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Differential behaviour of *Magnaporthe oryzae* in the vicinity and on the host surface of tolerant and susceptible Rice varieties

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This study characterizes the behaviour of *Magnaporthe oryzae* causing blast of rice in the vicinity and on the leaf surface of the tolerant host Satabdi and susceptible host Nipponbare. The pathogen showed more hyphal growth towards Nipponbare seedlings than towards Satabdi. In *in vitro* assays *M. oryzae* hyphae reached the Nipponbare seedling within 72 hours post inoculation. On the host leaf surface *M. oryzae* hyphae showed more profuse growth and intimate contact with host surface on Nipponbare leaf than Satabdi. Bulbous hyphopodia were formed on Nipponbare leaves from the hyphal tips within 48 hours post inoculation whereas on Satabdi leaves no hyphopodia were formed. Formation of hyphopodia on rice leaves from hyphae of *M. oryzae* during infection of Nipponbare leaves is being reported for the first time.

Key words: Hyphal behaviour, *Magnaporthe oryzae*, rice, hyphopodia, host-pathogen interaction

INTRODUCTION

The rice blast disease caused by, *Magnaporthe oryzae* (Cavara) is the most severe and economically significant fungal disease of cultivated rice worldwide (Campos-Soriano, 2013). It can cause upto 30% of annual yield loss of rice worldwide (Talbot, 2003). *Magnaporthe oryzae*-rice interaction is used as a model to understand plant-fungus interaction in monocotyledons due to established genetic manipulation and release of its draft genome sequence (Zhou *et al.* 2015). *M. oryzae* does possess different strategies while infecting different tissues of its host. While infecting the aerial parts of rice it undergoes hemibiotrophic life style whereas in roots it remains in prolonged biotrophic phase growing silently within rice cells (Paszkwsky *et al.* 2010). Three celled asexual spores or conidia come in contact with the hydrophobic surfaces of rice leaf blades, sheaths or culms to initiate the infection. The spore germinates to produce a specialized structure, known as appressorium by which the fungus can breach the rice physical cuticle barrier and obtain access to the underlying tissue (Talbot, 2003; Dean *et al.* 2005;

Valent and Khang, 2010; Zhang and Xu, 2014). Being a hyphopodiate fungi (Landschoot and Jackson, 1989) *M. oryzae* can produce hyphopodia, a simple hyphal swelling at the hyphal tip. Without forming appressoria they can invade rice by means of hyphopodia in rice roots (Tucker *et al.* 2010; Paszkowsky *et al.* 2010). Again *M. oryzae* infection has been studied in *Arabidopsis*, a dicot, where it is strictly necrotrophic (Lee *et al.* 2009).

Hence, the infection strategy of *M. oryzae* varies from host to host and even in different tissues of the same host. Here, in this study, comparative behaviour of *M. oryzae* to its compatible host a *japonica* rice variety, Nipponbare and an *indica* rice variety, Satabdi (IET 4786, CR-10-114 x CR-10115) and formation of hyphopodia on Nipponbare rice leaves when inoculated with *M. oryzae* mycelial discs are being reported.

MATERIALS AND METHODS

Plant materials and growth conditions

The *indica* rice variety Satabdi and the *japonica* rice variety Nipponbare were grown on commercial soilrites (Horticulture grade perlite: Irish peat

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moss : exfoliated vermiculite=1:1:1 ratio) under controlled environments of conviron (TM-48 C, Paragon, M/S Das Traders) at 28° C, 16/8 photoperiod. Leaves from two week old rice plants were taken for inoculation with *M. oryzae* mycelial discs.

Fungal material and growth conditions

The rice blast fungus, *M. oryzae* (MTCC No. 1477) was brought from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India. The phytopathogen was cultured on potato dextrose agar (PDA) within conviron at 28° C. Three week old mycelial mats were scooped by a sterilized cork borer as 3 mm uniform hyphal discs to perform the experiments.

