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Conidial Germination Study of *Fusarium fujikuroi* Causing Bakanae Disease of Rice

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Abstract Bakanae disease is caused by *Gibberella fujikuroi,* sawada, wollen worth (teleomorph) (anamorph: *Fusarium fujikuroi* Nirenberg), is emerging as a serious disease of rice in India. In this experiment we study the conidial germination of bakanae pathogen *F. fujikuroi* and the effect of different substrates such as rice root, leaf, stem and water on conidial germination. For which root, leaf and stem part of rice variety pusa basmati 1509 were washed and crushed. Later suspension were placed onto the cavity slides and the 100 µLdroplet of the 10^6 conidia/mL inoculum of F 250 was placed onto the cavity slides and germination of conidia was observed at 3, 6, 9, 12 and 24 h of incubation using light microscope. 3 h of incubation tips of conidia were slightly swollen, germ tube formation started from the tips of the conidia at 6 h of incubation later on the maximum germination of conidia occurred and the length of germ tube has also been increased at 9 h of incubation. The length of germ tube elongated, branching started and mycelium spread at 12 h and dense mycelium was observed at 24 of incubation. The conidial germination percentage was maximum in root suspension (88 %) followed by stem, leaf and water at 9 h of incubation. The maximum length of germ tube (103.2 µm) was observed in water followed by leaf, stem and root at 9 h of incubation.

Keywords Bakanae disease, *Fusarium fujikuroi,* Conidial germination, Rice.

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Introduction

Rice (*Oryza sativa* L.) is the staple food crop for more than 3 billion people around the world. India ranks first in the world for the production and export of basmati rice. The country has exported 40.0 lakh metric tonnes of basmati rice to the world for the worth of Rs 22 thousand crores during the year 2015-16 (APEDA). Presently, the rice disease scenario has been changed in response to changing in rice farming situations. Several diseases caused by fungal, viral and bacterial pathogens devastate rice crop all over the world. Among various fungal diseases affecting rice, bakanae (causal organism *Fusarium fujikuroi*) is an important emerging disease [1] which affects the crop production and causes losses up to 40%. Bakanae is Japanese name meaning foolish seed-

ling caused by *Gibberella fujikuroi,* sawada, wollen worth (teleomorph) (anamorph *:Fusarium fujikuroi* Nirenberg) is emerging as a serious disease of rice in India, Taiwan, Japan and Thailand [2–5].

F. fujikuroi causes different types of symptoms starting from pre-emergence seedling death to grain infection at maturity; elongation and stunting [6]. The disease occurs both, in seedbeds as well as in the field. Diseased seedlings are lanky, pale yellow and taller than the healthy ones. Such seedlings die either before or after transplanting.

In current years the disease has gained its importance in non-traditional rice growing areas also. The bakanae disease incidence has become serious concern in aromatic rice varieties in Northern parts of India viz.Punjab, Haryana and Uttar Pradesh. Both quality and quantity of the rice crop affected due to bakanae disease under field conditions.

As the first process of infection and colonization of the host is the germination of the spores, therefore spore germination for a plant pathogenic fungus may be seen as a critical developmental step in pathogenesis. It symbolizes a key step in the colonization of new environments by filamentous fungi. Once dormancy is broken, spores undergo a distinct set of morphogenetic events that lead to the formation of a polarized growth axis and the emergence of one or more germ tubes [7, 8]. Germination must occur at both the correct time and location in order for infection to be successful. Many studies have reported conidial germination of other fungal pathogen such as macroconidia of *F. graminearum* in vitro preferentially germinate at terminal cells [9]. conidial germination of the filamentous fungus *Fusarium graminearum* [10]. The microconidia germination of the tomato pathogens *F. axysporum f.* sp. *lycopersici* (Fol) and *F. axysporum f.* sp. *radicis-lycopersici* (Forl) in the presence of root exudates was studied by Steinkellner et al. [11]. Zhang et al [12]. studied the germination of microconidia in the rice blast fungus Magnaporthe oryzae [12]. The temporal and spatial regulation of these events usually reveals the biology and habit of a given fungus. For example germination of conidia produced by the saprophytic fungus *Aspergillus nidulans* is triggered by the presence of glucose and requires several hours for completion [13]. By contrast, in the plant pathogen *Magnaporthe grisea,* conidial germination is comparatively rapid and needs only prosence of water [14]. In exceptional cases (i.e. *Colletotrichum gloeosporioides*) [15], conidia display alternate patterns of morphogenesis depending on whether the fungus is initiating saprophytic or pathogenic growth. However, studies regarding conidial germination of *F. fujikuroi* have not been documented till now and the information regarding the effect of different substrates on conidial germination of *F. fujikuroi* is not available so the present study has been carried out.

