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Characterization of morpho-quality traits and validation of bacterial blight resistance in pyramided rice genotypes under various hotspots of India

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Abstract

Bacterial blight (BB) disease caused by *Xanthomonas oryza*e pv. *Oryzae* (*Xoo*) is the main yield limiting factor amongst the diseases of rice in India. Swarna is a very popular high yielding variety in India but highly susceptible to the disease. Six pyramided lines containing three BB resistance genes *xa5, xa13, Xa21* in the background of Swarna and IR64 were evaluated across the country under different hotspots to identify broad spectrum resistant line to promote as cultivar and donor for future breeding program. Characterizations for morphological and quality traits along with bioassay of the genotypes were performed across environments to know similarities of pyramided lines with the recurrent parents. Under the multi-location testing, pyramided line CRMAS2232-85 exhibited superior yield and related traits performance along with higher level of resistance to BB disease as compared to parental lines and check varieties. All the pyramided lines including CRMAS 2232-85 showed the presence of specific bands for *Xa21, xa13* and *xa5* resistance genes. The top yielding line also showed similar agro-morphologic characters like days to 50% flowering, panicles/m^2 and plant height. The milling%, head rice recovery, kernel length, L/B ratio, volume expansion ratio, water uptake, kernel length after cooking, alkali spreading value, kernel elongation ratio, amylose content and starch gel consistency quality parameters of best pyramided line were similar as like the recurrent parent.

Keywords: Bacterial leaf blight, molecular markers, multi-location testing, pyramided line.
Abbreviations: PCR_Polymerase Chain Reaction, BB_ Bacterial blight, *Xoo_Xanthomonas oryza*e pv. *Oryzae*, MAS_Marker-assisted selection, KLAC_ Kernel Length After Cooking, ASV_ Alkali Spreading Value, ER_ Elongation Ratio, AC_Amylose Content, GC_Gel Consistency, VER_Volume expansion ratio, WU_Water uptake.

Introduction

Rice (*Oryza sativa* L.) is an important food crop that serves as a major carbohydrate source for nearly half of the world's population. In India, it is grown in 43 million hectares accounting for 42% of food grain production and 55% of cereal production. To sustain self-sufficiency and to meet food grain requirement of future, India has to produce 135-140 million tones of rice by 2030. This has to necessarily meet from less land, less water, less labour and fewer chemicals, constant battle against new emerging pathogens and pests and possible adverse effects from climate change (Khush, 2005). Bacterial blight disease caused by *Xanthomonas oryza*e pv. *oryzae* is the main yield limiting factor amongst the diseases of rice in India. In some areas of Asia, it can reduce crop yield by up to 50% (Khush et al., 1989) or even up to 80% (Singh et al., 1977). *Xoo* affects photosynthetic areas thereby reduce the yield drastically and produce partial grain filling and low quality fodder yield. Host plant resistance offers the most effective, economical and environmentally safe option for management of BB pathogen in rice (Khush et al., 1989). Development of resistant cultivars carrying resistance genes have been the most effective and economical strategy to control BB disease and no environmental pollutions (Huang et al., 1997; Jena and MacKill, 2008; Singh et al, 2001; Sundaram et al., 2008; Rajpurohit et al., 2011; Dokku et al., 2013; Suh et al., 2013). Globally, thirty eight BB resistance genes have been identified from diverse sources (Bhasin et al., 2012; Natrajkumar et al., 2012; Suh et al., 2013). A number of these resistance genes have been tagged by closely linked molecular markers (Sonti, 1998; Rao et al., 2002). A few of these genes like *Xa4* have been incorporated widely in many high yielding varieties through conventional breeding (Khush et al., 1989). However, widespread cultivation of varieties with *Xa4* has led to predominance of *Xoo* races that can overcome this gene (Mew et al., 1992). The deployment of rice cultivars that have multiple BB resistance genes is expected to lead to more durable resistance. Gene pyramiding aims to assemble desirable genes from multiple parents into a single genotype. It requires much labour, time and material resource for gene pyramiding through conventional breeding. Molecular markers associated with the gene(s) responsible for the trait are integrated with backcross breeding program to choose precise segment and desired genotype containing only required gene(s). Pyramiding multiple R genes in a single line confers wide-spectrum and durable resistance. Tightly linked DNA markers have developed for several BB resistance genes. The BB resistance genes, *Xa1, xa5, xa13, Xa21, Xa26* and *Xa27* (Bhasin et al. 2012; Cheema et al. 2008; Gu et al. 2005; Liu et al. 2006; Natrajkumar et al. 2012; Song et al. 1997; Sun et al. 2003; Yang et al. 1998) have been cloned and used for breeding programs. With the exception of *xa5* and *xa13*, the BB resistance genes are dominant in nature and the markers developed from the sequencing information of these genes are widely used in

