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IDENTIFICATION OF BACTERIAL LEAF BLIGHT RESISTANCE GENES IN DIVERSE LOCAL PAKISTANI RICE (*ORYZA SATIVA*) GERMPLASM

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Abstract

Rice (*Oryza sativa*) is the most widely consumed staple foods worldwide, and Pakistan is an important rice-producing and exporting country. Among the various biotic and abiotic factors affecting rice yield, Bacterial Leaf Blight (BLB), caused by *Xanthomonas oryzae* (*Xoo*), is considered a major threat to rice production. Developing rice varieties with inherent genetic resistance is an economical and eco-friendly approach to managing the disease. Therefore, the current study is conducted to identify and characterize locally collected rice germplasm for BLB resistance. A total of 23 rice genotypes were collected from the Khyber Pakhtunkhwa (KPK) and Punjab regions and analyzed for the presence of the resistance genes *Xa5* and *Xa7*. The extracted DNA was further subjected to PCR amplification using gene-linked primers. The results revealed that Xa5 and Xa7 genes were present in all genotypes except UD-205 and UD-201. UD-205 lacked *Xa5* but had *Xa7* gene whereas UD-201 possessed *Xa5* but lacked the *Xa7* gene. Genetic similarity analysis based on the presence of these genes, divided the local germplasms into three main clusters showing genetic diversity in rice germplasm. Further dendrogram analysis revealed strong genetic relationships among rice germplasms. This study provides valuable insights into the genetic diversity of BLB resistance in local rice germplasm and contributes to the development of more resistant and climate-resilient rice cultivars, thereby contributing to sustainable rice production.

Key words: Bacterial leaf blight, Pakistan, *Xanthomonas oryza*, Rice, *Xa5*, *Xa7*

Introduction

For more than 3.5 billion people all over the world, rice serves as a staple food. Asia is ranked as number one in production and consumption of rice. Due to the rapid growth of the global human population, rice demand is expected to increase by approximately 70%. Pakistan contributes about 1.7% of Asia's total rice production, making it the tenth-largest rice-producing country and one of the leading rice exporters in the world (Naqvi, 2019). Numerous factors like soil composition, water availability, seed quality, land, weeds, pests, disease-causing microbes, etc., negatively affect rice production. Among these, plant diseases cause significant losses in rice yield and contribute to food insecurity across Asia. A disease known as bacterial leaf blight (BLB), caused by the bacterium *Xanthomonas oryzae* pv. *oryzae* (Xoo) is one of the destructive diseases of rice (Jan *et al.*, 2020). In South Asian lowland and irrigated rice fields, bacterial leaf blight (BLB) is considered one of the most severe rice diseases due to continuous rice cultivation and favorable environmental conditions, including high humidity, heavy rainfall, and strong winds. These conditions facilitate the rapid spread of the disease and result in significant losses in rice production (Qudsia *et al.*, 2019). The symptoms of BLB include small yellow lesions on the leaf margins, which progress through hydathodes and stomata. In the start the lesions have pale green, changing to yellowish white, last the whole leaf is covered. When tillering stage is reached, these yellow lesions cause necrosis and death of the leaf. Mainly it affects vascular tissues and may affect at any stage either the seedling, vegetative, and reproductive phases (Islam *et al.*, 2023).

There are about 45 resistance genes against BLB being identified. 11 of these genes are cloned and their function is assessed. These include *Xa1*, *Xa3*, *Xa26*, *Xa4*, *Xa5*, *Xa13*, *Xa21*, *Xa23*, *Xa25*, *Xa27*, and *Xa41* (Anik *et al.*, 2022). Nine of them are recessive. Eleven genes encode different proteins involved in different resistance mechanisms. A few of them are entered into commercial rice varieties in Asia. *Xa21*, *Xa7* which are dominant as well as *Xa5*, *Xa13* being recessive have broad spectrum resistance against many *Xoo* races. Therefore, these genes are widely used in rice breeding programs across Asia (Nanoukon *et al.*, 2023).

