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RESEARCH ARTICLE

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Virulence analysis of *Pyricularia grisea* on rice monogenic lines detected blast *R*-gene in Bangladesh

Abstract:

A total of 139 isolates representing 8 Agro Ecological Zones (AEZs of 1,2,9,11,13,19,23 and 28) of Bangladesh were characterized for their pathogenicity using 25 monogenic lines (MLs) as differential varieties (DVs) targeting 23 resistant genes and two susceptible checks LTH and US2 (having no blast resistant gene or *R*-gene).Considering significant higher plant height (28.7 cm) and higher leaf color chart reading (3.4), higher disease incidence (20.4%) and lower seedling mortality (0.7) the treatment T_8 ($3/4^{th}$ soil, $1/4^{th}$ CD and NPK of total volume) was considered as standard soil medium for screening of blast isolates against DVs. Virulence frequency of blast pathogens to DVs was higher for the isolates from AEZ11 (47%) followed by AEZ23 (46%) and lower for the isolates of AEZ28. The virulence spectrum of tested blast isolates differed significantly. Under control condition (controlled temperature and humidity) virulence frequencies of blast isolates, collected from rain fed ecosystem (Transplanted Aman season), against DVs was higher than the blast isolates those were collected from irrigated ecosystem (Boro season) specially for *Pii*, *Pi3*, *Pi5(t)*, *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p*, and*Pi7(t)* genes. Isolates clarified on MLs through pathogenecity test indicated that *Pi9*, *Pish*, *Pita* and *Pita2* were the major genes responsible for blast resistance in Bangladesh. Among them *Pi9*, *Pish* showed resistance frequencies of 90% and above while *Pita* and *Pita-2* showed 80-87% against all blast isolates.

***Keywords*:** Rice, Monogenic line, differential variety, blast, virulence, resistance

1. Introduction:

The main food Rice (*Oryza sativa* L.) provides about one-sixth of the national income of Bangladesh [1-3] and as more than 90 percent of all rice is produced in Asia it is called an Asian crop [4]. Rice blast caused by *Pyricularia grisea* is one of the most economically important and well-studied diseases of rice [5] which causes respectively 11% and 46.4% yield loss under low and medium disease pressure in Bangladesh [6] and causes worldwide 11-15% yield loss annually [7].

The climatic changes [8], such as water scarcity may increase many rice diseases particularly *Piricularia grisea.* Under such condition, use of resistant cultivars would be one of the promising ways to control blast. Discovery of 23 blast resistant genes (*R*-genes) in the rice genome has created an opportunity to develop durable resistant rice varieties against rice blast pathogen [9]. Multiple resistance or gene pyramiding is necessary for development of durable resistant cultivars against blast. Before that understanding population structure and distribution pattern of the pathogen variants, if any, is essential. Pathotype is the most widely used method of classifying virulence and until recently has been practical means to characterize field pathogen populations [10].

There is a little information on pathogenic races of blast pathogen in Bangladesh. Differential system can help to study the races of pathogen. Therefore, this project has been designed to know the virulence frequency and distribution pattern of blast pathogen. The project is also aims to find out blast resistant *R*-gene for Bangladesh.

2. Materials And Methods:

2.1. Collection, Isolation and purification of blast isolates

A total of 300 disease samples were collected from different locations of 8 AEZs of Bangladesh viz. AEZ 1, AEZ 2, AEZ 9, AEZ 11, AEZ 13, AEZ 19, AEZ 23 and AEZ 28 (Fig. 1) considering cropping patterns, seasons, locations and rice cultivars. The pathogen was isolated from panicles by tissue planting method following the procedure of Anon. [11].

Conidial formation was observed under a stereomicroscope. Single conidia were isolated from the samples after 3-5 days of incubation and transferred aseptically to glass petridishes containing water agar (WA) with a sterile needle. The culture was

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allowed to grow for 3-5 days. Contaminants other than *Pyricularia grisea* were eliminated through subsequent culture. Single hyphal tips were transferred to potato dextrose agar (PDA) plate for further purification of *P. grisea.*

2.2. Preservation and revival of blast isolates

Modified NIAS (National Institute of Advanced Studies) method [10] was used to preserve and purify blast pathogen. The survivality of preserved isolates was confirmed by sub-culturing at regular intervals.

2.3. Collection and multiplication of monogenic lines (MLs) as differential varieties (DVs)

Twenty five monogenic rice lines (MLs) having single blast *R*-gene (targeting 23 *R*-gene) collected from IRRI-Japan collaborative research project were used in this investigation [12]. The LTH and US-2 were used as susceptible checks (Table 1).

2.4. Preparation of for proper plant growth and disease development

As the amount of soil was small and rice seedlings had to survive for about one month, an appropriate soil medium was required. The amount of Urea (for N or nitrogen), TSP (for P or phosphorus) and MoP (for K or Potasium) fertilizer for per kilogram of soil was 0.2g, 0.1g and 0.1g, respectively.

The following treatments were used for appropriate soil media:

T1= only soil;
T2= only decomposed cow dung (CD);
T3= ½ soil+ ½ CD;
T4= ½ soil+ ½ CD+ NPK
T5=¼ soil+¾ CD;
T6=¼ soil+¾ CD+ NPK;
T7=¾ soil+¼ CD
T8=¾ soil+¼ CD+NPK

Experiment was laid out in Complete Randomized Design (CRD) with 3 replications. Treatments were analyzed using MSTAT-C [13] and compared by DMRT.

2.5. Preparation of plant materials for inoculation

Monogenic lines (ML) and susceptible checks (SC) were seeded in two seeding cell trays (having 35 holes of seeding options in each tray) for each isolate. Modified NIAS method was followed to prepare plant materials for inoculation [10].

