RESEARCH ARTICLE

Multi-location evaluation of gene pyramided lines of MTU 1010 and JGL 1798 against bacterial blight of rice

G. PADMAJA^{1,3}, CH. V. DURGA RANI², G. UMA DEVI³, A. YUGANDER¹, C. SRINIVAS⁴, R.M. SUNDARAM¹ and G.S. LAHA^{1*}

¹ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad 500 030, Telangana, India

²Institute of Biotechnology, ³Department of Plant Pathology, ⁴Department of Entomology, Prof. Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad 500 030, Telangana, India

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ABSTRACT: Cottondora Sannalu (MTU 1010) and Jagtial Sannalu (JGL 1798) are widely cultivated rice varieties in Telangana and Andhra Pradesh and very well-accepted among the farmers because of their high yield, slender grain and preferred cooking quality. Despite being popular, these varieties are susceptible to different pests and diseases including bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo). Two BB resistance genes viz., *xa13* and *Xa21* were introgressed in these two varieties using marker assisted backcross breeding. Homozygous lines possessing BB resistance genes *xa13* or *Xa21* and their combination were evaluated for BB resistance with multiple Xoo isolates under controlled condition in glass house and also at different hot spot locations in the states of Telangana and Andhra Pradesh, India. Both the recurrent parents (MTU 1010 and JGL 1798), TN1 (susceptible check variety) and Improved Samba Mahsuri (resistant check variety) were also subjected for resistance evaluation. All the gene-pyramided lines were highly resistant to BB both under glasshouse and field condition. Individually, BB resistance gene *xa13* showed higher level of resistance than *Xa21*. The resistance level was higher in the lines possessing both the BB resistance genes in comparison to the lines possessing single BB resistance genes. The results showed that combination of BB resistance genes *xa13* and *Xa21* can provide broad level of resistance against BB in Telangana and Andhra Pradesh.

Keywords: Bacterial blight, resistance, rice, *Xanthomonas oryzae* pv. *oryzae*

Rice is the principal staple food crop in India and rice production has so far kept pace with the growing population, primarily due to large scale cultivation of high yielding, high-input demanding, and semi-dwarf non-lodging rice varieties (Gnanamanickam, 2009). Rice varieties like Samba Mahsuri (BPT 5204), Cottondora Sannalu (MTU 1010), Jagtial Sannalu (JGL1798), Swarna (MTU 7029) and Tellahamsa are widely cultivated by the farmers in Telangana and Andhra Pradesh. These varieties are widely cultivated by the farmers because of their high yield potential, medium-slender grain type and desired cooking quality. In spite of their popularity, they are susceptible to many pests and diseases including bacterial blight (BB) of rice caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo).

BB has become a major production problem in rice cultivation in India and elsewhere (Devadath, 1992; Sridhar, 2002; Win *et al.*, 2013). In general, BB is a monsoon (wet) season problem of high yielding rice varieties cultivated with high nitrogen application, especially in irrigated and rainfed lowland ecosystems. The extent of yield loss due to BB can be as high as 50% or more depending on the crop stage during infection, environmental conditions, rice cultivars and

amount of nitrogenous fertilizers applied (Laha *et al.*, 2009). Epidemic form of the disease was reported from north-western India during 1979 and 1980 (Mew, 1987), Palakkad district of Kerala during 1998 (Priyadarishini and Gnanamanickam, 1999) and several rice growing areas of Andhra Pradesh during 2010 and 2013 (Yugander *et al.*, 2014). The disease appeared in very severe form in coastal Andhra Pradesh during 2014 following the severe cyclone 'HUDHUD'. The disease has become a serious constraint in several rice growing areas of Warangal, Nizamabad and other districts of Telangana (Laha *et al.*, 2016). As the chemical control of the disease is not very effective, cultivation of disease resistant varieties is the most successful and eco-friendly alternative for the management of BB (Mew, 1987; Sundaram *et al.*, 2014). Till date, at least 40 BB resistance genes have been identified from various sources (Sundaram *et al.*, 2014; Kim *et al.*, 2015) and few of these genes been used in resistance breeding program. However, Xoo is highly diverse in nature (Hunjan *et al.*, 2014; Mondal *et al.*, 2014; Yugander *et al.*, 2017) and resistant varieties particularly those carrying single BB resistance genes have become susceptible due to appearance of new virulence of the pathogen (Mew *et al.*, 1992; Laha *et al.*, 2007). The durability of resistance can be increased by pyramiding multiple BB resistance