Biotic and abiotic stresses affect morpho-biochemical properties of economically important crop species (Saleem *et al.*, 2025; Jan *et al.*, 2023; Khan *et al.*, 2023; Jan *et al.*, 2022; Qamar *et al.*, 2020). Biotic stress including *Xoo* negatively affects both morpho biochemical and molecular processes of economically important rice genotypes by causing BLB of rice. It negatively effects both its qualitative and quantitative traits. As rice is a globally important crop but due to BLB there is much loss in its

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yield. In Pakistan loss to rice production is a serious matter of concern as it is a staple food for most people. The present study was conducted to identify promising bacterial leaf blight resistant rice genotypes and to characterize diverse locally collected Rice genotypes for BLB resistance genes.

Material and Methods

Seed collection: 23 local rice genotypes were collected from different growing areas of Pakistan including Gujranwala (Punjab), Okara (Punjab), Upper Dir (KPK), Lower Dir (KPK), Swat (KPK), and Mansehra (KPK) (Table 1).

Table 1. Detail of local rice germplasm used (n=23).

Sr. No.	Acc. No.	Source
1.	GW-260	Gujranwala, Punjab
2.	GW-262	Gujranwala, Punjab
3.	OK-265	Okara, Punjab
4.	OK-270	Okara, Punjab
5.	UD-205	Upper Dir, KPK
6.	LD-167	Lower Dir, KPK
7.	LD-173	Lower Dir, KPK
8.	LD-175	Lower Dir, KPK
9.	SW-225	Swat, KPK
10.	SW-228	Swat, KPK
11.	SW-231	Swat, KPK
12.	SW-234	Swat, KPK
13.	SW-240	Swat, KPK
14.	SW-243	Swat, KPK
15.	SW-244	Swat, KPK
16.	MS-247	Mansehra, KPK
17.	MS-249	Mansehra, KPK
18.	MS-250	Mansehra, KPK
19.	LD-153	Lower Dir, KPK
20.	LD-155	Lower Dir, KPK
21.	LD-180	Lower Dir, KPK
22.	UD-201	Upper Dir, KPK
23.	UD-207	Upper Dir, KPK

Plant germination: The fresh and mature seeds of all rice genotypes were sown on disposable glass having fertile soil. The plants were monitored regularly and watered as needed. After 1 to 2 weeks seed germination was noted.

Genomic DNA extraction and DNA confirmation: Genomic DNA was extracted from young fresh leaves by using the standard protocol of Doyle and Doyle (1987). DNA samples were run on 1% agarose gel and visualized via UV based gel documented system.

Primer selection: Two primers (specific to BLB resistant gene) named as *Xa5* and *Xa7* were selected from previous literature and used for identification of BLB resistant genes.

PCR analysis: PCR profile for denaturation, annealing and extension was optimized for each primer. Two markers each of 100 bp were used. PCR reaction mixture was formulated using 2 μl PCR Master Mix, 1 μl of each forward and reverse Primer, 2 µl dNTPs, 0.2 µl Taq DNA polymerase,12.8 µl PCR water and 1 μl of genomic DNA. The PCR has: 4-mins initial denaturation step at 94ºC, 35 cycles of denaturation at 94ºC for 30s, annealing at 58ºC to 60ºC for 30s and extension at 72ºC for 1 min. PCR products were run in 1% agarose gel and visualized under UV Gel Doc System. PCR analysis was performed with *Xa5* primer set F:5-TGTTCTTTTCTCAGGGCCAC-3 and R:3-AGTTTGGAATCACAGGCCAC-5 and *Xa7*: F:5-CTGGATACGGAACCTTCTAAC-3 and R:3-AGAGAA CCTTCTCCTTCAGTG-5.

Data analysis: The recorded data were entered into an MS Excel sheet and subsequently analyzed using NTSYSpc version 2.1 software.

