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Isolation of *Curvularia affinis* Causing Rice Leaf Spot from West Bengal Rice Field and Optimization of Culture Conditions

Madhusree Halder[1], Surekha Kundu[2]

Molecular and Applied Mycology and Plant Pathology Laboratory, Department of Botany, University of Calcutta, Kolkata, India[1, 2]

A**bstract:** Typical *Curvularia* leaf spot disease of rice was observed in a rice field of Barasat area in West Bengal and the pathogen isolate was identified as *Curvularia affinis* Boedjin, 1933 (PP /2883) by ITCC. It is one of the six main pathogenic fungi that infect rice and cause yield loss. This is the first report of *C. affinis* infection from West Bengal. In this study we isolated, identified and optimized the vegetative growth media and sporulating media for *C. affinis.* The hyphal branching pattern on different media was also observed at specific time intervals. Here it has been found that potato dextrose agar (PDA) media was best suited for vegetative growth compared to oat meal agar (OMA), malt extract agar (MEA) and plain agar media whereas for conidia and conidiophore development OMA was found to be superior over the other three media. This basic information has relevance towards culture of *C. affinis* in the laboratory for further studies of the host pathogen interaction and disease development in rice.

Keywords: *Curvularia affinis*, rice disease, media, radial growth, hyphal branching.

I. INTRODUCTION

Fungi survive on diverse habitats in their own niche and are cosmopolitan in distribution requiring several specific elements for their growth and reproduction. Phyto-pathogenic fungi that infect different plant parts have been isolated by different methods [1]. [2]. In laboratory these are isolated and cultured on specific culture media and maintained under control condition for cultivation, preservation, biochemical-physiological-molecular characterization and other research works. For proper maintenance several parameters were standardized such as media composition [3]. [4]. pH of media and temperature [5].

Most of the filamentous fungi are heterotrophic in nature, they require external source of organic compounds to draw nutrition. Moreover different fungi have different nutritional requirement for their growth, *Aspergillus niger* showed better growth in soybean dextrose broth while *Rhizopus sotolonifer* preferred ground nut dextrose broth [6]. The organizing centre for hyphal growth and morphogenesis is called the spitzenkorper. It plays a crucial role in fungal growth at hyphal tips controlling polarized growth and branch formation, in Ascomycota as well as most of the families under the kingdom fungi [7]. Polarisomes control the position of spitzenkorper which partially dependent on water and ion influx [7]. Nutrients present in the medium is crucial to determine the growth pattern, sporulation and pigment production in fungi; 2% carbon and 2% nitrogen source is essential for sporulation and pigmentation [8]. Different pH of the same media can inhibit or promote the growth of fungi and thus can control the biomass production [9].

Till date some of *Curvularia* species have been reported as causal agent of leaf spot, leaf blight, black kernel, sheath blight, sheath rot and grain discoloration of rice from different part of the world [10], [11]. In 2002 de Luna reported the disease study of *C. tuberculata* with its compatible host Rice (*Oryza sativa*) and this strain was isolated from Cyperaceae family from Philippines and further subculture in half strength PDA.
One of the highly virulent strains of *C. lunata* was isolated from Maize and subsequent genome sequence analysis found that it was evolved from *Cochliobolus heterostrophus*. Two main genes of *C. lunata* polyketide synthase and non-ribosomal peptide synthase were responsible for production of secondary metabolites that facilitate production of melanin and host specific/ non-specific toxin production [12]. *C. lunata* was found to be pathogenic to dicots also. *Amaranthus spinosus*, aprotein rich herb, commonly known as pig weed found throughout India is infected by *C. lunata* at Rajasthan area [2].

II. MATERIALS AND METHODS

A. Collection of sample: Infected Rice leaves were collected from Barasat area North 24Pgs (S) West Bengal.

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B. Isolation of pathogen: Fungal pathogen from infected rice leaves was isolated according to the protocol of Sharma et al, 2011 with little modifications. Infected rice leaves were rinsed with sterile distilled water and then greyish brown area were cut into small pieces with sterile scissor and forceps and put into 0.5% Sodium hypochlorite (NaOCl) solution for 2-3 min. It was rinsed with several changes of sterile distilled water (each 2 min). Surface sterilized leaf pieces were put on agar media and kept in dark at 28 ± 1^0C. After 2-3 days fungal mycelia emerged from the leaf disc and it was allow to grow up to 7 days, then a little amount of mycelia were put into nutrient rich PDA media.

