Article

Weather parameters largely regulate the outbreak of the rice bacterial blight: an endemic disease poses a threat to food security in Bangladesh

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Abstract: Bacterial blight (BB) caused by Xanthomonas oryzae pv. oryzae is a destructive disease of rice causing significant yield losses. Surveys were conducted to know the influence of weather parameters on epidemic development of BB in the thirty agroecological zones of Bangladesh. The maximum (55.11%) BB incidence and severity were recorded in Mymensingh division (AEZs 8 and 9) while the minimum (3.25%) incidence was observed in Barishal division (AEZ 13) in both rainfed and irrigated seasons. In rainfed conditions, the maximum BB incidence and severity were recorded in hybrid varieties Aloron from Netrokona (AEZ 9) and the minimum incidence was observed in the local variety Sughondi from Jalhokhati (AEZ 13). On the other hand, in irrigated conditions, the highest BB incidence was recorded in the hybrid variety Hera-2 from Netrokona (AEZ 9) and the lowest incidence was observed in the local variety from Hobiganj (AEZ 20, 21, 22). Swarna, Puja, BRRIdhan28, BRRIdhan29, and BRRIdhan49 showed intermediate levels of infection, while hybrid Hera-2, Arize TejGold, Partex, Agrodhan, Aloron, ACI-2, and Dhani Gold showed higher level of infection (susceptibility) to BB. The data analysis on BB incidence with the weather parameters of rice growing seasons from 2015 to 2017 revealed that the rainfall distribution varied greatly within rice growing seasons over the years. The results indicated that weather parameters, viz. temperature (25-34°C), relative humidity (70-84%), and rainfall (>200mm), largely affected the prevalence, incidence and severity of BB. The correlation studies revealed that among the climate variables, minimum temperature exhibits a negative correlation. However, rainfall, maximum temperature, morning relative humidity and evening relative humidity were found positively correlated, while wind velocity imparted a significant positive correlation with BB infection. The regional outbreak of BB indicated a considerable yield loss of rice and give rise a threat for food security of the country.

Keywords: weather; bacterial blight; rice; food security

1. Introduction

Rice (Oryzae sativa) belongs to Poaceae and is one of the foremost food crops in the world. As such, it comprises a major source of calories for rural and urban people and is the staple food for over half of the world’s population (Khush, 2005). Among the rice producing countries, Bangladesh rank fourth to China, India
and Indonesia both in acreage and production (Nutrient Data Laboratory, ARS, 2018). The low yield of rice is caused by various factors. In Bangladesh, 43 diseases are known to occur on rice, whereas 27 are seed borne (Fakir, 2000). Bacterial blight (BB) of rice caused by the vascular pathogen *X. oryzae pv. oryzae* (Swings et al., 1990) is one of the most destructive diseases of rice throughout the world (Mew et al., 1982). *X. oryzae pv. oryzae* (Xoo) is a rod-shaped, round-ended, gram-negative bacterium whose cells vary in length and are motile by means of a single polar flagellum. Colonies on nutrient broth yeast extract media containing glucose are round, convex, mucoid and yellow in colour due to the production of the pigment Xanthomonadin, the characteristic of the genus (Bradbury, 1984).

BB is prevalent in both tropical and temperate areas and is endemic to much of Asia and parts of West Africa. In Asia, this disease occurs destructively during the monsoon (Ou, 1985) and it is a serious threat to agriculture and global food security (Verdier et al., 2012). The diseases become one of the most devastating of rice because of the extensive cultivation of nitrogen-responsive modern rice cultivars (Mew et al., 1993). Damage due to BB increased significantly following the widespread cultivation of high-yielding and nitrogen-responsive dwarf hybrid varieties of rice in the 1960s. In tropical countries BB is even more destructive in seedling stage known as Kresek (Reddy, 1984). Reports from the Philippines, Indonesia and India estimated the losses due to the kresek syndrome of BB, which affects recently transplanted seedlings, have reached 60-75%, depending on weather, location and rice varieties (Reddy et al., 1979). Besides reducing yield, BB may also affect grain quality by interfering with maturation (Goto, 1992). Plants are infected at the maximum tillering stage, resulting in a yield reduction from 20 to 40% (Mew et al., 1993). Prior to the more recent incorporation of resistant varieties and implementation of strict quarantine measures in Japan, BB damage reported to range from 20 to 30% and as high as 50% (Alam et al., 2016). The epidemiological factors such as high temperatures, low and high errant rainfall, high humidity, and anomalies of wind, play a crucial role in the establishment of BB, and therefore, rice yield is lower than its potential (Ali et al., 2017; Shakoor et al., 2015). Climatic conditions greatly influence the development of *X. oryzae pv. oryzae* infection on rice (Naqvi, 2019). The infection cycle is influenced by the dispersal of pathogen and transfer between host plant species. Environmental factors facilitating the pathogen dispersal may determine why susceptible crops in a region are regularly infected while others remain pathogen free (An et al., 2020). Adhikari et al. (1994) reported that, bacterial leaf blight progression was highly correlated with environmental factors (rainfall, Relative Humidity etc.). Kapoor et al. (2004) reported that, the rainfall and distribution varied significantly within growing seasons during 1979-1999. The average monthly temperature (18-28°C) and RH (>90%) for more than 9 hours was within the optimum range for disease development. Minogue and Fry (1983) referred both pathogens and host plants may be studied together under a specific environmental setting termed a plant disease classic triangle. It is imperative to study epidemiological factors which generally lead to the epidemic of disease. In Bangladesh the highest disease incidence and severity of BB was recorded in hybrid variety, Shakti 2. The lowest incidence and severity of disease BB were observed in BRRI Hybrid Dhan 2. The highest yield was found in BRRI Hybrid Dhan 2 and the lowest in hybrid varieties Aloran and Shakti 2. In case of inbred varieties, the highest incidence and severity of brown spot was found in BB was recorded in BRRI Dhan 28. The lowest incidence and severity of BB was found in BINA 10. The highest yield was recorded in BRRI Dhan 50 and BINA 10 while the lowest was recorded in BRRI Dhan 28 and BRRI Dhan 29. In case of both diseases, it was observed that disease incidence and severity was gradually increased with the age of the plant and minimum incidence and severity gave the maximum yield (Sania et al., 2015). The present study was carried out to know the influence of weather conditions conducive for BB incidence, severity and spread of BB of rice in both inbred and hybrid cultivars in both rainfed and irrigated season through field surveys in thirty Agroecological Zones (AEZs) of Bangladesh.

