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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 inbreed 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 inbreed 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*.

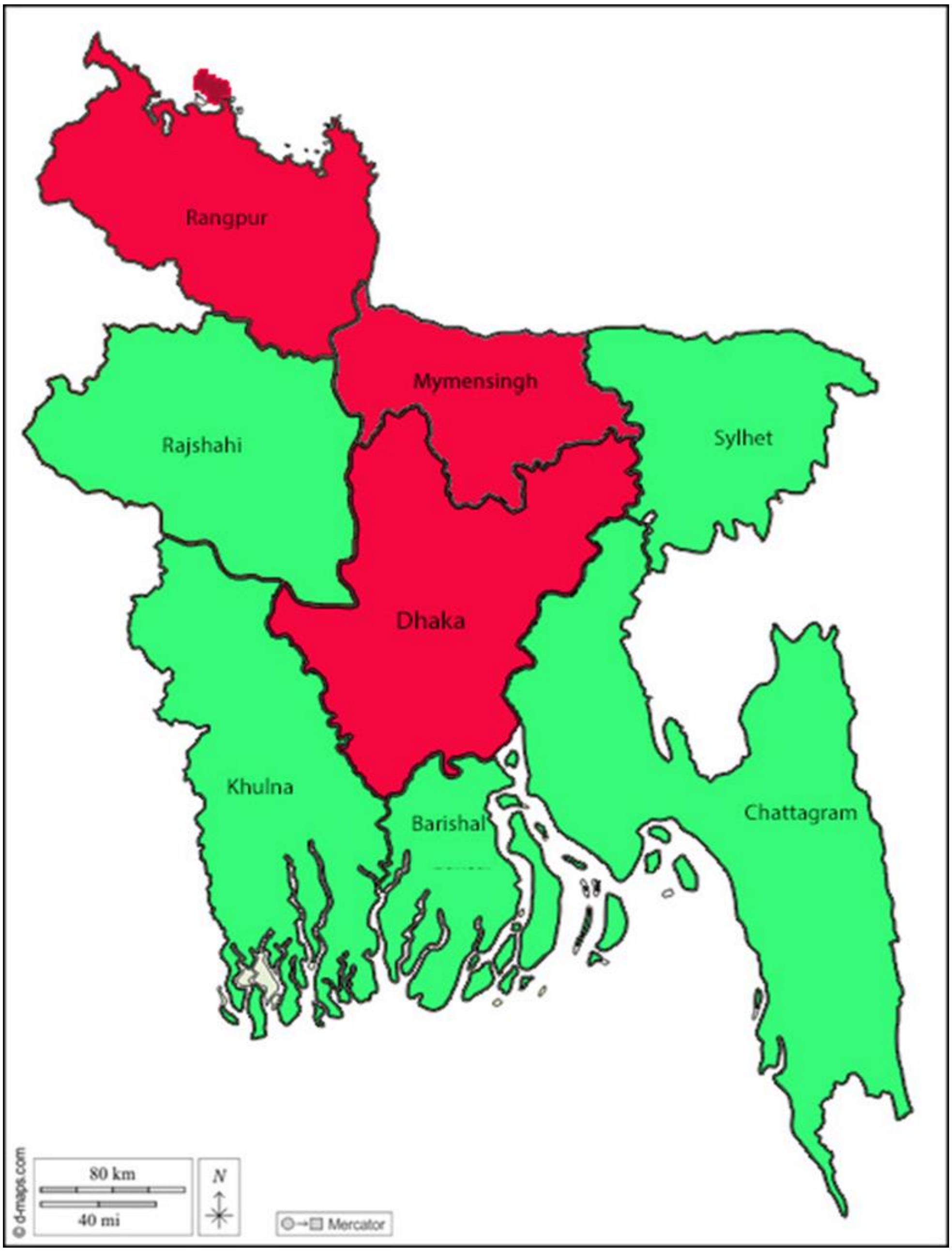

Figure 1. Bacterial blight prone areas of Bangladesh. Red colored divisions showed higher BB severity as compared to the divisions green colored. Status was assessed based on the incidence and severity of the surveyed districts representing thirty Agro-ecological zones (AEZs) of Bangladesh.

