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Selection of Rice Genotypes through Comparative Response to Bacterial Leaf Blight Disease Caused by *Xanthomonas oryzae pv. oryzae*

Waqar Islam[1,2,*], Muhammad Awais[3]

[1] College of Plant Protection, Fujian Agriculture & Forestry University, Fuzhou, Fujian 350002, China
[2] Fujian Province Key Laboratory for Plant Viruses, Institute of Plant Virology, Fujian Agriculture & Forestry University, Fuzhou, Fujian 350002, China
[3] Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan

Edited by:
Sagheer Atta, Ghazi University, D.G. Khan, Pakistan

Reviewed by:
M. Carmen Herrera Rodriguez, Universidade Federal de Viçosa, Brazil
Mona Mustafa Saleh, Agricultural Research Center, Egypt
Ali Noman, G.C. University, Faisalabad, Pakistan

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Abstract: Rice is one of the most important food crops all over the world and is consumed as staple food in many countries. The crop is attacked by several diseases during its life span which ultimately result in qualitative and quantitative losses. Among these diseases, bacterial leaf blight (BLB) is one of the most important biotic factors limiting the crop yield worldwide. Different rice genotypes were tested from time to time against the pathogen races to find resistant sources against (BLB) disease. Keeping in view dearth of tolerant genotypes, ten rice genotypes were screened against the BLB disease and disease severity was estimated according to the standardized method 1st, 2nd and 3rd week consecutively, after artificial inoculation. Seven, out of ten rice genotypes, were highly infected by bacterial leaf blight of rice. Resistance was not found against the disease in any of the genotypes. Only the genotype KSK-133 reacted as moderately resistant to BLB. Six genotypes, namely Basmati-515, Basmati-385, Basmati-370, Basmati-198, KSK-282 and IRRI-6 were categorized as moderately susceptible. Basmati Pak reacted as susceptible while Basmati super and Basmati-2000 proved to be highly susceptible to the disease. As there is no existing tolerant or resistant genotype against BLB, more efforts are required to screen the germplasm sources against the BLB.

Keywords: Bacterial disease, Blight, Germplasm, Resistance, Rice, Response, Screening.

Corresponding author: Waqar Islam, E-mail: ddoapsial@yahoo.com , waqarislam@m.fafu.edu.cn

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1 Introduction

Rice (*Oryzae sativa* L.) is abundantly grown in the heavy rainfall areas including tropical and subtropical zones of the globe (Chapagain and Hoekstra, 2011). This crop is highly nutritive for human beings and is source of food for more than half of the global population, especially Asian countries, where it as a staple food (Smith and Bruce, 2000; Zafar et al., 2004; Islam et al., 2016a). Therefore sustainable rice production is important for regional as well as global food security (Sharma et al., 2012). Pakistan economy is highly dependent upon the rice crop as the country is ranked second regarding rice export (Zahid et al., 2005; Islam and Ahmad, 2016). Total rice production area in Pakistan is 2850 thousand hectares with an annual production of 10351 thousands tons (USDA, 2015).

Diseases are major yield limiting factor for rice (Khan, 2015; Ke et al., 2017). More than forty genera of fungi and bacteria affect this highly important crop at various growth stages (Khan et al., 2009). Regarding bacterial attacks, bacterial leaf blight (BLB), caused by "*Xanthomonas oryzae pv. oryzae*", is one of the most destructive disease causing massive yield reductions, particularly in Asia (Islam et al., 2016a; Sharma et al., 2017; Wang et al., 2017). Crop yield losses by BLB vary depending upon stage of the infected crop and environmental conditions (Narayanan et al., 2002). The losses can climb up to 80% in case of the highly susceptible genotypes (Lee and Khush, 2000). "Kresek" or leaf blight symptoms are the most important symptoms produced by the pathogen (Akhtar et al., 2008). Diseased plants produce sterile panicles without grains (Akhtar et al., 2003; Khoa et al., 2017). Epidemics of the disease have occurred several times in Pakistan.

Consequently, all the highly aromatic and valuable basmati genotypes are categorized susceptible against the disease (Akhtar et al., 2008; Khan, 2009; Ali et al., 2009; Waheed et al., 2009).

