Pak. J. Bot., 56(4): DOI: http://dx.doi.org/10.30848/PJB2024-4(34)

MOLECULAR INVESTIGATION OF BACTERIAL BLIGHT OF RICE IN THE FOOTHILLS OF THE WESTERN HIMALAYAS, PAKISTAN

IRFAN ULLAH[1], TARIQ MAHMOOD[2], HAMID ALI[1], MUHAMMAD ISLAM[1], AZIZ-UD-DIN[1], MUHAMMAD HARIS[1] AND ASIF KHAN[3*]

[1]Department of Biotechnology and Genetic Engineering, Hazara University Mansehra, KP Pakistan
[2]Department of Agriculture/Bioinformatics, Hazara University Mansehra, KP Pakistan
[3]Laboratory of Phytochemistry, Department of Botany, University of São Paulo, São Paulo Brazil
**Corresponding author's email: asif.khan@usp.br*

Abstract

Bacterial blight (BB) is caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most devastating diseases in rice crops, which adversely affect the annual rice production (quantity and quality) in tropical and sub-tropical regions of the world.

The present study was conducted to identify and characterize *Xoo* strains obtained from infected rice leaves samples collected from foothills of the Himalayas at Mansehra District, Pakistan. A total of 20 samples were collected. The infected leaf samples were plated on nutrient agar and gave light yellow, circular, smooth, convex and viscous bacterial colonies. The collected samples were later on amplified, and their various morphological and genetic traits were accordingly checked on the Super Basmati and Basmati 385 seedlings. A total of 6 pure isolates were obtained, preserved, and were confirmed as *Xoo* using *Xoo* specific primers in PCR which showed 230 bp bands. Pathogenicity of *Xoo* isolates was confirm by Koch's postulates on rice varieties super basmati and basmati 385. The amplification of *16S rRNA* gene of these isolates was carried out using a pair of universal primers. Besides, the disease incidence (%) was also taken under consideration, in which super Basmati variety was found significant, *Xoo*-1 (65%), followed by *Xoo*-6 (48.10%) and *Xoo*-2 (53.30%) as compared to Basmati-385. The present study provides a base for rice breeders to initiate regional resistance breeding programs.

Key words: Bacterial blight, *Xanthomonas oryzae* pv. *oryzae,* PCR, Himalayas, Pakistan.

Abbreviations: BB (Bacterial blight), *Xoo* (*Xanthomonas oryzae* pv. *oryzae*), KP (Khyber Pakhtunkhwa), PCR (Polymerase Chain Reaction) and (RCBD) Randomized Complete Block Design.

Introduction

Rice (*Oryza sativa* L.) belongs to the family Gramineae, extensively growing in the tropical and sub-tropical regions of the world (Ezuka & Kaku, 2000). Around 90% rice is grown in the Asian's countries, including China (30%), India (24%), Indonesia and Bangladesh (7% respectively), Vietnam (5%), Thailand (4%) and Pakistan (3%), as one of the staple foods for 3 billion peoples across the world (Salim *et al*., 2003; Aldosari *et al*., 2019). In Pakistan, rice is the second most important cultivated cereal crop with a total production of 7.4 million metric ton (2018/2019) and is considered to be one of the main exports of the country (Gul *et al*., 2022; Ullah *et al*., 2023). Rice is cultivated all over Pakistan, predominantly in its plan land of Punjab and Sindh provinces. However, in the Khyber Pakhtunkhwa (KP) province of Pakistan, it is grown in cooler and high-altitude areas comprising western Himalayan regions like Mansehra, Swat, Chatral and Dir (Chaudhri, 1986; Aldosari *et al*., 2019).

Pathogenic microbes are considered to be one of the most devastating yields limiting factor that adversely affect the quantity as well as quality of cereal crops, especially rice (Deng *et al*., 2017). More than forty genera of fungi and bacteria are involved in causing sever diseases, which affects the production of rice crops at various growth stages (Khan *et al*., 2009). Bacterial Blight (BB) diseases, is considered the most destructive one, commonly found all over the world (Swings *et al*., 1990). In Pakistan, the diseases were first reported in 1977, which later on, spread all over the country and became a serious threat for rice cultivators (Mew & Majid, 1977; Bashir *et al*., 2010; Ali *et al*., 2016). BB mainly affects the plant vessels, which manifest itself either in the seedling resulting in severe wilting of the plantlet, or in leaves as leaf blight resulting in leaf drying (Jiang *et al*., 2020). In case of severe BB infection, plant either fails or produces sterile panicles that contain immature grains of bad quality and quantity (Waheed *et al*., 2009; Ali *et al*., 2016; Khoa *et al*., 2017).

A number of molecular approaches, including Polymerase Chain Reaction (Shivalingaiah & Sateesh, 2012), Western Blot analysis (Guo *et al*., 2015) and morpho-molecular screening have been used to identify the BB strains (Mubassir *et al*., 2016). However, biochemical tools can also be employed for detection of *Xoo* incidence (Samanta *et al*., 2014). It is very difficult to control the BB due to extraordinary *Xoo* mutability. Newly developed resistant varieties were easily broken down after three to four years (Ponciano *et al*., 2003). Conventional breeding to developed BB resistance variety is limited by the stretches of years it takes years before the release of such variety, necessitating conventional breeding to be associated with molecular markers (Sudir & Yuliani, 2016).