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):

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

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:

$$\text{Disease index (\%)}=\frac{\text{n(1) + n (3) + n (5) + n (7) + n (9)}}{\text{tn (9) cted plants}}\times 100$$

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 ºC 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 28ºC 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^{0}C. DNA concentration were adjusted to 100ng/µl and verified by comparing with a 100bp DNA ladder (Invitrogen, USA) on 1.5% agarose gel. For quantification of DNA concentration, the wave length of spectrophotometer (Model no. UH-5300, Hitachi, Japan) wave length was set at 260 nm after the spectrophotometer UV lamp was warmed up. Absorbance reading for other samples was recorded in the same way (Alam *et al.* 2016). Before conducting PCR analyses, DNA concentrations were adjusted to 25ng/µl by using the following formula: $V_1 \times S_1 = V_2 \times S_2$

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^{o}C for 2 minutes (once), 95^{o}C for 30sec, 63^{o}C for 30sec and 72^{o}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) 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	1, 2, 3, 27
2	40	Thakurghaon	17.78	2.53	Farmer	Swarna sumon	
3	49	Dinajpur	56.67	4.16	Farmer	Guti Swarna, Agrodhan	
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	4,5,6,26
8	95	Natore	10.00	3.11	Farmer	BR-11	
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,	8,9 parts of 28
11	S	Netrokona	78.89	5.51	Farmer	BR-11, Aloron,	
12	S9	Sherpur	55.56	4.50	Farmer	BRRI dhan-49, Dhaniglod	
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	11, 13, 14
16	147	Khulna	2.33	0.61	Farmer	Swarna	
17	113	Pabna	34.44	3.34	BADC	BR-11	
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	Barishal	5.00	2.00	Farmer	Local	13
21	131	Jhalokhati	1.00	1.00	Farmer	Sughondi	
22	135	Perojpur	2.00	0.50	Farmer	Balam	
23	144	Bagherhut	5.00	3.00	Farmer	Local	
Barishal Division			**3.25**	**1.63**			
24	192	MouloviBazar	8.33	1.87	Farmer	BRRIdhan19	20,21,22
25	201	Sylhet	14.44	2.31	Farmer	Local	
26	210	Hobiganj	6.11	1.91	Farmer	Local	
Sylhet Division			**9.63**	**2.03**			
27	219	Feni	8.89	2.24	Farmer	Local	16, 30, minor part 17, 18, 19, 22, 23, 24, 29
28	246	Loxmipur	11.67	2.11	Farmer	BRRIdhan49	
29	183	Cumilla	20.00	3.07	Farmer	BRRIdhan53	
30	254	Chandpur	10.00	3.00	Farmer	BRRIdhan49	
31	228	Chattagram	7.22	1.86	Farmer	Local	
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	15, 7, 8, 28
34	241	Gazipur	55.56	3.40	Farmer	BRRI dhan29, BR11	
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 Agrodhan-14, 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	
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	7, 8, 12, 14, 15, 28
20	305	Gazipur	20.00	2.00	Farmer	BRRI dhan29, BR11	
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), Chttagram (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), Chattagram (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).