MAS (Song et al., 1995; Yoshimura et al., 1998; Gu et al., 2005; Chu et al., 2006). Using the gene pyramid approach, improved *indica* rice cultivars with broad spectrum durable BB resistance have been developed by combining different genes (Huang et al., 1997; Sanchez et al., 2000; Singh et al., 2001; Joseph et al., 2004; Pha et al., 2004; Perez et al., 2008; Sundaram et al., 2008; Rajpurohit et al., 2011; Dokku et al., 2013; Suh et al., 2013). A three-gene combination appeared to be the most effective; with *Xa21* contributing the largest component of resistance. The usefulness of resistant cultivars for protection against bacterial blight suggests that MAB will be highly useful tool for breeders in developing adaptable varieties with resistant to BB. Rice varieties Swarna and IR64 are very popular among the rice growers in India for their quality and yield but these two varieties are highly susceptible to the BB disease. Therefore three bacterial blight resistance genes (*xa5*, *xa13* and *Xa21*) pyramided lines developed through marker-assisted selection in the background of varieties Swarna and IR 64 have been tested across multi-location and BB screening hotspots of the country to identify broad spectrum resistant line for use as cultivar and as donor for future breeding program.

Results

Grain yield and agro-morphological characters

The plants harboring three BB resistance genes in homozygous condition were identified and the progenies of these homozygous lines were later evaluated at nine locations for yield and other agro-morphologic and quality parameters. Under the multi-location testing, pyramided line CRMAS 2232-85 exhibited superior agronomic performance along with higher level of resistance to BB disease as compared to parental lines and check varieties (Table 3, 4 and 5). The promising line, CRMAS 2232-85 out yielded the recipient parent Swarna by 16.59% pooled over two years, in nine locations of the country (Table 3). In addition, it showed higher yield of 15.76% over the donor parent, IRBB60 and 9.3% over local check varieties. The next best BB pyramided line was CRMAS 2231-48 showing 9.9% increase over recurrent parent, 9.25 over donor parent and 9.3% over local check varieties. Days to 50% flowering varied from 97 to 114 days amongst the genotypes. The recipient genotype Swarna showed a pooled flowering duration of 112 days while the top yielding pyramided line also resembled recurrent parent with 113 days flowering. The best pyramided line exhibited more panicles/sq.mt (308) as compared to the recipient parent (287). Besides, the line also had almost similar plant height with the recurrent parent.

BB resistance of pyramided and parental lines

Results of multi-location screening of the genotypes for verification of BB resistance showed that pyramided line CRMAS 2232-71 had least bacterial blight infection as compared to the other studied genotypes. The highest yielding genotype CRMAS 2232-85 also exhibited a higher level of resistance (pooled SI score of 4.2) while the parental lines and susceptible check showed very high level of susceptibility (Table 5). Three pyramided lines namely CRMAS 2232-71, CRMAS 2232-85 and CRMAS 2232-66 had lower severity index than the resistant check variety Ajay, recipient parent Swarna and susceptible check TN1 for bacterial blight infection.

