Bangladesh Rice J.28(1): 57-68, 2024, doi.org/10.3329/brj.v28i1.86746

Diversity of Pathogenicity Among Rice Blast Fungus (*Pyricularia oryzae*) Isolates in Bangladesh

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ABSTRACT

A study was undertaken to determine the pathogenic and the genetic diversity of *Pyricularia oryzae* isolates causing blast disease of rice in Bangladesh. To find out pathogenic diversity of *P. oryzae*, 100 blast pathogen isolates were collected from five agro ecological zones (AEZ) abbreviated as AEZ23, AEZ19, AEZ13, AEZ28 and AEZ2 of Bangladesh. They were tested for their pathogenicity against 26 rice (*Oryza sativa* L.) differential varieties (DVs) having 23 resistance genes designated as *Pish, Pib, Pit, Pia, Pii, Pi3, Pi5(t), Pik-s, Pik-m, Pi1, Pik-h, Pik, Pik-p, Pi7(t), Pi9, Piz, Piz-5, Piz-t, Pita-2, Pita, Pi12(t), Pi19*, and *Pi20*(t). The virulence analysis showed that four genes, *Pish, Pi9, Piz* and *Pita-2* revealed a wide spectrum of moderate resistance to those isolates. The isolates were categorized into 94 races on the basis of the reaction patterns against rice differential varieties harboring twenty-three resistance genes and one susceptible variety, Lijiangxintuanheigu (LTH) by latest designation system. The findings demonstrate the existence of a wide variation in blast pathogens in Bangladesh. The average virulence of isolates from individual AEZ reveals that the maximum virulent isolates occur in AEZ2 (67%) followed by AEZ19 (63%) and AEZ28 (55%). Moreover, 100 *Pyricularia oryzae* isolates were grouped into five clusters viz. I, II, III, IV and V based on the results of the pathogenicity on 26 differential varieties including LTH on the basis of principal component analysis. Cluster I comprised of 18 isolates, Cluster II contained maximum of 31 isolates and cluster III contained 16 isolates. Twenty three isolates were placed in cluster IV and 12 isolates belonged to cluster V. The average virulence frequency of five Cluster I, II, III, IV and V showed 50%, 63%, 57%, 59% and 56% respectively. The findings of the present study reveal that the monogenic lines selected as differential varieties and the representative 25 blast pathogen isolates may be used to characterize the resistance of rice varieties.

Keywords: Rice blast fungus, Diversity of pathogenicity, *Pyrcularia oryzae*

INTRODUCTION

About half of the world's population relies on rice to achieve their daily caloric requirements (Wennberg, 2014). According to severity and incidence, 32 rice diseases have been identified in Bangladesh to far, blast (Nihad *et al.*, 2022), bacterial blight (Latif *et al.*, 2024a; Nihad *et al.*, 2021), sheath blight (Latif *et al.*, 2022), tungro (Nihad *et al.*, 2021) and false smut (Nessa *et al.*, 2015) considered as catastrophic disease throughout the nation. Rice blast caused by the fungal pathogen *Pyricularia oryzae* (telemorph: *Magnaporthe grisea* (Hebert) Barr.) is one of the most important diseases of rice in worldwide (Latif *et al.*, 2024b; Nihad *et al.*, 2022; Zeigler *et al.*, 1994). The use of resistant varieties is the most practical and economical method to control

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blast disease. But availability of effective blast resistant variety is limited due to breakdown of resistance by blast pathogen races virulent against the resistance (Koizumi, 2007). The dynamic interaction between host resistance and fungus virulence in the rice blast pathosystem can be explained by the gene-for-gene theory that every resistance gene in the host is corresponding with avirulence gene in the pathogen (Flor, 1971, Silue *et al.,* 1992). With the basis of the gene-for-gene theory, differential varieties and lines with various resistance genes against rice blast fungus have been developed to monitor the blast pathogen population structure and predict the emergence of new races *P. oryzae*.

The diversity of pathogenicity of blast pathogen isolates has been examined in many countries including Bangladesh, Indonesia, and China using differential varieties and lines. Over 100 blast resistance genes have been identified globally (Shahriar *et al.*, 2020; Yadav *et al.*, 2019), with *Pi9* and *Pb1* being the most prevalent. The *Pb1* gene, effective against a broad range of blast pathogen races, has demonstrated sustained resistance against panicle blast for over 35 years in Korea (Inoue *et al.*, 2017; Lee *et al.*, 2015b). Notably, *Pi9* and *Pb1* have also been shown to be effective against blast pathogen races in Bangladesh (Khan *et al.*, 2016; Nihad *et al.*, 2022). Noda *et al.* (1999) identified 12 blast pathogen races among 129 isolates collected from the Mekong River Delta area in Vietnam using 12 Japanese differential varieties carrying one of the resistance genes against blast pathogen (Yamada *et al.*, 1976; Kiyosawa, 1981; Kiyosawa *et al.*, 1984). Mekwatanakarn *et al.*, (2000) classified 527 blast pathogen isolates from Thailand into 175 races using differential near-isogeneic lines (NILs) developed from cultivars CO 39 and Lijiangxintuanheigu (LTH). Thinlay *et al.*, (2000) collected 110 isolates in Bhutan, and classified them into 53 races on the basis of the reactions of CO 39-based differential NILs. Chen *et al.*, (2001) collected 792 isolates in China, and classified them into 344 races using differential NILs derived from CO 39 and LTH. The pathogenicity of 119 blast pathogen isolates collected from the Philippines were categorized into 70 races (Telebanco-Yanoria *et al.*, 2008) using 18 Japanese differential varieties (Yamada *et al.*, 1976; Kiyosawa 1981, Kiyosawa *et al.*, 1984). These studies indicated that the variations in pathogenicity of blast fungus can be manifested by examining the reactions of differential varieties and lines to the pathogens.

