PSM Microbiology ISSN: 2518-3834 (Online) – An International Peer-reviewed Journal

Research Article 2018 | Volume 3 | Issue 2 | 55-61

Article Info

Open Access
Citation: Sandhu, A.F., Khan, J.A., Ali, A., Arshad, H.M.I., Saleem, K., 2018. Molecular Characterization of *Xanthomonas oryzae pv. oryzae* Isolates and its Resistance Sources in Rice Germplasm. PSM Microbiol. 3(2): 55-61.

Received: April 24, 2018

Accepted: June 30, 2018

Online first: July 1, 2018

Published: July 15, 2018

***Corresponding author:**
Ahmad Faraz Sandhu;
Email:
ahmadfarazsandhu@hotmail.com

Scan QR code to see this publication on your mobile device.

Molecular Characterization of *Xanthomonas oryzae* pv. *oryzae* Isolates and its Resistance Sources in Rice Germplasm

Ahmad Faraz Sandhu[1]*, Junaid Ahmad Khan[2], Safdar Ali[1], H. M. Imran Arshad[3], Kamran Saleem[3]

[1]Department of Plant Pathology, University of Agriculture Faisalabad, Pakistan.
[2]Pakistan Institute of Engineering and Applied Sciences, Islamabad, Pakistan.
[3]Plant Protection Division, Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan.

Abstract
Bacterial leaf blight (BLB) of rice, the most notorious and horrendous disease of rice, caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) andcontinue to evolve covering more area and rice cultivars. Keeping in view the escalating virulence in pathogen population, current study was conducted to determine the genetic diversity in pathogen population and to search new sources of resistance in rice germplasm. The samples collected during the survey from different rice growing areas of Punjab were provided by the Rice Pathology group of NIAB. Then these samples were plated to isolate and purify the pathogen causing bacterial blight disease *Xanthomonas oryzae* pv. *oryzae.* The genetic diversity of the pathogens from different areas of the Punjab was determined through the repetitive DNA element with primers JEL[1] and JEL[2]. During this study, twenty seven (27) varieties/lines were screened for resistance under artificial inoculation conditions against BLB. Plants were artificially inoculated by clipping method with the *Xoo* culture. After isolation and purification of pathogen, 9 isolates were selected for genetic diversity. DNA-Fingerprinting of 9 local isolates of *Xoo* generated with IS1112 based primers Jel[1]and Jel[2,] reveal great variability among themselves. The dendrogram showed two main groups A and B. In group A isolates 1, 3 and 5 have 86% similarity while in group B isolates 9 and 8 are 100% similar and 90% similar to 7 and isolate 6 is 87% similar to 4. Where isolate 2 is 82% similar to isolates 4,6,7,8 and 9. In comparison of group A and B they are 65% similar to each other. In search of resistance source against BLB, none of the variety/line out of 27 was found immune, highly resistant, resistant or moderately resistant to bacterial leaf blight disease. Only one variety/line was moderately susceptible, five were susceptible and twenty one were highly susceptible to BLB disease. The results of this study seem to be very helpful for deploying effective management of BLB disease. The information can be utilized in controlling the bacterial leaf blight disease in Pakistan that can save the economic and production losses of rice crop that are increasing every year.
Keywords: Virulence, Rice, Disease, Yield, Resistance.

INTRODUCTION

Among the cereals, rice and wheat share equal importance as leading food source for mankind. Rice has fed more people over a longer period of time than any other crop. Approximately 90% of the world's rice is grown in Asia continent and constitutes a staple food for 2.7 billion people worldwide (Salim *et al.,* 2003). Basmati rice is the premier food grain crop of Pakistan for domestic consumption and for exported. In Pakistan agrarian economy, rice plays multifarious role: it is 2nd staple food and contribute more than 2.5 million tones to our national food requirement. Annual per capita consumption of rice is 14 Kg/year in Pakistan (Economic Survey of Pakistan, 2009-10). Rice is an important staple food and cash crop of Pakistan. Rice industry is an important source of employment and income for rural people. Pakistan is the 5th largest exporter of rice in the world. It contributes 15 % to the total exchange earning of Pakistan (Anonymous, 2009).

