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Cross Infection between Rice and Wheat Blast Pathogen *Pyricularia oryzae*

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ABSTRACT

Cross infection between rice and wheat blast fungi was investigated in a series of experiments conducted under controlled glasshouse condition following a completely randomized design. Two rice (BRRI dhan29 and LTH) and two wheat (BARI Gom25 and BARI Gom26) varieties were grown in plastic trays as sole and rice-wheat mixed crop culture. Plants were artificially inoculated using virulent isolates of rice and wheat blast fungi. It was observed that irrespective of variety and crop culture technique, all the isolates of wheat blast fungus caused significant 100% plant infection on leaf typical leaf blast symptoms appeared on wheat seedlings but no blast symptom on rice. Conversely, the test-isolates of rice blast fungus did not produce any disease reaction on wheat seedlings, though leaf blast was observed on 100% rice plants. Therefore, we conclude that rice blast pathogen population is different from those of wheat blast pathogen (*Pyricularia oryzae*).

Keywords: Cross infection, rice, wheat blast, pathogen

INTRODUCTION

Magnaporthe oryzae (teleomorph) (Herbert) Barr (anamorph: *Pyricularia oryzae*) Couch and Kohn, 2002) is one of the most important plant pathogenic fungi having an exceptional capacity of rapidly changing its genetic make-up resulting in new pathogenic variants (races) (Dean *et al*., 2012; Khan *et al.,* 2016). It is the causal agent of rice blast, one of the most devastating diseases of rice (*Oryzae sativa* L.) observed in most of the rice growing-countries across the world (Kihoro *et al*., 2013). The pathogen can cause infection on leaves, stems, peduncles, panicles, seeds and even roots. This disease is the potential threat that may cause crop failure and yield loss. Thus it has been ranked among the most important rice diseases.

Blast is also a major disease of wheat (*Triticum aestivum* L.) in many South American countries (Goulart *et al*., 1992; Goulart and Paiva, 2000; Goulart *et al*., 2007; Kohli *et al*., 2011). However, on wheat, the disease is caused by *Magnaporthe oryzae* f. sp. *tritici*. The pathogen has the potential to infect all above-ground parts of wheat plant (Igarashi, 1991). However, while leaf infection, varietal characteristics may play important roles epidemiologically (Cruz *et al*., 2015). The most significant symptom of wheat blast in the field is the premature bleaching of spikelets (Igarashi, 1991; Urashima, 2010). In severe cases, the entire head is damaged. If head infection occurs early grain production can be critically lost. Spike infection caused by blast is often confused with Fusarium head blight of wheat. As the pathogen attacks the rachis, rather than attacking individual spikelets in case of head blight (Duveiller *et al*., 2012).

Wheat blast was first reported in 1985 in Paraná, Brazil (Igarashi *et al*., 1986) and has since spread throughout many wheat-producing areas of Brazil and to the neighboring countries such as Bolivia and Paraguay (Goulart *et al*., 2007; Kohli *et al*., 2011). Recently in 2016, in Bangladesh, wheat blast was reported for the first time in south-western districts such

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as Jessore, Kushtia, Chuadanga, Meherpur, Jhenaidah, Magura, Barisal and Bhola (The Daily Star, 2016). The disease caused severe yield loss in the affected areas. Blast infected field in Bangladesh Agricultural Development Corporation (BADC) seed producing farm was burnt even (Monsur *et al*, 2016).

Blast fungus is disseminated by air and seeds. Seed transmission is considered to be a feature that is significant for its dispersal by humans (Kato, 1994). Besides, there are several strains of *M. oryzae* which tend to display a degree of host specificity and they have been divided into pathotypes based on their host preference (Cruz *et al*., 2016). The strains that commonly occur on wheat in South America have been placed into the *Triticum* pathotype (MoT) which are genetically distinct from the strains of *Oryza* pathotype infecting rice. To date, the strains of *Oryza* pathotype is not known to cause disease on wheat and vice-versa (Prabhu *et al*., 1992; Orbach *et al*., 1996; Urashima *et al*., 1999; Farman, 2002; Maciel *et al*., 2014).

