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G × E Analysis of Rice Germplasm and NILs Having Bacterial Leaf Blight (BLB) Resistant Genes against Local Isolates of *Xanthomonas oryzae* at Diverse Agro-Ecological Zones

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Abstract

Rice is food for more than half of the world population and the most consumable cereal in most of the countries. Pakistan is the fifth largest exporter of rice. However, Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* is the most devastating and serious threat to rice production in many countries of the world including Pakistan. To combat this disease, innate genetic resistance of the plant plays vital role along with being environmentally friendly and economical. In this study, thirty-one (31) Near Isogenic Lines (NILs) having *Xa*4, *xa*5, *Xa*7, *xa*13 and *Xa*21 reported BLB tolerant genes and 34 locally developed rice lines were investigated under natural field conditions at three agro-ecologically different locations with highest disease occurrence records (BLB hotspots) viz., Sheikhupura, Hafizabad and Gujranwala, Punjab, Pakistan in order to assess their respective genetic resistance and G × E interactions against the disease. Thirty-one (31) lines were categorized under resistant cluster, twenty-eight (28) were moderately resistant, six (6) were moderately susceptible and one (susceptible check) was in susceptible category. Grouping of different lines/varieties under same cluster shows their significantly similar response against BLB disease in corresponding environment. Among the studied NILs, only one line showed polymorphism for all five resistant genes, two lines had four; seven lines had three genes, seven lines showed di-genic while five lines showed mono-genic polymorphism. These resistant lines with multiple-genes for BLB resistance can be evolved as a new BLB resistant variety and also be utilized as donor parent in breeding programs for developing new cultivars with horizontal resistance against more than one target pathotypes and environments. *Xa*4 and *xa*13

were found to deliver significant resistance against the local pathotypes in studied germplasm and NILs.

Keywords

Rice, *Oryza sativa* L., Genotypic Environment Interaction, Bacterial Leaf Blight, *Xanthomonas oryzae* pv. *oryzae*

1. Introduction

Rice is one of the most important staple food of over half of the world's population and about 90% of the world rice is grown and consumed in Asian continent [1]. Pakistan is the fifth largest rice exporter comprising mainly of basmati rice. Despite rice importance in Pakistan and all over the world, this crop is vulnerable to many biotic and abiotic stresses chiefly because of the absence of resistant/tolerant genes. Among the biotic stresses, bacterial leaf blight (BLB) disease is highly threatening all over the rice world. This disease is caused by rod-shaped bacterium *Xanthomonas oryzae* pv. *oryzae* [2]. In South Asia, BLB is the most serious disease in irrigated and lowland fields [3] because of conducive to environment and following repeated cultivation [4]. Management of many improved and high yielding varieties with high nitrogen levels and close spacing expose the crop defenseless against the disease [5]. This disease is the most devastating and serious threat to rice production. High humidity, wind and rainfall favor the development and spread of this disease. Symptoms of water-soaked, yellowish stripes along the leaf margins can be observed at all stages of rice plants leading to yield loss typically ranging from 20% - 30% but in severe cases as high as 80% reduction [3] [6].

High spread of disease in favorable conditions make BLB more difficult to manage through conventional control methods such as chemical sprays. To combat this disease, innate genetic resistance of the plant plays vital role along with being environmentally friendly and economical. Development of resistant cultivars against BLB force that the bacterium to modify itself hence breaking the host resistance. Therefore, it is very critical to continually explore new resistance sources against the ever-changing bacterial pathogen [7]. As chemical control of the disease is not much effective, and also causing environmental hazards, the deployment of resistant varieties has been considered as the most effective way to control the disease [8]. Using resistant varieties not only reduce the cost of chemicals being used for the management of the disease but also an environmentally friendly approach. In the case of BLB resistance, cultivated rice and the wild relatives have been identified with 35 BLB resistance genes [6] [8] [9].

Cluster analysis forms different groups or clusters on the basis of similar response of lines/varieties but it does not necessarily mean that all entries in a

group have same response or origin. However, this analysis provides helpful insight that could be used in the development of resistant varieties. Clusters can identify sources of new resistance along with high yield factor to overcome the altering pathogen and environment, and also study the mechanism of resistance.

