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Morphological, physiological and pathological variations among isolates of *Colletotrichum falcatum* that cause red rot of sugarcane

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Twenty-eight isolates were isolated from samples of different sugarcane varieties infected with red rot during this survey; the culture was maintained on slants and Petri plates at 29±2°C. These isolates showed variability in their optimum radial growth pattern. These isolates were morphologically differentiated into two distinct groups: fifteen had light colour and thirteen had dark colour. Cultural characters of the isolates were divided into five groups viz. light sporulating, sparsely light sporulating, light non-sporulating, dark sporulating and dark non-sporulating. The length of the conidia varied from 25.1 to 30.5 µm and the width of the conidia varied from 4.2 to 5.5 µm; however, all the conidia were found to be falcate. The virulence of various isolates on the cane stalk showed a wide range of variation. However, all the light sporulating and dark sporulating isolates showed more or less similar virulence against a set of standard susceptible cane varieties. In this study, 28 isolates were tested based on fourteen pathological standards, in which six (Cf208, Cf608, Cf908, Cf1508, Cf2109 and Cf2609) were found to be most virulent. We have found that *Colletotrichum falcatum* isolates were culturally, morphologically and pathologically dissimilar and six new races are found reported in India.

Key words: Red rot, *Colletotrichum falcatum*, *Saccharum officinarum*, races, virulence.

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.), belonging to the family *Poaceae*, is an economically important cash crop grown in the tropics and sub-tropical areas of India. Of all the sugarcane diseases such as fungal, bacterial, viral and phytoplasmal diseases, fungal diseases are gaining international importance (Bharti et al., 2012). Fungal diseases such as red rot, smut, wilt and pokkah boeng have become a major problem for the sugarcane growing countries. Red rot disease causes economic loss to the crop and it has a major incidence in sugarcane growing areas such as tropical and subtropical parts of India. Red rot disease often called 'Cancer' of sugarcane is caused by *Colletotrichum falcatum* Went. The varietal incidence of the disease varies from 2–64% depending upon the variety and locality. Red rot is responsible for the failure of many popular varieties in different countries (Satyavir, 2003). The disease was first described from Java (now Indonesia) by Went (1893), who called the fungus, *C. falcatum* and named the disease as 'het rood snot' meaning 'red smut'. Barber (1901) first observed this

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Abbreviations: **OMA**, Oat meal agar; **BOD**, biological oxygen demand; **IAA**, indole-3 acetic acid; **IBA**, indole-3 butyric acid; **RAPD**, random amplified polymorphic DNA; **URP**, universal rice primers.

disease in India and Butler (1906) coined the name 'Red Rot', the name by which it is known till date. The perfect stage of *C. falcatum* Went has been described as *Glomerella tucumanensis* (Von Arx & Muller) and is considered as the major constraint for sugarcane production in India (Viswanathan and Samiyappan, 2008). Red rot disease occurs when the plant withers by spores or ascospores and the pathogen makes its entry into the host tissues through any sort of injury injected by insects or borers or natural growth cracks. Red rot is widely distributed and has been reported in 68 sugarcane growing countries of the world (Bharti et al., 2012). 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 (Satyavir, 2003). This disease has been blamed for 5 to 10% cane yield and sugar recovery loss worldwide. It has been reported as damaging disease of sugarcane cultivars in Australia, Bangladesh, Pakistan, Taiwan, and USA (Viswanathan and Samiyappan, 2002).

The resistance of a variety is broken down after several years of cultivation probably due to the development of new races of *C. falcatum* Went. Therefore, continuous flow of new varieties resistant to red rot is the need of the day**. I**nformation is not available on virulence/pathotypes of *C. falcatum* prevalent in Uttar Pradesh and its correlation with morphological variability, if any. The isolates have been differentiated on the basis of morphology, physiology and host–reactivity parameters. Alvi et al. (2008) and Kumar et al. (2011) demonstrated that sugarcane resistant and susceptible genotypes to infection by *C. falcatum* had different genetic makeup using random amplified polymorphic DNA (RAPD) and Universal rice primers (URP) molecular markers. No information regarding morphological, physiological and pathological variations among the isolates is available in different states such as Uttar Pradesh and Bihar from India. The objective of the present study is to characterize the cultural, morphological and pathogenic variability among twenty-eight isolates of *C. falcatum* based on their morphological and pathological characteristics. In the present study, an attempt has been made to collect maximum number of isolates of *C. falcatum* Went prevalent in different parts of Indian states.

MATERIALS AND METHODS

Collection of diseased samples and isolation of *Colletotrichum falcatum* W.

A comprehensive survey of sugarcane growing areas of various districts in different Indian states was made during 2008-09 (Table 1). Twenty-one hybrid sugarcane disease symptoms of red rot were collected for 28 isolates of *C. falcatum*. Strains were isolated from lesions on infected stem pieces. 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 (Abbas et al., 2010). 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 (1999). 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.

