Short Communication
Advancements in Life Sciences – International Quarterly Journal of Biological Sciences

ARTICLE INFO

Date Received: 15/01/2021;
Date Revised: 24/04/2022;
Date Published Online: 31/07/2022;

Authors' Affiliation:
1. Department of Biotechnology, Virtual University, Lahore - Pakistan
2. Chughtai Laboratory, Lahore - Pakistan
3. University of Central Punjab Lahore - Pakistan
4. Centre of Excellence in Molecular Biology, University of the Punjab, Lahore - Pakistan

***Corresponding Author:**
Ishrat Aziz
Email: Ishrat.aziz@vu.edu.pk

How to Cite:
Aziz I, Ali S, Rehman Z, Riazuddin S, Shahid AA (2022). Biological movement of Xanthomonas oryzae pv. oryzae in Pakistan. A pioneer project of CEMB, Punjab, Pakistan. Adv. Life Sci. 9(2): 231-234.

Keywords:
Bacterial blight; *Xanthomonas oryzae*; Germ plasm; Genetic diversity' SAARC

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Biological movement of *Xanthomonas oryzae* pv. *oryzae* in Pakistan; A pioneer project of CEMB, Punjab, Pakistan

Ishrat Aziz*[1], Saadat Ali[2], Zia Rehman[3], Sheikh Riazuddin[4], Ahmad Ali Shahid[4]

Abstract

Background: In Asia, germ plasm exchange in different ecosystems, has been observed. This exchange causes movement of different pathogens. In present study, we worked on movement of *Xanthomonas oryzae* pv. *oryzae* (Xoo) causing bacterial blight of rice. In 1976, this disease was first recorded from Punjab (Pakistan) but the cultivars of that time showed resistance against bacterial blight. Then in 1984, the disease was seen on IRRI9. Tremendous increase of this pathogen has become prevalent due to increase in demand of Basmati 385 (cultivar), and now it is one of the most important rice diseases in Pakistan. Hypothesis of present study is "there is genetic diversity of Xoo in different rice growing areas of Punjab, Pakistan."

Methods: By fingerprinting; the movement pattern of *Xanthomonas oryzae* pv. *oryzae* were searched out in present study. The IS1112; a repetitive element of *Xanthomonas oryzae* pv. *oryzae* was used to fingerprint twenty-one bacterial strains.

Results: We compared local strains with that of provided by IRRI Philippine (International Rice Research Institute, Philippine). The obtained clusters were correlated with regional differentiation.

Conclusion: Present reported work is the pioneer study (1998-2000) which clued the occurrence of regional movement of pathogen via germ plasm exchange. This study may also provide help to forensics to watch the bioterrorism.

Introduction

The endemic disease bacterial blight of rice caused by the pathogen *Xanthomonas oryzae* pv. *oryzae*, is widespread in many parts of Asia. Primarily, it was controlled by using resistant cultivars. However, virulent pathogen strains overcame resistant cultivars of that time [1]. For development of germ plasm resistance, knowledge about dynamics and structure of local as well as distant population of pathogen is important. It is also important to have information about migration of pathogen. Because immigrant genotype is alien to our local pathogen control system. After studying different Asian ecosystems (India, China, Indonesia, Korea, Malaysia, Philippine and Nepal), geographic variations of bacterial blight pathogen have been observed [2].

Bacterial blight was first recorded in 1976 from Punjab, Pakistan. It is considered that the resistant cultivars of that time had shown no prominent effect. Only the IRRI9 had shown the visible effect in 1984. Tremendous increase of the said pathogen has become prevalent due to increase in demand of Basmati 385, and now it is one of the most important rice diseases in Pakistan [3]. Later in 2007, Muneer *et al.,* have collected different rice cultivar samples from lower Dir, Swat and Agricultural Research Station, Mingora in (Khyber Phkhtun Khuah) during 2002, and isolated *Xanthomonas oryzae* [4]. They also found genetic variability. In 2017, A mega project was started in Punjab, Pakistan [5] and confirmed pathogenic diversity of *Xanthomonas oryzae* isolates. They found that 39 isolates were widespread in 12 districts. From Bangladesh, Alam *et al.,* has also reported the genetic variability of *Xanthomonas oryzae* isolates [6].

