Pak. J. Bot., 51(1): 307-312, 2019. DOI: 10.30848/PJB2019-1(4)

INVESTIGATION OF BACTERIAL LEAF BLIGHT OF RICE THROUGH VARIOUS DETECTION TOOLS AND ITS IMPACT ON CROP YIELD IN PUNJAB, PAKISTAN

ROZINA SHAHEEN[1], MUHAMMAD ZAHID SHARIF [2]*, LUQMAN AMRAO[1], AIPING ZHENG [4], MUJAHID MANZOOR[2], DILAWAR MAJEED[2], HINA KIRAN[2], MUHAMMAD JAFIR[2] AND AMEER ALI[3]

[1]Department of Plant Pathology, University of Agriculture Faisalabad, Pakistan
[2]Integrated Genomic, Developmental and Biotechnology Laboratory,
Department of Entomology, University of Agriculture Faisalabad, Pakistan
[3]Department of Plant Breeding and Genetics (PBG), University of Agriculture Faisalabad, Pakistan
[4]Rice Research Institute, Sichuan Agricultural University. Chengdu, China
[*]Corresponding author's email: 2011ag4010@uaf.edu.pk

Abstract

Bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is an important bacterial disease in rice leading to heavy yield and economic losses. In current investigation, the rice leaf samples from infested regions were screened for *Xoo* strains linked to incidence of this disease. Subsequently, 17 different isolates were identified based on Gram staining, KOH assay and PCR analysis. Moreover, the percentage diseases incidence and weight loss of 1000-grains of each sample from four zones of rice production were recorded. Both biochemical test exhibited red stained and rod like Gram-ve bacteria. In addition, the molecular recognition by means of a 16S rRNA universal primer revealed DNA amplification in 15 out of 17 isolates which confirmed the pathogen as "*Xoo*". The data assessed for disease incidence in all investigated districts ranged between 70.12%- 49.23%. While, the maximum and minimum weight losses of 17.84% and 11.17% from Sialkot and Narowal were recorded respectively. The application of such tools for *Xoo* detection and its impact on crop yield are contested in this investigation.

Key words: Rice, Bacterial blight, Detection, Biochemical test, Molecular analysis, Yield losses, Pakistan.

Introduction

Rice (*Oryza sativa* L.) is principally grown in tropical and sub-tropical zones and is the staple food for about 9.3 billion by the year 2050 around the world (Salim *et al*., 2003; Sheehy & Mitchell, 2013). Rice is graded second position in Pakistan in terms of cultivation area and production next to wheat. Apart from significant crop of the country, rice is used to contribute major share in the export of Pakistan (Anon., 2004). Kernal and Super Basmati varities of rice are well-known in the world due to vitalizing aroma and taste (Muneer *et al.,* 2007). But now the position of rice export is less progressive for Pakistan, which maxim collective export to month of May is dropped by 19% year-on-year to value of 1.6 million tonnes. It is estimated that Pakistan has exported 3.8 million tonnes in 2017. This relates to a reviewed assessment of 3.9 million tonnes for year 2016 (Anon., 2017).

Every year 40% rice crop is lost worldwide due to biotic stresses including pests, insects, pathogens and weeds (Hossain, 1996). Multiple rice biotic stresses have been reported all over the world including Pakistan, such as Bacterial leaf blight, Paddy blast, Brown leaf spot, Stem rot, Foot rot/Bakanae and Rice yellow mottle disease (Anon., 2011; Sere *et al.,* 2013). Most importantly rice crop was found susceptible to some bacterial pathogens, and one of them is bacterial leaf blight (BLB) caused by *Xoo* that was regarded as the oldest rice disease of Asia (Jeung *et al*., 2006) and exerts severe losses in different rice growing regions of the globe (Xu *et al.,* 2010). This disease was first recognized by the farmer of Japan in 1884 (Tagami & Mizukami, 1962). In Pakistan, it was identified in 1977 (Mew & Majid, 1977). Previously, 10-20% crop losses were observed under moderate prevailing conditions, whereas under conducive conditions up to 50% crop losses were recorded in Asian countries (Mew *et al.,* 1993). In current years increased BLB attack was recorded in the rice growing areas of Pakistan (Ali *et al.,* 2009; Akhtar *et al.,* 2003; Bashir *et al.,* 2010). It was confirmed from conseqences of a research that amongst three rice varieties Super basmati was more susceptible to Xoo pathogen with maximum syndrome incidence of 89.5% for PXO 340 while Basmati 2000 variety was highly resistant at both growth phases and exhibited susceptibility at maximum tillering phase for PXO 280, with maximum disease incidence of 75.96% and PXO 340 with percentage incidence of 71.53% (Noor *et al.,* 2006).