2. Materials and Methods
2.1. Surveying and sampling
The experiment was conducted at Plant Bacteriology and Biotechnology Laboratory, Department of Plant Pathology, Seed Pathology Centre (SPC), Bangladesh Agricultural University, Mymensingh during August 2015 to December 2018. The surveys were conducted for the assessment of BB disease of rice in both irrigated and rainfed seasons in Bangladesh under 30 AEZs during 2015-2016 in the farmer’s field (Figure 1). The evaluation of the disease was done in terms of its prevalence, incidence, severity and disease index in 30 AEZs areas under field condition in Bangladesh. The leaf samples were randomly collected from different rice fields, experimental plots, or farmers’ plantings. Sample size within each zone ranged from 5 to 9. Three different locations of each zone were selected for sampling. Sample size varied depending on the prevalence and intense cropping intensity of the field. From each location upper 3 leaves of 10 plants were collected which formed...
composite sample and a representative sample was taken for the isolation of *X. oryzae pv. oryzae*. After collection, the diseased leaf samples were brought into the laboratory and preserved in the refrigerator for isolation of *Xoo*.

![Figure 1. Bacterial blight prone areas of Bangladesh. Red colored divisions showed higher BB severity as compared to the divisions green colored. Status was assessed based on the incidence and severity of the surveyed districts representing thirty Agro-ecological zones (AEZs) of Bangladesh.](image)

2.2. Assessment of incidence and severity

2.2.1. Prevalence

In each Agro-ecological zone, three locations were visited depending on the cropping intensity and more locations were visited with intense cropping. One acre (0.405 ha) of rice field was surveyed in each location for the presence or absence of BB disease (Rafi *et al.*, 2013). Prevalence was calculated using the following formula:

\[
\text{Prevalence (\%)} = \frac{\text{Locations showing bacterial blight disease}}{\text{Total locations visited}} \times 100
\]
2.2.2. Incidence

Visual incidence of the disease was recorded in terms of percentage of plants infected in a field. Plants were observed at five points along a diagonal transect. The points were five paces apart, starting ten paces into field. At each point four plants were examined. The disease incidence was calculated using the following formula (Rafi et al., 2013):

\[
\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants examined} \times \text{number of plants counted}} \times 100
\]

2.2.3. Severity and Disease Index

The severity of the disease was recorded as percentage of leaf area diseased out of the total area. The percentage of average lesion area of 15 leaves was taken for disease severity in each spot. For scoring BB severity in field, the following severity scale was used. Severity score was considered 1 if the lesion area is 1-5%, 3 if the area is 6-12%, 5 if the infected area is 13-25%, 7 if the lesion area is 26-50%, and maximum score 9 if 51-100% area of the leaf having lesion (SES, IRRI, 2013). For calculation of disease amount in an area following formula (SES, IRRI, 2013) was applied:

\[
\text{Disease index (\%)} = \frac{n(1) + n(3) + n(5) + n(7) + n(9)}{tn(9) \times \text{number of plants counted}} \times 100
\]

Where n (1), n (3), n (5), n (7) & n (9) = number of leaves showing severity scale

2.3. Metrological data collection for the surveyed area

Many environmental factors such as topography, climatic conditions, and cultural practices are related to disease development. Topographic factors or soil conditions are usually constant in contrast to the variable climatic conditions or cultural practices. Naturally, these non-variable and variable factors are interrelated (Mizukami et al., 1969). A meteorological data has recorded from meteorological department in Bangladesh for the period of 2015-2017 on temperature, rainfall and % RH for the surveyed area (BBS, 2017).

2.4. Isolation and purification of X. oryzae pv. oryzae from diseased leaves

Each diseased leaf sample was cut into small pieces, about 1-2 cm in length with the margin of typical lesions. The cut leaf pieces were sterilized by 10% Clorox solution for 1 minute and then with 70% ethanol for 1 minute followed by washing with sterilized deionized water. Each leaf sample was then homogenized with 1 ml of sterile distilled water. The resulting suspension was diluted serially (10^-5 to 10^-6) and then spreaded on Nutrient Broth Yeast Extract (NBY) agar medium. The plates were then incubated at 28ºC for 2-4 days (Alam et al., 2016). A pure culture is a population of cells or multicellular organisms growing in the absence of other species or types. A pure culture may originate from a single cell or single organism, in which case the cells are genetic clones of one another. The medium of NBY was used which can be used for the isolation and maintenance of thermophiles. The petridishes containing NBY medium was streaked using the pure culture of each group of the isolates of X. oryzae pv. oryzae with the help of sterilized loop (platinum wire dipping in rectified spirit and flaming over a spirit lamp). All the inoculated plates were incubated at 28 °C temperature for 48-72 hours to allow the growth of the bacteria. The bacterial growth obtained in the plates was recorded and those having morphological characteristics of X. oryzae pv. oryzae were picked up with sterilized loop and purified cultures were obtained by streaking on NBY media.

2.5. Confirmation of X. oryzae pv. oryzae isolates by pathogenicity test

The isolates of X. oryzae pv. oryzae were confirmed by the pathogenicity test using susceptible check rice cultivar IR24 in the net house, Seed Pathology Centre (SPC). Seeds of IR24 were sown in the plastic pot for raising the seedlings. The 30 days old seedlings were then transplanted in to the earthen pot into the net house for inoculation. The bacterial isolates were cultured in NBY agar medium at 28°C for 48 hours and then resuspend in sterile distilled water at cell density 10^8 cells/ml measured by spectrophotometer (Joshi et al., 2012). Secondary leaves of each plants were inoculated by clip-inoculation method of Kauffman et al. (1973) and then rice plants were kept in the net house for the development of BB symptoms (Rafi et al., 2013).
2.6. Confirmation of *X. oryzae* pv. *oryzae* isolates through Polymerase Chain Reaction (PCR)

The isolates with similar morphology and positive in pathogenicity test were used as representative isolates of *X. oryzae* pv. *oryzae* from each growing area for PCR confirmation. The PCR based identification of *X. oryzae* pv. *oryzae* was performed by using specific primers XOR-F and XOR-R2 as reported by Adachi et al. (2000).