Cultural growth of *C. falcatum* isolates

Pure cultures of all twenty-eight isolates maintained on slants were allowed to grow in Petri plates (90 mm diameter) containing 15 ml oat meal agar medium. The cultures were incubated at 29±2°C temperature on the BOD and growth of each isolate was measured at 48 h interval in mutually perpendicular directions up to 18 days; and average was calculated (Table 1).

Morphological characteristics

The mycelia discs (5 mm diameter) were taken from actively sporulating areas near the growing edge of 7 day old cultures, transferred to OMA medium and incubated at 29±2°C. The replicate cultures of each isolate were investigated and cultures were purified by single conidia, hyphae tip and identified on the basis of their morphological characters. Morphological characters like shape and size of mycelium, conidia and conidiophores of *C. falcatum* were examined. Colony characters, colour and growth diameter of all the isolates were observed.

For studying the degree of sporulation, the spores of each isolate were harvested in presterilized conical flask containing 100 ml of distilled water. The suspension was thoroughly mixed in warring blender for 3 min with the help of haemocytometer and the amount of conidia/ ml of distilled water was calculated. At least 3 replicates were maintained for each isolate and each observation was repeated twice and averages were calculated. The shape of the conidia was observed under high power of a microscope and the length and width of the conidia was measured after calibrating the microscope with oculars and stage micrometer in the Table 2.

Pathogencity assay

The experiment was carried out during 2010-2011 (Table 3) and the pathogenic variability of *C. falcatum* of twenty-eight isolates was tested on fourteen national pathological standard *viz*. Co419, Co975, Co997, Co1148, Co7717, Co62399, CoC671, CoS767, BO91, CoJ64, Baragua, Kakhai, SES594 and CoS8436 pathotypes. Artificial inoculation was done on healthy standing canes of 7 months old crop in the month of August using IISR inoculators developed by Kumar et al., (2010). Spore suspensions of all isolates were prepared in distilled water as described earlier for conidial germination. The concentration of spores was maintained to be 10^6spores/ml using a haemocytometer. The spore suspension was placed in a hole made with 20 ml hypodermic needle having 16-G size on the 3rd exposed internodes from bottom (Zai-ul-Hussnain et al., 2007). Spore suspension at a concentration of 10^6/mL was added, and the hole was sealed by modeling clay in order to prevent oxidation and contamination. Parameters for assessing the pathogenesis on the basis of the international scale (0 to 9) were adopted as suggested by Srinivasan and Bhatt (1961).

Table 1. Collection of infected cane varieties and cultural growth of 28 isolates of *C. falcatum* W.

States of India	Locality	Source of isolate	Isolate number	Radial growth (mm diam) in different days					
				03	06	09	12	15	18
Andhra Pradesh	Basar	Co1182	Cf108	06	18	36	59	74	90
	Farukhnagar	Co1251	Cf208	12	32	60	90	-	-
	Karimnagar	Co997	Cf308	06	22	46	86	90	-
Bihar	Betiya	CoSe01235	Cf408	02	14	36	56	82	90
	Champaran	BO99	Cf508	20	42	70	90	-	-
	Gopalganj	CoS8436	Cf608	08	18	40	70	90	-
	Raxaul	CoS767	Cf708	10	25	48	65	76	90
Haryana	Amballa	Co62268	Cf808	04	12	36	48	78	90
	Karnal	CoH15	Cf908	12	24	60	70	90	-
	Rohtak	CoH5	Cf1008	16	34	50	70	90	-
Punjab	Barnala	CoS767	Cf1108	18	40	70	90	-	-
	Jalandhar	Co7224	Cf1208	10	24	44	70	80	90
	Patiyala	Co975	Cf1308	11	24	45	66	70	90
Karnataka	Bagepali	Co6904	Cf1408	14	30	50	78	90	-
	Homanabad	Co62399	Cf1508	12	24	48	77	90	-
	Sira	Co7214	Cf1608	16	34	70	76	84	90
Maharashtra	Umari	CoC671	Cf1709	8	18	36	66	90	-
	Delur	Co997	Cf1809	10	34	70	90	-	-
Tamil Nadu	Coimbatore	Co8224	Cf1909	06	20	40	70	84	90
	Polachi	Co7214	Cf2009	14	30	70	84	90	-
Uttar Pradesh	Ballrampur	CoSe93232	Cf2109	10	25	40	65	84	90
	Balliya	CoS687	Cf2209	16	36	70	90	-	-
	Maharajganj	CoSe95422	Cf2309	12	26	56	69	70	90
	Pilibhit	CoS8436	Cf2409	07	14	30	45	76	90
	Saharanpur	CoSe01235	Cf2509	18	36	70	90	-	-
Uttarakhand	Nazibabad	Co975	Cf2609	10	24	56	72	90	-
	Pithauragharh	CoPant84214	Cf2709	05	14	39	56	67	90
	Tanakpur	CoS8436	Cf2809	08	16	46	60	75	90

For calculating the disease index, observations were recorded on the nature with condition of the top, lesion width, nodal transgression and white spots by splitting open the canes 60 days after inoculation.

