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Oryzae pathotype of *Magnaporthe oryzae* can cause typical blast disease symptoms on both leaves and spikes of wheat under a growth room condition

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Abstract

Blast diseases of rice and wheat are known to be caused by the specific pathotypes of *Magnaporthe oryzae* (syn. *Pyricularia oryzae*), *M. oryzae Oryzae* (*MoO*) and *M. oryzae Triticum* (*MoT*), respectively. Rice blast disease has been seen in Bangladesh from a very ancient time. However, Bangladesh's first epidemic outbreak of wheat blast was recorded in 2016. This study aimed to investigate the cross-infection reactions of *MoO* and *MoT* in rice and wheat in a growth room condition. Artificial inoculation was done at vegetative and reproductive phases of both wheat and rice plants in a completely randomized design using virulent isolates of *MoO* and *MoT*. Artificial inoculation with *MoO* resulted in foliar symptoms with typical eye-shaped lesions as well as partially bleached or completely white head symptoms in both wheat and rice plants. On the other hand, *MoT* produced blast symptoms only on the leaves and spikes of wheat. Molecular analyses using PCR amplification (with Pot2, MoT3 and MoT6099 primers) and a recently developed rapid detection PCRD strip confirmed the presence of *MoT* and *MoO* pathotypes in the symptomatic plant samples. Our results demonstrated that *MoO* pathotype can infect the leaves and spikes of wheat but *MoT* is unable to infect rice plants under the same controlled environment in Bangladesh. This study has revealed the vulnerability of wheat to *MoO* pathotype and an urgent need to understand the molecular mechanism underlying host-specificity of the blast fungus *M. oryzae*. Our results also provided evidence for a potential wheat blast epidemic by *MoO* in many rice–wheat inter-cropping regions as climate change intensifies. A comprehensive study is needed to have a better understanding on the variability in virulence of *MoO* and *MoT* isolates in infecting wheat and rice under controlled environment by the inclusion of a large number of isolates and crop varieties/genotypes.

Keywords: Cross inoculation, Host-specificity, Climate change, Blast fungus, *Magnaporthe oryzae Oryzae*, *M. oryzae Triticum*

Background

Wheat is a staple source of nutrients for around 40% of the world's population. Globally, wheat is considered as a widely grown crop providing 20–25% of daily protein and food calories (Curtis 2022). In Bangladesh, wheat is the second largest food crop after rice, which plays a vital role in feeding ca. 170 million people of this developing country. The consumption of wheat in this high-densely

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populated country is increasing gradually but there is a big gap between annual consumption and production (Islam et al. 2019). The yield and acreage of wheat were increasing steadily in Bangladesh before the first epidemic outbreak of wheat blast in 2016. The outbreak damaged approximately 15,000 hectares of wheat-cultivated area in eight districts with yield losses estimated up to 100%. Due to the panic, the infected wheat fields were burnt to kill the fungus, which decreased 15% of total wheat production in the country (Islam et al. 2016). Using field pathogenomics, open data sharing and open science approaches, the origin of wheat blast fungus in Bangladesh was determined as a lineage of South American *Magnaporthe oryzae* (Islam et al. 2016; Islam 2018; Islam and Kamoun 2018; Kamoun et al. 2019). The fungal pathogen *M. oryzae Triticum* (*MoT*) was likely to be introduced into Bangladesh through Brazilian grain import (Islam 2018; Ceresini et al 2018). Since its first emergence in the Paraná state of Brazil, wheat blast has been restricted to some South American countries, including Brazil, Argentina, Bolivia and Paraguay. However, this destructive wheat killer disease was recently introduced in an African country, Zambia (Tembo et al. 2020). Recent outbreaks have evidenced the prediction that wheat blast can be spread to other wheat-growing countries in Asia and Africa due to similar climatic conditions (CIMMYT 2016). Thus, wheat blast poses a serious threat to global food security (Islam 2018; Islam et al. 2020).

The filamentous fungus *M. oryzae* infects more than 50 species of Gramineae plants including the major food crops rice, wheat, maize, pearl millet and finger millet (Pordel et al. 2021). However, this fungus has many pathotypes for specific hosts. For example, rice and wheat blast diseases are caused by *M. oryzae Oryzae* (*MoO*) and *M. oryzae Triticum* (*MoT*) pathotypes, respectively (Gladieux et al. 2018). It is believed that the lack of cross infection by *MoT* and *MoO* is due to the fact that the adapted strains on one host lose their pathogenicity on the other host in the field conditions. The underlying molecular mechanisms regulating the host specificity of the pathotypes of *M. oryzae* are poorly understood (Gladieux et al. 2018). Wheat blast pathogen *MoT* usually attacks the base or upper part of the rachis to disturb spike formation or make the spike partially/completely bleached, resulting in wrinkled seeds or no grain (Islam et al. 2016, 2019, 2020; Surovy et al. 2020; Gupta et al. 2021). Monsur and his co-researchers investigated whether rice blast fungus can cause blast disease symptoms on wheat and *vice-versa* at the seedling stages of plants. They concluded that rice-infecting blast fungus (*MoO*) did not produce any characteristic symptoms on wheat plants by artificial inoculation (Monsur et al. 2016). However, a recent study demonstrated that some strains of *MoO* pathotype in China can infect wheat under certain environmental conditions (Wang et al. 2021). This prompted us to conduct an investigation in the context of Bangladesh where rice and wheat are cultivated in the same season side by side. In fact, the rice blast fungus has been a threat to rice cultivation in Bangladesh since the 1980s (Shahjahan 1994). This study aims to investigate the cross-infection reactions of *MoO* and *MoT* on rice and wheat under growth room conditions. The specific objectives of this study were to (1) assess the pathogenicity of *MoO* isolates on rice and wheat; (2) evaluate the pathogenicity of *MoT* isolates on wheat and rice; and (3) confirm the presence of a specific pathotype of *M. oryzae* in the infected plant samples by pathotype-specific primers, and also by PCRD strip (a rapid detection of wheat blast). Interestingly, we observed that artificial inoculation of wheat with *MoO* isolates resulted in typical wheat blast symptoms but the *MoT* isolates were unable to infect rice. This study provides evidence for a potential wheat blast epidemic by *MoO* to take place in many rice–wheat inter-cropping regions as the effect of climate change intensifies.