Results

Identification of resistant genotypes using molecular markers: Molecular analysis was performed using gene-specific primers. The amplification products appearing as bands in the electrophoresis gel are shown in Figs. 1 and 2. Banding patterns indicating the presence and absence of *Xa5* and *Xa7* genes in rice germplasm, amplified 141 bp and 294 bp, respectively. Gel electrophoretic bands of 23 rice genotypes (Table 1) generated for BLB resistance gene *Xa5* are shown in Fig. 1. Out of 23 genotypes, the *Xa5* gene fragment of size 141 bp was present in 22 genotypes and absent in 1 genotype (UD-205). Gel electrophoretic bands of 23 rice genotypes generated by using the marker for BLB resistance gene *Xa7* of size 294bp is shown in Fig. 2. The findings indicate that the *Xa7* gene was present in 22 genotypes and was absent in the UD-201 genotype.

Genetic similarity analysis among rice genotypes using similarity matrix: Among the 23 rice genotypes, similarity indices were computed for every pair that could exist. A scale from 0 to 1 was used to quantify similarity; a value of 1 indicates that different genotypes are similar, whereas values less than 1 indicate varying degrees of similarity. The range of genetic similarity was 0.00 to 1.00 (Table 2). The greatest similarity matrix of 1.00 was displayed by numerous combinations. Some combinations had the lowest similarity matrix value, 0.00 like UD-201 and UD-205. GW-260, GW-262, OK-265, OK-270, etc. were among the genotypes that showed strong resistance. This implies that significant resistance genes against BLB may be present in these genotypes. In many comparisons, genotypes such as UD-205 and LD-167 demonstrated moderate resistance with scores of 0.67. These genotypes might be useful in breeding initiatives meant to increase resistance to BLB. The similarity matrix among rice genotypes is shown in Table 2.

Cluster analysis of rice genotypes against BLB: Genotypes were classified into different groups through phylogenetic analysis. The dendrogram was generated using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA), which divided the 23 genotypes into three distinct clusters (Fig. 3).

Cluster A (highly resistant): This cluster consists of genotypes with similar patterns with coefficients 1.00. Clusters with high similarity scores indicate that these genotypes are very similar to one another. This group consists of 21 genotypes. These germplasms may share both the same BLB resistance genes (Fig. 3).

Table 2. Similarity matrix table showing similarity among 23 rice genotypes.

Acc. No.	GW-260	GW-262	OK-265	OK-270	UD-205	LD-167	LD-173	LD-175	SW-225	SW-228
GW-260	1.00									
GW-262	1.00	1.00								
OK-265	1.00	1.00	1.00							
OK-270	1.00	1.00	1.00	1.00						
UD-205	0.67	0.67	0.67	0.67	1.00					
LD-167	1.00	1.00	1.00	1.00	0.67	1.00				
LD-173	1.00	1.00	1.00	1.00	0.67	1.00	1.00			
LD-175	1.00	1.00	1.00	1.00	0.67	1.00	1.00	1.00		
SW-225	1.00	1.00	1.00	1.00	0.67	1.00	1.00	1.00	1.00	
SW-228	1.00	1.00	1.00	1.00	0.67	1.00	1.00	1.00	1.00	1.00
SW-231	1.00	1.00	1.00	1.00	0.67	1.00	1.00	1.00	1.00	1.00
SW-234	1.00	1.00	1.00	1.00	0.67	1.00	1.00	1.00	1.00	1.00
SW-240	1.00	1.00	1.00	1.00	0.67	1.00	1.00	1.00	1.00	1.00
SW-243	1.00	1.00	1.00	1.00	0.67	1.00	1.00	1.00	1.00	1.00
SW-244	1.00	1.00	1.00	1.00	0.67	1.00	1.00	1.00	1.00	1.00
MS-247	1.00	1.00	1.00	1.00	0.67	1.00	1.00	1.00	1.00	1.00
MS-249	1.00	1.00	1.00	1.00	0.67	1.00	1.00	1.00	1.00	1.00
MS-250	1.00	1.00	1.00	1.00	0.67	1.00	1.00	1.00	1.00	1.00
LD-153	1.00	1.00	1.00	1.00	0.67	1.00	1.00	1.00	1.00	1.00
LD-155	1.00	1.00	1.00	1.00	0.67	1.00	1.00	1.00	1.00	1.00
LD-180	1.00	1.00	1.00	1.00	0.67	1.00	1.00	1.00	1.00	1.00
UD-201	0.67	0.67	0.67	0.67	0.00	0.67	0.67	0.67	0.67	0.67
UD-207	1.00	1.00	1.00	1.00	0.67	1.00	1.00	1.00	1.00	1.00

Table 2. (Cont'd.).

