Assessment of potentiality of known bacterial blight resistant genes against *Xanthomonas oryzae* pv. *oryzae* pathotypes exist in Bangladesh

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**ABSTRACT**  
Bacterial blight (BB) caused by *X. oryzae* pv. *oryzae* is a destructive disease of rice and causes 30-50% losses to rice depending on the outbreak. Development BB resistant rice varieties have long been considered as one of the most effective approach to control the disease. However, the durability of host resistance is breaking down due to the change of pathotypes of *X. oryzae* pv. *oryzae* globally. Pathotypic analyses of 239 *X. oryzae* pv. *oryzae* Bangladeshi isolates on Near Isogenic Lines (NILs) containing resistance (*R*) gene(s) revealed the existence of eight pathotypes of *X. oryzae* pv. *oryzae*. Among eight pathotypes, pathotypes IV and V were considered as major comprising maximum number of isolates, (30.13% and 23.01%, respectively), whereas pathotype VIII considered as minor consisting only 2.51% of total isolates. Pathotype I showed highest virulence or aggressiveness compatible with all NILs, whereas pathotype VIII exhibited lowest virulence to these NILs. Bacterial blight resistant genes viz. *Xa1* (75.00%), *Xa11* (62.50%) and *Xa21* (50.00%) showed resistance to most of the pathotypes while *Xa4* performed worst as compared to all others *R*-genes. In pyramid lines, IRBBB63 (*Xa5*+*Xa7*+*Xa13*) and IRBB57 (*Xa5*+*Xa7*+*Xa21*) showed resistance reaction and IRBB61 (*Xa4*+*Xa5*+*Xa7*), IRBB60 (*Xa4*+*Xa5*+*Xa13*+*Xa21*), IRBB54 (*Xa5*+*Xa21*), and IRBB53 (*Xa4*+*Xa21*) showed susceptible reaction to *X. oryzae* pv. *oryzae* pathotypes. These results collectively indicated the deployment of *Xa1*, *Xa11*, *Xa4*, *Xa5*, *Xa7*, *Xa13* and *Xa21* either alone or in combination against BB would be a best choice for the development of BB resistant rice varieties in Bangladesh.

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**INTRODUCTION**  
Bacterial blight (BB) of rice caused by the vascular pathogen *Xanthomonas oryzae* pv. *oryzae* (Ishiyama, 1922; Swings et al., 1990) is one of the supreme devastating diseases of rice across the globe (Mew et al., 1982). 20 to 40 % yield lessened due to having gigantic infection at the highest tillering stage, resulting in a yield reduction from 20 to 40% (Mew et al., 1993). Highest 50% yield completely deteriorated by initial Bacterial blight infection (Ou et al., 1985; Alam et al., 2016). New races of this pathogens were detected in recent years in Bangladesh resulting considerable yield abatement (50-70%) mostly in irrigated hybrid varieties (Islam et al., 2016). Anyway, several control methods were practicing all across the globe including cultural, chemical, biological, disease forecasting and crucially host genetic resistance (Bharani et al., 2010) and Host plant resistance exhibited the highest elevation in controlling this disease compared to chemical control (Gautam et al., 2015).
Therefore, breeding method could be one of the fruitful approaches to develop resistant cultivars containing R (resistance) genes mitigating BB. Worldwide above 38 R genes (Xa/xa have been exploited (Nino Liu et al., 2006; Cheema et al., 2008). Moreover, 42 R-genes also have been developed from *japonica* varieties, *Oryza sativa* sp. *indica* cultivars and its relevant wild species viz. *O. minuta*, *O. longistaminata*, *O. officinalis* and *O. rufipogon* (Brar and Khush, 1997; Lee et al., 2003, Busungu et al., 2018) and in rice molecular markers have been associated with BB resistance (R) genes with a number of 25 or more amount (Lee et al., 2003; Yang et al., 2003). Lately, Xa1, Xa10, Xa21, Xa23, Xa3, Xa5, Xa13, Xa25, Xa27 and Xa26 genes are cloned and characterized which has been encoding several types of proteins and exhibiting numerous mechanisms of R-gene triggered Xoo resistance as well as three (Xa4, Xa7, and Xa13) have been mapped physically (Vikal et al., 2017). Depending on the availability of the several resistance genes, it is quite necessary to evaluate their effectiveness solely or in a combination for accurately introgression of these marker-assisted genes into well known cultivars (Sundaram et al., 2008). Space and times are the crucial factor to check the long-lasting effectiveness of new races of this pathogen (Adhikari et al., 1999; Noda et al., 2001; Lore et al., 2011) who revealed Xoo is awfully variable and over 30 pathotypes/races found across the globe.

New races of the pathogens continue to emerge and overcome the deployed resistance genes (Mew, 1987), majority of them fluctuate depending on regions, areas, and including fields within an area (Nelson et al., 1994). Thus, a significant approach is needed to develop particular resistant cultivar with the help of predominante races in that specific geographical area through breeding approaches. To unveil the complex connection between genotypes and pathotypes of the BB pathogen (*X. oryzae pv. oryzae*) in Bangladesh the diversity of pathotypic and genetic were exploited (Islam et al., 2016). However, some genes provide resistance to a wide spectrum of *X. oryzae pv. oryzae* races (e.g., Xa21, Xa23), whereas others are effective against only one or a few races that may be limited to a particular geographical location (e.g., Xa1). Most R-genes to BB are dominant, while some are recessive (e.g., xA5, Xa13) and some display semi dominance (e.g., Xa27). Most R-genes to BB have been introgressed into the background of the susceptible *indica* cultivar IR24 to develop a set of near isogenic lines (NILs) and some have been pyramided, either through classical breeding and marker-assisted selection or through genetic engineering, to develop new plant types and NILs (Narayanan et al., 2002; Sanchez et al., 2000; Singh et al., 2001). Similar research was conducted by Chen et al., (2020).