Wheat blast symptoms and its causal agent were not previously reported in Bangladesh. Therefore, there is a great deal of controversy how this disease invaded in Bangladesh. We considered three probable reasons might have influenced or caused wheat blast invasion in Bangladesh. Firstly, virulent strains of *M. oryzae tritici* might have been introduced from South America as a seed-transmitted pathogen escaping the quarantine regulations. Secondly, virulent strains of *M. oryzae tritici* have evolved from pre-existing avirulent strains in Bangladesh. Finally, strains of minor cereals blast fungus (*Pyricularia oryzae*) that is already diverse and widespread in Bangladesh might become pathogenic to wheat under changing climatic conditions. Most recently, the final hypothesis drawn significant attention when Tiedemann (2016) presented his experimental results in a conference held in Nepal showing that wheat blast pathogen infected rice plants in Germany. Therefore, the phenomenon of such cross infection of rice blast pathogen deserves detailed investigation in context of Bangladesh where rice blast fungus is present. Considering the above facts, this study was undertaken to investigate whether rice blast fungus can cause blast disease on wheat and *vice-versa*.

MATERIALS AND METHODS

Experimental site and period

A series of experiments were conducted in the laboratory and control facilities of Plant Pathology Division, Bangladesh Rice Research Institute (BRRI) during March-December 2016. Plants were exposed to relative humidity above 80% with constant temperature at 26°C ± 1.

Fungal isolates

Two rice blast isolates RBL332 (from BRRI dhan28) and RBL333 (from BRRI dhan64) were used in this experiment. Both the isolates were isolated from blast infected rice panicles collected from Gazipur, Bangladesh. Again, two wheat blast isolates WBL002 (from BARI Gom25) and WBL0011 (from BARI Gom26) were isolated from blast affected wheat head, which were collected from seed production farm of Bangladesh Agricultural Development Corporation (BADC) at Dottonagar in Jhinaedah district, Bangladesh.

Single conidia isolation and culture

Blast infected spikelets were sterilized by soaking with Clorox (5%) for 3-5 seconds and then in ethanol (70%) for 1 minute. Sterilized spikelets were gently rinsed with sterile water and dried samples with sterile tissue paper. Dried sterile spikelets were put on water soaked filter paper (Whatman International Ltd. Maidstone, England.) and incubated at 26°C for sporulation. Single spore were collected and cultured on prune agar medium.

Growing host plants

Two wheat varieties BARI Gom25 and BARI Gom26 and two blast susceptible rice varieties BRRI dhan29 and LTH (Lijiangxintuanheigu) were used in this study. LTH is recognized as universal blast susceptible rice variety. Apparently healthy and disease free seeds of rice and wheat were soaked on blotting paper in plastic Petridish and incubated at room temperature for 48 hours for germination. Germinated seeds were sown in a plastic tray (22 cm × 16 cm × 4 cm) containing sterile loamy soil. However, for first two trials, wheat and rice varieties (BARI Gom25, BARI Gom26, BRRI dhan29 and LTH) were planted as sole crops. For another two trials, wheat and rice were planted as mixed cropping (BARI Gom25 + BRRI dhan29 and BARI Gom26 + LTH). The plastic trays were then placed in a temperature controlled glass house operating at 26 ± 1°C. To ensure growth, the seedlings were watered daily and closely monitored for any insect infestation.

Experimental design

Experiments were laid out following completely randomized design (CRD) with three replicates in a glasshouse controlled temperature and relative humidity.

Inocula preparation and inoculation

Wheat blast isolates WBL002 and WBL0011 and rice blast isolate RBL332 and RBL333 were cultured separately on oat meal agar (1 liter distilled water, 50 g grinding oat powder, 20 g sugar, 20 g agar) for 17 days at 26°C. The surface of the oat meal agar media on Petri dish was gently rubbed with a sterile paint brush to remove aerial mycelia. The cultures were then exposed to fluorescent light for three days at 26°C for inducing sporulation (Fatemi and Nelson, 1978).