The rice crop is affected to more than thirty five diseases (fungal, bacterial and viral), which are one of the factors, for low yield of rice in the world. The diseases may appear at any stage of the growth and development of plant, attacking the seed sown, root system, foliage, stalk, leaf sheath, inflorescence and even the developing grain. All diseases are injurious in some areas, in some years and on some plant parts. All parts of plant are subject to disease and one or more diseases can occur on virtually every plant and in every field. All draw attention because of symptoms or signs and generate great concern because of their effects on the quality and quantity of plants, straw or grain. Bacterial leaf blight is one of the most destructive diseases of rice. This disease reduces grain yield to varying levels depending on the stage of the crop, degree of cultivar susceptibility/tolerance and a great extent to the conduciveness of the environmental conditions. BLB of rice can cause crop yield losses up to 50 % (Ou, 1985).

This disease is caused by a gram negative bacterium *Xanthomonas oryzae* pv. *oryzae* that belongs to family Xantho*monadaceae*. It was first recorded by the farmers in Japan in 1884. Crop loss assessment studies have revealed that this disease reduces grain yield to varying levels, depending on the stage of the crop, degree of cultivar's susceptibility and to great extent, the conduciveness of the environment in which it occur (Narayanan *et al.,* 2002). Subsequently, its incidence has been reported from different parts of Asia, Northern Australia, Africa and USA. In Japan yield losses normally ranges between 20-25 %, occasionally increasing up to 50% (Ou, 1985). BLB disease was reported for the first time in Pakistan in 1977 (Mew and Majid, 1977) and later on its occurrence was confirmed from all the provinces (Akhtar and Akram, 1987). BLB is increasing in "Kaller" belt that is famous for producing high quality rice and in 1997, Muridke, Narang, and adjoining areas were infected with BLB in patches showing 5-10 percent disease. BLB disease severity was found upto 40-50 % in Dhinga and Farkandabad and in some fields of the nearby villages upto 70-95 % infection was observed (Khan *et al.,* 2000).

Bacterial leaf blight of rice (BLB) is a devastating disease in rice growing areas of Pakistan (Islam *et al.,* 2016). Bacterial leaf blight (BLB) of rice created a serious situation in rice during the cropping year of 2002 in Pakistan. It affected the crop at all stages and showed either "Kresek" or leaf blight symptoms. A survey was conducted during the cropping year 2002 in Punjab, Sindh, Balochistan, NWFP and Azad Jammu & Kashmir to study the latest situation of this menace, in which high incidence of the disease was reported in all over the surveyed areas (Akhtar *et al.,* 2003). Rising trend of BLB is associated with change in the genetics of pathogen population and it provides the base of resistance cultivar development. In the current perusal both aspects i.e. genetic diversity and identification of resistance sources were unraveled.

MATERIALS AND METHODS

Collection of diseased samples

Surveys to collect the BLB infected leaves samples were conducted in 2009 during the rice season in different areas of Punjab, Pakistan. Diseased plants with BLB were identified by the visual observation of yellow to white stripes at the margins of infected leaves. Samples were placed in brown envelops and brought in fungal and bacterial diseases group laboratory, NIAB, Faisalabad.

Isolation and purification of pathogen

Infected leaves were cut into small pieces with the help of scissor and placed on nutrient agar (NA) plates and left overnight at 30^0C in incubator for the isolation of *Xanthomonas oryzae* pv. *oryzae*. Yellow colonies appeared on plates with the margins of placed samples. Single colonies were taken with the help of a loop and purified on fresh NA plates. Gram's staining was done of the isolates for the confirmation of the bacterium as negative and rod shaped. The isolated isolates were then transferred to slants for further use and long term preservation. Some biochemical tests like methyl red, citrate utilization, voges-proskaures and indole production for further confirmation. Biochemical tests were performed following Bergey's Manual of Determinative Bacteriology (Bergey, 1984; Iqbal *et al.,* 2015; Yunus *et al.,* 2016; Jabeen *et al.,* 2016). Pathogenecity of the isolates were checked by artificial inoculation in glass house to prove the Koch's postulates. Rice seeds were sown in pots and at three leaf stage they were artificially inoculated with the inoculum of the bacterium prepared in nutrient broth (2% nutrient broth in 30ml distilled water).