Targeting the disease management, there can be several options like chemical management (Rehman et al., 2013; Islam et al., 2016b; Islam et al., 2016c; Islam et al., 2017a; Islam et al., 2017b), cultural practices (Islam, 2017) and utilization of resistant varieties (Noman et al., 2016). From all these, the best option is adaptability of the resistant genotype (Ashraf et al., 2013; Ali et al., 2016;; Islam et al., 2017c; Islam et al., 2017d Islam, 2017). Unfortunately, we do not have any variety that may be reckoned as completely tolerant or resistant to the pathogen (Khan et al., 2008; Samiullah, 2015). Keeping in view of the importance of the resistant germplasm in management of the disease, the present study was carried out to find the BLB resistant rice genotypes from the available germplasm in the country.

2. Materials and Methods

2.1 Preparation of artificial inoculums of pathogen

Collection of the diseased leaf samples was carried out from districts Shiekhupura, Sialkot, Hafizabad and Gujranwala of Punjab province and composite sample was generated to draw a representative sample for bacterium isolation. 5mm leaf pieces were cut, placed in 75% ethyl alcohol for some seconds, distilled water rinsing was performed twice and were further dipped into 300 µl distilled water for 15-20 minutes in centrifuge tubes (Waheed et al., 2009). A loopful of bacterium containing water was streaked on potato starch agar (Sucrose 1.2%, agar agar 2%, peptone 1.2%) plates were streaked with a loopful of bacterial water and incubated at 28^0C for 72 hours. Using the sterilized wire loops, bacterial colonies were picked and again placed into fresh ager plates for purification and multiplication. Bacterium identification was carried out by morphological tests, technical bulletin on seed health tests and gram staining (Baker, 1962; Agarawal, 1989). Further confirmation was done by pathogenicity test for confirmation of koch's postulates upon susceptible varieties (Basmati-2000, Basmati-super).

2.2 Screening of rice germplasm against BLB disease

Ten aromatic rice genotypes, commonly sown all around in Punjab (KSK-133, KSK-282, Basmati-198, Basmati-370, Basmati-super, Basmati-2000, Basmati Pak, Basmati-385, Basmati-515 and IRRI-6) Pakistan, were obtained from Rice Research Institute Kala Shah Kaku, Pakistan. Nursery was grown in research area at Faculty of Agriculture, Faisalabad, Pakistan in 2014 and 2015. Seeds were broadcasted on dry land (1.5 ft. × 20ft, raised beds 1 ft.) for each entry, covered with wheat straw, then watered with hand sprinkler thrice a day. Nursery was flooded for 1st time after four days of sprouting. Rice seedlings, 30 days old, were transplanted (2014 and 2015) in 30 plots (10×6 feet P-P; 23cm L-L; 90cm Plot-Plot distance) with three replications. All the standard agronomic practices were applied according to crop requirements.

From the multiplied bacterial culture, dilution plate technique was followed to prepare the aqueous suspension of bacterium (Clifton, 1958). When the crop achieved its booting stage, with the help of the scissor inoculated with the bacterial suspension (1 x10^8 cfu/ml) and using the clipping method, two rows of each variety were inoculated in each plot artificially by cutting one fourth of top of the leaves (Kauffman et al., 1973). Humid conditions were artificially generated by spraying water around the inoculated plants.

Standard evaluation 0-9 scale, developed by IRRI for bacterial leaf blight disease (Anonymous, 1996), was followed for recording the data weekly as 0, immune; 1, Resistant (1 – 5% area affected); 3, Moderately resistant (6 – 12% area affected); 5, Moderately susceptible (13 – 25% area affected); 7, Susceptible (26 – 50% area affected); 9, Highly susceptible (51 – 100% area affected).

Gnanamanickam et al (1999) formula was used for determination of % disease severity.

$$\% \text{ Disease severity} = \frac{\text{Total lesion length}}{\text{Total leaf length}} \times 100$$

3. Results

Data of disease severity were regarded on weekly basis (consecutive for three weeks) after the inoculation. No resistance was recorded in a single tested variety. However, only one variety namely KSK-133 was categorized as moderately resistant, six genotypes (Basmati-385, Basmati-370, Basmati-515, Basmati-198, IRRI-6, KSK-282) were categorically stated as moderately susceptible. The other single variety called Basmati-Pak was scaled susceptible and two varieties (Basmati super, Basmati-2000) were categorized as highly susceptible to BLB diseases (Fig. 1).