Molecular marker analysis for bacterial blight resistance

The PCR amplification pattern using genomic DNA of Swarna BB pyramided lines, CRMAS 2232-85 and CRMAS 2232-66, susceptible check TN1, deepwater varieties, Jalmagna and CR Dhan 500 with the markers pTA248, RG136 and RG556 closely linked to bacterial blight resistance genes, *Xa21, xa13* and *xa5,* respectively are presented in Fig. 1A, B and C. All the pyramided lines showed the presence of resistance specific band of 1000bp (Fig. 1A), 530bp and 490bp (Fig. 1B) and 440bp, 410bp (Fig.1C), indicating the presence of BB resistance genes, *Xa21, xa13* and *xa5*, respectively (Table 6). The genotypes Swarna, CR Dhan 500 and Jalmagna and TN1 showed the absence of these bands, indicating absence of these three resistance genes (Fig. 1A, B, C and Table 6).

Analysis of grain and cooking quality characters of pyramided and parental lines

The grain and its cooking quality characters like milling %, head rice recovery %, kernel length (mm), L/B ratio, volume expansion ratio, water use (ml), kernel length after cooking (KLAC), alkali spreading value (ASV), elongation ratio, amylose content (AC) and gel consistency (GC) are presented in Table3. The milling % ranged from 68.1 to 73.6% among the studied genotypes and it revealed that the pyramided lines had almost similar value as like the values of both the parental lines. The highest head rice recovery was recorded from pyramided line CRMAS 2232-66 (67.4%) while the lowest value was obtained from line CRMAS 2231-36 (49%). The top yielding line CRMAS 2232-85 had HRR of 66.7% which is higher than the value obtained from both of its parents. Recurrent parent had kernel length of 5.28 mm. Similarly, the line CRMAS 2232-85 which had three BB resistant genes too had almost closer value with recurrent parent. The VER of CRMAS 2232-85 had value of 4.7 while the recipient parent exhibited 4.8. The quality parameter, water use varied from 152.5 (CRMAS 2232-71) to 275ml (local check). The pyramided line CRMAS 2232-85 had almost similar water use value like the recipient parent, Swarna. KLAC ranged from 8.0 to 10.6 mm among the studied genotypes. Highest KLAC of 10.6mm was observed in pyramided line CRMAS 2232-36. All the pyramided lines exhibited higher KLAC than the recipient parent, Swarna. Similarly, highest ER of 1.80 was recorded from CRMAS 2232-66 and all the derivatives showed better ER values than both the parental lines. Highest amylose content (%) was recorded from the recurrent parent Swarna (25.86) followed by CRMAS 2232-66 (25.73). On the other hand, all the pyramided lines had AC content in the desirable range of intermediate value (20-25). All the pyramided lines showed medium to high GC value while CRMAS 2232-85 and Swarna exhibited same GC value of 57.

Discussion

The pyramided line CRMAS 2232-85 produced highest yield of 4518 kg/ha pooled over two years and in nine locations exhibiting higher yield than both its parental lines, Swarna and IRBB60. The higher yield of the pyramided line CRMAS 2232-85 might be due to similar genome content of the line with recipient parent and lower severity index of bacterial blight disease of the genotype as compared to its parental lines. The other pyramided lines like CRMAS 2231-48, CRMAS 2231-37, CRMAS 2231-36 and CRMAS 2232-66 also showed low severity index and higher yield than the