RESULTS

Disease incidence and symptoms

Sugarcane leaves appeared as bright red rot with lesion on the mid rib of the leaves; and they were dry in the field. Infected canes splitted open, internodal tissues were red and interrupted with white streaks and gave out alcoholic odor. These symptoms were variable in appearance and were intense among cultivars, which led to this study. The different locations in tropical and subtropical parts of India had varietal incidence during 2008-09, *viz*. 0.5, 2, 5, 6, 8, 9, 12, 12, 14, 16, 18, 20, 24, 26 28, 30, 32, 34, 36, 38 and 46 on Co1182, Co1251, CoH5, CoS767, CoSe01235, CoS8436, CoS8224, CoSe95422, CoH15, Co62268, CoJ64, CoSe93232, Co997, C07214 Co975, CoPant84214, CoS687, Co7224, Co6904, CoC671 and BO99 sugarcane cultivars, respectively.

Cultural growth of *C. falcatum*

The results recorded in Table 1 revealed that there was considerable variation in the growth rate of 28 strains of different isolates. Six isolates exhibited maximum radial growth (90 mm diameter) in 12 days, while nine isolates attained the same growth in 15 days. The remaining thirteen isolates took 18 days to attain 90 mm diameter growth. However, on the 9^{th} day, the maximum radial growth of 70 mm diameter was obtained in isolates *viz*. Cf508 Cf1108, Cf1608 Cf1809, Cf2009, Cf2209 and Cf2509. While minimum growth of 36 mm diameter was obtained in Cf108, Cf408, Cf808, Cf1708 and Cf2409 during the same period.

Morphological characters

The former ones were designated as "Light type" and the latter as "Dark type". The colour and texture of the mycelia

nature and degree of sporulation appear to be inter-related to some extent with certain conidial characters as well as virulence. The details of the biotypes collected and their cultural and morphological grouping are summarized in Table 2. The colour and texture of mycelium, degree of sporulation, and granulation in conidia were recorded on 12th day under binocular microscope. The colour of mycelium of 15 isolates (Cf208, Cf2209, Cf1408, Cf908, Cf608, Cf2709, Cf308, Cf408, Cf2409, Cf2309, Cf2109, Cf1909, Cf2609, Cf508 and Cf108) was whitish to light grey, while 13 isolates (Cf1008, Cf2809, Cf1208, Cf1308, Cf2309, Cf1608, Cf708, Cf1809, Cf2009, Cf1709, Cf808, Cf2509 and Cf1508) were dark grey to olivaceous black. For example, in case of the 11 isolates (Cf208, Cf2209, Cf1408, Cf908, Cf608, Cf308, Cf2709, Cf408, Cf2109, and Cf2409 and Cf2309), of the highly light sporulating group, which showed high degree of virulence in the present study, the conidia were highly granulated but showed poor germination in water. Furthermore, conidia of these 11 isolates were generally longer with smaller ranges and frequency than those of isolates belonging to the other groups. Other conidial characters such as curvature, mutication to considerable extent were not found to be related to other characters. It is interesting to note that acervuli were formed in culture in only 7 out of 11 isolates of the highly light sporulating group. Their absence from the isolates of other groups shows this character is also related to some of the morphological characters as well as the virulence.

Variation in the morphological characters of all twenty-eight isolates of *C. falcatum*, out of 15 isolates of "light type", five isolates were sporulating with the presence of acervuli and setae. The degree of sporulation amongst these five isolates varied from 110x10^2 to 120x10^2 spores /ml and size of conidia (length x width) varied from 28.3x4.2 to 29.0x4.8 µm and was also granulated. Absence of acervuli with sporulation was found in 11 isolates, of which 7 were granulated viz. Cf908, Cf1408, Cf608, Cf208, Cf2209, Cf2708 and Cf308, and the remaining four *viz.* Cf808, Cf1508, Cf1308 & Cf2109 were slightly granulated. While, 4 isolates namely Cf1909, Cf2609, Cf108, and Cf408 were light non-sporulating and transparent population of conidia. The degree of lightness of sporulation amongst these six isolates varied from 36x10^2 to 105x 10^2 spores/ml and size of conidia also varied from 29.2 x 4.7 to 30.5 x 5.5 µm. Out of 13 isolates, 6 isolates *viz*. Cf1008, Cf708, Cf1709, Cf508, Cf1108 and Cf2309 were found to be dark sporulating; the degree of sporulation amongst these isolates varies from 96x10^2 to102 x 10^2 µm spores/ml and size of conidia (length x width) was from 25.4 x 4.5 to 30.4 x 4.8 µm; 7 isolates viz. Cf1608, Cf1809, Cf2409, Cf2809, Cf1208, Cf2009 and Cf1308 were dark non-sporulating whose size of conidia also varied from 22.4 x 4.2 to 25.5 x 4.6 µm, respectively. However, all the conidia were found to be falcate; 7 isolates were granulated, 4 isolates, slightly granulated and 17 isolates were of transparent conidial populations.