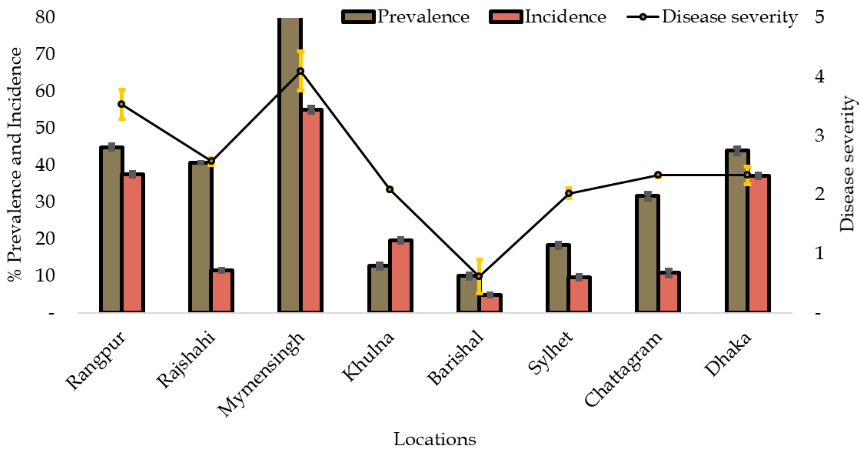

Figure 2. Percent Prevalence, incidence and diseases severity of bacterial leaf blight in eight divisions of Bangladesh in rainfed season; Rangpur (AEZ 1, 2, 3, 27); Rajshahi (AEZ 4, 6, 5, 26); Khulna (AEZ 11, 13, 14), Barishal (AEZ 14); Mymensingh (AEZ 7, 8, 9, 28); Sylhet (AEZ 20, 21, 22); Chattagram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

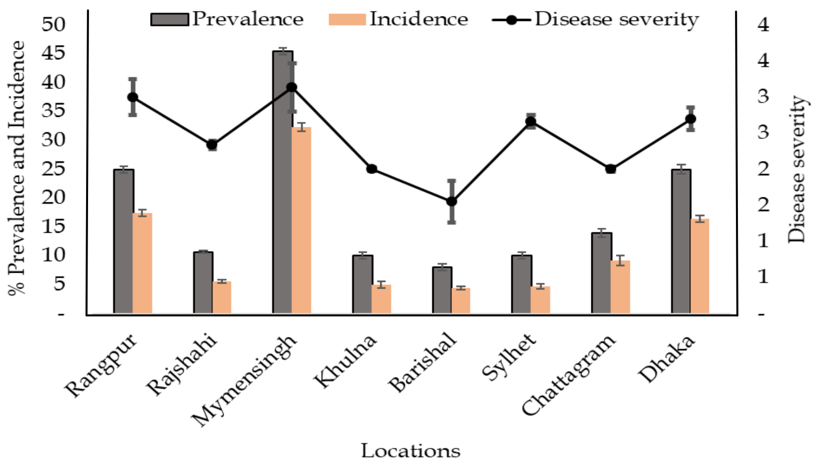

Figure 3. Percent Prevalence, incidence and diseases index of bacterial leaf blight in eight divisions of Bangladesh in irrigated season; Rangpur (AEZ 1, 2, 3, 27); Rajshahi (AEZ 4, 6, 5, 26); Khulna (AEZ 11, 13, 14), Barishal (AEZ 14); Mymensingh (AEZ 7, 8, 9, 28); Sylhet (AEZ 20, 21, 22); Chattagram (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.

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.