We have not only we worked on genetic diversity of *Xanthomonas oryzae* in Pakistan but also compared the results with internationally established strain of Xoo (*Xanthomonas oryzae*) for understanding the structure of genetic diversity in vast geographical scenario. A model strain (c2) was arranged (IRRI, Philippine). For achieving economic viability, simplification in the experimental steps was also an important objective of our study.

Methods

Collection of Bacterial Blight (BB) infected rice leaf samples

Infected rice leaves (BB) were collected from Basmati 385 fields of Punjab, Pakistan. Typical symptoms of BB (dry leaf whorls and amber colored ooze outs *etc*.) were verified. From each location most infected leaf was collected, cataloged, brought to center (CEMB) and preserved in specified refrigerator. A total of two hundred leaves were collected, isolates were purified which after molecular biology steps and statistical analysis restricted to twenty-one strains.

Bacterial isolates, culture media and isolation of genomic DNA

For Isolation of the pathogen *Xanthomonas oryzae* pv. *oryzae*, the amber colored oozed outs from each leaf were composited, rinsed with sterile deionized water and grown as sets of three in Nutrient Broth (Difco). Bacterial growth was achieved by Culturing bacteria at 28°C (48 hours). The obtained opaque bacterial growth was streaked on Luria Broth agar plates and incubated at 28°C (24 hours).

From these plates; single bacterial colony was taken and each selected colony was homogenized by using 650 µl lysis buffer (Tris;100 mM-pH 8; EDTA; 100 mM-pH 8, NaCl; 250 mM, Sodium Dodecyl Sulfate 1% and Polyvinyl Pyrrolidone 1% - molecular weight 40,000). Incubation was done at 65°C (30 min). 100 µl of potassium acetate (3M- potassium and 5M- acetate, pH 4.8) was used to remove cellular proteins. DNA was precipitated by using equal amount of isopropanol and air dried. The DNA was dissolved in sterile- distilled water and quantified.

Achievement of economic viability (direct DNA extraction from infected leaves)

For achieving economic viability, DNA from bacteria were directly amplified by leaching it from infected leaves. Leaf fragments (2 cm in length) with advanced lesion tips were cut into small pieces (with flame sterilized scissors), soaked in sterile distilled water (400 µl) for 60 min. After 1 hour, the leaf fragments were disposed, and the bacterial cells were obtained. The cells were concentrated by centrifugation at 10,000 × g (2 min). Extra water was removed. Left over solution (25 µl) having suspended cells was treated with 70% ethanol (200 µl). Spun for 10 seconds. Cell debris was pellet out. After transferring the supernatant to a new tube, content was spun for 5 minutes. DNA was precipitated, dissolved in 10 µl of sterile water. 5 ml of DNA solution was used as template for PCR.

Oligonucleotide synthesis and polymerase chain reaction (PCR)

The sequence of the repetitive element IS1112 (Yun, 1991) was used for oligonucleotide synthesis (software: PC/GENE of IntelliGenetics; Mountain View; CA). Two primers (JEL1 5′-CTCAGGTCAGGTCGCC-3′) and (JEL2 5′-GCTCTACAATCGTCCGC-3′) were designed (from each end of IS1112). Opposite orientation was followed (3′ ends were directed outwards from each element).

PCR amplification in a 25-µl volume was performed. Each of two opposite primers (0.5 µM), genomic DNA (20 ng), Each of four dNTPs (185 µM), Taq polymerase (2.5

U) and incubation buffer (Tris (100 mM) pH 8.3; KCl (500 mM); MgCl2 (1.5 mM), and gelatin (0.01% wt/vol). Dimethyl sulfoxide (10% vol/vol) and 1 M Tris pH 9.5 (7.5 µl) was added for long DNA fragments amplification (Cheng *et al,* 1994). For avoiding evaporation, reaction mixture was overlaid by one drop of mineral oil addition. Denaturation in PCR was done for 1 min (94°C). Subjected to 30 cycles of PCR (10 seconds denaturation; 94°C, 1 minute annealing; 62°C and 10 minutes extension; 65°C). Final extension was done for 15 minutes (65°C) in DNA thermal cycler of PerkinElmer Cetus, Foster City, CA. Each experiment was repeated twice.