In Bangladesh, blast disease severely affects rice production, especially that in rainfed lowland (July to December) and irrigated land (November to May) ecosystems (Nihad *et al.*, 2022; Islam *et al.*, 2001). In this study we examined the reactions of 25 monogenic lines carrying different resistance genes to *P. oryzae isolates* occurring in Bangladesh to find out variation in pathogenicity of the *P. oryzae isolates*, and to identify dominant races and resistance genes potentially effective to blast disease in Bangladesh.

MATERIALS AND METHODS

Differential lines for blast fungus isolates. The 25 differential monogenenic lines derived from LTH were used to evaluate the pathogenicity of blast fungus isolates (Tsunematsu *et al.*, 2000). Each of the 25 monogenic lines contains one of the 23 resistance genes, *Pish, Pib, Pit, Pia, Pii, Pi3, Pi5(t), Pik-s, Pik-m, Pi1, Pik-h, Pik, Pik-p, Pi7(t), Pi9, Piz, Piz-5, Piz-t, Pita-2, Pita, Pi12(t), Pi19,* or *Pi20*(t) (Table 1). LTH and a local variety, Nizersail of our native environmental check, were also included as susceptible controls in the evaluation.

Collection and isolation of blast pathogen isolates. Rice leaves, nodes and panicles infected with blast fungus were collected from 5 agro ecological zones (AEZs) of Northern (AEZ2), Barisal (AEZ13), Comilla (AEZ19), Chittagong (AEZ23) and Gazipur regions (AEZ28) of Bangladesh during Aman (80 isolates) and Boro (20 isolates) 2010 to 2012. The samples were cut into small pieces, and surface was sterilized in 0.001% $HgCl_2$. The sterilized samples were placed on moist filter paper in Petri dishes, and incubated for two days under near UV light (12 hrs. under light and 12 hrs. in dark) for sporulation. Conidia developed on the colony were transferred into Petri dishes containing 0.03% water agar medium containing streptomycin sulfate (40 mg/l). After 3 days of incubation, the individual colony was transferred to prune agar medium, and allowed to grow for 3-4 days at 28°C. Sterilized rice straw was put on the culture to allow the mycelium to grow on the straw. The straws were colonized with mycelium of *P. oryzae* were put under near UV light for sporulation as before. Conidia developed on the straw pieces were suspended in 5 ml sterilized distilled water, and poured into Petri dishes containing 20 ml liquid water agar medium. After two days of incubation, germinated conidium along with growing hyphae were transferred individually to prune agar medium in test tube slants and allowed to grow. Each of the single spore pure culture was noted as an isolate.

Preservation of blast isolates: Sterilized filter paper disks colonized with pure culture of blast isolates were made air dry for seven to ten days. Then colonized filter papers were preserved in -20°C under dry condition with silica gel at Seed Pathology and Molecular Lab.

Preparation of blast fungus inoculum. Filter paper disks colonized with isolates were transfer to prune agar plates and allowed to grow at about 25°C for 5 days. The blocks of mycelium (4 mm diameter) were transferred to oat meal agar plates. The oat meal culture was allowed to grow on the surface of the medium for 7 days at 25°C for sporulation. The crushing of mycelia was done with tooth brush, conidia produced were scraped from the surface of the culture, and suspended in sterilized distilled water. The spore concentration was measured and adjusted to 1×10^5 spores/ml. Exactly 50 ml of spore suspension of each isolate was used for inoculation of rice seedlings.

Evaluation for reactions of rice to blast fungus. Ten to twelve sprouted seeds/lines of the 25 monogenic lines, LTH, and Nizersail were sown in continuous lines in plastic trays (60 cm × 30 cm) filled with sandy loam soil. Inoculation of blast pathogen was done as described in Bonman *et al.*, (1986) and Hayashi *et al.*, (2009). About 21-25 days old seedlings were inoculated by spraying with 50 ml spore suspension of individual isolate using a hand sprayer in the afternoon at 5- 6 pm. All inoculated seedlings were incubated for 24 hours immediate after inoculation by covering polythene. The relative humidity and temperature of the net house were maintained to 70 to 80% and 25±1ºC, respectively for disease development. Severity of blast disease developed on inoculated rice plants was evaluated as described in Mackill and Bonman (1992) and Hayashi *et al.*, (2009) at 7 to 8 days after inoculation.

