Research Journal of Biology
Volume 1: 52 – 66 (Published: 14 October, 2013)
ISSN: 2322-0066

RESEARCH ARTICLE

Improvement of resistance to bacterial blight through marker assisted backcross breeding and field validation in rice (*Oryza sativa*)

Lalitha D. Guvvala, Pranitha Koradi, Vinay Shenoy and Lalitha S. Marella*

Barwale Foundation, Barwale Chambers, Street 10, Himayathnagar, Hyderabad 500 029, Andhra Pradesh, India
*Corresponding author's E-mail: shanti@barwalefoundation.org

ABSTRACT

Bacterial blight is one of the major constraints in rice cultivation. Breeding for durable resistance is most effective and economical method to combat disease. In present study, four resistance genes (*Xa4, xa5, xa13* and *Xa21*) were pyramided into popular cv. Mahsuri and two hybrid rice parental lines, PRR78 and KMR3 using marker assisted back cross breeding. Nine pyramid families of each background were evaluated under both artificial and natural disease conditions. Pyramids were inoculated with sixteen *Xanthomonas oryzae* pv. *oryzae* isolates collected from different parts of India. BF16 was highly virulent on recurrent parents with a yield loss of 23% in Mahsuri, 28% in PRR78 and 24% in KMR3. BF7 (10% loss in Mahsuri and 13% in KMR3) and BF10 (22% in PRR78) were least virulent. Under natural condition at Maruteru, disease showed a yield loss of 22% in Mahsuri, 28% in PRR78 and 26% in KMR3. Under both conditions, none of the pyramids were susceptible. There were no significant differences within pyramids with respect to any character evaluated. No pyramid was susceptible. The agronomic characters showed no significant difference between parent and pyramid. Yield and its related characters were statistically significant between parents and pyramids ($P< 0.05$). The pyramids insulated a heavy yield loss to the tune of ~28% and pyramiding of resistance genes (two dominant and two recessive) did not show any negative effect on agronomic performance of any pyramid. These pyramids can be used directly and also as valuable source of resistance in future breeding programs.

Key Words: Bacterial blight, molecular markers, resistance, rice yield, *Xanthomonas oryzae* pv. *oryzae*.
(Received: 18/09/2013; Accepted: 10/10/2013; Published: 14/10/2013)

INTRODUCTION

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) (Swings *et al.,* 1990) is one of the most devastating diseases of rice world-wide (Ou, 1985; Mew, 1987; Nino-Liu *et al*., 2006). It causes leaf wilting, affects rate of photosynthesis and lead to yield losses of up to 80-100% in severe cases (Singh *et al.,* 1997; Zai and Zhu, 1999). The disease being systemic, effective chemical control measures are lacking (Devadath, 1989) and the concern over health hazards of pesticides limited the utilization of chemical control agents (Guillebeau, 1998). Breeding for disease resistance is the most important approach for its management (Sanchez *et al*., 2000; Singh *et al.,* 2001; Zhang, 2005; Nino-Lui *et al*., 2006; Sundaram *et al*., 2008; Mamgain *et al.*, 2013). Conventional plant breeding mainly depends on phenotypic selection is time consuming process and less efficient in multiple gene pyramiding because of dominance and epistasis effect in multiple gene transfer (Collard and Mackill, 2008) and linkage drag (Young and Tanksley, 1989). Molecular markers have been successfully used in selecting for resistant varieties even in the absence of pathogens (Melchinger, 1990) and to reduce the likelihood of pathogens overcoming resistance (Yoshimura *et al*., 1995; Huang *et a*l., 1997). More than 30 genes imparting resistance to BB have been identified till now (Basavaraj *et al.,* 2010; Bhasin *et al.,* 2012; Natrajkumar *et al.,* 2012). Multiple resistance genes conferred broad spectrum and durable resistance by synergistic and complementary gene action to a wide range of races compared to one, two and three gene combinations (Ogawa and Khush, 1988; Huang *et al*., 1997; Adhikari *et al.,* 1999; Shanti and Shenoy, 2005).

Main aim of the present study was to pyramid four BB resistance genes and to assess the agronomic characters *viz*., days to 50% flowering (DFF), plant height (PH), number of tillers (NT), panicle length (PL), yield and its related characters namely filled grains per panicle (FG/P), 1000 grain weight (GW), yield (Y) and biomass (BM), of the nine pyramid families in the background of Mahsuri, PRR78 and KMR3.

Mahsuri is a first rain fed traditional variety of the Indian subcontinent with a significant yield advantage in the low–fertility soils and highly desirable grain quality (Collard *et al.,* 2008), but is highly susceptible to diseases and pests. Hybrid rice technology is one of the feasible options to increase rice production and productivity with 20% higher yields than inbred semi dwarf varieties (Virmani, 1996). The increased yield of rice hybrids alone does not ensure profitability to farmers, if their grain quality is not acceptable and if they are susceptible to the diseases (Virmani and Kumar, 2004). Hybrid vigor alone does not make rice hybrids more or less tolerant of biotic stresses than parental lines (Cohen *et al.,* 2003; Diwan *et al.,* 2013). Biotic stress resistance in a rice hybrid is determined by the resistance of its parental lines. PRR78 is a fertility restorer line having long slender grains and is one of the parents for Pusa RH10, the world's first and only superfine grained aromatic rice hybrid released from the Indian Agricultural Research Institute (IARI), New Delhi, India in 2001. This hybrid has maturity in 110 days and average yield of 7,000 kg per hectare (Siddiq *et al.,* 2009). The desirable characters of aroma, kernel elongation, fluffiness and longer shelf life than the other hybrids have made this hybrid commercially viable. KMR3 is the best fertility restorer for hybrid rice breeding programs and the male parent of KRH2, the most popular bred non aromatic hybrid.

A four gene combination (*Xa4, xa5, xa13* and *Xa21*) was most stable, conferring resistance against different isolates of the pathogen (Shanti and Shenoy, 2005). Based on these findings, Mahsuri, PRR78 and KMR3 pyramids with four BB resistance genes (*Xa4, xa5, xa13* and *Xa21*) were developed using marker assisted back cross breeding (MABB). Molecular markers were used for foreground selection to pick the four target genes in homozygous condition and conventional breeding was followed for background selection. Nine pyramid families were selected from each background.

The nine best performing pyramid families of Mahsuri, PRR78 and KMR3, were evaluated against their parents to compare their performance and to know the % of insulation against yield loss under disease pressure conditions inoculated with sixteen different *Xoo* isolates collected from different geographical locations of India. The pyramids were also tested under natural infection at Maruteru, West Godavari, Andhra Pradesh, India, a hotspot for bacterial leaf blight. This in turn would also show if any yield penalty existed due to pyramiding of four resistance genes at field level.

MATERIALS AND METHODS

Experimental treatments and site:

IRBB60, a near isogenic line in the background of IR24, carrying the four resistance genes, *Xa4, xa5, xa13* and *Xa21* served as the donor for all the crosses attempted with three recurrent parents (Mahsuri, PRR78 and KMR3) and a total of nine pyramid families with four BB resistance genes (*Xa4, xa5, xa13* and *Xa21)* in homozygous condition in the above three backgrounds. The recurrent parents were included as controls for susceptibility. All the pyramid families were evaluated for the agronomic, yield and yield related characters. In addition, resistance to BB was evaluated by measuring individual lesion length to the nearest centimetre. Malagkit Sung Song (MSS) an aromatic variety and IRBB60 were taken as controls for resistance and cultivar Taichung Native 1(TN1) was used as control for susceptibility. Sixteen *Xoo* isolates designated as BF1 to BF16 used in this study were procured from Central Rice Research Institute (CRRI), Cuttack (Table 1) and maintained in the Barwale Foundation's molecular biology laboratory on modified Wakimoto's semi synthetic agar 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). The pyramid families were evaluated in the wet season from June to October during 2009, 2010 and 2011 at the research farm of the Barwale Foundation, located at Maharajpet, Shankarpally Mandal, Hyderabad, Andhra Pradesh, India under artificial inoculation and at Maruteru under natural infection, India. Maharajpet area is located at 17º24' N, 78º12' E and an altitude of 536m above mean sea level and soil of the experimental site is clay loam. Maruteru is located at 16° 37' 60N, 81° 43' 60E and an altitude of 5m above the mean sea level. The soil type is black alluvial clay.

Table 1. *Xoo* isolates used for the validation of Mahsuri, PRR78 and KMR3 pyramids provided by Central Rice Research Institute, Cuttack, Orissa, India

Sl. No.	Isolate	Origin
1	BF1	Cuttack, Orissa
2	BF2	Cuttack, Orissa
3	BF3	Cuttack, Orissa
4	BF4	Raipur, Madhya Pradesh
5	BF5	Raipur, Madhya Pradesh
6	BF6	Raipur, Madhya Pradesh
7	BF7	Faizabad, Uttar Pradesh
8	BF8	Faizabad, Uttar Pradesh
9	BF9	Maruteru, Andhra Pradesh
10	BF10	Maruteru, Andhra Pradesh
11	BF11	Maruteru, Andhra Pradesh
12	BF12	Maruteru, Andhra Pradesh
13	BF13	Maruteru, Andhra Pradesh
14	BF14	Maruteru, Andhra Pradesh
15	BF15	Maruteru, Andhra Pradesh
16	BF 16	Maruteru, Andhra Pradesh

Experimental design and agronomic practices:

Each experiment was laid in a randomized complete block design with three replications under natural condition and for each culture-strain combination under artificial condition. Twenty one day old seedlings of the recurrent parents Mahsuri, PRR78 and KMR3, nine families each of the pyramids of Mahsuri, PRR78, KMR3, susceptible check TN1 and resistant checks MSS and IRBB60 were transplanted with one seedling per hill and a spacing of 20 X 20 cm. Each pyramid family, recurrent parent and checks consisted of 40 plants in four rows of 10 plants each. A spacing of 60cm was maintained between each replication. Agronomic observations were recorded on Mahsuri, PRR78 and KMR3 parents and nine pyramid families of Mahsuri, PRR78 and KMR3 whereas disease-related observations were recorded on the susceptible check TN1 and resistant check MSS and IRBB60 also. After transplanting, approximately 5 cm of standing water was maintained in the fields. 120:60:60 kg/hectare NPK was applied 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 brown plant hopper and blast under artificial inoculation condition. All plant protection measures were stopped fifteen days prior to inoculation studies to rule out the interactions and masking effects of these chemicals with *Xoo*. All the above practices were followed at Maruteru with no plant protection measures applied.