Pakistan is known worldwide for producing and exporting high-quality Basmati rice. Every year, about 5 million tonnes of Basmati rice are sold to countries including the United States, Europe, China, and the Middle East, generating a significant amount of revenue (Ali *et al*., 2016). Basmati rice, as high-yielding cultivars,

mainly grown in Punjab province and partially in KP province of Pakistan, is susceptible to BB incidences, thus resulting in significant yield loss every year (Nawaz *et al*., 2020; Noreen *et al*., 2020; Ullah *et al*., 2020). The scenario worsens as no BB resistance has been reported in the approved Basmati cultivars, indicating a lack of effective resistance genes in its genetic pool against geography specific contemporary *Xoo* strains (Sabar *et al*., 2016). Rice plant is also affected in the fields by subjected to heavy metal contamination (AL-Huqail *et al*., 2022). Rice grains tend to actively bio accumulate heavy metals originating from contaminated soils; therefore, soil properties are considered the most influencing factor (Xu *et al*., 2022). The industrial effluents resulted in dangerous soil degradation (decrease in soil quality by 100%), making these fields inappropriate for subsequent cultivation (Afrad *et al*., 2020). The purpose of the current study was to identify the BB strain in rice by employing a combinatorial approach of pathogenicity test and PCR based molecular techniques, from foothills of the Himalayas, District Mansehra Pakistan.

Material and Methods

Samples collection: Different areas of Mansehra District, Pakistan was visited and surveyed for the incidence of BB in the months of August and October 2020. The infected rice leaves were properly collected from different areas of the district during the growing season and preserved in paper envelopes, labelled with information about variety, location and sampling date (Table 1). The samples were brought to the Molecular Genetics laboratory, Department of Biotechnology and Genetic Engineering, Hazara University Mansehra, and were kept in the refrigerator for further analyses.

***Xanthomonas oryzae* pv. *oryzae* strains isolation:** The samples were surface disinfected in 70% ethanol, three times washed in sterile distilled water, and air -dried in laminar flow hood. The leaves were cut into small pieces of 2-4cm, washed with 70% ethanol, and kept in ethanol Eppendorf tube for 30 seconds with the help of sterilized forceps in order to remove the surface bacteria. The leaves were washed with distilled water to remove the ethanol and kept for 15 seconds in each separate Eppendorf tubes.

Similarly, the Eppendorf tubes, having a little amount of water (0.7 mL), and leaves were crushed with the help of sterilized blue tips and kept for one hour at room temperature. The oozed out bacterial strains originating from the infected leaves into the water, were streak on to the nutrient agar media (Peptic digest of animal tissue 5g, NaCl 5g, Water 1000ml, Beef extract 1.5g, Glucose 20g and Agar 17g) in the petri dish and incubated for four days at 28ºC. Mixed bacterial culture were streak on new petri dish for single round, smooth, golden-yellow and mucous colonies.

Confirmation of *Xanthomonas oryzae* pv. *oryzae* through Koch's postulates: For confirmation of *Xoo* isolates, clip method was used (Kauffman *et al*., 1973). Two-days old streaked *Xoo* plates were harvested to prepare 10-15 mL inoculum in autoclaved distilled water, with final concentration of 10^8 colony forming units (cfu) per /mL (Fig. 1). The bacterial strains were inoculated on two rice varieties; Super Basmati and Basmati 385 which were germinated in small pots for 48 hours, and later on transferred to the field for healthy plants growth in a randomized complete block design (RCBD) during the 2021, Kharif season. Each line was replicated thrice and sown in 1.5 m rows with a plant-plant spacing of 10 cm and row-row spacing of 20 cm. Young leaves of 25 days old plants were scissor- inoculated in triplicate. The disease symptoms were observed daily, and after 14 days of inoculation final data was recorded. The plants that possessed the diseased symptoms were cut into small pieces, surface-sterilized, and grown on nutrient agar. After 72 hours, the plates showed bacterial growth and colonies were compared with the mother culture for confirmation of Koch's postulates.

Preservation of *Xanthomonas oryzae* pv *oryzae* cultures: The bacterial isolates that proved to be *Xoo* using Koch's postulates were preserved for further studies using the following two methods.

Bloating paper method: Small pieces of blotting paper (sterilized) were used for the preservation of bacteria. Pure colonies of *Xoo* were touched with the paper via sterilized forceps and put into the Eppendorf tubes and stored at room temperatures.

Preservation in glycerol: 50% glycerol stock was prepared and autoclaved, and 1 mL of this stock solution was taken in 1.5mL Eppendorf tube. Pure colonies of *Xoo* were collected from the plate using wire loop or blue tip, mixed into the glycerol stock and preserved at -80°C.