Races of blast fungus. The pathogenic races of blast isolates were classified following the international designation system as detailed in Hayashi and Fukuta (2009) and Gilmorour (1973). The 25 differential monogenic lines and LTH were divided into five groups of U (4 lines and LTH), i (3 lines), k (7 lines), z (4 lines) and ta (7 lines) depending on the loci of resistance genes present in the respective lines (Hayashi and Fukuta, 2009). The monogenic lines and LTH within a group were further divided into subgroups of one to three lines. A number code of 1, 2, or 4 was assigned to each line within a subgroup. The codes (1, 2, or 4) assigned to the individual lines within a subgroup were added up if the lines were susceptible to a blast isolate to define the race number of the isolates. For

example, the race number of a blast fungus isolate causing a susceptible reaction in all the monogenic lines and LTH is U73-i7-k177-z17-ta773, while that of an isolate causing a resistance reaction in all the lines and LTH is U00-i0-k000-z00-ta000.

Data Analysis. A hierarchical clustering analysis for blast fungus isolates was done by the method by Ward (1963) using JMP 7.0.2 software (SAS Institute, Inc., Cary, NC, USA) based on the degree of infection also referred to the severity of infection of blast pathogen isolates to the 25 monogenic lines and LTH. Diversity of blast isolates within an AEZ was calculated based on their reactions to the groups of monogenic lines and LTH by the method of Simpson (1949). The diversity index varies from 0 to 1, where 0 represents no diversity and 1 for maximum diversity. Non-hierarchical clustering of blast fungus isolates based on their reactions to the monogenic lines was carried out by the GENSTAT 5.5 program (Rahman *et al.*, 2010). Distances (Mahalanobis distance) among the clusters of blast fungus isolates were obtained as a result of a canonical variate analysis using of the GENSTAT 5.5 program (Rahman *et al.*, 2010).

RESULTS AND DISCUSSION

Virulence spectrum of blast fungus isolates from Bangladesh

Frequencies of virulence of blast fungus isolates collected from different AEZs to the 25 differential monogenic lines was evaluated by inoculating the isolates to the lines and susceptible control plants. A large proportion (≥ 60%) of the isolates collected from AEZ23 were able to induce a susceptible (compatible) reaction in 14 monogenic lines carrying one of the resistance genes *Pib, Piz-5(Pi2(t)), Pit, Pia, Pik-s, Pik-m, Pik-h, Pik, Pik-p, Pi7(t), Piz-t, Pi12(t), Pita*, or *Pi20*(t) (Fig. 1A). In contrast, the proportions of isolates from AEZ23 showing a resistance (incompatible) reaction were only 20% or less in four lines for *Pita-2, Pish, Pi9* and *Piz* (Fig. 1A).

The proportions of the isolates from AEZ19 that caused a susceptible reaction were 60% or higher in 17 lines carrying one of the resistance genes *Pik-m, Piz-5(Pi2 (t)), Pi1, Pit, Pi5(t), Pi19, Pii, Piz-t, Pia, Pik-h, Pib, Pi7(t), Pi12(t), Pi20, Pik, Pik-p*, or *Pik-s* (Fig. 1B). Especially, all the isolates collected from AEZ19 showed a susceptible reaction in the line with *Pik-s*. The proportions of the isolates from AEZ19 caused a resistance reaction in three lines carrying *Piz* or *Pita-2* (Fig. 1B) were less than 20%.

The proportions of the isolates from AEZ13 showing a susceptible reaction were 60% or higher in ten lines carrying one of the resistance genes *Pi5(t), Pik, Piz-5(Pi2 (t)), Piz-t, Pi20, Pi12(t), Pit, Pib, Pia,* or *Pik-s* (Fig. 1C). The proportions of the isolates from AEZ13 causing a susceptible reaction in three lines carrying one of the resistance genes *Pita-2, Pi9* or *Pish* were less than 20% (Fig. 1C).

The proportions of the isolates from AEZ28 causing a susceptible reaction were 50% or less in 8 lines carrying one of the resistance genes *Piz, Pita-2, Pish, Pi9, Pit-2, Pita=Pi4(t), Pii,* or *Pi3(t)* (Fig. 1D). Especially 20% or less of the isolates from AEZ28 were able to cause a susceptible reaction in three lines for *Piz, Pita-2* and *Pish*.

The proportions of the isolates from AEZ2 causing a susceptible reaction were less than 40% in only five lines carrying one of the resistance genes *Pish, Piz, Pita-2,* or *Pi9* (Fig. 1E). The rest of the lines were found to show a susceptible reaction with more than 60% of the isolates from AEZ2 (Fig. 1E). These results indicate that the isolates from AEZ2 are more virulent than those from other AEZs.