Screening for BB resistance:

The recurrent parents and nine pyramid families of Mahsuri, PRR78 and KMR3 were evaluated for their resistance reaction to the BB pathogen under artificial inoculation condition with sixteen *Xoo* isolates at the research farm and natural infection at Maruteru. The cultures were maintained on modified Wakimoto's semi-synthetic medium. For long-term storage, the

cultures were maintained as 50% glycerol stocks at -20°C. 1mL of 50% (w/v) glycerol was aliquoted into 1.5ml eppendorf tubes. In these tubes, 48h old colonies of *Xoo* were taken in a sterile loop under aseptic conditions, suspended and stored. The stored cultures were revived and grown on modified Wakimoto's medium for inoculation experiments. The specific isolate to be used was inoculated on the susceptible cultivar TN1 and re-isolated in the lab before use in inoculation experiments, to maintain the virulence of the isolates.

Evaluation of resistance:

Under artificial condition - Approximately forty-day-old plants were clip inoculated (Kauffman *et al*., 1973) at maximum tillering stage with a pair of scissors every time dipped into the bacterial suspension prepared from 48h old actively growing bacterial culture of each isolate grown on modified Wakimoto's semi-synthetic agar medium with a cell density adjusted to 10^8 cfu (colony forming units) per ml, determined by a spectrophotometer at 620 nm (Kauffmann *et al*., 1973). A separate pair of sterilized scissors was used for inoculation of each isolate of *Xoo* to cut top five leaves of plants in each replication. Each test was replicated thrice. Disease reaction was recorded 15 days after inoculation. Lesion lengths were measured to the nearest centimetre.

Under natural condition - The pyramids and checks were allowed for natural infection at Maruteru. Disease reaction was measured 15 days after the disease started appearing. The lesion length of all the checks and pyramids was measured to the nearest centimetre for classify the disease response.

In both the above experiments, each plant was classified as resistant (0-4 cm) and susceptible (>4 cm) based on lesion length (Shanti *et al.*, 2001).

DNA extraction and polymerase chain reaction:

The recurrent parents (Mahsuri, PRR78 and KMR3) and their pyramids were screened to confirm that, pyramid families contain all the target genes in homozygous condition and to confirm that there were no off-types. Mini scale DNA isolation for PCR analysis of the parents and pyramid families of Mahsuri, PRR78 and KMR3 was carried out following Dellaporta *et al.* (1983). Three sequence-tagged-site (STS) markers Npb181, RG136 and pTA248 tightly linked to *Xa4, xa13* and *Xa21* genes respectively and one simple sequence repeat (SSR) marker RM122 tightly linked to *xa5* were used to confirm the presence of each gene and the gene combinations. The PCR mixture contained 50ng of template DNA, 5picomoles of each primer, 0.05mM dNTPs, 1X PCR buffer (10mM Tris, pH 8.4, 50mM KCl, 1.8mM MgCl2 and 0.01 mg/ml gelatin) and 1U Taq DNA polymerase in a reaction volume of 25µl. Template DNA was initially denatured at 94ºC for 5 min followed by 35 cycles of PCR amplification with the following parameters: 30 sec denaturation at 94ºC, 30sec annealing at 55ºC and 1min primer extension at 72ºC. A final extension was done at 72ºC for 5 min. The amplified product of pTA248 and Npb181 was electrophoretically resolved on a 1.4% and 2.5% agarose gel respectively and visualized under UV.

For the amplified products of RG136 since all the bands are monomorphic, before proceeding with digestion, 5µl of PCR product was used for gel electrophoresis to determine the success of amplification. The remaining product was used for restriction digestion. The reaction mixture consisted of 0.5µl (10U/µl) of restriction enzyme *Hinf I*, 2.0µl of 10X PCR buffer and 2.5µl of sterile distilled water added to 15µl of PCR product. The reaction mixture was incubated for 4h at 37ºC and the products of restriction digestion were separated by gel electrophoresis (1.4% agarose) and visualized under UV light after staining with ethidium bromide (10µg/ml). For *xa5*, PCR was carried out using 20ng DNA as template for amplification, 5picomoles of each primer, 0.05mM dNTPs, 1X PCR buffer, and 1U of Taq polymerase in a total volume of 15µl. Template DNA was initially denatured for 94ºC for 5min followed by 35 cycles of PCR amplification with the following parameters: 30sec denaturation at 94ºC, 30sec primer annealing at 55ºC and 1min primer extension at 72ºC. A final extension was done at 72ºC for 5min. PCR products were resolved in 3% agarose gel.

Data collection and statistical analysis:

Data were recorded on five plants from each entry, excluding the border plants. Data were collected for disease resistance along with the agronomic characters, *viz* days to 50% flowering (DFF), plant height (PH), number of tillers (NT), panicle length (PL), yield (Y) and its related characters namely filled grains per panicle (FG/P), spikelet fertility (SF %), 1000 grain weight (GW), biomass (BM) and harvest index (HI) was calculated. For data on inoculation studies log transformations were applied before subjecting it to statistical analysis. Data was analyzed combined with analysis of variance (ANOVA) using the Crop Stat Version 7.2. 2007. 3. Fisher's protected Least Significant Difference (LSD) at 5% probability was used where the F test was significant.

RESULTS

Marker-assisted selection for BB resistance:

Marker-assisted foreground selection for four BB resistance genes *Xa4, xa5, xa13* and *Xa21* was carried out using their respective tightly linked markers. F_1s of each cross (Mahsuri / IRBB60, PRR78/ IRBB60 and KMR3/ IRBB60) were tested for heterozygosity of target genes and selected true types were backcrossed to their respective recurrent parents. A total of 600 Mahsuri, 620 PRR78 and 580 KMR3, BC_1F_1s were produced and individuals heterozygous for all four genes were identified based on fore ground selection with linked markers. These were backcrossed to produce BC_2F_1 progenies which were subjected to same selection process and the selected individuals were backcrossed to produce BC_3F_1 progenies and the ones with true plant type and target genes in heterozygous condition were selfed to produce BC_3F_2 families. The BC_3F_2 families with true plant type and all target genes in homozygous condition were advanced to further selfing till F_6 generation. Phenotypic selection at field level for recurrent parent type was performed at each backcross and selfing generation to eliminate the plants with unwanted phenotype. We selected nine pyramid families at BC_3F_6 generation in Mahsuri, PRR78 and KMR3 backgrounds with the resistance alleles of the four BB genes in homozygous condition (Figure 1). The individual plants of each generation were first checked for the presence of *Xa21* resistance allele and the selected plants were

checked for the presence of *xa5* resistance allele. All the plants with the resistant allele of both the genes were screened for the *Xa4* gene followed by the presence of *xa13* resistance allele. The parents did not have the resistance allele but had the susceptibility allele of the four genes.

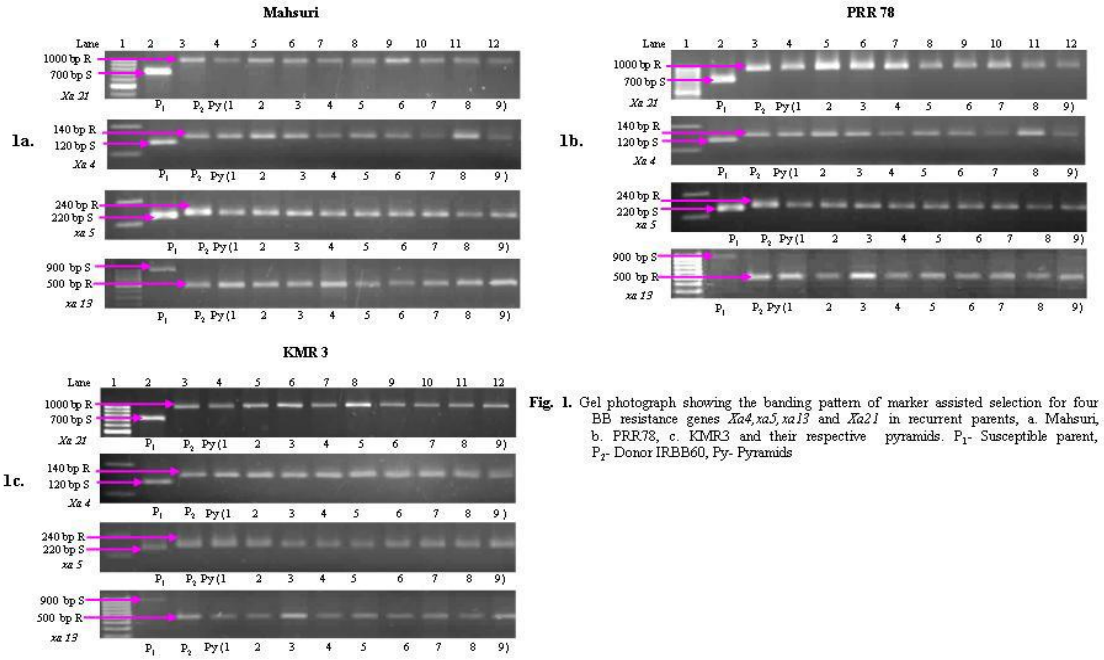

Fig. 1. Gel photograph showing the banding pattern of marker assisted selection for four BB resistance genes *Xa4, xa5, xa13* and *Xa21* in recurrent parents, a. Mahsuri, b. PRR78, c. KMR3 and their respective pyramids. P_1- Susceptible parent, P_2- Donor IRBB60, Py- Pyramids

Evaluation of BB resistance in pyramids:

Under artificial infection, there were significant differences (at 5% LSD) between isolate (I), genotype (G) and I*G interaction (Table 2) for lesion length (cm) evaluated. There was no significant difference between the seasons (S) and the interactions between S*I, S*G and S*I*G for the lesion length. Under natural infection, there were significant differences (at 5% LSD) between genotypes (G) for lesion length (cm) evaluated. There was no significant difference between the seasons (S) and the interactions between S*G for lesion length (Table 2).

Table 2. ANOVA results of log transformed lesion length data of Mahsuri, PRR78 and KMR3 pyramid families along with checks under artificial and natural conditions

Treatment	Genotype	Source of variation	d.f.	F. ratio	C.V.%	LSD (5%)
Artificial condition	Mahsuri pyramids	1. Season (S)	2	0.62	6.9	1.15
		2. Isolate (I)	15	518.01***		
		3. Genotype (G)	12	11863.92***		
		4.S*I	30	0.7		
		5. I*G	180	6.4***		
		6. S*G	24	1.09		
		7. S*I*G	360	0.93		
	PRR78 pyramids	1. Season (S)	2	0.12	6.2	0.147
		2. Isolate (I)	15	98.91***		
		3. Genotype (G)	12	11512.65***		
		4.S*I	30	0.61		
		5. I*G	180	14.42***		
		6. S*G	24	0.97		
		7. S*I*G	360	0.97		
	KMR3 pyramids	1. Season (S)	2	0.14	6.8	1.16
		2. Isolate (I)	15	196.20***		
		3. Genotype (G)	12	9442.34***		
		4.S*I	30	0.39		
		5. I*G	180	4.45***		
		6. S*G	24	0.97		
		7. S*I*G	360	0.63		
Natural condition	Mahsuri pyramids	1. Season (S)	2	0.79	2.7	1.06
		2. Genotype (G)	12	4033.22***		
		3. S*G	24	0.75		
	PRR78 pyramids	1. Season (S)	2	1.74	2.6	1.06

		2. Genotype (G)	12	4131.34***		
		3. S*G	24	1.28		
		1. Season (S)	2	0.95		
	KMR3 pyramids	2. Genotype (G)	12	2078.16***	3.2	0.03
		3. S*G	24	1.49		

*** Statistically significant at 0.05 probability level by LSD test, d.f. - degrees of freedom, M.S. -Mean squares, F. ratio - Fisher ratio, C.V. - Coefficient of variance, LSD-least significant difference at 5% level of significance

Artificial condition - Under inoculated condition, all the recurrent parents (Mahsuri, PRR78, and KMR3) and susceptible control exhibited highly susceptible reaction against all the isolates, whereas all pyramids showed resistance on par with the resistance checks across seasons. BF16 was highly virulent on all the recurrent parents, Mahsuri, PRR78 and KMR3 evaluated and the mean lesion length of 22.6cm in Mahsuri (control), 18.8cm in PRR78 (control) and 18.2cm in KMR3 (control) was recorded. The disease reaction of pyramids in all backgrounds was ranged between 1.5 and 3.0 cm. Individual pyramids showed varying degrees of lesion lengths but no pyramid family showed a lesion length more than 3 cm, proving that all families were resistant to all the isolates of *Xoo* collected from different parts of India.