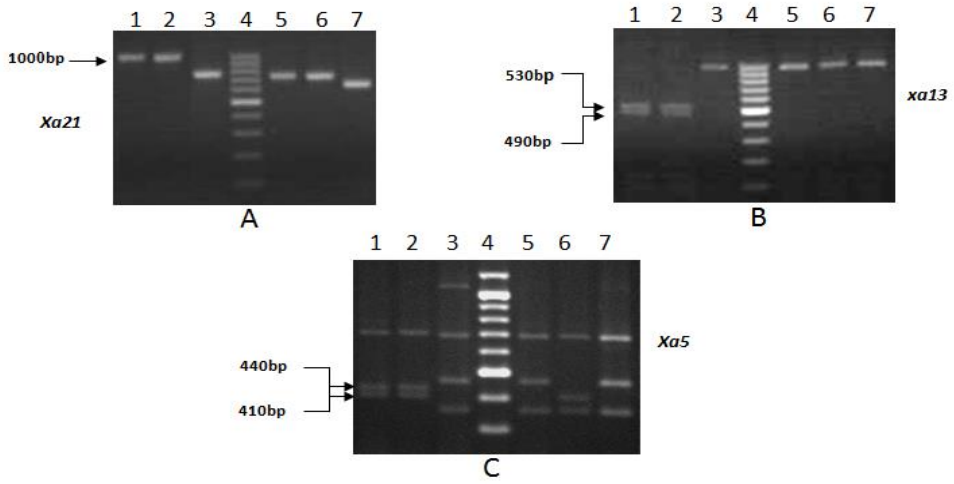

Fig 1. PCR amplification of markers linked to resistance genes, *Xa21*, *xa13* and *xa5* using primers A) pAT248 B) RG136 and C) RG556, respectively. Lane1-CRMAS 2232-85; Lane2-CRMAS 2232-66; Lane3-Swarna; Lane4-DNA ladder; Lane5-Jalmagna; Lane 6-TN1 and Lan7-CR Dhan 500.

recipient parent, Swarna. The plant height and flowering duration of the popular variety Swarna is also retained in the backcross derivative lines CRMAS 2232-85, CRMAS 2232-66 and CRMAS 2232-71. The major agro-morphologic traits of the mega variety Swarna are retained in the pyramided progenies. Hence, the better yielding pyramided line CRMAS 2232-85 may easily be accepted by the Swarna variety growers. Earlier reports indicate that many pyramided lines have been developed with incorporation of wanted character(s) without changing major traits of the popular varieties (Huang et al., 1997; Sanchez et al., 2000; Singh et al., 2001; Joseph et al., 2004; Pha et al., 2004; Perez et al., 2008; Sundaram et al., 2008; Rajpurohit et al., 2011; Dokku et al., 2013; Suh et al., 2013). However, in case of Swarna Sub1 development, the hull color and duration has been affected as compared to Swarna variety. This is due to linkage drag of the donor segments with the target trait and subsequently integrated in the backcross progeny (Neeraja et al., 2007). No such dragging effect is observed in the good pyramided lines characterized here. The best pyramided line showed higher panicles/m2 than the recurrent parent which might be manifesting for higher yield of pyramided lines. The pyramided lines homozygous for each of the individual genes and presence of the combinations were confirmed in the genotyping data using molecular markers linked to resistance (Fig.1). All the pyramided lines showed the presence of resistance specific band of 1000bp (Fig. 1A), 530bp and 490bp (Fig. 1B) and 440bp, 410bp (Fig.1C), indicating the presence of BB resistance genes, *Xa21*, *xa13* and *xa5*, respectively (Table 6). The genotypes Swarna, CR Dhan 500 and Jalmagna and TN1 showed the absence of these bands, indicating absence of these three resistance genes. The severity index of BB attack was very high for susceptible cultivar Swarna and TN1 while the indices are low for pyramided lines (Table 5). The grain and its cooking quality characters like milling %, head rice recovery %, kernel length (mm), L/B ratio, volume expansion ratio, water use (ml), kernel length after cooking (mm), alkali spreading value, elongation ratio, amylose content (%) and gel consistency (mm) almost remain same as like the recipient parent. The quality features of the mega variety Swarna have been retained along with high grain yield and durable bacterial blight resistance in selected pyramided lines. Hence, it is expected that the pyramid version of the mega varieties could be easily promoted among the farmers who prefers Swarna. Joseph et al. (2004) reported to have recovered favorable characteristics of Pusa Basmati 1 with two BB resistance genes through MAS in BC1 due to stringent phenotypic selection during segregating generation. Sundaram et al. (2008) recovered Sambha Mahsuri characteristics along with three BB resistance genes into the pyramided lines by using foreground and background selection. The key cooking and eating quality traits like KLAC, ER and KL values are higher in pyramided lines than the recurrent parent, Swarna suggests more possibility of acceptance of pyramided lines. Similarly, medium GC values of Swarna pyramided lines are more desirable than the hard GC value of recipient parent Swarna.

