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Molecular and Phenotypic Assay of Improved Lines Harboring Bacterial Blight Resistance

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ABSTRACT

Keywords

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Bacterial blight (BB) is one of the serious and wide spread diseases of many rice cultivating areas in India as well as world. Development of cultivars pyramided with more than two BB resistance genes increases durable resistance to prevailing and emerging biotypes and it offers environment safe approach to manage the disease. The present study was carried out to screen the improved lines with the foreground markers of *Xa21*, *xa13*, *xa5*, *Xa33* and *Xa38* and the confirmed plants were evaluated for phenotypic resistance against the local isolate of BB under the controlled conditions. Further, the lines harboring BB resistance genes were evaluated for key-morphological traits to identify the superior genotypes. The bioassay revealed, most of the lines harboring *Xa21*, *xa13*, *xa5* and *Xa33/Xa38* in homozygous conditions were displayed high degree of resistance to bacterial blight with a mean lesion length (LL) ranged from 2.13 ± 0.41 to 2.92 ± 0.65 . The bioassay results also suggest, the lines harboring *Xa21+ xa13 + xa5 + Xa33* resistance genes were observed to have less lesion length (2.13 ± 0.45) than *Xa21+ xa13 + xa5 + Xa38* resistance genes (2.81 ± 0.40). Cluster analysis of 24 improved lines based on co-efficient of genetic distance, identified the superior genotypes, which possess morphological characters as recurrent parents and also enriched with BB resistance genes.

Introduction

Bacterial blight is the most serious and destructive disease of many rice growing areas especially in south east Asia. It is caused by *Xanthomonas oryzae* pv. *oryzae* with the sign and symptoms of water-soaked lesions and wavy margins from tip of the leaf

to the edge. It occurs in two phases; one is at seedling stage and another at maximum tillering stage.

The seedling stage symptoms are termed as 'kresek' and second phase symptoms starts as blighting of leaves at maximum tillering stage with distinct ooze out, in the form of

yellow droplets and it spread through water (Yasmin *et al.*, 2017). The bacteria remain survive in the root of alternate host plant (*Leersia xandra*) and in infected paddy stubbles.

Rice is a staple crop for half of the world population with a production of 769.9 million tonnes and it is cultivated all over the world because of its wider adaptability to the vast environments. It is only the main source of carbohydrate for people below poverty line (Raveendra *et al.*, 2020) and it is regarded as strategic crop under the food security. It has been affected by fungi, bacterial and viral diseases. Though, many diseases have been reported in rice, BB (Bacterial Blight) got its own significance, by infecting more than 50% of cultivated area.

During the severe outbreak of BB, it may cause a yield loss upto 60-75% (Kumar *et al.*, 2012). For the control of BB, chemical method is effective only during the early stages, besides it also possesses the residual effect on soil. Host-plant resistance to BB is effective and it offers environment safe approach to control this disease (Ramalingam *et al.*, 2017).

About 44 BB resistance genes have been identified till date (Kim *et al.*, 2018) and some of them were cloned. The resistance conferred by single gene can't be sufficient to defend the pathogen effectively, pyramiding of more than two effective resistance genes will give broad-spectrum and durable resistance to BB than single gene resistance.

With this reference, the present study was carried out to evaluate the physical resistance of molecularly confirmed improved lines against bacterial blight disease under controlled conditions and also to assess the morphological traits to identify the phenotypically similar improved lines as recurrent parents.

Materials and Methods

Plant materials

Totally 24 lines of Improved BC₂F₃ population for bacterial blight resistance (*Xa21*, *xa13*, *xa5* and *Xa33/Xa38*) were developed in the backgrounds of ASD 16, ADT43 and ADT 47 at Tamil Nadu Agricultural University, Coimbatore. The present investigation was carried out at Agricultural College and Research Institute, Madurai to evaluate these improved lines for physical resistance against the BB under the controlled conditions as well as to evaluate the agro-morphological traits. The improved lines which possess these genes (*Xa21*, *xa13*, *xa5* and *Xa33/Xa38*) were confirmed by functional markers and were subjected to artificial bacterial blight resistant screening and same were assessed for agro-morphological traits.