DNA isolation and PCR amplification: Pure colonies of bacterial strains were used for the isolation of genomic DNA. A single colony from pure culture of bacterial strains was taken with the help of sterilized toothpicks and put into an Eppendorf tube having 100uL sterilized distilled water, the heat up to 96˚C for 6 minutes. For amplification, *16s rRNA* universal primers were used for the confirmation of isolates as *Xoo* and a pair of specific primers were also used. Amplification reactions were carried out in 20 uL reaction volumes containing 1 µL genomic DNA, 0.5 µL each of forward and reverse primers (10 µM/µL), 1.2 µL of dNTPs (25 mM each), 0.4 µL of Taq DNA Polymerase (2 units, Thermo Scientific), 1X Taq Buffer and 1.6 µl $MgCl_2$ (2.5 mM). PCR amplification was carried out in Thermal Cycler (Applied Bio System) the condition was set at an initial denaturation of 5 min at 94°C; 35 cycles of 94˚C for 1 min, 54 ˚C for 1 min for 16sRNA and 59 for 1 min for Xoo specific and 72°C for 2 minutes. One additional cycle of 7 min at 72°C was used for final extension. Amplification products were resolved by electrophoresis on 1.5% agarose gel run in 1X TAE buffer. The amplified products were observed under UV light after staining with ethidium bromide (10 ug/mL).

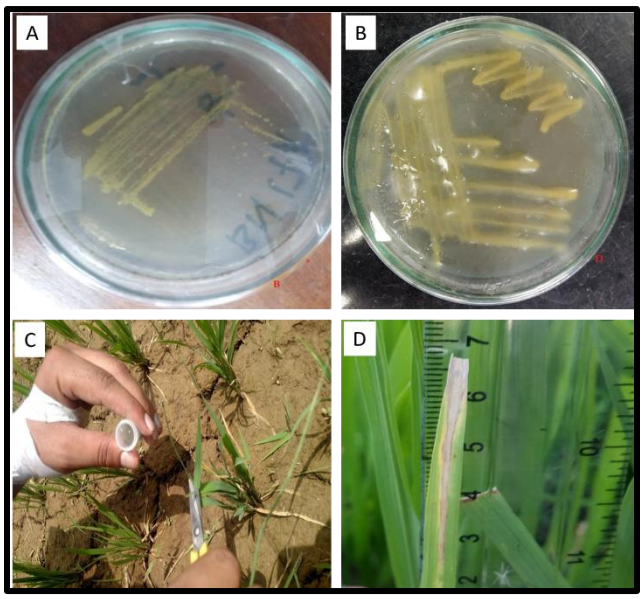

Fig. 1. **A** and **B,** Pure cultures of *Xoo* isolates on nutrient agar medium; **C** Rice inoculated with local *Xoo*; **D** Koch' postulates for confirmation of *Xoo* isolates.

Data analysis: The experiment was performed following a randomized complete block design (RCBD) with three replications. The data were analyzed through Tukey HSD test by means of statistical software package Statistix V. 8.10. The effects were considered significant at $p<0.001$. The disease incidence was monitored using the following formula (Anon., 2013);

$$\text{Disease incidence (\%)} = \frac{\text{Leaf lesion length}}{\text{Total leaf length}} \times 100$$

Results

Confirmation of *Xoo* isolates through specific primers: The polymerase chain reaction (PCR) is particularly useful for plant pathogen detection (Table 2). The specific primers JLXooF/R corresponding to putative glycosyltransferase gene were used, resulting in the amplification a 230 bp DNA fragments from all *Xoo* strains. Six samples, *Xoo*-1, *Xoo*-2, *Xoo*-3, *Xoo*-4, *Xoo*-4, *Xoo*-5 and *Xoo*-6 were confirmed as *Xoo* by amplification of 230 bp *Xoo* specific bands (Fig. 2).

Amplification of *16S rRNA* gene: Colony PCR was performed using universal primers of *16s rRNA* gene. Six isolates *Xoo*-1, *Xoo*-2, *Xoo*-3, *Xoo*-4, *Xoo*-4, *Xoo*-5 and *Xoo*-6 showed the amplification of 1500bp band. The PCR products of these isolates were sent to MACROGEN, Korea for sequencing (Fig. 3).

Maximum Parsimony analysis of taxa: The phylogenic tree was made using maximum parsimony method with 500 bootstrap values and calculated the genetic distance (Fig. 4). The tree is distinctly separated into two clades which were further divided into seven sub-clades. Clade A consisted of 19 elements and Clade B consisted of 13 elements. In Clade A, 4 sub-clades were present, consisting of clade IA, clade IIA, Clade IIIA, and clade IVA. Clade IA contained seven elements-*Xoo*-strain DP20 make a sister family with *Xoo*-strain XPO404 and *Xoo*-4, while *Xoo*-strains DXO, ZJT0002, LND0004, and GZ0008 share a common group and show more resemblance. Similarly, the Clade IIA contained three elements, *Xoo*-strains AUST2013, PX0513 and PXO602 that showed higher evolutionary resemblance. They were differentiated with their ancestor at 54-position. In sub-clade, IIIA contained two elements, *Xoo*-1 make a sister family with *Xoo*-strain CBE01; another specific group was observed in clade IVA-*Xoo*-strains JW11089, K2, K3a, YN24, CIAT, BXO1 share a common group with a reference *Xoo,* PX086, where BXO1 showed more resemblance to PX086 than other members of the group.