Therefore, the present study was carried out with the objective to evaluate 66 local and exotic rice lines/varieties and various Near Isogenic Lines (NILs) having known resistant genes against BLB. Three hot spot sites with higher disease incidence recorded in past years were selected for the screening under natural conditions.

2. Materials & Methods

2.1. Experimental Sites and Rice Lines/Varieties

Three hot spot sites of bacterial leaf blight disease with naturally higher disease incidence were selected for the experiment. These sites were in Gujranwala, Sheikhupura and Hafizabad, Punjab, Pakistan. In the experiment, sixty-five (65) rice lines/varieties including 34 locally developed rice lines, 31 NILs and one commercial variety Basmati Super as susceptible check was cultivated at farmer's fields in RCBD design.

2.2. Monitoring and Evaluation

Seeds were sown on dry land raised beds (1ft. × 1 ft. for each entry with 1 ft. interval). Thin layer of fine crushed decayed farmyard manure (FYM) was spread over them and then watered with a hand sprinkler thrice a day. After the 4th day of nursery sprouting, field was flooded for the first time. Thirty-five (35) days old plants were transplanted having 9 inches plant to plant and row to row distance. All the recommended agronomic practices followed throughout the season from sowing to harvest of the crop. The disease incidence was recorded by using scale given as Table 1.

2.3. Statistical Analysis

The replication means of each genotype response to disease were subjected to statistical analysis. Cluster Analysis (CA) and Principal Component Analysis (PCA) were performed for the genotypes grouping and to identify the response toward the prevalent BLB pathotypes at three different locations, respectively using Minitab software.

Table 1. Scale for BLB (for field test, lesion area).

Score	Percent Disease (%)	Host Response
1	1 - 5	Highly Resistant
3	6 - 12	Resistant
5	13 - 25	Moderately Resistant
7	26 - 50	Moderately Susceptible
9	51 - 100	Susceptible

2.4. Plant Materials

Sixty-seven (67) genotypes comprising of local and exotic origin, thirty near isogenic lines (NILs) as positive resistant gene checks and IR24 & Super Basmati as negative gene check were in this study. For this purpose, leaf samples were taken from rice seedling grown in the growth chamber of MAS lab of Rice Research Institute, Kala Shah Kaku, and Pakistan used for isolating genomic DNA.

2.5. Genomic DNA Extraction

Rice seeds of all the genotypes were grown in germination jars under controlled environment (temperature 35˚C and humidity 80% - 85%) of growth chamber. Young leaves were taken for DNA extraction at seedling stage using Miniprep protocol. Few leaves (10 - 15 days old) were ground in liquid nitrogen and then added 800 ul extraction buffers. Prepare 100 ml of DNA extraction buffer, 1 M Tris HCI (10 ml), 0.25 M EDTA (20 ml), NaCl (2.9 g), sodium bisulphate (0.38 g), 20% SDS (6.25 ml) and deionizedH_2O (63.75 ml) were used. The samples were incubated for 20 minutes at 65˚C in water bath. Then equal volume of Chloroform: Isoamyl alcohol (24:1) was added. Centrifugation was carried out it for 8 minutes at 11,000 rpm. In a new tube (1.5 ml), 500 ul of supernatant was taken and 1000 ul of chilled isopropanol (−20˚C) added. The samples were now centrifuged at 10,000 rpm for 10 minutes. Aqueous solution was discarded carefully so that pellet does not get damaged or lost. Pellet was then washed with 1000 ul of 70% ethanol and re-suspended in 100 µl of TrisEDTA (TE) buffer.

To check the DNA quality of isolated genomic DNA, 3 ul of stock DNA mixed with 2 ul of loading dye was loaded in 0.8% agarose gel prepared in 1X TAE buffer (pH 8.5). After 35 minutes run at 80 Volts, the genomic DNA concentration was observed using gel documentation system. Lambda DNA was used for estimation of DNA concentration of stock solution. Finally, the working dilutions were prepared with 30 - 50 ng/ul DNA concentration and stored at 4˚C.