Initially bacteria infect the leaf, then lead to leaf stalk and eventually symptoms reach to the center or base of the leaves. Pathogen enters the plant through water pores and wounds, produces water soaked irregular with yellow wavy margins lesions. Symptoms prevailing in crop at vegetative stage are known as Kresek while at generative stage are named as blight (Suparyono *et al.,* 2004; Akhtar *et al.,* 2008). Bacterial leaf blight disturbs the emergence of panicles and grain filling which leads to low production (Shanti *et al.*, 2010; Khan *et al.,* 2014).

Xoo can be detected based on molecular approaches, including Polymerase Chain Reaction (Shivalingaiah *et al.,* 2012), Western Blotting (Guo *et al.,* 2015) and morpho-molecular screening in case of BLB resistance in variant lines (Mubassir *et al.,* 2016). In addition, biochemical tools can also be employed for detection of *Xoo* incidence (Samanta *et al.,* 2014).

Previously, resistant variety was considered to be the most important strategy to control BLB. Unfortunately, technology was confronted by *Xoo* patho-types. It is very difficult to control the BLB due to extraordinary *Xoo* mutability. Newly developed resistant varieties were easily broken down after three to four years (Ponciano *et al.,* 2003; Sudir & Suprihanto, 2006). The limitations of this technology were increased with time and location as

onset of resistance against *Xoo* pathogen in rice and was carried out at vegetative phase only (Sudir & Yuliani, 2016; Wang *et al.,* 2006).

The purpose of the current study was to identify the BLB of rice by implementing biochemical and molecular techniques from the four districts of Punjab, Pakistan. It provides useful information regarding pathogen and its impact on yield variation in different zones of rice crop in Punjab Province.

Material and Methods

Survey and sampling: A comprehensive survey of major rice growing areas (Narowal, Nankana, Gujranwala, Hafizabad and Sialkot) of Pakistan was conducted to collect the rice leaves having the symptom of bacterial leaf blight (BLB) disease during the cropping year 2016-2017. The wheat leaves showing the symptoms of BLB were also collected. The samples were used for isolation, purification and identification of bacterium.

Isolation, purification and identification: The BLB infected samples were cut into small pieces of 3-4 mm size with sterilized scissors and sterilized with ethanol (70%) and 10 % chlorox for one minute. Then rinsed twice with sterilized water for 2-3 minutes for removal of any chemical on leaf surface and was left for air drying under sterilized conditions (inside laminar flow) using blotter paper. Samples were transferred on the fresh Luria Broth Agar (LBA) plates (taking precautions, plating should be done near the lamp, sterilized hands, forceps, heat the forceps but not too hot as it may kill the pathogen. The samples were placed on plates at equidistant and the touching to edges of plate was avoided. Then these plates were wrapped with cellophane tape in order to minimize the risk of contamination. Plates were marked with date and pathogen area and incubated at 30±1°C in an incubator. Within 2 days, bacterial growth was observed. Light yellow colonies were seen in the LBA medium after 48 hours. This yellowing of bacteria was due to pigment *Xanthomonadin.*

Streaking on plates: When the round bacterial colony appeared on the plates on the infected samples, the colony was picked with the help of sterilized loop and placed on freshly prepared media plates and were kept in incubator. The process until the pure colonies of bacteria were observed on the plates.

Preservation of pathogen cultures: After the purification these isolates were stored in 500 µl of 20% glycerol at -20°C for further studies as documented by Ahmad *et al.,* (2015). Glycerol stock is a good preservative as it doesn't allow the bacteria to freeze (Fig. 1).