DNA Isolation

Fresh leaves from the improved lines and parent lines were collected from 6-8 weeks old seedlings and DNA was isolated by using CTAB method with slight modifications (Dellaporta, Wood, & Hicks, 1983). Quality of DNA was checked by using 0.8% agarose gel as well as quantified with spectrophotometer and final quantity of DNA was adjusted to 60ng/μl and stored at -20°C for further use.

PCR amplification

The improved lines which contain targeted genes (*Xa21*, *xa13*, *xa5* and *Xa33/Xa38*) were confirmed by using closely linked functional markers (Table 1). 10 μl of PCR mixture contain 1 μl of template DNA, 0.75 μl of dNTPs (2mM), 1 μl of 10x PCR buffer, 0.2 μl of Taq polymerase, 0.5 μl of each forward and reverse primers and 7 μl of sterile water. The PCR reaction was performed by using

thermocycler (Eppendorf) with the following conditions: initial denaturation at 94°C for 5 min followed by 35 cycles of final denaturation at 94°C for 30sec; annealing at 56°C for 30sec (*Xa33*) and 59°C for 1min (*Xa38*); 72°C for extension; 72°C for final extension. The amplified PCR products were separated by using 3% agarose gel stained with ethidium bromide and visualized through BIO-RADGel DOC^{XR+}

Phenotypic screening for resistance against BB

From 24 lines of improved BC₂F₃ population only 13 lines shown homozygous condition for all the targeted genes (*Xa21*, *xa13* and *xa5*, *Xa33/Xa38*) were artificially inoculated with *Xoo* isolate. A total of three replications were maintained with 30 plants per each replication including parents as checks (IRBB60 as resistant; ASD 16, ADT 43 and ADT 47 as susceptible checks). To test the physical resistance of improved lines and parents against the BB, top five leaves were clipped off and leaves were dipped in to the suspension containing bacterial cells at a rate of 10⁹ cells/ml by using clip inoculation method Kauffman, H. E. (1973). The symptoms were recorded 15 days after inoculation based on mean lesion length (LL) and scored according to the IRRI, SES IRRI, (2002). The plants with a LL (cm) of less than 5cm were considered as resistant and the plants with more than 5cm LL were recorded as Susceptible.

Morphological traits

The improved lines possessing the targeted genes (*Xa21*, *xa13*, *xa5* and *Xa33/Xa38*) were assessed for agronomical traits to check the performance of the pyramided lines over the parents. The data on morphological characters viz., days to 50% of flowering (DFF), plant height (PH), flag leaf length (FL), flag leaf

width (FW), panicle length (PL), number of tillers (NT), number of productive tillers (NPT) and grain yield (SPY) were collected and statistically analyzed to identify the improved lines phenotypically similar to recurrent parents (ASD 16, ADT 43 and ADT 47).

Results and Discussion

The improved lines were screened for targeted genes (*Xa21*, *xa13*, *xa5* and *Xa33/Xa38*) by using linked/functional markers (Figure 1; Table 1) and the positive plants were inoculated with the local isolate of BB to check for physical resistance in the controlled conditions. The bioassay of BB reveals, 13 genotypically selected improved lines at BC₂F₃ generation (Table 4) possessing *Xa21*, *xa13*, *xa5* and *Xa33/Xa38* in homozygous conditions exhibited a high level of resistance with a mean lesion length (cm) of 2.2 ± 0.89 to 2.9 ± 0.33 with a diseases score of 0-1 (highly resistant). The rest of lines harboring *Xa21*, *xa13* and *xa5* without *Xa33/Xa38* resistance genes, exhibited a mean lesion length ranged from 3.33 ± 0.41 to 3.92 ± 0.65 with a diseases score of 1-2. Similarly, the resistant check, IRBB60 (*Xa21*, *xa13* and *xa5*) expressed a LL (cm) of 3.18 ± 0.44, while the susceptible checks ASD 16, ADT 43 and ADT 47 exhibited a mean lesion length of 15.12 ± 1.1, 15.57 ± 2.3 and 15.23 ± 1.3 respectively (Figure 4; Table 3). The evaluation of morphological traits revealed that, most of the improved lines were in similar with the recurrent parent morphological characters (Table 2). Some of the lines viz., IL-16/60-3 in the background of ASD 16, IL-43/60-8-1 and IL-43/60-91 in the background of ADT 43 and IL-47/60-36 in the background of ADT 47 were produced higher yield than its respective recurrent parent. The cluster of selected improved lines suggests, all the improved lines (Table 2) in this study were in similar to their recurrent parents (Figure 2). Some promise lines viz.,