Aseptic growth of rice seedlings

Satabdi and Nipponbare seeds were de-husked and surface sterilized in 2.5% Sodium hypochlorite added with Tween 20 for half an hour (Basu *et al.* 2016). Two varieties of surface-sterilized rice seeds were transferred into separate 1% water agar plates and were incubated within a conviron (TM-48 C, Paragon, M/S Das Traders) under 28° C and 16/8 photoperiodism.

In vitro set up for the comparative study of hyphal progression

Satabdi and Nipponbare seedlings were placed onto a separate 1% water agar plate at 1 cm. distance of either side of centrally placed 3 mm. *M. oryzae* mycelial discs. The hyphal progression from mycelial discs towards the seedlings of two varieties were observed at 24, 48 and 72 hours post inoculation (hpi). The number of hyphae reaching the seedlings at 72 hpi were also observed under the stereomicroscope (Radical Ltd.).

Inoculation of mycelial discs on rice leaves

Two week old Satabdi and Nipponbare rice leaves were harvested and placed on sterile wet tissue papers. 3 mm mycelial discs of *M. oryzae* were placed on those leaves. 48 hpi the infected leaves were collected for microscopic study.

Preparation of the rice leaves for compound microscopy

Aceto-ethanol (Acetic acid:ethanol= 1:3, v/v) was used to fix the infected rice leaf samples collected at 48 hpi. Leaf chlorophylls were also removed by overnight soaking of leaf samples in that solution (Basu *et al.* 2016). Bleached leaf samples were stained with lactophenol-cotton-blue. The cotton-blue stains the fungal cell wall chitin and

lactophenol serves as the mounting agent.

Scanning Electron Microscopy (SEM)

Inoculated Satabdi and Nipponbare leaves collected at 48 hpi, were fixed in 3% glutaraldehyde and subsequent dehydration of leaf samples through ethanol and iso-amyl acetate series as per standardized lab protocol (Basu *et al.* 2016). Fixed leaf samples were then further dehydrated as per Anderson's critical point drying (Anderson, 1951) before going into gold coating on a metal stub.

In vivo growth study of *M. oryzae* on host surfaces

The growth of *M. oryzae* stained with cotton-blue on Satabdi and Nipponbare rice leaves were observed under Leica DMLS light microscope. Development of infection related structures like hyphopodia was observed. SEM further revealed detailed growth of *M. oryzae* hyphae on Satabdi and Nipponbare leaves. The structure of hyphopodia at the hyphal tips were also observed.

RESULTS AND DISCUSSION

Hyphal behaviour of *M. oryzae* in vicinity of rice seedlings

Under stereomicroscope the gradual progression of hyphae from mycelial discs of *M. oryzae* were observed (Fig. 1 A). At 24 hpi *M. oryzae* hyphae started to grow towards Satabdi and Nippobare seedlings (Fig. 1 C-D). The length of hyphae growing towards the susceptible Nipponbare seedlings were more profuse than the tolerant Satabdi seedlings at all the time points, 24, 48 and 72 hpi (Fig. 1 B). Towards Satabdi the hyphae tended to accumulate within a periphery distant from the seedling in contrast to Nipponbare seedling.

The pattern of hyphal growth may be due to the exudates produced from the respective rice seedlings. Nipponbare, well-known as the compatible host of *M. oryzae* may exudes some inducers to facilitate the hyphal growth towards it whereas a retarded growth towards Satabdi suggests presence of some inhibitory substances in and around the Satabdi seedlings. Several compounds like phenolic acids, terpenes and indoles are found in and around the rice rhizosphere. Rice roots exude many allelochemicals like momilactone A, coumaric

acid, tetradecanoic acid under biotic stress (Amb and Ahluwalia, 2016). Among them momilactone A and B are diterpenoid compounds and their biosynthesis is found to be induced by blast infection. Momilactone A and B have been identified as potent phytoalexins having anti-fungal properties (Cho and Lee, 2015). In an earlier study from this laboratory, hyphal behaviour of *R. solani* towards tolerant Swarnadhan (IET 5656) and Swarna (MTU