When the virulence frequencies of the blast fungus isolates from the individual AEZs are combined, the proportions of the blast fungus isolates from five AEZs causing a susceptible reaction were less than 20% in the lines carrying *Pish, Pita-2* and *Piz*, and the proportions were between 20% and 60% in the lines carrying *Pi9, Pita-2, Pita=Pi4(t), Pi3(t)* and *Pi1* (Fig. 1F). The results suggested that rice cultivars carrying resistance genes such as *Pish, Pita-2, Pi9,* and *Piz* may serve as promising sources of resistance to blast in Bangladesh. Effectiveness of *Pish*,

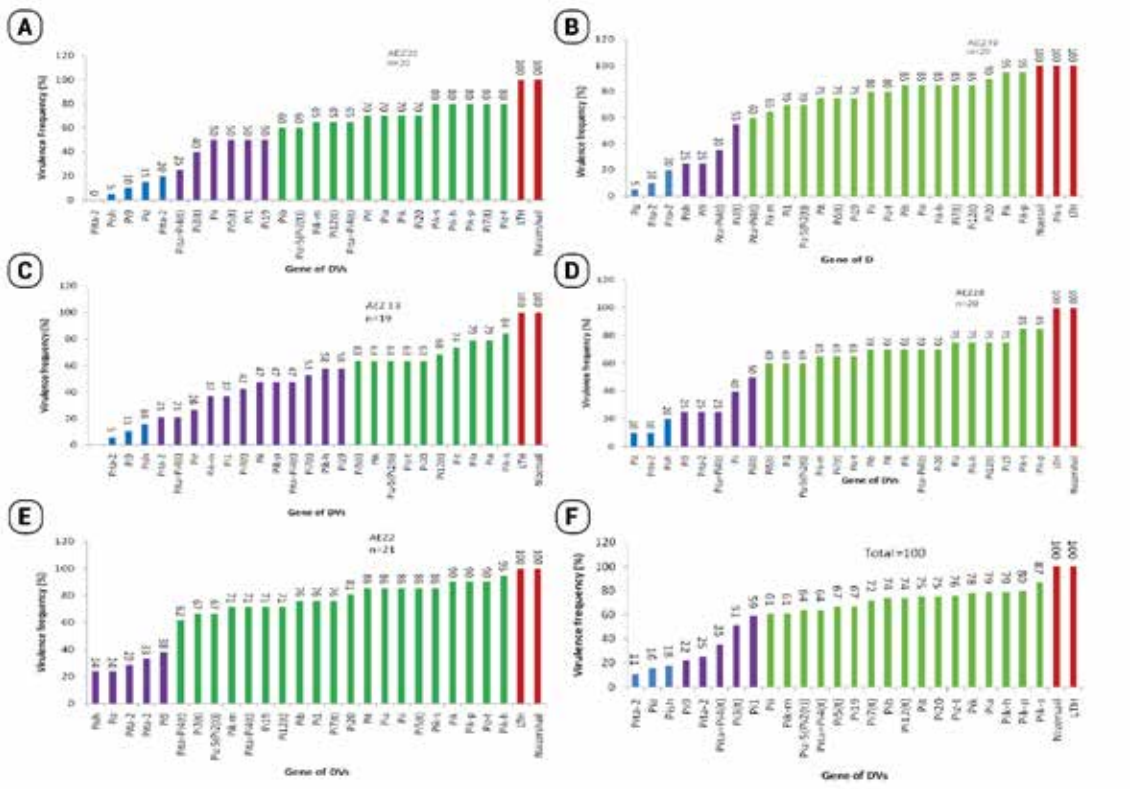

Fig. 1: Virulence frequencies of blast fungus isolates collected (Aman and Boro season) from A) AEZ23, B) AEZ19, C) AEZ13, D) AEZ28, and E) AEZ2, and F) all five AEZs combined against differential monogenic lines and susceptible checks.

Pita-2, Pi9 and *Piz* as a resistance gene against blast fungus were also documented in studies using blast fungus isolates in other countries. Mithrasena *et al.*, (2012) reported that rice plants carrying *Pish* or *Pi9* were resistant to a majority of blast pathogen isolates collected in Sri Lanka. Telebanco-Yanoria *et al.*, (2008) showed that the resistance genes *Pish, Piz-5*, and *Piz* showed a wide spectrum of moderate resistance to blast fungus isolates in Philippines. Koide *et al.*, (2011) also reported that a monogenic line carrying *Pi9* was resistant to all standard differential blast isolates in Philippines. Du *et al.*, (2007) reported that a rice plant carrying *Piz* showed effective resistance to 179 blast isolates in Vietnam. Sathya *et al.* (2013) reported that rice plants carrying four resistant genes *Pish, Piz, Pi9* and *Pita-2* showed broad spectra of resistance to Cambodian blast fungus isolates.