2.6. PCR Amplification

DNA fragment carrying *Xa*4, x*a*5 and *Xa*21 was amplified using tightly linked and co-segregated primers "MP1, RM122, and pTA 248" respectively (**Table 2**). The sequences (forward & reverse) of these marker primers are given in **Table 2**. A reaction volume of 10 µl was used for PCR amplification, containing 2 µl DNA (30 ng/µl), 0.75 µl of forward Primer and 0.75 µl of reverse primer (10 p moles/µl), 1.5 µl of double distilled water and 5 µl of green master mix (Thermo, USA). Amplification was performed in a programmed thermocycler (Kyratech, Australia) with initial denaturation at 95˚C for 5 min, followed by 35 denaturation cycles at 95˚C for 1min, annealing at 54˚C for 1 min, elongation at 72˚C for 2 min, and final extension at 72˚C for 5 min. Amplified products of *Xa*4, *xa*5, and *Xa*21 were subjected to electrophoresis in 3.5% agarose gel run in 0.5X TAE buffer. Ethidium bromide (10 µg/mL) was used to stain the gel, followed by gel documentation(UV Tech, USA) and identified for the presence (++), absence (−−) of BLB gene linked DNA fragment [10].

Table 2. Detailed information of markers and primer sequences for each studied gene.

Target Gene	Marker	Primer sequences	Annealing Temp.	Resistant Band (bp)	Susceptible Band (bp)	References
*Xa*4	MP1 + MP2	F-5'-ATCGATCGATCTTCACGAGG-3' R-5'-dTGCTATAAAAGGCATTCGGG-3'	55˚C	150	120	Ma Bo Jun *et al.*, 1992
*xa*5	RM122 (F + R)	F-5'-GAGTCGATGTAATGTCATCAGTGC-3' R-5'-GAAGGAGGTATCGCTTTGTTGGAC-3'	56˚C	240	230	Chen *et al.*, 1997
*Xa*7	M5 (F + R)	F-5'-CGATCTTACTGGCTCTGCAACTCTGT-3' R-5'-GCATGTCTGTGTCGATTCGTCCGTACGA-3'	55˚C	294,320	1170	Porter *et al.*, 2003
*xa*13	*xa*13 (F + R)	F-5'-CCTGATATGTGAGGTAGT-3' R-5'-GAGAAAGGCTTAAGTGC-3'	57˚C	560	250	Chu *et al.*, 2006
*Xa*21	pTA248 (F + R)	F-5'-AGACGCGGAAGGGTGGTTCCCGGA-3' R-5'-AGACGCGGTAATCGAAGATGAAA-3'	56˚C	925, 1040	730	Ronald *et al.*, 1992

2.7. Data Analysis

The amplified fragments of all the rice genotypes were scored by comparing with respective resistance (IRBB near-isogenic lines) and susceptible (IR24) bands. The data were scored using "++/−−" signs for presence/absence of target gene respectively [11] as depicted in Table 3.

3. Results and Discussions

Disease scoring was done by following the Standard Evaluation System (SES) of Rice developed by International Rice Research Institute (IRRI), Philippines.

Results of genotypic and environmental interactions among studied lines, genes and three hotspots were comprehended using Cluster Analysis (CA) and Genotypic and Environment International (GEI) analysis which divided the lines/varieties in four clusters or groups named as resistant, moderately resistant, moderately susceptible, and susceptible depending on disease scoring over environments/hotspots. Thirty-one (31) lines were categorized under resistant cluster, twenty-eight (28) were moderately resistant, six (6) were moderately susceptible and one (susceptible check) was in susceptible category (Figure 1 and Table 4). Grouping of different lines/varieties under the same cluster shows their significantly similar response against BLB disease in corresponding environment.

3.1. Genotypic Evaluation

Among the studied NILs, only one line showed polymorphism for all five resistant genes, two lines had four; seven lines had three genes, seven lines showed di-genic while five lines showed mono-genic polymorphism. These lines with multiple-genes for BLB resistance can be evolved as a new BLB resistant variety and also be utilized as donor parent in breeding programs for developing new cultivars with horizontal resistance against more than one target pathotypes and environments.