2.7. Extraction of genomic DNA from *X. oryzae* pv. *oryzae*

Genomic DNA of *X. oryzae* pv. *oryzae* was extracted by using wizard® genomic DNA purification kit solution: pH 8.0 (Promega, Madison, WI, USA). Extraction of genomic DNA from each strain of *X. oryzae* pv. *oryzae* set were quantified using an UV spectrophotometer absorbance at 260 nm with a model T-80 UV/VIS and stored at -20°C. DNA concentration were adjusted to 100ng/µl and verified by comparing with a 100bp DNA ladder (Invitrogen, USA) on 1.5% agarose UV lamp was warmed up. Absorbance reading for other samples was recorded in the same way (Alam et al. 2016).

2.8. PCR amplification with primers

For molecular confirmation of the pathogen polymerase chain reaction (PCR) was performed with *Xoo* specific primers, XOR-F 5’-GCATGACGTCATCGTCCTGT-3’ and XOR-R2 5’-CTCGGAGCTATATGCCGTGC-3’ (Adachi and Oku, 2000). The PCR reactions were performed in 25 µl of reaction mixture for each DNA template. The PCR Go taq G2 Green master mix included taq polymerase buffer and dNTPs. DNA amplification was performed in a T100 Thermal Cycler (Bio-Rad, Hercules, CA, USA) under the following conditions: 95°C for 2 minutes (once), 95°C for 30sec, 63°C for 30sec and 72°C for 60sec. The number of cycles was kept 30 at the end a final extension of 72°C for 7min was given for completion of the process (Adachi et al., 2000).

2.9. Gel electrophoresis and visualization of the PCR products

The PCR products were analyzed by gel electrophoresis using a 1.5% agarose in 1X TBE buffer (Tris base, boric acid and 0.5 M EDTA [pH 8.0]) containing ethidium bromide (0.5 µg/ml). After electrophoresis, the gel was placed under UV transilluminator using the Gel View Master, Dynamica, UK for visualization of DNA bands. The UV light of the apparatus switched on, the image of the desired bands on the gel was viewed on the monitor and saved on the computer disc (CD-R) for taking photograph.

3. Results

3.1. Incidence and severity of bacterial blight of rice in rainfed season

A total of 35 districts representing thirty Agroecological Zones (AEZs) were surveyed (Fig. 4) at tillering and reproductive stages of plants to know the status of BB of rice in terms of its incidence and severity. The highest (55.11%) BB incidence was found in AEZs 7, 8, 9 and part of 28 covering Mymensingh (AEZ 8) division under districts Netrokona (AEZ 9), Sherpur (AEZ 9), Jamalpur (AEZ 9) and Tangail (AEZ 9), while the lowest (3.25%) incidence was observed in AEZ 13 that covers districts of Barishal, Jhalokhati, Perojpur and Bagerhat (Table 1). The highest (4.10) BB average severity was recorded in Mymensingh (AEZ 8) division under districts Netrokona (AEZ 9), Sherpur (AEZ 9), Jamalpur (AEZ 9) and Tangail (AEZ 9) while the lowest (3.25%) incidence was observed in AEZ 13 that covers districts of Barishal, Jhalokhati, Perojpur and Bagerhat (Table 1). The highest (4.10) BB average severity was recorded in Mymensingh (AEZ 8) division under districts Netrokona (AEZ 9), Sherpur (AEZ 9), Jamalpur (AEZ 9) and Tangail (AEZ 9) districts and the lowest (1.63) BB severity was calculated in Barishal, Jhalokhati, Perojpur and Bagerhat that are belongs to AEZ 13 (Table 1). The highest BB incidence (78.89%) was observed in hybrid varieties Aloron followed Dhanigold (55.56%), Agrodhan-14 (47.78%) of Netrokona (AEZ 9), Sherpur (AEZ 9), Mymensingh (AEZ 8) districts, respectively and the lowest (1.00%) BB incidence was found in local varieties (Sughondi) of Jhalokhati (AEZ 13) district. The maximum (5.51) BB severity was recorded from BR11 and Aloron rice varieties of Netrokona (AEZ 9) and the lowest (0.50) severity was recorded from local varieties (Balam) in Perojpur (AEZ 13).
Table 1. Status of BB (percent incidence and severity) of rice in 30 AEZs of Bangladesh during rainfed season, 2015.