Races of blast isolates from Bangladesh

Based on the reactions of LTH and the four U group monogenic lines carrying *Pish, Pia, Pib,* or *Pit,* the 100 blast isolates were classified into ten pathotypes. The U63 type that can cause susceptible reactions in LTH and lines carrying *Pia, Pib,* or *Pit* was most predominant. The U63 type represented 41% of the total population and found in all the five zones (Supplementary Table 1).

Table 1. Characteristics of international standard differential monogenic lines derived from Lijiangxintuanheigu by Hayashi *et al.*, (2009).

Differential monogenic line	Resistance gene in monogenic lines		
	Gene name	Chromosome	Locus/group
IRBLsh-B	*Pish*	1	*Pish*/U
IRBLb-B	*Pib*	2	*Pib*/U
IRBLt-K59	*Pit*	1	*Pit*/U
IRBLa-A	*Pia*	11	*Pia*/U
IRBLi-F5	*Pii*	9	*Pii* / i
IRBL3-CP4	*Pi3*	9	*Pii* / i
IRBL5-M	*Pi5(t)*	9	*Pii* / i
IRBLks-F5	*Pik-s*	11	*Pik* / k
IRBLkm-Ts	*Pik-m*	11	*Pik* / k
IRBL1-CL	*Pi1*	11	*Pik* / k
IRBLkh-K3[LT]	*Pik-h*	11	*Pik* / k
IRBLk-Ka[LT]	*Pik*	11	*Pik* / k
IRBLkp-K60	*Pik-p*	11	*Pik* / k
IRBL7-M	*Pi7(t)*	11	*Piz* / z
IRBL9-W	*Pi9*	6	*Piz* / z
IRBLz-Fu	*Piz*	6	*Piz* / z
IRBLz5-CA	*Piz-5=Pi2(t)*	6	*Piz* / z
IRBLzt-T	*Piz-t*	6	*Piz* / z
IRBLta2-Pi	*Pita-2*	12	*Pita* / ta
IRBLta2-Re	*Pita-2*	12	*Pita* / ta
IRBL12-M	*Pi12(t)*	12	*Pita* / ta
IRBLta-K1	*Pita=Pi4(t)*	12	*Pita* / ta
IRBLta-CP1	*Pita=Pi4(t)*	12	*Pita* / ta
IRBL19-A	*Pi19*	12	*Pita* / ta
IRBL20-IR24	*Pi20(t)*	12	*Pita* / ta

The diversity indexes of the pathotypes determined by the U group lines in the respective AEZs were between 0.71 and 0.81.

Table 2. Pathogenic races of 100 blast fungus isolates collected in Bangladesh.

Clusters	Number of members	Races
I	18	U21-i7-k174-z07-ta623, U61-i1-k122-z06-ta400, U43-i1-k117-z00-ta423 U43-i1-k157-z14-ta021, U41-i0-k135-z02-ta022, U21-i4-k073-z06-ta622 U63-i7-k157-z06-ta423, U73-i7-k157-z14-ta733, U61-i0-k141-z07-ta403 U63-i7-k157-z06-ta423 , U63-i5-k122-z06-ta403, U63-i7-k177-z07-ta632 U63-i7-k135-z17-ta411, U63-i4-k176-z12-ta431, U63-i3-k167-z16-ta132 U21-i6-k157-z04-ta423,U63-i7-k177-z16-ta721, U43-i7-k177-z06-ta333,
II	31	U21-i0-k046-z04-ta032, U61-i6-k157-z06-ta403, U63-i7-k177-z06-ta603 U63-i7-k167-z06-ta423, U63-i7-k167-z07-ta733, U03-i1-k100-z00-ta002 U73-i5-k167-z16-ta433, U23-i1-k173-z06-ta003, U43-i7-k137-z14-ta433, U23-i4-k152-z04-ta003, U63-i4-k020-z02-ta622U63-i7-k166-z04-ta633, U23-i5-k122-z04-ta022, U41-i0-k040-z00-ta401,U73-i0-k177-z16-ta623, U73-i7-k167-z16-ta533, U03-i7-k127-z12-ta403,U73-i7-k177-z06-ta633, U01-i0-k042-z04-ta400, U61-i0-k040-z05-ta410 ,U63-i5-k175-z16-ta432, U63-i7-k177-z00-ta723, U23-i7-k177-z06-ta021,U43-i5-k173-z07-ta002, U43-i7-k137-z10-ta732, U63-i7-k167-z17-ta403,U73-i5-k177-z16-ta423, U63-i7-k177-z15-ta633, U73-i7-k177-z17-ta433,U21-i4-k143-z06-ta433, U43-i5-k053-z04-ta203,
III	16	U43-i0-k146-z06-ta400, U73-i4-k147-z04-ta422, U41-i6-k167-z00-ta400 U33-i5-k173-z00-ta623, U43-i0-k147-z00-ta422, U03-i3-k141-z02-ta421 U01-i0-k104-z04-ta000, U63-i1-k101-z02-ta403, U63-i0-k104-z06-ta000 U63-i6-k177-z04-ta423, U01-i0-k173-z06-ta203, U03-i0-k042-z00-ta021 U41-i6-k100-z10-ta502, U73-i3-k173-z06-ta423, U63-i5-k177-z06-ta633 U63-i5-k177-z06-ta633
IV	23	U61-i0-k057-z05-ta033, U03-i0-k020-z06-ta402,U43-i7-k177-z00-ta402, U63-i7-k167-z00-ta422, U03-i0-k146-z04-ta400, U63-i7-k157-z16-ta033, U03-i7-k177-z06-ta433,U63-i5-k157-z04-ta433, U63-i1-k157-z16-ta422, U63-i3-k177-z14-ta423,U63-i6-k105-z07-ta421, U73-i1-k143-z11-ta423, U23-i4-k114-z06-ta403,U73-i7-k177-z04-ta733, U63-i0-k057-z00-ta022, U43-i2-k127-z07-ta421, U41-i5-k173-z07-ta422, U73-i7-k147-z06-ta713, U63-i7-k177-z04-ta033, U63-i7-k177-z06-ta433, U63-i7-k177-z06-ta433, U63-i7-k177-z06-ta433, U63-i7-k177-z06-ta433
V	12	U03-i7-k175-z07-ta623, U63-i7-k173-z06-ta413, U63-i0-k157-z06-ta211 U63-i1-k177-z06-ta021, U61-i4-k127-z02-ta400, U63-i1-k157-z06-ta403 U63-i4-k141-z06-ta002, U01-i6-k042-z00-ta000, U73-i7-k077-z02-ta020 U63-i6-k157-z02-ta422, U63-i0-k114-z04-ta413, U73-i7-k077-z02-ta020