Results

Artificial inoculation with *MoO* strains causes blast symptoms in both wheat and rice

To investigate whether strains of *MoO* cause typical symptoms on both leaves and spikes of wheat and rice, we inoculated wheat and rice plants by spraying conidia of *MoO*. Artificial inoculation of wheat with three *MoO* isolates, RB13b, RBTa1849-2 and RBMe1819-3, resulted in typical leaf blast symptoms on the wheat variety BARI Gom-25 (Fig. 1a). All the isolates of *MoO* displayed similar results, and hence we presented the data representative of two different isolates.

The artificially inoculated wheat plants had partial or complete bleached spikes with dark gray to black-colored infection points on the rachis (Fig. 1c, e). Spikes infected at the flowering stage yielded no grains or had shriveled or distorted grains with very low test-weight (Table 1). Inoculation of wheat seedlings with *MoO* isolates resulted in typical blast symptoms on wheat leaves. The symptoms were elliptical or eye-shaped lesions with gray centers and dark brown margins on the leaves of *MoO*-inoculated wheat seedlings (Fig. 1a), and also on the flag leaves of the adult plants (Fig. 1c, e). The sizes and appearance of the developed lesions by two *MoO* strains were almost similar.

Meanwhile, we inoculated rice plants using three *MoO* strains, RB13b, RBTa1849-2 and RBMe1819-3, at both seedling and panicle stages maintained under controlled environmental conditions (28 ± 1 °C and minimum 80%

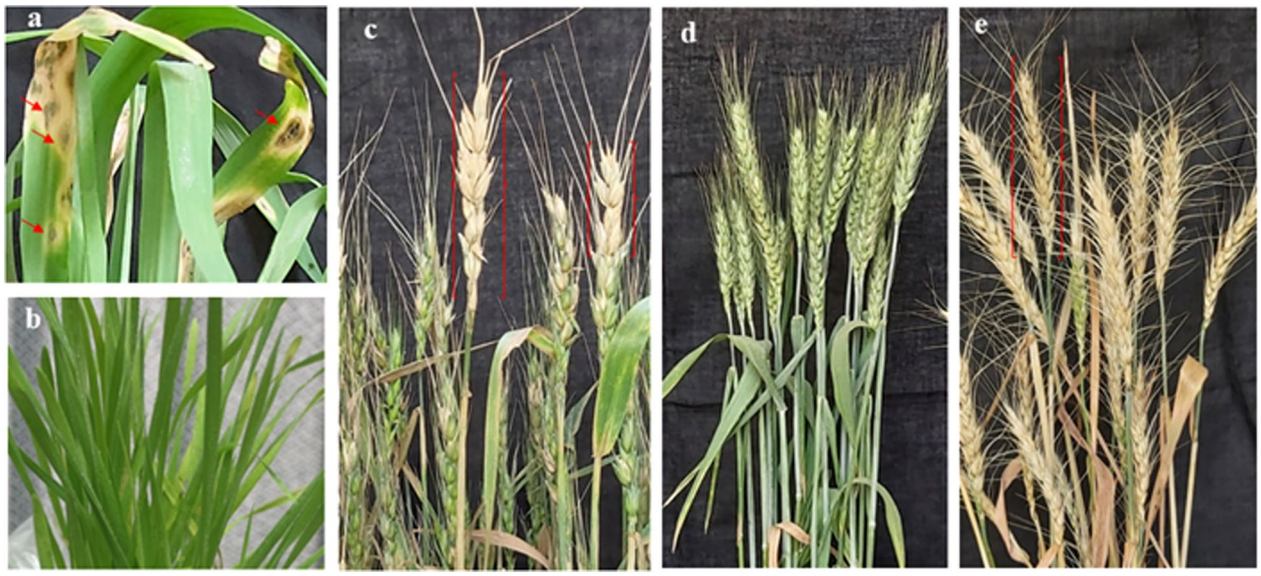

Fig. 1 Head and leaf blast symptoms developed in wheat leaves and spikes after artificial inoculation of plants with conidia of *MoO* strains RB13b and RBTa1849-2. **a** Typical eye-shaped lesions with gray center (arrow) on leaves of wheat seedling. **b** Mock-inoculated control wheat leaves showed no blast disease symptoms. **c**, **e** Fully and partially bleached wheat cv. BARI Gom-25 spikes inoculated with RB13b (**c**) and RBTa1849-2 (**e**). **d** No disease symptom appeared on the mock-inoculated wheat spikes in the control plants

Table 1 Yield or yield components of the wheat variety BARI Gom-25 under growth room condition after artificial inoculation with rice or wheat blast fungus

Treatment	Grain yield per hill (gm)*	1000-grain weight (gm)*	Disease severity (%)*
Healthy control	48.33 ± 4.06a	53.33 ± 1.45a	0b
RB13b (*MoO*)	10.33 ± 2.60b	30.00 ± 4.16b	77.00 ± 2.65a
RBTa1849-2 (*MoO*)	15.00 ± 3.06b	36.33 ± 1.20b	74.67 ± 5.36a
BTJP4-5 (*MoT*)	9.67 ± 1.20b	24.33 ± 2.60b	86.67 ± 2.96a
BTMaU(10b) (*MoT*)	8.33 ± 1.45b	24.67 ± 3.18b	86.33 ± 4.81a

*Any two means having a common letter are not significantly different at the 5% level of significance

relative humidity) (Fig. 2). All three rice blast strains developed typical leaf blast symptoms on rice variety BRRI dhan63 (Fig. 2a). The infected plants had partially bleached panicles with dark gray to black-colored infection points on the rachis (Fig. 2c–e). Some of the plants had completely bleached panicles. Panicles infected at the flowering stage resulted in no grains, or grains that were withered, distorted, and had a very low test-weight (Table 2). The characteristic blast symptoms on the leaves were elliptical or eye-shaped lesions with gray centers and dark-brown margins at the seedling stage and the similar symptoms on lower and flag leaves of the adult plants (Fig. 2a, c, d). The lesion sizes developed in leaves by the *MoO* strains were almost alike.