Natural condition - Under natural infection, the parents were at par with the susceptible check, TN1 in terms of disease reaction, whereas all the pyramids in Mahsuri, PRR78 and KMR3 backgrounds were on par with the resistance checks across seasons. The average lesion length of 24.1cm in Mahsuri (control), 23.9cm in PRR78 (control) and 19.1cm in KMR3 (control) was recorded. The disease reaction of the Mahsuri, PRR78 and KMR3 four gene pyramid families ranged from 1.4 -2.8 cm. Individual pyramids showed varying degrees of lesion lengths but no pyramid family showed a lesion length more than 3 cm.

Agronomic performance:

Under artificial infection there were significant differences (at 5% LSD) between isolate (I), genotype (G) and I*G interaction for agronomic characters, yield and its related characters evaluated. There was no significant difference between the seasons (S) and the interactions between S*I, S*G and S*I*G for the characters evaluated (Table 3). Under natural infection, there were significant differences (at 5% LSD) between genotypes (G) for agronomic traits, yield and its related characters evaluated. There was no significant difference between the seasons (S) and the interaction between S*G for the characters evaluated (Table 3).

Under artificial condition:

Mahsuri pyramid families - Within the nine pyramid families of Mahsuri, there was no significant difference for any of the characters evaluated against 16 isolates. There was no significant variation for the agronomic characters *viz.*, DFF, PH, NT and PL between parent and the pyramids across seasons (Figure 2a). Both parent and pyramid showed a DFF of 133 days, PH of 158cm, 14 tillers per plant and a panicle length of 24cm. There were significant differences for yield and its related characters (Table 4) at 5% LSD, between the parent and the pyramid.

Under artificial condition, the % change in yield and its related characters *viz.*, FG/P, SF, GW, BM and HI in recurrent parent are as shown in Figure 6a, whereas there was no reduction in these characters in any of the nine families of the pyramids. Among all the isolates, BF16 was the highly virulent one and showed a reduction of 22% in yield of Mahsuri.

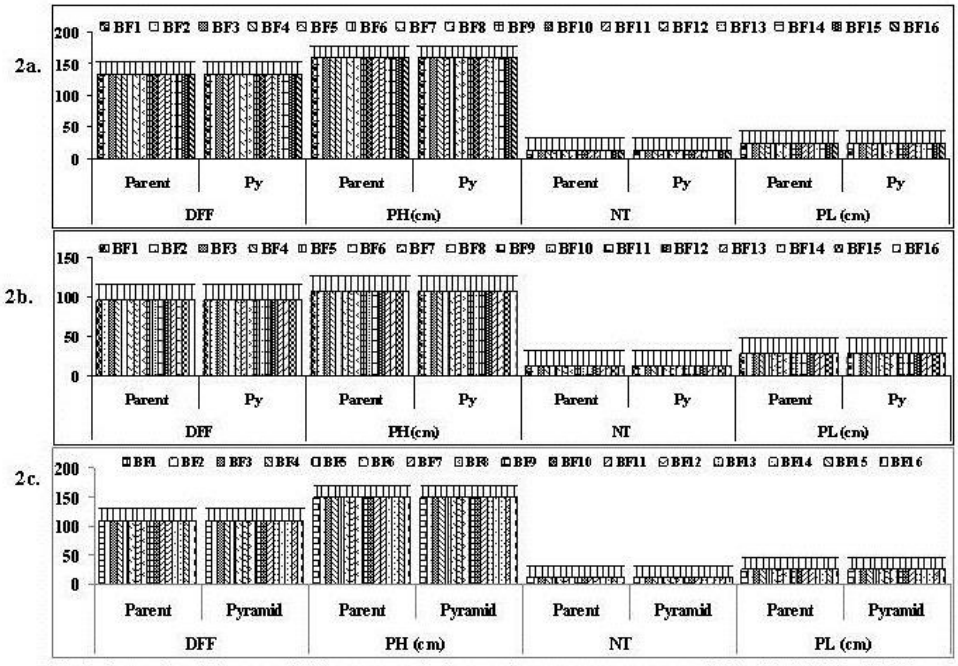

Fig. 2. Comparison of the means of different agronomic characters between recurrent parents, a. Mahsuri, b. PRR78, c. KMR3 and their respective pyramids under artificial condition; Where, DFF-Days to 50% flowering, PH-Plant height, NT-Number of tillers and PL-Panicle length

Table 3. Combined ANOVA results of agronomic performance of Mahsuri, PRR78 and KMR3 pyramids under artificial and natural conditions

Treatment	Genotype	Source of variation	d.f.	DFF	PH (cm)	NT	PL (cm)	FG/P	SF (%)	GW (g)	Y/P (g)	BM (g)	HI (g)
Artificial condition	Mahsuri pyramids	1. Season (S)	2	0.03	0.08	0.11	0.22	0.66	1.64	0.56	0.71	0.91	0.52
		2. Isolate (I)	15	0.39	0.52	0.3	0.27	173.35***	11.2***	612.81***	36.83***	35.44***	42.28***
		3. Genotype (G)	12	1.21	0.83	0.96	1.4	46427.89***	48811.11***	37519.86***	12035.12***	35206.39***	4480.11***
		4.S*I	30	0.38	0.31	0.3	0.4	0.34	1.38	0.46	0.66	0.44	0.78
		5. I*G	180	0.71	0.78	0.66	0.72	189.88***	11.22***	613.81***	33.58***	35.06***	37.45***
		6. S*G	24	0.47	0.43	0.43	0.45	0.26	0.42	0.34	0.47	0.28	0.68
		7. S*I*G	360	0.65	0.61	0.59	0.7	0.6	1	0.56	0.73	0.57	0.87
	PRR78 pyramids	1. Season (S)	2	0.02	0.03	0.1	0.08	0.44	1.43	1.66	0.99	0.67	0.94
		2. Isolate (I)	15	0.26	0.27	0.28	0.86	208.83***	15.41***	69.3***	36.92***	33.12***	44.26***
		3. Genotype (G)	12	0.79	0.9	0.97	0.96	24826.45***	28750.69***	20868.12***	49618.00***	33793.11***	37470.22***
		4.S*I	30	0.31	0.33	0.32	0.3	0.39	1.38	0.42	0.57	0.39	0.58
		5. I*G	180	0.71	0.66	0.72	0.65	212.13***	15.66***	73.83***	32.75***	33.18***	37.61***
		6. S*G	24	0.48	0.48	0.4	0.42	0.42	0.49	0.47	0.62	0.23	0.93
		7. S*I*G	360	0.65	0.6	0.63	0.57	0.61	0.98	0.67	0.76	0.54	0.95
	KMR3 pyramids	1. Season (S)	2	0.19	0.15	0.02	0.05	0.3	1.61	0.46	0.75	0.89	0.62
		2. Isolate (I)	15	0.28	0.57	1.19	0.3	90.20***	10.17***	222.57***	36.57***	36.61***	44.24***
		3. Genotype (G)	12	1.2	0.9	1.02	1.34	17879.48***	31851.44***	3497.81***	20728.89***	63033.53***	7727.49***
		4.S*I	30	0.36	0.32	0.35	0.34	0.32	1.37	0.32	0.63	0.41	0.75
		5. I*G	180	0.69	0.7	0.69	0.71	94.38***	10.04***	212.72***	33.35***	36.29***	39.28***
		6. S*G	24	0.47	0.5	0.34	0.43	0.29	0.38	0.5	0.47	0.24	0.69
		7. S*I*G	360	0.65	0.62	0.63	0.66	0.6	1.06	0.6	0.73	0.57	0.87
Natural condition	Mahsuri pyramids	1. Season (S)	2	0.13	0.15	0.02	0.71	0.25	0.66	0.22	0.06	0.17	0.13
		2. Genotype (G)	12	0.37	0.68	0.68	0.46	2333.88***	3623.79***	2624.44***	2462.80***	3821.68***	949.01***
		3. S*G	24	0.58	0.49	0.46	0.57	0.41	1.43	0.68	1.26	1.09	1.72
	PRR78 pyramids	1. Season (S)	2	0.31	0.38	0.13	0.01	0.18	0.12	0.29	0.88	0.03	1.02
		2. Genotype (G)	12	0.4	0.36	0.81	0.23	983.03***	3419.26***	581.47***	1695.85***	4292.40***	668.42***
		3. S*G	24	0.38	0.49	0.65	0.26	0.63	0.76	0.74	0.48	0.35	0.52
	KMR3 pyramids	1. Season (S)	2	0.12	1.88	0.52	0.3	0.74	0.18	2	2.05	0.42	1.49
		2. Genotype (G)	12	0.55	1.59	0.66	0.57	1854.64***	3379.96***	3039.11***	1498.52***	8103.00***	314.54***
		3. S*G	24	1.04	0.41	0.33	0.46	0.74	1.04	0.64	1.26	0.63	1.21

*** Statistically significant at 0.05 probability level by LSD test, d.f- degrees of freedom, M.S. - Mean squares, F. ratio - Fisher ratio, DFF-Days to 50% flowering, PH-Plant height, NT-Number of tillers/plant, PL-Panicle length, SF%- Spikelet fertility, FG/P-Filled , grains per panicle, GW-1000 Grain weight, Y/P-Yield/plant, BM-Biomass and HI- Harvest index

Table 4. Mean performance of yield and its related characters of the nine Mahsuri pyramid families as compared to the parent under artificial condition