2.6. Preparation of inoculum and inoculation of plant materials

A total of one hundred thirty nine blast isolates were used in this study. Among the tested isolates, a total of 17, 18, 17, 19, 16, 16, 16 and 20 were used respectively from AEZ1, AEZ2, AEZ9, AEZ11, AEZ13, AEZ19, AEZ23 and AEZ28. The NIAS method cited byHayashi et al. [10] was carried out to prepare inoculum and inoculation of plant materials.

2.7. Disease assessment based on infection type on individual differential lines

Susceptibility of the test plants was assessed by examining the leaves for blast symptoms. Six scales was used to rate the disease severity. Seven days after inoculation, infection type (0-5 scale) was rated following Hayashi et al. [10].Infection type, 'S' for susceptible and 'R' for resistant was recorded and converted to 1 and 0 respectively. The frequency of *P. grisea* isolates was calculated based on the number of differential rice lines infected by a particular isolates [14]. Distribution frequencies of virulent blast isolates were calculated in percent.

3. Results and Discussions:

To identify pathogenic variability of *P. grisea* using monogenic lines of rice, the experiment was conducted in a room under controlled temperature and humidity to provide favourable environment for proper disease development.

3.1. Collection, isolation and purification of blast isolates

From 300 disease samples, a total of 176 isolates were purified of which 100 isolates were from rain fed ecosystem (T. Aman season) and 76 isolates were from Boro season or irrigated ecosystem (Table 2).

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3.2. Preservation and revival of blast isolates

From the preserved isolates, 139 were revived and tested on differential varieties (DVs), of them 77 isolates (55.4%) were from rainfed ecosystem (Aman) and 62 isolates (44.6%) were from irrigated ecosystem (Boro). Maximum 15.3% isolates were taken from AEZ28 and minimum 10.7% from AEZ23 (Table 2 and Fig. 1). The rate of revival of blast isolates from storage was higher in thick paper-disk (thick paper box of blotter paper) than the thin paper disc (blotter paper) (Fig. 2). Similar observation was made by Ali et al. [21].

3.3. Preparation of soil media for proper plant growth and disease development

Table 3 represents the effect of different soil media on plant height, leaf color chart (LCC) reading, seedling mortality and % disease incidence (DI). Considering significant higher plant height (28.7 cm) andhigher LCC reading (3.4;higher greenness of leaves);higher disease incidence(20.4%) which is better for disease screening and lower seedling mortality (0.7) the treatment T8 ($3/4^{th}$ soil+ $1/4^{th}$ CD+ NPK) was considered as standard soil medium and used throughout the experimentation. Ali et al. [21] also observed that 3:1 portion of soil and decomposed cow dung (CD) along with additional chemical fertilizers acted as the best soil medium for proper plant growth and disease development. The present observation was in accordance with their finding.

3.4. Virulence analysis/reaction to differential varieties

The virulence spectrum of tested blast isolates to DVs and susceptible checks (LTH and US2) was divided into eight groups based on their origin of collection such as AEZ1 (blast isolates, n = 17), AEZ2 (n = 8), AEZ9 (n = 17), AEZ11 (n = 19), AEZ13 (n = 16), AEZ19 (n = 16), AEZ23 (n = 16) and AEZ28 (n = 20) of Bangladesh. Virulent frequency of those isolates was 100% on susceptible checks LTH and US2 in all the eight groups. The results were presented in Fig. 3-11.

3.4.1. Virulence frequencies in AEZ1: Virulence frequencies of the 17 blast isolates to DVs varied widely. Monogenic lines as DVs, IRBLsh-B (for *Pish*) and IRBLta2-Pi (for *Pita-2*), exhibited the highest resistant reaction against all the blast isolates from this location. Virulence frequencies of tested blast isolates to these two DVs for *Pish* and *Pita-2* genes respectively were 5.9% (Fig. 3). Thirteen DVs harboring *Pib, Pita= Pi4(t), Piz-5(pi-2(t)), Pi5, Pik-p, Pi19, Pia, Pik-m, Pi7(t), Piz, Pi20, Pi3* and *Pii* genes showed moderate frequencies (20-60%), and remaining Dvs for *Pish, Pita-2, Pi9, Pita-2* and *Pita= Pi4(t)* genes were recorded with lower frequencies which was less than 20% (Fig. 3). Seven DVs for *Pik-h, Pi12(t), Pi1(t), Pik, Piz-t, Pik*-s and *Pit* genes showed high frequencies (>60%) of virulent blast isolates. Two differential lines, IRBLks-F5 (for *Pik*-s gene) and IRBLt-K59 (for *Pit* gene) were very susceptible to the blast isolates similar to the checks.

3.4.2. Virulence frequencies in AEZ2: Eighteen blast isolates from AEZ2 were tested against all DVs including susceptible the check (Fig. 4). In this region, a total of five DVs for *Pi9, Pita=Pi4(t), Pita-2* and *Pish* genes showed lower frequencies of tested virulent blast isolates ranging from 11 to 17%. It means that *Pi9, Pita=Pi4(t), Pita-2* and *Pish* genes showed resistant reaction against the tested blast isolates. Fifteen DVs harboring *Pi3, Pi5, Pia, Pib, Pita-2, Piz-5(pi-2(t)), Pik-p, Pi19, Pi7(t), Pii, Pik-m, Piz, Pi1(t), Pik* and *Pik-h* genes showed moderate frequencies of compatible isolates with virulence ranging from 22 to 56%. The rest Dvs for *Pi12(t), Pi20, Pik-s, Pit* and *Piz-t* genes demonstrated high level of frequencies ranging from 67 to 89%.

