SCREENING OF RICE LINES OF DIVERSE ORIGIN RESISTANT TO BACTERIAL LEAF BLIGHT UNDER LOCAL CONDITIONS

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ABSTRACT

Current study comprised of 20 donor parent lines and four *indica* elite strains resistant against bacterial leaf blight having diversity in origin *viz.* Bangladesh, Philippine, Indonesia, Senegal and India. The lines were tested to check resistance against Bacterial Leaf Blight (BLB), having BLB resistance genes *Xa-4, xa-5, Xa-7* and *xa-13*. The experiment was conducted at Agronomic Research Station, Farooqabad, Sheikhupura. Artificial inoculation was done to study disease reaction. DNA marker analysis was also conducted to study the gene status of these lines. Results indicated that seven donor lines were completely resistant to Bacterial Leaf Blight whereas twelve lines exhibited moderate resistance. Among rest of the five susceptible genotypes, three were showing comparatively low susceptibility whereas two completely susceptible to BLB. Most of the lines with three or four gene combinations showing resistant behavior against BLB except AUS-298 that showed moderately susceptible behavior. Combination of genes for Bacterial Leaf Blight resistance can be explored further in targeted research program for disease resistance.

Keywords: Bacterial Leaf Blight, Resistance, Artificial inoculation, Diversity, Gene.

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INTRODUCTION

Rice is the staple food for one-third populace of the world (Tang *et al.,* 2001). Rice production is constrained by several factors but various diseases of fungal, bacterial and viral origin proved to be the most devastating biotic threat to its overall yield. Oldest know disease of rice, Bacterial leaf blight, caused by *Xanthomonas oryzae* pv. *Oryzae* (*Xoo*), was first reported in Japan in 1884 (Dinh *et al.* 2010). The disease incidence was observed in Northern Australia, Asia, USA and Africa while no record of disease was reported in Europe (Shanti *et al*., 2001).

Studies regarding yield losses due to BLB revealed varying levels of reduction in grain yield, depending on various factors like crop stage, susceptibility of strain and favorable environmental conditions. Due to its high damage to the rice field, a lot of studies and observations have already been done for controlling this disease but effective control measures are yet to be found (Mubassir *et al*. 2016). BLB caused 70-80% infestation in some African countries. Yield losses ranged from 20-30% with peak of 50-90% in some parts of the world (Sere *et al*. 2005). In rice-irrigated areas, BLB can cause severe yield losses of 20% and 70% due to varietal susceptibility, growth stage of crop, geographic situation and climatic conditions (Wonni *et al*. 2016). Generally, BLB disease is more prevalent in some Asian countries during monsoon season. Usually plants are affected at the later stage of tillering, reducing rice yield from 10 to 20% (Shazia *et al*. 2009). BLB incidence has started to rise in recent years in "Kaller" rice belt of Pakistan (Khan *et al*. 2000). Disease incidence on rice was recorded high during years between 1997-2008 in Central Punjab and Upper Northern areas (Khan *et al*. 2009). Susceptibility of basmati varieties to BLB is main contributing factor of its epidemic on wide scale. Development of resistant varieties had always been target of rice breeders. Screening of plants is common practice to develop resistant varieties against a particular stress (Ashfaq *et al*. 2016). Host-plant resistance would serve as a beneficial tool in managing BLB in rice crop (Banito *et al.* 2012). Host-plant resistance should be practiced in combination with other integrated practices to defend crop against menace of this disease (Singh *et al*. 2015). Severity of infection has stressed scientists to develop resistant strains to reduce yield losses.

MATERIALS AND METHODS

Seed of twenty parent lines having resistant genes against BLB belonging to different geographic locations *viz.* Bangladesh, India, Philippines, Indonesia and Senegal along with four indica elite lines was obtained from International Rice Research Institute, Philippines. The lines were screened for resistance against bacterial leaf blight at Agronomic Research Station Farooqabad, Sheikhupura, Pakistan. These lines were evaluated in augmented design during kharif 2012. Nursery was sown on 20-05-2012. Thirty-day old nursery

transplanted in field at P×P and R×R distance of 20 cm. All standard agronomic practices were adopted. To create conducive environment for disease occurrence, double dose (100 kg per acre) of nitrogen was applied (Ashfaq *et al*. 2016).