Achievement of economic viability (direct PCR of bacterial exudate from infected leaves)

For achieving economic viability, PCR was done directly from DNA of bacterial exudates (leached from lesions). So, the chemicals, time and human efforts involved in isolation and cultivation of the bacteria were reduced. The total process shrank from six to two days which may help in future use of present study for taking quarantine measurements and forensics.

DNA fingerprints

Product of Polymerase Chain Reaction (10 µl) was used for generating DNA fingerprints. Three techniques were compared for best resolution of DNA fingerprints.

1. 20 µl of PCR product was loaded in the gel (0.5% agarose and 0.75% Synergel). Electrophoresis conditions were: 0.5x Tris-borate buffer; 125 V for 6 hours; staining with ethidium bromide. Prints of gel photographs (20 x 25 cm) facilitated the scoring no. of bands (regardless of intensity).20-40 fragments were detected.
2. 20 µl of PCR product was loaded in the gel (2% agarose). Electrophoresis conditions were: 0.5x Tris-borate buffer; electrophoresis on 125 V for 6 hours; staining with ethidium bromide). 20-40 fragments were detected.
3. 5 µl of PCR product was loaded in the gel (10% polyacrylamide). Electrophoresis conditions were: 0.5x Tris-borate buffer; gels were run for about 25 cm, at 45 W, stained by silver staining and photographed. More than 80 fragments were detected through PAGE.

Numerical analysis of data and dendrogram formation

Reference sample (C2) of IRRI, Philippine was used for comparison. DNA fragments generated by PAGE were used for the banding pattern studies. Which was coded in binary form; presence of band was coded by 1 and the absence of band in comparison to C2 was coded by 0 (Yap, 1996). An index of genetic distance was calculated by using the unweighted pair-group method with arithmetic averages (UPGMA) method.

Results

Main objectives were achieved as given under:

1. Properly planned survey of Xoo affected rice growing areas of the Punjab, Pakistan was done, and diseased samples were collected.
2. For achieving economic viability, simplification in the experimental steps was done.

Based on bands similarity, twenty-one representative banding patterns were used for numerical analysis and comparison with IRRI strain. Dendrogram pattern was obtained and compared with IRRI strain C2 (fig.1).

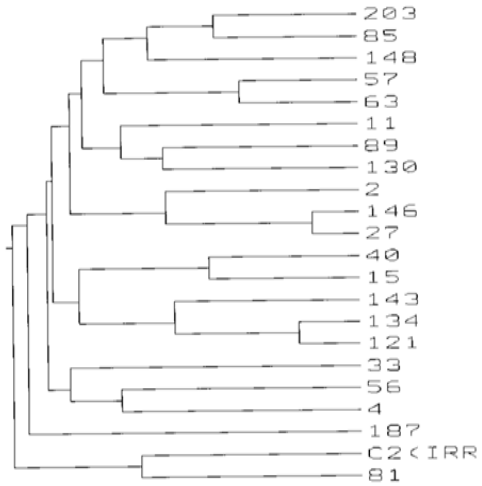

Figure 1: Dendrogram: Figure 1: Dendrogram showing genetic diversity of Xanthomonas oryzae pv. oryzae (Pakistani Xoo strains in comparison to the control strain from IRRI, Philippines). It could be seen in the dendrogram that sample No. 81 does not cluster with any other local isolate and is easily distinguishable along C2 (IRRI). Sample No. 187 is also quite distinct from rest of samples.

Discussion

In Asia, germ plasm exchange in different ecosystems, has been observed. This exchange causes movement of different pathogens [2]. In the present study, we worked on movement of *Xanthomonas oryzae pv. oryzae* causing bacterial blight of rice. We have done DNA fingerprinting of *Xanthomonas oryzae pv. oryzae* for understanding the movement pattern. We compared local strains with that of provided by IRRI Philippine (International Rice Research Institute, Philippine).