Compared to parental NILs with only one R-genes pyramidal lines manifested gigantic range of resistance to bacterial blight showing synergism as well as complementation within R-genes (Adhikari et al., 1999a; Huang et al., 1997). Testing of *X. oryzae pv. oryzae* isolates from each lineage or haplotype defined by DNA fingerprinting against near-isogenic lines (NILs) and pyramidal lines may allow the identification of suitable resistance genotypes and its deployment strategies as well as efficient characterization of the resistance spectra of the genes in relation to pathogen strains representing the population diversity of *X. oryzae pv. oryzae*. Mishra et al. (2013) showed numerous Xoo strains that exhibited compatible reaction with the xa13 R-gene and by pyramiding Xa4, Xa21 and xa5 genes were auspicious against strains from Eastern part of India. In total 224 Xoo strains coalesced between 1999-2006 from state of Punjab (North western India) bearing Xa genes on NRLs found any of these strains were not compatible with the Xa13 R-genes (Lore et al., 2011). Overall, Xa21 resistance gene was incompatible with 93% of the isolates as well as Xa5 resistance genes were showed 95% compatibility of the isolates (Singh et al., 2001). Pyramid lines have displayed higher levels and/or wider spectra of resistance to BB than the parental NILs with single R-genes (Adhikari et al., 1999a; Huang et al., 1997). Specifically, R-gene pyramiding is a fruitful approach to apply more R-genes which ultimately confer wide resistance as well as sustainable against various races or pathogens (Kim et al., 2018).

However, none of the breeding approaches found effective due to dynamic changes of pathogen population. The widespread repeated use of a few resistance genes might accelerate the selection of new pathogenic races of the isolates followed by selection of resistant host plant after a single crop cycle. This leads to a change in pathogen population structure through either mutation or recombination to adapt itself to the new resistant host plant or environmental changes. Thus, identification of the effective R-genes against BB of rice will be a good choice for the development of BB resistant rice cultivars in the country. All of these aforementioned studies were conducted within a limited condition or in a specific geographical area. Anyway, previously no such research was conducted to evaluate the genetic mitigation of resistance as well as pathogenic diversity analyses of Xoo isolates particularly for Bangladesh. Analysis of the virulence of the dominant Xoo isolates on a series of standard inequitable hosts and relevant information is inevitable to implement resistance transfer in a sustainable and systematic way. Considering all of these, the present work was experimented by using 239 strains of *X. oryzae pv. oryzae* obtained from 30 districts were carried out i) to get a more comprehensive idea of pathotype diversity analysis in Bangladesh including the existence of the dominant pathotype (s) using 10 near isogenic lines (NILs) and ii) to evaluate the performance of some selected R-genes against these pathotypes using some pyramided rice lines.

**MATERIALS AND METHODS**

**Collection of bacterial blight infected rice leaf samples**

Plant samples those were infected with bacterial blight exhibiting typical symptom were coalesced randomly from 30 districts of Bangladesh. Each district was comprised five to ten different locations and from each location, 10 plants were gathered and each of them had 3 leaves combine build composite sample and a representative sample was gathered for the isolation of
X. oryzae pv. oryzae, those were brought into laboratory and preserved those in the refrigerator for isolation.

Isolation and identification of X. oryzae pv. oryzae isolates
During 2015-2016, 239 strains of X. oryzae pv. oryzae were isolated from infected rice leaf samples as described previously by Alam et al. (2016). At each location, all X. oryzae pv. oryzae strains were independent isolates confirming by the pathogenicity test using susceptible check rice cultivar IR24 in the net house.

Identification and purification of X. oryzae pv. oryzae cultures
Plates containing bacterial growth was measured and from those which exhibited similar to the criterion of X. oryzae pv. Oryzae were pull out by sterilized loop and purified cultures were prepared by streaking on both YDC and NBY agar media.

Confirmation of X. oryzae pv. oryzae isolates by pathogenicity test
Rice cultivar IR24 which was susceptible used in the pathogenicity test to confirmed the isolates of X. oryzae pv. oryzae carried out in Professor Golam Ali Fakir Seed Pathology Centre, Bangladesh Agricultural University, Mymensingh, Bangladesh. Seedlings were emerged when seeds were sown in the plastic pot. Then seedlings were transplanted for inoculation in the net house. NBY agar medium were utilized to culture the bacterial isolates for 48 hours at 28ºC and then using Spectrophotometer for CFU/ml. Leaf tips of rice plants at the late tillering stage (45-50 days after sowing, three seedlings of each line were transplanted into 100-cm diameter plastic buckets in the net house. Each pot containing youngest leaves and subsequently plants were monitored for development of symptoms at net house.