Gram staining: A thinly spread air-dried bacterial film was fixed on clean glass slide by a light flame. The specimen was treated with 0.5% aqueous crystal violet and afterwards washed with running tap water, rinsed in water and decolorized with 95% ethanol. The specimen was again rinsed with tap water and counter-stained with safranin for approximately 10 seconds. It was eventually washed with water and observed under microscope at 10X and 40 magnifications (Gerhardt, 1981).

Fig. 1. *Xoo* culture preserved in glycerol stock.

Biochemical characterization

Potassium hydroxide (KOH) test: KOH test was applied to identify the biochemical characteristics of *Xoo* pathogen. Bacterial culture was taken on the glass slide and stirred (for 60 sec.) with 3% KOH solution. The DNA of bacteria came out of the bacterial cell in the form of thread indicating the presence of gram negative bacteria.

Molecular detection: Extraction of DNA (0.5 g) was carried out from rice leaf samples that were initially crushed with mortar and pestle by CTAB extraction protocol of Doyle & Doyle (1990). Amplification of a DNA product was performed by using the 16S rRNA universal primer (Forward: 5´ GAG TTT GAT CCT GC TCA G 3´; Reverse: 5´ GTT ACC TTG TTA CGA CTT 3´). The PCR profile used for this marker was as follows: 94°C for 1 min, 94°C for 30 sec, 46°C for 40 sec, 72°C for 1 min, (step 2–4 cycled 5 times), 94°C for 30 sec, 51°C for 40 sec, 72°C for 1 min, (step 5–7 cycled 35 times), and 72°C for 10 min. The PCR product was kept at -40 until loaded onto the gel. After gel electrophoresis it was pictured under UV light of gel documentation system.

Disease incidence (%) and Loss in grain weight (GW): In order to calculate disease incidence (%), five random spots of 1 m² area each were selected in the field, the infected plants were counted, and then the percentage of incidence was calculated from the total number of plants from that area. The formula applied for disease incidence is outlined below:

$$\text{Disease Incidence \%} = \frac{\text{Number of bacterial blight infected plants}}{\text{Total number of plants examined}} \times 100$$

For calculating loss in grain weight, samples were dried and their grains were separated from both healthy and diseased plants. Loss (%) in grain weight was calculated using the following formula:

$$\% \text{ loss in } 1000 \text{ GW} = \frac{W1 - W2}{W1} \times 100$$

where, GW= Grain weight, W1= Weight of 1000-grain obtained from healthy plants and W2= Weigh of 1000-grain obtained from the disease plants.

Results and Discussion

Symptoms of infected leaf: Infected leaf exhibited water soaked lesions (yellow in color) at the border of its leaf edge (Fig. 2). Such lesions were parallel along the leaf, later merged together and then covered overall leaf. Signal of the causative agent as bacterial exudation was noticed on the boundaries and veins of newly infected leaf.

The identical symptoms responsible for BLB of rice in West Bangal, India and also in Benin were also reported (Samanta *et al.,* 2014, Afolabi *et al.,* 2016). In the present study, sign of the causal agent as bacterial ooze was also noted on the veins or boundaries of the newly infected leaf under moist or humid conditions. Similar condition was also observed by Tagami & Mizukami (1962), Anon., (1970) and Ou (1985).

Isolation of *Xoo:* Five samples were taken from different areas including Sialkot, Gujranwala, Hafizabad, Nankana and Narowal in Pakistan. The growth of bacterial culture was studied in Luria Broth (LB) medium. They formed colonies having yellow, convex, mucoid and shiny texture (Fig. 3). Such kinds of colonies were constantly observed and tentatively recognized as the causative organism. Yellow coloring and mucoid colonies both are cultural features of *Xanthomonads* which were attributable to the EPS (extracellular polysaccharides slime) production in media comprising sugar. When such samples were streaked on LB media, they also produced light yellow, round, mucoid and smooth colonies of bacteria which were 1-2 mm in diameter (Fig. 3).

Biochemical test

Gram staining and Potassium hydroxide (KOH) test: Consequence of Gram staining (Table 1) verified that the pathogen was a gram negative rod, generating red color at the time of counter staining with safranin. Overall isolates reliably presented similar outcomes. While, KOH test was carried out to confirm Gram staining consequences. The particular isolates exhibited positive reaction in 3% KOH examination by producing the thread-like slime and confirmed the isolates as Gram negative (Table 1).

