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Morphological Studies on Red Rot of Sugarcane from Hardoi District of Uttar Pradesh

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Abstract **– Sugarcane is an important cash crop and used as the chief source of sugar grown tropical and subtropical region in India. Sugarcane production is challenged by various biotic and abiotic stresses among the biotic factors, red rot disease caused by Colletotrichum falcatum is a major disease leading to severe reduction in sugarcane production. Cultural, morphological studies conducted under in vitro in Oat Meal Agar (OMA) showed characteristic variation in their conidial and colony characters which were five isolates collected from various places in Hardoi district of Uttar Pradesh. Thus present study brings the cultural morphological variations and virulence characters of *Colletotrichum falcatum*.**

Keywords **– Red Rot, Morphology, Variation, Sugarcane.**

I. INTRODUCTION

Sugarcane (*Saccharum officinarum* L.), belong to the family Poaceae, is an economically important cash cum industrial crop grown in the tropical and sub-tropical region in India. Many biotic and abiotic stresses affected sugarcane quality and yield. Sugarcane diseases caused by fungi, bacteria, virus and mycoplasma, such as fungal diseases become major problems for the sugarcane growing countries. Red rots major fungal disease occur all sugarcane growing state in India. Red rot disease is the oldest serious fungal disease of sugarcane, generally called "Cancer" of sugarcane is caused by *Colletotrichum falcatum*. Disease incidence depends upon the varieties, localities and favourable environmental condition. The disease was first described from Java now Indonesia by Went [27], who called the fungus, *C. falcatum* and named the disease as "het rood snot" meaning "red smut". The sexual stage of *C. falcatum* was later reported by Spegazzini [23] in Argentina who named it *Physalospora tucumanensis*. Barber [4] first observed this disease in Bihar (India) and Butler [7] coined the name "Red Rot", the name by which it is known till date. Later, the red rot causal organism was reclassified by Von Arx and Muller [3] and included in the genus *Glomerella* as *G. tucumanensis*. In India, the first documented epidemic of red rot occurred in 1895-1901 and in subsequent years a number of major outbreaks have been recorded as a regular event in the sub-tropical and tropical regions of the country [22]. This disease has been hold accountable for 5 to 10% cane yield and sugar recovery loss worldwide. Red rot is considered as the major constraint for sugarcane production in India [26]. It has been reported as damaging disease of sugarcane cultivars in Australia, Bangladesh, Pakistan, Taiwan, and USA [25]. Red rot is widely distributed and has been reported in 68 sugarcane growing countries of the world [6]. Red rot infection in cane causes a loss of total weight to about 29.07% leading to 30.8% loss in sugar recovery [10]. The present study conducts cultural and morphological variability of *C. falcatum* based on their conidial and colony characteristics. In the present study, an attempt has been made to collect five isolates of *C. falcatum* Went prevalent in different sugarcane growing parts in Hardoi district of Uttar Pradesh in India.

II. MATERIAL METHODS

Survey and Collection of Disease Samples

An extensive survey of sugarcane growing areas various localities Loni, Hardoi, Baghauli, Rupapur and Hariyawan in Hardoi districts of Uttar Pradesh were conducted during July and August months of 2012-13. Varieties of sugarcane red rot disease symptoms of were collected for 5 isolates of *C. falcatum*. Strains were isolated from lesions on infected stem pieces. Symptoms of red rot disease on these cultivars were recorded. Red rot infected sugarcane sample collected from directly farmer's fields.

Table 1. Isolates selected place with cultivars of Hardoi district

Districts	Localities	Varieties	Isolates No.
Hardoi	Loni	CoS 98231	CFHALO
Hardoi	Hardoi	CoS 91269	CFHAHA
Hardoi	Baghauli	CoJ 64	CFHABA
Hardoi	Rupapur	CoSe 95422	CFHAHR
Hardoi	Hariyawan	Co 1148	CFHARO

Isolation of Colletotrichum falcatum:

Infected canes were split open by sterilized knife and observed for reddish tissue and white transverse band. The red rot pathogen was isolated by tissue segment methods as described by Rang swami [19], three 5-5 mm pieces of tissue were taken from the margin of infected tissues, surface sterilized by dipping in 1% sodium hypochlorite for 1 min, immersed in 70% ethanol for 1 min and rinsed three times with sterilized water and finally dried in sterilized tissue paper [1]. After 5 days of incubation, the plates having red sporulation were purified by sub-culturing. All the isolates were further purified by single spore technique [20]. The fungus from the pure cultures obtained was examined microscopically in order to match

it with the characters of the pathogen examined from the diseased samples. The pure cultures were maintained in Potato Dextrose Agar slants.

