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Demonstration of pathogenicity in three different *Magnaporthe oryzae* isolates

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Abstract
In present study, three *Magnaporthe isolates* were analyzed for understanding pathogenicity of blast disease in rice. *M oryzae 6808* and *M oryzae ch1* isolates produced pyriform shaped macroconidia and were able to form appressoria under artificial condition. *M oryzae 6926* produced crescent shaped microconidia but failed to produce appressoria in the similar condition. Pathogen inoculation assay results showed that all three *Magnaporthe isolates* were infective with varying degree of infection. Pathogen isolates produced spindle shaped lesion with necrotic borders on different cultivars of paddy lines with different disease intensity. Both types of sporulation contributed to disease incidence and disease severity in rice lines. Entry of pyriform shaped macroconidia and formation of appressoria in suitable host plant has been widely studied. Likely, there must be another mechanism for crescent shaped microconidia to make entry into host cell to cause disease symptoms in suitable host plant. It was observed that pathogenicity in rice was caused due to macroconidia and microconidia both. Microconidial infection needs to be further explored in understanding blast disease cycle.

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

Introduction

Blast caused by *Magnaporthe oryzae* is the most devastating disease that occurs to rice at any stages of plant's growth. It causes heavy losses in both quality and quantity of the produce. It is estimated about 14-18% of the yield reduction worldwide (Hajano, Pathan, Rajput, & Lodhi, 2011) [7]. Blast infestations could lead to complete failure of the crop causing severe losses. (Gavhane, Kulwal, Kumbhar, Jadhav, & Sarawate, 2019) [6].

Magnaporthe oryzae (teleomorph) (and its anamorph, *Pyricularia oryzae*) is a filamentous heterothallic ascomycete that causes blast disease mainly on cultivated gramene plants. It produces three-celled, pyriform macrospore or macroconidia in the imperfect stage, and four- celled, spindle-shaped ascospore or microspore i.e. microconidia in the perfect stage. (Chuma *et al*., 2009) [4]; (Kato *et al*., 1994) [9]; (Yaegashi & Nishihara, 1976) [14].

In general, more detailed information is available for macroconidia as most of the studies have been dealt exclusively with germination of macroconidia only. Rice blast infections are initiated when an asexual tri septate pyriform spore lands on the surface of a rice leaf in presence of dewdrops. Spore attaches itself to the

cuticle by release of an adhesive found in an apical compartment of the spore. Spore contains various types of endogenous reserves which are required for germination when there is no nutrient available. These include glycogen, trehalose, polyols such as glycerol, erythritol and mannitol etc. (Foster, Littlejohn, Soanes, & Talbot, 2016) [5]. In very short span after landing on the leaf, a polarized germ tube is formed from macrospore. Germ tube is formed from one of the apical cell of the conidium and extends only for short distance where pathogen is hooked on the surface then recognition of host occurs and then lastly appressorium formation takes place.

Developing appressorium has a cell wall which is made up of two layers i.e. outer layer of chitin and inner layer of melanin. These layers are required to generate turgor pressure of up to 8MPa. (Jenkinson *et al*., 2007) [8]. Cell wall acts as a barrier to the efflux of solute from the appressorium. The cellular turgor is then converted to mechanical force. This force is exerted by emerging penetration peg so as to forcibly get inside in to the leaf cuticle. (Wilson & Talbot, 2009) [12]. There are no reports of appressoria formation from microconidia as not much literature is available on microconidia

In the present studies, three types of *Magnaporthe oryzae* isolates were explored for appressoria formation event as it is widely considered that appressoria synthesis occurs in pathogenic strains only. Cytological analyses of different sporulation pattern among *Magnaporthe isolates* were evaluated for their role in demonstrating 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 692*6 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 for appressoria 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