| Sl No. | Sample No | Area       | *Incidence | *Severity | Source  | Variety               | AEZ covered in the region |
|--------|------------|------------|------------|-----------|---------|-----------------------|---------------------------|
| 1      | 31         | Panchaghar | 21.11      | 2.93      | Farmer  | Swarna sumon          |                          |
| 2      | 40         | Thakurghaon| 17.78      | 2.53      | Farmer  | Swarna sumon          |                          |
| 3      | 49         | Dinajpur   | 56.67      | 4.16      | Farmer  | Guti Swarna, Agrodhan | 1, 2, 3, 27              |
| 4      | 59         | Nilphamari | 44.44      | 3.60      | Farmer  | Tej                   |                          |
| 5      | 68         | Rangpur    | 48.89      | 4.64      | Farmer  | BR-11                 |                          |
| 6      | 77         | Gaibandha  | 36.67      | 3.36      | Farmer  | BR-11                 |                          |
|        |            | Rangpur Division |        |           |         |                       | 37.59, 3.54              |
| 7      | 86         | Bogura     | 17.78      | 2.73      | Farmer  | BR-11                 |                          |
| 8      | 95         | Natore     | 10.00      | 3.11      | Farmer  | BR-11                 | 4, 5, 6, 26              |
| 9      | 104        | Rajshahi   | 6.67       | 1.87      | Farmer  | SDV                   |                          |
|        |            | Rajshahi Division |        |           |         |                       | 11.48, 2.57              |
| 10     | 4          | Mymensingh | 47.78      | 3.71      | Farmer  | Pajam, Agrodhan-14,   |                          |
| 11     | S          | Netrokona  | 78.89      | 5.51      | Farmer  | BR-11, Aloron,        |                          |
| 12     | S9         | Sherpur    | 55.56      | 4.50      | Farmer  | BRRI dhan-49, Dhaniglod | 8,9 parts of 28         |
| 13     | 13         | Jamalpur   | 55.56      | 3.40      | Farmer  | BRRI dhan-49          |                          |
| 14     | 22         | Tangail    | 37.78      | 3.38      | Farmer  | BRRI dhan-49          |                          |
|        |            | Mymensingh Division |      |           |         |                       | 55.11, 4.10              |
| 15     | 156        | Satkhira   | 6.44       | 1.52      | Farmer  | Sada swarna           |                          |
| 16     | 147        | Khulna     | 2.33       | 0.61      | Farmer  | Swarna                |                          |
| 17     | 113        | Pabna      | 34.44      | 3.34      | BADC    | BR-11                 | 11, 13, 14               |
| 18     | 165        | Jashore    | 18.89      | 1.76      | Farmer  | Sorna                 |                          |
| 19     | 174        | Jhenaidah  | 22.78      | 2.64      | Farmer  | Tej                   |                          |
|        |            | Khulna Division |       |           |         |                       | 16.98, 1.98              |
| 20     | 122        | Barishtal  | 5.00       | 2.00      | Farmer  | Local                 |                          |
| 21     | 131        | Jhalokhati | 1.00       | 1.00      | Farmer  | Sughondi              | 13                       |
| 22     | 135        | Peroipur   | 2.00       | 0.50      | Farmer  | Balam                 |                          |
| 23     | 144        | Bagherhut  | 5.00       | 3.00      | Farmer  | Local                 |                          |
|        |            | Barishtal Division |     |           |         |                       | 3.25, 1.63              |
| 24     | 192        | MouloviBazar| 8.33     | 1.87      | Farmer  | BRRI dhan19           |                          |
| 25     | 201        | Sylhet     | 14.44      | 2.31      | Farmer  | Local                 | 20, 21, 22              |
| 26     | 210        | Hobiganj   | 6.11       | 1.91      | Farmer  | Local                 |                          |
|        |            | Sylhet Division |       |           |         |                       | 9.63, 2.03              |
| 27     | 219        | Feni       | 8.89       | 2.24      | Farmer  | Local                 |                          |
| 28     | 246        | Loxmipur   | 11.67      | 2.11      | Farmer  | BRRI dhan49           | 16, 30, minor            |
| 29     | 183        | Cumilla    | 20.00      | 3.07      | Farmer  | BRRI dhan53           | part 17, 18,             |
| 30     | 254        | Chandpur   | 10.00      | 3.00      | Farmer  | BRRI dhan49           | 19, 22, 23,              |
| 31     | 228        | Chattagram | 7.22       | 1.86      | Farmer  | Local                 | 24, 29                  |
| 32     | 237        | Khagrachori| 7.22       | 1.71      | Farmer  | Puja                  |                          |
|        |            | Chottagram Division |    |           |         |                       | 10.83, 2.33             |
| 33     | 239        | Narsingdi  | 8.33       | 0.60      | Farmer  | BRRI dhan29           |                          |
| 34     | 241        | Gazipur    | 55.56      | 3.40      | Farmer  | BRRI dhan29, BR11     | 15, 7, 8, 28            |
| 35     | 243        | Manikganj  | 47.78      | 3.00      | Farmer  | BRRI dhan29, BR12     |                          |
|        |            | Dhaka Division |      |           |         |                       | 37.22, 2.33             |

*Data are the averages of 9 locations of each the districts covering the AEZs surveyed
3.2. Incidence and severity of bacterial blight of rice in irrigated season
The status of bacterial leaf blight of rice was assessed in terms of its incidence and severity in irrigated season during 2016. The highest (32.33%) BB incidence was found in AEZs 7, 8, 9, and part of 28 covering Mymensingh division under districts Mymensingh (AEZ 8), Netrokona (AEZ 9), Sherpur (AEZ 9), Jamalpur (AEZ 9) and Tangail (AEZ 9), while the lowest (4.50%) incidence was observed in AEZ 13 that covers districts of Barishal, Jhalokhati, Perojpur and Bagerhat (Table 1). The highest (3.13) BB average severity was recorded in Mymensingh division under districts Mymensingh (AEZ 8) and Netrokona (AEZ 9) districts and the lowest (1.10) BB severity was calculated in Barishal, and Bhola that are belongs to AEZ 13 (Table 2). The highest BB incidence (45.00%) was observed in hybrid varieties Hera-2 (AEZ 9), ACI-2 (22.00%) of Netrokona (AEZ 9), Rangpur (AEZ 27) districts, respectively and the lowest (3.50%) BB incidence was found in local varieties of Hobiganj (AEZ 20, 21, 22) districts. The maximum (4.00) BB severity was recorded from Agrodhan14, BRRI dhan29 rice varieties in Mymensingh (AEZ 9), Gaibandha (AEZ 1. 2, 3, 27) and the lowest (0.50) severity was recorded from local varieties in Barishal (AEZ 13).