Mahsuri control												
Isolate	**FG/P**		**SF %**		**GW (g)**		**Y/P (g)**		**BM (g)**		**HI (g)**	
	Parent	**Pyramid**	**Parent**	**Pyramid**	**Parent**	**Pyramid**	**Parent**	**Pyramid**	**Parent**	**Pyramid**	**Parent**	**Pyramid**
BF1	212.00	239.84	72.79	95.45	16.71	17.41	16.64	18.97	56.59	61.97	29.40	30.61
BF2	193.11	239.79	70.41	95.34	14.81	17.30	15.39	18.96	54.97	61.96	27.99	30.60
BF3	188.89	239.86	69.85	95.31	14.31	17.41	15.15	18.94	54.67	61.94	27.72	30.58
BF4	207.11	239.78	72.03	95.44	16.19	17.42	16.31	18.96	56.04	61.95	29.10	30.60
BF5	198.11	239.90	70.81	95.36	15.31	17.42	15.69	18.94	55.30	61.94	28.37	30.58
BF6	201.89	239.81	71.32	95.46	15.67	17.42	16.02	18.97	55.58	61.97	28.82	30.61
BF7	215.00	239.78	73.15	95.42	16.84	17.42	17.05	18.97	56.90	61.97	29.96	30.61
BF8	183.22	239.73	69.18	95.51	13.59	17.42	15.00	18.97	54.50	62.00	27.52	30.60
BF9	182.67	239.78	68.90	95.39	13.41	17.41	14.91	18.95	54.37	61.97	27.42	30.59
BF10	209.67	238.28	72.42	94.56	16.49	17.42	16.47	18.98	56.39	61.98	29.20	30.62
BF11	199.67	239.85	70.99	95.47	15.39	17.42	15.84	18.97	55.46	61.98	28.56	30.62
BF12	196.22	239.77	70.55	95.45	15.08	17.41	15.59	18.97	55.18	61.97	28.26	30.61
BF13	185.78	239.75	69.40	95.40	13.89	17.41	15.11	18.98	54.52	61.98	27.71	30.62
BF14	192.00	239.78	70.19	95.43	14.58	17.41	15.29	18.98	54.91	61.98	27.85	30.62
BF15	205.11	239.80	71.60	95.39	15.89	17.41	16.17	18.97	55.78	61.97	28.98	30.61
BF16	181.11	240.47	68.04	95.50	13.08	17.43	14.69	18.95	54.17	61.96	27.13	30.59
LSD (5%)	1.21		0.69		0.07		0.18		0.22		0.20	
SE	0.44		0.25		0.03		0.07		0.08		0.07	

FG/P-Filled grains per panicle, SF %- Spikelet fertility, GW-1000 Grain weight, Y/P-Yield/plant, BM-Biomass, HI- Harvest index

PRR78 pyramid families - There was no significant variation among the pyramids of PRR78 for any of the characters evaluated. There was no significant variation for the agronomic characters *viz.*, DFF (95 days), PH (107cm), NT (12) and PL (27cm) between parent and the pyramids for any of the *Xoo* isolate (Figure 2b). There was a significant difference for the characters FG/P, SF, GW, Y and BM between parent and pyramids for all the 16 isolates across seasons at 5% LSD (Table 5). The order of % changes in yield and its related characters like, FG/P, SF, GW, Y and BM in the recurrent parent for 16 isolates are as shown in Figure 6b. Among all, BF16 was highly virulent with a yield loss of 28%.

Table 5. Mean performance of yield and its related characters of the nine PRR78 pyramid families as compared to the parent under artificial condition

PRR78 control												
Isolate	**FG/P**		**SF %**		**GW (g)**		**Y/P (g)**		**BM (g)**		**HI (g)**	
	Parent	**Pyramid**	**Parent**	**Pyramid**	**Parent**	**Pyramid**	**Parent**	**Pyramid**	**Parent**	**Pyramid**	**Parent**	**Pyramid**
BF1	182.00	199.77	76.10	94.52	20.69	24.87	19.02	23.98	59.40	64.98	32.03	36.90
BF2	160.00	199.86	72.30	94.36	17.89	24.86	17.59	23.94	57.82	64.94	30.43	36.87
BF3	161.78	199.77	72.43	94.50	18.31	24.83	17.69	23.98	58.02	64.98	30.50	36.90
BF4	185.00	199.84	76.38	94.52	20.88	24.84	19.17	23.97	59.59	64.97	32.17	36.89
BF5	177.00	199.80	75.43	94.45	19.98	24.85	18.47	23.97	59.13	64.97	31.23	36.89
BF6	174.11	199.81	74.14	94.52	19.69	24.88	18.37	23.97	59.02	64.97	31.13	36.89
BF7	178.89	199.78	75.71	94.50	20.29	24.87	18.64	23.96	59.29	64.95	31.44	36.88
BF8	154.89	199.73	71.71	94.59	17.39	24.87	17.34	23.97	57.59	65.00	30.10	36.88
BF9	156.89	199.77	72.20	94.50	17.69	24.86	17.48	23.98	57.69	64.98	30.31	36.90
BF10	187.22	199.78	76.80	94.49	21.27	24.88	19.29	23.97	59.70	64.97	32.32	36.89
BF11	171.67	199.84	73.82	94.55	19.42	24.88	18.20	23.97	58.64	64.98	31.04	36.90
BF12	168.89	199.90	73.39	94.40	19.11	24.88	18.08	23.94	58.47	64.94	30.93	36.87
BF13	153.11	199.78	71.41	94.46	17.21	24.85	17.23	23.95	57.39	64.97	30.02	36.87
BF14	164.22	199.79	72.87	94.40	18.49	24.84	17.84	23.96	58.17	64.96	30.67	36.88
BF15	165.89	199.78	73.14	94.52	18.76	24.85	17.92	23.97	58.31	64.97	30.73	36.90
BF16	151.11	199.96	71.09	94.65	16.93	24.98	17.14	23.94	57.27	64.96	29.93	36.85
LSD (5%)	1.21		0.69		0.07		0.18		0.22		0.20	
SE	0.44		0.25		0.03		0.07		0.08		0.07	

FG/P-Filled grains per panicle, SF %- Spikelet fertility, GW-1000 Grain weight, Y/P-Yield/plant, BM-Biomass, HI- Harvest index

KMR3 pyramid families - Within the nine families of KKR3 pyramids there was no significant difference for any of the characters evaluated. There was no significant variation for the agronomic characters *viz*., DFF, PH, NT and PL between parent and the pyramids (Figure 2c). They showed a DFF of 109 days, PH of 149cm, and 11 NT per plant and PL of 25cm. Significant differences were observed in yield and its related characters *viz.*, FG/P, SF, GW, BM and HI between KMR3 and its pyramids (Table 6) across seasons. The percent change in the characters showing significant variation across the seasons for 16 isolates is shown in Figure 6c. Among all, BF16 was highly virulent and showed a reduction of 24% in yield.

Table 6. Mean performance of yield and its related characters of the nine KMR3 pyramid families as compared to the parent under artificial condition

KMR3 control												
Isolate	**FG/P**		**SF %**		**GW (g)**		**Y/P (g)**		**BM (g)**		**HI (g)**	
	Parent	**Pyramid**	**Parent**	**Pyramid**	**Parent**	**Pyramid**	**Parent**	**Pyramid**	**Parent**	**Pyramid**	**Parent**	**Pyramid**
BF1	137.33	159.81	71.01	90.46	19.58	22.48	18.02	21.57	61.58	69.97	29.26	31.40
BF2	125.22	159.77	69.40	90.43	17.98	22.47	17.11	21.98	60.56	69.98	28.25	31.41
BF3	124.11	159.73	69.16	90.51	17.65	22.47	17.01	21.97	60.47	70.00	28.13	31.39
BF4	141.78	159.77	72.08	90.45	20.06	22.48	18.47	21.98	62.39	69.98	29.60	31.40
BF5	135.44	159.84	70.80	90.48	19.28	22.48	17.82	21.97	61.46	69.98	28.99	31.40
BF6	138.78	159.80	71.43	90.39	19.69	22.48	18.17	21.97	61.78	69.97	29.40	31.40
BF7	144.89	159.76	72.70	90.44	20.49	22.48	19.02	21.97	62.90	69.97	30.25	31.40
BF8	132.89	159.80	70.30	90.45	19.05	22.49	17.59	21.97	61.18	69.97	28.76	31.40
BF9	122.11	159.79	68.82	90.45	17.14	22.48	16.91	21.92	60.37	69.98	28.00	31.40
BF10	143.56	159.79	72.38	90.45	20.26	22.48	18.64	21.92	62.59	69.98	29.78	31.40
BF11	140.11	159.78	71.71	90.44	19.89	22.48	18.31	21.96	62.04	69.95	29.51	31.39
BF12	130.89	159.78	70.03	90.45	18.73	22.47	17.39	21.96	61.08	69.96	28.47	31.39
BF13	127.11	159.86	69.61	90.31	18.49	22.48	17.17	21.94	60.67	69.94	28.30	31.37
BF14	133.89	159.90	70.38	90.36	19.16	22.48	17.69	21.94	61.30	69.94	28.85	31.37
BF15	128.78	159.77	69.90	90.44	18.58	22.47	17.29	21.98	60.91	69.98	28.39	31.40
BF16	121.00	160.04	67.87	90.50	16.73	22.48	16.69	21.95	60.17	69.96	27.75	31.38
LSD (5%)	1.56		0.69		0.12		0.18		0.21		0.18	
SE	0.56		0.25		0.04		0.07		0.08		0.06	

FG/P-Filled grains per panicle, SF %-Spikelet fertility, GW-1000 Grain weight, Y/P-Yield/plant, BM-Biomass, HI- Harvest index