C. Identification of the isolate: Fungus was grown on PDA and PDB (Potato dextrose broth) media in 28 ± 1^0C for further studies. Some slant cultures were prepared for long term preservation and also sent for identification to Indian Type Culture Collection (ITCC), Agricultural Research Institute (IARI), New Delhi.

D. Chemicals: Agar powder, Oats powder, Malt extract, dextrose was obtained from HiMedia Laboratories Pvt. Ltd Mumbai, India.

E. Preparation of growth media: To optimize the growth condition of the fungus four different media, Agar media, PDA, Oat Meal Agar (OMA), and Malt Extract Agar (MEA) were prepared and inoculated with the isolated fungus.
Agar Media: 15 g/l agar powder was added to distilled water and sterilized it at 121^0C temperature under 15psi using autoclave.
PDA Media: 20 gm. of raw potato was cut into small pieces, boiled into distilled water for 20 min, and add 60 gm. of dextrose into it, volume make up to 1litre then add 15gm /l agar powder as solidifying agent then sterilize in autoclave.
OMA media: Prepared as instructed by HiMedia, 60gm. of Oat meal powder was added to distilled water and boiled for 10-15 min to melt the oats in water, volume made up to 1 litre. 15 gm/l of agar powder was added and then sterilized.
MEA media: 20 gm. of Malt extract was added to 1 litter of distilled water, after dissolving the powder 15 gm. of agar powder was added followed by sterilization of media.

F. Experimental set-up: 20ml of each media was poured into 90mm diameter petriplates and allowed to solidifying, followed by inoculation of each media with 3mm of inoculum disc with the help of sterile cork borer. These were incubated in dark at 28 ± 1^0C and observed under regular time intervals up to 4 days. For detailed observations, the mycelia was observed after 24 and 48 Hrs. of inoculation, under compound microscope (Lieca DFC450C).

III. RESULTS AND DISCUSSION

Isolation of fungus from leaf spot: The spots which were oval to irregular in shape, outer periphery was dark brown while central part of the spot was greyish white in colour were cut out and placed on media. After 7 days of inoculation on PDA plate profuse mycelial growth was observed.

Identification of the isolate: Isolated fungus has been identified as *Curvularia affinis* (PP /2883) by the Indian Type Culture Collection (ITCC), Identification/ Culture Supply Service, Division of Plant Pathology. Indian Agricultural research Institute (IARI). New Delhi.

Optimization of growth on different media: The fungus shows proliferated growth on Potato Dextrose Agar media as the radial diameter is 60mm followed by 54mm in agar media after 4days post inoculation but in case of agar media not much aerial hyphae was observed and the culture is hyaline. Whereas in case of OMA and MEA media radial growth was very small i.e. 29mm and 29.4mm respectively, but more aerial hyphae grown and the fungus is greyish black in colour within 48hrs post inoculation (**Figure 1 A-E**).

The data revealed that the mycelial growth preferred PDA media followed by Agar media, but in agar media flat type of hyphae were seen to grow. These two types of colony morphology, both in terms of produced hyphae and colour of the colony depends on growth media and their ingredients [8]. In case of OMA and MEA mycelial growth was slower but colour of colony was dark gray. After 48 hours, microscopic observation revealed conidiogenesis and conidia development in those two media. OF the two media, frequency of sporulation was much higher in OMA than MEA. No conidiophore development was found in PDA and agar media after 48 hrs post incubation (**Figure 1 F-I**). From this data we can conclude that OMA and MEA promote spore formation or sporulation in *C. affinis* rather than extensive vegetative growth.

Branching Pattern on different media:
The same trend was also found while studying the branching pattern of mycelia on those different media after 24 and 48 hrs post incubation (hpi). In case of Agar media and PDA media primary, secondary and even tertiary branching were also found only after 24 hpi, but in case of OMA and MEA media only primary and secondary branching were

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found an 48hpi (**Figure 2 A-H**). In water agar the hyphae found had less elasticity and looked feeble and grow solely in close contact with the surface of media with no cottony mass formation. While on PDA media 3 types of branches were very much prominent and the number of branches was much higher than that on agar media. In OMA and MEA tertiary branching was completely absent and the number of first and second order branches was less at 24 and 48hpi (**Figure 2I-J)**.