Confirmation of X. oryzae pv. oryzae isolates by hypersensitivity test
Hypersensitivity response of the isolates was assessed on tobacco (cv. virginia) plant grown in earthen pots containing loamy soil. Approximately 10^8-10^9 CFU ml^-1 of freshly cultured bacteria were injected onto the abaxial surface of tobacco leaf with a hypodermic syringe at 5-6-leaf stage. Inoculated plants were then kept in a moist chamber for a few hours to promote symptom development which was later shifted to the screen house. Controls were similarly inoculated with Sterile distilled water. Complete collapse of tissue after 24 hrs, followed by necrosis was interpreted as positive reaction (Klement and Goodman, 1967).

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

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 (Promega, Madison, WI, USA) from 1 ml of liquid culture of each isolate and was quantified using an UV spectrophotometer absorbance at 260 nm with a model T-80 UV/VIS and stored at -20°C. DNA concentration was adjusted to 100ng/µl and verified by comparing with a 100bp/1kb plus DNA ladder (Invitrogen, USA) on 1.5% agarose gel. After electrophoresis, the gel was placed under UV trans illuminator 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.

Determination of pathotypes of X. oryzae pv. oryzae isolates
Pathotypic analyses of X. oryzae pv. oryzae were experimented with a number of NILs (Near-isogenic lines) of rice viz. IRBB1, IRBB2, IRBB4, IRBB5, IRBB7, IRBB8, IRBB10, IRBB11, IRBB13, IRBB14 and IRBB21 conveying single known bacterial blight resistance gene (R-gene) in the background of IR24 (susceptible check cultivar). Each pot comprising three hills where plants were raised. Three pots were designated as replications for individual isolate.

Plant materials
Three sets of bacterial blight differential rice lines were used in the study for inoculation with a total of 239X. oryzae pv. oryzae isolates (180 isolates from rainfed and 59 isolates obtained from irrigated condition) to monitor the pathotypic changes of X. oryzae pv. oryzae isolates as compared to the pathotypes reported in Bangladesh previously by Alam et al. (2016). The first set consists of ten NILs with a single R gene in an IR24 background viz. NILs (IRBB-1, 2, 4, 5, 8, 10, 11, 13, 14 and 21). The second set included 11 different gene pyramid lines (IRBB- 52, 53, 54, 57, 58, 59, 60, 61, 63, 64 and 65), carrying R-genes (Xa4, Xa5, Xa7, Xa13, and Xa21) in all possible combinations in an IR24 background. Seeds of the near-isogenic and pyramid lines were obtained from the Bangladesh Rice Research Institute (BRRI), and sown in plastic pots. Thirty days after sowing, three seedlings of each line were transplanted into 100-cm diameter plastic buckets in the net house. Each pot was filled with 8kg of soil and farm yard manure mixed at a ratio of (1:1) wt/wt (N/P/K was applied approximately at the proportion of 1:1:1:2:3) 10g, mixer of N/P/K per pot (as the basal dose in the form of ammonium sulphate, triple super phosphate, and muriate of potash). Plants were watered daily and were top dressed with urea 46% N (at the rate of 5g per pot at 30 days after transplanting. Isolates of X. oryzae pv. oryzae that were maintained in 20% glycerol at -80°C were revived on NBY plates and incubated for 72h at 28°C

Method of inoculation
Inoculums of each strain was prepared by suspending the bacterial cells in 50 ml of sterile distilled water and adjusting (optical density at 600nm=0.3) to approximately 1.2 X 10^5 CFU/ml. Leaf tips of rice plants at the late tillering stage (45-50 days after sowing) were covered with sterile gauze. Bacteria were suspended in sterile water at a concentration of 10^8 CFU/ml. The leaves were lightly dressed with urea 46% N (at the rate of 5g per pot at 30 days after transplanting). The leaves were lightly dressed with urea 46% N (at the rate of 5g per pot at 30 days after transplanting). The leaves were lightly dressed with urea 46% N (at the rate of 5g per pot at 30 days after transplanting).
### Table 1. Response of near-isogenic lines to the eight pathotypes of *X. oryzae* pv. *oryzae* isolates in irrigated and rainfed season during 2016.

| Pathotype | % of Isolates | Total no. of Isolates | IR24 (susceptible) | IRBB21 (Xa21) | IRBB22 (Xa22) | IRBB4 (Xa4) | IRBB5 (Xa5) | IRBB10 (Xa10) | IRBB11 (Xa11) | IRBB13 (Xa13) | IRBB14 (Xa14) | IRBB1 (Xa1) | IRBB8 (Xa8) | IRBB7 (Xa7) | IRBB6 (Xa6) |
|-----------|---------------|----------------------|-------------------|---------------|---------------|-------------|-------------|---------------|---------------|---------------|---------------|-------------|-------------|-------------|-------------|
| I         |               | 17                   | S                 | S             | S             | S           | S           | S             | S             | S             | S             | S           | S           | S           | S           |
| II        |               | 71.11                | S                 | S             | S             | S           | R           | R             | R             | S             | S             | S           | S           | S           | S           |
| III       |               | 3.69                 | R                 | R             | R             | R           | R           | R             | R             | R             | R             | R           | R           | R           | R           |
| IV        |               | 15.06                | R                 | S             | S             | S           | S           | S             | S             | S             | S             | S           | S           | S           | S           |
| V         |               | 30.13                | R                 | S             | S             | S           | R           | S             | S             | S             | S             | S           | S           | S           | S           |
| VI        |               | 23.01                | R                 | S             | S             | S           | R           | R             | R             | S             | S             | S           | S           | S           | S           |
| VII       |               | 2.51                 | S                 | R             | R             | S           | R           | R             | R             | S             | S             | S           | S           | S           | S           |
| VIII      |               | 25.11                | S                 | S             | S             | R           | R           | S             | S             | S             | S             | S           | S           | S           | S           |

S: Susceptible and R: Resistant.

after transplanting) were clip-inoculation method with scissors that had been dipped in the inoculum (Mew and Vera Cruz, 1978). Individual isolate was used for inoculation on all the leaves of five plants which was around a total of 10 leaves. The plants were cultivated in a net house under natural photoperiodic conditions. Twenty one days after inoculation, the lesion length formed due to inoculation was measured with a ruler.