Sixty-seven (67) genotypes including local cultivars, IRBB lines carrying BLB

Table 3. *Xa* genes status of IRBB lines: Presence (++); Absence (−−).

Sr. No	DESIGNATION	*Xa*4	*xa*5	*Xa*7	*xa*13	*Xa*21
1	IRBB1	−−	−−	−−	−−	−−
2	IRBB3	−−	−−	−−	−−	−−
3	IRBB4	++	−−	−−	−−	−−
4	IRBB5	−−	++	−−	−−	−−
5	IRBB7	−−	−−	++	−−	−−
6	IRBB8	−−	−−	−−	−−	−−
7	IRBB10	−−	−−	−−	−−	−−
8	IRBB11	−−	−−	−−	−−	−−
9	IRBB13	−−	−−	−−	++	−−
10	IRBB14	−−	−−	−−	−−	−−
11	IRBB21	−−	−−	−−	−−	++
12	IRBB23	−−	−−	−−	−−	−−
13	IRBB50	++	++	−−	−−	−−
14	IRBB51	++	−−	−−	++	−−
15	IRBB52	++	−−	−−	−−	++
16	IRBB53	−−	++	−−	++	−−
17	IRBB54	−−	++	−−	−−	++
18	IRBB55	−−	−−	−−	++	++
19	IRBB56	++	++	−−	++	−−
20	IRBB57	++	++	−−	−−	++
21	IRBB58	++	−−	−−	++	++
22	IRBB59	−−	++	−−	++	++
23	IRBB60	++	++	−−	++	++
24	IRBB61	++	++	++	−−	−−
25	IRBB62	++	−−	++	−−	++
26	IRBB63	−−	++	++	++	−−
27	IRBB64	++	++	++	−−	++
28	IRBB65	++	−−	++	++	++
29	IRBB66	++	++	++	++	++
30	IRBB67	++	−−	++	−−	−−

resistant genes as single/in combinations; and F1 crosses along with resistant and susceptible controls were screened against 5 BLB resistance genes under epiphytotic conditions of hotspot locations during the year. Results depicted in Table 3 show the gene status of IRBB lines being used for studying the interaction of single or multiple genes for BLB disease resistance in three different environmental conditions. Forward and reverse sequences of the primers used for

Table 4. Grouping of rice lines/varieties in different clusters based on their response to disease.

Cluster No.	Score	Host Response	Lines/Varieties
1	3	Resistant (Cluster I)	00521/IRBB5-16, 00521/IRBB5-17, Super Bas./IRBB-21//Super Bas.-19, Bas.2000/IRBB-58//IRBB-58-20, Super Bas./IRBB-21//Super Bas.-23, RRI-7-1, RRI-7-2, RRI-7-3, RRI-7-5, RRI-7-6, RRI-7-8, RRI-7-12, RRI-7-13, PKBB 1501, PKBB 1502, PKBB 1503, PKBB 1506, IRBB1, IRBB3, IRBB5, IRBB11, IRBB14, IRBB51, IRBB52, IRBB53, IRBB55, IRBB58, IRBB66
2	5	Moderately Resistant (Cluster II)	00515/IRBB13, 00515/IRBB21-13, 00515/IRBB21-14, 00515/IRBB59, 00515/IRBB7, Bas.2000/IRBB-58//IRBB-58-21, Bas.2000/IRBB-58//IRBB-58-22, RRI-7-10, RRI-7-11, PKBB 1504, PKBB 1505, PKBB 1507, PKBB 1508, PKBB 1509, PKBB 1510, IRBB4, IRBB8, IRBB10, IRBB13, IRBB23, IRBB50, IRBB54, IRBB56, IRBB57, IRBB59, IRBB60, IRBB61, IRBB62, IRBB63, IRBB65, IRBB67
3	7	Moderately Susceptible (Cluster III)	Bas.2000/IRBB-58//IRBB-58-20, RRI-7-4, RRI-7-7, RRI-7-9, IRBB64, RRI-7-9, PKBB 1507, IRBB23, IRBB57, PKBB 1508, IRBB8, PKBB 1509, PKBB 1510
4	9	Susceptible (Cluster IV)	IRBB7, IR24 & Check (Super Basmati)