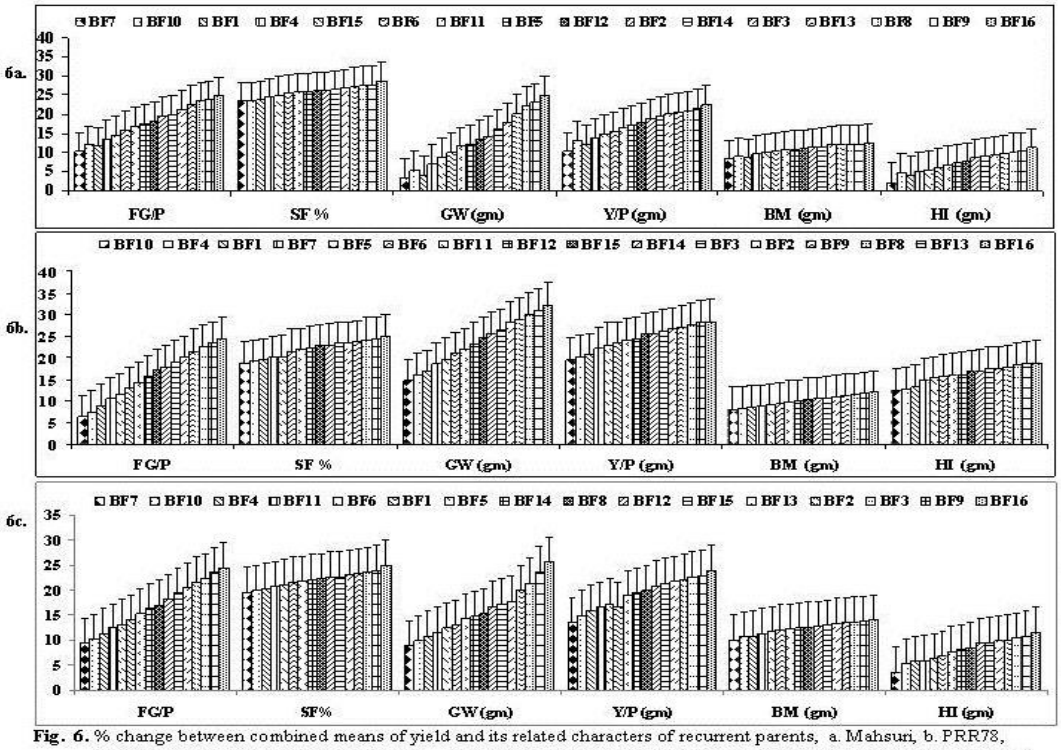

Fig. 6. % change between combined means of yield and its related characters of recurrent parents, a. Mahsuri, b. PRR78, c.KMR3 under artificial condition, Where, FG/P-Filled grains per panicle, SF %- Spikelet fertility, GW-1000 Grain weight, Y/P-Yield/plant, BM-Biomass, HI- Harvest index

Virulence spectrum of *Xoo* isolates - Sixteen *Xoo* isolates showed their characteristic significant virulence and affected the yield and its related characters *viz*., SF%, FG/P, GW, Y/P, BM and HI in all the recurrent parents evaluated. The virulence spectrum of isolates used in this study is showed in Figure 3 in terms of yield loss.

The ANOVA (Table 2 and 3) indicated that there was significant interaction between the isolates and genotypes. The isolate, BF16 was highly virulent across seasons on all the recurrent parents with a yield loss ranging from 23-28% in different genotypes. The isolates, BF7 (in Mahsuri and KMR3) and BF10 (in PRR78) are the least virulent ones in different genotypes. BF7 caused a yield loss of 10% in Mahsuri and 13% in KMR3 whereas BF10 showed a yield loss of 20% in PRR78. All the pyramids showed resistance and no isolate could breakdown any of the four gene pyramid across three wet seasons in any background tested.

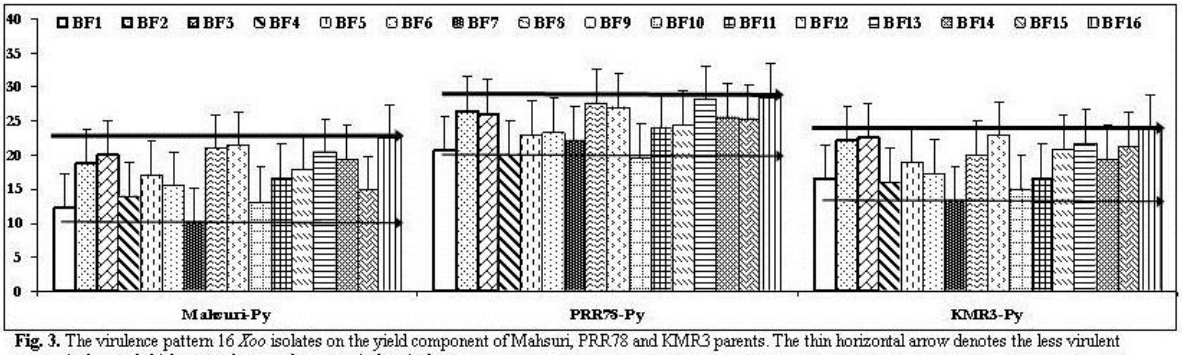

Fig. 3. The virulence pattern 16 *Xoo* isolates on the yield component of Mahsuri, PRR78 and KMR3 parents. The thin horizontal arrow denotes the less virulent isolate and thick arrow denotes the most virulent isolate

Under natural condition:

Mahsuri pyramid families - There was no significant variation among the pyramids for any of the characters evaluated. There was no significant variation in any of the agronomic characters *viz*., DFF, PH, NT and PL between parent and the pyramids under disease pressure across the seasons evaluated (Figure 4a). There was significant difference in yield and its related characters *viz*., FG/P, SF, GW and BM across seasons (Table 7) at 5% LSD between parent and pyramid. Under natural infection the % reduction in yield and its related characters *viz*., FG/P, SF, GW, Y and BM in the parent are as shown in Table 7. There was a reduction of 22% in yield of Mahsuri parent comparing to pyramid. Mahsuri parent showed a reduction of 25% in FG/P, 27% in SF%, 24% in GW, 13% BM and 11% in HI.

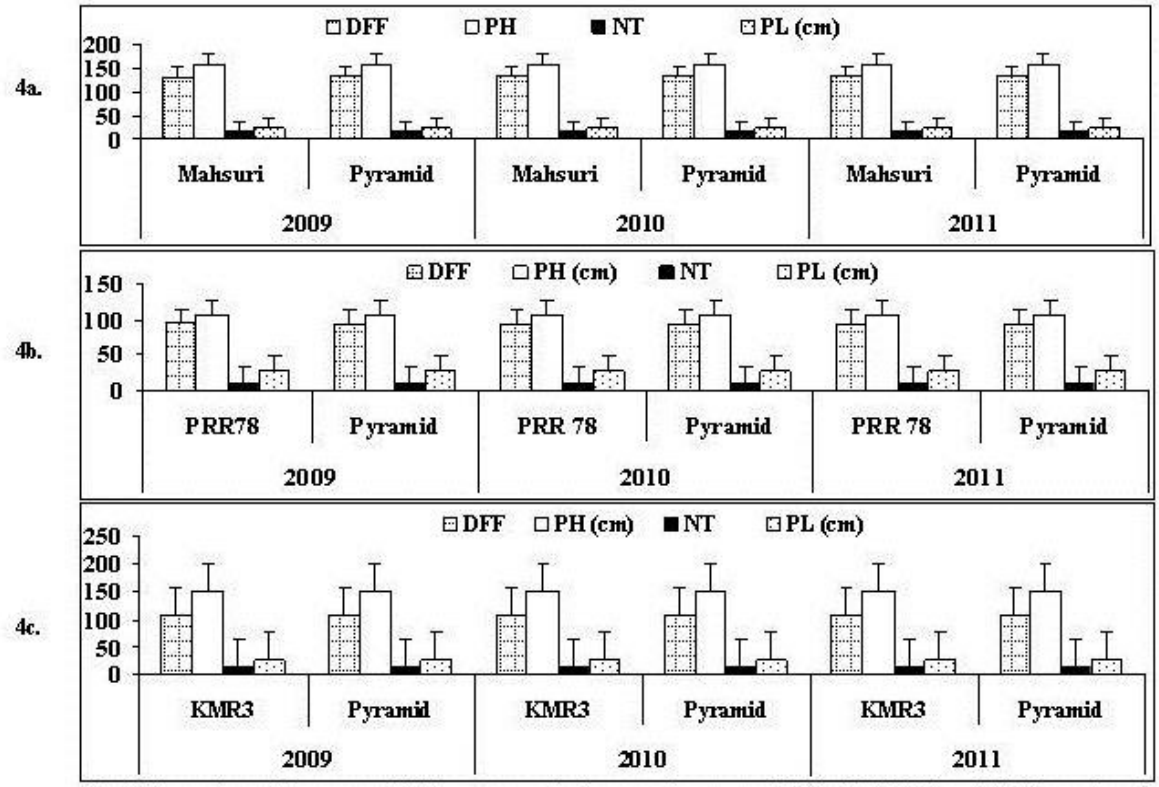

Fig. 4. Comparison of the means of different agronomic characters between a. Mahsuri, b. PRR78, c. KMR3 controls and their respective pyramids under natural disease condition Where, DFF-Days to 50% flowering, PH-Plant height, NT- Number of tillers and PL-Panicle length

PRR78 pyramid families - The recurrent parent showed significant difference comparing with pyramid in yield and its related characters under natural infection. There was no significant difference in the agronomic characters *viz*., DFF, PH, NT and PL across seasons (Figure 4b). ANOVA Table showed a significant difference at 5% LSD, between parent and pyramid for yield and its related characters *viz.,* FG/P, SF, GW, Y and BM across seasons (Table 3). There was no such difference among the pyramids for any of these characters. There was a decrease of 28% yield loss across the seasons. PRR78 showed a decrease of 24% in FG/P and SF%, 31% in GW, 13% BM and 17% in HI (Table 7).

KMR3 pyramid families - There was no significant difference within the nine families of the pyramids for any of the characters evaluated under natural infection with BB pathogen (Table 7). There was no significant variation for agronomic characters *viz.*, DFF, PH, NT and PL between parent and pyramids across season's evaluated (Figure 4c). ANOVA (Table 3) showed a significant difference for the characters, FG/P, SF, GW, Y, BM and HI across seasons between parent and pyramid. At 5% LSD, there was no such variation for the above characters among pyramids across seasons. In the parent there was a reduction of 25% in FG/P, GW and Y, 24% in SF%, 15% in BM and 12% in HI which finally leads to a yield loss of 24% (Table 7).

Table 7. Mean performance of agronomic, yield and its related characters of the nine pyramid families of Mahsuri, PRR78 and KMR3 as compared to the parent under natural condition