*Corresponding author: lahags@yahoo.co.in

genes through marker assisted selection (MAS). Some of the major BB resistance genes which have been widely used in gene-pyramiding programs in India are *xa13* and *Xa21* (Sundaram *et al.*, 2014). Several studies in India have reported that combination of *Xa21* and *xa13* provides high level and broad spectrum resistance to Indian Xoo population (Joseph *et al.*, 2004; Mishra *et al.*, 2013; Mondal *et al.*, 2014; Ellur *et al.*, 2016a). Keeping in view the broad spectrum effectiveness of *Xa21* and *xa13* combination, these two BB resistance genes were selected for pyramiding in these two popular rice cultivars. In this study, we report the performance of MAS-derived lines of MTU 1010 and JGL 1798 possessing BB resistance genes *xa13* and *Xa21*, either individually or in combination.

MATERIALS AND METHODS

Plant materials and Xoo isolates

The experimental materials consisted of 5 homozygous NILs (near isogenic lines) in the background of popular rice variety MTU 1010 (C1-20-3 possessing BB resistance gene *xa13*, C1-13-3 possessing *Xa21* and C1-20-2, C1-20-4 and C1-20-5, all possessing both *xa13* and *Xa21*) and 3 homozygous NILs in the genetic background of JGL 1798 (C5-42-47, C5-45-48 and C5-49-44, all possessing both *xa13* and *Xa21*). The recurrent parents MTU 1010 and JGL 1798, TN1 (susceptible check variety) and Improved Samba Mahsuri (resistant check variety) were also included for resistance evaluation. The NILs were developed at the Institute of Biotechnology, PJTSAU, Hyderabad using marker assisted backcross breeding.

Resistance evaluation of these NILs was carried out both in the glasshouse under controlled condition and also at different hot spot locations. Under glasshouse condition, the NILs were evaluated against 10 different Xoo isolates. The Xoo isolates included IX-006 (Warangal, Telangana), IX-015 (Aduthurai, Tamil Nadu), IX-020 (ICAR-IIRR, Telangana), IX-027 (Chinsurah, West Bengal), IX-050 (Maruteru, Andhra Pradesh), IX-148 (Cuttack, Odisha), IX-133 (Raipur, Chhattishgarh), IX-266 (Nandhiyal, Andhra Pradesh), IX-281 (Tanuku, Andhra Pradesh) and IX-372 (Guntur, Andhra Pradesh). These Xoo isolates were obtained from ICAR-IIRR stock cultures maintained at the Department of Plant Pathology, ICAR-IIRR, Hyderabad. The resistance evaluation of the NILs at different hot spots was done by artificially inoculating the entries with local Xoo isolates. The Xoo strains from those locations were isolated

following the standard procedures (Yugander *et al.*, 2014) and maintained on modified Wakimoto's Agar (MWA) (Sucrose 20g; peptone 5g; Calcium nitrate 0.5g; Na_2HPO_4 1.82 g; Ferrous sulphate 0.05g; Agar 20g; Distilled water 1000 ml; pH 6.8-7.0) at 4°C for regular use and in 15% glycerol at -70°C for long term preservation. The pathogenicity of all the Xoo isolates was checked before use by inoculating them on 40 days old TN1 (susceptible variety) plants in glass house using 3-day old bacterial suspension (10^{8-9} cfu/ml).