Artificial inoculation with *MoT* strains results in blast symptoms in wheat but not in rice

Inoculation of wheat plants with *MoT* strains, BTJP4-5, BTMaU(10b) and BTMP1845-3, developed typical leaf blast symptoms on the wheat variety BARI Gom-25 (Fig. 3a). The infected plants had partially bleached spikes with dark gray to black-colored infection points on the rachis (Fig. 3c, d). Some of the plants showed completely bleached spikes (Fig. 3c middle image). Spikes inoculated at the flowering stage yielded no grains, or shriveled or distorted grains that had a very low test-weight. However, inoculation at the seedling stage of wheat resulted in characteristic blast symptoms which include elliptical or eye-shaped lesions with gray centers and dark-brown margins on the leaves of wheat seedlings. The symptoms developed in wheat by the three *MoT* strains were almost similar.

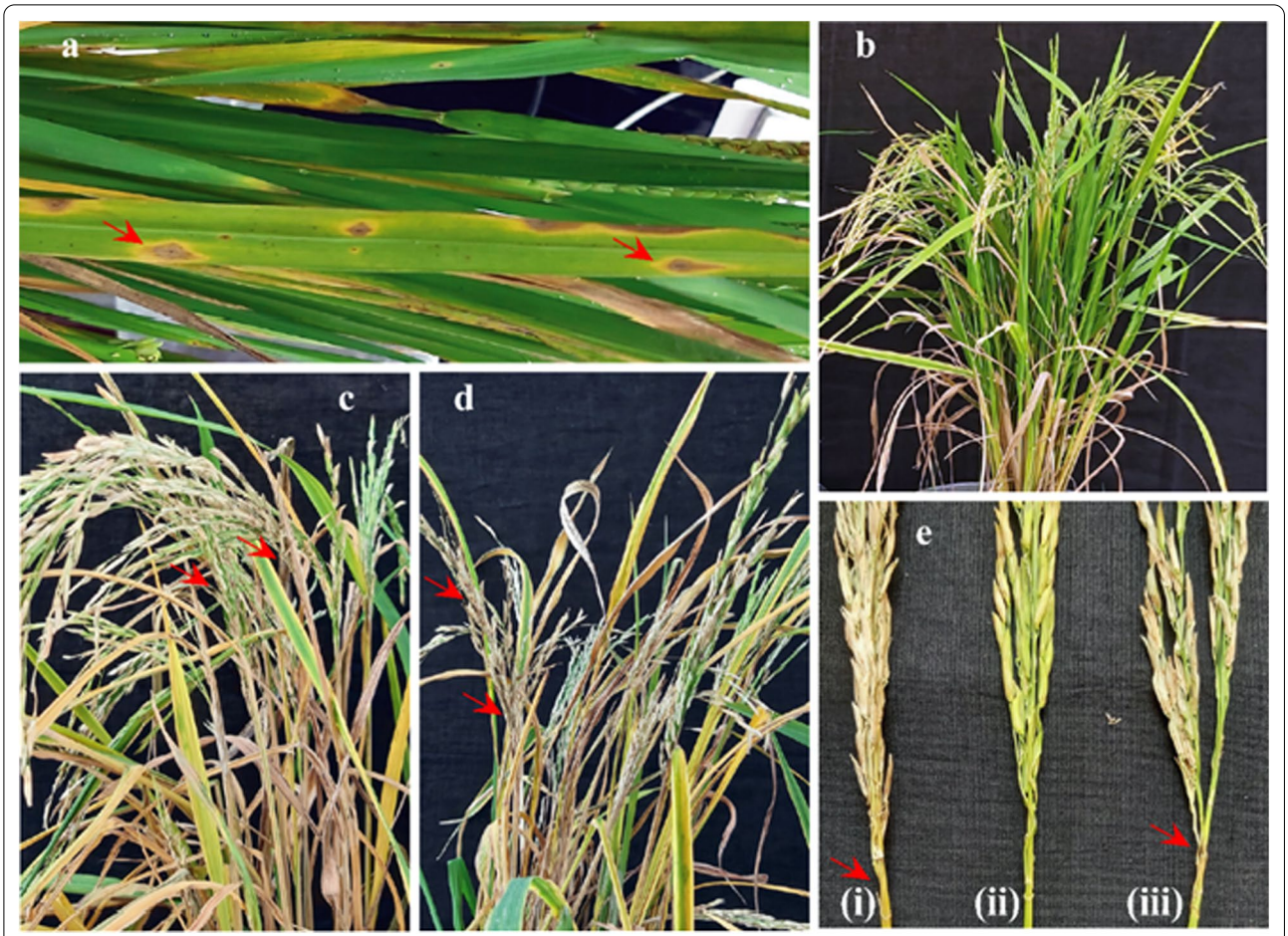

Fig. 2 Neck and leaf blast symptoms developed in rice cv. BRRI Dhan63 leaves and panicles after artificial inoculation of plants with conidia of *MoO* strains RB13b and RBTa1849-2. **a** Typical eye-shaped lesions with gray center (arrows) on the leaf of rice seedling. **b** Mock-inoculated control plants showed no blast disease symptoms on leaves and panicles. **c**, **d** Partially bleached rice panicles by *MoO* strains RB13b (**c**) and RBTa1849-2 (**d**). **e** Black-pigmented infection appeared on panicle of rice plant inoculated with *MoO* strains, RB13b (i) and RBTa1849-2 (iii), whereas middle panicle collected from mock-inoculated control plant (ii) had no sign of infection

Table 2 Yield or yield components of the rice variety BRRI Dhan63 under growth room condition after artificial inoculation with rice blast fungus

Treatment	Grain yield per hill (gm)*	1000-grain weight (gm)*	Disease severity (%)*
Healthy control	51.00 ± 6.08a	20.67 ± 0.67a	0b
RB13b	13.67 ± 2.40b	16.33 ± 0.88b	86.33 ± 2.60a
RBTa1849-2	14.67 ± 2.33b	17.67 ± 0.88ab	75.33 ± 4.41a

*Any two means having a common letter are not significantly different at the 5% level of significance

To see whether *MoT* isolates can infect rice plants simultaneously under the controlled growth room conditions, we inoculated rice plants cv. BRRI dhan63 with three virulent *MoT* strains viz., BTJP4-5, BTMaU(10b) and BTMP1845-3. Herein, no blast disease symptoms

Fig. 3 Head and leaf blast symptoms developed in wheat leaves and spikes after artificial inoculation of plants with conidia of *MoT* strains BTJP4-5 and BTMaU(10b). **a** Typical eye-shaped lesions with gray center (arrows) on the leaves of wheat seedling. **b** Mock-inoculated control wheat leaves showed no blast disease symptoms. **c**, **d** The middle spikes in **c** and **d** were completely bleached when inoculated with *MoT* strains, BTJP4-5 and BTMaU(10b), respectively, whereas mock-inoculated control (the right and left spikes) were free from blast symptom

were developed in leaves and spikes of rice plants by artificial inoculation with the strains of *MoT* (Fig. 4).