#### Classification of pathotypes or races

The different *X. oryzae* pv. *oryzae* isolates were classified into pathotypes/races based on their virulence on the differential NILs, pyramid lines and cultivated varieties. Reaction showing lesion length <3 cm was considered as resistant and >3 cm was considered as susceptible (Adhikari et al., 1995). The collected *X. oryzae* pv. *oryzae* pathotypes in 2015-2016 was compared based on their reactions against each NILs and the pyramid lines.

#### Data analyses

Data sets of measured lesion lengths for each set of rice lines or varieties against the selected pathotypes of *X. oryzae* pv. *oryzae* were used to know the effect of single R-genes and their combined effects on BB resistance. A dendrogram was constructed based on the reactions (lesion lengths) of each line against the tested isolates to evaluate and compare the discriminating powers of cultivated rice varieties and single-R-gene NILs on the *X. oryzae* pv. *oryzae* isolates. Disease severity patterns of cultivated rice varieties and IR24 against the selected *X. oryzae* pv. *oryzae* isolates were compared by using correlation analysis.

#### RESULTS AND DISCUSSION

#### Determination of pathotypes of *X. oryzae* pv. *oryzae* field isolates

Pathotypic analyses of 239 *X. oryzae* pv. *oryzae* isolates were conducted based on their reactions against 10 Near Isogenic Lines (NILs) and a total of 8 pathotypes (I-VIII) were recorded. Among 8 pathotypes, pathotypes IV and V considered as major, containing maximum number of isolates, (30.13% and 23.01% respectively) whereas pathotype VIII considered as minor, containing only 2.51% isolates recorded from Khulna and Barishal districts. Pathotype I showed highest virulence or aggressive ness compatible to all NILs, whereas pathotype VIII exposed lowest virulence incompatible to NILs. One study conducted by Suparyono et al. (2003) that at panicle initiation and tillering growth stages the most influential bacterial pathotype was VIII and at maturity, the most eminent was pathotypes III and IV. Pathotype VIII also exhibited a virulence reactions to almost all R-genes except *Xa1, Xa4 & Xa5*. Pathotype II performed better virulence by showing maximum compatible reaction to NILs. Pathotype II showed a virulence reaction only with *Xa1, Xa8* and *Xa21*. Pathotypes III performed a virulence interactions to 4 R-genes and showed virulence reactions to *Xa4, Xa5, Xa8, Xa13, Xa14 & Xa21*. The major pathotype IV exposed virulence reactions to *Xa2, Xa4, Xa8, Xa10, Xa14 and Xa21*, whereas
pathotypes V showed virulence reactions to Xa2, Xa5, Xa8, Xa13, Xa13 and Xa14. Pathotypes VI exhibited virulence reactions to Xa2, Xa4, Xa5, Xa11 and Xa13, whereas VII pathotypes showed virulence reactions with Xa4, Xa10, Xa13 and Xa14. Similarly, all other pathotypes showed distinct virulence and a virulence reactions to each other with NILs tested (Table 1). From 1980s the highest exploited R-genes are Xa4, Xa7, Xa3 and Xa21 for rice breeding purposes to enhance BB resistance. The most utilized R genes are Xa3, Xa4, Xa7, and Xa21 for rice breeding to improve BB resistance from the 1980s (Wang et al., 2020). Moreover, a crucial factor was analysed by a number of researchers named gene for gene theory exhibiting pathogen and host interaction (Alam et al., 2016) and a similar approach was conducted by Wolfe et al. (1976); Browder and Eversmeyer, (1977); Lebeda et al. (1982), based on gene for gene theory. Dynamic changes of X. oryzae pv. oryzae field isolates were also observed in Bangladesh by Khan et al. (2009); Alam et al. (2016); Rashid et al. (2021). This occurred due to expansion of overused a number of resistance genes which also triggered completely unknown pathogenic races with an increasing rate of 1.64 times compared to the level of virulence in the particular isolate following resistance host-plant selection pressure (Nayak, 1986). Another study conducted by focused that the diversity in Vietnam exhibited due to comparatively wide rage of diverse rice cultivars which was traditional and also been suggested that cultivar difference plays a crucial role to make the changes in the Xoo pathogen structure (Furuya et al., 2012) and breakdown of R-genes was detected when these genes have been applied frequently for a number of years in a gigantic population (Chen et al., 2020). A hypothesis was adopted by Bai and Shi, (1993) and Leung et al. (1993) that applying cultivars throughout many decades share the similar genetic background with resistance genes, however, in China they proposed that planting susceptible hybrid line as well as signification infection by leaf blight pathogen, besides, cultivar diversity and mass propagation of the bacteria, all are the potential causes of frequently diverse virulence mutation (Li et al., 2009). Thus, these incidents will enhance the possibility of population of pathogens structure through mutation or modification to adapt itself within unfavorable environmental changes or in the new host plant which is resistance.