Genotyping of the NILs

The NILs were screened with gene-specific markers to detect the presence of target BB resistance genes viz., *xa13* and *Xa21*. The primers used were *xa13*-prom for *xa13* (Sundaram *et al.*, 2011) and pTA248 for *Xa21* (Ronald *et al.*, 1992). The details of the primers used are presented in the Table 1. The NILs, recurrent parents (MTU 1010 and JGL 1798) and donor parent (Improved Samba Mahsuri) were raised in plastic pots and leaf samples (5-7 cm leaf pieces) were collected from 20 days old seedlings of each entry for DNA isolation. Extraction of plant genomic DNA was carried out following Zheng *et al.* (1995). The DNA was washed with chilled ethanol (70%), dried at room temperature overnight and dissolved in 50 µl of sterile distilled water or TE buffer (10 mM Tris-HCl, pH 8.0 and 1 mM EDTA, pH 8.0) and preserved at -20°C for future use. The quality of the DNA was checked by running in 0.8% agarose gel.

Presence of BB resistance genes in the NILs was detected through PCR using the gene-specific markers. The reaction mixture (20 µl) consisted of 20 ng of template DNA, 5 pmoles of each primer, 0.5 µl dNTPs (2.5 mM), 2 µl PCR buffer (10 X) and 1 µl of Taq DNA polymerase (1 u/ml). PCR amplification was performed in a thermal cycler (Applied Biosystems, USA) using initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 1 min, followed by a final extension at 72°C for 7 min and then finally incubating at 4°C. The PCR products (20 µl) were mixed with gel loading buffer (40% sucrose and 0.25% bromophenol blue) and loaded in a gel containing 2% agarose in 1X TBE buffer. Electrophoresis was carried out for 1 hour at a constant voltage of 100V and stained using ethidium bromide (0.5 µg/ml) and documented (Alpha Image Analysis, USA). Confirmation of BB resistance genes, *xa13* and *Xa21* in the NILs was done based on the amplicon size corresponding to the donor parent, Improved Samba Mahsuri possessing three BB resistance genes *Xa21*, *xa13* and *xa5*.

Table 1. Details of the primers used for detection of BB resistance genes *xa13* and *Xa21*

BB resistance genes used	Markers	Chromosomal location	Sequence of the marker	Amplicon size	Reference
<i>xa13</i>	<i>xa13</i> prom	8	F-5'-TCCCAGAAAGCTACTACAGC-3' R-5'-GCAGACTCCAGTTTGACTTC-3'	S allele: 250 bp R allele: 530 bp	Sundaram <i>et al.</i> (2011)
<i>Xa21</i>	pTA248	11	F-5'-AGACGCGGGAAGGGTGGTTCCCGGA-3' R-5'-AGACGCGGGTAATCGAAAGATGAAA-3'	S allele: 650 bp R allele: 960 bp	Ronald <i>et al.</i> (1992)

Raising of NILs, pathogen multiplication and resistance evaluation

For glasshouse evaluation, all the entries (NILs, recurrent parents and check varieties) were line-sown in plastic trays (dimensions- 60 × 40 × 7 cm). A mixture of soil and farm yard manure (3:1) was prepared and the trays were filled up. The trays were fertilized with N @ 2.4 g/tray and P₂O₅ @ 1.2 g/tray. Part of N and entire amount of P was applied as basal while remaining N was applied as top dressing. Each tray was used for inoculation with individual Xoo isolate, 35-40 days after sowing. These isolates were cultured on MWA at 28 ± 2°C. Using 3 days old bacterial suspension (10⁸⁻⁹ cfu/ml), 2-3 leaves of every plant of each entry was inoculated by leaf clipping method (Kauffman *et al.*, 1973). Observations on disease reactions were made 15 days post inoculation by measuring the lesion length. For each entry, 3 observations were taken. The disease reactions were categorized as resistant (lesion length up to 5 cm), moderately resistant (5-10 cm lesion length), moderately susceptible (10-15 cm lesion length) and susceptible (lesion length more than 15 cm) (Mondal *et al.*, 2014).