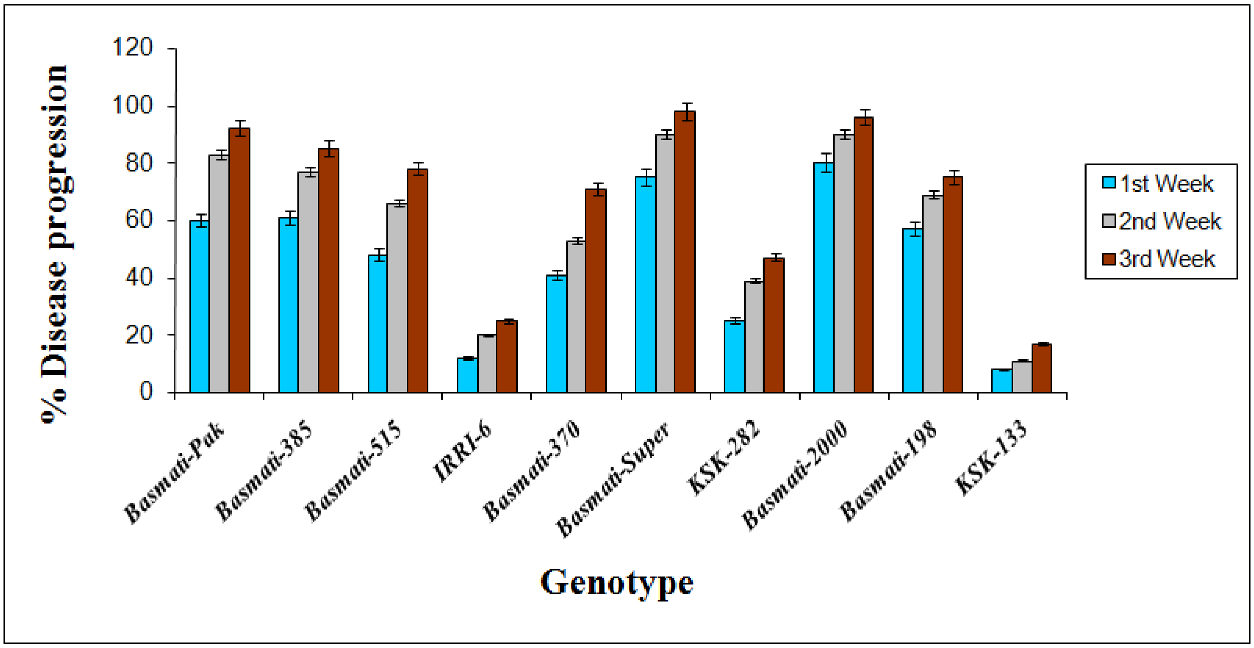

Fig. 1. Weekly mean percentage of bacterial leaf blight disease progression in rice genotypes recorded in September 2014-15.

Progression of disease severity of bacterial leaf blight was monitored at weekly intervals till maximum infection was attained. In the first week, 7 out of 10 rice genotypes were infected by bacterial leaf blight of rice. This represented 70% of the tested genotypes being infected at very early stage. BLB infection in this week ranged between 0-50%. Maximum infection was observed in Basmati-Pak, Basmati-2000, Basmati-super and Basmati-385. The disease progressed and spread fast in the second week as 80% of the tested genotypes, while only two genotypes remained with no symptoms. BLB infection in this week ranged between 0-60%. In the third week, only one tested entry escaped from BLB infection as all the ten genotypes were infected and disease symptoms became highly conspicuous (Fig, 2).

