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Pathogenicity shown in three distinct Magnaporthe oryzae isolates

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Research Article

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Abstract
To further understand the pathogenesis of blast disease in rice, three isolates of Magnaporthe were examined in this work. Under controlled laboratory conditions, M. oryzae 6808 and M. oryzae ch1 isolates generated appressoria and macroconidia with pyriform shapes. In the same environment, M. oryzae 6926 did not develop appressoria but did produce crescent-shaped microconidia. All three of the Magnaporthe isolates tested positive in the pathogen inoculation assay, but to different degrees. Different cultivars of paddy lines showed varying degrees of disease severity as a result of pathogen isolates producing spindle-shaped lesions with necrotic margins. In rice lines, both forms of sporulation increased the frequency and severity of illness. A lot of research has focused on the process of pyriform macroconidia entering a suitable host plant and developing appressoria. In order for crescent-shaped microconidia to infect plants that are a good fit, they will need to find another way to enter host cells. Both macroconidia and microconidia were shown to produce pathogenicity in rice. The role of microconidial infection in the progression of blast illness requires more research.

Keywords: *Magnaporthe oryzae*, microconidia, appressoria, disease intensity

Introduction

Magnaporthe oryzae blast is the worst disease that may happen to rice plants at any point in their life cycle. It drastically reduces yields in terms of both quality and quantity. Worldwide, it is believed to be responsible for around 14% to 18% of the yield decline (Hajano, Pathan, Rajput, & Lodhi, 2011—7]. The crop might collapse entirely due to blast infestations, resulting in significant losses. In their 2019 publication, Gavhane, Kulwal, Kumbhar, Jadhav, and Sarawate mentioned [6]. The blast disease mostly affects cultivated gramene plants and is caused by the filamentous heterothallic ascomycete Magnaporthe oryzae (teleomorph) and its anamorph Pyricularia oryzae. When it's in its imperfect state, it makes macrospores, which are three-celled and pyriform, and microspores, which are four-celled and spindle-shaped, or microconidia. This is supported by research conducted by Choha et al. (2009), Kato et al. (1994), Yaegashi and Nishihara (1976), and others. Because most research have focused only on macroconidia germination, there is often more extensive information available for macroconidia. An asexual triseptate pyriform spore may infect rice when it rests on a leaf when dewdrops are present. The adhesive produced by the apical compartment of the spore allows it to cling to the cuticle. For germination to occur in the absence of a nutrient, the spores carry a variety of endogenous reserves. Glycerol, trehalose, and other polyols like erythritol and mannitol are examples of such substances (Foster, Littlejohn, Soanes, & Talbot, 2016) [5]. A polarized germ tube is produced from macrospores in a very little amount of time after their arrival on the leaf. After one of the conidium's apical cells forms a germ tube, which only extends a short distance, the pathogen attaches to the surface, the host is recognized, and finally, the appressorium is created. The cell wall of a developing appressorium consists of two layers: an outside chitin layer and an interior melanin layer. In order to achieve a turgor pressure of 8 MPa or higher, these layers are necessary (Jenkinson et al., 2007) [8]. To prevent the outflow of solute from the appressorium, the cell wall functions as a barrier. Next, mechanical force is generated from the cellular turgor. In order to forcefully penetrate the leaf cuticle, the developing penetration peg applies this force. The referenced work is Wilson and Talbot's 2009 [12]. Since there is little literature on microconidia, there are no accounts of their development into appressoria. It is often believed that only pathogenic strains of Magnaporthe oryzae synthesize appressoria, so the current study tested three different kinds of isolates for this occurrence. Various sporulation patterns among Magnaporthe isolates were examined cytologically to determine their potential for pathogenicity.

Material & Methods

Appressoria formation assay: *Magnaporthe oryzae* 6808 and 6926 cultures, obtained from Plant Pathology Department, Indian Agricultural Research Institute, Delhi and field isolate *Magnaporthe oryzae ch1* culture (Kulkarni & Peshwe, 2019) [10]. were subjected for appressoria formation assay. Hydrophobic surface of coverslip was used for formation of appressoria. Conidial suspension of 100-200 ul (1*10^3/ml) of *Magnaporthe oryzae ch1, 6808 and 6926* isolates were placed on different sterile coverslips (Xu, Liu, Zhuang, Zhu, & Lin, 2011) [13]. After each 12 hours, 20 ul conidial suspension was taken on another glass slide and lactophenol blue staining was conducted. Germ tube formation, appressoria formation was observed from first few hours till 48 hours.