The entries were also evaluated for their reaction to BB at four hot spot locations in Telangana and Andhra Pradesh viz., Agricultural Research Institute, Hyderabad, Telangana, Regional Agricultural Research Station, Warangal, Telangana, Regional Agricultural Research Station, Jagtial, Telangana and Agricultural Research Station, Nellore, Andhra Pradesh. Each entry was transplanted in field using 25 days old seedlings. Local susceptible varieties were also included for comparison. Plants were inoculated with Xoo isolate specific to that location, 25-30 days after transplanting following clip inoculation as described above. Data were recorded by measuring the lesion length 15 days after inoculation. For each entry 3 observations were taken and disease reaction was categorized as described earlier.

Data analysis

The data from glass house and field tests were analyzed by Randomized Complete Block Design using ANOVA

technique using the statistical package Statistix version 8.1. Further, least significant difference (LSD) test was applied to test the significance of treatment difference.

RESULTS

Genotyping of the NILs to re-confirm the presence of BB resistance genes

The NILs were screened with gene-specific primers *xa13*-prom and pTA248 for detecting rice BB resistance gene *xa13* and *Xa21*, respectively. Presence of target genes, *xa13* and *Xa21* in the NILs was identified based on the amplicon size (For *xa13*: ~ 250 bp for susceptible allele in MTU 1010 and JGL 1798 and ~ 530 bp for resistance allele in Improved Samba Mahsuri and for *Xa21*: ~ 650 bp for susceptible allele in MTU 1010 and JGL 1798 and ~ 960 bp for resistance allele in Improved Samba Mahsuri). The line C1-20-3 in the genetic background of MTU 1010 was confirmed to possess single BB resistance gene *xa13* while NIL C1-13-3 was confirmed to possess single BB resistance gene *Xa21* (Fig. 1a, 1b). All the remaining lines in the background of MTU 1010 (C1-20-2, C1-20-4 and C1-20-5) and JGL 1798 (C5-42-47, C5-45-48 and C5-49-44) revealed the presence of both *xa13* and *Xa21* (Fig. 1a, 1b).

Glasshouse evaluation of the NILs against multiple Xoo isolates

The NILs, recurrent parents, susceptible and resistant checks were evaluated under glasshouse condition with 10 diverse Xoo strains belonging to different rice growing regions and different virulence groups. The lesion length of the NILs against different Xoo strains is presented in the Table 2. All the pyramided lines possessing *xa13* or *Xa21* or their combination exhibited resistant reaction with significantly smaller lesion lengths when compared to their recurrent parents. However the NILs with single BB resistance genes produced relatively larger lesions compared to the lines possessing both the BB resistance genes (Table 2). The NIL C1-20-3 possessing single BB resistance gene *xa13* produced a lesion length varying

Fig. 1a. Verification of near isogenic lines for the presence of *Xa21* gene with pTA248 marker; The marker pTA248 produces an amplicon size of ~ 650 bp for susceptible allele in MTU 1010 and JGL 1798 and ~ 960 bp for resistance allele in Improved Samba Mahsuri; M-50 bp ladder; D-Donor (Improved Samba Mahsuri); P1-MTU 1010; P2-JGL 1798; 1-C1-20-3 (*xa13*); 2-C1-13-3 (*Xa21*); 3-C1-20-2; 4-C1-20-4; 5-C1-20-5; 6-C5-42-47; 7-C5-45-48; 8-C5-49-44

