derived lines should not possess the major fertility restorer genes (*Rf3* and *Rf4*). All the progenies of maintainers were screened with DRR-CMS-10 for R*f3* (Balaji *et al.*, 2012) and RM 6100 for *Rf4* (Sheeba *et al.*, 2009). Negative selection was used and plants found containing the above genes in homozygous recessive condition, were advanced further.

The grain quality characters of all the selected lines were on par with that of the original hybrid. In addition, replicated yield multilocation trials conducted in the three states showed that the yield levels of the value-added hybrids were on par with that of the original in some entries was higher than the original hybrid. Under BB infection, there was a significant yield reduction in the hybrids while this was negligible in the value-added entries and would be of great advantage in the endemic areas.

These value-added hybrids are undergoing another season of multilocation testing and in future these can replace the original hybrid, whereby the farmers are benefitted with insulation against yield loss due to bacterial blight. In addition, these lines are being used as pre-breeding donor lines for transfer of BB resistance into other parental lines and varieties under the rice breeding program at GK. The restorers of GK5017 and GK5003 containing *Xa21+xa13* are being used as the parents to stack BPH resistant genes.

This work demonstrates the successful application of marker assisted backcross breeding to develop value added resistant hybrids. These hybrids can help the agricultural community insulate against the yield losses due to BB.

ACKNOWLEDGEMENTS

The authors thank the Ganga Kaveri Management and DBT SBIRI for their encouragement and financial support. The encouragement and support of Dr. K. Krishnaiah is thankfully acknowledged. The authors are thankful to Mr. Ramprasad for the statistical inputs.

REFERENCES

Adhikari T.B., Vera Cruz C.M., Zhang Q., Nelson R.J., Skinner D.Z., Mew T.W., 1995. Genetic diversity of resistance of *Xanthomonas oryzae* pv. *oryzae* in Asia. *Applied Environmental Microbiology* **61**: 966-971.

Balachiranjeevi C.H., Bhaskar N.S., Abhilash V., Akanksha S., Viraktamath B.C., Madhav M.S. *et al.*, 2015. Marker-assisted introgression of bacterial blight and blast resistance into DRR17B, elite, fine-grain type maintainer line of rice. *Molecular Breeding* **35**: 15.

Balaji S.P., Srikanth B., Hemanth K.B., Subhakar Rao I., Vemi Reddy L.R. *et al.*, 2012. Fine mapping of *Rf3* and *Rf4* fertility restorer loci of WA-CMS of rice (*Oryza sativa* L.) and validation of the developed marker system for identification of restorer lines. *Euphytica* **187**: 421-435.

Basavaraj S.H., Singh V.K., Singh A., Singh A., Singh A., Anand D. *et al.*, 2010. Marker-assisted improvement of bacterial blight resistance in parental lines of Pusa RH10, a superfine grain aromatic rice hybrid. *Molecular Breeding* **26**: 293-305.

Chen S.C., Xu G., Lin X.H., Zhang Q., 2001. Improving bacterial blight resistance of 6078, an elite restorer line of hybrid rice by molecular marker-aided selection. *Plant Breeding* **120**: 133-137.

Debabrata N., Shanti M.L., Bose L.K., Singh U.D., Nayak P., 2008. Pathogenicity association in *Xanthomonas oryzae* pv. *oryzae*, the causal organism of rice bacterial blight disease. *ARPN Journal of Agricultural and Biological Science* **3**: 12-27.

Dellaporta S.L., Woo J., Hicks J.B., 1983. A plant DNA mini preparation: version II. *Plant Molecular Biology Reporter* **1**: 19-22.

Devadath S., 1989. Chemical control of bacterial blight of rice. In: IRRI (ed.). Bacterial Blight of Rice, pp. 89-98. IRRI, Manila, Philippines.

Ellur R.K., Khanna A., Krishnan G.S., Bhowmick P.K., Vinod K.K., Nagarajan M., Mondal K., Singh N.K., Singh K., Prabhu K.V., Singh A.K., 2016. Marker-aided Incorporation of Xa38, a novel bacterial blight resistance gene, in PB1121 and comparison of its resistance spectrum with xa13+Xa21. *Scientific Reports* **6**: 29188.

Gopalakrishnan S., Sharma R.K., Rajkumar K.A., Joseph M., Singh V.P., Singh A.K., Bhat K.V., Singh N.K., Mohapatra T., 2008. Integrating marker assisted background analysis with foreground selection for identification of superior bacterial blight resistant recombinants in Basmati rice. *Plant Breeding* **127**: 131-139.

Gupta V.S., Raja Bhosale M.D., Sodhi M.S., Singh S., Gnanamanickam S.S., Dhaliwal H.S., Ranjekar K., 2001. Assessment of genetic variability and strain identification of *Xanthomonas oryzae* pv. *oryzae* using RAPD-PCR and IS1112 based PCR. *Current Science* **80**: 1043-1049.