DNA extraction of *Xoo* isolates

The 9 cultures of purified *Xoo* isolates, representing different location, were grown in NB with 10 % glycerol (in replicate), for 72 hrs on shaker at 30° C at room temperature. 1.5 ml 72 hrs *Xoo* culture (in NB medium) was taken in eppendorf tubes, centrifuged at 13,000 xg for 5 min and supernatant was discarded and pellets were air dried. 200 ul of Tris 0.5M was added in each tube and dissolved the pellet by gentle tapping. When pellets were dissolved, 200 ul of lysis buffer was added and tubes were left for 5-6 min for lysis procedure. Then 700 µl of chloroform and Isoamyl alcohol (24:1) was added in eppendorf tubes and centrifuged again at 13,000 xg for 10 min. Supernatant was taken and 95% ethanol was added in each tube again centrifuged for 10 minutes to get DNA pellets. DNA palette was washed with 70% ethanol and centrifuged as above again supernatant was discarded and samples were allowed to dry at room temperature. 200 µl distilled water was added and left overnight at 4°C in incubator. 0.8 gm agarose gel was prepared in 100 ml TBE buffer and poured in casting tray to allow it to polymerize. After half an hour 10ul of samples along with 2 ul of loading dye were loaded in the wells. After complete running of gel, samples were stained with Enthidium bromide and DNA documentation was done. After documentation 200 ul of acetate solution was added in each tube and centrifuged at 14,800 rpm. Discard the supernatant, added 200 ul of Isopropanol again centrifuged at 14,800 rpm. Again discarded the supernatant and 200 ul of autoclaved water was added and stored at 4 ^{0}C for further analysis.

Polymerase chain reaction (PCR)

Required volume of reagent mixture (Master mix. 12.5ul, PCR water. 4.5ul, Taq polymerase 0.5ul, Primer 1(P^{JEL}1, 5/CTCAGGTCAGGTCGCC-3/) and Primer 2 (P^{JEL}2, 5/GCTCTACAATCGTCCGC-3/) each of 1.25ul having 5ul of the DNA sample were mixed in PCR tubes and placed in PCR apparatus. Programme of PCR machine was adjusted to initially denaturation for 1 min at 94^{0}C and then subjected to 30 cycles of PCR (10 sec denaturation at 94^{o}C, 1 min annealing at 62^{0}C and 10-min extension at 65 ^{0}C) and a final extension for 15min at 65^{0}C. After the completion of PCR reaction, samples were loaded in 1.2% agarose gel along with loading dye. Electrophoresis was done at 90 Volt. The gel was stained with Enthidium bromide for 3-4 min and documented under UV-pro.

Statistical Analysis

With the help of STATISTICA software a tree diagram was drawn that showed the genetic distances within the isolates collected from different areas of Punjab, Pakistan.

Screening of rice varieties/lines against BLB disease

Seed nursery of twenty two varieties/lines along with five commercial varieties was sown to determine the resistance source against bacterial leaf blight disease in field conditions according to Randomized Complete Block Design (RCBD) along with control, spreader and border rows. Inoculum of Xoo was prepared in nutrient broth and kept at 37^{0}C for 48 hrs in shaker. Plants before their booting stage were artificially inoculated by dipping scissor in inoculum and one forth of top 3-4 leaves were cut with it. After 3 weeks of artificial inoculation data was recorded for the disease severity using the standard BLB disease rating scale as described in Table 1 (IRRI, 1993).

Table 1. Bacterial leaf blight of rice disease rating scale

BLB Severity	Score	Host Behvaiour
No incidence	0	Immune
Less than 1 %	1	Highly Resistant
1-3 %	2	Resistant
4-5%	3	Moderately
6-10 %	4	Resistant
11-15 %	5	Moderately Susceptible
16-25 %	6	
26-50 %	7	Susceptible
51-75 %	8	Highly Susceptible
76-100 %	9	

RESULTS

Isolation and Purification of Xoo isolates

In visual observation the colonies appeared on NA plates were circular, entire, convex, whitish yellow at first, yellow later. All isolates were confirmed to be Xoo after biochemical and pathogenicity tests. Among all purified Xoo isolates, 9 were selected based on different location for genetic diversity (Table 2).

Table 2. Xoo isolates used for genetic diversity along with their location.