The multi location trials have confirmed the similarity of morphological and grain quality features of Swarna and IR64 with resistance for the bacterial blight disease in the pyramided lines. The high levels of resistance to BB and the absence of any yield penalty due to accumulation of resistance genes in the pyramids provides us a successful example of the integrated approach of selection at both molecular and phenotypic levels for transfer of the desired trait(s) and recovery of the recurrent parental genome. The multi-location data suggests that pyramid line CRMAS 2232-85 is better than its recurrent parent with respect to yield, agro-morphological traits and grain quality features and better than recurrent parent in terms of BB resistance. Development of broad-spectrum resistance against BB in the Indian subcontinent is a major challenge due to many agro-climatic zones in the country in the rice is cultivation areas along with the presence of a number of genetically distinct virulent *Xoo* strains in different geographical areas of the country. The study demonstrated that deployment of a three gene combination xa5+ xa13 + Xa21 can achieve durable and broad-spectrum resistance in many BB prone rice growing areas in India.

Materials and Methods

Plant materials and experimental design

Six near isogenic lines (NILs) in the background of Swarna and IR 64 varieties were developed through marker assisted backcrossing (Sundaram et al., 2014) and evaluated at nine locations of the country to validate the agronomic worth and

Table 1. List of pyramided lines, parents and check varieties used in the study

Pyramided line/Cultivar	Description	Cross	Gene	Remarks
CRMAS 2232-66	Pyramided line in Swarna background	Swarna*4/IRBB60	xa5+xa13+Xa21	Three BB resistance genes pyramided line in Swarna background
CRMAS 2232-71	Pyramided line in Swarna background	Swarna*4/IRBB60	xa5+xa13+Xa21	Three BB resistance genes pyramided line in Swarna background
CRMAS 2232-85	Pyramided line in Swarna background	Swarna*4/IRBB60	xa5+xa13+Xa21	Three BB resistance genes pyramided line in Swarna background
CRMAS 2231-36	Pyramided line in IR 64 background	IR64*4/IRBB60	xa5+xa13+Xa21	Three BB resistance genes pyramided line in IR64 background
CRMAS 2231-37	Pyramided line in IR 64 background	IR64*4/IRBB60	xa5+xa13+Xa21	Three BB resistance genes pyramided line in IR 64 background
CRMAS 2232-48	Pyramided line in IR 64 background	IR64*4/IRBB60	xa5+xa13+Xa21	Three BB resistance genes pyramided line in IR64 background
Swarna	Recurrent parent	Mahsuri/Vasistha	Unknown	Mega variety in India
IR64	Recurrent parent	-	Unknown	Mega variety in India
IRBB60	Donor parent	-	Xa4+xa5+xa13+Xa21	Donor parent
Local checks	Pantdhan4 for Pantnagar;HKR47 for Kaul; PAU 201 for Ludhiana; Tapaswini for CRRI; Karma Mahsuri for Raipur; Rajendra Sweta for Patna; GR 11 for Nawagaon; Karjat 6 for Karjat and BPT 5204 for Maruteru	-	Unknown	Popular variety of each location

Table 2. Polymerase chain reaction primers used for the identification of major BB resistance genes