Dendrogram was generated by UPGMA (Unweighted pair group method and arithmetic means) analysis in order to determine the grouping of Xoo isolates. In the

dendrogram sample no. 81 does not cluster with any other local isolate and is easily distinguishable along C2 (IRRI). Isolates belonging to sample no. 81 were collected from Kala shah Kaku. Sample no. 187 is also quite distinct from rest of samples. Sample no. 15 is more than 85% similar to most of local varieties. Interestingly, sample no. 15 was also collected from Kala shah Kaku and the latter from another location, (Mureedkay). This fact may provide us a clue about dispersal path of disease. The coefficient of similarity of most of the other isolates ranges between 70% to 90%. From the dendrogram, two clusters represented as A and B, are very distinct. 20 isolates are in cluster A, they are all local isolates. In cluster B, there are 2 isolates and their similarity range from 43.5% to 99.9%. These results are according to present hypothesis, which states that, there is genetic diversity of Xoo in different rice growing areas of Asia.

Same close relation has also been found by George *et al*., who compared Philippines and Indonesian strains of Xoo, 42% similarly level was observed [7]. Strain collected from Philippines made five clusters, only one cluster was formed by strains collected from Indonesia. While one cluster included the strains collected from both countries.

Present study is evident for the movement of strains between countries. South Asian Association for Regional Cooperation (SAARC) is the forum which can actively guide the regional cooperation in resistance disposition of seeds and in taking quarantine measures. It may also be helpful in forensics field for focusing bioterrorism. The utility of our adopted and modified methods may help in the identification of sequences unique to the pathogen and could be useful for routine seed testing.

Competing Interest

The author declares that there is no conflict of interest regarding the publication of this paper.

Funding

We are thankful to Centre of Excellence in Molecular Biology for whole funding of this research.

Acknowledgements

We are thankful to Dr. Naz Abbas for her technical guidance. We are also thankful to Dr. John Bennett, Philippine for provision of *Xanthmonas oryzae pv. Oryzae* strain (IRRI) for comparison of genetic diversity of our local stains from that of Philippine and Dr. M. Akhter (RRI, KSK, Lahore).

Authors' Contribution

Conception or design of the work: Sheikh Riaz ud Din
Data collection: Ishrat Aziz
Data analysis and interpretation: Ishrat Aziz
Drafting the article: Ishrat Aziz, Saadat Ali
Critical revision of the article: Zia-ur-Rehman
Final approval of the version to be published: Ahmed Ali Shahid

References

1. Mew T. Current status and future prospects of research on bacterial blight of rice. Annual review of phytopathology, (1987); 25(1): 359-382.
2. Adhikari TB, Cruz C, Zhang Q, Nelson R, Skinner D, et al. Genetic diversity of Xanthomonas oryzae pv. oryzae in Asia. Applied and Environmental Microbiology, (1995); 61(3): 966-971.
3. Mahajan G, Matloob A, Singh R, Singh VP, Chauhan BS. Basmati rice in the Indian subcontinent: Strategies to boost production and quality traits. Advances in Agronomy, (2018); 151159-213.
4. Muneer N, Rafi A, Akhtar MA. Isolation and characterization of Xanthomonas oryzae pv. oryzae isolates from North West Frontier Province (NWFP), Pakistan. Sarhad Journal of Agriculture, (2007); 23(3): 743.
5. Arshad HMI, Saleem K, Khan JA, Rashid M, Atiq M, et al. Pathogenic diversity of Xanthomonas oryzae pv. oryzae isolates collected from Punjab Province of Pakistan. European journal of plant pathology, (2017); 147(3): 639-651.
6. Alam MS, Islam MR, Hossain I, Bhuiyan M, Khan M. Pathotypic variation of Xanthomonas oryzae pv. oryzae in Bangladesh. Archives of Phytopathology and Plant Protection, (2016); 49(1-4): 31-42.
7. George M, Bustamam M, Cruz W, Leach J, Nelson R. Movement of Xanthomonas oryzae pv. oryzae in Southeast Asia detected using PCR-based DNA fingerprinting. Phytopathology, (1997); 87(3): 302-309.