Pathogencity assay

The pathogenic variability recorded in Table 3 revealed 28 isolates disease reaction on 14 national pathological standards of sugarcane varieties in comparative studies using plug method. Isolate number Cf208 (source-Co1148 national pathotype) had similar reaction with Cf608, Cf908, Cf1408, Cf2209 and Cf2709 (source-CoS8436, CoH15, Co6904, CoSe01235 and CoPant2708, which were found most virulent). The rest isolates gave dissimilar reaction in all pathological standards, in which isolates Cf408, Cf808, Cf1909 and Cf2509 gave similar reaction in the same differentials. One genotype, SES594 resistant and moderately resistant, proved to be the same with all 28 isolates and the rest genotypes had variable reaction, respectively. 6 races belonging to the most virulent isolates are reported for the first time in tropical and subtropical belt of Indian states.

Physiological characters

Radial growth of *C. falcatum* in different solid media

The effect of different solid media viz. Asthana and Hawkers, Brown's agar, Capek's agar, Czapek's Dox, Elliots agar, Oatmeal agar, Potato dextrose agar, Richard's agar and Sucrose ammonium nitrate prepared as described on the growth of the selected isolates was calculated. The averages of the diametric growth calculated from 5 replicates are given in Figure 1. The results revealed that oatmeal medium followed by potato dextrose agar medium was best suited for the growth of all isolates. It was observed that oatmeal medium followed by PDA was best for the growth of all the isolates of *C.falcatum* under study.

Growth on liquid media of *C. falcatum*

The effect of different liquid media viz. Elliot's medium, dextrose yeast extract, Richard's medium, Asthana and Hawker's medium, Sucrose Ammonium Nitrate, Czapek's Dox, Brown's medium, Czapek's II medium, Dextrose Ammonium nitrate and Pfeffer's medium prepared as described in materials and methods and the average of 5 replicates are given in Figure 3. Result recorded for Richard's medium followed by Czapek's Dox liquid media was found to be the best medium for the growth of all the isolates of *C. falcatum* Went.

Effect of different temperatures on the growth of *C. falcatum*

Oat meal agar medium was prepared and 15 ml of the molten

Table 2. Morphological and cultural characters of twenty eight isolates of *C. falcatum* Went.

Isolate number	Colour and texture of mycelium	Degree of sporulation spore/ml	Conidial shape	Conidial size (LxW) µm	Population of conidia
Light porulating (presence of acervuli and setae)					
Cf2109	Mycelium scanty, white turning slightly grayish, numerous pink spore masses, Zonation absent.	120×10^2	Falcate	28.3µm×4.2µm	Granulate
Cf208	Thin cottony, grayish, whitish, numerous pink slimy masses of spores' donation complete black stomata and bodies absent.	113×10^2	Falcate	28.4µm× 4.6µm	Granulate
Cf1408	Mycelium scanty, whitish turning slightly grayish, numerous pink spore masses, donation partial.	112×10^2	Falcate	28.5µm×4.4 µm	Granulate
Cf908	Mycelium scanty, light grayish, flutty whitish mummeries pink slimy spore masses, zonation absent.	110×10^2	Falcate	29.0µm×4.7 µm	Granulate
Cf608	Mycelium scanty whitish, turning slightly greyish, numerous pink spore masses, zonation absent.	118×10^2	Falcate	28.6µm×4.2 µm	Granulate
Light sparsely sporulating (absence of acervuli and setae)					
Cf2709	Ashy grey mealy aerial growth, no pink spore masses, zonation, partial, black stromatoid bodies absent.	105×10^2	Falcate	29.2µm× 4.9µm	Granulate
Cf308	Thin cottony growth, grayish white, numerous pink slimy masses of spore, Zenation complete black stromatoid bodies.	103×10^2	Falcate.	29.2µm× 4.7µm	Granulate
Cf808	Cottony and floccose, white later grey slimy pink masses of conidia, zonation partial	36×10^2	Falcate	30.1µm× 5.1µm	Slightly granulate
Cf1508	Grey fully aerial growth, with few pink spore masses, zonation partial black stromatoid bodies.	40×10^2	Falcate	30.4µm× 5.3µm	Slightly granulate
Cf1308	Ashy grey mealy aerial growth, no pink-spore masses, zonation absent black stromatoid bodies.	42×10^2	Falcate	30.5µm× 5.0µm	Slightly granulate
Cf2209	Grey fluffy aeriod growth with very few pink spore masses, zonation complete black stromatoid bodies.	36×10^2	Falcate	30.5µm× 5.5µm	Slightly granulate
Light non-sporulating					
Cf1909	Ashy grey mealy aerial growth, spore mass meagre, zonation partial.	1×10	Falcate	25.4µm×4.6 µm	Transparent
Cf2609	Cottony and fluccose, white later turning slightly grayish slimy pink, spore masses absent zonation partial.	1×10	Falcate	25.6µm× 4.6µm	Transparent
Cf408	Loose and milky, turning slightly grayish zonation complete, spore mass meager	1×10	Falcate	25.2µm× 4.5µm	Transparent
Cf108	Grey fluffy aeriod growth, zonation partial, and spore mass meager.	1×10	Falcate	25.1µm× 4.4µm	Transparent
Dark sporulating					
Cf1008	Denser, more velvetry, dark grey turning darker, numerous pink spore masses, zonation absent.	100×10^2	Falcate	30.4µm ×4.6µm	Transparent
Cf708	Velvety olivaceous black, numerous slimy pink masses, zonation absent.	96×10^2	Falcate	25.6µm× 4.8µm	Transparent

Table 2. Contd.