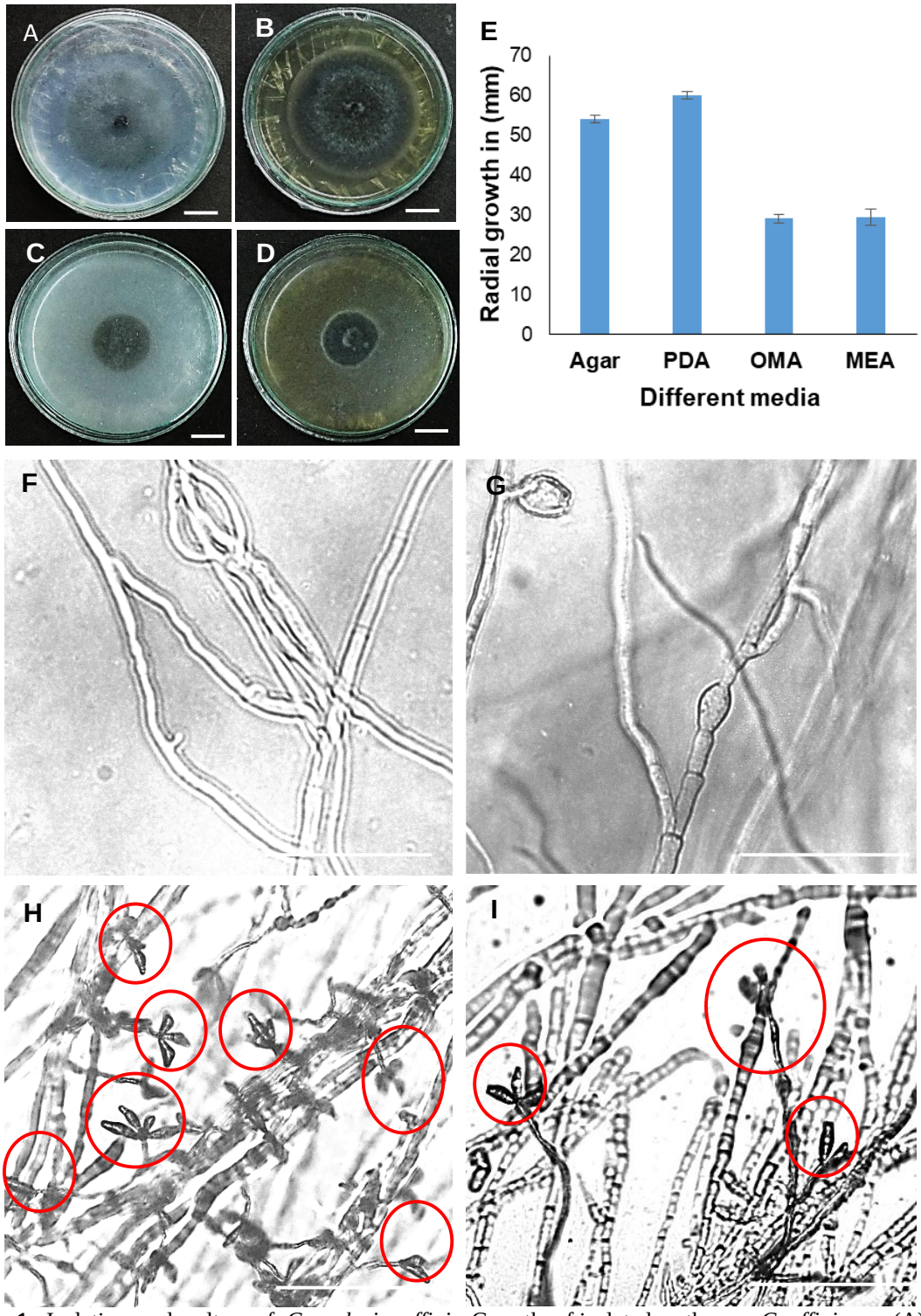

Figure 1: Isolation and culture of *Curvularia affinis*. Growth of isolated pathogen *C. affinis* on (A) Agar (B) potato dextrose agar (PDA) (C) oat meal agar (OMA) (D) malt extract agar (MEA). (E) Graphical representation of comparative radial growth of *C. affinis* of different media, Bar=1.5mm. (F,G) No sporulation seen on Agar and PDA media after 48 Hrs.(I,J) Sporulation occurred on OMA and MEA media after 48 Hrs. Bar=50µM.