Isolation of bacteria: Diseased leaf samples of rice were cut into 5-10 mm and sterilized with 70% ethanol for 10 seconds. Samples were washed twice with distilled water and left in a Petri plate dipped in distilled water for 15 minutes. Bacteria were streaked out on artificial nutrient media (Joint *et al*., 2016) with the help of sterilized wire loop. Yellow colonies appeared on streaked plates after three days incubation at 30 °C. The bacterial culture was then identified by gram's staining method (Gerhardt, 1981).

Inoculation: Aluminium wrapped flasks were used to protect inoculum from sunlight. Inoculation was carried out using clip method (Kauffman *et al.* 1973). 1- 2 cm tips of expanded leaves of each plant were clipped with scissors dipped in inoculum. Sterilized distilled water was used to inoculate control plants.

Disease scoring: Fourteen days post inoculation, the plants were surveyed for 21 days with 24 hours' time interval to record onset of disease symptoms. At 21 DAI (days after inoculation), final data was recorded. Disease incidence (%) was calculated by following formula (Ganamanickam *et al.* 1999).

$$\% \text{ Disease Incidence} = \frac{\text{Lesion Length}}{\text{Total Leaf Length}} \text{ X } 100$$

Following scale was used as standard to estimate host plant response (Sanchez *et al*. 2000).

Table 1. Score chart for Bacterial Leaf Blight infection

Infection %	Score	Host Response
0	0	Highly Resistant
1-10	1	Resistant
10-30	3	Moderately Resistant
30-50	5	Moderately Susceptible
50-75	7	Susceptible
75-100	9	Highly Susceptible

DNA Marker Analysis: At tillering, tissues from healthy leaves were harvested and kept at -80 ºC to extract DNA. CTAB method was used to extract genomic DNA (Sambrook and Russell, 2001 and Dellaporta *et al*. 1983). Extracted DNA was examined on agarose gel (Fig-I). Status of BLB resistant genes was examined through previously published DNA markers (Table-2).

Polymerase Chain Reaction (PCR): Amplification reactions were carried out in 20 µl volume using the following recipe.

Table 2. Microsatellite (SSR) markers to evaluate BLB genes

Gene	Marker	Type of Marker	Primer sequence(5'-3')	Reference
Xa4	MP 1	STS	ATCGATCGATCTTCACGAGG	Ma *et al*., 1999
	MP2		TCGTATAAAAGGCATTCGGG	
xa5	RM122 F	SSR	GAGTCGATGTAATGTCATCAGTGC	Chen *et al*., 1997
	RM122 R		GAAGGAGGTATCGCTTTGTTGGAC	
Xa7	M5 F	STS	CGATCTTACTGGCTCTGCAACTCTGT	Porter *et al*., 2003
	M5 R		GCATGTCTGTGTCGATTCGTCCGTACGA	
xa13	RGI36 F	STS/	TCCCAGAAAGCTACTACAGC	Zhang *et al*., 1996
	RG136 R	HinfI	GCAGACTCCAGTTTGACTTC	

Table-3. Volume and Concentration of Reagents.

Reagents	Conc.	Volume Used (µl)			
		Xa-4	***xa-5***	***Xa-7***	***xa-13***
PCR buffer	10 X	2	2	2	2
$MgCl_2$	25 mM	1.6	1.6	1.6	1.6
dNTPs	2.5 mM	1	2	2	2
Primer Forward	15 ng/µl	1	2	2	2.5
Primer Reverse	15 ng/µl	1	2	2	2.5
Taq DNA polymerase	5 units/µl	0.2	0.2	0.2	0.2
Template DNA	15 ng/µl	2	3	3	4
d_3H_2O	-	11.2	7.2	7.2	4.2

The products of *xa5, Xa7, xa13* were run on 1.5% agarose gel, stained with Ethidium bromide and documented by NYXTECHNIK gel documentation system.