Such techniques were documented by Jabeen *et al.,* (2012) which exhibited similar consequences in support of present investigation. Samanta *et al.,* (2014) also studied such tests with matching results but beside these test they also applied some other biochemical examinations which include Endospore staining, KOH test, Starch hydrolysis test, Egg yolk reaction, Gelatin hydrolysis test, Tetrazolium salt tolerance test, Oxidase test, Catalase test and Conc. sulfuric acid test.

Molecular detection: Universal primers for 16S rRNA gene were utilized for the purpose of amplification of confirmed bacterial DNA in overall particular positive isolates to approve the pathogens. The polymerase chain reaction products of 1500 bp was achieved in 15 out of 17 isolates (Table 1 & Fig. 4). The isolates at well 4 and 10 and those which were used as negative control (shown as zero "0") did not exhibit any amplification.

Same the consequences of PCR detection were documented in Malaysia by Jonit *et al.,* (2016). Furthermore, polymerase chain reaction analysis conducted by Shivalingaiah *et al.,* (2012) also detected this infection in India. In the present investigation, 17 isolates of *Xoo* were identified from the various zones of Punjab where rice was a main produce. Such isolates were also detected via biochemical investigation and further confirmed by PCR employing a particular primer (Lang *et al.,* 2010).

Fig. 2. Rice field outlook with bacterial leaf blight (BLB) infection.

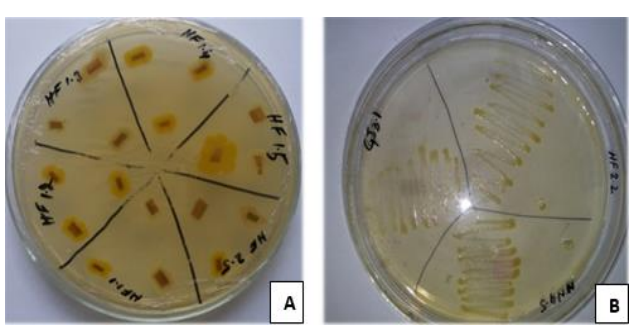

Fig. 3. Picture showing bacterial growths on LB media after 48 hours (A); Streak plates from the overnight culture (B).

Disease incidence (%) and Loss in grain weight (GW): In recent investigation not any single field surveyed were harmless from BLB infection. Data regarding disease incidence of bacterial leaf blight (BLB) from various districts of Punjab during year 2017 is shown (Table 2). The peak disease incidence (70.12%) was recorded for Sialkot, and lowest (49.23%) was recorded for Nankana. While, the percentage disease incidence for the rest of the districts including Gujranwala, Hafizabad, Narowal, was 51%, 51.82% and 37%, respectively. Similar results were

obtained by Rafi *et al*., (2013) who evaluated that BLB syndrome stimulated by means of *Xoo* pathogen had generated a severe situation in overall provinces of Pakistan including Punjab (36.8-74.6%), Khyber pakhtukhwa (35-80.2%), Sindh (11.67-46.67%) and Baluchistan (12-21.67%). They also assessed same consequences of disease incidence (% age) in each district of Punjab including Gujranwala (50.6%), Hafizabad (50.8%), Narowal (36.8%) and Sialkot (69.6%). Moreover, disease incidence was evaluated in Khyber Pakhtunkhwa that ranged between 66.6%-75% (Khan *et al.,* 2015). According to Ou (1985), the disease ensues at different stages in the host plantations, but the yield losses depends on multiple factors including weather, location and specific rice cultivar so such factors may also contribute in the yield variations.