Yield or yield components of wheat and rice after artificial inoculation with rice blast fungus

The wheat variety BARI Gom-25 was severely affected by all the three *MoO* isolates (RB13b, RBTa1849-2 and RBMe1819-3). Artificial inoculation with RB13b and RBTa1849-2 resulted in 77.00% and 74.67% of disease severity (DS) in BARI Gom-25, respectively, significantly higher than that (0% DS) in mock-inoculated control (Table 1). Moreover, 1000-grain weight and grain yield per hill were also significantly ($P \leq 0.05$) reduced after inoculation with these *MoO* isolates (Table 1).

We also inoculated the rice variety BRRI Dhan63 with the same *MoO* isolates viz., RB13b, RBTa1849-2 and RBMe1819-3. Herein, 86.33% and 75.33% of DS were observed for rice plants artificially inoculated with RB13b and RBTa1849-2, respectively (Table 2). In the case of healthy control, the disease severity (DS) was 0%. Additionally, a significant reduction in 1000-grain weight and grain yield per hill was observed in BRRI Dhan63 infected by the *MoO* isolates (Table 2).

Features of wheat-infecting rice blast fungus

We used three isolates of *MoO*, RB13b, RBTa1849-2 and RBMe1819-3, collected from naturally infected rice field for artificial inoculation of wheat in the growth room. After the development of typical blast symptoms, we reisolated and obtained wheat-infecting *MoO* isolates. Isolates were grown on PDA culture media to study growth characteristics. All of the isolates exhibited almost identical cultural features of the parent isolates used for the artificial inoculation. Moreover, similar characteristics of conidia were found under microscope (Additional file 1: Figure S1).

Confirmation of the presence of *M. oryzae* pathotype in infected tissues of rice and wheat by specific molecular markers

We reisolated the fungus from the symptomatic tissues of artificially inoculated rice and wheat plants. To confirm their genetic identity, we used a general primer Pot2 (Fig. 5a) which amplifies any pathotypes (*MoO* or *MoT*) of *M. oryzae*, and primers MoT3 and MoT6099 that specifically amplify only *MoT* pathotype of *M. oryzae* fungus (Fig. 5). Reasonably, the Pot2 primer clearly amplified both *MoO* and *MoT* pathotypes (Fig. 5a). On the other

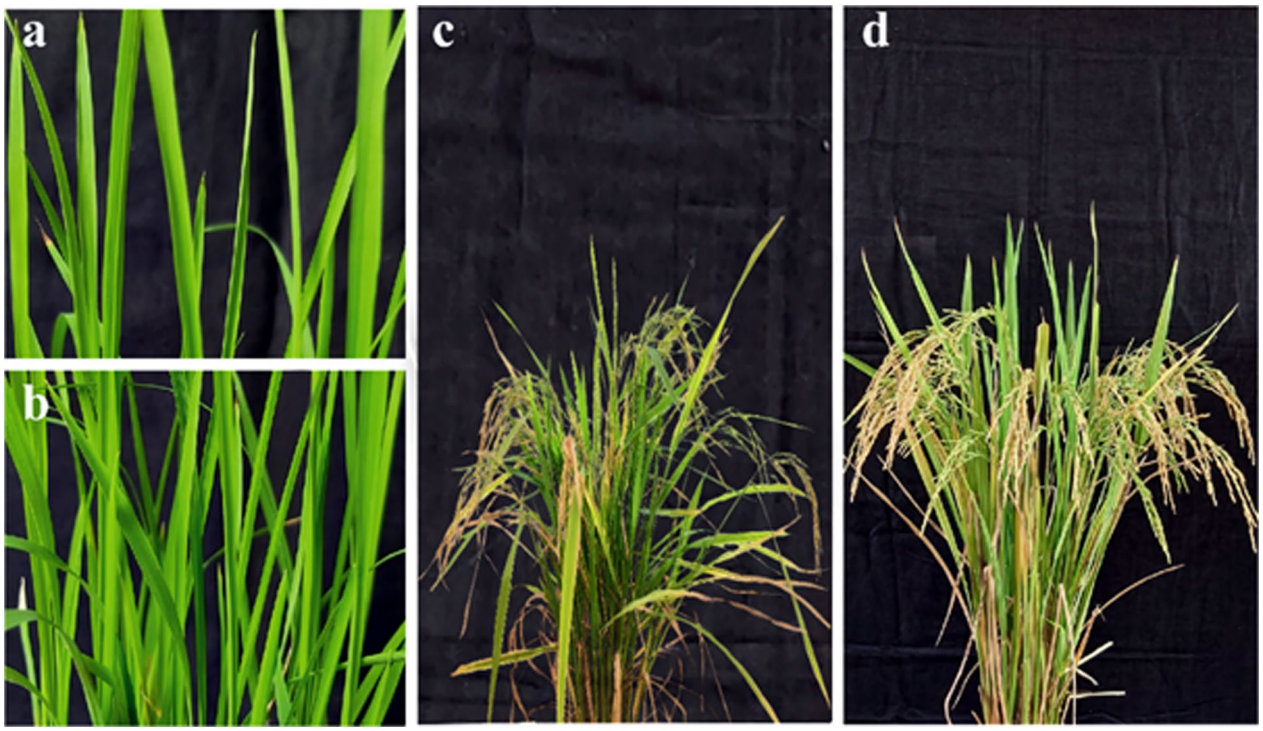

Fig. 4 Inoculation of rice cv. BRRI dhan63 with two *MoT* strains viz. BTJP4-5 and BTMaU(10b) developed no blast disease symptoms on the leaves and panicles. **a**, **c** No blast disease symptoms in rice leaves and panicles artificially inoculated by the conidia of *MoT* strain BTJP4-5. **b**, **d** No blast disease symptoms in artificially inoculated rice plants with *MoT* strain BTMaU(10b)

hand, both MoT3 and MoT6099 amplified only *MoT* pathotype but not *MoO* (Fig. 5b, c).