3.4.3. Virulence frequencies in AEZ9: Seventeen blast isolates from AEZ9 were also tested against all DVs including susceptible the check (Fig. 5). It was noted that only five DVs viz. IRBL9-W, IRBLsh-B, IRBLta-K1, IRBLta2-Re and IRBLZ5-CA harboring *Pi9, Pish, Pita= Pi4(t), Pita-2* and *Piz-5(pi-2(t))*, respectively exhibited lower frequencies of virulent blast isolates which was <20%. Eight DVs for *Pi20, Pi5, Pit, Piz-t, Pi12(t), Pi19, Pia* and *Pik-s* genes showed higher frequencies of compatible blast isolates which ranged from 61 to 76%. The remaining DVs showed moderate compatible reactions to the isolates (23 to 46% frequency). This result indicated that virulence spectrum in this region was narrow among most of the isolates compared to other region.

3.4.4. Virulence frequencies in AEZ11: Lower virulence frequency was observed in IRBL9-W, IRBLta-K1, IRBLta-CP1, IRBLta2-Pi and IRBLta2-Re, whereas higher virulence frequencies (68 to 93%) of the isolates were recognized with only four DVs IRBLt-K59, IRBL12-M, IRBL7-M, IRBL20-IR24 and IRBLks-F5. The remaining DVs for *Pish, Piz, Piz-5(pi-*

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2(t)), *Pib Pi3*, *Pi5*, *Pia*, *Piz-t*, *Pik*, *Pik-p*, *Pi1(t)*, *Pi19*, *Pii*, *Pik-h* and *Pik-m* genes showed moderate compatible reaction to the tested blast isolated of 21 to 58% virulence frequencies (Fig. 6). No compatible reaction was observed on the cultivar IRBL9-W (*Pi9* gene).

3.4.5. Virulence frequencies in AEZ13: Sixteen isolates from AEZ13 were tested on all the DVs. In this region, the highest level of virulence frequencies (100%) of the tested blast isolates was observed in the control followed by IRBLks-F5 (for *Pik*-s gene), IRBLi-F5 (*Pii*), IRBL1-CL (*Pi1(t)*) and IRBLkh-k3(LT) (*Pik-h*) having 93 to 64% virulence frequency (Fig. 7). No virulence interaction of the isolates to DV was recorded in IRBL9-W (*Pi9* gene). Lower virulence frequencies of isolates were noted in DVs for *Pish*, *Pita-2*, *Pita-2*, *Pib* and *Pita*= Pi4 (t) genes ranging from 7 to 14%, while DVs for *Pi20*, *Pi3*, *Pia*, *Pi7(t)*, *Pik-p*, *Pik*-s, Pit, *Piz*, *Pi12(t)*, *Pi19*, *Pik-m*, *Pi5*, *Pik* and *Piz-t* genes showed moderately compatible reaction with 28-57% virulent frequency of the isolates.

3.4.6. Virulence frequencies in AEZ19: Virulence frequencies of 16 blast isolates to DVs were presented in Figure 8. Except susceptible checks, it was recognized that virulent frequencies of the blast pathogen varied from the lowest 6.3 with IRBLsh-B (*Pish*) to the highest 81.3 with IRBLks-F5 (*Pik*-s) in this location. Higher virulence frequencies (75%) were recorded against *Pi12(t)*, *Pi19*, *Pik-p*, *Pit* and *Piz-t* genes followed by *Pi1(t)*, *Pik*, *Pik-h*, *Piz*, *Pi3*, *Pi5*, *Pib*, *Pii*, *Pik-m*, *Pia*, *Pi20* and *Pi7(t)* genes (25-56%) while genes *Pi9*, *Pita= Pi4(t)*, *Pita-2*, *Pita= Pi4(t)* and *Piz-5(pi-2(t))* showed lower level of compatible reaction with the isolates having 25 to 56% frequencies.

3.4.7. Virulence frequencies in AEZ23: The result of AEZ23 was presented in Fig. 9. The IRBLt-K59 (*Piz-t*), IRBL19-A (*Pik*-s), IRBL12-M (*Pi20*), IRBL20-IR24 (*Pi12(t)*), IRBLks-F5 (*Pi19*) and IRBLzt-T (Pit) showed 93 to 71% virulence frequency. The DVs IRBLz-Fu (*Piz*), IRBL3-CP4 (*Pi3*), IRBLZ5-CA (*Piz-5(pi-2(t))*, IRBL1-CL (*Pi1(t)*), IRBL7-M (*Pi7(t)*), IRBLi-F5 (*Pii*), IRBLta-CP1 (*Pita= Pi4(t)*), IRBLkh-K(LT) (*Pik*), IRBLkm-Ts (*Pik-m*), IRBL5-M (*Pi5*), IRBLa-A (*Pia*), IRBLkh-k3(LT) (*Pik-h*) and IRBLkp-K60 (*Pik-p*) exhibited moderate compatible reaction against 16 blast isolates with 21-57% virulent frequency. Lower virulent frequency (14.3%) of the blast isolates was recorded in IRBLta-K1 (*Pita= Pi4(t)*) while the lowest (7.1% virulent frequency) was observed in IRBL9-W (*Pi9*), IRBLb-B (*Pib*), IRBLsh-B (*Pish*), IRBLta2-Pi (*Pita-2*), IRBLta2-Re (*Pita-2*0) and IRBLta-K1 (*Pita= Pi4(t)*).

3.4.8. Virulence frequencies in AEZ28: In AEZ28, virulence frequencies of 20 blast isolates to DVs varied widely (Figure 10). Lower virulence frequencies of all the blast isolates of this location was found in three DVs for *Pish*, *Pita= Pi4(t)* and *Pita-2* genes ranged from 10 to 15% frequency. Nineteen DVs showed moderate compatible reaction against blast isolates having 20-60 % virulent frequency, and remaining three DVs for *Piz-t*, *Pit* and *Pik*-s genes exhibited higher frequencies which were 75 to 90% of the blast isolates (Fig. 10). Reaction of virulent isolates on the DV IRBLks-F5 (for *Pik*-s gene) was similar to susceptible checks.