Xoo Isolates	Locations
1	Pindi Machian
2	Harmoey Bangla
3	Sangla hill
4	Satiana
5	Chaniot
6	Phalia
7	Jandiala Sher Khan
8	Pakpatan
9	Mern Shah Gogaria

DNA Extraction from *Xoo*

By adopting the method for the extraction of DNA from the bacteria, DNA of the nine isolates was extracted. To confirm the extraction DNA samples were run in agarose gel (0.8 %) along with the loading dye at 75 Volt. After electrophoresis gel was stained with enthidium bromide and documented under UV-pro that showed the clear bands of the extracted DNA (Figure 1).

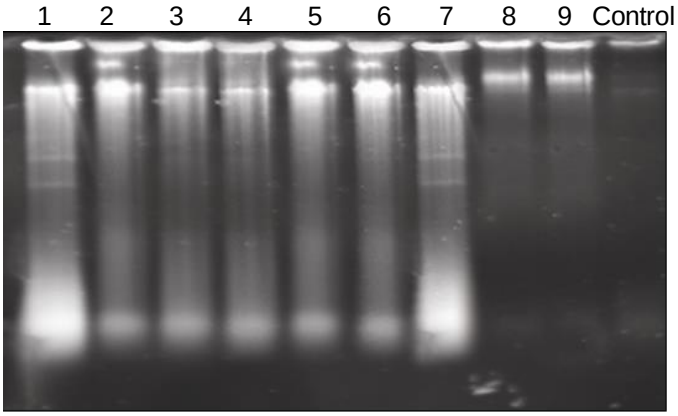

Fig. 1. Bands of DNA extracted from Isolates of *Xoo.* Sample 1-9 are described in table 2 and control having no DNA.

PCR Analysis and Genetic Diversity within the Isolates

After the confirmation of extraction of DNA from *Xoo* isolates, PCR analysis for amplification of DNA of all the isolate was done (George *et al.,* 1997). After completion of PCR reaction samples were again loaded on agarose gel and run at 85 Volt. After electrophoresis gel was stained with Enthidium bromide and documented under UV-pro. Different banding pattern showed DNA polymorphism among local population of *Xoo* isolates (Figure 2).

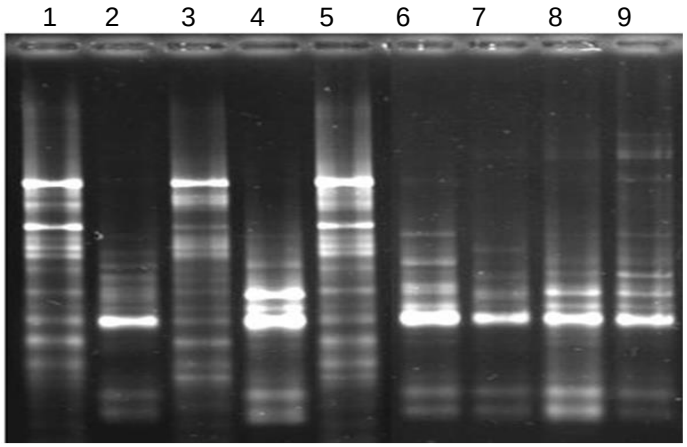

Fig. 2. Jel[1]and Jel[2] Primers based DNA Fingerprint of *Xoo*.

After documentation of the gel, cluster analysis was done (Figure 3). The tree diagram showed two main groups A and B. In group A isolates 1, 3 and 5 have 86% similarity where in group B isolates 9 and 8 are 100% similar and 90% similar to 7 and isolate 6 is 87% similar to 4. Where isolate 2 is 82% similar to isolates 4,6,7,8 and 9. In comparison of group A and B they are 65% similar to each other from different districts.