Samples were placed on water agar and incubated at room temperature (26 to 31°C). The growing edges of any fungal hyphae developing from the tissues were then transferred aseptically to oatmeal agar medium and fungi were identified following sporulation. Single spore subcultures were obtained for each isolate using the procedure described by Goh [9].

When the fungus showed sporulation, spore masses were pieced off with a sterilized weir loop and streaked on the surface of water agar. After inoculating overnight at 29 ± 2°C on biological oxygen demand (BOD), single germinated spores were picked with a sterilized needle and transferred to oat meal agar (OMA) medium. The cultures of different isolates were maintained on OMA slants at 4°C for further studies.

Morphological characters of the colony viz., colony colour, substrate colour, margin of colony and topography were recorded through naked eye and spores viz., size, colour and shape of the conidia were observed in binocular microscope with oculars lens. The three replicate mean values examined and the range was determined.

III. Results

In order to find morphological variations of Colletotrichum falcatum among the different parts of Uttar Pradesh, an extensive survey was conducted in major sugarcane growing areas of Uttar Pradesh covering (Loni, Hardoi, Baghauli, Rupapur and Hariyawan) Hardoi, 2012-2013.

Cultural, Morphology Characteristic of Colletotrichum Falcatum

The morphological characteristics of different isolates of Colletotrichum falcatum on OMA medium were studied; significant variations were observed with respect to Conidial and Colony characteristics base.

Table 2. Conidial characteristic of Colletotrichum falcatum

Isolates No.	Conidial Characteristics			
	Length (µm)	Width (µm)	Colour	Shape
CFHALO	25.2	4.7	Hyaline	Falcate
CFHAHA	25.4	4.4	Hyaline	Falcate
CFHABA	26.2	4.5	Hyaline	Falcate
CFHAHR	25.6	4.6	Hyaline	Falcate
CFHARO	25.5	4.5	Hyaline	Falcate

Table shows that, the highest conidial length of *C. falcatum* was recorded for isolate CFHABA as 26.2 µm, which was followed by CFHAHR as 25.6 µm, CFHARO as 25.5 µm, CFHAHA as 25.4 µm; whereas the shortest conidial length of *C. falcatum* was recorded for isolate CFHALO as 25.2 µm.

The highest conidial width of *C. falcatum* was recorded for isolate CFHALO as 4.7 µm, which was followed by CFHAHR as 4.6 µm, CFHABA as 4.5 µm, CFHARO as 4.5 µm; whereas the shortest conidial width of *C. falcatum* was recorded for isolate CFHAHA as 4.4 µm. All conidia were falcate shaped with hyaline colour in the base.

Table 3. Colony characteristic of Colletotrichum falcatum

Isolates No.	Colony Characteristics					
	Colony colour	Substrate colour	Margin	Topography	Colony (mm)	Sporulation
CFHALO	Greyish White	Black	Smooth	Raised Fluffy	87.8	+++
CFHAHA	White	White	Smooth	Raised Fluffy	85.7	+++
CFHABA	Greyish White	Black	Smooth	Raised Fluffy	89.6	+++
CFHAHR	Greyish White	Black	Irregularly	Raised Fluffy	85.5	+++
CFHARO	Greyish	White	Smooth	Raised Fluffy	86.4	+++

Note: +Poor sporulation: 1-10 spores / microscopic field (100X); ++ Medium sporulation: 11-50 spores/ microscopic field (100X), +++ Good sporulation: More than 100 spores/ microscopic field (100X)

Table shows that the maximum colony growth of *C. falcatum* was recorded for isolate CFHABA as 89.6 mm, which was followed by CFHALO as 87.8 mm, CFHARO as 86.4 mm, and CFHAHA as 85.7 mm, whereas the minimum colony growth of *C. falcatum* was recorded for isolate CFHAHR as 85.5 mm. The colony colour of *C. falcatum* was recorded for isolates CFHALO, CFHABA, CFHAHR greyish white, and CFHAHA white, CFHARO greyish. The substrate colour of *C. falcatum* was recorded for isolates CFHALO, CFHABA, CFHAHR, black and CFHAHA, CFHARO, white. The margin of *C. falcatum* was recorded for isolates CFHALO, CFHAHA, CFHABA, CFHARO, Smooth and CFHAHR, Irregularly. All isolates sporulation recorded well. Similar morphological variation revealed that there exists a wide variation among the isolates which is the basic method for characterization of different thirty isolates [15].