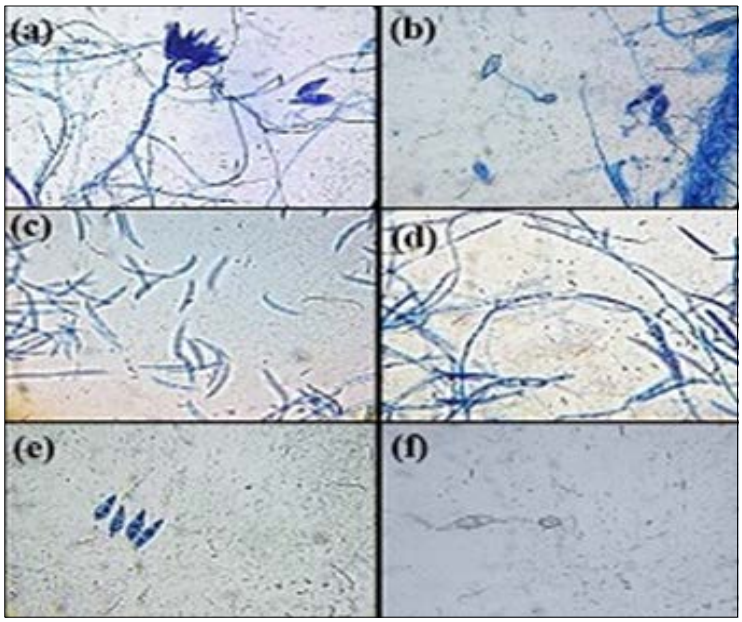

Fig 2: (**a)** Sporulation: Triseptate pyriform shaped macroconidia in *M.oryzae 6808* isolate **(b)** Appressoria formation: Oval shaped appressoria seen along with germ tube after 24 hours in *M.oryzae 6808* isolate **(c)** Sporulation: Crescent shaped microconidia seen in *M. oryzae 6926* isolate **(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

Pathogen inoculation assay on host determines the infectivity of pathogen, mode of action of pathogen and response of the host towards the pathogen strain. Pathogen inoculation assay was performed with five types of rice cultivars by spraying of conidial suspension of three types of *M oryzae isolates* in controlled laboratory condition. Virulence of the pathogen was measured in terms of disease scale from 0-9 (based on Standard Evaluation System of Rice, International Rice Research Institute (IRRI), Rice research organization, Phillipines). Pathogenicity test had been used in various studies.(Zellerhoff, Jarosch, Groenewald, Crous, & Schaffrath, 2006) [15] (Babu *et al*., 2013) [2] (Chi, Park, Kim, & Lee, 2009) [3]All *Magnaporthe isolates* used in experiment i.e. *Magnaporthe oryzae ch1*, 6808 and 6926 isolates produced spindle shaped lesion with necrotic borders on different cultivars with varying disease intensity. Statistical analysis was performed. Percent disease incidence and percent disease severity were estimated according to (Asafaha M. *et al.,* 2015) [1] and (Hajano *et al.,* 2011) [7] as in Figure3