Cf1709	Compact and velvety dark grey to olivaceous black numerous pink spore masses, zonation absent.	98×10^2	Falcate	25.5µm× 4.6µm	Transparent
Cf508	Velvety, numerous conidia, pink spore masses dark grey turning darker zonation absent.	100×10^2	Falcate	25.4µm× 4.5µm	Transparent
Cf1108	Velverty dark grey zonation absent, spore masses in concentric sings.	97×10^2	Falcate	28.3µm× 4.5µm	Transparent
Cf2309	Fealty texture dark grey turning darker numerous pink slimy spore masses, zonation absent.	102×10^2	Falcate	26.5µm× 4.7µm	Transparent
Dark non-sporulating					
Cf1608	More velvety texture darker grey zonation absent.	1×10	Falcate	25.2µm× 4.5µm	Transparent
Cf1809	Denser texture, dark grey, velvety, zonation absent	1×10	Falcate	25.4µm× 4.5µm	Transparent
Cf2409	More velvety texture, dark grayish zonation absent.	1×10	Falcate	25.4µm× 4.5µm	Transparent
Cf2809	Velvety dark grey to olivaceous black numerous pink spore masses, zonation absent.	1×10	Falcate	25.5µm× 4.6µm	Transparent
Cf1208	Velvety texture, dark grey, zonation absent	1×10	Falcate	25.5µm× 4.5µm	Transparent
Cf2009	Fealty texture, dark grey, zonation absent	1×10	Falcate	25.3µm× 4.4µm	Transparent
Cf2509	Velvety texture, dark grey, zonation absent	1×10	Falcate	25.2µm× 4.6µm	Transparent

medium was aseptically poured in presterilized petriplates (90 mm diameter). 5 mm discs of each isolates, cut from 7 days old cultures on OMA were inoculated in assay plates. To evaluate the effect of temperature, the assay plates were inoculated at different temperatures 10, 15, 20, 25, 30, 35 and 40°C and incubated. The assay plates used as controls were incubated at room temperature (24±2°C). The growth of the fungi was recorded in mutually perpendicular directions of intervals of 2, 4, 6,8,10 and 12 days and averages were recorded (Figure 2). It is apparent that the growth of all isolates was maximum at 30°C and interestingly, very minimum growth was observed at 40°C in any test isolates of *C. falcatum*.

Effect of growth regulator on the growth of *C. falcatum*

Richards's broth medium was proved in presterilized conical flasks containing 0.5 ml absolute alcohol and requisite quantities of indole-3 acetic acid (IAA) and indole-3 butyric acid (IBA), separately so as to procure 1, 5, 10, 25 and 50 ppm of the growth regulators with respect to the volume of the medium. A mycelial disc of 5 mm in diameter cut from 7 days old cultures of test isolate was aseptically inoculated in each assay flasks. Control sets contained Richard's medium and 0.5 ml absolute alcohol but were without growth regulator. The control and treatment assay flasks were incubated at 30±1°C temperature. On 15th day of incubation, the dry weight of the mycelial mat was recorded and averages of 5 replicates are given. The results recorded in Figure 4 revealed that lower concentration of IAA and IBA proved stimulatory to the growth of selected isolates, while higher concentration produced adverse effect on the growth of all the isolates.

DISCUSSION

Sugarcane is not only a cash crop for the growers, but it is the main source of white crystal sugar; it has been recognized as an old energy source for human beings and more recently it is a replace-ment of fossil fuel for motor vehicles also. Sugar-cane is the second largest cash crop of India and is being cultivated on 28 million tones contributing around 3.6% of Gross Domestic Production (G.D.P). The sugar industry plays a pivotal role in the na-tional economy of our country. Major production limiting factors are insects and diseases, particu-larly red rot caused by *C. falcatum* (Hussnain and Afghan, 2006a). The red rot disease was first re-ported from Java (Now Indonesia) by Went (1893) as "red smut" and coined as red rot by Butler (1906). Variety released for commercial cultivation soon becomes susceptible to red rot due to deve-lopment of new races of the pathogen causing breakdown of resistance (Viswanathan et al, 2003). In the subtropical plains of North Uttar Pradesh, the high relative humidity and temperatures during the monsoon period in the month of July-August

Table 3. Pathogenic behavior of 28 isolates of *C. falcatum* on reaction in sugarcane pathological standards.