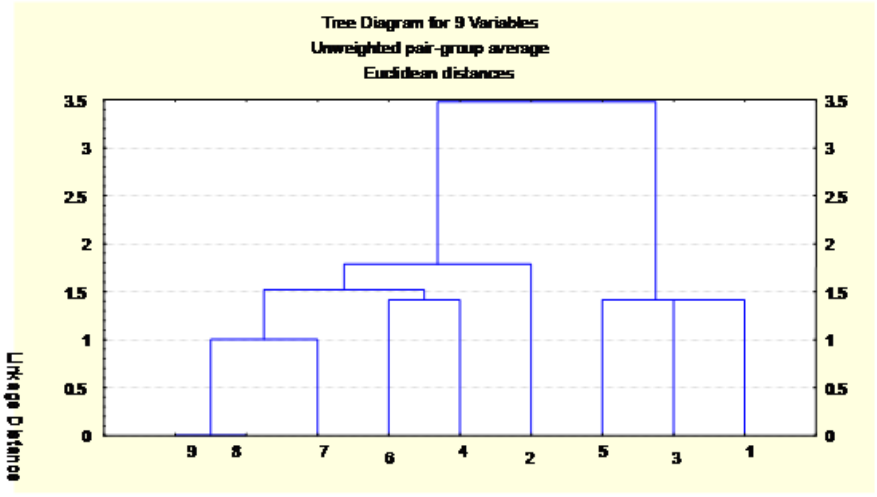

Fig. 3. Dendrogram of nine isolates of Xoo

Key: 1: Pindi Machian**, 2:** Harmoey Bangla, **3:** Sangla hill**, 4:** Satiana, **5:** Chaniot , **6:** Phalia, **7:** Jandiala Sher Khan**, 8:** Pakpatan, **9:** Mern Shah Gogaria

Screening of Rice varieties for resistance against BLB

None of the variety/line was found immune, highly resistant, resistant or moderately resistant against BLB disease (Table 3). Only one line (PS-2) was moderately susceptible, five varieties/lines (Basmati Pak, DM-1-30-34-99, P-2-02, MG Basmati and RSP-1) were susceptible and twenty one (Basmati 370, Basmati 385, Basmati super, Basmati 2000, EF-1-20-119-02, PK-7429-5-14-1-1, PK-7392-10-1-1-1-1, PK-790-9-4-1-1, PK-7375-2-3-7-1, JAJAI 25/A, GUARD-5015, KSK-431, KSK 433, KSK-436, KSK-439, IR-6-15/A, RSP-1, RSP-3, RSP-4, DR-65 and KSK-133) were highly susceptible to bacterial leaf blight disease. During the past years in the experiments no highly resistant or resistance varieties were found against this disease (Khan *et al.,* 2000).

Table 3. Response of varieties/lines against BLB disease.

Variety/line	R-1	R- 2	R-3	Mean Incidence	Response
Basmati-370	55	48	61	54.7	HS
Basmati-385	55	39	70	54.7	HS
Basmati-Super	39	57	62	52.7	HS
Basmati-Pak	44	48	55	49.0	S
Basmati-2000	67	55	84	68.7	HS
DM-1-30-34-99	39	55	47	47.0	S
EF-1-20-119-02	85	36	61	60.7	HS
P-2-02	40	18	45	34.3	S
PK-7429-5-14-1-1	55	56	83	64.7	HS
PK-7392-10-1-1-1-1	71	65	84	73.3	HS
PK-790-9-4-1-1	85	78	83	74.5	HS
PK-7375-2-3-7-1	75	70	62	76.0	HS
PS-2	14	25	35	24.7	MS
JAJAI 25/A	39	73	53	55.0	HS
MG Basmati	41	52	35	42.7	S
Guard-5015	53	67	60	60.0	HS
KSK-431	84	89	88	87.0	HS
KSK-433	95	82	73	83.3	HS
KSK-436	79	89	84	84.0	HS
KSK-439	91	83	73	82.3	HS
IR-6-15/A	62	77	70	69.7	HS
RSP-1	99	95	93	95.7	HS
RSP-2	44	31	48	41.0	S
RSP-3	96	91	89	92.0	HS
RSP-4	67	72	79	72.7	HS
DR-65	53	69	70	64.0	HS
KSK-133	93	89	91	91.0	HS

HS: Highly Susceptible S: susceptible MS: Moderately Susceptible
MR: Moderately Resistant R: Resistant HR: Highly Resistant

DISCUSSION

Purpose of present study was to isolate and confirmation of the bacterium *Xoo*, to find the resistance source to BLB and to determine the genetic diversity in the isolates of *Xanthomonas oryzae* pv. *oryzae* collected from different areas of Punjab, Pakistan. This work would help in reducing the loss of crop production caused by bacterial blight disease. The samples collected during the previous crop season from different rice growing areas of Punjab were provided by the Rice Pathology group of NIAB that helps me to conduct this study. Then these samples were plated to isolate the casual organism causing bacterial blight disease *Xanthomonas oryzae* pv. *oryzae.* Then these isolated cultures of *Xoo* were purified.