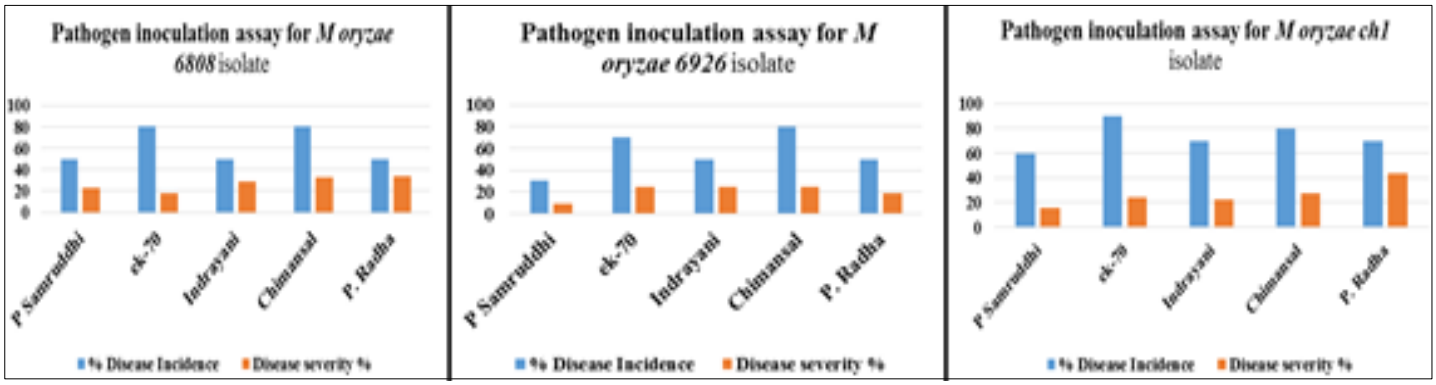

Fig 3: Pathogen inoculation assay of *M oryzae 6808, 6926 and ch1* isolates with different rice cultivars along with percent disease incidence and percent disease severity values

In general, *M oryzae ch1* had more disease incidence and was more severe with different rice cultivars as compared to other two isolates. *M oryzae* 6926 produced microconidia but showed approximate similar percent disease incidence though severity of disease found to be towards little lower side, this might be due to lack of appressoria formation.

According to Hajime Kato *et al.,* (1994) [9]. and Chuma *et al.,*(2009) [4]. *M oryzae* isolates from various graminous plants which possessed mating ability produced phialide and microconidia potentially. Microconidia were hyaline, cylindrical and rounded at end first then lunate with thin cell wall with 5-8 um long, 0.5 to 0.8 um wide with one nucleus. Microconidia were crescent shaped and more in number which forms globose mass. In the present study, crescent shaped microconidia in *M oryzae 6926* isolate and macroconidia in *M oryzae 6806* and *ch1 isolates* were observed (as shown in figure 2). Thus macroconidia and microconidia production in present study found be specific to the type of isolate in controlled laboratory conditions. Appressoria formation was reported in *Magnaporth*e isolates producing macroconidia. Microconidia failed to produce appressorial structure which is considered to be important factor for breaching the host cell surface and allowing entry of pathogen into host cell. Pathogen inoculation assay results showed that all three types of isolates were infective with varyiing degree of infection. Thus, there must be another mechanism for microconidia to make entry in host cell to cause disease and produce symptoms. Zhang *et. al.,* (2014) [16]. demonstrated germination and infectivity of microconidia and macroconidia. Colonies produced by microconidia were normal and pathogenic. In infection assays with rice and barley seedlings, microconidia could colonize and develop necrotic lesions on wounded leaves and stem. Microconidia were found to cause disease symptoms on inoculated spikelets in barley and brachypodium heads. These microconidia were detected inside the rice plants and produced blast lesions under laboratory and field conditions. Microconidia could germinate and become infectious, thus play important role in rice blast cycle. Due to their smaller size, microconidia may allow *M. oryzae* to spread readily through the vascular system, which could be an important but overlooked factor in the disease cycle and outbreaks of rice blast and other important diseases caused by Magnaporthe species.

Conclusion

Three *Magnaporthe isolates* were analyzed for demonstrating pathogenicity in blast disease in different rice cultivars in controlled laboratory conditions. *M oryzae 6808* and *M oryzae ch1* isolates produced pyriform shaped macroconidia and were able to form appressoria under artificial condition. *M oryzae 6926* produced crescent shaped microconidia but failed to produce appressoria in the similar condition. Pathogen inoculation assay results showed that all three *Magnaporthe isolates* were infective with varying degree of infection in different rice cultivars. They produced spindle shaped lesion with necrotic borders on different cultivars of paddy lines with different disease intensity. *M oryzae ch1* isolate showed more percent disease incidence. Disease caused by *M oryzae ch1* was more severe as compared to other two blast isolates. *M oryzae* 6926 produced microconidia but showed approximate similar percent disease incidence, though severity of disease was found to be lower than disease severity caused by other two isolates, this may be due to lack of appressoria formation in *M oryzae 6926* isolate. Pathogen entry mechanism of crescent shaped microconidia to cause disease in suitable host plant needs to be further studied which can open up another aspect of blast disease cycle caused by microconidial transmission

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