Isolate number	Disease reaction by plug method /disease rating													
	Co 419	Co 975	Co 997	Co 1148	Co 7717	Co 62399	CoC 671	CoJ 64	BO 91	CoS 767	Baragua	Kakhai	SES 594	CoS 8436
Cf108	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	R	R	R	MR
Cf208	S	S	HS	S	S	S	HS	S	S	S	MS	S	MR	S
Cf308	MR	MR	HS	MS	S	MS	MS	MS	MS	MS	R	R	R	MS
Cf408	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	R	MR
Cf508	S	MS	MS	MS	MS	S	MS	MS	S	MS	MR	MS	R	MS
Cf608	HS	S	S	S	HS	S	S	S	S	HS	MS	S	MR	S
Cf708	MS	MR	MS	MR	MS	MS	MR	MS	S	HS	MR	MS	R	MS
Cf808	MS	MR	MS	MR	HS	MR	MR	MS	MR	MR	R	MR	R	MS
Cf908	S	S	HS	S	S	HS	S	HS	S	S	MS	MS	MR	HS
Cf1008	MS	MS	MS	S	MS	HS	MS	MS	MR	MS	MR	MR	R	MS
Cf1108	HS	MS	MR	MS	MS	MS	MS	HS	MR	HS	MS	MR	R	MS
Cf1208	MS	R	MS	MR	MR	MS	MR	MS	MS	MR	R	R	R	MS
Cf1308	MS	HS	MS	MR	MS	MR	MR	MS	MS	MS	MR	MR	R	S
Cf1408	MR	MS	MR	MS	MR	S	MS	MS	MS	MR	MS	MS	R	MS
Cf1508	HS	S	S	HS	S	S	MS	S	HS	S	MS	S	MR	S
Cf1608	MS	S	MS	MS	MS	MS	MR	MR	MR	MS	MR	R	R	MS
Cf1709	MS	MS	S	MS	MS	MS	HS	MS	MS	MS	MR	R	R	MS
Cf1809	S	MS	MS	MR	S	MS	MS	MR	MS	MR	MS	MS	R	MS
Cf1909	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	R	MR
Cf2009	MR	R	MR	MS	MR	R	MR	MS	R	R	MR	MR	R	MS
Cf2109	S	HS	S	S	S	HS	S	S	S	S	HS	S	MR	HS
Cf2209	MR	MS	MR	MR	MR	MS	MR	MR	MR	MR	MS	MR	R	MS
Cf2309	MS	MS	S	MS	MS	S	MS	MS	MS	MR	MR	R	R	MS
Cf2409	MS	MR	MS	MR	MS	MR	MR	MS	S	MS	S	MS	R	S
Cf2509	R	MR	MR	MS	MR	MR	MR	MR	MR	MS	MR	MR	R	MR
Cf2609	HS	S	HS	S	HS	S	S	HS	MS	HS	S	S	MR	HS
Cf2709	S	MS	MS	MS	S	MR	S	MS	MR	S	MR	MS	R	S
Cf2809	MS	MR	MS	MS	MR	HS	MS	MR	MS	S	MS	MR	R	MS

R, Resistant (0.0 - 2.0); MR, moderately resistant (2.1 - 4.0); MS, moderately susceptible (4.1 - 6.0); S, susceptible (6.1 - 8.0); HS, highly susceptible (8.1 – 9).

make genotypes very vulnerable to the attack of *C. falcatum*, resulting in complete devastation of the standing crop. We observed variable symptoms on genotypes infected by *C. falcatum* and variation may be related to development of new races. The spores germinate and the mycelium gets established in bud scales, root primordial or leaf scars and later within the plant tissues. Since new races of red rot pathogen appear regularly in nature

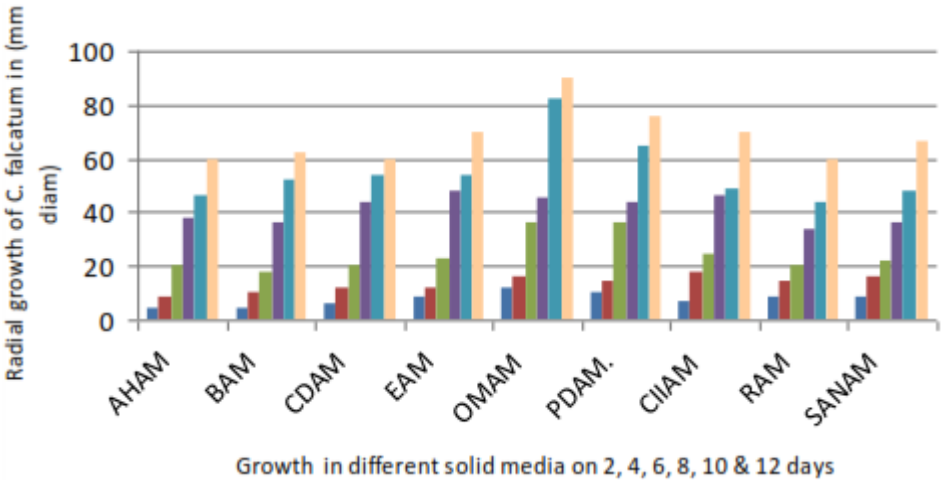

Figure 1. Radial growth in solid media of *C .falcatum* Went.