Rapid detection of *MoT* using PCRD strip

We also used our recently developed PCRD strip method for the rapid detection of *MoT*, which integrated the Cas12a protein with RPA as well as NALFIA technology for the detection of *MoT* in the symptomatic plants (Kang et al. 2020). The NALFIA detection was carried out by loading the reaction volume onto the PCRD strips. The results displayed that the ssDNA band (the second band from top) was obvious in two *MoO* samples and in the reisolated *MoO* samples that were collected from the symptomatic plant tissues of wheat plants artificially inoculated with *MoO*. However, no ssDNA band was shown in the two *MoT* samples (Fig. 6).

Discussion

M. oryzae, a hemibiotrophic filamentous fungal pathogen, infects multiple grasses and cereals including three staple food crops, namely rice, wheat and maize. The existence of rice blast was reported nearly three centuries ago in China and Japan and is now found in over 85 rice-growing countries (Talbot 2003). Several host-specific pathotypes of *M. oryzae* have already been described, among which *MoO* infects rice and *MoT* mainly infects wheat (Gladieux et al. 2018). However, recently, it was demonstrated that some strains of *MoO* cause blast disease symptoms in wheat via characteristic appressorium-mediated infection processes at both seedling and heading stages of plants under certain environmental conditions (Wang et al. 2021). The researchers concluded that the strain of *MoO* and also temperature are critical factors for successful infection of wheat by *MoO* pathogen. In the current study, we demonstrated that artificial inoculation of wheat plants with some *MoO* strains in Bangladesh led to blast symptoms in both leaves and spikes of wheat under the growth room conditions (Fig. 1). However, no disease symptoms were developed in rice plants inoculated with the strains of *MoT* (Fig. 4). Artificial infection of wheat by *MoO* isolates also significantly reduced grain yield of wheat (Table 1). In addition to cause typical infection in rice, pearl millet and finger millet, the rice blast fungus is claimed to be a major threat to wheat, barley and oat (Kumar et al. 2017). On the other hand, wheat is potentially susceptible not only to rice-infecting *Magnaporthe* but also to *Magnaporthe* infecting other cereal hosts, such as pearl millet and *Lolium*. Pearl millet-infecting blast fungus infects wheat, barley and oat under artificial conditions

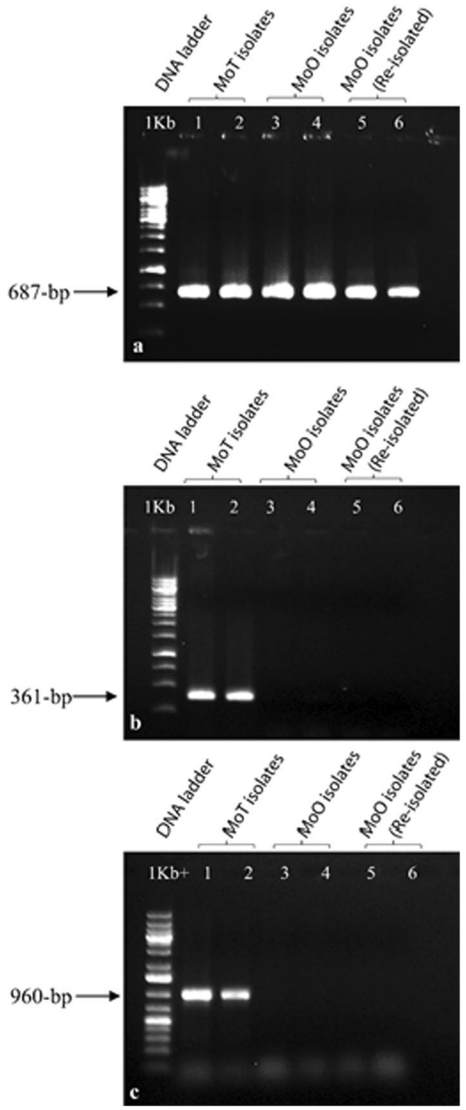

Fig. 5 PCR detection of *MoT* and *MoO* pathotypes. The gel images of PCR products amplified by Pot2 (**a**), MoT3 (**b**) and MoT6099 primers (**c**) to confirm the *M. oryzae* pathotype. The gDNA used in this study was isolated from mycelia of the respective fungus. Lane 1, DNA ladder; Lanes 2 and 3 represent DNA isolated from *MoT* strains BTJP4-5 and BTMaU(10b), respectively, which were collected from naturally blast infected wheat field; Lanes 3 and 4 denote DNA isolated from *MoO* isolates RB13b and RBTa1849-2, respectively, which were collected from naturally blast infected rice field; Lanes 5 and 6 represent DNA reisolated from the symptomatic tissues of wheat spikes artificially inoculated with the *MoO* strains RB13b and RBTa1849-2, respectively

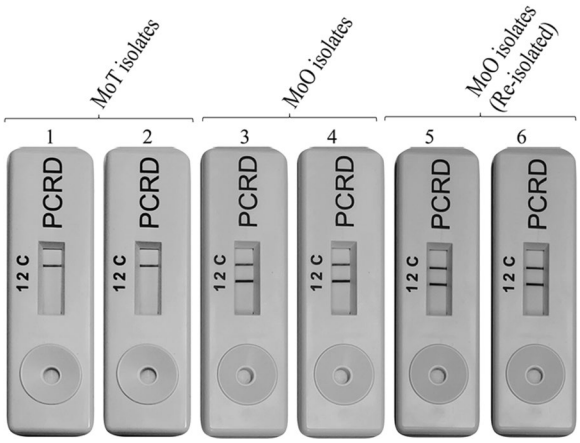

Fig. 6 Rapid detection of *MoT* by PCRD strip. Lanes 1 and 2 signify DNA isolated from *MoT* strains BTJP4-5 and BTMaU(10b), respectively. The *MoO* isolates, RB13b and RBTa1849-2 specify lane 3 and 4 separately, which were collected from naturally blast infected rice panicles (double band indicate *MoT* negative results). Moreover, lanes 5 and 6 denote DNA reisolated from the symptomatic tissues of wheat spikes artificially inoculated with *MoO* strains RB13b and RBTa1849-2, respectively (double band indicate *MoT* negative results)