3.4.9. Virulence frequencies irrespective of location: Virulence frequencies of total (139) blast isolates collected from eight AEZs were shown in Fig. 11. Virulent frequencies (%) ranged from the lowest 8 with IRBL9-W (*Pi9*) to 87 with IRBLks-F5 (*Pik*-s). It was noted that higher virulent frequencies (>60%) were observed against the DVs for *Pi20*, *Pi12(t)*, Pit, *Piz-t* and *Pik*-s genes while lower virulent frequencies (<20%) were recorded against the DVs for *Pi9*, *Pish*, *Pita* and *Pita-2* genes. Moderate and almost similar virulence pattern was found against *Pita= Pi4(t)*, *Piz-5(pi-2(t))*, *Pib*, *Piz*, *Pi3*, *Pik-m*, *Pi5*, *Pia*, *Pik-p*, *Pi7(t)*, *Pi1(t)*, *Pii*, *Pik-h*, *Pik* and *Pi19* genes with virulence frequencies ranging from 20-60%. Among the *R*-gene *Pi9* and *Pish* showed resistance frequencies of 90% and above while *Pita* and *Pita-2* showed 82-83% against all tested blast isolates.

3.4.10. Virulence frequencies irrespective of isolates: Virulence frequency of blast pathogens to DVs was higher for the isolates of AEZ11 (47%) followed by AEZ23 (46%) and lower (37%) for the isolates of AEZ28 (Fig. 12).

3.4.11. Virulence frequencies in rice growing ecosystem: The virulence spectrum of tested 139 blast isolates against DVs differed significantly. Under control condition (controlled temperature and humidity) virulence frequencies of blast isolates, collected from rain fed ecosystem (Transplanted Aman season), against DVs was higher than the blast isolates those were collected from irrigated ecosystem (Boro season) specially for *Pii*, *Pi3*, *Pi5(t)*, *Pik-m*, *Pi1*, *Pik-h*, *Pik*, *Pik-p*, and *Pi7(t)* genes (Fig. 13).

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The diverse virulence frequencies were distributed in all agro-ecological zones (AEZs) under study. There were differences in virulence frequencies of tested blast isolates on DVs collected from different AEZs. Two susceptible checks did not show any resistance against blast isolates in all the eight AEZs. The MLs IRBLsh-B (for *Pish* gene), IRBL9-W (*Pi9* gene), IRBLZ5-CA (*Piz-5*), IRBLta2-Pi (*Pita-2*), and IRBLta-K1 (*Pita*) showed higher resistance in all the studied locations (AEZs) which means blast isolates were less virulent to those genes. In AEZ11 and AEZ13, IRBL9-W (for *Pi9* gene) showed complete resistance against blast isolates. The results of this investigation showed that most of the isolates were able to overcome the resistance of twenty two differential varieties and few isolates were able to overcome the resistance of four DVs for *Pish*, *Pita*, *Pita*-2 and *Pi9* genes. In this study, no isolate was found to be incompatible to LTH. Similar report was also made by Lei et al. [15] and Mithrasena et al. [16]. These four genes, therefore, can be used for the development of blast resistance rice cultivars. These genes present in above mentioned four lines can provide long lasting resistance to rice blast if they are effectively pyramided in presently recommended rice cultivars in Bangladesh using marker assisted selection [17].

In the present study, there were difference in soil status, climatic factors, host factors and cultivation practices in different locations (AEZs). Blast pathogens in the different locations can change their virulence over time and space [18]. This might be the reason for the blast pathogens reacting differently against the tested DVs. Similar results were also observed by Shahjahan [19], Ali and Fukuta [20, 21] and BRRI [22]. Salim [23] observed higher virulence frequencies in Chittagong region (AEZ23). The present studies were in accordance with their results.

No isolate was found having incompatible reaction to the susceptible checks LTH and US2. In Figure 11 (when n=139), virulent frequencies (%) ranged from the lowest 8 with IRBL9-W (*Pi9*) to 87 with IRBLks-F5 (*Pik-s*). Except four DVs for *Pi9*, *Pish*, *Pita* and *Pita*-2 genes (resistance frequency 82 to more than 90%), all other DVs were susceptible to all tested isolates. These indicated that the virulence genes of blast isolates against blast resistance genes for *Pib*, *Pit*, *Pia*, *Pi5(t)*, *Pik-s*, *Pik-h*, *Pik*, *Pik-p*, *Pi7(t)*, *Piz*-5, *Piz*-t *Pil2*(t), *Pil*9(t) and avirulence genes of blast isolates against those of *Pish*, *Pi9*, *Pita*, and *Pita*-2 were distributed widely in the blast fungus population in the eight AEZs. Khan et al. [24] observed that four DVs for *Pish*, *Pi9*, *Pita*-2 and *Pita* genes showed low reaction against isolates. They suggested those four genes for developing a blast-resistant fragment rice cultivar. We observed similar results in our study which were in accordance with their study. The results of pathogenicity tests provided important information for selecting useful genes to develop blast resistant rice cultivars and gene-based markers for molecular study. Liu et al. [25] similarly observed that *Pi9*-bearing lines were highly resistant to all tested isolates confirming the broad-spectrum resistance of those genes to diverse blast isolates.

Virulence frequencies against DVs were higher for the pathogens collected from rain fed lowland ecosystem (T. Aman) than irrigated ecosystem (Boro). Similar result was observed by Hayashi et al. [26]. Hossain et al. [28] reported that rice in the rain fed lowland ecosystem suffered more by this pathogen than rice in the irrigated ecosystem. In the observation of BRRI [22], virulence frequencies were higher in cluster group I (isolates from rain fed low land ecosystem) than in cluster group II (isolates from irrigated ecosystem). The present results were supported by their observations.

Based on the application of differential system, the pathogenicity of blast isolates and genotypes of resistance genes in rice cultivars would be enhanced in breeding and pathological studies, and then a durable protection system for this disease would be built up in Bangladesh. Since the durability of single resistance genes to blast is very limited, efforts in the development of resistant cultivars are geared towards stacking of genes to develop gene pyramids [27]. Although the blast isolates used in this experiment were limited, this study identified well blast *R*-genes in Bangladesh by using monogenic lines.