The maximum weight loss discerned in 1000 rice grains was 17.84% at Sialkot while the minimum loss (11.17%) because of BLB infection was noted at Narowal. Other percentage losses noted at Hafizabad, Gujranwala and Nankana were 15.46%, 14.73% and 13.56% respectively (Fig. 5). Maximum and minimum percentage losses in Grain Weight (GW) due to BLB infection were noticed in Sialkot and Narowal were 17.84% and 11.17% respectively. Such range of percentage weight losses (15.59%-11.94%) due to this pathogenic infection in Khyber Pakhtonkhwa province of Pakistan are also reported by Khan *et al*., (2015). Intensification in disease severity is the main cause of 1000 grain weight reduction (Fig. 5). Among all other pathogenic factors, BLB instigated due to *Xoo* interference is economically much important and cause considerable yield forfeiture each year in rice cultivating countries including Pakistan as well (Swing *et al.,* 1990). It is surely a severe infection resulting annual grain losses of million tones. In case of Pakistan, the occurrence of BLB infection has increased in current years particularly in Kaller belt which is eminent for the production of high quality rice (Akhtar *et al.,* 2003; Ali *et al.,* 2009; Bashir *et al.,* 2010).

Table. 1. Consequences of variant tests and PCR examines for *Xoo* detection.

Isolates (no.)	Gram staining reaction (+ve/-ve)	3% KOH test (+ve/-ve)	PCR analysis (+ve/-ve)
1	-ve	+ ve	+ ve
2	-ve	+ ve	+ ve
3	-ve	+ ve	+ ve
4	-ve	+ ve	- ve
5	-ve	+ ve	+ ve
6	-ve	+ ve	+ ve
7	-ve	+ ve	+ ve
8	-ve	+ ve	+ ve
9	-ve	+ ve	+ ve
10	-ve	+ ve	- ve
11	-ve	+ ve	+ ve
12	-ve	+ ve	+ ve
13	-ve	+ ve	+ ve
14	-ve	+ ve	+ ve
15	-ve	+ ve	+ ve
16	-ve	+ ve	+ ve
17	-ve	+ ve	+ ve