For foliar spray inoculation, conidia from surface of sporulated oat meal culture were scraped gently with sterile paint brush and suspended in sterile water containing 0.01% Tween 20. The suspension was filtered through two layer cheese cloth and conidial concentration was adjusted to 1×10^5 µL water. The conidial suspension was sprayed using a hand sprayer on 21-day-old wheat and rice seedlings. Inoculated seedlings were incubated in a humid chamber at 25°C temperature for 24 hrs. Then the seedlings were transferred into a temperature controlled glass house operating at 28± 1°C.

Data collection and analysis

A week after inoculation, data were collected on plant infection (%), leaf infection (%), number of lesion per leaf, lesion size (mm^2) and disease severity scale. Size of elliptical leaf blast lesion was calculated from lesion length and width using the following formula: Lesion size = 3.14 × (Lesion length/2) × (Lesion width/2). Disease severity scale was measured by using Standard Evaluation System (SES, IRRI, 2014). Data were average of 20 plants. Data were analyzed using a statistical software package Minitab version 16 (Tukeys test).

RESULTS

Pathogenicity of wheat isolates on wheat and rice plants (single crop culture)

Wheat blast isolates WBL002 and WBL0011 developed typical blast lesions on leaves of the wheat varieties BARI Gom25 (Fig. 1) and BARI Gom26. Both of the isolates caused the highest level (100%) of plant and leaf infection on both wheat varieties used in this study (Table 1). Disease severity was also high (8.8 in 9 scale on BARI Gom25 with WBL002 and 8.0 in 9 scale on BARI Gom26 with WBL0011) for wheat blast isolates. However, the test-isolates varied to some extent in terms of the average number of lesion per leaf. On average, the isolate WBL002 developed 13.70 blast lesions per leaf on BARI Gom26 whereas it was 15.70 by WBL0011 isolate. Lesion size recorded with both of the wheat blast isolates were almost identical (~12 mm^2 on BARI Gom25 and ~10 mm^2 on BARI Gom26). On the other hand, the wheat blast isolates WBL002 and WBL0011 did not develop any blast symptom (Plant infection=0, Leaf infection =0, No. of lesion per leaf=0, Lesion size=0 and Disease score=0) on the rice variety BRRI dhan29 (Table 1).

Table 1. Effect of wheat isolates on wheat and rice single cropping.

Isolate	Parameter	Wheat		Rice	
		BARI Gom25	BARI Gom26	BRRI dhan29	LTH
WBL002	% plant infection	100 a	100 a	0.00 b	0.00 b
	% leaf infection	100 a	83.33 b	0.00 c	0.00 c
	No. of lesion/leaf	13.70 a	10.55 b	0.00 c	0.00 c
	Lesion size (mm²)	12.05 a	10.85 a	0.00 b	0.00 b
	Disease severity	8.80 a	7.65 b	0.00 c	0.00 c
WBL0011	% plant infection	100 a	100 a	0.00 b	0.00 b
	% leaf infection	100 a	88.33 b	0.00 c	0.00 c
	No. of lesion/leaf	15.70 a	16.15 a	0.00 b	0.00 b
	Lesion size (mm²)	12.47 a	10.66 a	0.00 b	0.00 b
	Disease severity	7.95 a	8.00 a	0.00 b	0.00 b

*In a row, means followed by the same letter are not significantly different at the 5% level.