but not rice and finger millet (Prakash et al. 2019). In a 17 genetic loci-based analysis, wheat isolates were clustered with *Lolium* pathotype (*Lolium* was considered as the suspected original host of wheat blast isolate in South America), but rice-infecting isolates showed a separate clustering pattern (Sheoran et al. 2021). Infection of wheat by the *Lolium* pathotype of *M. oryzae* has been reported by Farman and his co-researchers (Farman et al. 2017). Earlier, researchers demonstrated the evolutionary mechanism underlying the host-jump of native *Lolium* isolate to wheat to cause the world's first wheat blast outbreak in the Parana state of Brazil in 1985 (Igarashi et al. 1986; Inoue et al. 2017). Occurrence and severity of plant diseases are dependent on three major factors viz., the host plant, the pathogen and the environmental conditions. We also think all these three factors are important for *MoO* isolates to infect wheat and cause wheat blast. A further comprehensive study is needed for better understanding about the cross-infection of *MoO* and *MoT* pathotypes by the inclusion of a high number isolates from diverse geographical locations and also differential blast resistant genotypes/varieties of wheat and rice.

Although rice blast has been a serious problem in Bangladesh since 1984 (Shahjahan 1994), the first epidemic outbreak of wheat blast in Bangladesh by a clonal population of a South American lineage of *M. oryzae* was reported in 2016 (Islam et al. 2016). In Bangladesh, rice and wheat are cultivated side by side in the same season. Therefore, there is a high chance of genetic recombination of *MoO* and *MoT* pathotypes as they may overwinter in some common grasses. One of the interesting findings of this study is that three virulent *MoO* strains equally produced typical blast symptoms on wheat and rice plants in a growth room condition. The experimental

results indicated that *MoO* can develop blast symptoms on rice leaves and neck of the panicles as well as produce identical disease symptoms on leaves and spikes of wheat. Conversely, artificial inoculation with three *MoT* strains only produced disease symptoms on wheat leaves and base/rachis of the spikes but did not develop any blast symptoms on rice plants. Our experimental findings unambiguously supported the previous results (Wang et al. 2021). Wang and co-workers found that some of the *MoO* strains can develop typical blast symptoms on wheat in a temperature-dependent manner. As global climate is changing, they opined that some *MoO* strains may evolve as a pathogen of wheat in future when the environment matches to the requirements for infection. It has also been reported that some strains of *M. oryzae Lolium* (*MoL*), which cause gray leaf spot disease in turf grasses, can also infect wheat (Cruz and Valent 2017; Islam et al. 2019). The wheat blast fungus *MoT* has high genetic and phenotypic diversity, which may enable this pathogen to move back and forth between wheat and other grass hosts under suitable environmental conditions (Ceresini et al. 2018). The interlineage gene flow has contributed to the genetic makeup of multiple *M. oryzae* lineages within the same species, especially in regions where multiple lineages of this fungus are in contact with one another (Gladieux et al. 2018). It could even happen in Bangladesh where wheat and rice are grown in the same field side by side in the same season (Islam et al. 2019). It is well known that both rice and wheat can also grow in the same area in many other regions such as Pelotas in Brazil, Eastern China and Arkansas in the USA. The findings of the current study indicate that a potential wheat blast epidemic by *MoO* will prevail in many rice–wheat inter-cropping regions as climate change intensifies and becomes more widespread in Bangladesh and also in many other wheat-growing regions in the world.

In this study, we reisolated the fungus from the symptomatic plant tissues, which showed identical morphological features with the original strains used for the plant inoculation (Additional file 1: Figure S1). We also checked the presence of sporulation of the fungus on the lesions of infected leaves and spikes by microscopic observation (data not shown). Furthermore, we confirmed their genetic and pathotype identities using a general primer, Pot2 (687-bp fragment) (Harmon et al. 2003) for *M. oryzae*, and two *MoT*-specific primers, MoT3 (Pieck et al. 2017) and MoT6099 (Kang et al. 2020) (Fig. 6). We also used a novel NALFIA technology, which can rapidly, sensitively and inexpensively identify *MoT*-specific DNA segments in blast-affected wheat plants through a PCRD strips (Kang et al. 2020), to reconfirm our findings (Fig. 6). All of these molecular diagnostic tools unambiguously confirmed that artificial inoculation with *MoO* strains in Bangladesh resulted in typical blast symptoms in wheat under the growth room conditions. However, based on our findings using limited number of strains, we cannot rule out other unknown mechanism leading to the gain of virulence of the particular isolates of *MoO* against wheat plants.

Here, we used only three isolates for each *M. oryzae* pathotype and a single variety of wheat and rice. As high variability in pathogenesis of *MoO* strains in response to different wheat cultivars and temperature has been reported (Wang et al. 2021), a further cross-inoculation study is needed by the inclusion of a large number of *MoO* and *MoT* isolates and rice and wheat varieties under varying environmental conditions. Our reproducible pathosystem developed for artificial inoculation of wheat by *MoO* strains would facilitate further cell biological and molecular biological investigations for shedding light on host-specificity among the pathotypes of *M. oryzae*. Therefore, our results have provided helpful information for wheat extension specialists and epidemiologists to examine a possible outbreak of wheat blast disease in future.

Conclusions

We demonstrated that artificial inoculation of wheat with *MoO* strains produced typical blast symptoms on both leaves and spikes under the controlled growth room conditions. However, inoculation of rice with *MoT* strains didn't induce disease symptoms in any parts of the plants under the same environmental conditions. We confirmed the genetic identity of the re-isolated fungal pathotypes of *M. oryzae* from the symptomatic tissues by both PCR method and rapid detection PCRD strip for *MoT*. These research findings indicate the possibility of cross infection of rice and wheat by contrasting pathotypes of *M. oryzae* under the prevailing suitable environment due to global climate change. As rice and wheat are cultivated side by side in the same season in Bangladesh, there is a risk of genetic recombination among the *MoO* and *MoT*. Taken together, our study has provided evidence for a potential wheat blast epidemic by *MoO* in many rice–wheat inter-cropping regions as the climate change worsens. Our findings would facilitate further in-depth research by the inclusion of a large number of blast fungal isolates and wheat and rice genotypes with differential blast resistance to better understand the host-specificity in *MoO* and *MoT* isolates in Bangladesh.