Fig. 1b. Verification of near isogenic lines for the presence of *xa13* gene with *xa13*-prom Marker; The marker *xa13*-prom produces an amplicon size of ~ 250 bp for susceptible allele in MTU 1010 and JGL 1798 and ~ 530 bp for resistance allele in Improved Samba Mahsuri; M-50 bp ladder; D-Donor (Improved Samba Mahsuri); P1-MTU 1010; P2-JGL 1798; 1-C1-20-3 (*xa13*); 2-C1-13-3 (*Xa21*); 3-C1-20-2; 4-C1-20-4; 5-C1-20-5; 6-C5-42-47; 7-C5-45-48; 8-C5-49-44

Table 2. Evaluation of NILs for BB resistance with different isolates of *Xanthomonas oryzae* pv. *oryzae* under glasshouse

NILs	Genes	Xoo isolates/Mean lesion length (cm)									
		IX-006	IX-015	IX-020	IX-027	IX-050	IX-133	IX-148	IX-266	IX-281	IX-372
C1-20-3	<i>xa13</i>	3.80 ^e	3.23 ^e	3.83 ^e	2.4 ^e	3.03 ^e	3.3 ^d	2.0 ^e	5.00 ^e	3.47 ^e	4.23 ^e
C1-13-3	<i>Xa21</i>	7.67 ^d	5.77 ^d	6.27 ^d	3.33 ^d	6.00 ^d	7.73 ^c	5.5 ^d	6.77 ^d	5.73 ^d	6.00 ^d
C1-20-2	<i>xa13</i> + <i>Xa21</i>	0.33 ^g	0.20 ^g	0.43 ^f	0.43 ^{gh}	0.60 ^g	0.6 ^f	0.57 ^g	0.53 ^{ghi}	0.33 ^f	0.50 ^g
C1-20-4	<i>xa13</i> + <i>Xa21</i>	0.43 ^{fg}	0.33 ^g	0.27 ^f	0.57 ^{gh}	0.57 ^g	0.367 ^f	0.47 ^g	0.20 ⁱ	0.33 ^f	0.33 ^g
C1-20-5	<i>xa13</i> + <i>Xa21</i>	0.47 ^{fg}	0.43 ^{fg}	0.63 ^f	0.93 ^{fg}	1.10 ^{fg}	0.5 ^f	0.37 ^g	0.70 ^{ghi}	0.30 ^f	0.60 ^g
C5-42-47	<i>xa13</i> + <i>Xa21</i>	0.80 ^f	3.00 ^e	1.4 ^f	1.0 ^f	1.83 ^f	1.2 ^{ef}	1.33 ^f	2.30 ^f	1.00 ^f	2.10 ^f
C5-45-48	<i>xa13</i> + <i>Xa21</i>	0.37 ^g	0.93 ^f	0.57 ^f	0.87 ^{fg}	1.23 ^{fg}	2.27 ^{de}	1.67 ^{ef}	1.13 ^g	0.67 ^f	0.43 ^g
C5-49-44	<i>xa13</i> + <i>Xa21</i>	0.43 ^{fg}	0.43 ^{fg}	0.4 ^f	0.47 ^{gh}	1.20 ^{fg}	0.43 ^f	0.5 ^g	1.23 ^{gh}	0.33 ^f	0.33 ^g
MTU 1010 (Recurrent parent)		11.0 ^c	9.27 ^c	13.00 ^b	13.00 ^b	7.0 ^c	14.00 ^b	11.0 ^b	12.33 ^b	10.00 ^c	12.33 ^b
JGL 1798 (Recurrent parent)		11.5 ^b	10.00 ^b	12.17 ^c	11.00 ^c	9.0 ^b	10.00 ^c	15.0 ^a	10.67 ^c	11.00 ^b	11.00 ^c
ISM (<i>xa5</i> + <i>xa13</i> + <i>Xa21</i>) (R Check)		0.4 ^g	0.20 ^g	0.20 ^g	0.20 ^f	0.23 ^h	0.47 ^g	0.2 ^f	0.37 ^g	0.37 ^{hi}	0.27 ^f
TN 1 (S Check)		13.0 ^a	11.97 ^a	21.00 ^a	21.67 ^a	25.0 ^a	21.67 ^a	14.17 ^a	22.33 ^a	21.33 ^a	20.00 ^a
CV (%)		5.42	6.27	6.6	17.07	7.54	9.4	19.58	10.26	10.07	16.7
LSD (<i>P</i> =0.05)		0.37	0.41	0.57	1.44	0.5451	0.82	1.57	0.87	0.86	1.31