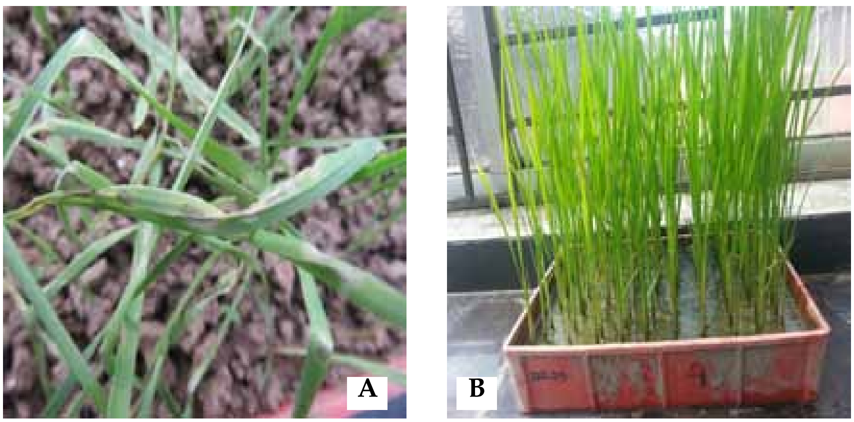

Fig. 1. Blast infected wheat leaves and disease free rice A. Blast symptom on wheat (BARI Gom25) leaves B. No blast symptom on rice (BRRI dhan29) leaves.

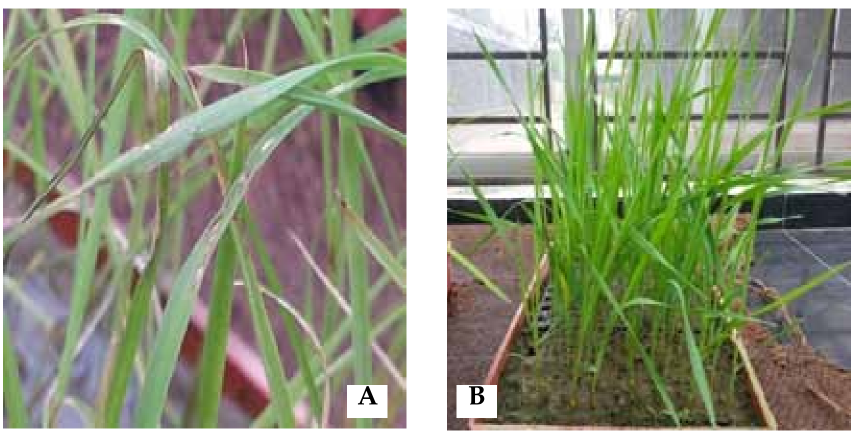

Fig. 2. Blast symptom on rice leaves and disease free wheat plants A. Blast symptom on rice (LTH) leaves B. No blast symptom on wheat (BARI Gom26) leaves.

Pathogenicity of rice blast isolates on rice and wheat plants (single crop culture)

Both of the rice blast isolates RBL332 and RBL333 developed typical leaf blast symptoms on rice varieties BRRI dhan29 and LTH (Fig. 2) and also caused maximum (100%) plant infection (Table 2). However, the isolate RBL332 produced significantly higher degree of leaf infection (83.33%) in a typical blast susceptible rice variety LTH compared to BRRI dhan29 (52.50%). On the other hand, the isolate RBL333 caused significantly higher degree of leaf infection (74.17%) in BRRI dhan29 compared to LTH (52.50%). Average lesion number per leaf was 4.65 with RBL332 and 5.25 with RBL333 (Table 2). However, the lesion size was almost similar (~4.00 mm^2) with both the isolates. The highest disease severity index was for the isolate RBL332 was 7.50 in the scale of 9 and that for RBL333 was 6.20 (Table 2).

Pathogenicity of wheat blast isolates in rice-wheat mixed crop culture

In rice-wheat mixed crop culture, the wheat blast isolates WBL002 and WBL0011 caused severe blast infection on both the wheat varieties BARI Gom25 and BARI Gom26 only (Fig. 3), which was similar to that observed in case of single cropping. Both of the isolates caused the highest level (100%) of wheat plant infection (Table 3). Leaf infection was also high (97.50-100%). Average numbers of leaf blast lesions were almost identical (~13.00) for both of the wheat blast isolates. However, larger (10.94 mm^2) lesion was observed with WBL0011 and compared to the fungal isolate WBL002 (7.79 mm^2). Disease severity index were recorded as 7.85 and 8.60 in the scale of 9 with the isolates WBL002 and WBL0011, respectively. Wheat isolate WBL0011 showed virulent reaction when compared with WBL002 isolate in case of percent leaf infection 100 and 84.17 in BARI Gom25 and BARI Gom26, respectively. However, wheat isolate WBL0011 produced the highest number of lesion per leaf 18.55 in BARI Gom26 and bigger lesion size (10.55 mm^2) was found in BARI Gom25. In contrast, the wheat isolates WBL002 and WBL0011 did not develop any blast symptom on the blast susceptible rice varieties BRRI dhan29 and LTH.