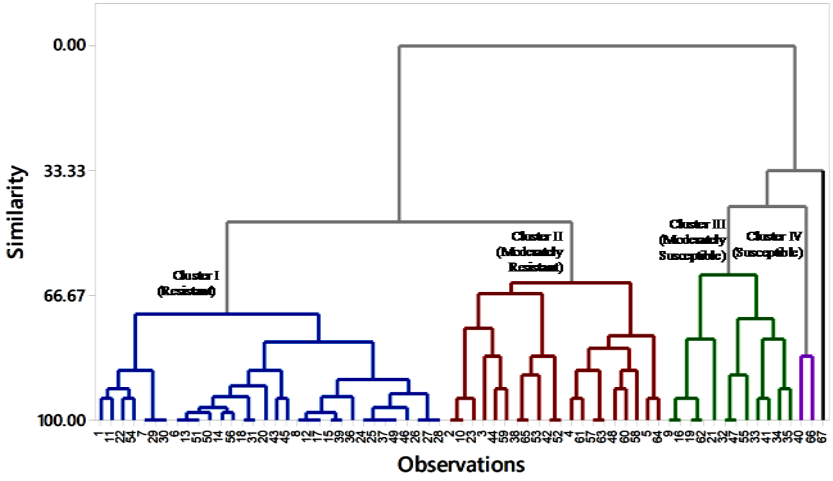

Figure 1. Dendrogram showing clusters of studied genotypes on the basis of response against BLB disease at three locations; entry 67 is Super Basmati as susceptible check

the confirmation of respective BLB resistance genes is also given in Table 2. All the studied genotypes were screened for the presence or absence of five BLB resistance genes using PCR based markers respectively linked to these genes. The PCR results for the BLB resistant genes estimated by visualizing amplicons in different base pairs of positive fragments respectively. Results of the gene status of IRBB lines clearly showed that the primer pairs used in this study can be effectively used for the confirmation of the BLB resist resistance genes. Data depicted in Table 3 to clearly confirm that IRBB 1, 3, 8, 10, 11, 14 and 23 did not show polymorphism indicating that these lines do not possess the target genes. During the polymorphic survey out of total 30 studied near isogenic lines, only one line showed polymorphism for all five resistance genes, 2 lines were tri-genic, 7 lines showed digenic while 5 lines showed monogenic polymorphism.

As depicted in Table 5, BLB disease incidence (DI) ranged from 2.67% - 46.67% for varieties and NILs at three locations on average basis, where variety

Table 5. Genotypes with their disease incidence (%) record at studied three locations.

	Genotypes name	Hafizabad	Kala Shah Kaku	Gujranwala	Average (genotypes)
1	00515/IRBB13	20	5	15	13.33
2	00515/IRBB21	3	30	20	17.67
3	00515/IRBB21	10	35	30	25.00
4	00515/IRBB59	20	20	10	16.67
5	00521/IRBB 5	10	20	10	13.33
6	00521/IRBB 5	3	5	5	4.33
7	00515/IRBB 7	20	10	5	11.67
8	Super Bas./IRBB-21// Super Bas.	10	3	10	7.67
9	Bas.2000/IRBB-58//IRBB-58	40	30	30	33.33
10	Bas.2000/IRBB-58//IRBB-58	3	30	20	17.67
11	Bas.2000/IRBB-58//IRBB-58	20	5	10	11.67
12	Super Bas./IRBB-21//Super Bas.	10	3	10	7.67
13	RRI-7-1	3	5	5	4.33
14	RRI-7-2	3	3	3	3.00
15	RRI-7-3	10	5	10	8.33
16	RRI-7-4	40	30	30	33.33
17	RRI-7-5	10	3	10	7.67
18	RRI-7-6	3	0	5	2.67
19	RRI-7-7	40	35	30	35.00
20	RRI-7-8	10	3	5	6.00
21	RRI-7-9	40	20	40	33.33
22	RRI-7-10	20	10	15	15.00
23	RRI-7-11	3	25	20	16.00
24	RRI-7-12	3	5	10	6.00
25	RRI-7-13	3	5	10	6.00
26	PKBB 1501	3	5	15	7.67
27	PKBB 1502	3	5	15	7.67
28	PKBB 1503	3	5	15	7.67
29	PKBB 1504	20	10	5	11.67
30	PKBB 1505	20	10	5	11.67
31	PKBB 1506	3	0	5	2.67
32	PKBB 1507	40	30	10	26.67
33	PKBB 1508	40	10	15	21.67
34	PKBB 1509	40	15	20	25.00
35	PKBB 1510	40	20	25	28.33
36	IRBB 1	10	5	15	10.00