Resistance gene	Chromosome number	Marker	Primer sequences used for gene detection Forward(5'-3')	Reverse(5'-3')	Expected size (bp)	Band type	reference
xa5	5	RG556	TAGCTGCTGCCGTGCTGTGC	AATATTTCAGTGTGCATCTC	440bp, 410bp	STS	Huang et al.,1997
xa13	8	RG136	TCCCAGAAAGCTACTACAGC	GCAGACTCCAGTTTGACTTC	530bp, 490bp	STS	Huang et al.,1997
Xa21	11	pTA248	AGACGCGGAAGGGTGGTTCCCGGA	AGACGCGGTAATCGAAGATGAAA	1000bp	STS	Huang et al.,1997

Table 3. Grain yield (kg ha^{-1}) of pyramided lines and parents across locations and over years.

Genotypes	Year	PNT	KUL	LDH	CRR	RPR	PTN	NGW	KJT	MTU	Mean	Pooled Mean
CRMAS 2231-36	1st	2425	5777	6106	3716	2880	3981	9000	3958	4816	4740	4174
	2nd	2939	4521	4451	2309	2051	2222	5328	3590	5058	3608	
CRMAS 2231-37	1st	2500	5148	6209	4900	2607	4490	6166	3690	4908	4513	4205
	2nd	3250	4078	3985	2524	2678	2222	6802	4535	5000	3897	
CRMAS 2231-48	1st	2723	6000	5450	4043	2768	4768	8000	3541	4645	4660	4262
	2nd	3384	5008	5050	2729	2735	1999	5668	3476	4728	3864	
CRMAS 2232-66	1st	2709	3037	-	3310	4098	4444	6500	4136	5934	4271	4064
	2nd	1687	3812	4173	3677	3133	3259	5442	4157	5366	3856	
CRMAS 2232-71	1st	2446	2888	-	2760	4074	4120	6166	3988	5363	3976	3867
	2nd	1666	4078	5621	3624	2877	2444	5328	2877	5300	3757	
CRMAS 2232-85	1st	2744	3629	-	3600	4537	5138	9000	4672	5579	4862	4518
	2nd	2053	3546	4941	4119	3532	2740	6802	4572	5250	4173	
Swarna (Recurrent Parent)	1st	2446	3777	-	3026	4722	4305	6666	4285	5815	4380	3875
	2nd	928	3280	2435	3739	3874	3703	3174	3874	5310	3369	
IR 64	1st	2482	5481	6120	3836	2785	3703	7833	3601	4584	4492	4001
	2nd	2918	3324	4176	2569	2165	2222	5555	3817	4927	3519	
IRBB60 (Donor)	1st	2354	5814	6125	4170	2750	3194	6000	3720	4795	4325	3903
	2nd	2652	4476	2294	2621	2421	2296	5668	4043	4860	3481	
Local check	1st	3390	6037	7233	2836	3578	2731	6333	3690	4172	4444	4135
	2nd	3372	4388	4619	3045	3504	2222	4308	3892	5078	3825	
$CD_{5\%}$		274	768	1351	705	529	513	130	649	342		
CV%		6.2	10.7	19.5	19.7	9.7	9.5	8.9	9.8	4.1		

PNT-Pantnager; KUL-Kaul; LDH-Ludhiana; CRR-CRRI, Cuttack; RPR-Raipur; PTN-Patna; NGW-Nawagaon; KJT-Karjat; MTU-Maruteru

Table 4. Overall performance of agro-morphological and quality traits of the pyramided lines and parents across locations and year.

