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Evaluation of Indigenous Rice Germplasm for Resistance to Bacterial Leaf Blight and Yield Performance

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Abstract
The present study was conducted at the University of Agriculture Peshawar, in the rice growing season of the year 2012 to investigate the genetic potential against bacterial leaf blight and to study yield potential of indigenous rice germplasm under natural condition. Twenty three indigenous rice germplasm and two commercial varieties i.e. Kashmir Basmati and IR-8 were studied. Bacterial suspension of concentration 10^8 CFU/ml was prepared from mixture of *Xanthomonas oryzae* pv. *oryzae.* Clip method of artificial inoculation was used. Out of all the tested genotypes thirteen were moderately resistant, five were moderately susceptible, six were susceptible and Kashmir Basmati showed highly susceptible response. None of the genotype was resistant to bacterial leaf blight. Among the tested genotypes Acc. 6505, 6508, 6509, 6515 6535 performed well for quantitative yield parameters along with moderately resistance response to BLB. Being highly susceptible variety Kashmir Basmati still showed good result for grain yield (0.78 kg/plot) relative to others.

Keywords: *Xanthomonas oryzae*, germplasm, indigenous, leaf blight, rice

1. Introduction
Rice (*Oryzae sativa* L.) belongs to grass family Poaceace. Tropical as well as subtropical regions are best suited for its cultivation [1]. Rice is the best source of food and provides enough energy (calories) to the consumers all over the world especially in Asian countries like Pakistan, India, China, Bangladesh and Korea. In Pakistan it is considered the second important food after wheat. Rice has a key role in the total export of Pakistan that account for 1.27 billion US dollar annually [2]. Basmati rice produced and exported by Pakistan is famous worldwide for its aroma and is prevalent in international market having greater price. Pakistan shares 14 % in the total export of rice to the world market [3].

In order to further improve the export volume and to reach the country demand, rice production has to be enhanced. However it is clear that in Pakistan rice yield per unit area is lower than most of the rice growing countries. Unfortunately this valuable economic crop is facing a number of biotic and abiotic stresses. Rice diseases caused by fungi, bacteria, viruses, nematodes have negative effect on yield performance. Among them bacterial leaf blight caused by a bacterium *Xanthomonas oryzae* pv. *oryzae* is the most devastating [4].

In Pakistan the disease (BLB) of rice was 1st reported in Kala Shah Kaku in Punjab province [5]. Later on it was reported in rice varieties i.e. in Basmati 198, IR-6, and Palman at Kala Shah Kaku Agriculture Research Institute [6]. In 1986 the disease incidence was recorded in all the provinces of Pakistan except Baluchistan (Sindh 10-15 %, Punjab 15-20%, and Khyber Pakhtunkhwa 20-25%). In the farmer fields of Dhing and Fakandabad the disease incidence was 40-45% while it was recorded as high as 70-80% or even 90-95% in nearby areas. In a survey conducted in different districts of Punjab province i.e. in the districts of Gujranwala, Hafizabad, Sheikhupura and Gujrat during the rice growing season of the year 1999 the disease incidence was recorded as 15%, 25%, 28% and 29% respectively [7].

Presently, all commercial varieties are susceptible to BLB, however wild rice land races and indigenous germplasm has shown encouraging results. Keeping in view as a source of variation and resistant genes present study was conducted to study resistance of indigenous rice germplasm against bacterial leaf blight and yield performance.

2. Materials and Methods

The study was conducted during the year 2012.

2.1. Seed Source and Nursery Raising

Seeds of Indigenous rice germplasm were obtained from National Agricultural Research Center (NARC), Islamabad and nursery was raised in the first week of June, 2012 in the experimental field of the Department of Plant Breeding and Genetics at New Development farm, University of Agriculture, Peshawar. Seeds were sown manually on well puddled seed bed and watered instantly. For each genotype separate seed bed of 1×1 meter was prepared. To test germination, genotypes were checked periodically and other agronomic requirements such as irrigation, fertilization and weeding were fulfilled on time. Forty days old rice seedlings were uprooted carefully early in the morning and then transplanted into well puddle field already prepared. The genotypes were planted in Half Square Lattice design replicated three times with row length of two meters and plant to plant and row to row distance of 20 cm and 30 cm respectively.