4. Conclusion:

Thick paper-disk (thick paper box of blotter paper) could revive more blast pathogen from storage efficiently than that of thin paper disc (blotter paper that was used generally). In all the studied locations, diverse virulence spectrum of blast pathogen was present. Rice in the rain fed lowland ecosystem (Transplanted Aman rice season) faced more virulent blast pathogen than that of irrigated ecosystem (Boro rice season). Four genes, *Pi9*, *Pish*, *Pita* and *Pita2* were identified as major blast resistant genes for Bangladesh. Resistance breeding program would be undertaken to pyramid those identified blast *R*-genes in promising rice cultivar to obtain durable blast resistance in Bangladesh.

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Table 1: Characteristic of international standard differential varieties (DVs) developed by IRRI – Japan Collaborative Research Project [10]

Differential varieties (as MLs)	Target resistant gene and location on chromosome			Glume colour	Remarks
	Harboring gene	Chromosome	Locus		
IRBLsh-B	*Pish*	1	U^a	Brown	
IRBLb-B	*Pib*	2	U	Purple	
IRBLt-K59	*Pit*	1	U	Purple	
IRBLa-A	*Pia*	11	U	Purple	
IRBLi-F5	*Pii*	9	Pii / i	Brown	
IRBL3-CP4	*Pi3*	9	Pii / i	Purple	
IRBL5-M	*Pi5(t)*	9	Pii / i	Brown	
IRBLks-F5	*Pik-s*	11	Pik / k	Purple	
IRBLkm-Ts	*Pik-m*	11	Pik / k	Brown	
IRBL1-CL	*Pi1*	11	Pik / k	Brown	
IRBLkh-K3[LT]	*Pik-h*	11	Pik / k	Purple	
IRBLk-Ka[LT]	*Pik*	11	Pik / k	Purple	
IRBLkp-K60	*Pik-p*	11	Pik / k	Brown	
IRBL7-M	*Pi7(t)*	11	Piz / z	Purple	
IRBL9-W	*Pi9*	6	Piz / z	Brown	
IRBLz-Fu	*Piz*	6	Piz / z	Brown	
IRBLz5-CA	*Piz-5=Pi2(t)*	6	Piz / z	Purple	
IRBLzt-T	*Piz-t*	6	Piz / z	Brown	
IRBLta2-Pi	*Pita-2*	12	Pita / ta	Purple	
IRBLta2-Re	*Pita-2*	12	Pita / ta	Brown	
IRBL12-M	*Pi12(t)*	12	Pita / ta	Purple	
IRBLta-K1	*Pita=Pi4(t)*	12	Pita / ta	Purple	
IRBLta-CP1	*Pita=Pi4(t)*	12	Pita / ta	Purple	
IRBL19-A	*Pi19*	12	Pita / ta	Brown	
IRBL20-IR24	*Pi20(t)*	12	Pita / ta	Brown	
LTH	-	-	-	Purple	Susceptible
US2	-	-	-		Susceptible

U^a = locus of multiple allele. Chr. = Chromosome

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Table 2: Preservation and revival of pure blast isolates collected from different AEZs of Bangladesh

AEZ	Rain fed low land (# isolate)			Irrigated land (# isolate)		
	Collected sample	Pure isolates preserved	Revived & tested on MLs	Collected sample	Pure isolates preserved	Revived & tested on MLs
1	40	24	17	0	0	0
2	20	11	10	20	10	8
9	15	0	0	30	19	17
11	35	28	19	0	0	0
13	10	5	4	20	18	12
19	10	5	3	30	14	13
23	15	5	4	15	15	12
28	40	22	20	0	0	0
Total	185	100	77	115	76	62
Grand total: Collected Sample: 300; Pure Isolates Preserved: 176; Revived and tested on MLs: 139						

Table 3: Effect of treatment on plant height, leaf color chart (LCC) reading, seedling mortality and % disease incidence (DI)

Treatment	Plant height (cm)	LCC Scale	Seedling mortality (no.)	% Disease incidence
T1= only soil	26.0bc	2.4bc	2.1	13.0
T2= only cow dung (CD)	25.1bc	2.0c	2.5	10.0
T3= 1/2 soil+ 1/2 CD	25.8bc	2.4bc	1.8	9.0
T4= 1/2 soil+ 1/2 CD+NPK	25.4bc	2.6bc	1.5	10.0
T5=1/4 soil+3/4CD	24.4c	2.4bc	1.5	11.0
T6=1/4 soil+3/4CD+ NPK	25.9bc	2.6bc	1.8	11.0
T7=3/4 soil+1/4CD	27.0ab	3.0ab	1.4	17.0
T8=3/4 soil+1/4CD+NPK	28.7a	3.4a	0.7	20.4
LSD	2.47	0.69	ns	ns

In a column means followed by the same letter do not differ significantly at the 5% level of DMRT. (N= nitrogen, P = phosphorus, K = potassium, CD = cow dung).