ISM-Improved Samba Mahsuri; Figures in a column with same letters are not significantly different at 5% level of significance

from 2 cm (with Xoo strain IX 148) to 5 cm (with Xoo strain IX 266) while the NIL C1-13-3 possessing single BB resistance gene *Xa21* exhibited a lesion length of 3.3 cm (with Xoo strain IX 027) to 7.4 cm (with Xoo strain IX 133) (Table 2). Among these two lines, line C-20-3 with *xa13* showed shorter lesion lengths compared to line C-13-3 possessing *Xa21* (Table 2).

The NILs possessing both *xa13* and *Xa21* in the genetic background of MTU 1010 (C1-20-2, C1-20-4 and C1-20-5) showed highly resistant reaction with significantly reduced lesion length (Table 2, Fig. 2). In these NILs, the lesion length varied from 0.2 cm (C1-20-2 with IX-015 and C1-20-4 with IX-266) to 1.1 cm (C1-20-5 with IX-050) (Table 2). The NILs in the background of JGL 1798 (C5-42-47, C5-45-48 and C5-49-44) possessing both the BB resistance genes (*xa13* and *Xa21*) also showed highly resistant reaction with significantly reduced lesion length (Fig. 2). In these NILs, the average lesion length ranged from 0.33 cm (C5-49-

44 with IX-281 and IX-372) to 2.30 cm (C5-42-47 with IX-266) (Table 2). Though 2-gene pyramided lines of both MTU 1010 and JGL 1798 showed highly resistant reaction, the average lesion lengths were slightly less in NILs of MTU 1010 compared to NILs of JGL 1798 (Table 2). Both the recurrent parents were moderate to highly susceptible to the Xoo isolates used in the present study with average lesion length ranging from 7-14 cm in MTU 1010 and 9-14 cm in JGL 1798. Improved Samba Mahsuri showed high level of resistance to the Xoo isolates with an average lesion length ranging from 0.2-0.4 cm while the susceptible check TN1 showed highly susceptible reaction with average lesion length ranging from 13-15 cm (Table 2).

Resistance evaluation of NILs at hot spot locations

Pyramided lines, possessing *xa13* and *Xa21*, either singly or in combination in the genetic background of MTU 1010 and JGL 1798 were also evaluated under field conditions

Fig. 2. BB reaction of 2-gene (*xa13* and *Xa21*) pyramided lines of MTU 1010 and JGL 1798 to Xoo isolate IX-020 under glasshouse condition; 1-MTU 1010; 2-JGL 1798; 3-Improved Samba Mahsuri; 4-C1-20-2; 5- C1-20-4; 6- C1-20-5; 7- C5-42-47; 8- C5-45-48; 9- C5-49-44

at four different hot spot locations in Telangana and Andhra Pradesh having different weather conditions. The Plants were clip-inoculated at maximum tillering stage (Kauffman *et al.*, 1973) with local representative Xoo isolates which were established from BB infected leaves collected from research stations or nearby farmer's fields. The observations were taken on lesion length (cm) 15 days after inoculation. Data on lesion length were subjected to combined analysis to study the performance