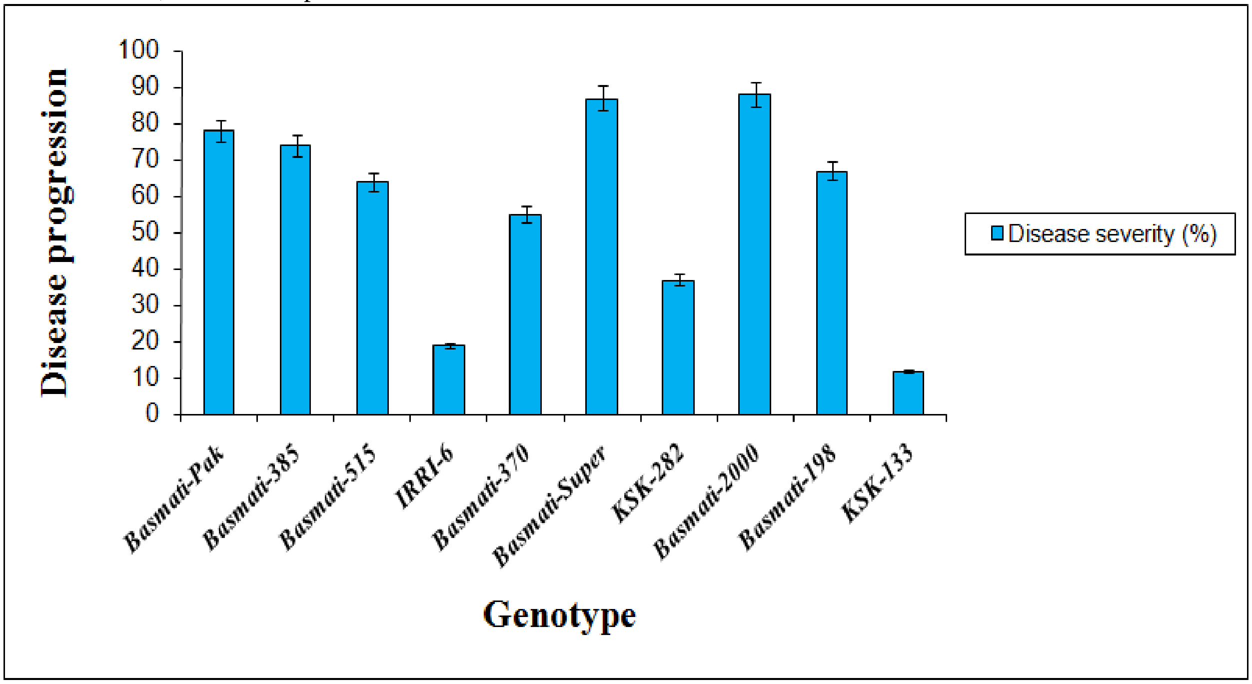

Fig. 2. Overall mean disease severity of bacterial leaf blight of rice in 2014-15.

4. Discussion

The studies were carried out to find out sources of resistance in available rice germplasm or varieties against the BLB disease, but none of the genotypes was categorized as resistant or tolerant to the disease. Results were strengthened by the work of different scientists who conducted similar investigations and reported no completely tolerant varieties against this particular disease. Previous reports explain that all the famous and aromatic basmati varieties were in susceptible category regarding to the BLB disease. Based on the results, Basmati super and Basmati-2000 exhibited most susceptible reaction against BLB which are in line with the results of Nabeela et al (2004), Shah et al (2009) and Jabeen et al (2011) who also categorized these varieties as highly susceptible. Accordingly to Khan (2000), Basmati-365 is the only genotype that was recorded as moderately resistant.

In our findings, Basmati-365 proved to be moderately susceptible as the variety lost its resistance against the pathogen with the passage of time. Similar findings were in context with the efforts of Tasleem et al., (2000) as in his trails at KSK Rice Research Institute in 1997-98, he could not find any resistant lines out of 104 new genotypes screened against BLB disease. Correspondingly, Khan et al., (2009) in his field experiments of evaluation of 50 rice genotypes against BLB in 2003-04, none of the varieties was under categorization of resistant but he also considered Basmati-365 and Basmati Pak as moderately susceptible. Furthermore, experiments of Khan et al (2009) during 1997, 2002, 2006, 2007 and 2008 revealed that no variety in Pakistan exhibits the tolerance against the pathogen. We further recorded that IRRI-6 showed some resistance against the disease. Our results coordinate with those of Akhtar et al. (2011) who evaluated 47 rice accessions and observed resistance against bacterial leaf blight isolates in IRRI-6and KSK-282. But our findings declare that KSK-282 has lost some resistance against the pathogen as we categorized the variety as moderately susceptible. Non aromatic varieties (such as KSK-133) had some resistance against the pathogen.

Waheed et al (2009) reported resistance in some non aromatic, least famous newly evolving genotypes against BLB disease i.e. PARC-298, PARC-299 and PARC-301. The results are more correlated with findings of Naqvi et al (2015), who tested 40 rice genotypes, and found only six as moderately resistance, while the others were moderately susceptible or susceptible ones. Supporting our views, Samiullah, (2015) also reported that none of the genetic entry was tolerant and all were in context of susceptible or moderately susceptible.

5. Conclusion

Our results illustrate that rice cultivars commonly growing for cultivation in Pakistan are lacking immunity against bacterial leaf blight. Tested rice cultivars are categorized as moderately susceptible to highly susceptible against bacterial leaf blight. Basmati super and Basmati-2000 appears to be highly susceptible, whereas Basmati-515, Basmati-385, Basmati-370, Basmati-198, KSK-282 and IRRI-6 are categorized as moderately susceptible to bacterial leaf blight. These results highlighted urgency or efforts required to screen the available rice germplasm sources against the bacterial leaf blight and use for future breeding program. These efforts will be helpful to develop rice varieties against bacterial leaf blight and also possessing good aroma and better yield potential.

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List of Abbreviations:- BLB: Bacterial leaf blight

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