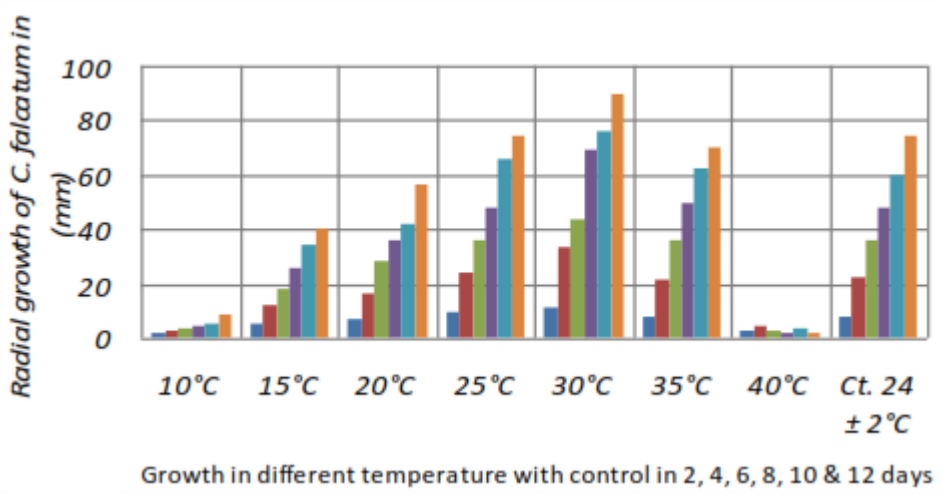

Figure 2. Effect of different Temperatures on the growth of *C. falcatum* Went.

due to mutation, it is necessary for pathologists to keep a constant watch on such new races, so that information may be exploited for breeding resistance against the evolved mutant. Keeping this in view, the present study was undertaken in order to collect the various isolates of *C. falcatum* prevalent in Uttar Pradesh, a place already reported as region of 'hot spot' for red rot epiphytotics due to its physiographic and climatic situations so that breeders may use the information for developing varieties resistant to red rot for this region. The objective of this study is to understand cultural, morphological and pathological diversity of the red rot pathogen inducing variable symptoms on sugarcane cultivars. Morphological diversity has been found in four isolates of *C. falcatum* from SPF234, CO1148, BF162 and SHF242 (Abbas et al., 2010). *Colletotrichum* spp. has been differentiated based on phenotypic traits such as mycelia growth rate, character of conidia and appressoria, colony appearance and production of setae. It was reported that the isolates of *Colletotrichum* pathogen attacking various crops differ in mycelia growth. *Colletotrichum acutatum* grew slower than *Colletotrichum gloeosporioides* (Talhinhas et al., 2002a) common anthracnose pathogen of trees, chili, and pepper (Kim et al., 2006; Kim and Hong, 2008; Kim et al., 2008). Viswanathan et al. (2003) have grouped the isolates mainly on the basis of their morphological characters into 'Dark type' having darker mycelium with spores sporulation and 'Light type' having light colored mycelium with profuse sporulation. The isolation of the former types has been reported to be virulent in nature and those of the latter, highly virulent. In the present study an attempt has been made to group the two collected isolates type as dark coloured mycelium with poor sporulation probable avirulent type and light coloured mycelium with profuse

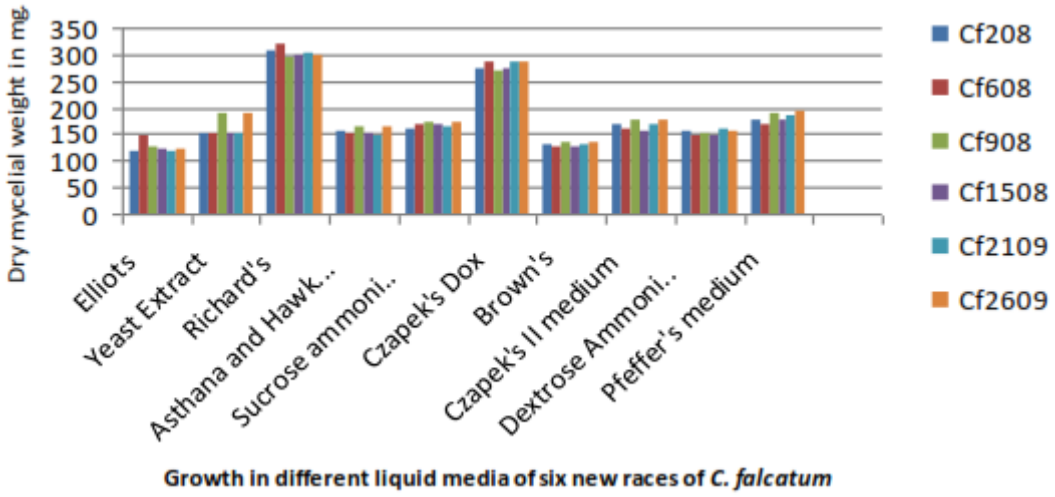

Figure 3. Dry weight of mycelium in liquid media of *C. falcatum* Went.