Pathogenicity of rice blast isolates in rice-wheat mixed crop culture

The rice blast isolates RBL332 and RBL333 caused severe rice leaf blast symptom on BRRI dhan29 and LTH but no disease reaction on BARI Gom25 and BARI Gom26 (Fig. 4). They caused 100 percent plant infection in BRRI dhan29 and LTH (Table 4). Number of lesions per leaf was 3.95 in BRRI dhan29 and 5.10 in LTH with RBL332 isolate. Lesion number and

Table 2. Effect of rice isolates on wheat and rice varieties with single cropping method.

Isolate	Parameter	Wheat		Rice	
		BARI Gom25	BARI Gom26	BRRI dhan29	LTH
RBL332	% plant infection	0.00 b	0.00 b	100 a	100 a
	% leaf infection	0.00 c	0.00 c	52.50 b	83.33 a
	No. of lesion/ leaf	0.00 b	0.00 b	4.65 a	4.00 a
	Lesion size (mm^2)	0.00 b	0.00 b	4.00 a	3.38 a
	Disease severity	0.00 c	0.00 c	7.50 a	6.20 b
RBL333	% plant infection	0.00 b	0.00 b	100 a	100 a
	% leaf infection	0.00 c	0.00 c	74.17 a	52.50 b
	No. of lesion/leaf	0.00 b	0.00 b	5.25 a	4.00 a
	Lesion size (mm^2)	0.00 b	0.00 b	3.98 a	4.03 a
	Disease severity	0.00 b	0.00 b	5.65 a	6.20 a

*In a row, means followed by the same letter are not significantly different at the 5% level.

Table 3. Effect of wheat isolates on rice wheat mixed cropping.

Isolate	Parameter	Rice + wheat		Rice + wheat	
		BARI Gom25 (Wheat)	BRRI dhan29 (Rice)	BARI Gom26	LTH (Rice)
WBL002	% plant infection	100 a	0.00 b	100 a	0.00 b
	% leaf infection	97.50 a	0.00 c	84.17 b	0.00 c
	No. of lesion/leaf	13.45 a	0.00 b	14.70 a	0.00 b
	Lesion size (mm^2)	7.79 b	0.00 c	10.83 a	0.00 c
	Disease severity	7.85 a	0.00 b	8.00 a	0.00 b
WBL0011	% plant infection	100 a	0.00 b	100 a	0.00 b
	% leaf infection	100 a	0.00 b	89.17 b	0.00 b
	No. of lesion/leaf	13.30 b	0.00 c	18.55 a	0.00 c
	Lesion size (mm^2)	10.94 a	0.00 b	9.98 a	0.00 b
	Disease severity	8.6 a	0.00 b	8.00 a	0.00 b

*In a row, means followed by the same letter are not significantly different at the 5% level.

Table 4. Effect of rice isolates on rice-wheat mixed cropping.

Isolate	Parameter	Rice + wheat		Rice + wheat	
		BARI Gom25 (Wheat)	BRRI dhan29 (Rice)	BARI Gom26 (Wheat)	LTH (Rice)
RBL332	% plant infection	0.00 b	100 a	0.00 b	100 a
	% leaf infection	0.00 b	77.50 a	0.00 b	84.17 a
	No. of lesion/leaf	0.00 b	3.95 b	0.00 b	5.10 a
	Lesion size (mm^2)	0.00 b	2.30 b	0.00 b	5.69 a
	Disease severity	0.00 b	6.2 a	0.00 b	5.10 b
RBL333	% plant infection	0.00 b	100 a	0.00 b	100 a
	% leaf infection	0.00 b	80.83 a	0.00 b	89.25 a
	No. of lesion/leaf	0.00 c	4.65 b	0.00 c	7.55 a
	Lesion size (mm^2)	0.00 c	2.92 b	0.00 c	5.28 a
	Disease severity	0.00 b	6.20 a	0.00 b	7.4 a

*In a row, means followed by the same letter are not significantly different at the 5% level.

