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Studies on morphological and cultural characters of *Colletotrichum lindemuthianum* inciting Anthracnose of *Dolichos* bean

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The experiment was conducted to know the morphological and cultural characters of *Colletotrichum lindemuthianum* (Sacc & Magn.) Scriber causal organism of an thracnose disease of *Dolichos* bean. It produced hyaline, single celled, oblong, sickle shaped, sometimes with one end slightly pointed spores. The length of conidia ranged between 10.5-15.5 µm and with a breadth of 3.5-4.5 µm. The maximum spore germination of 56 per cent was recorded at 2 per cent sucrose solution after 24 h of incubation where as the least spore germination (15.0 %) was observed in distilled water at 24 h of incubation. The maximum mean mycelial growth of 81.00 mm was recorded on potato dextrose agar followed by Richard's agar (79.00 mm), whereas least mean mycelial growth of 60.00 mm was recorded in Sach's agar medium. Pathogen produced brownish white, uniform, circular, fluffy growth in center with concentric rings and appressed growth at periphery on potato dextrose agar.

Key words: Acervuli, spore germination, sucrose solution

INTRODUCTION

The food legumes play a very important role in diet of poor people throughout the world. Among legumes, *Dolichos* bean (*Dolichos lablab* L.) is an important pulse-cum-vegetable crop. Anthracnose is an important disease of *Dolichos* bean caused by *Colletotrichum lindemuthianum* (Sacc & Magn.) Scriber, which is a fungal disease of a great significance of affecting all plant parts *viz.*, leaves, stems, branches and seeds and may cause considerable damage when infected seeds and susceptible cultivars are used under weather conditions favourable to the development of epidemics. This pathogen distributed worldwide, causes economic losses. The variation in terms of symptom expression on different parts of the plant depends on the virulence of pathotypes. In India, it is prevalent in all *Dolichos* bean growing states and causes an annual loss of up to 26.11 per cent (Rajesha *et al.*, 2010). *Colletotrichum* exhibits different morphological and cultural variation among species under different environmental conditions. The race structure of *C. lindemuthiaum* is highly variable and new ones reportedly keep emerging time after time. The existence and emergence of this variability leads to change in morphological and cultural characteristics of pathogen. Therefore, information on the morphological and cultural characters of *C. lindemuthianum* is less and requires for an accurate method for identification and characterization of pathogens for effective disease management. Hence, there is a need to study the morphological and cultural characteristics of pathogen. Therefore in the present investigations, an attempt has been made to know the spore morphology, spore germination and cultural characters of the pathogen.

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MATERIALS AND METHODS

Isolation of C. lindemuthianum

Anthracnose-infected bean seeds of 'Hebbal Avare-3' variety were obtained from AICRP on Pigeonpea, UAS, GKVK, Bangalore. The seeds were surface sterilized by immersing them in 0.5% NaOCl for 3 minutes. After which they were rinsed in sterile distilled water, blotted dry on sterile filter paper, and placed on potato dextrose agar medium. Streptomycin sulphate (0.1 g/l) was added to suppress bacterial growth (Dillard and Cobb 1993). The cultures were incubated in darkness at 20-21°C. The hyphal tips of fungi growing from the seeds were transferred aseptically to PDA slants and *C. lindemuthianum* was identified based on descriptions by Mordue (1971).

Morphological characters of C. lindemuthianum

Seven day old culture plate, grown on PDA medium was used for study of spore morphology and measurements of *C. lindemuthianum*. Spore characteristics such as size, shape and colour were observed under the microscope, after preparing slide. Micrometric technique (Tuite and John, 1969) was followed to measure spore length as well as spore breadth. For measurement of spore size, a total of 20 observations were taken and the average of all the observation was recorded.