Based on the reactions to the three i group monogenic lines carrying *Pii, Pi3,* or *Pi5(t)*, the 100 isolates were classified into seven pathotypes (Supplementary Table 2). The i7 type that can cause susceptible reactions to all I group lines was most dominant, and represented 36 isolates. Especially 13 isolates among 21 from AEZ2 were the i7 type, and thus the diversity index among the isolates from AEZ21 (0.57) were notably lower than the indexes of isolates from other AEZs (0.71 to 0.81) (Supplementary Table 2). The i0 type that can cause resistance reactions in all the i group lines was not found among the isolates from AEZ19, suggesting that rice plants carrying the i locus resistance genes are vulnerable to the isolates from AEZ19.

Based on the reaction to the seven k group monogenic lines for *Pik-s, Pik-m, Pi1, Pik-h, Pik, Pik-p*, or *Pi7(t)*, the 100 isolates were classified into 31 pathotypes (Supplementary Table 3).

Table 3. Pathogenic races selected for the standard differential blast fungus isolates in Bangladesh.

Blast Isolates				Differential variety and susceptible control																										
				U					i			k							z				ta							
				IRBLsh-B	IRBLb-B	IRBLt-K59	USV, LTH	IRBLa-A	IRBLi-F5	IRBL3-CP4	IRBL5-M	IRBLks-F5	IRBLkm-Ts	IRBL1-CL	IRBLkh-K3	IRBLk-Ka	IRBLkp-K60	IRBL7-M	IRBL9-W	IRBLz-Fu	IRBLz5-CA-	IRBLzt-T	IRBLta2-Pi	IRBLta-Re	IRBL12-M	IRBLta-K1	IRBLta-CP1	IRBL19-A	IRBL20-		
Sl. No.	Isolate Code	Name of the race	Virulent frequency (%)	Resistant gene harboring in the genetic background																										
				Pish	Pib	Pit	None	Pia	Pii	Pi3(t)	Pi5(t)	Pik-s	Pik-m	Pi1	Pik-h	Pik	Pik-p	Pi7(t)	Pi9(t)	Piz	Piz-5(Pi2(t))	Piz-t	Pita-2	Pita-2	Pi12(t)	Pita=Pi4(t)	Pita=Pi4(t)	Pi19(t)	Pi20(t)		
1	BS-97	U63-i7-k177-z07-ta632	85	R	s	s	s	s	s	s	s	s	s	s	s	s	s	s	R	s	s	s	R	s	s	s	s	R	s	
2	NS-109	U43-i7-k137-z10-ta732	73	R	R	s	s	s	s	s	s	s	s	s	R	s	s	s	s	R	R	R	s	s	s	s	s	R	s	
3	CS-135	U63-i3-k177-z14-ta423	69	R	s	R	s	s	s	s	R	s	s	s	s	s	s	s	s	R	R	s	R	R	s	R	s	s	s	
4	CS-84	U41-i6-k167-z00-ta400	46	R	s	s	s	R	R	s	s	s	R	s	s	s	s	s	R	R	R	R	R	R	s	R	R	R	R	
5	GS-14	U63-i6-k157-z02-ta422	62	R	s	s	s	s	R	s	s	s	s	R	s	s	s	s	R	R	s	R	R	R	s	R	s	R	s	
6	CS-124	U73-i5-k167-z16-ta433	81	s	s	s	s	s	s	R	s	s	R	s	s	s	s	s	s	R	s	s	R	R	s	s	s	s	s	
7	GS-98	U73-i3-k173-z06-ta423	77	s	s	s	s	s	s	s	R	s	s	s	s	s	s	R	R	R	s	s	R	s	s	R	s	s	s	
8	BS-98	U73-i3-k173-z06-ta423	62	s	s	s	s	s	s	s	s	R	s	s	s	s	s	s	R	R	s	R	R	R	R	R	s	R	R	
9	CS-142	U73-i7-k157-z14-ta733	92	s	s	s	s	s	s	s	s	s	R	s	s	s	s	s	s	R	s	s	s	s	s	s	s	s	s	
10	CS-136	U63-i7-k157-z06-ta423	73	R	s	s	s	s	s	s	s	s	s	R	s	s	s	s	R	R	s	s	R	R	s	R	s	s	s	
11	BS-30	U63-i6-k105-z07-ta421	58	R	s	s	s	s	R	s	s	s	R	R	R	s	R	s	R	s	s	s	R	R	s	R	s	s	R	
12	BS-88	U63-i5-k122-z06-ta403	54	R	s	s	s	s	s	R	s	s	R	s	R	R	s	R	R	R	s	s	R	R	s	R	R	s	s	