Continued

37	IRBB3	3	5	10	6.00
38	IRBB4	3	10	30	14.33
39	IRBB5	10	5	10	8.33
40	IRBB7	70	30	20	40.00
41	IRBB8	40	10	10	20.00
42	IRBB 10	3	20	30	17.67
43	IRBB 11	3	10	0	4.33
44	IRBB13	20	30	25	25.00
45	IRBB 14	3	10	5	6.00
46	IRBB 21	3	8	10	7.00
47	IRBB 23	40	30	10	26.67
48	IRBB 50	20	20	25	21.67
49	IRBB 51	3	5	10	6.00
50	IRBB 52	3	3	5	3.67
51	IRBB 53	3	5	5	4.33
52	IRBB 54	3	20	30	17.67
53	IRBB 55	3	10	20	11.00
54	IRBB 56	20	10	10	13.33
55	IRBB 57	40	30	20	30.00
56	IRBB 58	3	5	3	3.67
57	IRBB 59	20	30	15	21.67
58	IRBB 60	10	20	20	16.67
59	IRBB 61	20	25	30	25.00
60	IRBB 62	20	15	20	18.33
61	IRBB 63	20	25	10	18.33
62	IRBB 64	40	35	30	35.00
63	IRBB 65	20	30	15	21.67
64	IRBB 66	10	15	10	11.67
65	IRBB 67	3	10	30	14.33
66	IR 24	70	40	30	46.67
67	Check	20	50	30	33.33
	Average (locations)	16.84	15.39	15.54	15.92

Highly Resistant = 1% - 5%; Resistant = 6% - 12%; Moderately Resistant = 13% - 25%; Moderately Susceptible = 26% - 50%; Susceptible = 51% - 100%.

IR 24 showed maximum (46.27%) disease incidence percentage in average at three locations followed by IRBB7 (40%), IRBB64 & RRI7-6 (35%) and susceptible check (33.3%) among the studied germplasm and NILs. On the other hand, minimum disease incidence percentage was recorded for PKBB1506 & RRI7-6 (2.67%) on average at three locations, followed by RRI7-2 (3%), IRBB52 & IRBB58 (3.67%), four lines with 4.33% DI percentage, 5 lines with 6% DI and 7

lines with 7% DI on average basis. Likewise, percentage DI for each location on average basis for all studied genotypes was ranged from 70% for IR64 at Hafizabad to minimum of 0% (no DI observed) for IRBB11 (at Gujranwala location) while RRI7-6 and PKBB1506 (at KSK location). On average, the maximum DI percentage (16.84) was recorded at Hafizabad location, followed by Gujranwala (15.54%) and KSK (15.39%).

3.2. GEI and Biplot Analysis

Biplot view (Figure 2) of GEI analysis shows the significantly different performances of all the three target hotspot locations with respect to the disease responses of all the studied genotypes. Two locations *i.e.* Shiekhupura and Gujranwala, among all the three locations, showed close proximities in disease response and out of these two locations, Gujranwala location showed more variability while the sheikhupura location showed more stability as compared to one another. The third locations, showed maximum variability for the disease incidence as indicated by the longest vector as depicted in biplot (Figure 2).