Over the past two decades, population analyses of *Xoo* have advanced from the description of variation to application of pathogen population genetics to disease management. The discovery of repetitive DNA elements in *Xoo* has provided the tools for analyses of genomic variation of the pathogen. Analysis of genetic diversity of bacteria through PCR has also been carried out by several workers throughout the world. The study conducted to determine the genetic diversity of the pathogens from

different areas of the Punjab through DNA polymorphism with the repetitive DNA element in JEL [1] and JEL[2] and they showed great variability among themselves. The results showed two main groups A and B. In group A isolates 1, 3 and five have 86% similarity where in group B Isolates 9 and 8 are 100% similar and 90% similar to isolate 7 and isolate 6 is 87% similar to isolate 4. Where isolate 2 is 82% similar to isolates 4,6,7,8 and 9. In comparison of group A and B on the whole similarity is 65%. This variability of the pathogen is due to some genetic factors and more often due to some environmental changes. Results are in line with other studies (Leach *et al.,* 1992) wherein 98 strains of *Xanthomonas oryzae* pv. *oryzae* for DNA polymorphism with the repetitive DNA element in JEL[101] were evaluated and twenty-seven distinct banding patterns were observed. Different studies reported different number of clusters of Xoo population depending upon the primer used. Like 5 clusters in Sri Lanka were reported through evaluation of 29 Xoo isolates (Ochiai *et al.,* 2000).

To find the resistance source against BLB disease twenty seven varieties/lines were tested against the disease during this study. None of the twenty seven varieties/lines showed immunity of resistance against the disease. Out of twenty seven varieties/lines one was moderately resistant, five susceptible and twenty one were highly susceptible. It was already reported in one study that most of the basmati rice varieties cultivated in Pakistan susceptible to bacterial leaf blight disease (Khan *et al.,* 2000). In the same year some 104 local rice varieties/lines for resistance to bacterial leaf blight pathogen (*Xanthomonas oryzae* pv. *oryzae*) were evaluated under field conditions at Kala Shah Kaku during 1996-98. None of the varieties/lines showed complete resistance to bacterial leaf blight (Tasleem *et al.,* 2000). In a recent study, 29 new pathotypes of Xoo were indentified which emphasized to find new sources of resistance (Arshad et al., 2017). Biochemical and pathological characterization of pathogen along with genetic diversity study is the baseline for deploying appropriate management strategies. Biochemical characterization of Xoo along with pathogenicity was also well documented previously (Arshad *et al.,* 2013) but along with current results of genetic diversity provides baseline for selection of resistant rice cultivars.

CONCLUSION

Host plant resistance has been the primary control strategy for this disease; however, resistant varieties grown over large areas are vulnerable to genetic adaptation by the pathogen population. So the study conducted and results generated are satisfactory and are being strengthened by the work of different scientists all over the world and there results are closely matched with the study conducted. Therefore this work done in my opinion can be utilized in controlling the bacterial leaf blight disease in Pakistan that can save the economic and production losses of rice crop that are increasing every year.

ACKNOWLEDGEMENT

We are thankful to Department of Plant Pathology, University of Agriculture Faisalabad, Pakistan, for supporting this study.

CONFLICT OF INTEREST

The authors declare that no competing interests exist.

REFERENCES

Akhtar, M.A., Akram, M., 1987. Incidence of bacterial blight of rice in the Punjab (Pakistan). Int. Rice Res. Newslett., (5): 5.

Akhtar, M.A., Zakaria, M., Abbasi, E.M., Masood, M.A., 2003. Incidence of bacterial blight of rice in Pakistan during 2002. Pak. J. Bot., 35(5): 993-997.

Anonymous, 2009a. IRRI World Rice Statistics (WRS). IRRI, Manila, Philippines. http://beta.irri.org/solutions/index.php?option=com_content&task=view&id=

Arshad, H.M.I., Naureen, S., Saleem, K., Ali, S., Jabeen, T., Babar, M.M., 2013. Morphological and biochemical characterization of *Xanthomonas oryzae* pv. *oryzae* isolates collected from Punjab during 2013. Adv. Life Sci., 2(3):125-130.