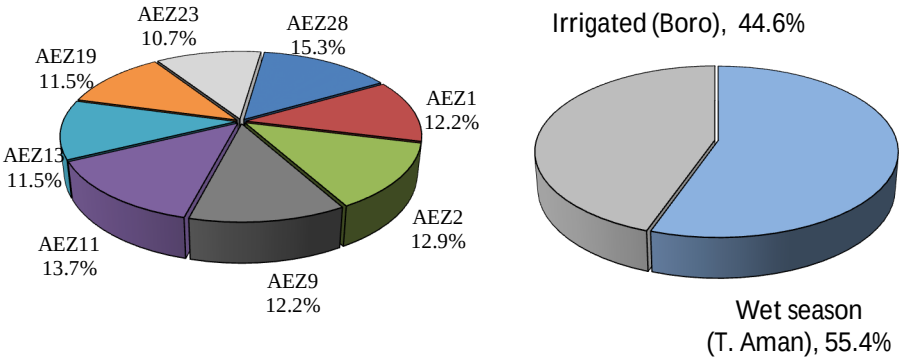

Figure 1: Regional and seasonal distribution of blast isolate tested

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RESEARCH ARTICLE

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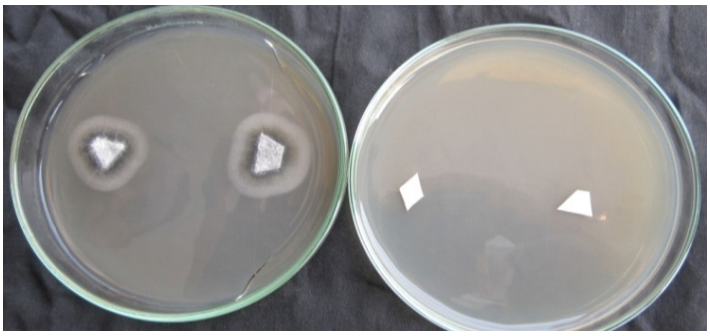
A  B

Figure 2: Revival of blast isolate from dry paper disk on PDA media. A. Cover of blotter paper, B. Blotter paper (Six month after storage).

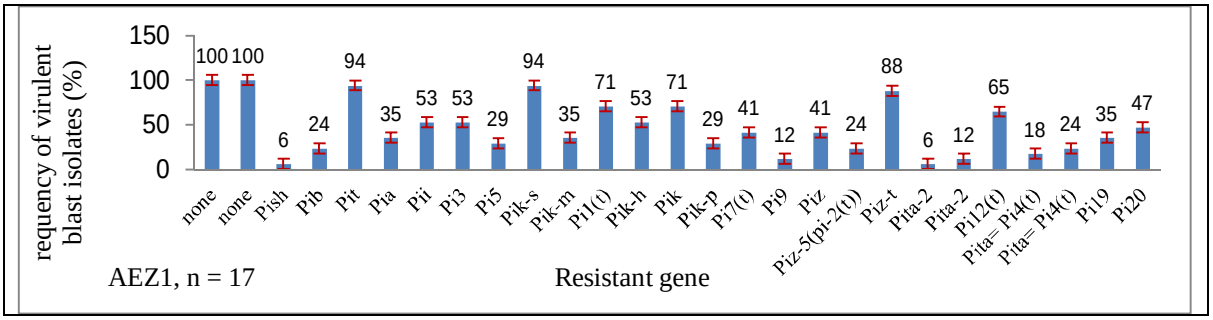


Figure 3: Virulence frequencies of blast isolates against DVs and susceptible check in AEZ1

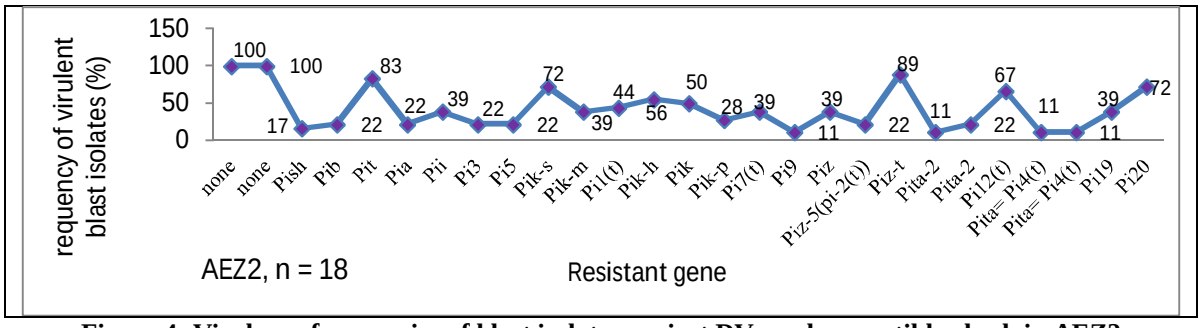


Figure 4: Virulence frequencies of blast isolates against DVs and susceptible check in AEZ2

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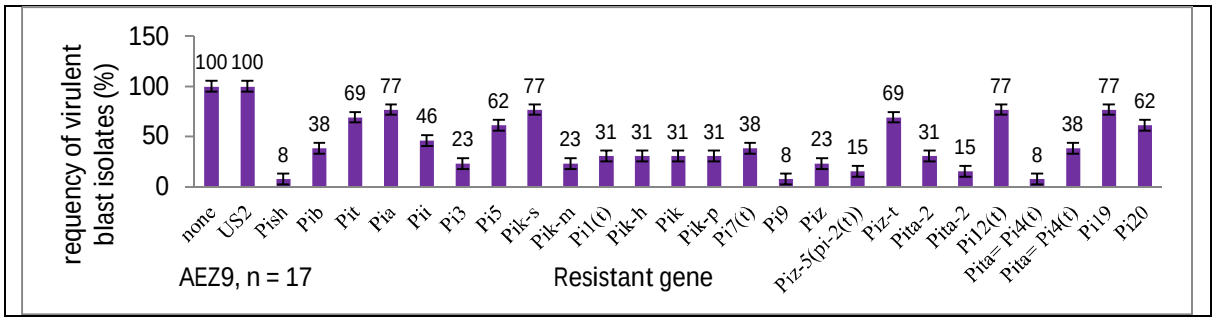

Figure 5: Virulence frequencies of blast isolates against DVs and susceptible check in AEZ9