Acc. No.	SW-231	SW-234	SW-240	SW-243	SW-244	MS-247	MS-249	MS-250	LD-153	LD-155	LD-180	UD-201	UD-207
SW-231	1.00												
SW-234	1.00	1.00											
SW-240	1.00	1.00	1.00										
SW-243	1.00	1.00	1.00	1.00									
SW-244	1.00	1.00	1.00	1.00	1.00								
MS-247	1.00	1.00	1.00	1.00	1.00	1.00							
MS-249	1.00	1.00	1.00	1.00	1.00	1.00	1.00						
MS-250	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00					
LD-153	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00				
LD-155	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00			
LD-180	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
UD-201	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67		
UD-207	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
UD-201												1.00	
UD-207												0.67	1.00

Cluster B (moderately resistant): This cluster represents the moderate resistance genotype UD-205. The genotype in this cluster possesses only one resistance gene, *Xa5* (Fig. 3).

Cluster C (moderately resistant): This cluster represents the moderate resistance genotype UD-201. The genotype in this cluster possesses only one resistance gene, *Xa7* (Fig. 3).

Discussion

One of the most destructive diseases of rice is BLB, which is caused by Xoo. The use of resistant cultivars is the most efficient strategy against BLB (Das *et al.*, 2021). We identified the presence of *Xa5* and *Xa7* resistance genes in 23 local rice Pakistani genotypes using molecular markers and genetic analysis. As many studies in the past have documented the use of gene-linked molecular markers to identify the genotype of resistance genes in rice. Similar resistant genes were identified in other local Pakistani rice germplasm by Sabar *et al*., (2016).

Our study verified the presence of the *Xa5* and *Xa7* genes in the studied genotypes. The results revealed that both *Xa5* and *Xa7* genes were present in the majority of the rice genotypes, suggesting that these resistance genes are highly prevalent in the local germplasm. The consistent banding patterns observed across most genotypes further confirmed the role of these genes in providing resistance against BLB. These findings are consistent with those of Naveed *et al*., (2010), who identified the *Xa5* gene in 45 out of 88 rice germplasm lines, and Abbasi *et al*., (2011), who detected the *Xa5* gene in 31 out of 60 rice lines using specific primers.