2.2. Inoculum Production and inoculation of Plants

Bacterial suspension was prepared from pure culture of *X. oryzae* pv. *oryzae* obtained from Plant Pathology Laboratory, University of Agricultural Peshawar. The bacterium was grown overnight on Luria Bertani (LB) growth medium at 27 ^{0}C. The growth was scrapped off the plates, resuspended in 0.85% saline solution and adjusted to a concentration of 10^8CFU/ml having OD_{600}= 2.14 A using spectrophotometer. The suspension was used as inoculum. Plants were inoculated four to five days before panicle emergence. The clip method was used for inoculation process. Scissor was dipped in the inoculum (bacterial suspension) and one/fourth of top three to four leaves was cutted through it. Inoculated plants were regularly observed for symptoms appearance and bacterium was identified through morphological characterization (colony color, shape and texture) and different biochemical tests. The data was recorded after three weeks of inoculation.

Disease Scoring

On the basis of mean lesion length, the genotypes were grouped into different categories of resistance and susceptibility using standard International Rice Research Institute (IRRI) procedure [8].

Table 1: IRRI standard used for adult plant response against Bacterial Leaf Blight (Anonymous, 1996)

Disease rating	Lesion length (%)	Category
1	1-5	Resistant
3	6-12	Moderately Resistant
5	13-25	Moderately Susceptible
7	26-50	Susceptible
9	51-100	Highly susceptible

2.3. Identification of Bacterium

In order to confirm the disease, certain cultural and biochemical tests were carried out in Plant Pathology research laboratory in the month of September-October 2012. Cultural characteristics (colony shape, colony color, colony texture, margin and elevation etc.) were studied after the incubation of pure culture of *X. oryzae* pv. *oryzae* on LB growth medium for 48 hours at 27 ^{0}C. Certain biochemical tests like gram staining, KOH (3%), starch hydrolysis and Tween 80 hydrolysis were also conducted according to the standard procedures [9, 10, 11, 12, 13].

2.4. Yield Parameters

Data on various quantitative parameters was taken time to time using the standard procedure. Culm length was measured (cm) from the ground level to the base of panicle after heading. Panicle length was measured (cm) from the base of the panicle to the tip. Data on other panicle traits such as primary branches panicle $^{-1}$, secondary branches panicle $^{-1}$ and number of spikelet panicle $^{-1}$ were also taken. Data on the various grain traits such as grain length, grain width, 1000 grain weight, biological yield, grain yield, were also recorded.

2.5. Data Analysis

The data collected were analyzed using analysis of variance technique with the help of statistical package MSTATC [14]. Further, least significant difference (LSD) test was applied to test the significance of treatment difference.

3. Results and Discussion

3.1. Identification of *Xanthomonas oryzae* pv. *Oryzae*

The bacterium was isolated from infected leaves of rice plants showing typical symptoms of bacterial blight and cultured on Luria Bertani (LB) medium. The pathogen was identified through morphological and biochemical tests. On LB medium the colony was looking yellow, round and dome shaped, slippery and raised. Biochemical tests results were; bacterium was gram negative, Lugol's iodine formed clear zone around individual colony and a milky white precipitate was developed around the colonies in Tween-80 hydrolysis test.

3.2. Disease Severity and Quantitative Yield Parameters

Highly significant difference was observed for disease severity and quantitative yield parameters studied. Acc. 006509 showed minimum disease severity (5.76%) while Kashmir basmati showed maximum disease severity (55.33%) presented highly susceptible response. Average culm length recorded was 109.266 with a minimum level of 58.000 cm (Kashmir Basmati) maximum level of 131.333 (accession 006527). Lower and higher range for panicle length was 25.267 and 33.583 cm for Kashmir Basmati and acc.0065025 respectively with overall average of 29.618. Minimum number of primary branches panicle^{-1} (8.000) was observed in commercial genotype IR-8 while maximum number of primary branches panicle^{-1} (12.667) was observed in 006509. Commercial variety IR-8 showed minimum number of secondary branches panicle^{-1} and acc.006517 showed maximum number of secondary branches panicle$^{-1.}$ Minimum number of Spikelets panicle^{-1} (113.000) was studied in acc. 006505 and maximum number of spike lets panicle $^{-1}$ (246.333) was observed in Kashmir Basmati. The average grain length for all tested genotypes was 8.523mm. The minimum grain length (8.303 mm) was observed in acc. 006531 while acc. 006515 showed maximum grain length (9.527 mm). The maximum grain width was recorded in Kashmir basmati (8.217 mm) while the minimum grain width was recorded in acc. 006523 (2.017 mm). The overall average for grain width was observed as 2.402 mm. Average 1000 grain weight was recorded as 24.837 g. The minimum level (20.543 g) was observed in acc. 006512 and higher level (44.463 g) was observed in acc. 006515. Acc. 006531 showed minimum value of biological yield per plot i.e. 1.433 kg while acc. 006509 showed maximum level of biological yield per plot i.e. 3.100 kg. Overall average was 2.090 kg. The difference is highly significant ($p<0.01$) having a minimum value of 0.323 kg for acc. 006505 and higher value of 0.783 kg for Kashmir basmati for grain yield.