Genotype	Mahsuri						PRR78						KMR3					
	FG/P ***	SF % ***	GW ***	Y/P ***	BM ***	HI ***	FG/P ***	SF % ***	GW ***	Y/P ***	BM ***	HI ***	FG/P ***	SF % ***	GW ***	Y/P ***	BM ***	HI ***
parent	182.00	70.02	13.23	14.7	54.13	27.15	152.33	72.01	17.07	18.01	58.13	31.00	122.33	69.80	16.90	17.00	61.00	27.88
Pyramid 1	241.67	95.48	17.39	18.99	62.02	30.62	201.67	95.05	24.93	25.11	67.02	37.50	163.00	91.69	22.51	22.80	72.10	31.65
Pyramid 2	241.89	95.24	17.43	18.96	61.94	30.61	201.22	94.98	24.94	24.99	67.04	37.30	163.11	91.56	22.49	22.90	72.00	31.78
Pyramid 3	242.11	95.26	17.40	18.93	61.97	30.55	200.78	94.44	24.80	25.14	67.04	37.50	163.22	91.50	22.47	23.00	72.00	31.92
Pyramid 4	242.33	95.33	17.39	18.88	61.89	30.51	201.22	94.69	24.99	24.89	66.99	37.20	163.11	91.26	22.48	23.10	72.00	32.05
Pyramid 5	241.56	95.40	17.38	18.91	61.97	30.52	200.78	94.91	24.86	25.03	67.03	37.30	163.22	91.30	22.50	23.00	72.10	31.86
Pyramid 6	242.33	95.57	17.42	18.91	61.97	30.52	201.33	95.17	24.90	25.09	67.00	37.50	163.00	91.64	22.49	23.00	72.00	31.88
Pyramid 7	241.33	95.45	17.41	18.97	61.94	30.63	201.00	94.78	24.80	25.07	67.05	37.40	163.00	91.92	22.51	22.80	72.00	31.66
Pyramid 8	241.89	95.32	17.42	18.94	61.92	30.59	200.56	94.69	24.89	25.11	67.03	37.50	163.22	91.79	22.52	22.80	72.00	31.69
Pyramid 9	242.33	95.61	17.39	18.97	61.98	30.60	200.89	95.06	24.94	25.04	67.03	37.40	163.33	91.38	22.51	22.80	72.00	31.66
LSD (5%)	1.99	0.67	0.13	0.14	0.20	0.18	1.29	1.13	0.50	0.27	0.21	0.38	1.09	0.78	0.16	0.240	0.19	0.34
SE	0.69	0.24	0.05	0.05	0.07	0.06	0.46	0.4	0.18	0.09	0.07	0.14	0.38	0.28	0.06	0.08	0.07	0.12
% Change between parent and pyramid																		
2009	24.67	26.57	24.17	22.42	12.61	11.23	24.07	24.07	31.35	27.98	13.07	17.20	25.20	24.13	24.90	25.70	15.30	12.19
2010	24.76	26.66	24.00	22.45	12.64	11.22	24.21	24.05	31.68	28.20	13.38	17.10	24.81	23.51	25.05	25.70	15.30	12.31
2011	24.90	26.60	23.72	22.35	12.64	11.12	24.41	24.16	31.24	28.20	13.35	17.10	25.02	23.65	24.74	25.90	15.30	12.47

FG/P-Filled grains per panicle, SF %-Spikelet fertility, GW-1000 Grain weight, Y/P-Yield/plant, BM-Biomass, HI- Harvest index

DISCUSSION

The main aim of the present study was to pyramid four BB resistance genes (*viz*., *Xa4, xa5, xa13* and *Xa21*) into the popular cv. Mahsuri and two hybrid rice parental lines, PRR78 and KMR3 and to evaluate their nine best performing pyramid families with *Xa4, xa5, xa13* and *Xa21* genes in homozygous condition for agronomic, yield and its related characters along with disease resistance both under artificial and natural conditions across seasons. Marker assisted back cross breeding with the tightly linked markers in conjunction with phenotypic selection at field level has made it possible to pyramid four BB resistance genes simultaneously into multiple backgrounds. Marker assisted back cross breeding for BB resistance has been successfully utilized in several studies (Huang *et al*., 1997; Hittalmani *et al*., 2000; Sanchez *et al*., 2000; Narayanan *et al.,* 2004; Perez *et al*., 2008; Perumalsamy *et al*., 2010).

The disease causes leaf wilting, affects photosynthesis, rate of seed setting and reduces grain weight (Ou, 1985). It also affects the grain quality. Hybrid rice production faces more severe problem with bacterial blight as it needs to cut the flag leaves of the primary tillers of 'A' and 'R' lines at booting stage to facilitate pollination, which paves a way for pathogen entry.

The pyramid families in the background of Mahsuri, PRR78 and KMR3 were on par with their respective parents in agronomic performance and also exhibited a high level of resistance against BB by insulating the yield losses due to this disease both under artificial and natural conditions. Phenotypic selection was done very critically to retain all the characters of the recurrent parent as much as possible. Studies conducted earlier have shown similar results with other genotypes and different races of the pathogen (Singh *et al*., 2001; Joseph *et al.,* 2004; Sundaram *et al.,* 2008; Basavaraj *et al.,* 2010; Salgotra *et al.,* 2012; Suh *et al.,* 2013).

Screening disease resistance under natural infection alone is not a feasible option due to seasonal variation and the absence of adequate inoculums to initiate uniform disease reaction. Artificial inoculation simulating natural environment under controlled field conditions minimizes such problems and generates larger variations in disease severity.

Under artificial condition the parents showed a high degree of disease incidence on par with the susceptible check and all the pyramids showed a lesion length of 1.5 to 3cm on par with the resistant check (Figure 5). There were significant differences in characters like FG/P, SF and GW in the recurrent parents against all the sixteen isolates (Table 3). One isolate designated as BF16 showed high virulence comparing to other isolates in all the three parents across the seasons causing highest yield loss of up to 23 to 28% (Figure 3). BF7 (in Mahsuri and KMR3) and BF10 (in PRR78) showed least virulence across the seasons. BF7 caused a yield loss of 10% in Mahsuri parent and 13% in KMR3 parent, whereas BF10 showed a yield loss of 20% in PRR78 parent.

The isolate BF16 affected yield and its related characters heavily in all the recurrent parents. The mean performance of the Mahsuri parent across seasons inoculated with BF16 showed 181 FG/P, 68% SF%, 13gm 1000GW, 15gm/plant Y, 54gm BM and 27gm HI (Table 4), whereas the pyramid showed 240 FG/P, 96% SF%, 17gm 1000GW, 19gm/plant Y, 62gm BM and 31gm HI. The mean performance of the PRR78 parent across seasons with BF16 was, 151 FG/P, 71% SF%, 17gm 1000 GW and Y, 57gm BM and 30gm HI under diseased condition (Table 5) whereas the pyramid showed 200 FG/P, 95% SF%, 25gm 1000GW, 24gm/plant Y, 65gm BM and 37gm HI and in case of KMR3 parent with BF16 it was 121 FG/P, 68% SF%, 17gm 1000GW and Y, 60gm BM and 28gm of HI across the seasons and its pyramid showed 160FG/P, 91% SF%, 22gm 1000GW and Y, 70gm BM and 31gm HI (Table 6).

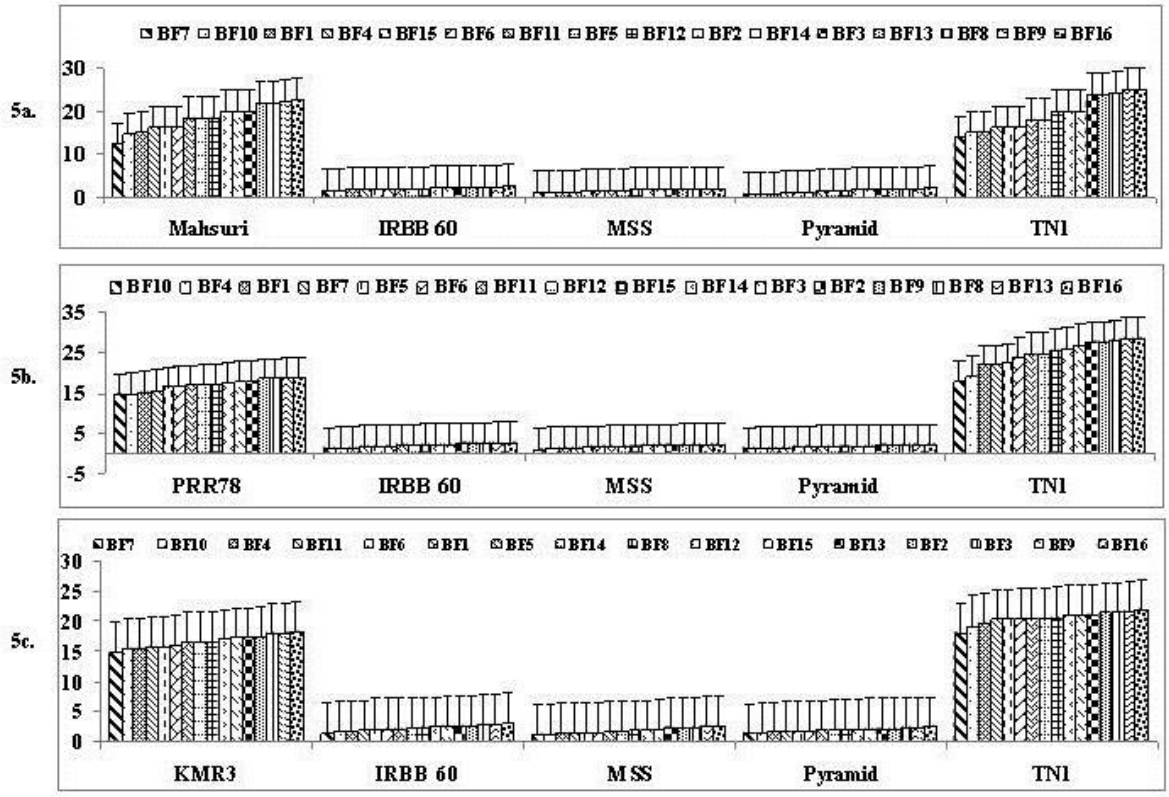

Fig. 5. Lesion length of recurrent parents, a. Mahsuri, b. PRR78, c. KMR3, along with their respective pyramids and checks under artificial condition

Results have shown that pyramids have yielded 23% in Mahsuri, 28% in PRR78 and 24% in KMR3 backgrounds more than their respective parents under artificial disease pressure with highly virulent isolate BF16 across seasons. The agronomic characters *viz*. days to fifty percent flowering, plant height, number of tillers and panicle length did not show any significant differences with any of the challenged isolate in any of the parents tested (Table 3).

The studies conducted in Eastern India have shown that the gene combinations *Xa4+xa5*, *xa5+Xa21* and *Xa4 + xa5 + Xa21* conferred a broad spectrum of resistance (Shanti *et al.*, 2001). Two gene combinations confer a less degree of resistance than the three gene combinations and so use of three gene combinations was the most feasible option. Over the years there were reports on *xa5+xa13+Xa21* being a suitable gene combination for different locations in India and popular varieties were pyramided with this combination (Singh *et al.*, 2003 and Sundaram *et al.*, 2008). Earlier results (Shanti and Shenoy, 2005) and AICRIP trials (Directorate of Rice Research, 2006a) have shown that there was a breakdown of resistance in some of the test locations. The pathogen population being highly dynamic, apparently co-evolves with the host and novel strains arise which lead to the breakdown of resistance of the pyramids. Hence, there is an urgent need to understand the contemporary pathogen population and the effective resistance genes.

The four gene combination of *Xa4*, *xa5*, *xa13* and *Xa21* is the most effective in combating the pathogen population (Shanti and Shenoy, 2005). Resistance conferred is due to the complementary action of the resistance genes. This corroborates to the earlier reports showing that multiple genes have higher levels of resistance as compared to those with single genes (Yoshimura *et al.*, 1996; Huang *et al.*, 1997; Shanti *et al.*, 2001 and Sundaram *et al.*, 2008). Multiple genes have an additive effect on overall levels of resistance.