IL-16//60-3 in the background of ASD 16, IL-43/60-16-1 in the background of ADT 43 and IL-47/60-16-4 in the background of ADT 47 were highly similar to their recurrent parents (Figure 2). Correlation of yield and yield contributing traits suggests, the number of productive tillers ($r = 0.68$) and number of tillers ($r = 0.60$) were correlated with grain yield (Figure 3). Development of cultivars with broad-spectrum resistance to BB highly essential to produce stable and higher yields. Developing cultivars with single gene is not recommended as it is overcome by newly evolved pathotypes.

To enhance the resistance levels to prevailing as well as emerging pathotypes, pyramiding of multiple genes in a single cultivar is highly essential for durable and broad-spectrum resistance (Pradhan *et al.*, 2015; Ramalingam, Savitha, Ganesh, & Ranganathan, 2017; Sundaram *et al.*, 2011). In the present investigation, physical resistance of selected improved lines possessing *Xa21*, *xa13*, *xa5* and *Xa33/Xa38* exhibited a mean lesion length (cm) of 2.81 ± 0.41 to 2.92 ± 0.65 ,

while the resistant check, IRBB60 expressed 3.18 ± 0.44 lesion length (cm). In addition, the improved lines with *Xa33/Xa38* resistance genes were displayed lesser lesion length than the lines with three resistance gene combination (*Xa21*, *xa13* and *xa5*). While, in comparison of lines with *Xa21 + xa13 + xa5 + Xa33* and *Xa21 + xa13 + xa5 + Xa38*, the *Xa33* combination has given good results with lesser lesion length (2.13 ± 0.45 to 2.25 ± 0.25) than the lines with *Xa38* combination (2.81 ± 0.40 to 2.92 ± 0.65). Similar studies on NIL population in PB1121 for improved resistance against BB through introgression of *Xa21+xa13* and *Xa38* resistance genes and they evaluated for phenotypical screening against *Xoo*.

They have observed lesser lesion length of 3.83 ± 0.64 , in the lines harboring *Xa38* component in addition to *Xa21 + xa13* (Ellur *et al.*, 2016). Gidamo, 2015 has observed lesser lesion length in the lines harboring *Xa33* resistance genes (0.4 ± 0.6) than *Xa38* resistance genes (0.95 ± 0.69) phenotypical screening against BB (unpublished data).

Table.1 Molecular markers used for foreground selection

Markers	Gene	Chr	Primer sequence	AT (°C)	Reference
pta-248	Xa21	11	F ATAGCTAGTTCATAGAGG	65	(Iyer-Pascuzzi & McCouch, 2007)
			R ACATCCGTCACTCTGCCA		
xa-13- prom	xa-13	8	F GAGCTCCAGCTCTCCAAATG	59	(Perumalsamy et al., 2010)
			R GGCCATGGCTCAGTGTTTAT		
xa-5-1	xa-5	5	F CGGATAGCAGCATTTC CAAGAG	56	(Chu et al., 2006)
			R GATTCCTTTAGCAAGGTGTG		
RMWR 7.1	Xa33	7	F 5-TTTTACCCCTTCTTCTTTC-3	55	(Kumar et al., 2012)
			R 5-CGTGTTTTGTGTGTCTTTTG-3		
RMWR7.6	Xa33	7	F 5-CAACAAACACCTCCATGGTC-3	56	(Kumar et al., 2012)
			R 5-GGGAATGAGCAAAAATTGG-3		
Os04g53050-1	Xa38	4L	F 5TCTTCTATTGCTAACATTGGTA-3	59	(Bhasin et al., 2011)
			R 5-TCGCATTCATTTTCAGAG-3		