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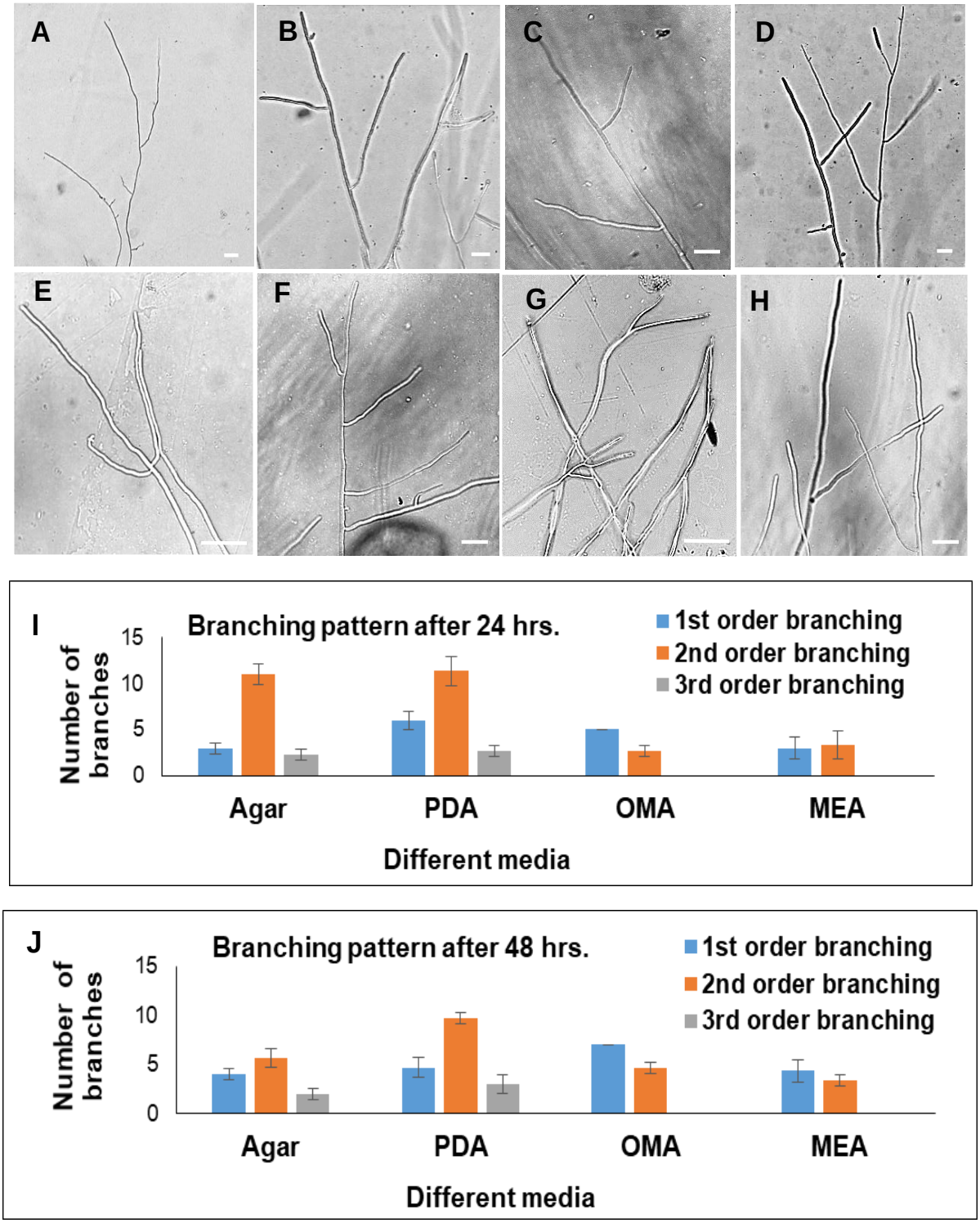

Figure 2: **Branching pattern of *C. affinis* hyphae on different media.** (A,E) Branching on Agar media, (B, F) On PDA, (C, G) On OMA, (D, H) On MEA after 24 and 48 hrs respectively. (I,J) Graphical representation shows number of different types branching on different media at 24 and 48 hrs post incubation.

CONCLUSION

The result indicate that different growth media are suitable for different growth purposes for *C. affinis* and have relevance for choosing culture conditions for better vegetative growth or early sporulation. Conidia are the main agents for spread of infection in *C. affinis*. This basic information has relevance towards culture of *C. affinis* in the laboratory for further studies of the host pathogen interaction and disease development in rice.

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