Center of the co-centric rings shows the average of the disease incidence data at all the three studied locations. Farther the lines from the center, the more diverse with respect to disease incidence. The check genotype showed maximum disease incidence in all the three locations while the disease incidence was more in other two locations as compared to Hafizabad location. Likewise, entry no 66, showed

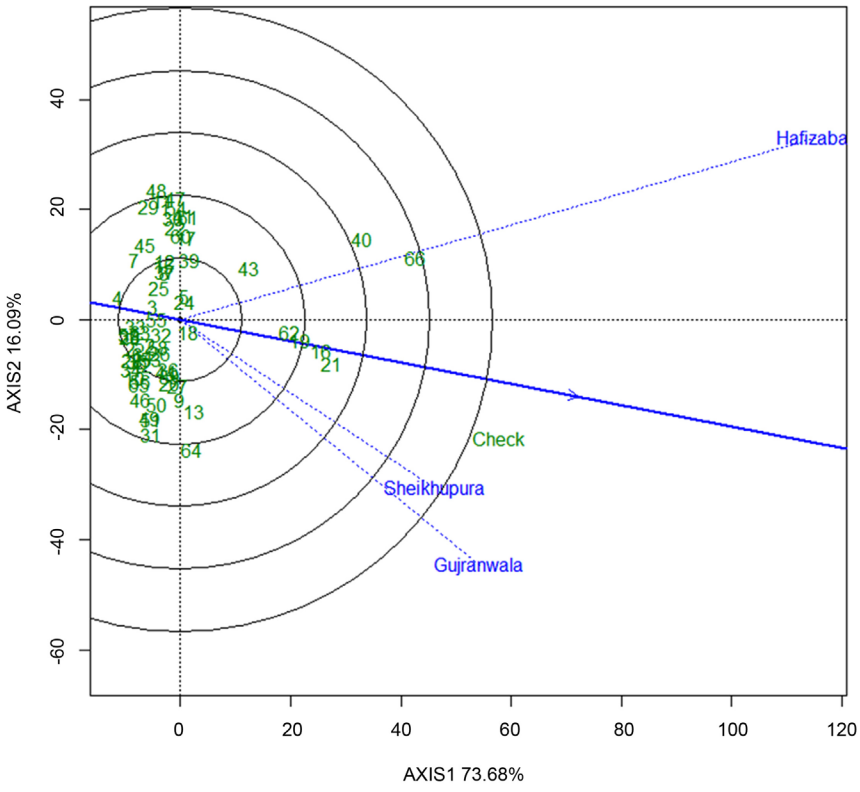

Figure 2. Performance of genotypes and IRBB lines against the disease (BLB) at three hotspots.

maximum disease incidence in Hafizabad location as compared to other two locations, followed by entry no 40, as indicated by farthest distance of these genotypes in biplot from center towards the vector of Hafizabad location.

The figure further emphasizes the performance of each genotype in different locations. Genotypes can be further divided into group according to their locations in GEI biplot. The biplot can be seen divided into four parts (**Figure 2**) by two axis, vertical mean axis and horizontal mean axis. The genotypes near the horizontal mean axis (HMA) are more stable as compared to those genotypes which are far from the HMA. Keeping in view the whole scenario, it can be depicted that performance of the genotype entry no 64 was the least stable, followed by entry no 64, 40 and 66 as indicated by their distance from HMA in the GEI biplot.

On the other hand, vertical mean axis is the measure of average disease incidence in all the target locations. The genotypes falling left to this axis showed less disease incidence than the average of disease incidence while the genotypes falling right to this axis are more susceptible. Check variety is the located far right position in the GEI biplot indicating its maximum disease incidence and maximum susceptibility to BLB disease, followed by entry no. 66, 40, 21, 16 and 19 and so on. Check variety showed unstable performance and disease incidence was higher in Gujranwala and Sheikhupura locations as compared to Hafizabad location; likewise, the susceptibility of entry 66, and 40 was also unstable in different environments and its disease incidence likelihood was more in Hafizabad location as compared to other two locations. Entry no 21 followed by 16, 19 and 62 also among the most susceptible lines with stability in susceptible performance to BLB disease in all the three locations indicating their usefulness as stable susceptible check for these locations in further experimentations.