Table 2. Status of BB (percent incidence and severity) of rice in 30 AEZs of Bangladesh during irrigated season, 2016.

| Sl No. | Sample No | Area       | *Incidence | *Severity | Source       | Variety       | AEZ covered in the region |
|--------|-----------|------------|------------|-----------|--------------|---------------|--------------------------|
| 1      | 281       | Gaibandha  | 25.33      | 4.00      | Farmer       | BRRI dhan28   | 1,2,3,27                 |
| 2      | 287       | Rangpur    | 22.00      | 3.00      | Farmer       | BRRI dhan29, Hera-2, ACI-2 |                         |
| 3      | 290       | Dinajpur   | 5.00       | 2.00      | Farmer       | BRRI dhan29   |                          |
|        |           | Rangpur Division | 17.44      | 3.00      |              |               |                          |
| 4      | 264       | Natore     | 5.27       | 2.00      | Farmer       | BRRI dhan29   | 4,5,6,26                 |
| 5      | 277       | Bogura     | 6.50       | 3.00      | Farmer       | Binadhan29    |                          |
| 6      | 269       | Sirajganj  | 5.00       | 2.00      | Farmer       | BRRI dhan29   |                          |
|        |           | Rajshahi Division | 5.59      | 2.33      |              |               |                          |
| 7      | 307       | Mymensingh | 22.00      | 4.00      | Farmer       | Agrodhan14    | 8,9,28                   |
| 8      | 312       | Netrokona  | 45.00      | 3.00      | Farmer       | Hera-2,       |                          |
| 9      | 315       | Fulphur    | 30.00      | 2.40      | Farmer       | Partex hybrid |                          |
|        |           | Mymensingh Division | 32.33      | 3.13      |              |               |                          |
| 10     | 321       | Bagerhat   | 5.00       | 2.00      | Farmer       | local         | 13                       |
|        |           | Khulna Division | 5.00      | 2.00      |              |               |                          |
| 11     | 316       | Bhola      | 4.00       | 1.60      | Farmer       | local, BRAC hybrid | 13                    |
| 12     | 319       | Barishal   | 5.00       | 0.50      | Farmer       | local         |                          |
|        |           | Barishal Division | 4.50      | 1.10      |              |               |                          |
| 13     | 293       | Sylhet     | 6.50       | 2.00      | Farmer       | BRRI dhan29   | 20, 21, 22               |
| 14     | 295       | Hobiganj   | 3.50       | 3.00      | Farmer       | Local         |                          |
| 15     | 297       | Sunamganj  | 4.33       | 3.00      | Farmer       | BRRI dhan29   |                          |
|        |           | Sylhet Division | 4.78      | 2.67      |              |               |                          |
| 16     | 299       | Brahmanbaria| 10.00      | 2.50      | Farmer       | BRRI dhan29   | 16, 17, 18, 19, 22, 30        |
| 17     | 305       | Cumilla    | 7.50       | 2.00      | Farmer       | BRRI dhan29, ACI-2 | 7, 8, 12, 14, 19, 22, 30 |
| 18     | 274       | Feni       | 10.00      | 2.00      | Farmer       | BRRI dhan29   |                          |
|        |           | Chattagram Division | 9.17      | 2.00      |              |               |                          |
| 19     | 302       | Narsingdi  | 15.00      | 0.60      | Farmer       | BRRI dhan29   |                          |
| 20     | 305       | Gazipur    | 20.00      | 2.00      | Farmer       | BRRI dhan29, BR11 | 7, 8, 12, 14, 15, 28 |
| 21     | 310       | Manikganj  | 20.5       | 3.00      | Farmer       | BRRI dhan29, BR12 |                          |
| 22     | 323       | Gopalganj  | 10.00      | 2.40      | Farmer       | local         |                          |
|        |           | Dhaka Division | 16.38     | 2.70      |              |               |                          |

*Data are the averages of 9 locations of each the districts covering the AEZs surveyed
3.3. Prevalence of bacterial blight disease in rice varieties during rainfed and irrigated seasons 2015-16

In rainfed season, surveyed results indicated that the highest (86.00%) prevalence and disease severity (4.10) were observed in Mymensingh (AEZ 7, 8, 9 part of 28) division followed by Rangpur (AEZ 1, 2, 3, 27), Dhaka (AEZ 7, 8, 15, 28), Rajshahi (AEZ 4, 5, 6, 26), Chittagong (AEZ 16, 17, 19, 22, 23, 29, 30), Sylhet (AEZ 20, 21, 22), Khulna (AEZ 11, 13, 14) and lowest prevalence (10.00%) and disease severity (0.61) were found in Barishal (AEZ 14) (Figure 2). In irrigated season, the results indicated that the highest (45.50%) prevalence and disease severity (3.13) were observed in Mymensingh (AEZ 7, 8, 9 part of 28) division followed by Rangpur (AEZ 1, 2, 3, 27), Dhaka (AEZ 7, 8, 15, 28), Chittagong (AEZ 16, 17, 19, 22, 23, 29, 30), Rajshahi (AEZ 4, 5, 6, 26), Sylhet (AEZ 20, 21, 22), Khulna (AEZ 11, 13, 14) and lowest prevalence (8.00%) and disease severity (1.55) were found in Barishal (AEZ 14) (Figure 3).

![Figure 2. Percent Prevalence, incidence and diseases severity of bacterial leaf blight in eight divisions of Bangladesh in rainfed season; Rangpur (AEZ 1, 2, 3, 27); Rajshahi (AEZ 4, 6, 5, 26); Khulna (AEZ 11, 13, 14), Barishal (AEZ 14); Mymensingh (AEZ 7, 8, 9, 28); Sylhet (AEZ 20, 21, 22); Chittagong (AEZ 23, 24, 29, 16, 17, 19, 22, 30); Dhaka (AEZ 7, 8, 28, 15).](image1)

![Figure 3. Percent Prevalence, incidence and diseases index of bacterial leaf blight in eight divisions of Bangladesh in irrigated season; Rangpur (AEZ 1, 2, 3, 27); Rajshahi (AEZ 4, 6, 5, 26); Khulna (AEZ 11, 13, 14), Barishal (AEZ 14); Mymensingh (AEZ 7, 8, 9, 28); Sylhet (AEZ 20, 21, 22); Chittagong (AEZ 23, 24, 29, 16, 17, 19, 22, 30); Dhaka (AEZ 7, 8, 28, 15). * Data are the average of the districts of each division.](image2)

3.4. Effects of environment factors on disease development

3.4.1. Climatic conditions

Different environmental factors that greatly influence disease development are temperature, relative humidity, rainfall and a little sunshine and strong winds influenced BB during rice growing season. When mean temperature more than 24°C, relative humidity ranges from 64-84 percent, rainfall more than 200 mm, little sunshine and strong winds coincide, this disease appear in the form of epidemic. A combination of
meteorological factors such as high temperature, high humidity, heavy rainfall, high light intensity and frequent typhoons favoured the outbreak of the disease. In both rainfed and irrigated season, all the environmental factors prevailed in Mymensingh division and BB development were greatly influenced followed by Rangpur, Dhaka, Rajshahi, Chattagram, Khulna, Sylhet, and Barishal (Figure 4, 5). Survey results indicated that environmental factors viz. temperature (25-34°C), Relative humidity (70-84%), and rainfall (>200mm) largely affected the prevalence, incidence and severity of BB division wise.