### District-wise distribution of X. oryzae pv. oryzae strains collected from thirty districts of Bangladesh

The frequent distributions of major pathotypes within each district along with total number of isolates are shown. Maximum Four (4) pathotypes were recorded from Dinajpur, Gaibandha, Natore, Bholo and Habigonj districts, whereas only single pathotype was recorded from Jamalpur, Moulovibazar, Gazipur and Manikganj districts. Three (3) pathotypes were recorded from Thakurgaon, Nilphamari, Bogura, Mymensingh, Netrokona, Jhalokhati, Perorjup, Bagerhat, Satkhira, Feni, and Loxmipur. Two (2) pathotypes were recorded from Panchagar, Rangpur, Rajshahi, Sirajganj, Sherpur, Tangail, Pabna, Jessore, Jhenaidah, Gopalganj, Barishal, Khulna, Sylhet, Sunamganj, Cumilla, and

| Pathotypes | IRBB64 | IRBB65 | IRBB63 | IRBB61 | IRBB59 | IRBB58 | IRBB57 | IRBB54 | IRBB53 | IRBB52 |
|------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Xa4-Xa5    | S      | R      | R      | R      | R      | R      | R      | R      | S      | S      |
| Xa4-Xa6    | S      | R      | R      | R      | R      | R      | R      | R      | S      | S      |
| Xa4-Xa7    | S      | R      | R      | R      | R      | R      | R      | R      | S      | S      |
| Xa4-Xa8    | S      | R      | R      | R      | R      | R      | R      | R      | S      | S      |
| Xa4-Xa9    | S      | R      | R      | R      | R      | R      | R      | R      | S      | S      |
| Xa4-Xa10   | S      | R      | R      | R      | R      | R      | R      | R      | S      | S      |
| Xa4-Xa11   | S      | R      | R      | R      | R      | R      | R      | R      | S      | S      |
| Xa4-Xa12   | S      | R      | R      | R      | R      | R      | R      | R      | S      | S      |
| Xa4-Xa13   | S      | R      | R      | R      | R      | R      | R      | R      | S      | S      |
| Xa4-Xa14   | S      | R      | R      | R      | R      | R      | R      | R      | S      | S      |
| Xa4-Xa15   | S      | R      | R      | R      | R      | R      | R      | R      | S      | S      |
| Xa4-Xa16   | S      | R      | R      | R      | R      | R      | R      | R      | S      | S      |
| Xa4-Xa17   | S      | R      | R      | R      | R      | R      | R      | R      | S      | S      |
| Xa4-Xa18   | S      | R      | R      | R      | R      | R      | R      | R      | S      | S      |
| Xa4-Xa19   | S      | R      | R      | R      | R      | R      | R      | R      | S      | S      |
| Xa4-Xa20   | S      | R      | R      | R      | R      | R      | R      | R      | S      | S      |
| Xa4-Xa21   | S      | R      | R      | R      | R      | R      | R      | R      | S      | S      |

**Note:** The Xa-genes that are present in the gene pyramid line is indicated in parentheses. **Based on mean lesion length, the nature of responses was classified into susceptible (S), resistant (R) (up to 3 cm).**
Pathotypes against which these genes confer resistance. Reactions showing lesion length <3 cm were considered as resistant (R) and >3 cm were considered as susceptible (S).

**Figure 2.** Performance of Xa genes against X. oryzae pv. oryzae pathotypes. The X-axis indicates Pathotypes and the Y-axis indicates the frequency of pathotypes against which these genes confer resistance. Reactions showing lesion length <3 cm was considered as resistant (R) and >3 cm was considered as susceptible (S).

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**Figure 3.** Performance of Xa genes against X. oryzae pv. oryzae isolates. The X-axis indicates Pathotypes and the Y-axis indicates the frequency of pathotypes against which these genes confer resistance. Reactions showing lesion length <3 cm were considered as resistant (R) and >3 cm were considered as susceptible (S).

Pathotypes against which these genes confer resistance. Reactions showing lesion length <3 cm were considered as resistant (R) and >3 cm were considered as susceptible (S).

**Figure 4.** Performance of Xa gene combinations against X. oryzae pv. oryzae pathotypes. The X-axis indicates Pathotypes and the Y-axis indicates the frequency of pathotypes against which these genes confer resistance. Reaction showing lesion length <3 cm was considered as resistant (R) and >3 cm was considered as susceptible (S).

Pathotypes against which these genes confer resistance. Reactions showing lesion length <3 cm were considered as resistant (R) and >3 cm were considered as susceptible (S).

**Figure 5.** Performance of Xa gene combinations against X. oryzae pv. oryzae isolates. The X-axis indicates Pathotypes and the Y-axis indicates the frequency of pathotypes against which these genes confer resistance. Reaction showing lesion length <3 cm was considered as resistant (R) and >3 cm was considered as susceptible (S).

Chandpur, Brahmanbaria, Chattagram, Khagrachari and Narsingdi. Others pathotypes also frequently distributed in all surveyed 40 districts recorded from both irrigated and rainfed seasons 2016–2017 (Figure 8). This relationship formed due to a high level of DNA polymorphism within all isolates which were collected district wise. Islam et al. (2016) also conducted similar approaches reporting 17 molecular lineages were detected from 96 Xoo isolates under 10 clusters covering 19 several geographic districts.