Entry no 4, located infar left position of the GEI biplot (**Figure 3**) indicated its maximum resistance towards BLB disease in all the three target locations. Furthermore, the shortest vector from HMA also confirms the stable performance in all the three locations. Therefore, this line can be utilized as the most stable BLB resistant line or check in these studied three target locations.

It was further emphasized by this study that *Xa*4 and *xa*13 were found to deliver significant resistance against the local pathotypes of disease bacteria in studied germplasm and NILs. Among the resistant germplasm, these two genes were found in the majority as compared to other genes studied. However, some other lines as well as NILs were also found resistant carrying no gene among these studied five genes. These lines may be further studied to investigate genes that can be utilized further in these locations to breed resistant rice lines in the future.

4. Conclusion

The study confirms that NILs having different combination of introgressed BLB resistant (*Xa*) genes can be effectively employed to incorporate horizontal resistance in local germplasm against a number of local isolates of bacterial diseases

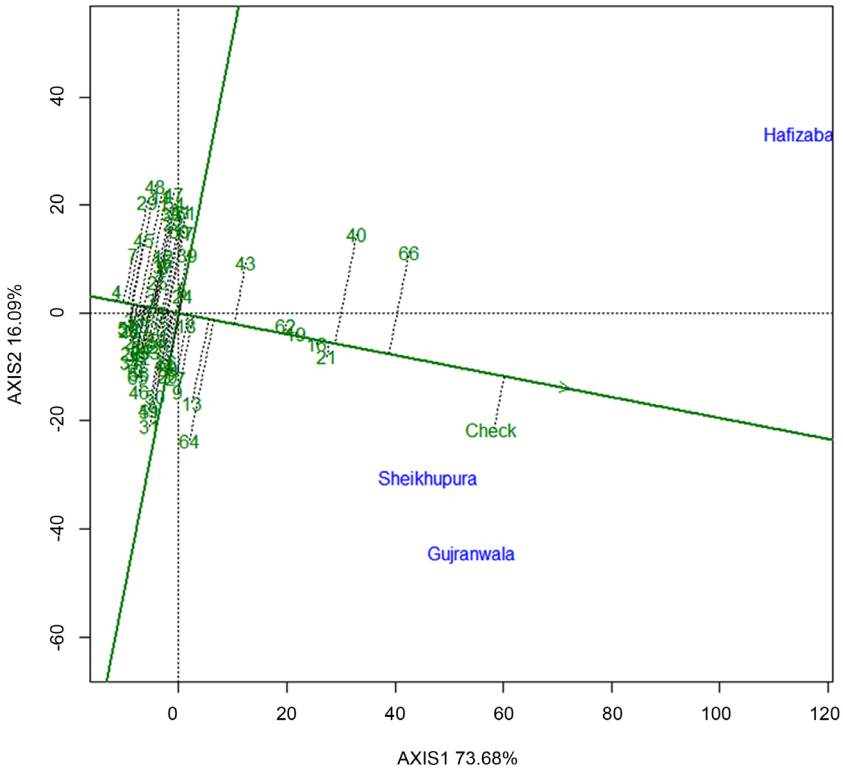

Figure 3. Stability and diversity studied of rice germplasm and BLB hotspot locations.

in crops. Furthermore, these *Xa* genes (*Xa*4, *xa*5, *Xa*7, *xa*13, and *Xa*21) successfully developed resistance in rice germplasm and can be exploited in breeding programs to develop BLB resistant germplasm and varieties. Thirty-one (31) lines were categorized under resistant cluster, twenty-eight (28) were moderately resistant, six (6) were moderately susceptible and one (susceptible check) was in susceptible category. Grouping of different lines/varieties under same cluster shows their significantly similar response against BLB disease in corresponding environment. Among the studied NILs, only one line showed polymorphism for all five resistant genes, two lines had four; seven lines had three genes, seven lines showed di-genic while five lines showed mono-genic polymorphism. These lines with multiple-genes for BLB resistance can be evolved as a new BLB resistant variety and also be utilized as donor parent in breeding programs for developing new cultivars with horizontal resistance against more than one target pathotypes and environments. It was further emphasized by this study that *Xa*4 and *xa*13 were found to deliver significant resistance against the local pathotypes in studied germplasm and NILs.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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