Table.2 Agro-morphological characters of selected improved lines

Genotype	DFF	PH (cm)	FL (cm)	FW (cm)	PL (cm)	NT	NPT	SPY (gm)
IL-16/60-3	86	90.2	29.3	1	20	18	16	29.9*
IL-43/60-37	82*	88	32	1.2*	26*	14	12	26.3
IL-43/60-16-1	82*	87.5	35.6*	1.3*	21.7	14	11	26.3
IL-43/60-13-1	86	87.3	31.5	1	20.5	12	10	26.2
IL-43/60-35	82*	86.3	39.2*	1.2*	24.9*	16	13	27.1*
IL-43/60-2	82*	84.3*	44.1*	1.6*	29*	17*	14*	27.2*
IL-43/60-14-1	81*	85.4*	36*	1.2*	26.3*	16	12	27.3*
IL-43/60-8-1	81*	86.3	33.5*	1.2*	25.4*	12	10	27.9*
IL-43/60-1-2	82*	87.3	37.6*	1.3*	22.3	23*	20*	27.2*
IL-43/60-4-1	81*	88.1	34.5*	1	24.1*	14	10	27.3*
IL-43/60-15-2	82*	83.3*	35.4*	1.2*	23.5*	15	14	27.1*
IL-43/60-91	81*	84.4	36.1*	1.2*	20.9	15	12	27.7*
IL-43/60-37-1	83*	86.2*	34.1*	1	20.5	23*	18*	26.8*
IL-43/60-31	83*	86.3	35.9*	1.2*	23.9*	14	12	26.9*
IL-43/60-33	84	85.5*	32.1	1.2*	21.5	16	14*	26.9*
IL-43/60-29	81*	86.1*	33.2*	1	21.9	14	12	27.1*
IL-47/60-31	88	87.4	24.3	1.2*	17.3	20*	15*	25.1
IL-47/60-3	88	83.4*	28.6	1	24.2*	18*	15*	25.2
IL-47/60-36	89	85.7*	28.3	1	22.6	18*	15	26.9*
IL-47/60-4	89	87.3	28.5	1.2*	25.3*	13	10	25.2
IL-47/60-8	86	87.5	28.2	1	23	21*	19*	25.3
IL-47/60-18	85	86.4	32.1	1.2*	25.6*	16	13	25.5
IL-47/60-32	88	86.3	27.3	1	23.5	14	12	25.1
IL-47/60-36	88	86.2*	29.8	1.2*	27.5*	18*	16*	25.3
ASD 16	86	86.3	29.3	1	20	19	17	29.9
ADT 43	85	87.5	35.6	1.3	21.7	18	16	26.3
ADT 47	88	86.2	29.8	1.2	27.5	18	16	25.3
Mean	84.4074	86.3963	32.66296	1.151852	23.35556	16.11111	13.18519	26.67778
SD	2.87241	1.474522	4.276245	0.142425	2.726273	2.965615	2.842494	1.277116
CV	3.40303	1.706696	13.09203	12.36488	11.67291	18.40726	21.55824	4.787191