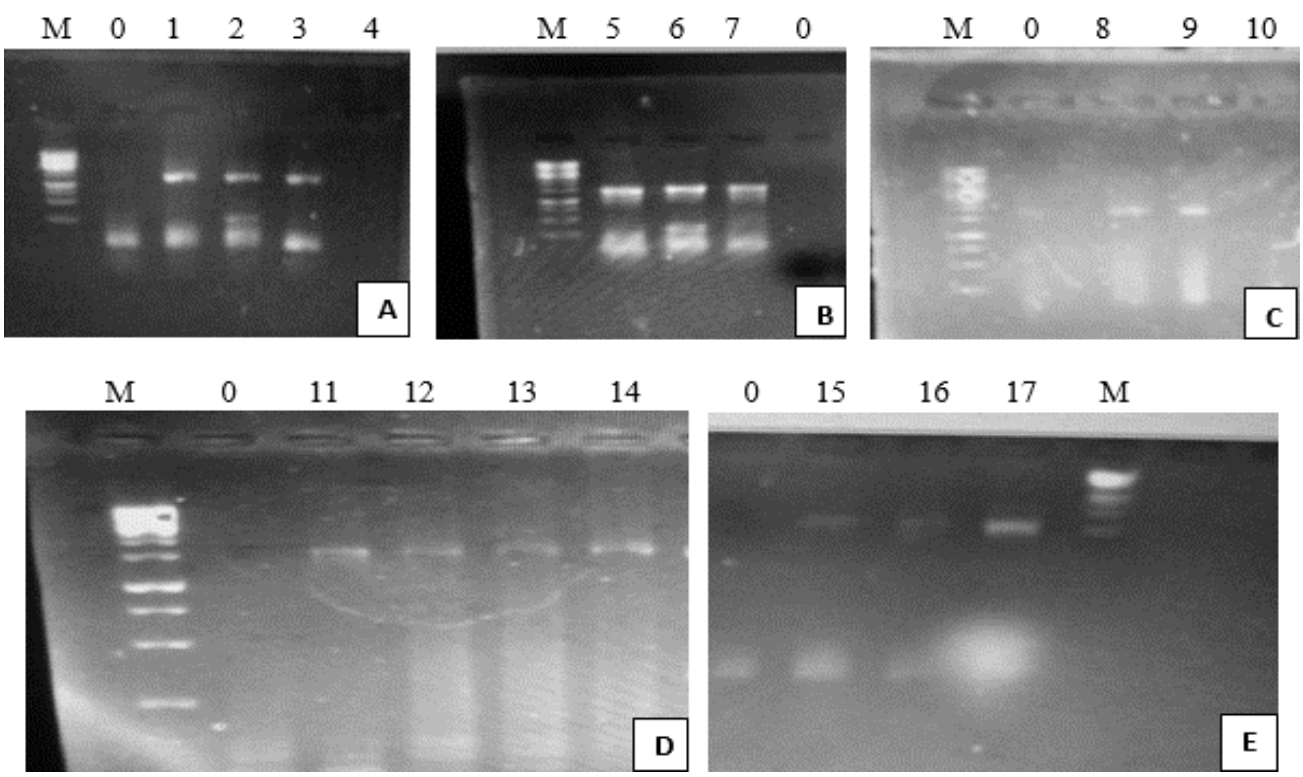

Fig. 4. PCR amplified of 1500 bp bands from 17 *Xoo* isolates (with universal primer 16S rRNA), M= 1kb ladder as marker, 0= negative control, Lane 1-16= showing *X. oryzae* pv. *oryzae* isolates from variant regions of Punjab including Sialkot (A), Narowal (B), Gujranwala (C), Nankana (D) and Hafizabad (E).

Table 2. Data exhibiting percentage incidence of disease from different districts of Punjab, Pakistan during year 2017.

S. No.	Location	Coordinates	Disease incidence (%age)
1.	Gujranwala	32.1544° N, 74.1842° E	51
2.	Hafizabad	32.0717° N, 73.6857° E	51.82
3.	Narowal	32.0995° N, 74.8747° E	39.53
4.	Sialkot	32.4925° N, 74.5310° E	70.12
5.	Nankana	31.4508° N, 73.7037° E	49.23

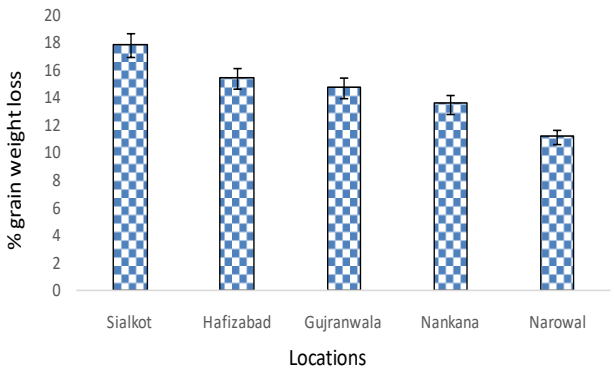

Fig. 5. Graph presentation of rice grains weight loss at different districts of Punjab.

Conclusions

On the basis of disease indications, biochemical test and consequences of PCR analysis, the overall isolates were considered as "*Xoo"*. BLB of rice is a disparaging, wide-ranging disease and also a warning to rice production in various rice growing zones of Punjab. According to our understanding, the existence and incidence of this disease in rice from different geographical zones of Punjab have not been examined using any molecular technique. Current investigation will provide molecular facts of *Xoo* incidence producing BLB of rice in Pakistan. In present experiment, it is suggested that steps must be taken to control such bacterial disease which consequently increase the yield losses of this crop.

References

Afolabi, O., R. Amoussa, M. Bile, A. Oludare, V. Gbogbo, L. Poulin, R. Koebnik, B. Szurek and D. Silue. 2016. First report of bacterial leaf blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* in Benin. *Plant Dis.,* 100: 515-515.

Ahmad, I., M. Zakria, A. Rehman, A. Munir and M.T. Khan. 2015. Screening of rice (*Oryza sativa* L.) germplasm against *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) in green house condition. *Eur. Acad. Res.,* 2: 12591-12597.

Akhtar, M.A. 2003. Study on genetic variation in *Xanthomonas oryzae* Pv. *oryzae* in relation to resistance in rice. 1st Annual Progress Report. Institute of Plant and Environmental Protection, NARC, Islamabad, Pakistan. pp. 10.

Akhtar, M.A., A. Rafi and A. Hameed. 2008. Comparison of methods of inoculation of *Xanthomonas oryzae* pv. *oryzae* in rice cultivars. *Pak. J. Bot.,* 40: 2171-2175.