Hanging drop method was followed to study the spore germination in different substrates. Five different substrates *viz.*, 1, 2 and 5 per cent sucrose solutions, sterile distilled water and tap water were selected to study the germination of spores. One drop of spore suspension was prepared from seven day old culture and mixed with a drop of substrate solution and placed on the centre of a clean cavity slide and covered with a cover slip. Those cavity slides were placed in a Petriplates lined with four fold moist blotting paper and incubated at room temperature of 28±1°C. The experiment was replicated thrice. Slides were examined after 24 h of incubation and recorded the number of spore germinated. The total number of spores and the number of spores germinated were recorded in different five microscopic fields under l0X objective. The germination percentage of spores in different substrates was calculated using the following formulae.

$$\text{Germination percentage (\%)} = \frac{\text{Number of spores germinated}}{\text{Total number of spores}} \times 100$$

Growth of Colletotrichum lindemuthianum on different media

The study was conducted to describe the cultural characters such as colony colour, growth pattern, type of margin and texture on different solid media. For this study, nine different media *viz.*, Brown's agar, Czapek's agar, Mathur's medium, Potato dextrose agar (PDA), Richard's agar, Sach's agar, Tochina's agar and Yeast extract agar were used. In each medium, all the ingredients of media except agar were dissolved in 500 ml of distilled water. The agar was melted in 500 ml distilled water. Both solutions were added and media were autoclaved at 121°C for 20 minutes.

Approximately 15 ml of each of the sterilized medium was poured in to the sterilized Petriplates under aseptic conditions and allowed to solidify. Inoculation was made by placing 5 mm mycelial discs obtained from seven days old culture and placed in the centre of each Petriplates on solidified surface in each media. The Petriplates were incubated at 28±1°C. The cultural characters and the colony diameter (mm) were recorded from all the treatments.

RESULTS AND DISCUSSION

Morphological studies of the C. lindemuthianum

The morphological characteristics and measurements of the spore of *C. lindemuthianum* were recorded from the fully grown culture in the Petriplates. Spore size was measured with the help of ocular micrometer after calibrating in the microscope. The fungus produced acervuli fruiting bodies. The spores of *C. lindemuthianum* were hyaline, single celled, oblong, sickle shaped, some times with one end slightly pointed end. The length of conidia ranged between 10.5-15.5 µm and with a breadth of 3.5-4.5 µm.

The per cent spore germination of *C. lindemuthianum* was studied on different substrates. The maximum spore germination of 56 per cent was recorded at 2 per cent sucrose solution after 24 hrs. of incubation and 35 per cent, 22 per cent of spore germination at 18 and 12 h of

Table 1 Conidial germination of *Colletotrichum lindemuthianum* in different substrates.

Sl. No.	Substrates	Per cent spore germination		
		12 h	18 h	24 h
1	Sucrose (1 %)	16	24	49
2	Sucrose (2 %)	22	35	56
3	Sucrose (5 %)	13	19	32
4	Sterile water	8	13	27
5	Distilled water	3	9	15
6	Tap water	16	23	39

incubation respectively, followed by 39 per cent of spore germination was recorded at 1 per cent sucrose solution at 24 h of incubation. The per cent spore germination was least in distilled water (15.0 %) at 24 h of incubation (Table 1).

Table 2 Growth of *Colletotrichum lindemuthianum* on different solid culture media

Name of the media	Mean Colony diameter in mm *
Czapek's agar	69.67
Richard's agar	79.00
Sach's agar	60.00
Tochina's agar	65.67
Mathur's medium	65.33
Yeast extract agar	61.33
Potato dextrose agar	81.00
Brown's agar	72.33
SEm+	0.69
CD (0.01)	2.09
CV (%)	1.74

* Mean of three replication

Cultural studies

The growth and cultural characteristics of *C. lindemuthianum* was studied on eight different media the data were furnished in Table 2. There were significant differences among the media requirement of *C. lindemuthianum*. The maximum mean colony diameter of 81.00 mm was recorded on potato dextrose agar which was statistically at par with Richard's agar (79.00 mm) and significantly differed with Brown's agar (72.33 mm) and Czapeck's agar (69.67 mm). The least mean colony diameter of 60.00 mm was noticed on Sach's agar medium. Since the maximum mean colony diameter of *C. lindemuthianum* was recorded on PDA, the culture of the anthracnose fungus was maintained on this medium.