While the second clade B, composed of 3 sub-clades consisted of Clade IB, Clade IIB, and Clade IIIB. The elements *Xoo*-5 and *Xoo*-6 were similar elements detected in Clade IB; they were differentiated with their ancestor at 50-position. Clade IIB contained six elements, *Xoo*-strains XO704, PXO404, LND0004, LND0003, *Xoo*2 and DX321 sharing the same group and showing higher evolutionary resemblance. *Xoo*2 made a sister family with *Xoo*-strain DX321. While Clade IIIB consisted of five elements, *Xoo*-strains PXO51 showed more resemblance to strain PXO364, while strains *Xoo*3, XO704 and ZJT0004 showed the closest evolutionary relationship among each other.

Table 1. Sample collections from different localities of Mansehra District, Pakistan.

S. No.	Sample	Location	Variety
1	X00-1 (IR-1)	Tanto Pull Shinkiari	BEGAMMI
2	X00-2 (IR-2)	Tanto Pull Shinkiari	CHINA (BEGAMMI)
3	X00-3 (IR-3)	Gulli Bagh Baffa	JP-5
4	X00-4 (IR-4)	Khan Dari Shinkiari	GHARAH
5	X00-5 (IR-5)	Khan Dari Shinkiari	CHINA (BEGAMMI)
6	X00-6 (IR-6)	Shahzeeb Jeel	CHINA (BEGAMMI)

Table 2. List of primers used in this study.

S. No.	Primer	Primer name	Sequence (3' – 5')	Product size (bp)
1	16s rRNA	9F	GAGTTTGATCCTGGCTCAG	1500
		1510R	GGCTACCTTGTTACGA	
2	*Xoo*-specific	JLXooF	CCTCTATGAGTCGGGAGCTG	230
		JLXooR	ACACCGTGATGCAATGAAGA	

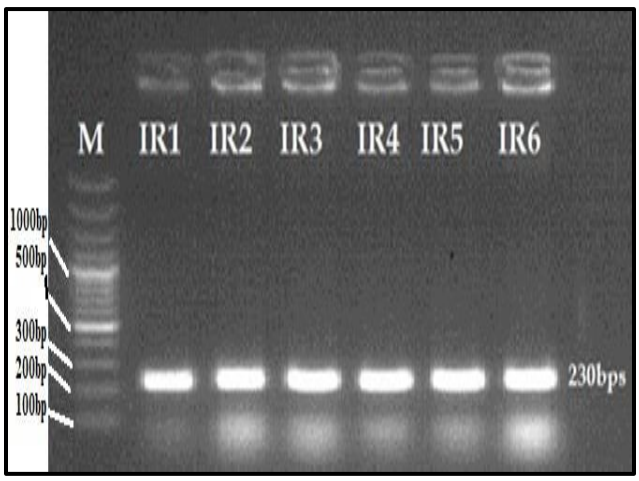

Fig. 2. Confirmation of *Xoo* isolates using specific primer. Where, M= DNA ladder 100 bp, IR1=*Xoo*1, IR1=*Xoo*1, IR2=*Xoo*2, IR3=*Xoo*3, IR4=*Xoo*4, IR5=*Xoo*5 and IR6=*Xoo*6.

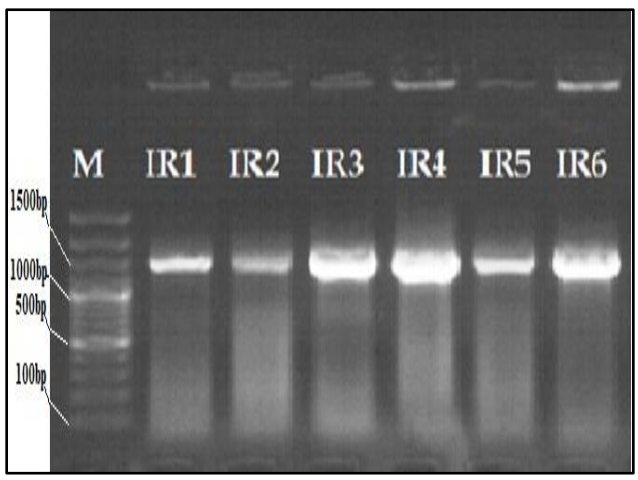

Fig. 3. PCR amplified gel documentation of 16s rRNA gene; where, M is the DNA ladder 100bp, IR1 is *Xoo*1, IR1 is *Xoo*1, IR2 is *Xoo*2, IR3 is *Xoo*3, IR4 is *Xoo*4, IR5is *Xoo*5, IR6 is *Xoo*6.