Table 2: Response of rice genotypes against bacterial leaf blight

Category	Disease rating	Genotypes
Resistant	1	
Moderately Resistant	3	Acc. 6505, 6508, 6509, 6512, 6514, 6515, 6516, 6517, 6519, 6521, 6523, 6529, 6536
Moderately Susceptible	5	Acc. 6507, 6520, 6525, 6527, 6535
Susceptible	7	Acc. 6506, 6522, 6526, 6531, 6537, IR-8
Highly Susceptible	9	Kashmir Basmati

Table 3: Mean Data for Disease Severity (DS %), Culm Length and Panicle parameters of 25 rice genotypes

S.NO	Genotype	DS (%)	CL (cm)	PL (cm)	PBP	SBP	SKP
01	Acc.006505	9.200	101.667	33.583	11.000	25.333	113.000
02	Acc.006506	32.733	108.667	32.290	10.333	29.000	163.000
03	Acc.006507	19.767	99.000	29.313	12.000	36.333	172.333
04	Acc.006508	7.553	107.333	29.207	12.000	31.667	217.000
05	Acc.006509	5.767	111.333	31.833	12.667	29.000	196.000
06	Acc.006512	8.000	112.667	31.817	10.000	29.000	183.000
07	Acc.006514	10.567	106.333	29.383	11.000	28.667	171.667
08	Acc.006515	10.250	117.333	31.123	9.000	28.000	198.333
09	Acc.006516	6.573	119.000	30.687	10.667	40.000	205.333
10	Acc.006517	9.527	120.667	31.280	10.667	45.667	195.333
11	Acc.006519	11.573	128.667	28.987	9.333	31.667	201.333
12	Acc.006520	14.523	113.000	31.267	9.000	28.333	183.667
13	Acc.006521	10.233	118.667	30.247	10.000	28.000	158.333
14	Acc.006522	38.367	101.667	27.800	9.667	34.000	179.667
15	Acc.006523	7.667	111.667	29.260	11.333	29.667	159.000
16	Acc.006525	24.233	97.667	27.981	9.333	20.667	140.000
17	Acc.006526	45.157	125.333	28.567	10.333	34.000	182.667
18	Acc.006527	20.333	131.333	31.500	10.667	32.667	206.333
19	Acc.006529	10.283	121.000	29.860	10.000	30.667	174.667
20	Acc.006531	44.000	105.000	27.760	10.667	21.667	176.667
21	Acc.006535	21.900	110.333	28.553	10.000	31.333	162.667
22	Acc.006536	8.033	105.000	27.253	11.000	27.000	179.667
23	Acc.006537	31.333	105.333	29.867	11.000	27.667	123.667
24	Kashmir Basmati	55.33	58.000	25.267	8.667	25.000	246.333
25	IR-8	40.667	95.000	25.780	8.000	16.000	230.000
	Average	20.144	109.266	29.618	10.333	29.640	180.786
	LSD (0.01)	1.4742	4.8912	1.0742	0.8652	1.7120	19.5034

DS Diseases severity CL Culm length
PL Panicle length PBP Primary Branches panicle-1
SBP Secondary Branches panicle-1 SKP Number of spikiest panicle-1

Table 4: Mean Data for yield parameters of 25 rice genotypes

S.NO	Genotype	GL (mm)	GW (mm)	TGW (g)	BY (kg)	GY (kg)
01	Acc.006505	9.300	2.407	29.0000	2.333	0.323
02	Acc.006506	8.967	2.400	25.950	1.733	0.460
03	Acc.006507	9.017	2.467	25.317	2.167	0.653
04	Acc.006508	9.233	2.893	27.207	2.167	0.730
05	Acc.006509	8.900	2.337	22.150	3.100	0.560
06	Acc.006512	9.073	2.400	20.543	1.700	0.390
07	Acc.006514	8.840	2.400	22.467	1.633	0.493
08	Acc.006515	9.527	2.170	44.463	2.367	0.603
09	Acc.006516	8.520	2.233	27.280	1.900	0.527
10	Acc.006517	8.060	2.247	23.253	2.233	0.457
11	Acc.006519	8.500	2.313	22.297	1.667	0.347
12	Acc.006520	9.200	2.313	21.527	1.567	0.430
13	Acc.006521	8.467	2.483	21.250	2.067	0.491
14	Acc.006522	8.040	2.163	24.790	2.200	0.367
15	Acc.006523	8.800	2.017	21.853	2.400	0.580
16	Acc.006525	8.583	2.763	25.187	2.100	0.607
17	Acc.006526	9.317	2.627	22.347	2.500	0.591
18	Acc.006527	9.283	2.300	21.950	2.267	0.510
19	Acc.006529	8.513	2.377	21.847	2.167	0.481