**Hypersensitive Reaction (HR) test**

The representative isolates of X. oryzae pv. oryzae collected major growing areas in Bangladesh were tested for hypersensitivity reaction (HR) test on tobacco plant (cv. MT 95). Tobacco leaves infiltrated with bacterial suspension induced a distinct yellow zone at the spreading edge of the lesion. Tobacco leaves infiltrated with sterile water were maintained as control and found not infected. Slight localized chlorosis followed by necrosis and collapse of whole tissue was evident in HR positive isolates. The result showed that all the 8 tested pathotypes were able to induce HR (death of local cells of tissues) between veins of tobacco leaves within 24 hrs (Figure 6). One study exposed by Li et al. (2009) that tested X. oryzae pv. oryzae strains had lessened their capability to engender a non-host HR (hypersensitivity reaction) when experimented with a syringe inoculation without needle in Nicotiana tabacum L as well as in Philippines same kind of experiment was also executed with other strains (Gonzalez et al., 2007).

**Confirmation of X. oryzae pv. oryzae by PCR**

To test the specificity of the pathogen, amplification was carried out with genomic DNA of all the 8 representative isolates and negative control was maintained with nuclease free water instead of DNA template. 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 X. oryzae pv. oryzae isolates (Figure 7). These results are in accordance with the PCR confirmatory test of X. oryzae pv. oryzae by using primers XOR-F and XOR-R2 as reported by Adachi et al. (2000).

**Performance of R genes against X. oryzae pv. oryzae pathotypes**

Considering the performance of individual R-genes against the X. oryzae pv. oryzae pathotypes, R-genes Xa1 (75.00%), Xa11, Xa21 (62.50% each) and Xa8, Xa10 (50.00% each) performed better resistance followed by Xa2 (37.50%), Xa5, Xa13 and Xa14 (25.00% each), whereas Xa4 (12.50%) containing lowest resistance value. Xa1 gene showed resistance to around 75.00% of total pathotypes followed by Xa11, Xa21, Xa8 and Xa10 while Xa4 showed resistance to only around 12.50% of pathotypes (Figure 2). Over time Xa4 gene might become ineffectual in rice producing countries of South Asia, thus it’s quite substantial to pyramid this gene with other striking genes like Xa5, Xa13, Xa21 and Xa7 (Khush et al., 1989). Numerous studies revealed that resistance denoted by Xa8 varies within the areas of China (Li et al., 2009; Liu et al., 2007) and almost 85% virulent on Xa8 producing countries of South Asia, thus it's quite substantial to pyramid this gene with other striking genes like Xa5, Xa13, Xa21 and Xa7 (Khush et al., 1989). Numerous studies revealed that resistance denoted by Xa8 varies within the areas of China (Li et al., 2009; Liu et al., 2007) and almost 85% virulent on Xa8.
found from 33 strains, suggesting that Xa8 cannot provide effectual against BB disease of rice in Fangchenggang, China. In case of individual R-genes, Xa21 depicted wider resistance against 88% of X. oryzae pv. oryzae isolates in India (Mishra et al., 2013). When screening genotypes of 57 basmati rice, Xa5, Xa7, Xa4 and Xa13 detected for resistance activity and chronicity of Xa7 in the experimental phase (Ullah et al., 2012), and same outcomes found has also been confirmed by Jeung et al. (2006) in Korea, Khan et al. (2012) in Pakistan and Shanti et al. (2001) in eastern India and these findings were quite matched in our studies.

**Performance of Xa genes against X. oryzae pv. oryzae isolates**

Considering the performance of individual R-genes against the X. oryzae pv. oryzae isolates, R-genes Xa1 (92.89%), Xa11, Xa21 (47.70% each) and Xa10 (45.61%), Xa5 (35.15%) performed better resistance followed by Xa13 (32.64%), Xa8 (24.69%), Xa4 (23.01%) and Xa2 (20.08%), whereas Xa14 (7.95%) containing lowest resistance value. Xa1 gene showed resistance to around 92.89% of total isolates and Xa14 showed resistance to only around 7.95% of total isolates (Figures 3). The IRBB-MR (pyramid lines) + IR24 containing R genes (Xa4, Xa5, Xa7, Xa13, and Xa21) in different combinations exhibited higher resistance levels than IRBB-NILs. In a number of countries Xa21 gene was also manifested extensive resistance significantly (Song et al., 1995; Wang et al., 1996) and in Fangchenggang, China Xa5 gene depicted efficacious activity in case of resistance (Yang et al., 2012) and Xa21 unveils much utter resistance than rest of the R-genes (Li et al., 2009). Considering isolates, IRBB63 showed resistant to around 85.77% of total isolates followed by IRBB58, IRBB64, IRBB65 and IRBB52 while IRBB60 and IRBB61 showed susceptibility to more than 95% of total isolates. These results are agreement with the findings of Li et al. (2001). They reported that there is a strong interaction leading to increased resistance between Xa13 and Xa5 and between either of them Xa4 or Xa21. Isolates in Bangladesh, Nepal, Pakistan, Korea and Sri Lanka exhibited on Xa21 due to having mutation in pathogen of avrXa21 gene (Lee et al., 1999) and developing wider and persistent impact (Jeung et al., 2006). In this study we observed the interaction effect between combined R-gene and pathotypes on lesion length was statistically significant. Numerically, the highest (12.50 cm) lesion length was found in (IRBB60 × pathotypes I) and the lowest (0.66 cm) lesion length was found in (IRBB57 × pathotype I) and (IRBB60 × pathotype VIII). Khan et al. (2010) reported that the IRBB65 (Xa4, Xa7, Xa13 and Xa21) was the best among the tested entries for gene pyramiding against the BB pathogen in Bangladesh. Jeung et al. (2006) found that the pyramid line containing gene Xa4, Xa5 and Xa21 would be the most promising and valuable genotype for improving Korean cultivars for BB resistance. These increased resistance levels might be due to the synergistic effects of the R-genes, and each R-gene combination expressed a unique genetic effect against the corresponding X. oryzae pv. oryzae isolates. The pathotypic reactions of eight pathotypes against