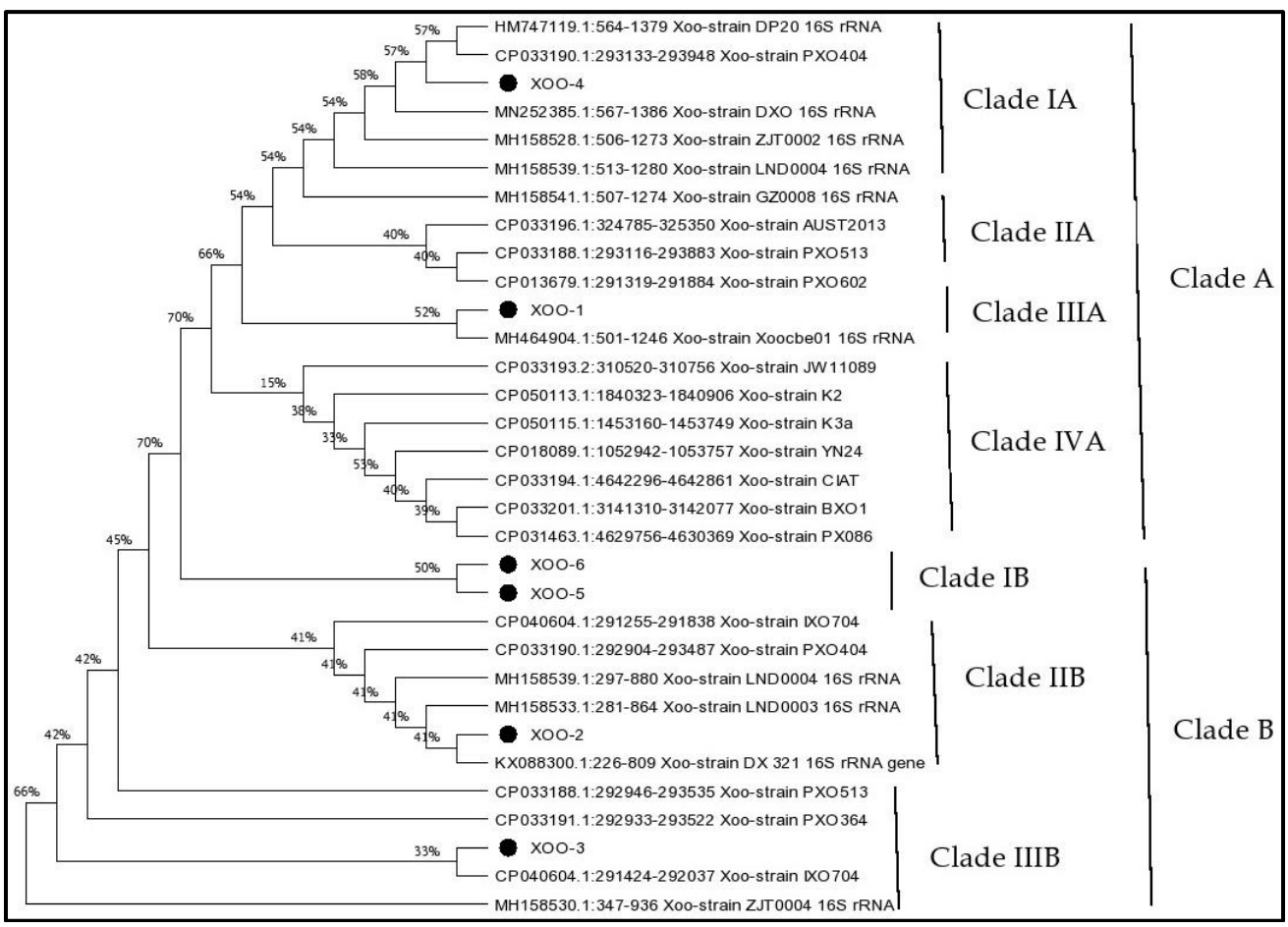

Fig. 4. Phylogenetic analysis of *Xoo* isolates.

Confirmation of *Xoo* isolates through Koch's postulates: In order to confirm the isolates as *Xoo* by Koch's postulates, bacteria were inoculated on two varieties of rice: Basmati 385 and Super Basmati. The selected plants for pathogenicity test were perfectly healthy. For the preparation of inoculums, pure cultures of *Xoo* were performed on nutrient agar. The young leaves were clipped at the tips with the help of scissors dipped in inoculums. After 14 days, data was recorded. The BB-infected leaves were cut into small pieces and cultured on the nutrient agar medium. After 4-5 days, plates were observed for bacterial growth and colonies were similar to mother cultures.

Analysis of variance: The analysis of variance showed highly significant (p<0.001) effect was observed, among the means lesion length developed by *Xoo* isolates on Super Basmati as well as Basmati-385 (Tables 3 and 4).

Table 3. Analysis of variance for % diseases incident on Super Basmati.

Source of variation	Degree of freedom	Sum of square	Mean square	F value	P value
Treatments/Isolates	5	5915.78	1183.16	85.80	0.0000
Error	10	137.89	13.79		
Total	17	6077.78			
Overall mean	63.111		Coefficient of variation		5.88 %

Table 4. Analysis of variance for % diseases incident on Basmati-385.