Ali, A., M.H. Khan, R. Bano, H. Rashid, NI. Raja and Z. Chaudhry. 2009. Screening of Pakistani rice (*Oryzae sativa*) cultivars against *Xanthomonas oryzae* pv. *oryzae*. *Pak. J. Bot.,* 41: 2595-2604.

Anonymous. 1970. OCTA. Bacterial leaf blight of rice in Southern Asia. Overseas Technical Cooperation Agency. Tokyo, Japan. 71p.

Anonymous. 2004. Agriculture Statistics of Pakistan. Govt. of Pakistan, Ministry of Food, Agric. & Livestock (Economic Wing), Islamabad. 17 p.

Anonymous. 2011. CSISA (Central system initiative for South Asia). Ayub Agricultural Research Institute (Punjab) Pakistan.

Anonymous. 2017. FAO (Food and Agriculture Organization of United States). Rice Market Monitor (RMM). 20(2). http://www.fao.org/economic/RMM.

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. Agric. Sci.,* 47: 361-365.

Doyle, J.J and Doyle. J.L. 1990. Isolation of plant DNA from fresh tissues. *Focirs.* 12: 13-15.

Gerhardt, P. (Ed.). 1981. Manual of Methods of General Bacteriology. *Amer. Soc. of Microbiol.,* Washington, D.C.

Guo, M., J. Lan, J. Shi, M. Guan and J. Wei. 2015. Western Blot Detection of *Xanthomonas Oryzae* pv. *oryzae* in Rice. *J. Plant Pathol. and Microbiol.,* doi: http://dx.doi.org/ 10.4172/2157-7471.S4-00.

Hossain. M. 1996. Recent developments in the Asian rice economy: challenge for the rice research. In Rice research in Asia: progress and priorities, Wellington, UK. p. 17.

Jabeen, R., T. Iftikhar and H. Batool. 2012. Isolation, characterization, preservation and pathogenicity test of *Xanthomonas oryzae* pv. *oryzae* causing BLB disease in rice. *Pak. J. Bot*., 44: 261-265.

Jeung, J.U., S.G. Heu, M.S. Shin, C.M.V. Ruz and K.K. Jena. 2006. Dynamics of *Xanthomonas oryzae* pv. *oryzae* populations in Korea and their relationship to known bacterial blight resistance genes. *Phytopathol.*, 96: 867-875.

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

Khan, J.A. 2014. Biochemical basis of resistance in rice against Bacterial leaf blight disease caused by *Xanthomonas oryzae* pv. *oryzae*. *Adv. Life Sci.,* 1: 181-190.

Khan, M., A. Rafi, A. Abbas, T. Ali and A. Hassan. 2015. Assessment of yield lossess caused by bacterial blight of rice in Upper Dir, Khyber Pakhtunkhwa Province. *Asian J. Agri. Biol.,* 3: 74-78.

Lang, J.M., J.P. Hamilton, M.Q. Diaz, M.A.V. Sluys, G. Ruby, M.R.G. Burgos, C.M.V.V. 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: 312-319.

Mew, T.W. and A. Majid. 1977. Bacterial blight of rice in Pakistan. *Int. Rice Res Newsletter*. 2: 50-51.

Mew, T.W., A.M. Alvarez, J.E. Leach, J. Swings. 1993. Focus on bacterial blight of rice. *Plant Dis.,* 77: 5-12.

Mubassir, M.H.M., S.N. Begum, M.K. Saha and A.Q.M. Bazlur Rashid. 2016. Morphomolecular screening for bacterial leaf blight resistance in some rice lines and varieties. *J. Plant Sci*., 4: 146-152.

Muneer, N., A. Rafi and M.A. Akhtar. 2007. Isolation and characterization of *Xanthomonas oryzae* pv. *oryzae* isolates from North West Frontier Province (NWFP) Pakistan. *Sarhad J. Agric.,* 23: 743-751.

Noor, A.M., Z. Chaudhry, H. Rashid and B. Mirza. 2006. Evaluation of resistance of rice varieties against bacterial blight caused by Xanthomonas oryzae pv. *oryzae*. *Pak. J. Bot.,* 38: 193-203.