Hari Y., Srinivasarao K., Viraktamath B.C., Hariprasad A.S., Laha G.S., Ilyas Ahmed M. *et al.*, 2011. Marker-assisted improvement of a stable restorer line, KMR-3R and its derived hybrid KRH2 for bacterial blight resistance and grain-quality. *Plant Breeding* **130**: 608-616.

Hari Y., Srinivasarao K., Basavraj C., Viraktamath A., Hari Prasad S., Gouri S. *et al.*, 2013. Marker assisted introgression of bacterial blight and blast resistance into IR58025B, an elite maintainer line of rice. *Plant Breeding* **130**: 586-594.

Hutin M., Sabot F., Ghesquière A., Koebnik R., Szurek B., 2015. A knowledge-based molecular screen uncovers a broad-spectrum OsSWEET14 resistance allele to bacterial blight from wild rice. *The Plant Journal* **84**: 694-703.

Joseph M., Gopalakrishnan S., Sharma R.K., 2004. Combining bacterial blight resistance and basmati quality characteristics by phenotypic and molecular marker assisted selection in rice. *Molecular Breeding* **13**: 377-387.

Karaganilla A., Natural M.P., Ou S.H., 1973. A comparative study of culture media of *Xanthomonas oryzae* pv. *oryzae*. *Philippines Agriculture* **57**: 141-152.

Kauffman H.E., Reddy A.P.K., Hsieh S.P.Y., Merca S.D., 1973. An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. *Plant Disease Reporter* **57**: 537-541.

Khush G.S., Mackill D.J., Sidhu G.S., 1989. Breeding rice for resistance to bacterial leaf blight. In: IRRI (ed.). Bacterial Blight of Rice, pp. 207-217. IRRI, Manila, Philippines.

Khush G.S., 2005. What it will take to feed 5.0 billion rice consumers in 2030. *Plant Molecular Biology* **59**: 1-6.

Khush G., Jena K.K., 2009. Current status and future prospects for research on blast resistance in rice (*Oryza sativa* L.). In: Wang G.L., Valent B. (eds). Advances in Genetics, Genomics and Control of Rice Blast Disease, pp. 1-10. Springer, New York, USA.

Kim S.M., Suh J.P., Quin Y., Noh T.H., Reinke R.F., Jena K.K., 2015. Identification and fine-mapping of a new resistance gene, *Xa40*, conferring resistance to bacterial blight races in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* **128**: 1933-1943.

Kumar P.N., Sujatha K., Laha G.S., Srinivasa Rao K., Mishra B., Viraktamath B.C., Hari Y., Reddy C.S., Shaikh H., Sundaram R.M., 2012. Identification and fine mapping of *Xa33*, a novel gene for resistance to *Xanthomonas oryzae* pv. *oryzae*. *Phytopathology* **102**: 222-228.

Lalitha Devi G., Pranitha K., Vinay S., Lalitha Shanti M., 2013a. Improvement of resistance to bacterial blight through marker assisted backcross breeding and field validation in rice (*Oryza sativa*). *Research Journal of Biology* **1**: 52-66.

Lalitha Devi G., Pranitha K., Vinay S., Lalitha Shanti M., 2013b. Marker Aided Selection (MAS): evaluation of bacterial blight resistance pyramid lines of popular fertility restorer lines. *Journal of Plant Pathology* **95**: 299-311.

Lalitha Shanti M., Mohan Kumar Varma C., Premalatha P., Lalitha Devi G., 2010. Understanding the bacterial blight pathogen - Combining pathotyping and molecular marker studies. *International Journal of Plant Pathology* **1**: 58-68.

Liu S.P., Li X., Wang C.Y., Li X.H., He Y.Q., 2003. Improvement of resistance to rice blast in Zhenshan 97 by molecular marker aided selection. *Acta Botanica Sinica* **45**: 1346-1350.

Liyong C., Jie-yun Z., Shou-jiang Y., Xiao-deng Z., Kang-le Z., Shi-hua C., 2003. Hybrid rice resistant to bacterial leaf blight developed by marker assisted selection. *Rice Science* **11**: 68-70.

Ma B.J., Wang W.M., Zhao B., Zhou Y.L., 1999. Studies of PCR marker for the rice bacterial blight resistance gene *Xa-4*. *Hereditas* **21**: 9-12.

McCouch S.R., Teytelman L., Xu Y., Lobos K.B., Clare K., Walton M., Fu B., Maghirang R., Li Z., Xing Y., Zhang Q., Kono I., Yano M., Fjellstrom R., DeClerck G., Schneider D., Cartinhour S., Ware D., Stein L., 2002. Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). *DNA Research* **9**: 257-279.

Mew T.W., Alvarez A.M., Leach J.E., Swings J., 1993. Focus on bacterial blight of rice. *Plant Disease* **77**: 5-12.

Ou S.H., 1985. Rice Diseases. Commonwealth Mycological Institute, Kew, Surrey, UK.