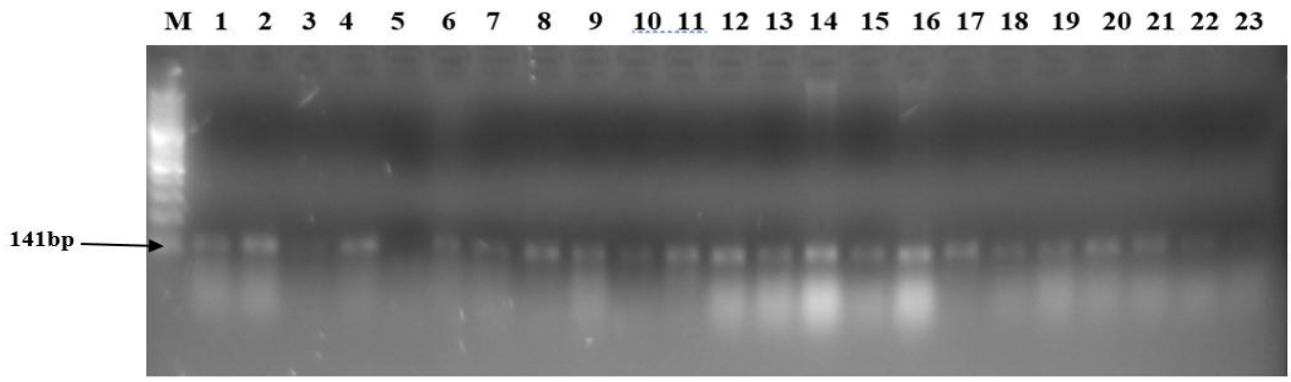

Fig. 1. DNA banding patterns of 23 rice accessions for identification of the BLB resistance gene *Xa5*
M = Marker(100bp), band 1 = GW-260, band 2 = GW-262, band 3 = OK-265, band 4 = OK-270, band 5 = UD-205, band 6 = LD-167, band 7 = LD-173, band 8 = LD-175, band 9 = SW-225, band 10 = SW-228, band 11 = SW-231, band 12 = SW-234, band 13 = SW-240, band 14 = SW-243, band 15 = SW244, band 16 = MS-247 , band 17 = MS-249, band 18 = MS-250, band 19 =LD-153 , band 20 = LD-155, band 21 = LD-180 , band 22 = UD-201 , band 23 = UD-207

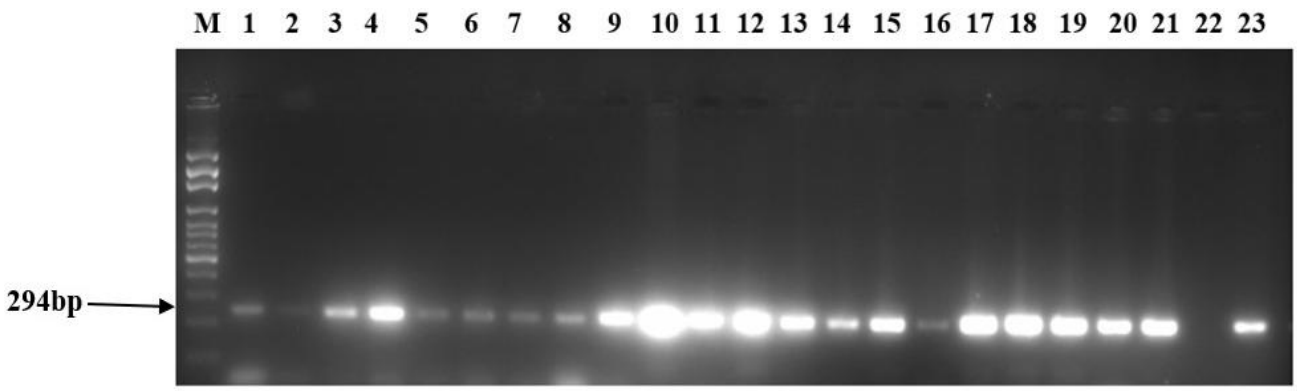

Fig. 2. DNA banding patterns of 23 rice accessions for identification of the BLB resistance gene *Xa7*
M = Marker(100bp), band 1 = GW-260, band 2 = GW-262, band 3 = OK-265, band 4 = OK-270, band 5 = UD-205, band 6 = LD-167, band 7 = LD-173, band 8 = LD-175, band 9 = SW-225, band 10 = SW-228, band 11 = SW-231, band 12 = SW-234, band 13 = SW-240, band 14 = SW-243, band 15 = SW244, band 16 = MS-247 , band 17 = MS-249, band 18 = MS-250, band 19 =LD-153 , band 20 = LD-155, band 21 = LD-180 , band 22 = UD-201 , band 23 = UD-207.