The pyramid families of Mahsuri, PRR78 and KMR3 along with their respective recurrent parents and checks were also subjected to natural disease pressure in Maruteru in Andhra Pradesh, India during wet seasons of 2009, 2010 and 2011. It is a hotspot for BB with a very high incidence of BB with a location severity index as high as 8.6 (Directorate of Rice Research, 2006b). Natural infection results in typical disease symptoms. It also showed same result as under artificial infection. The pyramids showed resistance on par with donor and resistance check whereas recurrent parents were highly susceptible. Mahsuri and PRR78 parents showed a lesion length of 24cm (Figure 7a and b) and KMR3 showed 19cm (Figure 7c). No pyramid showed susceptible reaction and lesion length of 1.5 cm to 2.8cm not exceeding 3cm.

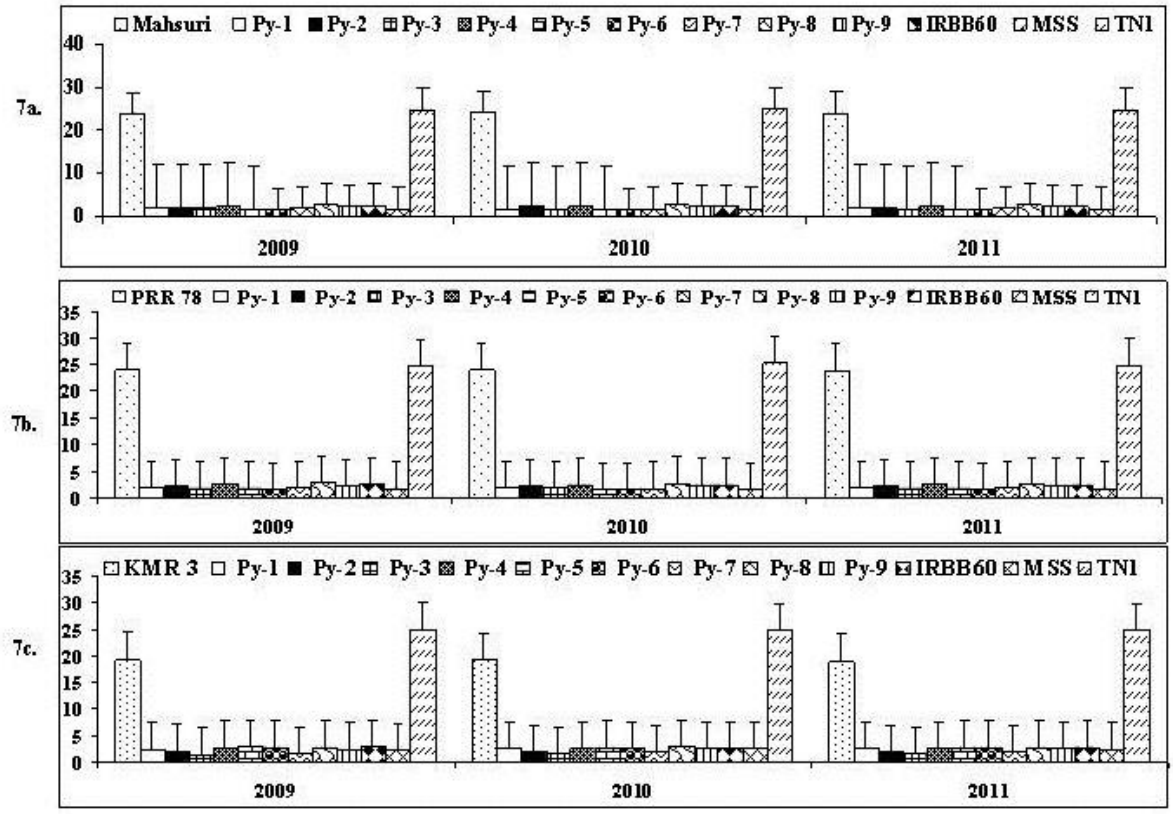

Fig.7. Lesion length of recurrent parents, a. Mahsuri, b. PRR78, c. KMR3 along with their respective pyramids and checks under natural condition

The agronomic characters *viz*., days to 50% flowering, plant height, number of tillers and panicle length did not show any significant difference between the recurrent parents and pyramids of Mahsuri, PRR78 and KMR3 under natural condition (Table 3) across seasons. Yield and its related characters *viz.*, FG/P, SF%, GW, BM and HI showed significant difference between the parents and pyramids across seasons. Mahsuri parent showed 182 FG/P, 70% SF%, 13g 1000GW, 15gm/plant Y, 54 g BM and 27 g HI (Table 7), PRR78 parent showed 152 FG/P, 72% SF%, 17 g 1000GW, 18gm/plant Y, 58 g BM and 31 g HI (Table 7) and KMR3 parent showed 122 FG/P, 70% SF%, 17 g 1000GW and Y, 61 g BM and 28 g of HI (Table 7). Under natural infection no pyramid showed significant difference in the agronomic, yield and its related characters. There was a yield reduction of 22% in Mahsuri parent, 28% in PRR78 parent and 26% in KMR3 parent comparing to their respective pyramids.

Environmental conditions also play an important role in phenotyping and agronomic performance (Garrett *et al*., 2006). During the experimental period, across the three seasons, the environmental conditions were measured both under artificial field condition at Barwale Foundation research farm and also natural condition at Maruteru (Figure 8). The interaction between season, treatment and genotype has no effect on yield loss due to the disease under both the environments.

The pyramid families in the backgrounds of Mahsuri, PRR78 and KMR3 under artificial condition have shown that there was varying degrees of resistance to a very diverse set of *Xoo* isolates but none of the isolates could breakdown the resistance in any of pyramid families. Pyramids were also highly resistant under natural condition. Earlier studies (Bharatkumar *et al.*, 2008; Prasad *et al*., 2013) and the present study prove that the four gene combination is the most effective in combating the dynamic pathogen population.

This study shows the stable performance of the four gene pyramids across the locations and seasons. All the pyramids could show the broad spectrum resistance with synergistic effect on multiple *Xoo* isolates across seasons. These pyramids are insulating against yield loss to the tune of 25-30%, which goes a long way in reducing yield losses, thereby increasing the rice production. There was no yield loss among the pyramids for any of the isolate inoculated. This study has also indicated that incorporation of four genes has not led to any penalty on the yield since the parent and pyramids showed same average yield under control condition.

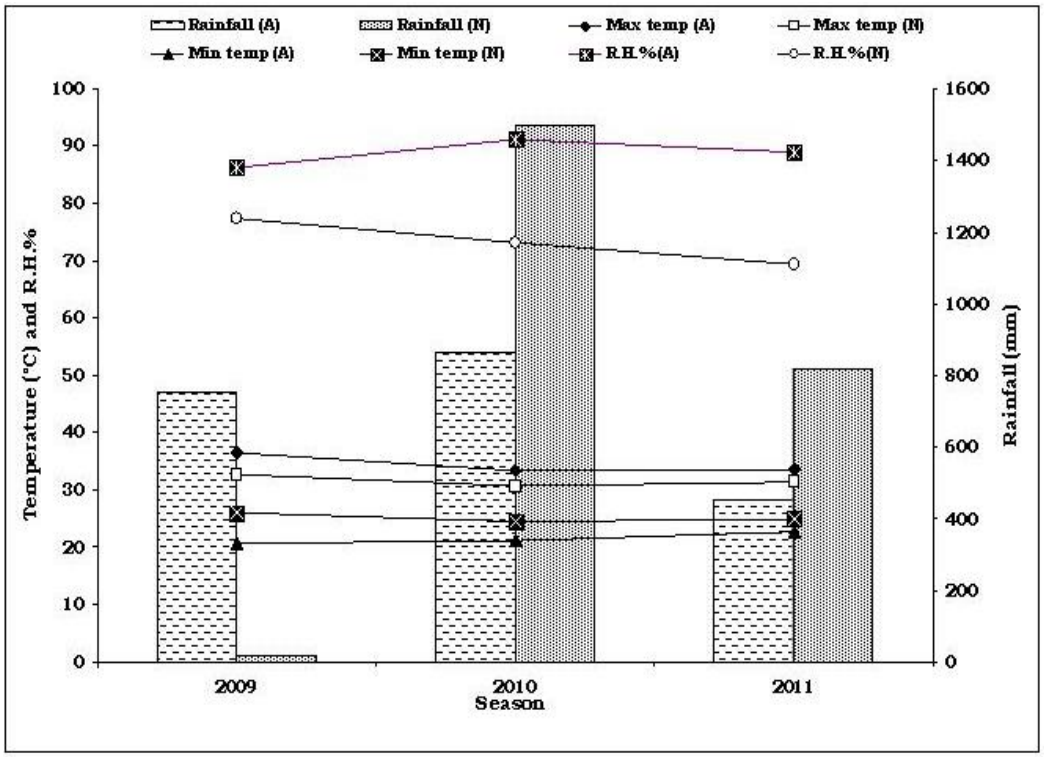

Fig. 8. Profiles of mean maximum and minimum temperature (Max. and Min. temp), mean rainfall and relative humidity under both artificial (A) and natural (N) conditions

CONCLUSION

From the present experiment it is evident that the four gene pyramid in multiple backgrounds could combat the *Xoo* pathogen effectively without any yield penalty and also showed no negative effect on the agronomic performance of any pyramid. These pyramids can be used directly in the breeding programs or as potential donors of bacterial blight resistance in future breeding programs. The evolution of *Xoo* pathogen is a dynamic process. In future the new race/s may defeat this four gene combination. So, the study of pathogen population structure is a continuous process to know the correct gene combination effective against new race and that gene combination to be pyramided to the susceptible lines.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. Usha B. Zehr, Director and Mr. Dinesh C. Joshi, Executive Director, Barwale Foundation for their encouragement and financial support.

REFERENCES

Adhikari TB, Basnyat RC and Mew TW. 1999. Virulence of *Xanthomonas oryzae* pv. *oryzae* on rice lines containing single resistance genes and gene combinations. Plant Disease, **83 (1):** 46-50.

Basavaraj SH, Singh VK, Sing AT, Singh AS, Singh AN, Anand D, Yadav S, Ellur RK, Singh D, Gopalakrishnan S, Nagarajan M, Mohapatra T, Prabhu KV and Singh AK. 2010. Marker-assisted improvement of bacterial blight resistance in parental lines of PusaRH10, a superfine grain aromatic rice hybrid. Molecular Breeding, **26:** 293-305. DOI**:** 10.1007/s11032-010-9407-3

Bharatkumar S, Paulraj RSD, Brindha PV, Kavitha S and Gnanmanickam SS. 2008. Improvement of bacterial blight resistance in rice cultivars Jyothi and IR50 via marker assisted backcross breeding. Journal of Crop Science, **21:** 101-116. DOI: 10.1300/J411v21n01_07

Bhasin H, Bhatia D, Raghuvanshi S, Lore JS, Sahi GK, Kaur B, Vikal Y and Singh K. 2012. New PCR-based sequence-tagged site marker for bacterial blight resistance gene *Xa38* of rice. Molecular Breeding, **30:**607-611. DOI: 10.1007/s11032-011-9646-y

Cohen M, Bernal C, Virmani SS. 2003. Do rice hybrids have heterosis for insect resistance? A study with *Nilaparvata lugens* (Hemiptera: Delphacidae) and *Marasmia patnalis* (Lepidoptera: Pyralidae). Journal of Economic Entomology, **96(6):** 1935-1941. DOI: 10.1603/0022-0493-96.6.1935

Collard, Iftekharuddaula, Thomson, Pamplona and Mackill. 2008. Relevance of backcross breeding in rice GCP McClintock. Bioinformatics Resource – MAS.