Pathogen inoculation assay: Three blast isolates i.e. *Magnaporthe oryzae ch1, 6808 and 6926 isolates* were used as source of pathogen for inoculation against

Paddy lines *viz*. Phule Samruddhi, Indrayani, Chimansal, Ek-70 and Phule Radha, obtained from Mahatma Phule Krishi Vidyapeeth

(MPKV), Rahuri, Maharashtra. Paddy plants of 4 weeks old age were sprayed with spore suspension ($1*10^3$/ml) of all three isolates on different lines. Experiment was done in duplicates for each cultivar with respect to each pathogen isolate. Control plants were kept along with experimental plants. After spraying,each cultivar was sealed by transparent polybags from top. (Nagao Hayashi *et al*, 2009) [11]. All the plants were kept in dark for 24 -48 hours which would favour disease setting on the plants. Every day monitoring was done for disease symptom observations

Result and discussion

Mycelial growth of *M oryzae 6808* and *M oryzae ch1* isolates on oat meal agar was dark pigmented cottony mycelia due to secretion of melanine where as in *M orzae 6926 isolate* mycelia growth was white colored, which showed lack of melanine pigmentation as shown in Figure1.

Fig1: Mycelial growth of *M.oryzae 6808, M.oryzae 6926* and *M.oryzae ch1* isolates on Oat meal agar

*Magnaporthe oryzae ch1 and Magnaporthe oryzae 6808 isolate*s produced tri-septate pyriform macroconidia. *Magnaporthe oryzae* 6926 produced microconidia at 28ºC in controlled laboratory conditions. Conidial suspension were subjected forappressoria formation. *Magnaporthe oryzae ch1 and 6808 isolate*s showed germ tube formation and later appressoria were prominently seen when observed under 40X Microscope in specific time intervals

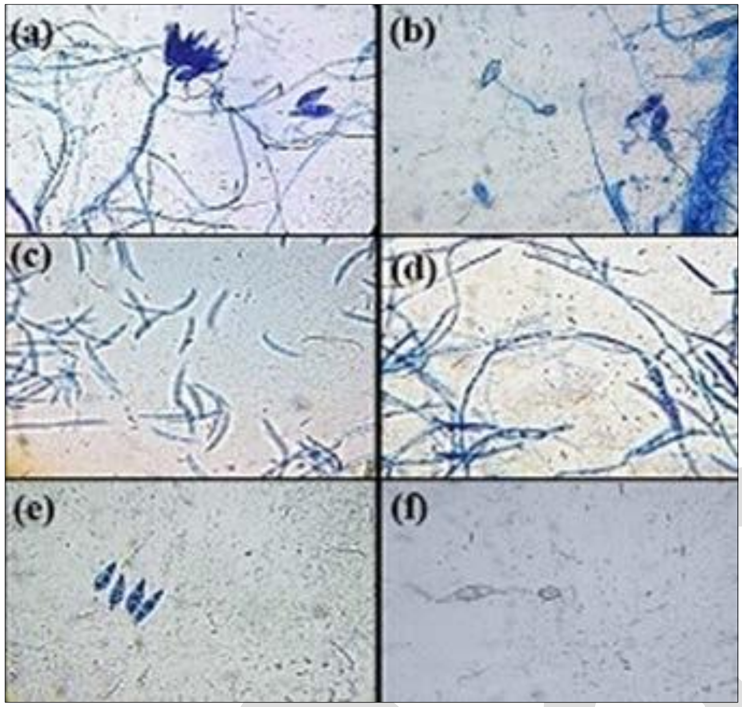

Fig 2: **(a)** Sporulation: Triseptate pyriform shaped macroconidia in *M.oryzae 6808* isolate **(b)** Appressoria formation: Oval shaped appressoria seen along with germ tube after 24 hours in *M.oryzae 6808* isolate **(c)** Sporulation: Crescent shaped microconidia seen in *M. oryzae 6926* isolate **(d)** Appressoria were not formed after 24 hours but microconidia grow longer in *M. oryzae 6926* isolate **(e)** Sporulation: Triseptate pyriform shaped macroconidia in *M.oryzae ch1* isolate (**f)** Appressoria formation: Oval shaped appressoria seen along with germ tube after 24 hours in *M.oryzae ch1* isolate

The pathogen's infectiousness, mode of action, and host's reaction to the pathogen strain are all determined by the results of the pathogen inoculation test. Under controlled laboratory conditions, a pathogen inoculation study was conducted using five different rice cultivars. The assay included spraying a conidial slurry containing three different strains of M oryzae isolates. A pathogenicity test that has been used in several studies was used to quantify the pathogen's virulence on a disease scale ranging from 0 to 9. This scale was developed by the International Rice Research Institute (IRRI) and the Rice Research Organization in the Philippines.According to various studies, including those by Zellerhoff et al. (2006), Babu et al. (2013), Chi et al. (2009), and others, all of the Magnaporthe oryzae ch1, 6808, and 6926 isolates caused spindle-shaped lesions with necrotic borders on various cultivars, albeit the severity of the disease varied. Data was analyzed statistically. (Asafaha M. et al., 2015) [1] and (Hajano et al., 2011) [7] were used to estimate the percentages of illness incidence and severity, as shown in Figure 3.