Materials and Methods

Fungal strain and inoculum preparation

Virulent isolates of *Fusarium fujikuroi* F 250 was used throughout the experiment. Conidial inoculum was produced in minimal medium incubated for 7 days at 25^0C. The culture solution was filtered using a sterilized muslin cloth and centrifuged at 3000 g for 5 min. The pellet was washed once and re-suspended in sterile distilled water. The conidial suspension was quantified using a counting chamber (Heamocytometer) and diluted to a concentration of 1×10^6 conidia/ml.

Plant material

Rice genotypes pusa basmati 1509 taken from Division of Genetics, Indian Agricultural Research Institute (IARI), New Delhi, were used throughout the experiments. Experiments were conducted in laboratories and glass house of Division of Plant Pathology, IARI, New Delhi, situated at 28^0 38´23´´ N and $77^0$9´27´´E.

Examination of conidial germination of *F.. fijikuroi*

Conidial germination was observed by following methodology of Apoga et al. [16] in which 100 µL droplet of the 10^6 conidia/mL. inoculum of F 250 were applied on pre-cleaned glass slide smeared with 2% water agar. Then glass slides were kept in para film seraled petri dish and were incubated at 25^0C later on the germination of conidia was observed at 3, 6, 9, 12

Table 1. Germination (%) and length of germ tube of *Fusarium fujikuroi* in different rice tissue suspension at different time of incubation. *SD: standard deviation, hoi-hours of incubation.

Treatments	Germination (%)				Germ tube length (µm) at different hour of incubation	
	3 hoi	6 hoi	9 hoi	Mean	6 hoi	9 hoi
Water	76	76	82	78	42.00 (SD ± 20.17)*	103.2 (SD ± 34.01)
Root	80	91	95	88	26.20 (SD ± 5.05)	26.00 (SD ± 8.15)
Stem	65	89	97	83	44.8 (SD ±11.34)	52.4 (SD ± 15.36)
Leaf	69	85	92	82	53 (SD ± 17.82)	76.80 (SD ± 17.02)

and 24 h of incubation and each slide were observed at single time point. The effect of different substrate such as water, root, leaf and stem on conidial germination was studied. For which root, leaf and stem part of rice variety pusa basmati 1509 were washed in running tap water and dried. Individual plant parts were crushed with the help of mortar and pestle in 100 ml of distilled water. Plant extracts were filtered through muslin cloth. Later suspension were placed onto the cavity slides and the 100 µL. droplet of the 10^6 conidia/mLinoculum of F 250 was placed onto the cavity slides and germination of conidia was observed at 3, 6, 9, 12 and 24 h of incubation and para film sealed petri dish were incubated at 25^0C. Each slide was observed at single time point.

Results and Discussion

The microconidia are 1-2 celled with fusiform oval shape. The macroconidia are of slightly sickle or sometime straight in shaped [17]. 3 h of incubation conidial suspension onto a cavity slide, tips of conidia were slightly swollen. At 6 h of incubation germ tube formation started from the tips of the conidia (Fig. 1a, b),