of the entries across different locations (Table 3). The analysis revealed a wide variation in the lesion length of different gene pyramided lines across different locations indicating virulence of the Xoo isolates used and influence of weather factors in expression of BB symptoms. Perusal of mean lesion length data revealed that all the NILs possessing *xa13* and *Xa21* either singly or in combination in the background of MTU 1010 or JGL 1798 produced significantly smaller lesions compared to recurrent parents and susceptible checks (Table 3). Mean data across the locations indicated that among the NILs possessing single BB resistance genes, line C1-20-3 possessing BB resistance gene *xa13* performed better (mean lesion length 3.38 cm) compared to NIL C1-13-3 possessing BB resistance gene *Xa21* (mean lesion length 6.7 cm). The mean data revealed that the NILs possessing combination of both *xa13* and *Xa21* showed significantly shorter lesion length (ranging from 0.91 cm in C5-49-44 to 1.73 cm in C5-42-47) compared to NILs possessing single genes (Table 3). Analysis of mean data revealed that 2-gene pyramided lines, C1-20-2 and C1-20-4 in the genetic background of MTU 1010 were on par with each other and performed slightly better than C1-20-5 (another 2-gene pyramided lines in the background of MTU 1010). Similarly, 2-gene pyramided line C5-49-44 in the background of JGL 1798 performed slightly better than other two 2-gene pyramided lines *i.e.* C5-42-47 and C5-45-48 (also in the background of JGL 1798). The lines C1-20-5, C5-42-47 and C5-45-48 possessing BB resistance genes *Xa21* and *xa13* showed comparatively larger lesions at Nellore which could be due to variation in the Xoo strains.

Table 3. Mean lesion length (cm) of NILs against BB at different hot spot locations in Telangana and Andhra Pradesh

NILs	Genes	Locations/Lesion length (cm)				Mean
		WGL	RNR	JGL	NLR	
C1-20-3	<i>xa13</i>	2.87 ^{mn}	3.70 ^{kl}	2.77 ⁿ	4.17 ^k	3.38 ^e
C1-13-3	<i>Xa21</i>	5.37 ^j	7.47 ⁱ	5.73 ^j	8.23 ^h	6.70 ^d
C1-20-2	<i>xa13</i> + <i>Xa21</i>	0.67 ^{rstuvw}	0.93 ^{rstuv}	1.07 ^{qrst}	1.87 ^{op}	1.13 ^g
C1-20-4	<i>xa13</i> + <i>Xa21</i>	0.77 ^{rstuvw}	0.53 ^{tuvw}	0.87 ^{rstuvw}	2.00 ^o	1.04 ^g
C1-20-5	<i>xa13</i> + <i>Xa21</i>	0.63 ^{rstuvw}	0.87 ^{rstuvw}	1.20 ^{pqrst}	3.53 ^{klm}	1.56 ^f
C5-42-47	<i>xa13</i> + <i>Xa21</i>	1.73 ^{opq}	1.30 ^{opqr}	0.83 ^{rstuvw}	3.07 ^{lmn}	1.73 ^f
C5-45-48	<i>xa13</i> + <i>Xa21</i>	1.27 ^{pqrs}	1.20 ^{pqrst}	0.57 ^{stuvw}	3.17 ^{lmn}	1.55 ^f
C5-49-44	<i>xa13</i> + <i>Xa21</i>	1.10 ^{qrst}	0.50 ^{tuvw}	1.03 ^{qrst}	1.00 ^{rstuv}	0.91 ^g
MTU-1010	Recurrent parent	10.33 ^g	12.33 ^e	11.67 ^{ef}	14.17 ^c	12.13 ^b
JGL-1798	Recurrent parent	10.67 ^g	10.50 ^g	10.40 ^g	11.43 ^f	10.75 ^c
ISM	Resistant check	0.47 ^{uvw}	0.43 ^{uvw}	0.20 ^w	0.30 ^{vw}	0.35 ^h
TN-1	Susceptible check	13.17 ^d	15.00 ^b	12.33 ^e	18.33 ^a	14.71 ^a
Mean		4.09 ^c	4.56 ^b	4.06 ^c	5.94 ^a	