Ou, S.H. 1985. Bacterial leaf blight in rice diseases. Common wealth Microbiological Institutes, Cambrian News, Aberystwyth, U. k. 70-74.

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: 1272-1282.

Rafi, A, A. Hameed, M.A. Akhtar, S.M.A. Shah, M. Junaid, M. Shahid and S.F. Shah. 2013. Field based assessment of rice bacterial leaf blight in major rice growing zones of Pakistan. *Sarhad J. Agric.,* 29: 415-422.

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

Samanta, T.S., P. Samanta and A. Das. 2014. Isolation and characterization of *Xanthomonas oryzae* isolates from different regions of Midnapore district of West Bangal and their ecofriendly management by some medicinal plant extracts. *Int. J. Phytomed.,* 6: 29-42.

Sere, Y., D. Fargette, M.E. Abo, K. Wydra, M. Bimerew, A. Onasanya and S.K. Akator. 2013. 17 Managing the major diseases of rice in Africa. *Realizing Africa's Rice Promise*, 213-228.

Shanti, M.L., G.L. Devi, G.N. Kumar and H.E. Shashidhar. 2010. Molecular marker assisted selection: a tool for insulating parental lines of hybrid rice against bacterial leaf blight. *Int. J. Plant Pathol.,* 1: 114-123.

Sheehy, J.E. and P.L. Mitchell. 2013. Designing rice for the 21st century: The three laws of maximum yield. Discussion Paper Series 48, International Rice Research Institute, Los Banos, Philippines.

Shivalingaiah, S. Umesha and M.K. Sateesh. 2012. Molecular detection of *Xanthomonas oryzae* Pv. *Oryzae* in rice seeds by molecular techniques. *Asian Aust. J. Plant Sci. Biotechnol*., 6: 44-47.

Sudir and Suprihanto. 2006. Changes in strains virulence of *Xanthomonas oryzae* pv. *oryzae*, causing of bacterial leaf blight in rice plants (in Indonesian). *J. Penelitian Pertanian Tanaman Pangan.*, 25: 100-107.

Sudir and Y. Dini. 2016. Composition and distribution of *Xanthomonas oryzae* pv. *oryzae* pathotypes, the pathogen of rice bacterial leaf blight in Indonesia. *Agrivita J. Agri. Sci.,* 38: 174-185.

Suparyono, Sudir and Suprihanto. 2004. Pathotype profile of *Xanthomonas oryzae* pv. *oryzae* isolates from the rice ecosystem in Java. *Ind. J. Agric. Sci.,* 5: 63-69.

Swings, J., M.V.D. Mooter, L. Vauterin, M. Hoste, T.W. Gillis and K. Kerestes. 1990. Reclassification of the causal agents of bacterial blight (*Xanthomonas compestrise* pv. *oryzae*) and bacterial leaf streak (*Xanthomonas oryzae* pv. *oryzacola*) of rice as pathovars of *Xanthomonas oryzae* (ex Ishiyama 1922) sp. nov., nom. rev. *Int. J. Syst. Bacteriol.,* 40: 309 - 311.

Tagami, Y. and T. Mizukami. 1962. Historical review of researches on bacterial leaf blight of rice caused by *Xanthomonas oryzae* (Uyeda et Ushiyama) Dowson. Special report of the plant diseases and insect pest forecasting service No. 10. Plant protection Division, Ministry of Agriculture and Forestry, Tokyo, Japan. 112 p.

Wang, YS., L.Y. Pi, X. Chen, P.K. Chakrabarty, J. Jiang, A.L. De Leon, G.Z. Liu, L. Li, U. Benny, J. Oard, P.C. Ronald and W.Y. Song. 2006. Rice XA21 binding protein 3 is a ubiquitin ligase required for full Xa21-mediated disease resistance. *Plant Cell*., 18: 3635-3646.

Xu, Y., X.F. Zhu, M.G. Zhou, J. Kuang, Y. Zhang, Y. Shang and J.X. Wang. 2010. Status of Streptomycin resistance development in *Xanthomonas oryzae* pv. *Oryzae* and *Xanthomonas oryzae* pv. *Oryzicola* in China and their resistance characters. *J. Phytopathol*., 158: 601-608.

(Received for publication 26 December 2017)