Figure 4. Environment factors (rainfall, temperature and relative humidity) on BB disease development with relation to disease incidence in rainfed season during 2015.

Figure 5. Environment factors (rainfall, temperature and relative humidity) on BB disease development with relation to disease incidence in irrigated season during 2016.

3.5. Identification of X. oryzae pv. oryzae with specific molecular marker and by pathogenicity test
The PCR based confirmation of X. oryzae pv. oryzae was performed by using primers XOR-F and XOR-R2 markers. Results showed that the primers XOR-F and XOR-R2 amplified a specific DNA fragment in the size of 470 bp with DNA of all representatives X. oryzae pv. oryzae isolates (Figure 6). The isolates of X. oryzae pv. oryzae were confirmed by the pathogenicity test using susceptible check rice cultivar IR24. All 239 isolates produced typical disease symptom on susceptible check variety IR24. The BB symptom first appeared as tiny water-soaked spots at the margin of the rice leaf blade. Then, it rapidly engulfed surrounding tissue and the rice plants turned yellow and wither. Finally, on leaf tissue, yellowish-white or straw-colored lesion with wavy edges exhibited bacterial leaf blight symptoms which was caused by X. oryzae pv. oryzae (Figure 7).
Figure 6. Agarose gel electrophoresis of PCR products from *X. oryzae* pv. *oryzae* using primer XOR-F and XOR-R2, M= DNA ladder (100bp Invitrogen). Lanes 1-8 are the PCR products from isolates BDXO91, BDXO68, BDXO34, BDXOS6, BDXO210, BDXO251 and BDXO309, control (Water, lane 9) respectively. The molecular size marker is a 100bp DNA ladder (Invitrogen).

Figure 7. Typical symptoms of BB on mature leaves of rice (cv. IR24) caused by *X. oryzae* pv. *oryzae* isolates.

4. Discussion

The study was undertaken to know the status of BB of rice in terms of its incidence and severity in thirty Agroecological Zones (AEZs) of Bangladesh. Findings of survey during two seasons showed that the disease was prevailed throughout the major rice growing areas with varying degree of incidence. A total of thirty Agroecological Zones (AEZs) were surveyed to know the status of BB of rice in terms of incidence and severity. In rainfed season, survey results showed that the highest (55.11%) BB incidence was found in AEZs 7, 8, 9, and part of 28 covering districts Mymensingh (AEZ 8), Netrokona (AEZ 9), Sherpur (AEZ 9), Jamalpur (AEZ 9) and Tangail (AEZ 9), while the lowest (3.25%) incidence was observed in AEZ 13 that covers districts of Barishal, Jhalokhati, Perojpur and Bagerhat. The highest (4.10) BB average severity was recorded in Mymensingh (AEZ 8), Netrokona (AEZ 9), Sherpur (AEZ 9), Jamalpur (AEZ 9) and Tangail (AEZ 9) districts and the lowest (1.63) BB severity was calculated in Barishal, Jhalokhati, Perojpur and Bagerhat that are belongs to AEZ 13. The severity scale was calculated by the randomly selected three from each farmer field for each location. The highest (5.51) BB average severity was recorded in hybrid varieties Aloron in Netrokona (AEZ 9) districts and the lowest BB severity (0.50) was calculated in local varieties Balam in Perojpur. In irrigated season, the highest (32.33%) BB incidence was found in AEZs 7, 8, 9, and part of 28 covering districts Mymensingh (AEZ 8), Netrokona (AEZ 9), Sherpur (AEZ 9), Jamalpur (AEZ 9) and Tangail (AEZ 9), while the lowest (4.50%) incidence was observed in AEZ 13 that covers districts of Barishal, Jhalokhati, Perojpur and Bagerhat. The highest (4.00) BB severity was recorded in Mymensingh (AEZ 8), Gaibandha (AEZ 3) districts and the lowest (0.50) severity was calculated in Barishal that belongs to AEZ 13. The present findings were supported by Miah *et al.* (1985) reported that *Aus*, the most humid and warmest season, and the coastal humid areas experienced more major diseases. Seasonal and regional differences in the disease incidence appeared related to agro-climatic variations. Faruq *et al.* (2015) who reported that in all growth stages, the highest incidence and severity was recorded in hybrid variety Aloron whereas the lowest incidence and severity was observed in
hybrid variety Hera-2. These results agreed with the findings of Sania *et al.* (2015) found that, all the varieties were developed BB disease under natural condition. Among the hybrid varieties, the highest incidence and severity of BB was found in Shakti-2 at different days after transplanting (40 DAT, 60 DAT and 80 DAT). The lowest incidence of BB was found in Aloran (40 DAT and 80 DAT) and BRRI Hybrid dhan 2 (60 DAT and 80 DAT). The lowest Incidence and severity in leaf of BB was recorded in BRRI Hybrid dhan 2 at all of the investigation (40 DAT, 60 DAT and 80 DAT). In case of inbred varieties, the highest incidence and severity of BB in both cases of hill and leaf, was recorded in BRRI dhan 28 and lowest in Binadhan-10 at all of the investigation (40 DAT, 60 DAT and 80 DAT). The incidence of BB disease of rice at maximum tillering and reproductive stage ranged from 2.33 to 78.89%. The surveyed results indicated that the highest (78.89%) incidence was observed in hybrid variety Hera-2 found in Mymensingh division followed by Agrodhan (56.67%), ACI hybrid (55.56), BRAC hybrid (22.78%) found in rangpur, Cumilla and Khulna divisions and the lowest (2.33%) incidence was found in local and HYV variety Swarna followed by BRRI dhan29 (48.89%), Pajam (47.78%), area covered in Khulna, Rangpur and Mymensingh divisions. Similar findings of the study Rahman *et al.* (2013) reported that out of 15 tested hybrid rice varieties, Tej-1 and Krishan-2 showed the highest incidence and severity of BB at all the growth stages. The lowest disease incidence and severity of BB was recorded from Hera-1 followed by BRRI hybrid dhan 2, Hera-2 and ACI-1. Latif *et al.* (2011) reported that in the irrigated rice during 1999 to 2000, most of the tested hybrid and inbred varieties were found moderately susceptible, while hybrid variety Aalock6201, IR69690H, IR68877H and inbred variety Anamika and BRRI dhan28 were found susceptible against BB. The yield of these varieties varied significantly due to brown spot and BB disease. Among the hybrid varieties, the highest yield was recorded in BRRI hybrid dhan 2 and the lowest yield was recorded in Aloran and Shakti 2. In inbred varieties, the highest yield was found in BRRI dhan 50 and Binadhan-10 while the lowest was found in BRRI dhan 28 and BRRI dhan 29. It should be noted that the highly infected varieties give the lowest yield and the lowest infected varieties gives the higher yield. Rahman *et al.* (2013) reported that Hera-1 followed by BRRI hybrid dhan 2, Hera-2 and ACI-1 produced the higher panicle length, number of filled grains/panicle, number of rachis/panicles, 1000-seed weight, grain yield and showed lowest disease incidence and severity of BB. The studies were conducted to evaluate the effect of different varieties of rice on incidence and severity of brown spot, bacterial leaf blight and tungro disease which cause serious damages in rice cultivation in worldwide. The survey results implied that there were a considerable variation of BB incidence and severity from one region to another region. Differences of blight incidence and severity related to the great diversity of host plants affected by this pathogen, phenotype and genotype of *X. oryzae pv. oryzae*, its wide geographical distribution in the country (Alam *et al.*, 2016; Haque *et al.*, 2022; Islam *et al.*, 2016; Haque *et al.*, 2021) and the range of environmental (temperature, relative humidity, rainfall, sunshine) conditions conducive to BB. In rainfed and irrigated season, Mymensingh division favours all the environmental factors that greatly influence BB diseases development followed by rangpur, Dhaka, Rajshahi, Chittagram, Khulna, Sylhet, and Barisal. Survey results indicated that environmental factors viz. temperature (25-34°C), Relative humidity (70-84%), and rainfall (>200mm) largely affect the prevalence, incidence and severity of BB disease wise. For more confirmation of pathogenic bacteria, *X. oryzae pv. oryzae* up to species, the species-specific primer XOR-F and XOR-R2 was used. The polymerase chain reaction (PCR)-based identification technique is able to detect as few as one single copy of target DNA of the test pathogen. However, the DNA from 8 representative pathotypes of *X. oryzae pv. oryzae* isolate was extracted, quantified and amplified in PCR by using specific primers of XOR-F and XOR-R2. All the isolates gave 470 bp band size for XOR-F and XOR-R2 primers that corresponded to *X. oryzae pv. oryzae*. This band pattern was in agreement with the result of Adachi *et al.* (2000), they reported that *X. oryzae pv. oryzae* produced 470 bp band size when DNA of that pathogen was amplified with the specific primer XOR-F and XOR-R2 and Raff *et al.* (2013) also found that identity of the all candidate Xoo isolates was verified through Polymerase Chain Reaction (PCR) using primer XOR-F and XOR-R2 and produced 470 bp band size.