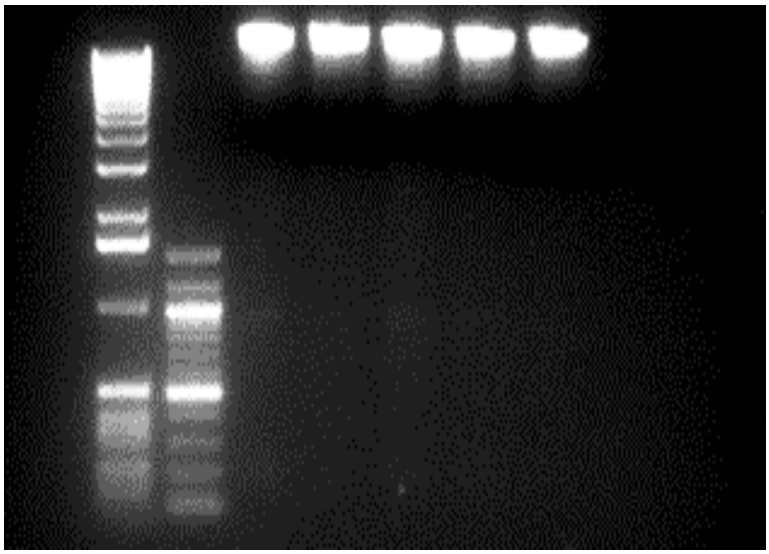
RESULTS AND DISCUSSION

Twenty donor parents for BLB and four Indica elite lines along with the resistance gene combination present in these lines were studied for their disease reaction against bacterial leaf blight. As indicated in Table-4, seven genotypes showed resistance against BLB. Among the resistant genotypes five lines including SPONJONA *(xa5, Xa7, xa13*), PURANUKNA *(Xa4, xa5, Xa7)*, TUPA-501, JAWA-14 and IR02A127 with *(Xa4, xa5, xa13)* had three genes in combination. The line IR04A421 was also among resistant category having four genes in pyramid *(Xa-4, xa-5, Xa-7, xa-13)*, whereas genotype CAS-209 possessed *Xa-4* and *xa-5* genes for BLB resistance. Twelve lines including KALIMERIKRI *(Xa-4, xa-5, xa-13*), PULUT NANGKA *(Xa-4, xa-5, Xa-7, xa-13*), ARC 11204 *(Xa-4, xa-5, Xa-7, xa-13)*, BERI *(Xa-4, xa-5, Xa-7, xa-13)*, INGRA *(Xa-4, xa-5, Xa-7, xa-13)*, LAKSMILOTA *(Xa-4, xa-5, Xa-7, xa-13)*, AUS-307 *(Xa-4, xa-5, Xa-7, xa-13)*, AUS-361 *(Xa-4, xa-5, Xa-7, xa-13)*, RATA 21-3 *(Xa-4, xa-5, Xa-7, xa-13)*, TUPA-730 *(Xa-4, xa-5, xa-13)*, TRM-6 *(Xa-4, xa-5, xa-13)* and IR05A272 *(Xa-4, xa-5, xa-13)* had moderately resistant behavior against *X. oryzae pv oryzae*. Most of the MR lines had three or four gene combination. The genotypes AUS-80, AUS-298 and DJ-2 were found moderately susceptible in their response. Two genotypes BJ-1 and IR06A150 were susceptible to bacterial leaf blight.