Source of variation	Degree of freedom	Sum of square	Mean square	F value	P value
Treatments/Isolates	5	2482.28	496.456	71.03	0.0000
Error	10	69.89	6.989		
Total	17	2592.28			
Overall mean	38.611		Coefficient of variation		6.85 %

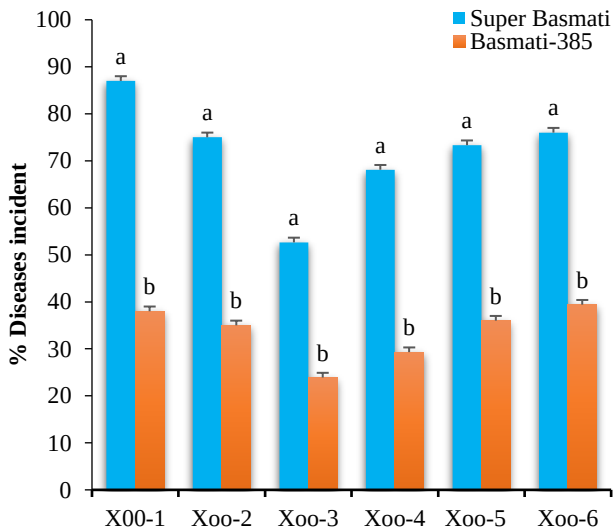

Fig. 5. Tukey HSD test for % diseases incident produced by *Xoo* isolates.

Disease incidence: Tukey HSD test on Super Basmati showed that the disease incidence was significantly higher in the Super Basmati variety compared to Basmati-385 variety (Fig. 5). Among all, *Xoo*-1 was found significant with 65% increase, followed by *Xoo*-6 (48.10%), *Xoo*-2 (53.30%), *Xoo*-5 (50.90%), *Xoo*-4 (56.98%) and *Xoo*-3 (53.40%) as compared to Basmati-385 variety at $p<0.001$.

Discussion

Rice is an important cereal crop in the world, provides more than 21% of the staple food for the world population and up to 76% of the caloric intake in Southeast Asia (Fitzgerald *et al*., 2009; Miura *et al*., 2011). Protein, fats, carbohydrates, and sensible quantity of iron containing compounds, niacin, thiamine, calcium, as well as riboflavin are found in rice (Villareal *et al*., 1994). Although rice is very important, its quantitative as well as qualitative yields are adversely affected by a bacterial pathogen namely called bacterial blight which is caused by *Xoo* (Ali *et al*., 2016).

During the isolation of bacteria from foliar sample of rice, different types of bacterial colonies were obtained. It was noticed that the isolation of bacteria (*Xoo*) from freshly collected samples is easy. As time passed, *Xoo* quickly lost its viability. Therefore, the use of freshly collected samples was recommended for isolation of *Xoo*, as previously documented by (Ullah *et al*., 2020). In this study, 20 samples were collected from different areas of Mansehra District with typical BB symptoms. Furthermore, 6 isolates were recovered from these samples. The isolates were confirmed as *Xoo,* using *Xoo* specific primers. The pathogenicity of the isolates was confirmed through Koch's postulate. The *16s rRNA* gene of these isolates was amplified using a pair of universal primers. Same PCR detection findings were documented in Malaysia by (Jonit *et al*., 2016). Furthermore, the polymerase chain reaction (PCR) analysis led by (Shivalingaiah & Sateesh, 2012), also detected same infections in India. In the present investigation, 6 isolates of *Xoo* were identified from the various location of Mansehra District, where rice was the main and preferred crop economically. Such isolates were also detected via biochemical investigation, and further confirmed by PCR employing a particular primer (Lang *et al*., 2010). The *16s rRNA* gene is the central key used for phylogeny-based recognition amid the several thousand genes inside a bacterial genome (Nogales *et al*., 2001). With distinctive house-keeping features, *16s RNA* gene is often marked by (1) its presence in all bacterial strains often as an operon (2) its conserved or slightly changed sequence over the time and (iii) its 1500 bp sequence being sufficiently large enough for informatics purposes (Patel, 2001).

Conventionally, the identification or detection of a plant pathogen requires pathogen isolation, cultivation, and verification based on bacteriological characteristics, colony morphology, electron microscopic observation, thus a time-consuming process (Fang & Ramasamy, 2015; Kaur & Sharma, 2021). In addition, the detection process requires much equipment and chemicals, which increases the costs (Fang & Ramasamy, 2015).

Conclusion

In this investigation a molecular approach was carried out for the detection of *Xoo* and its adverse effect on the rice crops at Mansehra District KP, Pakistan. Infected leaves samples of local rice were properly collected, in which 6 different *Xoo* strains detected using molecular approach. Disease incidence was also observed very high in the Super Basmati variety as compared to Basmati-385 variety. Therefore, the present study provides a base for the rice breeders to initiate a regional resistance breeding programs as well as *Xoo* susceptible varieties like Super Basmati.

Acknowledgement

The authors are thankful to the local peoples for their support and help during the survey. We are also thankful to the Department of Biotechnology and Genetic Engineering, Hazara University Mansehra, KP, Pakistan, for assistance and technical support in the experiment.

References

Afrad, M.S.I., M.B. Monir, M.E. Haque, A.A. Barau and M.M. Haque. 2020. Impact of industrial effluent on water, soil and rice production in bangladesh: A case of turag river bank. *J. Environ. Health Sci. Eng.*, 18: 825-834.