**Figure 6.** Representative photographs showing hypersensitive response (HR) test on tobacco leaves (cv.) of different X. oryzae pv. oryzae pathotypes. Infiltrated tobacco leaves with sterile water (A) and with bacterial cell suspension of X. oryzae pv. oryzae (B).

**Figure 7.** Agarose gel electrophoresis of PCR products amplified from X. oryzae pv. oryzae using primer XOR-F and XOR-R2. Lanes 1-8 are the PCR products of isolates BDXO91, BDXO68, BDXO34, BDXO56, BDXO210, BDXO251, BDXO309 and BDXO319 control W (Lane 9) respectively. The molecular size marker is a 100-bp DNA ladder (Invitrogen).

**Figure 8.** Distribution of X. oryzae pv. oryzae pathotypes in the 40 districts covering 30 Agro-ecological Zones (AEZs) of Bangladesh.
11 pyramided lines have been shown in Table 2. Pathotypes I, II and III showed that highest virulence or aggressiveness compatible with all gene pairs, whereas pathotype VII and VIII exposed lowest virulence to pyramided lines. Pathotype VIII also exhibited a virulence reaction with almost all R-genes except IRBB52, IRBB61, IRBB63, IRBB65. Pathotype IV, V and V showed a virulence reaction only with IRBB52, IRBB58, IRBB59, IRBB63, IRBB64, and IRBB65. Pathotypes VI showed virulence reactions to IRBB53, IRBB54, IRBB59, IRBB60, and IRBB61). Significant reductions in lesion length were not frequently observed between the gene pairs Xa4 and Xa5 or Xa7, probably because of the effective resistance spectrum of Xa5, Xa7 and Xa13 against all tested X. oryzae pv. oryzae isolates (Table 2). Therefore, in the gene pairs Xa5+Xa7+Xa13 (IRBB63), Xa4+Xa5+Xa21 (IRBB57), and Xa4+Xa13+Xa21 (IRBB58), the R genes interacted with each other independently and additively. Expression of the three (Xa5 + Xa13 + Xa21) gene pyramid as the impact of genetic background expressing resistance was also discussed previously (Yoshimura, 1989; Gautam et al., 2005). Inoculation experiments on IR24 pyramid lines with a newly evolved field isolates showed high susceptibility to bacterial blight in IRBB61 (Xa4+Xa5+Xa7), IRBB60 (Xa4+Xa5+Xa13+Xa21), IRBB54 (Xa5+Xa21), and IRBB53 (Xa4+Xa21), and highly resistance to IRBB63 (Xa5+Xa7+Xa13), IRBB57 (Xa5+Xa5+Xa21), IRBB58 (Xa4+Xa13+Xa21), and IRBB64 (Xa4+Xa5+Xa7+Xa21). Around 2 -5 resistance genes from 16 gene pyramids derived in 10 NILs (near isogenic lines) and IRBB lines were detected against 61 isolates that Xa21 and Xa13 genes accorded resistance to more than average Xoo isolates in Pakistan (Khan et al., 2012). A combination of three R-genes Xa1, Xa3, and Xa4 in promising lines could lead to a significant outcome controlling BLB in suitable areas of Mali reported by Tekete et al. (2020).

Figure 9. Phenogram of X. oryzae pv. oryzae strains based on virulence to 11 near isogenic lines containing a single gene for resistance. A data matrix was generated for the virulence data by scoring avirulence as 0 and virulence as 1. From these data, a similarity matrix was derived with the SIMQUAL program (NTSYSpc, version 2.02i, Exeter Biological Software, Setauket, NY) using Jaccard’s coefficient of similarity. A phenogram was reproduced by the unweighted pair group method for arithmetic average in the SAHN Program.

Pyramiding line bearing Xa2, Xa4, Xa5 would be the highest fruitful genotype for developing Korean cultivars against BB resistance (Jeung et al., 2006) as similar to our findings. IRBB63 showed resistance to around 75% of total pathotypes followed by IRBB57, IRBB58, IRBB64 (62.50% each) and IRBB65, IRBB52 (50.00% each), while IRBB61 showed susceptibility to all of the pathotypes followed by IRBB60, IRBB53, IRBB54. Other lines showed moderate performance. Considering the performance of pyramiding lines against the X. oryzae pv. oryzae isolates, pyramid lines IRBB63 (85.77%), IRBB58 (76.15%), IRBB65 (70.29%), IRBB52 (63.60%) and IRBB64 (58.16%) performed better resistant followed by IRBB59 (42.26%), IRBB57 (31.80%), IRBB53 (14.64%) whereas IRBB61 (1%), IRBB60 (2.51%), containing lowest resistance value. IRBB63 showed resistant to around 85.77% of total isolates followed by IRBB58, IRBB65, IRBB52 and IRBB64 while IRBB61 and IRBB60 showed susceptibility to more than 98% of total isolates (Figures 4 and 5).