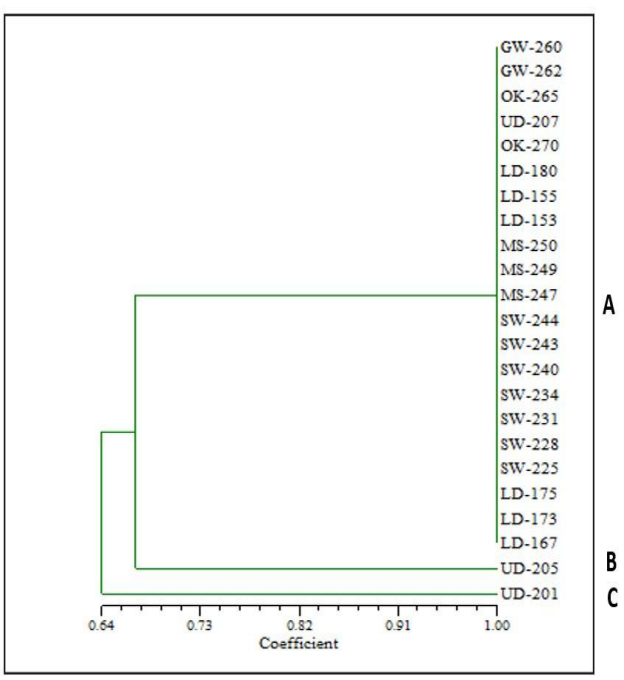

Fig. 3. A dendrogram based on scoring data revealed patterns of bacterial blight resistance among Pakistani rice germplasm.

In a similar study Muhammad *et al.*, (2015) screened six rice varieties and 52 lines for *Xa13* gene. Only four varieties and 23 lines were found positive for this gene. Phylogenetic analysis divided all 23 germplasm in three clusters according to their genetic similarity. A similar study by Javed *et al.*, (2022) analysed genomic 38 rice cultivars from Malaysia and examined for allelic variation using one STS marker and thirteen SSR markers. Using a dendrogram, cultivars were grouped into seven clusters according to their genetic closeness.

The lack of *Xa5* in UD-205 and *Xa7* in UD-201 draws attention to the genetic diversity present in the germplasm and marks these genotypes as special situations that require additional research like Gautam *et al.*, (2023) study used marker-assisted backcross breeding (MABB) to introduce many resistance genes (*Xa4, Xa5, Xa13*, and *Xa21*) into salt-tolerant rice variants. The study showed that resistance gene combinations offer durable defense against BLB. Similarly, Babar *et al.*, (2022) transferred three BLB resistance genes, *Xa4*, *Xa5*, and *Xa21*, from the coarse but BLB-resistant variety IRBB57 into Super Basmati through marker-assisted breeding. As a result, several Super Basmati lines carrying different combinations of BLB resistance genes were developed.

Conclusion

Bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), remains a significant threat to rice production worldwide, resulting in yield losses. One of the effective and sustainable ways to control the BLB is development of rice cultivars having resistant genes. The present study aimed to identify and analyse the BLB resistance genes *Xa5* and *Xa7* in 23 rice genotypes collected from different rice growing areas of Pakistan including KPK and Punjab. Molecular screening and genetic analysis gave valuable insights into the genetic similarity, genetic diversity, and potential breeding applications of these germplasms. Our findings showed that maximum rice accessions contained both *Xa5* and *Xa7* resistance genes. No accession was found to lack both genes, indicating a strong prevalence of BLB resistance traits in the studied germplasm. The identification of accessions like UD-205 and UD-201, which show genetic distinctiveness, opens ways for further genomic studies to discover additional resistance loci and determine the molecular mechanisms involved in BLB resistance. This study gives a comprehensive understanding of BLB resistance gene presence in local rice germplasm, emphasizing the importance of genetic diversity in resistance breeding. In addition to *Xa5* and *Xa7*, there is a need to identify and incorporate additional resistance genes to enhance resistance against a broader range of BLB strains and diverse climatic conditions. Expanding the screening of rice genotypes to include a larger and more diverse set of varieties, both local and exotic, would also give valuable findings into genetic diversity and the overall effectiveness of these resistance genes. Modern molecular breeding techniques, such as CRISPR/Cas9 gene editing, gives an opportunity to enhance the development of rice varieties with increased resistance, allowing for precise modification of resistance genes.

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