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VIRULENCE ANALYSIS OF *PYRICULARIA ORYZAE* ISOLATES CAUSING WHEAT BLAST IN BANGLADESH

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Abstract

Pyricularia oryzae pathotype *Triticum*, causal agent of wheat blast, has emerged in Bangladesh as a serious threat for wheat production. Virulence analysis of plant pathogen can reveal the pathogenic nature of that pathogen. In the present study, twenty-four monoconidial isolates of *P. oryzae* from Chuadanga, Meherpur, Kustia and Jhenaidaha districts of Bangladesh were analyzed to observe their pathogenic potential. Based on the disease reactions, all the isolates were grouped into 3 pathotypes. Present investigation revealed that the isolates with high virulence were prevalent in the studied *P. oryzae* population. Again, Pathotype 1 that was identified as the most virulent can be used as reference for screening resistant wheat varieties.

Introduction

Wheat (*Triticum aestivum* L.) is one of the oldest and most widely cultivated cereal grain in the world belongs to the family Poaceae. World trade in wheat is greater than for all other crops combined. Although Bangladesh is one of the principal rice-consuming countries, the consumption of wheat in Bangladesh has intensely increased over the years[1].

Wheat blast was first reported in Paraná province of Brazil in 1985[2] and then it was subsequently spread in Brazil, Bolivia, Paraguay, Argentina and Uruguay[3-6]. Deadly wheat blast attacked Bangladesh for the first time in 2016 which was its first epidemic outside South America[7-9]. Before its emergence in Bangladesh in 2016, it was confined in some south American countries[7,10]. Wheat blast, caused by ascomyceteous fungus *Pyricularia oryzae* Cavara (Teleomorh: *Magnaporthe oryzae* B. C. Couch) pathotype *Triticum*, is an explosive pathogen of wheat. Although this pathogen can affect all above-ground parts, symptoms are generally seen on leaves and spikes of wheat plant. The most common symptoms of wheat blast on spikes are premature bleaching of spikelets and entire heads[11,12]. On the leaves, initially diamond shaped water-soaked lesions may be formed. However, those lesions gradually turn into typical eye shaped necrotic lesions with grey centers.

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Pathogen diversity has a great importance in disease dynamics and also in the success of disease management strategies including the development of cultivars resistant to diseases. Pathogenic variation plays a crucial role in resistance breakdown in cultivar[13]. This also aids the pathogen to evolve as a fungicide resistant one. Virulence diversity of *P. oryzae* populations were previously studied in different countries and different results had been found[14-16]. As wheat blast pathogen is a recent incidence in Bangladesh, an understanding on virulence potential of this pathogen is important. Furthermore, knowledge on the pathogenic nature of this pathogen can speed up the control process. Therefore, the present investigation was undertaken to evaluate the virulence nature of *P. oryzae* isolates from major wheat blast affected districts of Bangladesh.

Materials and Methods

Wheat blast infected leaves and panicles were collected from the four major wheat blast affected districts of Bangladesh *viz.,* Chuadanga, Meherpur, Kustia and Jhenaidaha during the tenure of 2017-18 wheat season. A total of 24 monoconidial isolates were obtained through single conidium isolation technique. All the isolates were maintained in PDA slants at 4°C. For long term storage, isolates were grown on sterile filter paper disks and kept at -80°C.

For virulence analysis, all the *Pyricularia oryzae* isolates were tested against two commonly cultivated wheat varieties (BARI Gom- 21 and BARI Gom- 24) in the study areas. Seed samples of these two wheat varieties were collected from Bangladesh Agriculrural Research Institute (BARI), Joydebpur, Gazipur, Bangladesh.

For inoculum preparation, stored cultures were allowed to grow on oat meal agar (OMA) medium (Oat meal 60 g, sucrose 2 g, agar 16 g and distilled water 1000 ml) at 25°C for two weeks. Pathogenicity test for all the isolates was performed following the method described by Hayashi *et al*. (2009)[17]. Disease scoring was done 7 to 8 days after inoculation. Before scoring, re-isolation of the pathogen from the lesions was done to fulfill the criteria of Koch's postulates.

Reaction types of selected wheat varieties against each isolate were recorded after 7 days of inoculation. Infection types were rated on a 0 - 5 disease assessment scale[16,18] where 0 = no evidence of infection; 1 = brown specks smaller than 0.5 mm in diameter, no sporulation; 2 = brown specks about 0.5 - 1.00 mm in diameter, no sporulation; 3 = roundish to elliptical lesions about 1 - 3 mm in diameter with gray center surrounded by brown margins, lesions capable of sporulation; 4 = typical spindle-shaped blast lesions capable of sporulation, 3 mm or longer with little or no coalescence of lesions; 5 = lesions as in 4 but about half of 1 - 2 leaf blades killed by coalescence of lesions. Reaction types 0 - 3 were considered as resistant while 4 and 5 were considered as susceptible.