Arshad, H.M.I., Saleem, K., Khan, J.A. et al. 2017. Pathogenic diversity of *Xanthomonas oryzae* pv. oryzae isolates collected from Punjab Province of Pakistan. Eur. J. Plant Pathol., 147: 639. https://doi.org/10.1007/s10658-016-1032-5.

Bergey, S.A., 1984. Bergey, Manual of Determinative Bacteriology, 9th edition, Williams & Wilkins., Philadelphia.

Economic Survey of Pakistan. Agriculture, (2009-10); Pp. 13.

George, M.L.C., Bustamam, M., Cruz, W.T., Leach, J.E., Nelson, R.J., 1997. Movement of *Xanthomonas oryzae* pv. *oryzae* in southeast Asia detected using PCR-based DNA fingerprinting. Phytopathol., 87: 302-309.

Iqbal, M.N., Anjum, A.A., Ali, M.A., Hussain, F., ALI, S., Muhammad, A., Irfan, M., Ahmad, A., Irfan, M., Shabbir, A., 2015. Assessment of microbial load of un-pasteurized fruit juices and in vitro antibacterial potential of honey against bacterial isolates. Open Microbiol. J., 9: 26-32. DOI: 10.2174/1874285820150601E001.

IRRI. 1996. Standard Evaluation System for Rice. INGER Genetic Resources center, IRRI, Manila, Philppines. 4th Ed.19-20.

Islam, W., Awais, M., Noman, A., Wu, Z., 2016. Success of Bio Products against Bacterial Leaf Blight Disease of Rice caused by *Xanthomonas oryzae pv. oryzae*. PSM Microbiol., 01(2): 50-55.

Jabeen, T., Arshad, H.M.I., Saleem, K., Ali, S., Ullah, E., Naureen, S., Babar, M.M., 2016. Morphological and Biochemical Characterization of *Xanthomonas axenopodis* pv.\][p citri Isolates Causing Citrus Canker Disease in Pakistan. PSM Microbiol., 01(1): 10-17.

Khan, J.A., Jamil, F.F., Gill, M.A., 2000. Screening of rice varieties/lines against bakanae and bacterial leaf blight (BLB). Pak. J. Phytopathol ., 12 (1): 6-11.

Leach, J.E., Rhoads, M.L., Vera Cruz, C.M., White, F.F., Mew, T.W., Leung, H., 1992. Assessment of genetic diversity and population structure of *Xanthomonas oryzae* pv. *oryzae* with a repetitive DNA element. Appl. Environ. Microbiol., 58(7): 2188-95.

Mew, T.W., Majid, A., 1977. Bacterial blight of rice in Pakistan. Int. Rice Res. Newslett., (2): 5.

Narayanan, N.N., Baisakh, N., Vera Cruz, C.M., Gnanamanickam, S.S., Datta, K., Datta, S.K., 2002. Molecular breeding for the development of blast and bacterial blight resistance in rice cv. IR50. Crop Sci.,(42): 2072–2079.

Ochiai, H., Horino, O., Miyajima, H., Kaku, H., 2000. Genetic Diversity of *Xanthomonas oryzae* pv. *oryzae* Strains from Sri Lanka. J. Bacteriol., 90: 415-421.

Ou, S.H., 1985. Rice Diseases. 2nd ed. Commonwealth Mycological Institute, Kew, Surrey, England, 6 (1): 96pp.

Salim, M., Akram, M., Akhtar, M.E., Ashraf, M., 2003. A Production Handbook, Balaced Fertilization for Maximizing economic Crop yields. Pakistan Research Council, Islamabad, 1-8.

Tasleem, U.Z.K., Gill, M.A., Khan, M.G., 2000. Screening of rice varieties/lines for resistance to bacterial leaf blight. Pak. J. Phytopathol., 12(1): 71–72.

Yunus, F.N., Kanwal, F., Rashid, F., Ashraf, A., Iqbal, M.N., Xiao, S., 2016. A Comparative Study on Isolation and Identification of *Bacillus thuringiensis* from Different Localities of Gujranwala City. PSM Biol. Res., 01(1): 34-38.