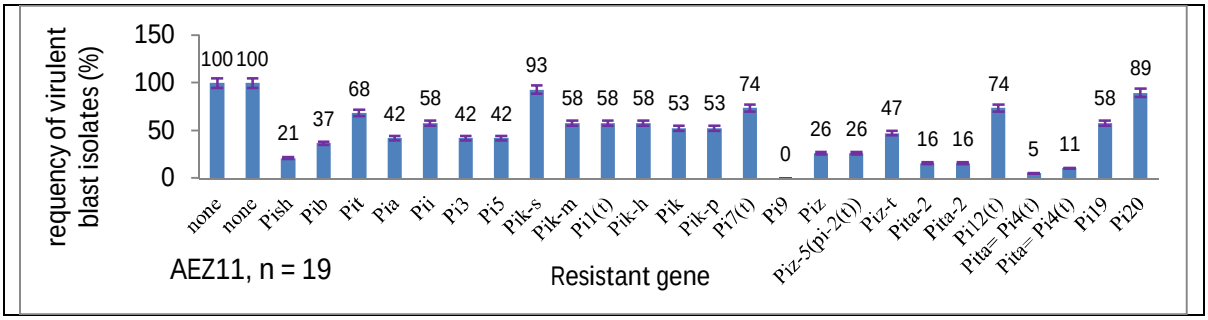

Figure 6: Virulence frequencies of blast isolates against DVs and susceptible check in AEZ11

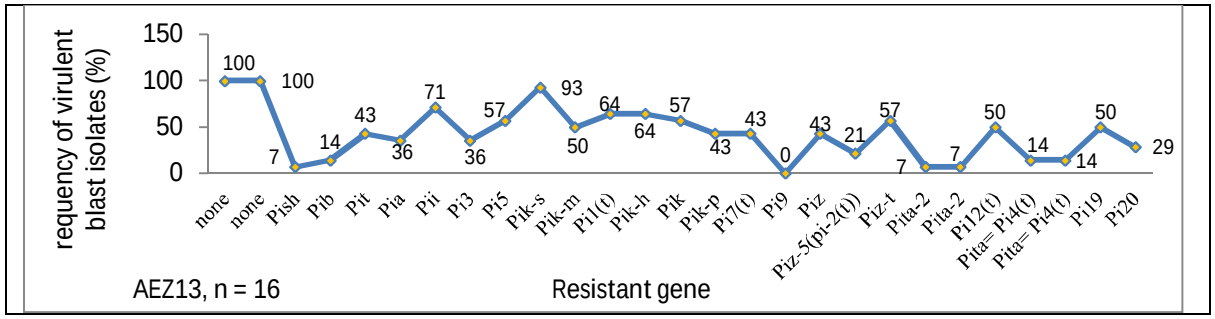

Figure 7: Virulence frequencies of blast isolates against DVs and susceptible check in AEZ13

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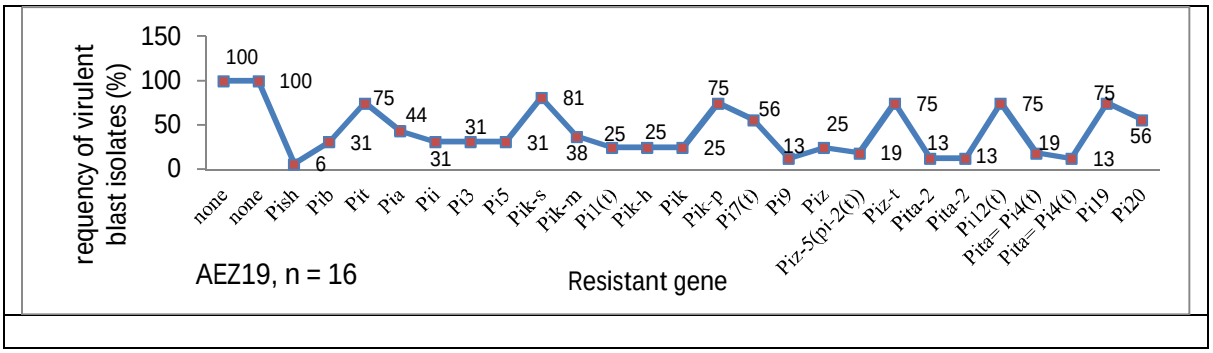

Figure 8: Virulence frequencies of blast isolates against DVs and susceptible check in AEZ19

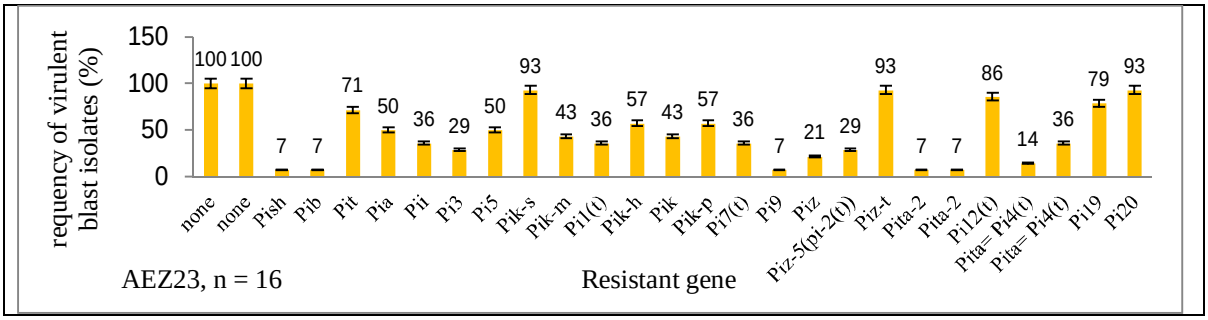

Figure 9: Virulence frequencies of blast isolates against DVs and susceptible check in AEZ23