Overall outcomes from one study suggested that several isolates on IRBB lines revealed the sequential order of resistance genes based on their effectiveness was Xa4~Xa4 + Xa5~Xa4 + Xa21~Xa4 + Xa5 + Xa13 + Xa21 > Xa5 + Xa13 + Xa21 > Xa4 + Xa13 + Xa7 > Xa4 + Xa13 + Xa21 (Gautam et al., 2015). However, in Punjab province of Pakistan, Xa4 + xa13 + Xa21 followed by Xa5 + Xa21, xa13 + Xa21, Xa4 + Xa21, Xa4 + Xa13 and Xa4 + Xa5 were found effective in combating the Xoo pathogen (Khan et al., 2012). With results on major aspects of our quantitative observations and statistical interpretation strong and broad resistance of the Xa1, Xa11, Xa21 gene and combined R-gene (Xa5+Xa7+Xa13) and a strong quantitative complementation effect between the Xa4, Xa5 and Xa13 genes deduced that the R -gene pyramid of Xa1, Xa5, Xa7, Xa11 and Xa21 would be the most prospective genotype for improving Bangladeshi cultivars for BB resistance. A comparison of resistance spectra between the NILs with (Xa5 + Xa7 + Xa13) gene combinations and rice lines with known R-genes that have been used as genetic sources for improving BB resistance by Bangladeshi breeders also confirmed the effectiveness of the three R-gene pyramid against the BB population of Bangladeshi represented by the eight X. oryzae pv. oryzae pathotypes. Considering the above facts, Xa1, Xa11 and Xa21 genes (including Xa5, Xa7, and Xa13) may recommend for breeding rice cultivars resistant to BB disease in Bangladesh. The problems of understanding the biology of X. oryzae pv. oryzae and controlling bacterial blight have long been challenging. The known geographic distribution of the haplotypes provides a baseline for monitoring the future spread of X. oryzae pv. oryzae in other rice producing areas in Bangladesh.

Relationship between pathotypes and haplotypes of X. oryzae pv. oryzae in Bangladesh
A phenogram was constructed based on the virulence of X. oryzae pv. oryzae isolates to the 10 Near Isogenic Lines (NILs) of rice containing single gene for resistance to interpret the relationship between the pathotypes and the molecular
haplotypes (Figure 9). In the phenogram, constructed based on the findings of inoculation where, four haplotypes were observed at 63% similarity level. Among these haplotypes, haplotype II considered as major which comprises number of pathotypes III, V containing 91 isolates out of 239. Haplotype I contained number of isolates (89) belongs to pathotypes I and IV. Haplotype III comprises 2 pathotypes (II and VI) containing 41 isolates and haplotype IV represented by pathotypes VII, VIII, diverse than other haplotypes. From the phenogram, i) group of X. oryzae pv. oryzae isolates from different populations with same pathotypes showed multiple haplotypes, ii) group of X. oryzae pv. oryzae isolates from different populations had identical haplotypes in multiple pathotypes and iii) group of X. oryzae pv. oryzae isolates from different populations had identical pathotypes and haplotypes. These pathogenic strains showed an intricate relationship between molecular haplotypes and pathotypes as similar to the experimentation of Adhikari et al. (1999); Nelson et al. (1994); Ochiai et al. (2000) and Yashitola et al. (1997). Adhikari et al. (1999) delineated those 31 molecular haplotypes were found from 171 strains from 8 rice producing areas in Nepal by reacting under two PCR based assays, among them three were found highest intermittent withing a vast range of geographical population and some of them were dispersed geographically. Furthermore, Shanti et al. (2001) revealed that several haplotypes positioned in same pathotype belonging to several isolates and they also pointed out the genetic and pathotypic modification exits within the X. oryzae pv. oryzae isolates. Genetically similar haplotypes were identified from several districts of Bangladesh speculating due to national distribution or regional dispersion of contaminated germplasm (Islam et al., 2016) and same projection was speculated by George et al. (1997).

**Conclusion**

Potential R-genes viz. *Xa1, Xa4, Xa5, Xa7, Xa11, Xa13 and Xa21* against the *X. oryzae* pv. oryzae Bangladeshi pathotypes can be deployed in a suitable combination in the promising rice varieties through pyramiding for example *Xa5+Xa7+Xa13* or *Xa5*Xa7*Xa2* or *Xa4*Xa5*Xa7* or *Xa4*+*Xa5*+*Xa13*+*Xa21* or *Xa5*Xa21 or *Xa4*Xa21 in developing BB resistant rice varieties in both rainfed and irrigated seasons of Bangladesh. However, frequent monitoring of the i) performance of these R’ genes against BB and ii) pathotypic changes of *X. oryzae* pv. oryzae population would be crucial to manage BB successfully through national rice resistant breeding programs under the global climatic change’s scenario in the forthcoming years for ensuring food security in Bangladesh.

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