Results and Discussion

All the isolates of *Pyricularia oryzae* were tested on BARI Gom- 21 and BARI Gom- 24 wheat varieties to assay their virulence potential. Data on pathogenicity test of the isolates are given in Table 1. No symptoms were observed on control leaves inoculated with sterile distilled water. Some disease reactions during pathogenicity test are shown in Fig. 1. The present study found evidence for diversity in virulence among *P. oryzae* isolates. Based on the disease reactions, isolates were grouped into 3 pathotypes (Table 2). From this analysis, Pathotype 1 was comprised of 56.14% of the total isolates which

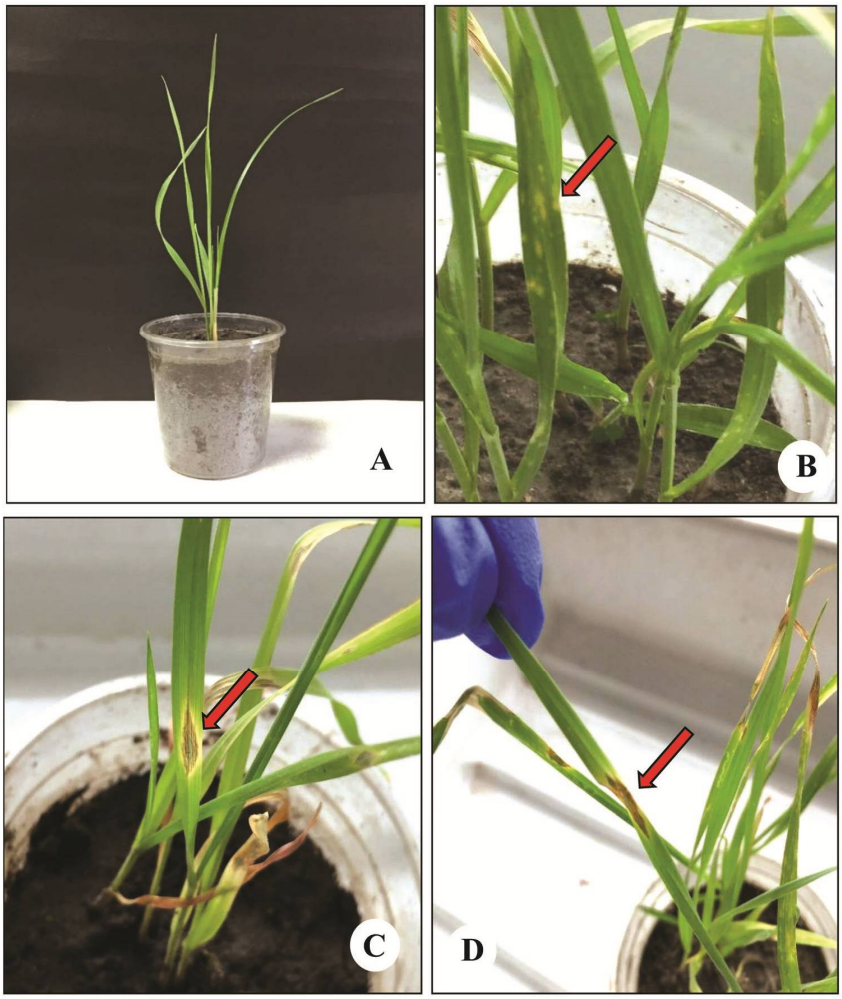

Fig. 1. Some photographs of pathogenicity test. A. Control plant showing no symptom. B. Lesion (Red arrow; Scale-2) produced by M15 isolate on BARI Gom- 24. C. Lesion (Red arrow; Scale-5) produced by C8 isolate on BARI Gom- 24. D. Lesion (Red arrow; Scale-5) produced by M20 isolate on BARI Gom- 21 wheat variety after 7 days of inoculation.