* Significantly deviating from mean

Table.3 Phenotypic scoring of improved lines against bacterial blight resistance

Genotypes	Bacterial blight	
	Lesion length(cm)	Score
IL-16/60-3 (<i>Xa21+xa13+xa5 +Xa33</i>)	2.21 ± 0.33	0
IL-43/60-4 (<i>Xa21+xa13+xa5 +Xa33</i>)	2.13± 0.41	0
IL-43/60-91 (<i>Xa21+xa13+xa5 +Xa33</i>)	2.25 ± 0.25	0
IL-47/60-36 (<i>Xa21+xa13+xa5 +Xa38</i>)	2.87 ± 0.89	0
IL-43/60-16-1 (<i>Xa21+xa13+xa5 +Xa38</i>)	2.92 ± 0.65	0
IL-47/60-16-4 (<i>Xa21+xa13+xa5 +Xa38</i>)	2.81± 0.40	0
IL-43/60-8-1 (<i>Xa21+xa13+xa5</i>)	3.60 ± 0.50	1
IL-43/60-33 (<i>Xa21+xa13+xa5</i>)	3.47± 0.54	1
IL 43/60-29 (<i>Xa21+xa13+xa5</i>)	3.64 ± 0.7	1
IL-47/60-8 (<i>Xa21+xa13+xa5</i>)	3.83 ± 0.45	1
ASD 16	15.12 ± 1.1	9
ADT 43	15.57 ± 2.3	9
ADT 47	15.23± 1.3	9
IRBB60	3.18 ± 0.44	1

Lesion length (LL) of <5 cm is considered as resistant and lesion length of > 5 cm is considered susceptible.0-1 (Highly resistant); 9 (highly susceptible)

Table.4 Genotypic data of improved lines harboring BB resistance genes

Population	<i>Xa21</i>	<i>xa13</i>	<i>xa5</i>	<i>Xa38</i>	<i>Xa33</i>
IL-47/60-3 (<i>Xa21+xa13+xa5 +Xa38</i>)	R	R	R	S	--
IL-47/60-4 (<i>Xa21+xa13+xa5 +Xa38</i>)	R	R	R	S	--
IL-47/60-8 (<i>Xa21+xa13+xa5 +Xa38</i>)	R	R	R	R	--
IL-47/60-36 (<i>Xa21+xa13+xa5 +Xa38</i>)	R	R	R	R	--

IL-47/60-31 (<i>Xa21+xa13+xa5 +Xa38</i>)	R	R	R	R	--
IL-47/60-32 (<i>Xa21+xa13+xa5 +Xa38</i>)	R	R	R	R	--
IL-43/60-18 (<i>Xa21+xa13+xa5 +Xa38</i>)	R	R	R	R	--
IL-43/60-91 (<i>Xa21+xa13+xa5 +Xa38</i>)	R	R	R	S	--
IL-16/60-1 (<i>Xa21+xa13+xa5 +Xa33</i>)	R	R	R	--	R
IL-16/60-3 (<i>Xa21+xa13+xa5 +Xa33</i>)	R	R	R	--	S
IL-43/60-1 (<i>Xa21+xa13+xa5 +Xa33</i>)	R	R	S	--	R
IL-43/60-2 (<i>Xa21+xa13+xa5 +Xa33</i>)	R	R	R	--	R
IL-43/60-8 (<i>Xa21+xa13+xa5 +Xa33</i>)	R	R	R	--	R
IL-43/60-14 (<i>Xa21+xa13+xa5 +Xa33</i>)	R	R	R	--	R
IL-43/60-15 (<i>Xa21+xa13+xa5 +Xa33</i>)	S	S	S	--	R
IL-43/60-16 (<i>Xa21+xa13+xa5 +Xa33</i>)	R	R	R	--	R
IL-43/60-25 (<i>Xa21+xa13+xa5 +Xa33</i>)	R	R	S	--	S
IL-43/60-28 (<i>Xa21+xa13+xa5 +Xa33</i>)	R	R	R	--	S
IL-43/60-29 (<i>Xa21+xa13+xa5 +Xa33</i>)	R	R	R	--	S
IL-43/60-31 (<i>Xa21+xa13+xa5 +Xa33</i>)	R	R	R	--	R
IL-43/60-33 (<i>Xa21+xa13+xa5 +Xa33</i>)	S	S	S	--	S
IL-43/60-35 (<i>Xa21+xa13+xa5 +Xa33</i>)	R	R	S	--	S
IL-43/60-37-1 (<i>Xa21+xa13+xa5 +Xa33</i>)	R	R	R	--	S
IL-43/60-37-2 (<i>Xa21+xa13+xa5 +Xa33</i>)	R	R	R	--	S