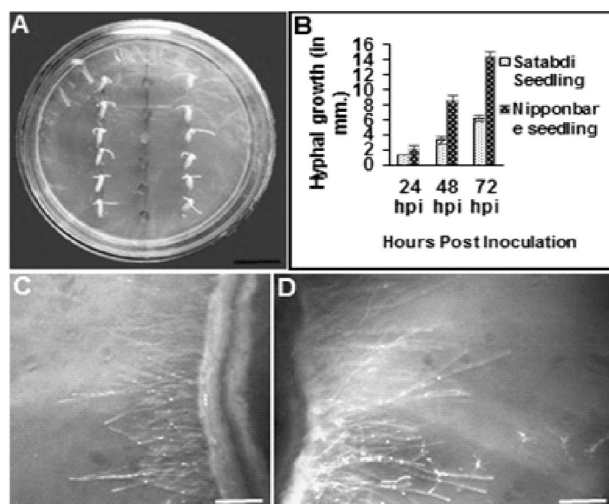


Fig. 1 : *In vitro* growth of *Magnaporthe oryzae* towards its tolerant host (Satabdi) and susceptible host Nipponbare).

A. The agar plate setup, with Satabdi and Nipponbare seedlings were placed *M. oryzae* mycelial discs (3mm.) Bar= 2cm.

B. Graphical representation of the length of hyphae growth towards the tolerant and susceptible host of *M.oryzae* at 24, 48 and 72 hpi. Stereomicroscopic study of lesser mycelial growth of *M. oryzae* towards Satabdi seedling on water agar 24 hpi (C) while more mycelial growth towards the Nipponbare seedling at 24 hpi (D).

7029) showed the same trend of faster growth of hyphae towards the susceptible variety than the tolerant one (Basu *et al.* 2016). By our research group, other plant-pathogen interactions like the charcoal rot pathogen, *Macrophomina phaseolina* produced secondary branches in the rhizosphere of susceptible sesame (VRI-1) roots while no secondary hyphal branches near the resistant sesame variety, Nirmala 0S-SE1-164 (Chowdhury *et al.* 2014).

Number of *M. oryzae* hyphae reaching the susceptible seedlings is more than the resistant one

At 72 hpi no *M. oryzae* hyphae were able to reach the tolerant Satabdi seedlings (Fig. 2 A) whereas on unit area of Nipponbare seedlings an average

of 11.67 ± 0.57 hyphae had reached the surface of seedlings at the same point of time (Fig. 2 B-C). This suggests the inability of Nipponbare to inhibit *M. oryzae* growth and thus the susceptibil-

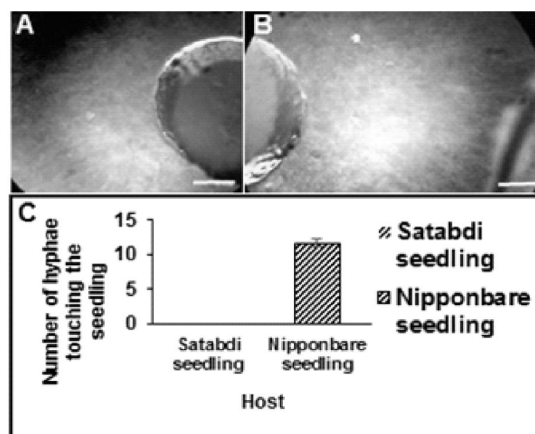


Fig. 2 : Number of *M. oryzae* hyphae reaching the seedlings at 72 hpi.

A. the *M. oryzae* hyphae are restricted to a certain periphery on the water agar towards the resistant Satabdi seedlings. Bar = 1mm.

B. The *M.oryzae* hyphae reached the susceptible Nipponbare seedling at 72 hpi. Bar = 1 mm. C. Graphical representation of the hyphae reaching to the tolerant and susceptible hosts.

ity of Nipponbare to the pathogen and its efficiency to colonize on Nipponbare becomes evident. But on the tolerant *indica* variety Satabdi, *M. oryzae* was unable to colonize at 72 hpi just like its inability to reach Satabdi seedlings on water agar.