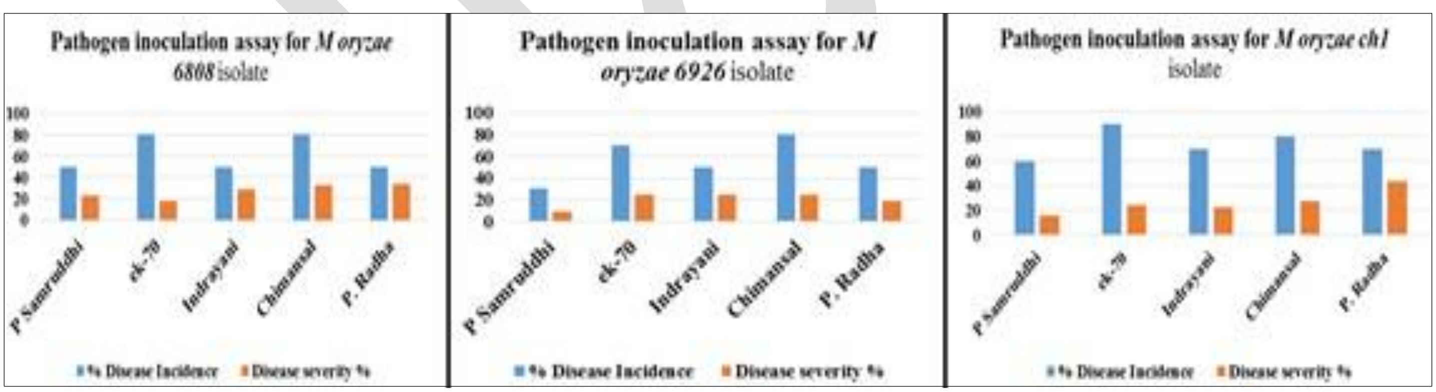

Fig 3: Pathogen inoculation assay of *M oryzae 6808, 6926 and ch1* isolates with different rice cultivars along with percent disease incidence and percent disease severity values

Disease incidence and severity were both higher with M oryzae ch1 when compared to the other two isolates and other rice cultivars. This could be because M. oryzae 6926 did not develop appressoria, but it still formed microconidia and had a nearly same percentage of illness incidence, although with a somewhat lower severity level. Both Hajime Kato et al. (1994) [9] and Chuma et al. (2009) [4] support this idea.

Potentially producing phialide and microconidia, M. oryzae isolates from a variety of graminous plants shown mating capacity. The microconidia had a thin cell wall, were 5-8 um long, 0.5 to 0.8 um broad, and had one nucleus; they were hyaline, cylindrical, and rounded at the end initially, and later lunate. More microconidia formed a globose mass, and they were crescent shaped. Figure 2 shows that the microconidia in the M. oryzae 6926 isolate were crescent-shaped, whereas the macroconidia in the M. oryzae 6806 and ch1 isolates were larger. Therefore, under controlled laboratory circumstances, the current investigation discovered that the formation of macroconidia and microconidia was isolate type specific. It was shown that Magnaporthe isolates that produce macroconidia also produce appressoria. Microconidia were unable to enter host cells because they lacked the appressorial structure necessary to penetrate cell membranes. All three isolate types were infectious to varying degrees, according to the findings of the pathogen inoculation experiment. Therefore, microconidia must find another way to infect host cells and create symptoms. Both microconidia and macroconidia were shown to germinate and infect in a study by Zhang et al. (2014) [16]. Normal and pathogenic colonies were formed by microconidia. Microconidia were able to colonize injured leaves and stems in infection tests with barley and rice seedlings, leading to the development of necrotic lesions. On inoculated spikelets in barley and brachypodium heads, microconidia were discovered to generate disease signs. Both in the lab and out in the field, these microscopic parasites caused blast wounds in rice plants. An important part of the rice blast cycle is the potential for microconidia to germinate and spread disease. It is possible that the diminutive size of microconidia enables Magnaporthe oryzae to more easily traverse the circulatory system. This may be a crucial but often disregarded aspect of the disease cycle and epidemics involving rice blast and other significant illnesses produced by Magnaporthe species.

Conclusion

In a controlled laboratory setting, three isolates of Magnaporthe were examined for their ability to cause blast disease in several rice varieties. Under controlled laboratory conditions, M. oryzae 6808 and M. oryzae ch1 isolates generated appressoria and macroconidia with pyriform shapes. In the same environment, M. oryzae 6926 did not develop appressoria but did produce crescent-shaped microconidia. The findings of the pathogen inoculation experiment demonstrated that all three Magnaporthe isolates were infectious, infecting various rice cultivars to diverse degrees. Depending on the severity of the illness, they caused spindle-shaped lesions with necrotic margins on various rice varieties. The illness incidence was higher in the M. oryzae ch1 isolate. When compared to the other two blast isolates, M oryzae ch1 produced a more severe disease. The microconidia generated by M. oryzae 6926 were comparable in percentage to those of the other two isolates, but the severity of the illness was reduced; this could be because the M. oryzae 6926 strain did not develop appressoria. Additional research on the pathogen entrance mechanism of crescent-shaped microconidia in order to generate disease in appropriate host plants is necessary, as it might provide light on another facet of the blast disease cycle brought about by microconidial transmission.

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