5. Conclusions
In rainfed condition, the highest BB incidence and severity were recorded in hybrid varieties “Aloron” from Netrokona and the lowest incidence was observed in local variety “Sughondi” from Jalhokhati. Considering the BB severity, the highest severity was recorded in Aloron from Netrokona while the lowest severity was obtained in local variety “Balam” from Perojpur. In irrigated condition, the highest BB incidence was recorded in hybrid varieties Hera-2 from Netrokona and the lowest incidence was observed in local varieties from Hobiganj. Considering the BB severity, the highest severity was recorded in Agrodhan-14, BRRI dhan28 from Mymensingh and Gaibandha while the lowest severity was obtained in local variety from Barishal. Variety
Swarna, Puja, BRRIdhan28, BRRIdhan29, BRRI dhan49 showed a moderate resistant and hybrid Hera-2, Tej, Partex, Agrodhan-14, Aloron, ACI-2, Dhanigold etc. were showed a susceptible against BB. The findings of the present study clearly indicated that close monitoring is required to prevent inoculum buildup of BB pathogen in major rice growing areas and search for region specific sustainable management strategies to minimize yield losses that occurred due to BB for ensuring food security of the country.

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Data availability
The tables, figures and texts in this article contain the data that support the findings of this study.

Conflict of interest
None to declare.

Authors' contribution
Conceptualization and methodology: Md. Rashidul Islam; Methodology: Md. Mahbubul Haque, Mamuna Mahjabin Mita, Samrin Bashar; Data collection: Md. Mostafa Masud; Laboratory work: Md. Mostafa Masud, Mamuna Mahjabin Mita, Samrin Bashar, Uttam Kumar Mozumdar; Writing-original draft: Md. Mahbubul Haque; Review and editing: Md. Rashidul Islam. All authors have read and approved the final manuscript.