Methods

Pot preparation, growing of plants and recording of experimental data

The pots used for the experiment are 30 cm in length and 24 cm in diameter. Soil samples were collected from

the Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) Research field at a depth of 0–15 cm. The pots were filled with soil and cowdung in 2:1 ratio. For wheat, nitrogen, triple super phosphate, muriate of potash and gypsum were applied at a ratio of 70:28:50:11 kg/ha of N:P:K:S (FRG 2012). Wheat seeds were surface-sterilized with 70% ethanol for 10 min, soaked in 1.5% active chlorine for 1 h, and rinsed five times in sterile distilled water (SDW) (Robinson et al. 2016). Five wheat seeds of BARI Gom-25 were sown and finally one healthy plant per pot was allowed to grow. Weeding and watering were done as regular management practices.

For rice cv. BRRI dhan63, the plants were grown in plastic pots containing approximately 12 kg of clayed soil. Initially, each pot was filled with 10 kg dry soil followed by soil test-based fertilizer (Iqbal et al. 2019). Except N, fertilizer doses of 18 kg P, 90 kg K, 20 kg S, and 3.5 kg Zn per ha in the form of triple super phosphate, muriate of potash, and gypsum fertilizers were applied prior to transplanting. Optimum dose of 120 kg nitrogen per ha for modern variety was applied in the form of urea in three splits at 10, 25 and 50 days after transplanting (BRRI 2020). Rice seeds were surface-sterilized with 70% ethanol for 10 min, soaked in 1.5% active chlorine for 1 h, and rinsed five times in sterile distilled water (SDW) (Robinson et al. 2016). Seeds were first germinated on wet filter paper in petri-dishes at 28 °C for 5 days. After the emergence of the radicle, seeds were transferred to plastic pots and each pot had one seedling. Several cultural practices, such as weeding and fertilizing, were done when necessary. Standing water of 2 cm above the soil was maintained until the crops attained hard dough stage.

For both wheat and rice, data were collected on total tiller, effective tiller and infected tiller per hill, full length and infected part of spike or panicle, seeds per spike or panicle, 1000-grain weight and grain yield per hill. Blast disease severity assessment was done using a 0–4 scale in which % infection means length of the spike/panicle infected by blast. The scales were 0 = no lesions; 1 = 1–25% infection; 2 = 26–50% infection; 3 = 51–75% infection and 4 = 76–100% length of the spike or panicle was infected by blast (Suryadi et al. 2013). The severity of blast is calculated using the formula: $\mathrm{DS}(\%) = \frac{\sum n \times \mathrm{v}}{\mathrm{N} \times \mathrm{V}} \times 100$ (DS, disease severity; n, number of panicles infected by blast; v, value score of each category attack; N, number of panicles observed; V, value the highest score).

In the cases of rice and wheat blast diseases, head or neck blast is predominant. The neck and/head blast are more vulnerable than the leaf blast in both rice and wheat. In the field conditions, we observed that with the presence of wheat blast symptoms in the leaves, the yield of wheat was not remarkably decreased. That is why we used only data related to the blast severity at the reproductive stage.

Environmental conditions of growth room

Five replicated pots were arranged in a growth room according to a completely randomized design. In the case of wheat, fluorescent and incandescent lamps were used in growth room to provide a light intensity of 275 µmol/m^2s on the surface of pots. Light and dark periods were adjusted to keep 10 h (21 °C) and 14 h (16 °C), respectively (Abbas et al. 2017). The relative humidity for wheat plants was kept at ca. 70% throughout the day and night. On the other hand, the photoperiod for rice growth room was 14-h day at 27 °C and 10-h night at 25 °C, and the relative humidity was kept at 75% throughout the day and night. Light provided by tungsten lamps was 600 µmol photons/m^2s at the top of the plants (Khan et al. 2021).

Culture of wheat and rice blast isolates

Blast-infected wheat spikes and rice panicles were collected from fields (Table 3). The diseased plant samples were put inside brown paper bags and brought to the Institute of Biotechnology and Genetic Engineering (IBGE) laboratory of BSMRAU, Gazipur for further analysis. Isolation and filter paper storage of pure fungal

Table 3 List of *Magnaporthe oryzae* isolates used in this study

Isolate	Crop	Variety	Location	Collection time	Source
BTJP4-5	Wheat	Prodip	Jhenaidah	March, 2016	Wheat leaf
BTMaU(10b)	Wheat	Unknown	Magura	February, 2017	Wheat spike
BTMP1845-3	Wheat	Prodip	Meherpur Sadar, Meherpur	March, 2018	Wheat spike
RB13b	Rice	BRRI Dhan28	Khulna	May, 2017	Rice panicle
RBTa1849-2	Rice	BRRI Dhan29	Nolua, Sakhipur, Tangail	May, 2018	Rice panicle
RBMe1819-3	Rice	BRRI Dhan28	Monohorpur, Meherpur	May, 2018	Rice panicle

isolates were done by picking up a single conidium following the method described by Gupta and his co-researches (Gupta et al. 2020). For this study, isolates of *MoT* and *MoO* were retrieved from the storage in potato dextrose agar (PDA) media and incubated at 26 °C.

Preparation of spore suspension and cross inoculation of leaf and head of wheat and rice

Wheat and rice blast isolates (Table 3) were cultivated separately on PDA medium for 7 days at 26 °C. Then, the fungal mycelia in Petri dishes were flooded with 5 mL of sterilized water, and aerial parts of the fungal colony were washed by gentle rubbing with a sterilized paint brush. The rubbed culture plates were incubated at 25 °C for 24 h in a laminar air flow cabinet for inducing sporulation. The lids were kept closed loosely to allow entry of air to the Petri plates. For foliar spray of inoculum, spores (conidia) from the surface of sporulated mycelia on PDA medium was scraped gently with sterilized glass spreader and suspended in sterilized water containing 0.01% Tween 20. The suspension was filtered through miracloth (pore size 22–25 µm) and spore concentration was adjusted to 5×10^4 conidia/mL.