IV. Discussion

Conidial length; the isolate CFHABA recorded highest conidial length of *C. falcatum* which was 26.2 µm, while shortest conidial length of *C. falcatum* was recorded for isolate CFHALO as 25.2 µm. Conidial width; isolate

CFHALO recorded highest width of *C. falcatum* which was 4.7 µm, while shortest conidial width of *C. falcatum* was recorded for isolate CFHAHA as 4.4 µm. All conidia were falcate shaped with hyaline colour in the base.
Colony radial growth; isolate CFHABA recorded maximum colony radial growth of C. falcatum which was 89.6 mm, while the minimum colony radial growth of *C. falcatum* was recorded for isolate CFHAHR as 85.5 mm. The colony colour was recorded greyish white, white and greyish. The substrate colour was recorded black and white. The margin was recorded smooth and irregularly. All isolates sporulation recorded well.

Red rot caused by the fungus *C. falcatum* is dreadful and seed transmissible stalk disease which spreads from place to place through the infected sugarcanes [18], [13]. On the basis of the cultural characters of *C. falcatum*, Abbott [2] distinguished two races a light one producing white to light-grey, cottony mycelia, and a dark one with compact, velvety, dark-grey mycelia. Morphological diversity has been found in four isolates of *C. falcatum* from SPF234, CO1148, BF162 and SHF242 [1]. Variability in cultural and morphological characters and virulence and development of physiological races has been attributed to hybridization, mutation conidial and hyphen fusions [5]. Morphological variation revealed that there exists a wide variation among the isolates which is the basic method for characterization of different thirty isolates [17]. Similar results have been obtained by Malathi *et al*. [13], in a study with large number of isolates the growth of isolates has direct correlation with pathogencity and it revealed that the tropical isolates were light colored, fast growing and highly sporulating types. Prema *et al*. [15] observed wide variation in *C. musae* isolates with respect to cultural and morphological characters, the isolates produced blackish white, light pink and dark orange coloured colonies. Chona and Srivastava [8] sub-divided each of these two races into groups and sub-groups based on the texture of the mycelium and the degree of sporulation. They found that isolations made from diseased canes from localities affected with the red rot epidemic invariably yielded light, highly sporulating strains whether isolated from the diseased stalk or midrib lesions and the dark sparsely sporulating isolates were only rarely encountered in epidemic areas. Recent study, two types of colony morphology was classified in which the light type was observed more frequently than the dark type in 15 isolates of Thailand [21]. The light type was isolated from the stalks of sugarcane in both localities affected and not affected with the red rot epidemic. However, the dark type could be isolated from the mid-rib lesions and stalks of sugarcane and was found only in localities without the red rot epidemic. Red rot morphological characterization, the 15 isolates reported in the current study had conidial size differences from previous reports. Sutton [24] reported that the conidial size of *C. falcatum* ranged between 15.5 and 26.5 µm in length and from 4 to 5 µm in width. Kalaimani [11] examined six isolates of *C. falcatum* and found variation in the length and width between 30.62 to 37.65 µm and 6.69 to 8.46 µm, respectively. Mishra and Behera [14] revealed significant variation in the size of conidia of *C. falcatum* from India where the dimensions varied between 23.94 and 30.83 µm in length and from 3.28 to 3.69 µm in width. The current study found the length of the conidia of the 15 isolates varied between 21.42 and 28.56 µm which was less than in the earlier findings of Kalaimani [11] and Mishra and Behera [14]. In addition, the width of the conidia of the 15 isolates varied between 2.38 and 4.76 µm which were shorter than in the previous reports by Sutton [24], Kalaimani [11] and Mishra and Behera [14]. Prihastuti *et al.* [17] reported differences in the size of conidia (16–35 µm long, 4–5 µm wide).

V. Conclusion

The length of conidia ranged from 26.2 -25.2 µm. Width of the conidia ranged from 4.7-4.4 µm. Conidia were falcate shaped with hyaline colour in the base. The colony growth raged from 89.6 -85.5 mm. Different colony colours *viz.*, greyish white, white and greyish colours were observed. All the isolates showed variation regarding substrate colour, margin, topography, colony diameter and sporulation.

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