CV (%): 9.5

LSD ($P=0.05$) (Treatment): 0.36

LSD ($P=0.05$) (Location): 0.21

LSD ($P=0.05$) (Treatment x Location): 0.72

WGL-Warangal, Telangana; RNR-Rajendranagar, Telangana; JGL-Jagtial, Telangana; NLR-Nellore, Andhra Pradesh; Figures in a column with same letters are not significantly different at 5% level of significance

DISCUSSIONS

Bacterial blight is a major disease of rice in India which can significantly reduce the yield. Though, more than 40 BB resistance genes have been reported from various sources, the effectiveness of individual BB resistance genes varies from region to region, due to difference in pathogen population structure. Pyramiding multiple resistance genes for different biotic stresses provides in-built resistance to the popular and widely adopted cultivars and thereby reduces the yield loss due to biotic stresses. A number of research laboratories are carrying out gene pyramiding program by introgressing multiple BB resistance genes in popular rice varieties to provide in-built resistance against this disease (Sundaram *et al.*, 2008; 2009; Salgotra *et al.*, 2012; Hajira *et al.*, 2014; Ellur *et al.*, 2016a; Ellur *et al.*, 2016b). In this study, two broad-spectrum BB resistance genes, *Xa21* and *xa13* were pyramided in two popular rice varieties, MTU 1010 and JGL 1798 to provide in-built BB resistance.

Evaluation of NILs against multiple Xoo isolates under glasshouse conditions revealed that NILs with both *xa13* and *Xa21* provided broader and higher level of resistance to Xoo compared to the NILs which have single BB resistance gene. The lesion length in lines possessing both the BB resistance genes ranged from 0.2-2.3 cm compared to the lines possessing single BB resistance gene (lesion length ranged from 2-7.4 cm). However, among the NILs possessing single BB resistance genes, line C1-20-3 with *xa13* showed slightly better level of resistance compared to line C1-13-3 with *Xa21*. In a similar study, Laha *et al.* (2007) showed high level of resistance of 2 and 3-gene pyramided lines in the background of popular rice variety Swarna to multiple isolates of Xoo under glasshouse condition. Hari *et al.* (2011) demonstrated broad spectrum resistance of KMR-3R (restorer line of rice hybrid KRH 2) lines possessing BB resistance gene *Xa21* to multiple Xoo strains under glasshouse condition. Many earlier studies have reported that BB resistance gene *xa13* provides broad-spectrum resistance to Xoo (Lore *et al.*, 2011; Mishra *et al.*, 2013; Yugander *et al.*, 2017).

A large number of studies have shown the performance of different gene pyramided lines to multiple isolates of Xoo under field conditions. Basmati rice varieties Pusa Basmati 1121 and Pusa Basmati 6 were improved for blast and BB resistance by incorporating two BB resistance genes (*xa13* and *Xa21*) and two blast resistance genes (*Pi2* and *Pi54*) using marker assisted backcross breeding (Ellur *et al.*, 2016a). Sundaram *et al.* (2008) evaluated four 3-gene pyramided (*xa5* + *xa13* + *Xa21*) lines of Samba Mahsuri under AICRIP (All India Coordinated Rice Improvement Project) trials at different hot spot locations and reported a high degree of resistance of the gene pyramided lines indicating broad spectrum resistance conferred by combination of three BB resistance genes. Sundaram *et al.* (2009) again showed that NILs possessing a combination of *xa13* and *Xa21* in the background of rice variety, Triguna showed high level of resistance to Xoo. Combination of BB

resistance genes, *xa13* and *Xa21* in the background of popular basmati rice variety, Pusa Basmati 1 provided broad-spectrum resistance to BB (Joseph *et al.*, 2004). It can be concluded from the study that 2-gene pyramided lines of popular rice varieties MTU 1010 and JGL 1798 performed better compared to lines possessing single BB resistance gene and can be deployed in the state of Telangana and Andhra Pradesh to combat bacterial blight disease of rice.

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