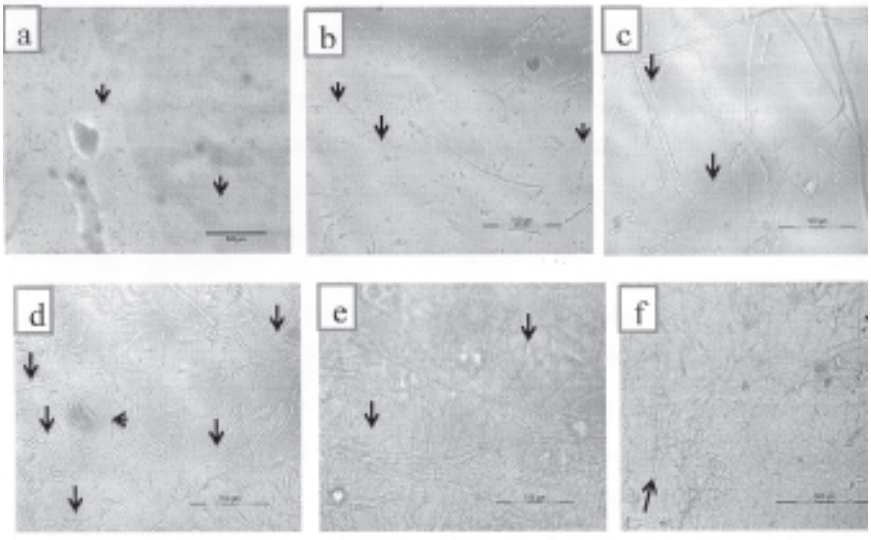

Fig. 1. (a-f) Conidial germination of *F. fujikuroi* iosolate F 250 in water at different hours interval : a) 3 h of incubation (hoi) tips of conidia were slightly swollen, b) 6 hoigerm tube formation started from the tips of the conidia, c) and d) 9 hoimaximum germination of conidia occurred and the length of germ tube increased, e) 12 hoilength of germ tube elongated, branching started and mycelium spread, f) 24 hoi dense mycelium was observed. Arrowheads represents germ tube emergence in Fig. 1 a, b, c, d and in Fig. 1 e, f represents dense mycelium.

later on at 9 h of incubation the maximum germination of conidia occurred and the length of germ tube has also been increased (Fig. 1 c, d). The length of germ tube elongated, branching started and mycelium spread after 12 h and dense mycelium was observed 24 h of incubation (Fig. le, f).

We also study the effect of different substrate on conidial germination and germ tube length in which we found that the conidial germination percentage was maximum in root suspension (88%) followed by stem (83%), leaf (82%) and water (78%) at 9 h of incubation. The maximum length of germ tube (103.2 µm) was observed in water followed by leaf (76.80 µm), stem (52.4 µm) and root (26.00 µm) at 9 h of incubation (Table 1).

Spore developmental processes are critical for the reproduction and survival of fungi. By way of conidial formation and dispersal, fungi proliferate, expand in range and can explore different niches. Once dispersed, dormant fungal spores detect appropriate activation signals and initiate the process of germination. The spore germination for a plant pathogenic fungus may be seen as pivotal step in pathogenesis as well as in establishment of the fungus on a suitable substrate or host. The present study on conidial germination of *Fusarium fujikuroi* causing bakanae disease of rice was performed using light microscope. 3 h of incubation tips of conidia were slightly swollen, germ tube formation started from the tips of the conidia at 6 h of incubation, later on the maximum germination of conidia occurred and the length of germ tube has also been increased at 9 h of incubation. The length of germ tube elongated, branching started and mycelium spread after 12 h and dense mycelium was observed at 24 h of incubation. Similar kind of results also found in the conidial germination of the filamentous fungus *Fusarium graminearum* i.e. swelling of conidia at 2 h of incubation, germination tube emergence and elongation from conidia at 8 h of incubation and hyphal branching at 24 h of incubation by Seong et al. [10]. The conidia of *F. fujikuroi* started germinating at 6 h of incubation and maximum germination was found at hours of incubation. Maximum conidial germination percentage was observed in root but lower the length of germ tube, which was found maximum in water at 9 h of incubation. The host signal may have role in the conidial germination and length of germ tube formation. Root exudates are an integral part of the concept of inoculum potential for the stimulation of plant pathogens [18]. Propagules of several plant pathogenic fungi have been shown to germinate in the presence of root exudates [19].

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