Fig.1 Agarose gel images representing the alleles of (a) *Xa21* (b) *xa13*(c) *xa5* (d) *Xa33* and (e) *Xa38*. L – 100 bp ladder; P1 – ASD 16; P2 – IRBB60; R – Resistant;S – Susceptible; H – Heterozygous; L – 100 bp ladder

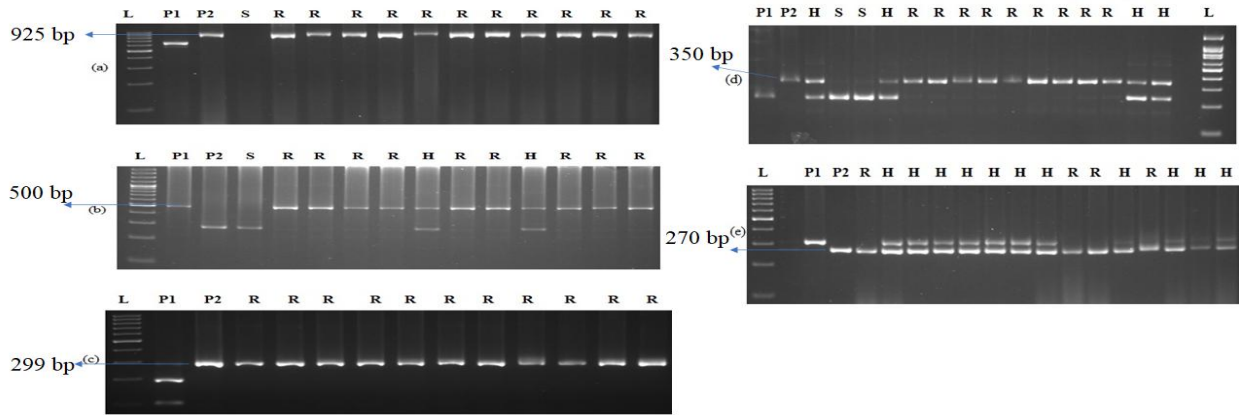


Fig.2 Cluster dendrogram showing the genetic relationship among the improved lines

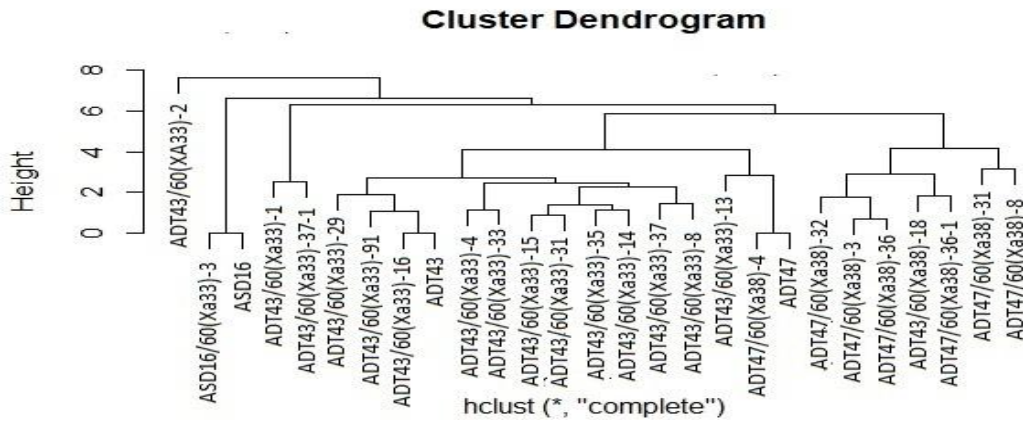


Fig.3 Correlation of yield and yield contributing traits of selected improved lines