Table 1. The isolates and disease reactions of BARI Gom-21 (Shatabdi) and BARI Gom-24 (Prodip) wheat varieties against 24 *Pyricularia oryzae* isolates. (Mackill and Bonman 1992[18], Sharma *et al.* 2002[16])

Isolates	Origin	Variety of the host	Reaction types*	
			BARI Gom- 21 (Shatabdi)	BARI Gom- 24 (Prodip)
M1	Meherpur	BARI Gom- 24	S	R
M2	Meherpur	BARI Gom- 24	S	S
M3	Meherpur	BARI Gom- 24	S	S
M4	Meherpur	BARI Gom- 21	S	S
M5	Meherpur	BARI Gom- 21	R	R
C6	Chuadanga	BARI Gom- 24	S	R
C7	Chuadanga	BARI Gom- 24	R	S
C8	Chuadanga	BARI Gom- 24	S	S
C9	Chuadanga	BARI Gom- 24	S	S
C10	Chuadanga	BARI Gom- 24	S	S
K11	Kustia	BARI Gom- 26	R	R
K12	Kustia	BARI Gom- 26	S	R
K13	Kustia	BARI Gom- 24	S	S
K14	Kustia	BARI Gom- 24	R	R
M15	Meherpur	BARI Gom- 24	R	R
M16	Meherpur	BARI Gom- 24	S	S
J17	Jhenaidaha	BARI Gom- 24	R	S
J18	Jhenaidaha	BARI Gom- 24	S	S
M19	Meherpur	BARI Gom- 21	S	S
M20	Meherpur	BARI Gom- 21	S	S
M21	Meherpur	BARI Gom- 21	S	S
C22	Chuadanga	BARI Gom- 24	S	S
J23	Jhenaidaha	BARI Gom- 24	S	R
J24	Jhenaidaha	BARI Gom- 24	R	R

* [S = Susceptible, R = Resistant] Disease reaction types were based on 0–5 disease assessment scale

were virulent on both the wheat varieties and 25% of the isolates were virulent on any one of the two varieties and categorized as Pathotype 2 (Fig. 2). However, 20.84% of the isolates could not infect any of the two varieties and categorized as Pathotype 3 (Fig. 2). Present investigation revealed that the isolates with high virulence frequency were

prevalent in the studied *P. oryzae* population. Maciel *et al.* (2014)[5] also found similar result while studying population structure and pathotype diversity of the wheat blast pathogen in Brazil and reported that *P. oryzae* isolates with high virulence were the most common group in that population. However, Sharma *et al.* (2002)[17] found majority of the isolates tested were avirulent on all the differential lines in a study on pathotype analyses of *P. grisea* populations from the north-western Himalayan region of India.

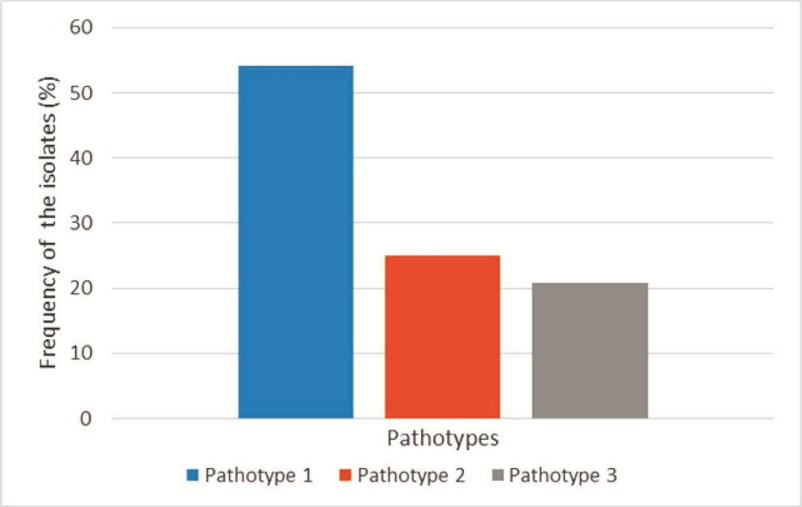

Fig. 2. Frequency of the *Pyricularia oryzae* isolates comprising the pathotypes.

Table 2. *Pyricularia oryzae* pathotypes obtained based on disease reactions.

Pathotype	Criteria	No. of isolates
1	Virulent on both the varieties tested (Highly virulent)	13 (M2, M3, M4, C8, C9, C10, K13, M16, J18, M19, M20, M21, C22)
2	Virulent on any one of the two variety tested (Moderately virulent)	6 (M1, C6, C7, K12, J17, J23)
3	Avirulent on both the varieties tested (Low virulent)	5 (M5, K11, K14, M15, J24)

This study also indicated that BARI Gom- 21 and BARI Gom- 24 wheat varieties were found to be vulnerable against wheat blast as most of the isolates could severely infect both the varieties. Furthermore, Pathotype 1 that was identified as the most virulent can be used as reference for screening resistant wheat varieties. Overall, knowledge generated in this study will be helpful in understanding pathogenic nature of *P. oryzae* isolates causing wheat blast in Bangladesh.

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