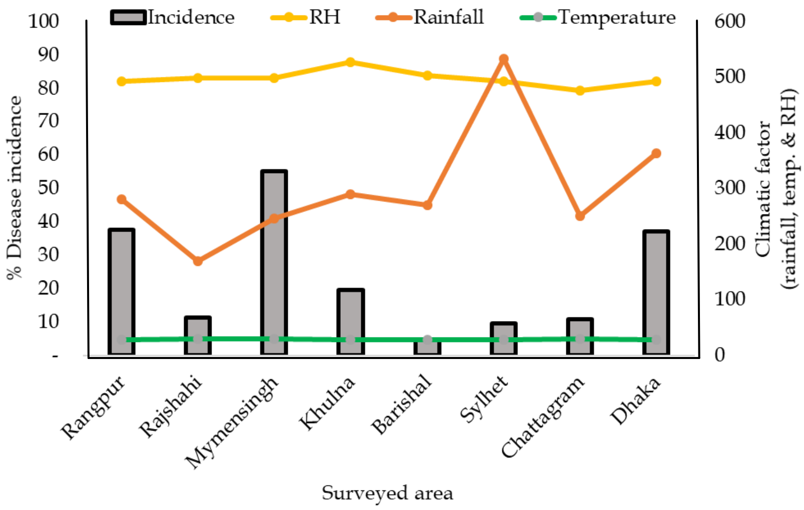

Figure 4. Environment factors (rainfall, temperature and relative humidity) on BB disease development with relation to disease incidence in rainfed season during 2015.

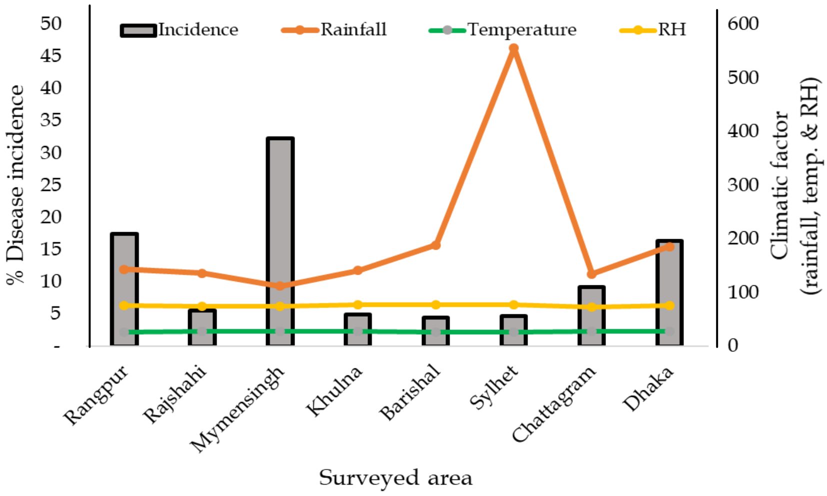

Figure 5. Environment factors (rainfall, temperature and relative humidity) on BB disease development with relation to disease incidence in irrigated season during 2016.

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).

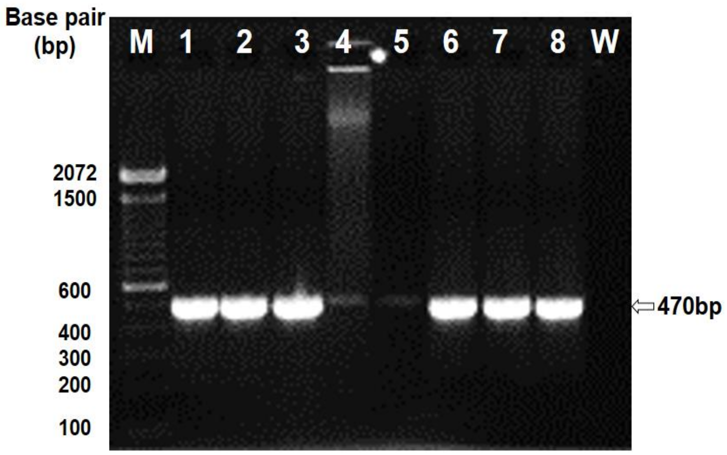

Figure 6. Agarose gel electrophoresis of PCR products from *X. oryzae* pv. *oryzae* using primer XOR-F and XOR-R2, M= DNA ladder (100bp Invitrogen). Lanes 1-8 are the PCR products from isolates BDXO91, BDXO68, BDXO34, BDXOS6, BDXO210, BDXO251 and BDXO309, control (Water, lane 9) respectively. The molecular 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 varities 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) reportedthat 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, Chattagram, Khulna, Sylhet, and Barishal. 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 division 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 Rafi *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.

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