Dellaporta SL, Wood J and Hicks B. 1983. A plant molecular DNA mini preparation. Plant Molecular Biology Reporter, **1:** 19-21. DOI: 10.1007/BF02712670

Devadath S. 1989. Chemical control of bacterial blight of rice. *In:* Proceedings of the International Workshop on Bacterial Blight of Rice [International Rice Research Institute (ed.)], 14th-18th March 1988, IRRI, Manila, Philippines. p. 89-98.

Directorate of Rice Research. Progress Report. 2006a. Vol.2, Crop Protection (Entomology, Plant Pathology): p.3.55.

Directorate of Rice Research. Progress Report. 2006b.Vol.2, Crop Protection (Entomology, Plant Pathology): pp.3.53.

Diwan J, Channbyregowda M, Shenoy V, Salimath P and Bhat R. 2013. Molecular mapping of early vigour related QTLs in rice. Research Journal of Biology, **1:** 24 - 30.

Garret K, Deny S, Frank E, Rouse M and Travers S. 2006. Climate change effects on plant disease: Genomes to ecosystems. Annual Review of Phytopathology, **44:** 489-509. DOI: 10.1146/annurev.phyto.44.070505.143420

Guillebeau, P. 1998. What to do about the food quality protection act? Or how can we protect the pesticides we need? Proc. S.E. Pecan Growers Association, **91:**65-69.

Hittalmani S, Parco A, Mew TV, Zeigler RS and Huang N. 2000. Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. Theoretical and Applied Genetics, **100:** 1121-1128. DOI: 10.1007/s001220051395

Huang N, Angeles ER, Domingo J, Magpantay G and Singh S. 1997. Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. Theoretical and Applied Genetics, **95:** 313-320. DOI: 10.1007/s001220050565

Joseph M, Gopalakrishnan S, Sharma RK, Singh VP, Singh AK, Singh NK and Mohapatra T. 2004. Combining bacterial blight resistance and Basmati quality characteristics by phenotypic and molecular marker-assisted selection in rice. Molecular Breeding, **13:** 377-387. DOI: 10.1023/B:MOLB.0000034093.63593.4c

Karaganilla A, Natural MP and Ou SH. 1973. A comparative study of culture media of *Xanthomonas oryzae* pv. *oryzae*. Philippines Agriculture, **57:** 141-152.

Kauffman HE, Reddy APK, Hsieh SPY and Merca SD. 1973. An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae.* Plant Disease Report, **57:**537-541.

Mamgain A, Roychowdhury R and Tah J. 2013. *Alternaria* pathogenicity and its strategic controls. Research Journal of Biology, **1:** 1 - 9.

Melchinger AE. 1990. Use of molecular markers in breeding for oligogenic disease resistance. Plant Breeding, **104:** 1-19. DOI: 10.1111/j.1439-0523.1990.tb00396.x

Mew TW. 1987. Current status and future prospects of research on bacterial blight of rice. Annual Review of Phytopathology, **25:** 359-382. DOI: 10.1146/annurev.py.25.090187.002043

Narayanan NN, Baisakh N, Oliva NP, Vera Cruz CV, Gnanmanickam SS, Datta K and Datta SK. 2004. Molecular breeding: Marker-assisted selection combined with biolistic transformation for blast and bacterial blight resistance in indica rice (cv.Co39). Molecular Breeding, **14:** 61-67. DOI: 10.1023/B:MOLB.0000037995.63856.2d

Natrajkumar P, Sujatha K, Laha GS, Srinivasarao K, Mishra B, Viraktamath BC, Hari Y, Reddy CS, Balachandran SM, Ram T, Sheshumadhav M, Shobharani N, Neeraja CN, Ashokreddy G, Shaik H, Sundaram RM. 2012. Identification and fine-mapping of *Xa33*, a novel gene for resistance to *Xanthomonas oryzae* pv. *oryzae*. Phytopathology, **102:**222-228. DOI: 10.5376/mpb.2012.03.0012

Nino-Liu DO, Ronald PC, Bogdanove A.J. 2006. *Xanthomonas oryzae* pathovars: model pathogens of a model crop. Molecular Plant Pathology, **7:**303–324. DOI: 10.1111/J.1364-3703.2006.00344.X

Ou SH. 1985. Rice Disease. Kew, England: Commonwealth Mycological Institute (CMI), 2nd Edn, p: 61-63.

Ogawa T and Khush GS. 1988. Major genes for resistance to bacterial blight. *In:* Proceedings of the International Workshop on Bacterial Blight of Rice [International Rice Research Institute (ed.)], 14^{th}-18^{th} March 1988, IRRI, Manila, Philippines. p. 177-192.

Perez ML, Redona DE, Mendioro SM, Vera Cruz MC and Leung H. 2008. Introgression of *Xa4*, *Xa7* and *Xa21* for resistance to bacterial blight in thermo sensitive genetic male sterile rice (*Oryza sativa* L.) for the development of two line hybrids. Euphytica, **164:** 627-636. DOI: 10.1007/s10681-008-9653-1

Perumalsamy S, Bharani M, Sudha M, Nagarajan P, Arul L, Saraswathi R, Balasubramanian P and Ramalingam J. 2010. Functional marker-assisted selection for bacterial leaf blight resistance genes in rice (*Oryza sativa* L.). Plant Breed, **129:** 400-406. DOI: 10.1111/j.1439-0523.2009.01705.x

Prasad D, Das K M and Rao GJN. 2013. Pyramiding of four resistance genes of bacterial blight in Tapaswini, an elite rice cultivar, through marker-assisted selection. Euphytica, **192 (1):** 87 – 96. DOI: 10.1007/s10681-013-0878-2

Salgotra RK, Gupta BB, Millwood RJ, Balasubramanian M and Stewart CNJr. 2012. Introgression of bacterial leaf blight resistance and aroma genes using functional marker-assisted selection in rice (*Oryza sativa* L.). Euphytica, **187:**313-323. DOI: 10.1007/s10681-011-0599-3

Sanchez AC, Brar DS, Haung N, LiZ and Khush GS. 2000. Sequence tagged site marker assisted selection for three bacterial blight resistance genes in rice. Crop Science, **40:**792-797. DOI:10.2135/cropsci2000.403792x

Shanti ML, George MLC, Cruz CMV, Bernardo MA and Nelson RJ. 2001. Identification of resistance genes effective against rice bacterial blight pathogen in Eastern India. Plant Disease, **85:**506-512.

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

Siddiq EA, Singh VP, Zaman FU, Sadananda AR, Abraham MJ, Hari Prasad AS, Mahendrum A, Natrajan US, Nagarajan M, Atwal SS, Sinha SN, Chopra NK, Seth R, Mohapatra T, Prabhu, KV and Singh AK. 2009. Development of high yielding Basmati quality rice varieties: a success story. Indian Farming, **59:** 13-17.

Singh S, Sodhi M, Vikal Y, George MLC, Bala GS, Mangat GS, Garg M, Sidhu JS and Dhaliwal HS. 2003. DNA fingerprinting and virulence analysis of *Xanthomonas oryzae* pv. *oryzae* isolates from Punjab, northern India. Euphytica, **130:** 107-115. DOI: 10.1023/A: 10223 290 24651

Singh GP, Srivastava MK, Singh RV and Singh RM. 1997. Variation and qualitative losses caused by bacterial blight in different rice varieties. Indian Phyto pathology**, 30:** 180-185.

Singh S, Sidhu JS, Huang N, Vikal Y, Li Z, Brar DS, Dhaliwal HS, Khush GS. 2001. Pyramiding three bacterial blight resistance genes (*xa5, xa13* and *Xa21*) using marker-assisted selection into indica rice cultivar PR106. Theoretical and Applied Genetics, **102:**1011–1015 DOI: 10.1007/s001220000495

Suh JP, Jeung JU, Noh TH, Cho YC, Park SH, Park HS, Shin MS, Kim CK and Jena KK. 2013. Development of breeding lines with three pyramided genes that confer broad-spectrum bacterial blight resistance and their molecular analysis in rice. Rice**, 6:** 5. DOI: 10.1186/1939-8433-6-5

Sundaram RM, Vishnupriya MR, Biradar SK, Laha GS and Reddy GA. 2008. Marker-assisted Introgression of bacterial blight resistance in Samba Mahsuri, an elite indica rice variety. Euphytica**, 160:** 411-422. DOI: 10.1007/s10681-007-9564-6

Swings J, Van den Mooter M, Vauterin L, Hoste B, Gillis M, Mew TW and Kersters K. 1990. Reclassification of the causal agents of bacterial blight (*Xanthomonas campestris* pv. *oryzae*) and bacterial leaf streak (*Xanthomonas campestris pv. oryzicola*) of rice as pathovars of *Xanthomonas orzae* (ex Ishiyama, 1922) sp. nov., nom. rev. International Journal of Systematic Bacteriology**, 40:** 309-311.

Virmani SS and Kumar I. 2004. Development and use of hybrid rice technology to increase rice productivity in the tropics. International Rice Research Notes**, 29(1):** 10-19.

Virmani SS. 1996. Hybrid rice. Advances in Agronomy**, 57:** 377-462.

Yoshimura S, Yoshimura A, Iwata N, Mc. Couch SR, Abenes ML, Baraoidan MR, Mew TW and Nelson RJ. 1995. Tagging and combining bacterial blight resistance genes in rice using RAPD and RFLP markers. Molecular Breeding**, 1:**375-387. DOI: 10.1007/BF01248415

Yoshimura S, Umehera Y, Kurata N, Nagamura Y, Sasaki T, Minobe Y and Iwata N. 1996. Identification of a YAC clone carrying the *Xa-1* allele, a bacterial blight resistance gene in rice. Theoretical and Applied Genetics**, 93:** 117–122. DOI: 10.1007/BF00225736

Young ND and Tanksley SD. 1989. RFLP analysis of the size of chromosomal segments retained around the Tin-2 locus of tomato during backcross breeding. Theoretical and Applied Genetics**, 77:**353-359.

Zhai WX and Zhu LH. 1999. Rice bacterial blight resistance genes and their utilization in molecular breeding. Advanced BioTech, **19:** 9-15.

Zhang Q. 2005. Utilization and strategy of gene for resistance to rice bacterial blight in China. Chinese Journal of Rice Science, **19(5):** 453-459. (In Chinese with English abstract).