The cross inoculation of *MoT* and *MoO* isolates (Table 3) was done by spraying spore suspension using a hand sprayer on 14-day-old wheat cv. BARI Gom-25 and rice cv. BRRI dhan63 seedlings. Inoculated seedlings were incubated in a humid chamber (95% relative humidity) at 25 °C and kept in dark for 24 h after inoculation. Then, the seedlings were transferred into a growth room maintained at 28 ± 1 °C, 80% relative humidity and a 12-h photoperiod (Ha et al. 2016). At the reproductive phase, after emergence of head, spore suspension was sprayed using a hand sprayer on wheat and rice plants following the procedure as applied at the seedling stage. Sterilized water was sprayed on the heads of the plants 5–7 times a day to give a conducive environment for disease development in the growth room conditions. During the seedling stage, data were recorded at a 6-h interval up to five days and for heading stage, data were recorded up to 12 days of inoculation. Each treatment was replicated for five times and the experiment was laid in a complete randomized design in the growth chamber mentioned above.

Re-isolation of *MoO*, production of conidia and microscopy

Re-isolation of rice blast fungal strains viz. RB13b, RBTa1849-2 and RBMe1819-3 was done from the symptomatic tissues of the artificially infected leaves of wheat cv. BARI Gom-25 (Gupta et al. 2020), and conidial suspension was adjusted to a final concentration of 5×10^4 conidia/mL. Features of conidia were observed with Zeiss Axiocam ERc 5 s. The experiments were repeated five times and with five replications per treatment.

Detection of *MoT* and *MoO* by specific primers

The isolates were cultivated on PDA medium for 10 days at 26 °C, and then the mycelia were collected by scrapping. The scraped mycelia were crushed using mortar and pastel. Extraction of genomic DNA was performed from *MoT* and *MoO* isolates using Promega Kit (Cat# A1125) following the manufacturer's protocol. DNA quantification was done using a nano-drop spectrophotometer and was diluted with sterile distilled water as required.

The polymerase chain reaction (PCR) amplification of 687-bp region of the Pot2 transposon (a general primer for the detection of any pathotype of *M. oryzae*) were performed using primers pfh2a (5-CGTCACACGTTCTTC AACC-3) and pfh2b (5′-CGTTTCACGCTTCTCCG-3′) (Harmon et al. 2003). To amplify a 361-bp of DNA segment from *MoT* isolates, forward primer MoT3F (5′-GTCGTCATCAACGTGACCAG-3′) and reverse primer MoT3R (5′-ACTTGACCCAAGCCTCGAAT-3′) were used (Pieck et al. 2017). Moreover, a recently discovered *MoT*-specific forward primer MoT6099F (5′-TCTGTA TTTCACACTTGGGCTTTGG-3′) and reverse primer MoT6099R (5′-AACGTCATGTAGTGCGTCTTGTTG A-3′) were used to amplify a 960-bp of DNA segment (Kang et al. 2020). For all primers, PCR amplification was performed in a 50-µL reaction mixture which contained 0.5 µL of DNA *Taq* polymerase (2.5 U), 5 µL of 10 × polymerase buffer, 3 µL of 25 mM $MgCl_2$, 1 µL of 10 mM dNTP, 2 µL of 20 pmol/µL of each primer, and 1 µL of the template (extracted genomic DNA at 50 ng/µL). The PCR reaction for amplification of Pot2 was carried out in a thermal cycler (Applied biosystems, Thermo Fisher Scientific, USA) following previously described protocol (Harmon et al. 2003). In the case of MoT3, specific gene sequence was amplified following the described protocol (Pieck et al. 2017). On the other hand, MoT6099-specific gene sequence was amplified using previously described methods (Kang et al. 2020). The amplification products were subjected to electrophoresis in a 1% agarose gel and stained for 10 min in an ethidium bromide solution (10 µg/mL). Gel pictures were achieved using a digital imaging system (Alpha Imager MINI, Protein Simple, Santa Clara, CA).

Nucleic acid lateral flow immunoassay (NALFIA) through PCRD strips

Extraction of genomic DNA from *MoT* and *MoO* isolates was performed using Promega Kit (Cat#A1125) following the manufacturer's protocol. The recombinase polymerase amplification (RPA) with Cas12a was performed by using a previously described method (Chen et al. 2018). sgRNA was mixed equimolarly with Cas12a in reaction buffer (Kang et al. 2020). Then, the Cas12a mixture was incubated at room temperature for ~ 10 min. After that,

amplified target DNA from RPA reaction was incubated with the Cas12a mixture at 37 °C for around 10 min. Due to incubation, the Cas12a ssDNA digestion process was activated. Finally, the designed ssDNA and the activated Cas12a protein were combined for ssDNA digestion. The visualization of DNA was carried out by using PCRD strips (Abingdon Health PCRD test cassettes, # FD51673, UK). For this stage, 5 µL reaction mixture from the RPA process was added with 70 µL of PCRD extraction buffer. The total 75 µL volume was transferred to the sample well of the PCRD text cassette. The consequence was measured after 3–5 min (Kang et al. 2020).

Data analysis

All statistical analyses were conducted using the statistical software package (IBM SPSS Statistics 25) and Microsoft Office Excel 2015 program package. Analysis of means comparison of the treatments was accomplished by LSD test ($P \leq 0.05$).

Abbreviations

BARI: Bangladesh Agricultural Research Institute; BRRI: Bangladesh Rice Research Institute; RPA: Recombinase polymerase amplification; CTAB: Cetyltrimethylammonium bromide; EDTA: Ethylene diamine tetraacetic acid; *MoO*: *Magnaporthe oryzae Oryzae*; *MoT*: *Magnaporthe oryzae Triticum*; PCR: Polymerase chain reaction; PDA: Potato dextrose agar.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-022-00114-4.

Additional file 1: Figure S1. Isolation of conidia from purified *MoO* isolates. The upper panels show the major steps for the isolation of conidia from RB13b (**a**), RBTa1849-2 (**b**) and RBMe1819-3 (**c**) collected from naturally infected rice plants; The lower panels show the major steps for the re-isolation of conidia from wheat plants artificially inoculated with rice blast isolate RB13b (**a**), RBTa1849-2 (**b**) and RBMe1819-3 (**c**). In both cases, cultures were gown on PDA media from a single conidium. Bar = 10 µm.

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Authors' contributions

Conceptualization, writing-review, editing and supervision, TI; investigation, visualization, writing-original draft and editing, SKP; methodology and software, NUM; investigation and formal analysis, KR and DRG; writing, review and editing, HK, GLW and LJ; project administration, TI; funding acquisition, LJ, GLW and TI. All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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