Aldosari, F., M.S. Al Shunaifi, M.A. Ullah, M. Muddassir and M.A. Noor. 2019. Farmers' perceptions regarding the use of information and communication technology (ICT) in khyber pakhtunkhwa, northern pakistan. *J. Saudi Soc. Agric. Sci.*, 18(2): 211-217.

AL-Huqail, A.A., P. Kumar, E.M. Eid, B. Adelodun, S. Abou Fayssal, J. Singh, A.K. Arya, M. Goala, V. Kumar and I. Siric. 2022. Risk assessment of heavy metals contamination in soil and two rice (*Oryza sativa* L.) varieties irrigated with paper mill effluent. *Agriculture*, 12(11): 1864.

Ali, H., F.M. Abbasi and H. Ahmad. 2016. Bacterial blight, a serious threat to productivity of rice (*Oryza sativa* L.), an overview. *Int. J. Biosci.*, 9(1): 154-169.

Ali, H., F.M.K. Abbasi, Abdullah, M.A. Khan, I. Ullah, A. Zeb and A. Sarwar. 2016. Identification of fragrance gene in some elite advance lines of rice cultivated in foothills of the himalayas. *Int. J. Biosci.*, 8(1): 47-54.

Anonymous. 2013. Standard evaluation system for rice.: International rice research institute. (Manila, Philippines), *PO Box*., 933: 1099.

Bashir, M.U., N. Akbar, A. Iqbal and H. Zaman. 2010. Effect of different sowing dates on yield and yield components of direct seeded coarse rice (*Oryza sativa* L.). *Pak. J. Agri. Sci.*, 47(4): 361-365.

Chaudhri, M. 1986. Problems and prospects of rice cultivation in pakistan. *Progressive farming*, 6: 6-11.

Deng, Y., K. Zhai, Z. Xie, D. Yang, X. Zhu, J. Liu, X. Wang, P. Qin, Y. Yang and G. Zhang. 2017. Epigenetic regulation of antagonistic receptors confers rice blast resistance with yield balance. *Science*, 355(6328): 962-965.

Ezuka, A. and H. Kaku. 2000. A historical review of bacterial blight of rice. *Bulletin of the national institute of agrobiological resources,* (15): 1-207.

Fang, Y. and R.P. Ramasamy. 2015. Current and prospective methods for plant disease detection. *Biosensors*, 5(3): 537-561.

Fitzgerald, M.A., S.R. McCouch and R.D. Hall. 2009. Not just a grain of rice: The quest for quality. *Trends in plant sci.*, 14(3): 133-139.

Gul, A., W. Xiumin, A.A. Chandio, A. Rehman, S.A. Siyal and I. Asare. 2022. Tracking the effect of climatic and non-climatic elements on rice production in pakistan using the ardl approach. *Environ. Sci. Pollut. Res.,* 1-15.

Guo, M., J. Lan, J. Shi, M. Guan, J. Wei, L. Liu, L. Li, S. Dou and G. Liu. 2015. Western blot detection of *xanthomonas oryzae* pv. *oryzae* in rice. *J Plant Pathol. Microbiol S.*, 4: 1-6.

Jiang, N., J. Yan, Y. Liang, Y. Shi, Z. He, Y. Wu, Q. Zeng, X. Liu and J. Peng. 2020. Resistance genes and their interactions with bacterial blight/leaf streak pathogens (*Xanthomonas oryzae*) in rice (*Oryza sativa* L.)—an updated review. *Rice*, 13(1): 1-12.

Jonit, N., Y. Low and G. Tan. 2016. *Xanthomonas oryzae* pv. *oryzae*, biochemical tests, rice (oryza sativa), bacterial leaf blight (BLB) disease, sekinchan. *Appl. Environ. Microbiol.*, 4: 63-69.

Kaur, L. and S.G. Sharma. 2021. Identification of plant diseases and distinct approaches for their management. *Bull. Natl. Res. Cent.,* 45: 1-10.

Khan, J.A., H.M.I. Arshad, F.F. Jamil and S. Hasnain. 2009. Evaluation of rice genotypes against bacterial leaf blight (BLB) disease. *Pak. J. Phytopathol.*, 21(1): 26-30.

Khoa, N.Đ., T.V. Xạ and L.T. Hào. 2017. Disease-reducing effects of aqueous leaf extract of kalanchoe pinnata on rice bacterial leaf blight caused by *xanthomonas oryzae* pv. *Oryzae* involve induced resistance. *Physiol. Mol. Plant Pathol.*, 100: 57-66.

Lang, J.M., J.P. Hamilton, M.G.Q. Diaz, M.A. Van Sluys, M.R.G. Burgos, C.M. Vera Cruz, C.R. Buell, N.A. Tisserat and J.E. Leach. 2010. Genomics-based diagnostic marker development for *xanthomonas oryzae* pv. *Oryzae* and *x. Oryzae* pv. *Oryzicola*. *Plant Dis.*, 94(3): 311-319.