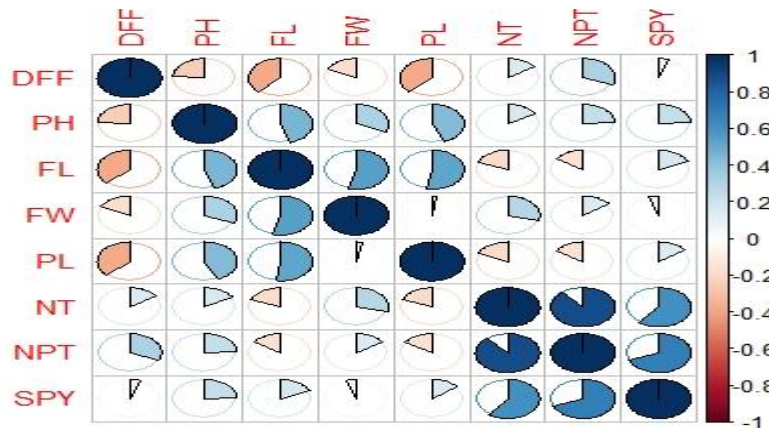
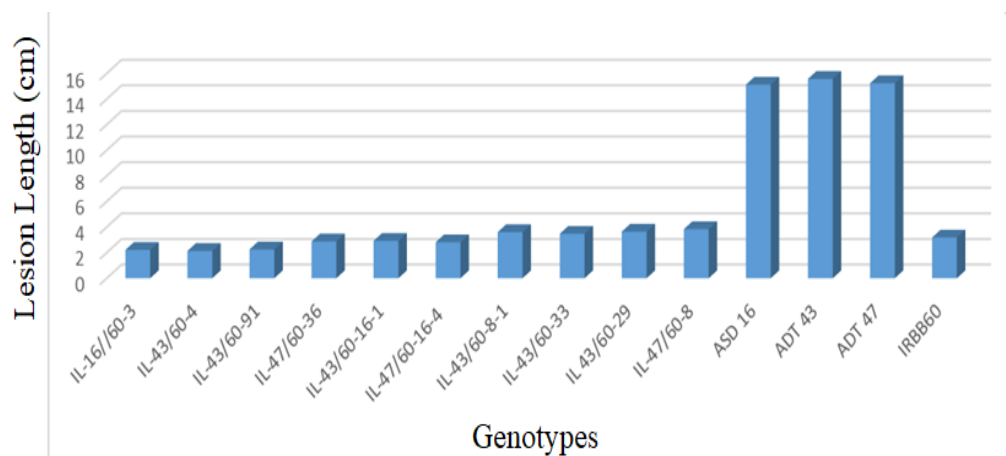


Fig.4 Graphical representation of Phenotypic evaluation of improved lines against bacterial blight resistance



The results of Gidamo, 2015 supports the present study results, as the lines harboring *Xa33* components was given lesser lesion length against isolates of *Xoo*. The resistance reaction of improved lines is due to the inheritance of resistant alleles from the donor parent, which were confirmed by using functional DNA markers (Figure 1). The morphological characters of the most of the improved lines were in similar with recurrent parent, due to the transfer of all the recurrent parent alleles for superior morphological characters as recurrent parents. The similar results were also obtained by (Hsu, Chiu, Yap, Tseng, & Wu, 2020), while introgressing *Xa4*, *xa5*, *Xa7*, *xa13* and *Xa21* in to the TNG82 cultivar through marker-assisted backcross breeding (MABB). Further, the improved lines with superior morphological characters and having physical resistance to BB were tested in multilocational trails to be release as new improved variety for BB prone rice cultivating areas or can be used as a potential donor in breeding programmes.

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