References
Adachi N and T Oku, 2000. PCR-mediated Detection of X. oryzae pv. oryzae by amplification of the 16S–23S rDNA Spacer Region Sequence. J. Gen. Plant Pathol., 66: 303.
Adhikari TB, TW Mew and PS Teng, 1994. Phenotypic diversity of Xanthomonas oryzae pv. oryzae in Nepal. Plant Dis., 78: 68–72.
Alam S, R Islam, I Hossain and MR Bhuiyan, 2016. Pathotypic variation of X. oryzae pv. oryzae in Bangladesh. Arch. Phytopathol. Pflanzenschutz, 49: 1-12.
Ali S, Y Liu, M Ishaq, T Shah, Abdullah, A Ilyas and IU Din, 2017. Climate change and its impact on the yield of major food crops: evidence from Pakistan. Foods, 6: 39.
An, SQ, N Potnis, M Dow, FJ Vorhölter, YQ He, A Becker, D Teper, Y Li, N Wang, I Bleris and JL Tang, 2020. Mechanistic insights into host adaptation, virulence and epidemiology of the phytopathogen Xanthomonas. FEMS Microbiol. Rev., 44: 1-32.
BBS, 2017. Statistical yearbook of Bangladesh, Bangladesh Bureau of Statistics, Government of People’s Republic of Bangladesh.
Bradbury, 1984. Genus II. Xanthomonas Dowson. In: Bergey’s Manual of Systematic Bacteriology (Krieg, N.R. and Holt, J.G., eds), pp. 199–210.
Fakir GA, 2000. An annotated list of seed borne disease in Bangladesh. Seed Pathology Laboratory. Dept. of Plant Pathology, BAU, Mymensingh. p. 41.
Faruq AN, MM Rahman, N Akhtar, MT Islam, MM Uddin and N Ora, 2015. Evaluation of imported hybrid rice varieties against three field diseases under natural epiphytic conditions of Bangladesh. Adv. Agric. Biol., 4: 1-7.
Goto M, 1992. Fundamentals of Bacterial Plant Pathology. San Diego: Academic Press inc. San Diego, California (US).
Haque MM, MM Masud, MI Hossain, MM Rashid, MZ Alam and MR Islam, 2021. Assessment of potentiality of known bacterial blight resistant genes against Xanthomonas oryzae pv. oryzae pathotypes exist in Bangladesh. Arch. Agric. Environ Sci., 6: 257-267.
Haque MM, MM Masud, S Bashar, MI Hossain, MZ Alam and MR Islam, 2022. Avirulence gene based RFLP and rep-PCR distinguish the genetic variation of Xanthomonas oryzae pv. oryzae pathotypes in Bangladesh. Plant Sci. Today, 9: 29-40.
IRRI, SES, 2013. Standard evaluation system for Rice.
Islam MR, MS Alam, AI Khan, I Hossain, LR Adam and F Daayf, 2016. Analyses of genetic diversity of bacterial blight pathogen, X.oryzae pv. oryzae using IS1112 in Bangladesh. C. R. Biol., 339: 399-407.
Joshi LT, DS Phillips, CF Williams, A Alyousef and L Baillie, 2012. Contribution of spores to the ability of Clostridium difficile to adhere to surfaces. Appl. Environ. Microbiol., 78: 7671–7679.
Kapoor S, NSK Harsh and SK Sharma, 2004. A new wilt disease of Acacia nilotica caused by Fusarium oxysporum. J. Trop. For. Sci., 16: 453-462.

Kauffman HE, APK Reddy, SPY Hsieh and SD Merca, 1973. An improved technique for evaluating resistance of rice varieties to X. oryzae. Plant Dis. Rep., 57: 537-541.

Khush GS, 2005. What it will take to feed 5.0 billion rice consumers in 2030. Plant Mol. Biol., 59: 1-6.

Latif MA, MA Badsha, MI Tajul, MS Kabir, MY Rafii and MAT Mia, 2011. Identification of genotypes resistant to blast, bacterial leaf blight, sheath blight and tungro and efficacy of seed treating fungicides against blast disease of rice. Sci. Res. Essays, 6: 2804-2811.

Mew TW, AM Alvarez, JE Leach and J Swings, 1993. Focus on bacterial blight of rice. Plant Dis., 77: 5-12.

Mew TW, SZ Wu and H Horino, 1982. Pathotypes of X. campestris pv. oryzae in Asia. IRRI Research Paper Series 75. International Rice Research Institute, Manila, the Philippines.

Miah SA, AKM Shahjahan, MA Hossain and NR Sharma, 1985. A survey of rice disease in Bangladesh. Trop. Pest Manag., 31: 208-213.

Minogue KP and WE Fry, 1983. Models for the spread of disease: model description. Phytopathol., 73: 1168-1173.

Mizukami T and S Wakimoto, 1969. Epidemiology and control of bacterial leaf blight of rice. Annu. Rev. Phytopathol., 7: 51-72.

Naqvi SAH, 2019. Bacterial leaf blight of rice: an overview of epidemiology and management with special reference to Indian sub-continent. Pak. J. AgricRes, 32: 359.

Nutrient Data Laboratory, ARS, 2018. USDA National Food and Nutrient Analysis Program Wave 6m, Beltsville MD.

Ou SH, 1985. Rice Diseases. 2nd ed., Commonwealth Mycological Institute, Kew, Surrey, England. p. 61-96.

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

Rahman MM, MT Islam, AN Faruq, N Akhtar, N Ora and MM Uddin, 2013. Evaluation of some cultivated hybrid boro rice varieties against BLB, ShB and ALS diseases under natural epiphytic conditions. Middle East J. Sci. Res., 15: 146-151.

Reddy APK, DR Mackenzie, DI Rouse and AV Rao, 1979. Relationship of bacterial leaf blight X. oryzae severity to grain yield of rice Oryza sativa. Phytopathol., 69: 967–969.

Reddy PR, 1984. Kresek phase of bacterial blight of rice. Oryza, 21: 179-187.

Sania AR, MB Hossain, FM Aminuzzaman, PS Amith and SM Sydujjaman, 2015. Incidence and severity of Brown Spot (BS) and Bacterial Leaf Blight (BLB) in hybrid and inbred rice varieties in Bangladesh. AASCIT J. Biol., 1: 55-64.

Shakoor U, A Saboor, I Baig, A Afzal and A Rahman, 2015. Climate variability impacts on rice crop production in Pakistan. Pak. J. Agric. Res., 28: 19-27.

Swings J, M Van Den Mooter, L Vauterin, B Hoste, M Gillis, TW Mew and K Kersters, 1990. Reclassification of the causal agents of bacterial blight X. campestris pathovar oryzae and bacterial leaf streak X. campestris pathovar oryzicola of rice as pathovars of X. oryzae new species Ex Ishiyama 1922 Revived Name. Int. J. Syst. Bacteriol., 40: 309–311.

Verdier V, V Cruz and JE Leach, 2012. Controlling rice bacterial blight in Africa: needs and prospects. J. Biotechnol., 159: 320–328.