20	Acc.006531	8.303	2.293	20.067	1.433	0.387
21	Acc.006535	9.077	2.717	27.847	2.533	0.773
22	Acc.006536	9.040	2.073	26.063	2.200	0.597
23	Acc.006537	9.020	2.333	20.827	2.600	0.580
24	Kashmir Basmati	9.167	2.817	28.683	2.433	0.783
25	IR-8	8.330	2.520	26.767	0.800	0.313
	Average	8.523	2.402	24.837	2.090	0.521
	LSD (0.01)	0.3313	0.1234	0.7774	0.3300	0.0877

GL Grain length GW Grain width
TGW 1000 grain weight BY Biological yield
GY Grain yield

Table 4: Mean squares for Disease severity and yield traits of 25 rice genotypes

SOV	DF	DS	CL	PL	PBP	SBP	SKP	GL	GW	TGW	BY	GY
Replications	2	10.218	53.373	8.374	0.333	6.880	74.094	0.034	0.031	0.671	0.071	0.004
Treatments	24	651.0**	615.0**	12.1**	3.6**	107.3**	2728.8**	0.4**	0.1**	22.6**	0.6**	0.1**
Blocks	12	0.318	15.090	0.380	0.167	1.197	181.993	0.100	0.009	0.404	0.037	0.001
Error	36	0.969	7.783	0.444	0.315	1.034	129.367	0.034	0.005	0.195	0.041	0.004

DS Diseases severity CL Culm length PL Panicle length
PBP Primary Branches panicle-1 SBP Secondary Branches panicle-1 SKP Number of spikiest panicle-1
GL Grain length GW Grain width TGW 1000 grain weight
BY Biological yield GY Grain yield

* = Significant at 95% (P value = 0.05-0.01)
** = Significant at 99 % (P<0.01)
NS = Non Significant (P>0.05)

Among twenty three indigenous accessions, thirteen showed moderately resistance response which showed that these might have certain resistant genes against the disease. These results are compatible with the findings of Kaushal *et al.* (1998), who screened 167 wild rice accessions in the same environment in which nine accessions were identified with resistance against bacterial blight [15]. Pha and Lang (2004) screened 166 local accessions and 25 lines of hybrid rice against 10 international bacterial races. Some of the local accessions showed resistant response against all the races. The overall results for the disease severity of the indigenous accessions showed that minimum level of disease occurrence might be due to low rainfall that year [16]. In our study a total of ten indigenous accessions and two commercially cultivated varieties i.e. Kashmir Basmati and IR-8 gave moderately susceptible to highly susceptible response. The susceptible response of this germplasm might be due to absence of resistance genes against BLB. These findings are in line with Shah *et al.* (2009) who tested 14 wild rice accessions and three cultivated varieties against BLB. Among these tested genotypes, 11 accessions and all the cultivated varieties were found susceptible to the disease [17]. Similarly Akhtar *et al.* (2011) tested 47 rice accessions and two commercial varieties against 25 isolates of *Xoo.* Among them most of the accessions and the tested commercial varieties Basmati-385 and KS-282 were found susceptible to BLB [18].

Highly significant differences were observed among the rice genotypes for panicle traits. This difference among the germplasm might be due to difference response against BLB and other genetic factors. Roy and Panwar (1993) previously evaluated ninety nine rice genotypes and observed significant variation in spikelets panicle^{-1}, grains panicle^{-1} and panicle plant^{-1} [19]. Highly significant difference was also observed among rice genotypes for grain yield and grain weight which was similar with the findings of Sumi and Katyama (1994) [20]. The reason of variation for grain traits might be due to resistance and susceptibility against BLB and might be due to yield potential of different germplasm. The rice genotypes used in the study also showed significant diversity for primary branches panicle^{-1}, secondary branches panicle^{-1}, grain length and grain width as well. Different response to BLB and genetic potential of indigenous germplasm seems to be the most important reason for variability among these traits. Previously Nabeela *et al.* (2004) and Bajracharya *et al.* (2006) screened rice genotypes and found similar results [21, 22].

4. Conclusion

It is concluded that indigenous rice germplasm has the potential to resist bacterial leaf blight and showed better performance for quantitative yield parameters as well. Based on the results, Acc. 006505, 006508, 006509 and 006515 were more prominent in yield parameters along with moderately resistance response to BLB.

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