Genotypes	Days to 50% flow.	Panicles/ Sq.mt.	Plant Height (cm)	Milling (%)	Head Rice Recovery (%)	KL (mm)	L/B Ratio	VER	WU (ml)	KLAC (mm)	ASV	ER (mm)	Amylose %	Gel consistency of starch (mm)
CRMAS 2231-36	97	301	96	68.1	49.0	6.54	3.16	5.0	172.5	10.6	4.5	1.63	22.83	65
CRMAS 2231-37	98	306	95	70.0	51.0	6.45	3.11	4.7	177.5	10.0	4.5	1.54	22.59	63
CRMAS 2231-48	97	302	95	68.4	51.0	6.35	3.10	5.5	160.0	9.8	4.5	1.54	23.37	51
CRMAS 2232-66	114	300	86	70.8	67.4	5.39	2.59	4.4	172.5	9.7	6.5	1.80	25.73	42
CRMAS 2232-71	112	297	85	70.5	67.2	5.38	2.53	4.8	152.5	8.0	7.0	1.49	25.58	48
CRMAS 2232-85	113	308	86	69.9	66.7	5.24	2.57	4.8	155.0	8.8	4.5	1.65	22.42	57
Swarna (recurrent Parent)	112	287	89	68.5	63.5	5.28	2.44	4.7	157.5	8.0	6.0	1.51	25.86	57
IRBB60 (Donor)	100	315	94	69.0	52.8	6.45	3.11	4.7	177.5	10.1	4.5	1.57	22.42	67
Local check	104	282	94	73.6	60.2	6.47	3.21	5.3	275.0	10.4	7.0	1.6	23.97	52

KL- Kernel length (mm);L/B:Length breadth ratio; VER- Volume expansion ratio; WU- Water uptake(ml);KLAC- Kernel length after cooking; ASV- Alkali spreading value; ER- Elongation ratio

bacterial blight resistance of the lines. The donor parent IRBB60, recipient parent Swarna, resistant check Ajay and susceptible check TN1 were compared along with the Swarna and IR64 backcross progenies (Table 1). The multi-location evaluations of the entries were conducted in 9 locations representing nine states and four regions of the country in a randomized block design with three replications. Pantnagar in Uttaranchal state, Kaul in Haryana, Ludhiana in Punjab, Cuttack in Odisha, Raipur in Chhattisgarh, Patna of Bihar, Nawagaon of Gujarat, Karjat in Maharashtra and Maruteru of Andhra Pradesh were taken during wet season, 2007 and 2008.

Screening for bacterial blight resistance

For field evaluation against BB, the mixed inoculums of eight predominant *Xoo* isolates prepared by suspending the bacterial mass in sterile water to a concentration of approximately 10^9 cells/ml (Kauffman et al., 1973) were clip inoculated. Four leaves from three different plants of each

Table 5. Reaction of the pyramided lines and parents against BB at multiple locations and over years.

Genotypes	Year	PNT	KUL	LDH	CRR	RPR	PTN	NGW	KJT	MTU	Severity Index(SI)	Pooled SI
CRMAS	1st	-	-	-	-	-	-	-	-	-	-	5.0
2231-36	2nd	-	-	3.0	-	9.0	-	-	3.0	5.0	5.0	
CRMAS	1st	-	-	-	-	-	-	-	-	-	-	5.0
2231-37												
	2nd	-	-	3.0	-	9.0	-	-	3.0	-	5.0	
CRMAS	1st	-	-	-	-	-	-	-	-	-	-	5.5
2231-48	2nd	-	-	3.0	-	9.0	-	-	3.0	7.0	5.5	
CRMAS	1st	1.0	7.0	1.0	3.0	5.0	5.0	1.0	4.0	3.0	3.3	4.15
2232-66	2nd	-	-	3.0	-	7.0	-	-	3.0	7.0	5.0	
CRMAS	1st	1.0	7.0	1.0	3.0	5.0	5.0	1.0	4.0	3.0	3.3	3.9
2232-71	2nd	-	-	3.0	-	7.0	-	-	3.0	5.0	4.5	
CRMAS	1st	1.0	7.0	3.0	3.0	7.0	7.0	5.0	4.0	3.0	4.4	4.2
2232-85	2nd	-	-	3.0	-	9.0	-	-	3.0	1.0	4.0	
Swarna	1st	1.0	7.0	5.0	7.0	5.0	7.0	3.0	4.0	5.0	4.9	5.45
(Recurrent	2nd	-	-	9.0	-	7.0	-	-	3.0	5.0	6.0	
Parent)												
Ajay	1st	3.0	7.0	3.0	7.0	7.0	3.0	3.0	3.0	5.0	4.6	4.95
	2nd	-	-	3.0	-	7.0	-	-	6.0	5.0	5.3	
TN1	1st	5.0	9.0	7.0	9.0	7.0	9.0	7.0	5.0	9.0	7.4	7.2
	2nd	-	-	9.0	-	7.0	-	-	7.0	5.0	7.0	