Pandey M.K., Rani N.S., Sundaram R.M., Laha G.S, Madhav M.S., Srinivasa Rao K., Sudharshan I., Hari Y., Varaprasad G.S., Subba Rao L.V., Suneetha K., Sivaranjani A.K. P., Viraktamath B.C., 2013. Improvement of two traditional Basmati rice varieties for bacterial blight resistance and plant stature through morphological and marker-assisted selection. *Molecular Breeding* **31**: 239-246.

Perumalsamy S., Bharani M., Sudha M., Nagarajan P., Arul L., Saraswathi R., Balasubramanian P., Ramalingam J., 2010. Functional marker-assisted selection for bacterial leaf blight resistance genes in rice (*Oryza sativa* L.). *Plant Breeding* **129**: 400-406.

Ronald P.C., Albano B., Tabien R., Abenes L., Wu K., McCouch S., Tanksley S., 1992. Genetic and physical analysis of the rice bacterial blight resistance locus, *Xa21*. *Molecular and General Genetics* **235**: 113-120.

Shanti M.L., George M.L.C., Vera Cruz C.M., Bernardo M.A., Nelson, R.J., Leung H., Reddy J.N., Sridhar R., 2001. Identification of resistance genes effective against rice bacterial blight pathogen in eastern India. *Plant Disease* **85**: 506-512.

Shanti M.L., Shenoy V.V., 2005. Evaluation of resistance genes and their pyramids against rice bacterial leaf blight pathogen *Xanthomonas oryzae* pv. *oryzae*. *Oryza* **42**: 169-173.

Shanti M.L., Shenoy V.V., Devi G.L., Kumar V.M., Premalatha P, Kumar G.N., Shashidhar H.E., Zehr U.B., Freeman W.H., 2010. Marker-assisted breeding for resistance to bacterial leaf blight in popular cultivars and parental lines of hybrid rice. *Journal of Plant Pathology* **92**: 495-501.

Sheeba N.K., Viraktamath B.C., Sivaramakrishnan S., Gangashetti M.G., Khera P., Sundaram R.M., 2009. Validation of molecular markers linked to fertility restorer gene(s) for WA-CMS lines of rice. *Euphytica* **167**: 217-227.

Singh K., Vikal Y., Singh S., Leung H., Dhaliwal H.S., Khush G.S., 2002. Mapping of bacterial blight resistance gene *xa8* using microsatellite markers. *Rice Genetics Newsletter* **19**: 94-96.

Singh S., Sidhu J.S., Huang N., Vikal Y., Li Z., Brar D.S. *et al.*, 2001. Pyramiding three bacterial blight resistance genes (*xa5*, *xa13* and *Xa21*) using marker-assisted selection into indica rice cultivar PR-106. *Theoretical and Applied Genetics* **102**: 1011-1015.

Singh S., Sodhi M., Vikal Y., George M.L.C., Bala G.S., Mangat G.S., Garg M., Sidhu J.S., Dhaliwal H.S., 2003. DNA fingerpriting and virulence analysis of *Xanthomonas oryzae* pv. *oryzae* isolated from Punjab, Northern India. *Euphytica* **130**: 107-115.

Suh J., Jeung J.U., Noh T.H., Cho Y.C., Park S.H., Park H.S. *et al.*, 2013. Development of breeding lines with three pyramided resistance genes that confer broad-spectrum bacterial blight resistance and their molecular analysis in rice. *Rice* **6**: 5.

Sundaram R.M., Vishnupriya M.R., Biradar S.K., Laha G.S., Reddy G.A., Shobha Rani N., Sarma N.P., Sonti R.V., 2008. Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite *indica* rice variety. *Euphytica* **160**: 411-422.

Sundaram R.M., Vishnupriya M.R., Laha G.S., Shobha Rani N., Srinivas Rao P., Balachandaran S.M., Reddy G.A., Sarma N.P., Sonti R.V., 2009. Introduction of bacterial blight resistance into Triguna, a high yielding, mid-early duration rice variety. *Biotechnology Journal* **4**: 400-407.

Sundaram R.M., Laha G.S., Viraktamath B.C., Sujatha K., Natarajkumar P., Hari Y., Srinivasa Rao K., 2011. Marker assisted breeding for development of bacterial blight resistant rice. In: Muralidharan K., Siddiq E.A. (eds). Genomics and Crop Improvement: Relevance and Reservations, pp 154–182. Institute of Biotechnology, Acharya NG Ranga Agricultural University, Hyderabad, India.

Van Berloo R., 1999. GGT: software for display of graphical genotypes. *Journal of Heredity* **90**: 328–330.

Virmani S.S., 1996. *Hybrid Rice Advances in Agronomy* **57**: 378-462.

Yashitola J., Krishnaveni D., Reddy A.P.K., Sonti R.V., 1997. Genetic diversity within the population of *Xanthomonas oryzae* pv. *oryzae* in India. *Phytopathology* **87**: 760-765.

Received April 13, 2017
Accepted July 8, 2017