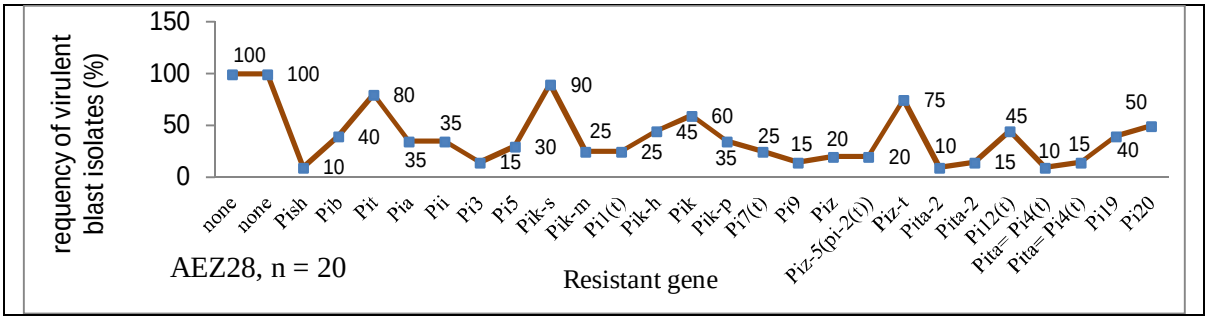

Figure 10: Virulence frequencies of blast isolates against DVs and susceptible check in AEZ28

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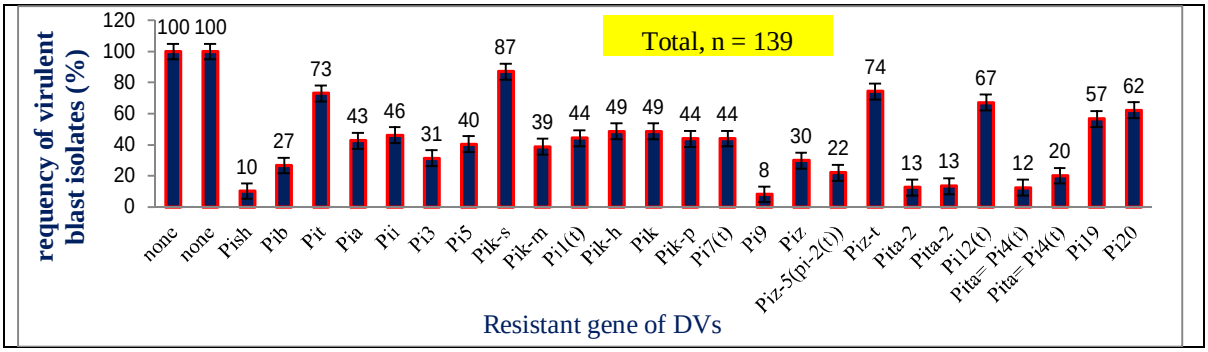

Figure 11: Virulence of all tested isolates against blast R-gene in all selected AEZs of Bangladesh

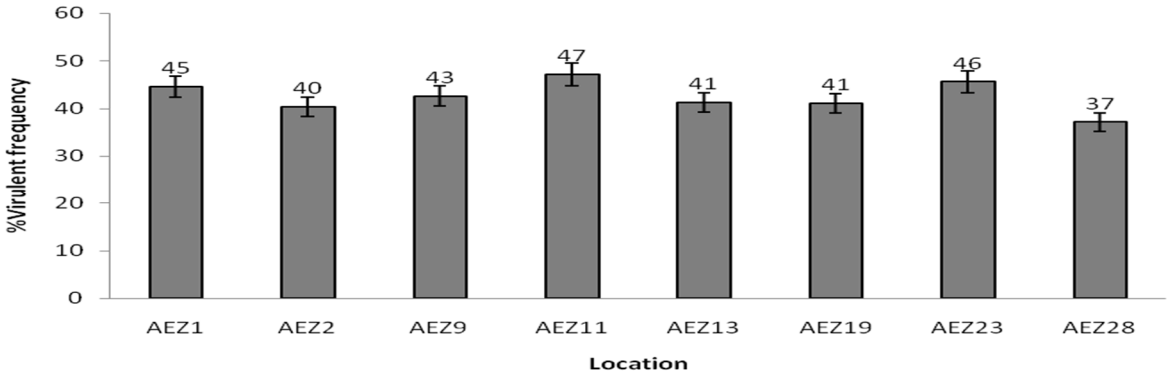

Figure 12: Average virulent frequency of blast isolates in different AEZs

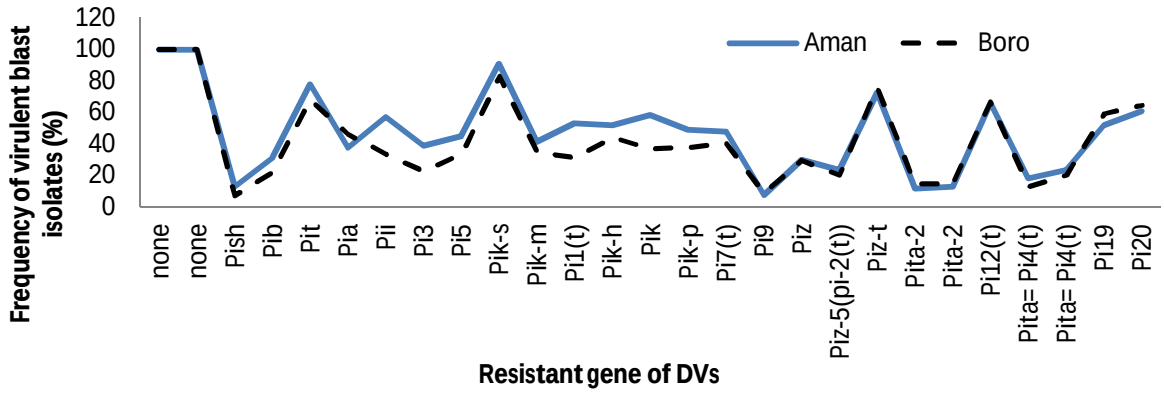

Figure 13: Virulence frequencies of blast isolate against DVs and susceptible check in rain fed low land (T. Aman season) and irrigated land (Boro season) ecosystems.

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Authors' contributions:

All authors are contributed equally in this research work.

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