Fig. 3. Infected wheat leaves (red arrow) and healthy rice leaves (blue arrow) in mixed rice and wheat cropping method.

Fig. 4. Infected rice leaves (red arrow) and healthy wheat leaves (blue arrow) in mixed cropping method.

lesion size were higher in both the isolates in LTH rice variety. Lesion number per leaf was 5.10 for RBL332 isolate and 7.55 for RBL333 isolate in LTH (Table 4). In case of RBL333 isolate, lesion size was 2.92 (mm^2) and 5.28 (mm^2) respectably for in BRRI dhan29 and LTH. Disease severity scale was 5.10 with RBL332 and 7.4 with RBL333 isolate in LTH.

DISCUSSION

Rice blast is one of the most devastating diseases of rice in Bangladesh and throughout rice growing areas of the world. It threatens the stability of rice production worldwide. Plant pathogenic fungi have been suggested or demonstrated to have strategies for generating genetic variations, which enable them to adapt on resistant host by gaining pathogenicity to new hosts (Oliver, 2012). *Pyricularia* spp. possesses higher genetic changing ability and host diversification, for this reason degree of yield loss level is much more than other fungal pathogen. Normally blast disease found in rice but this year in March, 2016 blast disease were found in wheat in north-western part of Bangladesh. Wheat blast was first found in South America (Kohli *et al*., 2011).

In glasshouse experiments, variations on expression of blast disease symptoms were found in wheat and rice seedlings by artificial inoculation. It was found that wheat blast pathogen developed blast symptoms on wheat leaves, stem and junction of leaf blade and leaf sheath, but did not develop any blast symptom on any part of rice plants. Similarly, rice blast fungus developed blast symptoms only on rice leaves but not produced any disease symptom in any part of wheat plants. Couch *et al*., 2005 and Tredway *et al*., 2005 claimed that fungal isolates collected in nature belong to the pathotype specialized for the host species from which they were collected, indicating that cross infection is rare in nature.

Although host specific blast symptoms were observed with wheat and rice blast fungi, disease severity were higher in wheat seedlings when compared with rice seedlings. All parameters such as percent plant infection, percent leaf infection, lesion number per leaf, lesion size and disease score were higher in wheat seedlings comparatively to rice. Chromosomal constituent of wheat and rice blast pathogen bears some difference, so they do not cause compatible reaction on wheat and rice plants. Farman (2002), Tosa *et al*., 2004 and Viji *et al*., 2001 concluded that rice isolates differed from wheat isolates in containing a distinct set of transposable elements, but wheat and ryegrass isolates share the same set of transposable elements and are extremely closely related. Klaubauf *et al*. (2014) suggested that wheat blast pathogen and rice blast pathogen may represent distinct species. Paulo *et al.* (2015) have sequenced 10 genes in 121 *Pyricularia* spp. strains showing divergence between strains infecting wheat (97 strains) and rice (24 strains) and concluded that wheat blast isolates do not infect rice, but they all infect barley. In a project report of Kansus State University B. Valent (2016) claimed that *P. grisea*, *P. oryzae Tritici* and *P. oryzae Lolium* isolates are closely related and relatively distant from *P. oryzae* populations. They also found that *P. oryzae Tritici* isolates show a surprising degree of SNP (Single Nucleotide Polymorphism) ability relative to other blast pathogens.

CONCLUSION

Wheat blast disease was observed in the South-western Part of Bangladesh in 2016. This is also the first report of the disease in Asia. In this study, it was investigated whether the rice blast fungus could cause wheat blast symptoms and *vice-versa*. The study found that the isolates of rice blast fungus produced blast symptoms only on rice plant but not on wheat under both single and rice-wheat mixed crop culture. Similarly, the wheat blast fungus developed typical blast symptoms only on wheat varieties but not on rice. These preliminary findings discard the hypothesis of cross infection between rice and wheat blast fungi and also indicate the potential introduction or evolution of wheat blast fungus in Bangladesh.

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