Variations in colony characters of *C. lindemuthianum* on various media were recorded and the data were presented in Table 3. Observations on colony characters like colour, growth pattern, type of margin and texture of the colonies on different media were recorded.

The results indicated that *C. lindemuthianum* produced brownish white, uniform, circular, fluffy growth in center with concentric rings and appressed growth at periphery on potato dextrose agar. On Richard's agar, fungus produced whitish brown, uniform, circular, regular and partially fluffy growth. Whereas in case of Sach's agar the fungus produced dull white, circular, regular, ununiform, poor and appressed growth.

Table 3 Colony characteristics of *Colletotrichum lindemuthianum* on different solid media

Media	Colony Characters			
	Colour	Growth	Margin	Texture
Brown's agar	Brownish white	Uniform and circular	Regular	Appressed
Czapek's agar	Blackish white	Uniform and circular	Regular	Appressed with concentric rings
Mathur's medium	Dull brownish white	Uniform and circular	Regular	Appressed with concentric rings
Potato dextrose agar	Brownish white	Uniform and circular	Regular	Fluffy in center with concentric rings and appressed at periphery
Richard's agar	Whitish brown	Uniform and circular	Regular	Partially fluffy
Sach's agar	Dull white	Ununiform, circular and Poor	Regular	Appressed
Tochina's agar	Brownish white	Uniform and circular	Regular	Appressed
Yeast extract agar	Dull white	Uniform and circular	Regular	Appressed with concentric rings

The morphology of *C. lindemuthianum* was studied. The spores of the fungus were hyaline, single celled, oblong, sickled shaped sometimes with one end was slightly pointed. The result of spore morphology of our experiment was similar to the study conducted by Tiffany and Gilman (1954) who reported the similar result of hyaline, single celled, oblong, sickled shaped sometimes with one end was slightly pointed end spores and length of conidia ranged between 9.50-11.50 µm and with a breadth of 3.50-4.50 µm. Similar report also have been made by Kulshrestha *et al.* (1976). In mycological literature, the morphological characters of fungi often are described without adequate definition of the medium used and other growth conditions. In our experiment, the conidia were found to vary widely in size and shape, because the description of conidia varied based on measurements of conidia produced not on natural substrate under variable environmental conditions.

Germination is the initial stage in the development of a fungus mycelium from its spore. The germination of a fungal spore is a complex process involving spore swelling and then emergence and growth of the germ-tube. But before emergence of a germ-tube many spores will depends primarily on its stored reserves and an exogenous supply of nutrients to be available for metabolism and consequently spores will germinate (Plascencia-Jatomea *et al.*, 2003). Study on spore germination of *C. lindemuthianum* was done on different substrates. Spore germination was highest in 2 per cent sucrose solution followed by 1 per cent sucrose solution. Distilled water was less efficient substrate for spore germination. Because it seems that besides water, there are other chemical stimulates or factors which trigger the spore germination which are present in sucrose. But, higher per cent of sucrose concentration was found to be toxic for the spore germination. Lower per cent of spore germination in distilled water was due to absence of certain nutrients required for spore germination. The similar report by Mahuku and Goodwin (1998) showed that the sucrose solution stimulated significant germination of *C. gloeosporioides* spores.

Fungi grow on diverse nutritional requirement for their growth and reproduction. A wide range of media are used for isolation that influence the vegetative growth and colony morphology, pigmentation and sporulation depending upon the composition of specific culture media (Sharma and Pandey, 2010). In this experiment, the maximum growth of *C. lindemuthianum* occurred on Potato dextrose agar and it was best medium for growth of the fungus followed by Richard's Agar but no growth was reported in Sach's agar medium. Hiremath *et al.* (1993) also noticed the significantly higher radial growth on Richard's agar and Potato dextrose agar. Venkatravanappa *et al.* (2006) also reported that maximum radial growth was found on PDA. The present study established that Potato dextrose agar and Richard's agar media supported maximum growth of the fungus.

There is a great difference between the cultural characters on different nutrients. The *C. lindemuthianum* produced brownish white, uniform, circular, compact at periphery but raised center, regular and fluffy growth with concentric rings were observed on PDA medium.

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