13	HS-4	U03-i7-k175-z07-ta623	73	R	R	R	s	s	s	s	s	s	s	s	s	s	R	s	R	s	s	s	R	s	s	R	s	s	s	
14	HS-53	U63-i7-k173-z06-ta413	73	R	s	s	s	s	s	s	s	s	s	s	s	s	s	R	R	R	s	s	R	R	s	s	R	s	s	
15	NS-112	U63-i7-k177-z06-ta433	81	R	s	s	s	s	s	s	s	s	s	s	s	s	s	s	R	R	s	s	R	R	s	s	s	s	s	
16	GS-129	U73-i7-k177-z06-ta633	88	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	R	R	s	s	R	s	s	s	s	s	s	
17	BS-141	U73-i7-k177-z04-ta733	88	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	R	R	R	s	s	s	s	s	s	s	s	
18	NS-106	U63-i7-k177-z00-ta723	80	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	R	R	R	R	s	s	s	R	s	s	s	
19	NS-117	U73-i7-k177-z17-ta433	92	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	R	R	s	s	s	s	s	
20	CS-76	U63-i7-k177-z06-ta433	84	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	R	R	s	s	R	R	s	s	s	s	s	
21	HS-101	U63-i7-k157-z16-ta033	77	R	s	s	s	s	s	s	s	s	s	R	s	s	s	s	s	R	s	s	R	R	R	s	s	s	s	
22	GS-79	U41-i5-k173-z07-ta422	69	R	s	s	s	s	s	R	s	s	s	s	s	s	s	R	R	s	s	s	R	R	s	R	s	R	s	
23	NS-103	U73-i7-k147-z06-ta713	81	s	s	s	s	s	s	s	s	s	R	R	s	s	s	s	R	R	s	s	s	s	s	s	R	s	s	
24	CS-133	U63-i1-k157-z16-ta422	65	R	s	s	s	s	s	R	R	s	s	R	s	s	s	s	s	R	s	s	R	R	s	R	s	R	s	
25	GS-13	U63-i6-k177-z04-ta423	65	R	s	s	s	s	R	s	s	s	s	s	s	s	s	s	R	R	R	s	R	R	s	R	s	s	R	

The k177 type that can cause susceptible reactions to all the k group lines was most dominant, and represented 24% of the isolates. The proportion of the k177 type was especially high among the isolates from AEZ2, representing 52% of the isolates, and thus the diversity index of the isolates from AEZ2 (0.70) was notably lower than the indexes of the isolates from other AEZs (0.79 to 0.91) (Supplementary Table 2).

Based on the reactions of the four z group lines for *Pi9, Piz, Piz-5,* or *Piz-t*, the 100 isolates were classified into13 pathotypes (Supplementary Table 4). The z06 type that shows incompatible (resistance) reactions in the lines for *Pi9* and *Piz* was most dominant, representing 33% of the isolates. Especially, the z06 type represented 45% of the isolates from AEZ19, which showed the lowest diversity index (Supplementary Table 2) The isolates of the z00 type that can cause

susceptible reactions in all the z group lines represented 12% of the isolates, and found in all the five AEZs.

Based on the reactions of the seven ta group lines for *Pita-2, Pi12(t), Pita, Pi19(t),* or *Pi20(t)*, the 100 isolates were classified into 38 pathotypes (Supplementary Table 5). The ta403 type showing incompatible reactions in the lines for *Pita-2* and *Pita* and ta423 types showing incompatible reactions in the lines for *Pita-2* and one of the lines for Pita were the major types, each representing 9% of the isolates.