Multiple resistance genes were used in crop breeding strategies to develop high resistance level (Samis *et al.* 2002; Huang *et al.* 1997). In a study of soybean crop, Shi *et al.* (2009) observed that high resistance was observed by three resistance genes (Rsv1, Rsv3, and Rsv4) against soybean mosaic virus. Wider resistance level was observed in combination of 4 resistance genes (*Xa4, xa5, xa13, and Xa21*) against Xoo strains (Kumar *et al.* 2008), whereas, increased lesion length was observed at 21 DAI in lines having two-gene combination, while three-gene combination lines depicted no increase in lesion length. In a study, Sundaram *et al.* (2008) made similar observations by indicating that possibility of critical mass of genetic resistance may inherent in such cases. This depicts importance of combining more than two genes for long-lasting resistance against pathogens. Gene interaction may exhibit higher resistance level (Sanchez *et al.* 2000; Yoshimura *et al.* 1995). High level of resistance in combination of genes was observed in previous studies (Sundaram *et al.* 2008; Singh *et al.* 2001; Sanchez *et al.* 2000; Huang *et al.* 1997). This anomaly may be due to mutual combination of resistant genes in pyramid lines. Quantitative complementation involves high resistance level against single race by more than one gene (Sanchez *et al.* 2000). Similar studies were used to evaluate the best gene combinations showing wider resistance. These studies concluded that combination of four genes (i.e., *Xa4, xa5, xa13, Xa21)* was having durable resistance against various pathogen strains (Nayak *et al.* 2008; Shanti and Shenoy 2005).

Table 4. Rice germplasm under different reaction grades after inoculated by *Xanthomonas oryzae pv Oryzae.*

Sr. No.	Varieties/Lines	Gene Status	Disease Incidence (%)	Score
1	KALIMERIKRI	*Xa-4, xa-5, xa-13*	28.13	MR
2	PULUT NANGKA	*Xa-4, xa-5, Xa-7, xa-13*	29.27	MR
3	SOPONJONO	*xa-5, Xa-7, xa-13*	8.55	R
4	ARC 11204	*Xa-4, xa-5, Xa-7, xa-13*	21.01	MR
5	BERI	*Xa-4, xa-5, Xa-7, xa-13*	29.91	MR
6	PURA NUKNA	*Xa-4, xa-5, Xa-7*	9.43	R
7	INGRA	*Xa-4, xa-5, Xa-7, xa-13*	25.00	MR
8	LAKSMILOTA	*Xa-4, xa-5, Xa-7, xa-13*	28.85	MR
9	AUS-80	*Xa-4, xa-13*	49.17	MS
10	AUS-298	*Xa-4, xa-5, xa-13*	46.22	MS
11	AUS-307	*Xa-4, xa-5, Xa-7, xa-13*	25.85	MR
12	AUS-361	*Xa-4, xa-5, Xa-7, xa-13*	29.73	MR
13	RATA 21-3	*Xa-4, xa-5, Xa-7, xa-13*	29.66	MR
14	TUPA-501	*Xa-4, xa-5, xa-13*	9.93	R
15	TUPA-730	*Xa-4, xa-5, xa-13*	29.41	MR
16	JAWA-14	*Xa-4, xa-5, xa-13*	8.33	R
17	TKM-6	*Xa-4, xa-5, xa-13*	23.64	MR
18	DJ-2	*Xa-4, xa-13*	41.18	MS
19	CAS-209	*Xa-4, xa-5*	9.40	R
20	BJ-1	*xa-5, xa-13*	60.38	S
21	IR05A272	*Xa-4, xa-5, xa-13*	27.66	MR
22	IR02A127	*Xa-4, xa-5, xa-13*	8.63	R
23	IR04A421	*Xa-4, xa-5, Xa-7, xa-13*	9.24	R
24	IR06A150	*xa-5*	52.86	S



Ladders **Genomic DNA**

Fig. 1. Genomic DNA on Agarose gel

The study revealed that most of the combinations of genes exhibited strong resistance against Bacterial Leaf Blight. Therefore the lines having three gene pyramid and four gene pyramid could be selected further in breeding programs for incorporation of durable resistance in our local lines.

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