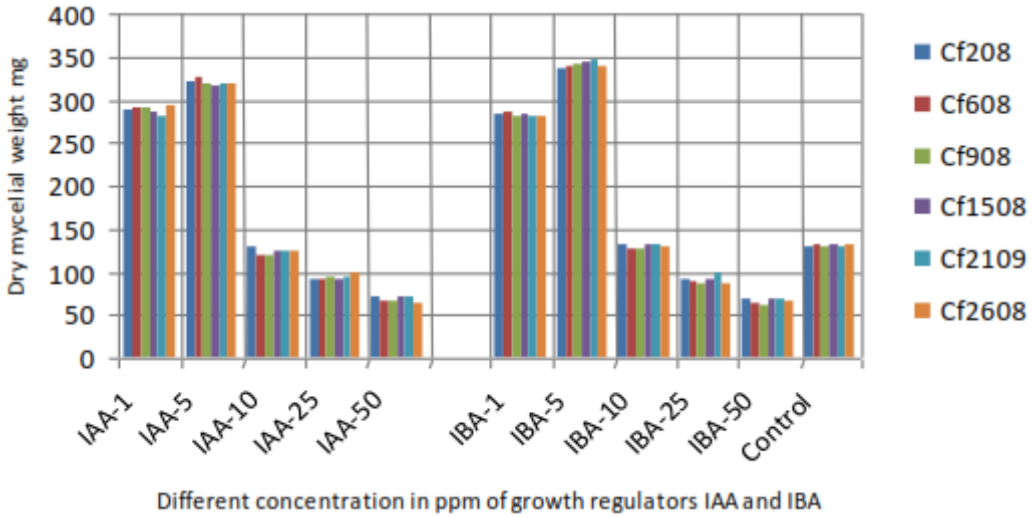

Figure 4. Effect of Growth regulator on the growth of *C. falcatum* Went.

sporulation probable virulent types.

During our survey, 28 isolates of *C. falcatum* from sugarcane showed the same tendency in growth rate, of which five isolates were light sporulating, 6 isolates, light sparsely sporulating and 4 isolates, light non-sporulating. 6 isolates that were dark sporulating and 7 isolates, non-sporulating were found, respectively. Different isolates of the fungus showed variability in cultural characteristics, fruiting structures and virulence. In the occurrence of two distinct strains, the dark and light types were recognized (Vishwanathan et al., 2003). Later presence of many strains has been reported by pathologists working in different places (Suman et al., 2005; Alvi et al., 2008; Kumar et al., 2011).Variability in cultural and morphological characters and virulence and development of physiological races have been attributed to hybridization, mutation conidial and hyphen fusions (Bharti et al., 2011). Variability in virulence among the pathotypes has also been reported that red rot pathogen undergoes adaptive changes in relation to the host cultivars cultivated in subsequent alterations in the virulence patterns of the fungus. Further, it was found that isolates of red rot fungus are often unstable in their pathogenicity and have a tendency to pass irreversibly into a virulent phase. This study showed existence of 5 major races in the country and higher virulence of the tropical isolates as compared to subtropical isolates. Kumar et al. (2011) reported that the isolates from sub-tropical regions were more virulent than the then existing tropical isolates. Later, different pathotypes were grouped based on serological protein profiles and DNA studies (Alvi et al., 2008). Development of new races/pathotypes is responsible for the removal of many

ruling varieties from cultivation. A combination of molecular diagnostic tools with traditional morphological techniques is an appropriate and reliable approach for studying of *C. falcatum* isolates complex (Kumar et al., 2010). Variation in red rot pathogen led to a horizontal breeding programme for red rot resistance. Pathogenic variability in red rot has generally been assigned to be the main cause for breakdown of resistance in sugarcane varieties (Duttamajumdar, 2002). The results on pathogencity test as observed in the present study prove that one isolate pathogenic to one variety may not necessarily be the same in reaction with another variety, confirming that sugarcane genotype differd in reaction with different isolates of the pathogen (Satyavir, 2003). The present study also suggested that the isolates of red rot pathogen with different pathogencity are in existence in the tropical and sub-tropical areas of Indian states and it is desirable to make regular collection of prevailing isolates of red rot pathogen in this region. The results indicated that plug method of inoculation followed by nodal with stripping was found most effective displaying characteristic symptoms like full lateral spread of lesions, white spots, nodal transgression producing acrylic on rind area and emitting alcoholic smell. Among the other methods, nodal without stripping and nodal whorl methods were less effective because of more dependence on environmental conditions at the time of inoculation. The findings of this investigation have found cultural, morphological and pathological variations in the isolates of *C. falcatum* collected from sugarcane varieties. To confirm the genetic diversity among these isolates of *C. falcatum* at DNA level and the specific protein responsible for disease resistance using molecular approaches should be the plan for future study.

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