Comparative colonization of *M. oryzae* hyphae on host leaves and infection structure formation

The differential behaviour of the pathogen on the susceptible and tolerant rice varieties were further corroborated by compound microscopy studies. On Nipponbare leaves at 48 hpi profuse hyphal growth (Fig. 3 B) was observed while scanty hyphal growth was seen on Satabdi leaf (Fig. 3 A). At the hyphal tips, globular hyphopodia were formed and scattered on the Nipponbare leaf (Fig. 3 B). The Scanning Electron Micrographs (SEM) also validated the same (Fig. 3 C-D).

M. oryzae generally infects the aerial plant parts by means of asexual spores germinating into an appressorium (Talbot *et al.* 2009). The hyphopodia formation from vegetative hyphae had been studied on the underground parts i.e. roots (Sesma

and Osbourn, 2004; Paszkowsky *et al.* 2010). But our report suggests that just like on roots, hyphopodia formation also takes place in aerial parts like leaves of compatible host like Nipponbare. Limited hyphal growth on Satabdi leaf and absence of hyphopodia formation from hyphal

nutrients for saprophytic life style. For example, presence of sugar, aspartic acid, phenylalanine in leaf exudates induce the pathogenicity and growth of phytopathogens like *Alternaria alternata*. Again leaf exudates of resistant cultivars produce amino acids like methionine, serine and arginine having inhibitory effect on *A. alternata* (Migahed and Nofel, 2001).

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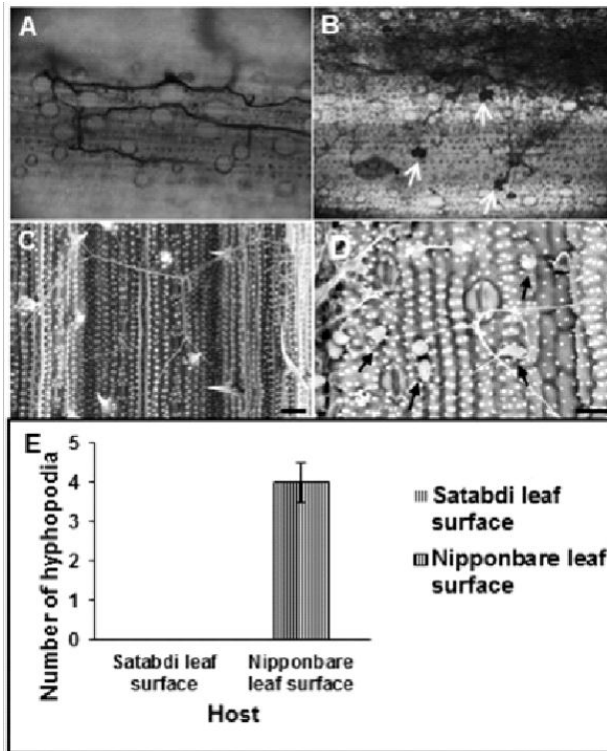


Fig. 3 : Hyphal growth of *M. oryzae* on the Satabdi and Nipponbare leaf surface. A scanty growth of *M. oryzae* mycelia on the susceptible host Nipponbare and hyphopodia formation after 48 hpi. Bar = 10 μ m. C-D Scanning Electron Microscopy showing the differential hyphal growth on Satabdi and Nipponbare leaf respectively and also the abundant hyphopodia formation on Nipponbare leaf. Bar = 10 μ m. E. Graphical representation of the number of hyphopodia formed on Satabdi and Nipponbare leaf surface.

tips correlates with the tolerance of Satabdi. The differences in *M. oryzae* colonization and hyphopodia formation on the susceptible Nipponbare and tolerant Satabdi leaves indicate the differences in the level of infection in two rice varieties.

Differential behaviour of *M. oryzae* on susceptible and tolerant host surfaces may be the result of presence of stimulatory and inhibitory substances produced by the susceptible and tolerant host respectively (Basu *et al.* 2016). Chromatographic profiles have shown the differences in the amino acid profiles of susceptible and resistant plants. Leaf exudates of susceptible leaves induce spore germination, pathogenicity of fungi by providing

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