When the reactions to all the 25 lines were considered together, the 100 blast isolates from the five AEZs were represented by 94 pathogenic races that were classified into five clusters (Table 2 and Fig. 2). Four isolates were classified as the same race U63-i7-k177-z06-ta433 in cluster IV. Each of three races U63-i5-k177-z06-ta633 in cluster III, U63-i7-k157-z06-ta423 in cluster I, or U73-i7-k077-z02-ta020 in cluster V represented two different isolates. The rest of the races represented single isolates. Cluster II was the largest one comprised of 33 isolates followed by cluster IV comprised of 23 isolates. A canonical variate analysis showed that the inter-cluster distance was maximum between clusters I and V, and was minimum between clusters III and IV (Fig. 2). The highest average virulence frequency (63%) was observed in Cluster II and cluster I showed lowest virulence frequency (50%). The virulence frequency of cluster III, IV and V showed 57%, 59% and 56% respectively.

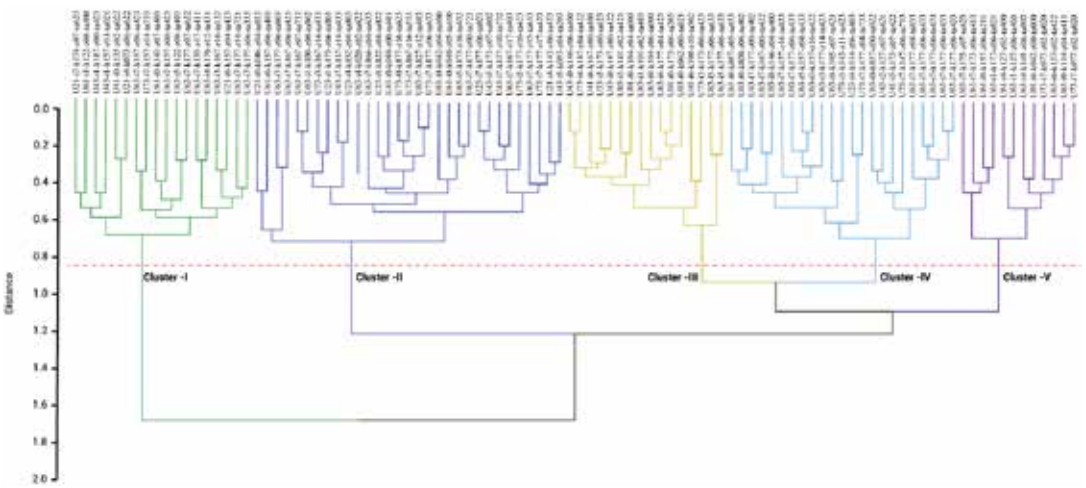

Fig. 2. Dendrogram showing Pathogenic races of 100 blast fungus isolates collected in Bangladesh.

Standard differential blast isolates in Bangladesh

As the result of our evaluation of blast fungus isolates for the reactions to 25 differential monogenic lines, twenty-five isolates were selected as the standard differential blast fungus isolates in Bangladesh based on their virulence to differentiate 23 resistance genes in the 25 monogenic lines, revive capacity and sporulation potentiality (Table 3). A standard set of differential blast fungus isolates has been used to characterize the resistance of rice breeding lines and varieties in Philippines (Telebanco-Yanoria *et al.*, 2008; Koide *et al.*, 2011). We expect the standard differential blast fungus isolates can also be used to characterize the resistance spectra of new rice varieties, and to examine pathogenicity of blast causing fungus isolates from other AEZs of Bangladesh.

CONCLUSION

Based on the reaction patterns against 25 LTH-derived differential monogenic lines carrying one of the 23 resistance genes, 100 isolates from five AEZs in Bangladesh were classified into 94 pathogenic races. Races designated by pathotypes of U63, i0, i7, k157, k177, z00, z04, z06, ta403, ta423, and ta433 were found as the dominant types, among which races designated by pathotypes of U63, i7, k177, z00, z04, z06 and ta423 were commonly found in all the five AEZs of Bangladesh. The results indicated that any rice varieties relying on *Pib, Pit, Pia, Pii, Pi3, Pi5(t), Pik, Pik-h, Pik-m, Pi1, Pik-p, Pik-s, Pi7(t), Piz-t, Piz-5, Pi12(t), Pita(2), Pi19(t)*, and *Pi20(t)* might be at the risk of blast fungus infection in Bangladesh, especially in areas such as AEZ2 and AEZ19 where such races were found to present at a high frequency. In contrast, for a number of isolates incompatible to lines carrying *Pish, Pi9, Piz, Pita-2,* and *Pita* were also found among the 100 isolates examined, suggesting these genes are promising as the sources of robust resistance in Bangladesh. These genes can be used in breeding program for development of blast resistant variety. The standard set of 25 blast fungus isolates selected would be a useful tool in developing rice varieties, resistant to blast disease in Bangladesh.

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