Table 6. Amplification of genotypes with BB resistance gene specific markers.

Sl. No.	Genotypes	Target genes		
		Xa21 *(*1000bp)	*xa13* *(*530bp, 490bp)	*xa5* (440bp, 410bp)
1	CRMAS 2232-85 (IET 20672)	+	+	+
2	CRMAS 2232-66	+	+	+
3	Swarna	-	-	-
4	Jalmagna	-	-	-
5	TN1	-	-	-
6	CR Dhan 500	-	-	-

entry were clip inoculated at the maximum tillering stage and observations were recorded after 15 days. Disease reaction was based on visual scoring and measurement of lesion length (LL) and the threshold level employed for distinguishing resistant and susceptible reaction was 3 cm (resistant) and 5 cm (susceptible) lesion length.

Characterization for morphological and quality traits

Thirty-day-old seedlings of the pyramided lines, the parents (IRBB60, Swarna) and check varieties were transplanted with 15 × 20 cm spacing at various BB hotspot locations of the country. Data were recorded on five plants from each line for agronomic traits like plant height, tiller no./plant, panicle length, number of filled grains/panicle and 1,000-grain weight while whole genotype on plot basis for days to 50 % flowering (DFF). The head rice was prepared according to the methods described by Tan et al. (1999). The amylose content of the genotypes was estimated according to Juliano (1996). GC analysis followed the methods described by Cagampang et al. (1973). ASV was assayed according to the method of Little et al. (1958). Cooking qualities were estimated by cooking 25 intact polished grains. The grains were soaked in 20 ml distilled water for 30 min in test tubes. The tubes were placed in vigorously boiling water for 10 min and than cooled in cold water. The average length and width of 10 intact cooked kernels was measured in mm and the length/width (L/W) ratio calculated from the average.

DNA isolation, PCR amplification and marker analysis

To establish/ confirm the presence of the three BB resistance genes, the DNA was isolated from young leaf for PCR analysis following Dellaporta et al. (1983). The PCR reaction mixture contained 50 ng templates DNA, 5 pico mole of each of the primers, 200 µM dNTPs, 1 X PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% TritonX-100) and 1 unit of Taq DNA polymerase in a volume of 20 µl and amplification of target sequences were as per earlier reports (Table 2). The PCR products of CAPS markers RG 556 and RG 136 were digested with restriction enzymes *Dra*I and *Hinf*I respectively as per manufacturer's instructions. The PCR products and the DNA fragments produced by restriction digestions were separated by gel electrophoresis and gel images were analyzed on gel documentation system (SynGene) for establishing the presence of BB resistance genes, *Xa21*, *xa13* and *xa5*, respectively. Amplified products were scored for presence (1) or absence (0) for each primer genotype combination.

Conclusion

The three BB genes (*xa5, xa13, Xa21*) pyramided lines in the background of Swarna and IR64 were observed to be superior in agronomic performance along with higher level of resistance to BB disease as compared to parental lines. Besides, the present study confirmed the presence of BB resistance genes *Xa21*, *xa13* and *xa5*. Also, these pyramided

lines showed similar agro-morphologic and quality traits as like the recipient parent. Hence, development and release of pyramided lines with broad-spectrum resistance against bacterial blight disease to tackle different virulent strains present in the different agro-climatic zones of the country can provide better resistance against the disease.

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