Mew, T. and A. Majid. 1977. Bacterial blight of rice in pakistan. *IRRN.*, 2(1): 5.

Miura, K., M. Ashikari and M. Matsuoka. 2011. The role of qtls in the breeding of high-yielding rice. *Trends Plant Sci.*, 16(6): 319-326.

Mubassir, M., K.M. Nasiruddin, N.H. Shahin, S.N. Begum, M.K. Saha and A. Rashid. 2016. Morpho-molecular screening for bacterial leaf blight resistance in some rice lines and varieties. *J. Plant Sci.*, 4(6): 146-152.

Nawaz, A., J.A. Tariq, A.M. Lodhi and R.M. Memon. 2020. Studies on characteristics of *xanthomonas oryzae* isolates associated with rice crop. *J. Appl. Res. Plant Sci.*, 1(1): 30-35.

Nogales, B., E.R. Moore, E. Llobet-Brossa, R. Rossello-Mora, R. Amann and K.N. Timmis. 2001. Combined use of 16s ribosomal DNA and 16s rRNA to study the bacterial community of polychlorinated biphenyl-polluted soil. *Appl. Environ. Microbiol.*, 67(4): 1874-1884.

Noreen, R., S. Khan, A. Rabbani and A. Kanwal. 2020. Screening of different rice (*Oryza sativa* L.) varieties for genetic diversity and bacterial blight resistance gene. *Pak. J. Bot.*, 52(3): 1087-1096.

Patel, J.B. 2001. 16s rrna gene sequencing for bacterial pathogen identification in the clinical laboratory. *J. Mol. Diagn.*, 6: 313-321.

Ponciano, G., H. Ishihara, S. Tsuyumu and J.E. Leach. 2003. Bacterial effectors in plant disease and defense: Keys to durable resistance? *Plant Dis.*, 87(11): 1272-1282.

Sabar, M., T. Bibi, H.U. Farooq, Z. Haider, I. Naseem, A. Mahmood and M. Akhter. 2016. Molecular screening of rice (*Oryza sativa* L.) germplasm for *xa4, xa5* and *xa21* bacterial leaf blight (BLB) resistant genes using linked marker approach. *Afr. J. Biotechnol.*, 15(41): 2317-2324.

Salim, M., M. Akram, M. Akhtar and M. Ashraf. 2003. Rice, a production hand book. *Pakistan Agricultural Research Council, Islamabad*, 70.

Samanta, T.T., P. Samanta and A. Das. 2014. Isolation and characterization of *xanthomonas oryzae* isolates from different regions of midnapore district of west bengal and their ecofriendly management by some medicinal plant extracts. *Int. J. Phytomed*., 6 (1): 29-42.

Shivalingaiah, U.S. and M. Sateesh. 2012. Detection of *xanthomonas oryzae* pv. *Oryzae* in rice seeds by molecular techniques. *Asian Australas. J. Biosci. Biotechnol.,* 6: 44-47.

Sudir, S. and D. Yuliani. 2016. Composition and distribution of *xanthomonas oryzae* pv. *Oryzae* pathotypes, the pathogen of rice bacterial leaf blight in indonesia. *AGRIVITA, J Agric Sci.*, 38(2): 174-185.

Swings, J., M. Van den Mooter, L. Vauterin, B. Hoste, M. Gillis, T. Mew and K. Kersters. 1990. Reclassification of the causal agents of bacterial blight (*xanthomonas campestris* pv. *Oryzae*) and bacterial leaf streak (*xanthomonas campestris* pv. *Oryzicola*) of rice as pathovars of *xanthomonas oryzae* (ex ishiyama 1922) sp. Nov., nom. Rev. *Int. J. Syst. Evol. Microbiol.*, 40(3): 309-311.

Ullah, I., H. Ali, M. Islam, W. Ullah, M. Haris, M.Q. Khan, K.K. Shafiq-ur-Rehman, K. Khan and B. Ghani. 2020. Molecular analysis of bacterial blight resistance gene *Xa*7 in advance population of rice using sts markers. *Int. J. Biosci.*, 17(2): 1-10.

Ullah, I., H. Ali, T. Mahmood, M.N. Khan, M. Haris, H. Shah, A. Mihoub, A. Jamal, M.F. Saeed and R. Mancinelli. 2023. Pyramiding of four broad spectrum bacterial blight resistance genes in cross breeds of basmati rice. *Plants*, 12(1): 46.

Villareal, C.P., N.M. De La Cruz and B.O. Juliano. 1994. Rice amylose analysis by near-infrared transmittance spectroscopy. *Cereal Chem.*, 71(3): 292-296.

Waheed, M., A.H. Inamullah, A.H. Sirajuddin, A. Khan and A. Khan. 2009. Evaluation of rice genotypes for resistance against bacterial leaf blight. *Pak. J. Bot.*, 41(1): 329-335.

Xu, D., Z. Shen, C. Dou, Z. Dou, Y. Li, Y. Gao and Q. Sun. 2022. Effects of soil properties on heavy metal bioavailability and accumulation in crop grains under different farmland use patterns. *Sci. Rep.*, 12(1): 9211.

(Received for publication 16 November 2022)