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Pathogenic Behaviour Pattern of *Colletotrichum falcatum* Isolates of Sugarcane in Sub-tropical India

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Abstract

Red rot caused by *Colletotrichum falcatum* is the most serious disease of sugarcane affecting cane production in different states in India. Since the pathogenic variants very frequently emerge in the field we have assessed pathogenic variation in 12 *C. falcatum* isolates collected from subtropical India along with previously designated pathotypes on a set of 16 host differentials for the eight seasons. By and large, a variable virulence pattern in *C. falcatum* isolates was recorded on most of the host differentials. The results showed that the predominant sub-tropical pathotypes (CF09 and CF08) isolated from then the ruling cultivars CoS 767 and CoJ 64, respectively showed less virulence and the pathotype CF02 isolated from Co 7717 showed higher virulence over the period of time on the host differentials. The higher aggressiveness exhibited by CF02 over on the differentials such as Co1148, Co 975 and Khakai and known susceptible differentials Co 997, Co 62399, CoC 671, CoJ 64 and another variety Co 89003 clearly revealed higher virulence of CF11 pathotype. Even though the pathotype CF08 remained virulent on its host cultivar CoJ 64, it could not cause same type of virulence on the recently developed cultivars and it suggests that the pathotype no longer maintains its virulence once a popular cultivar CoJ 64 removed from the cultivation. Among the 11 new *C. falcatum* isolates evaluated, the isolate Cf8436 Karnal was found to be virulent and the differential CoS 8436 succumbed to this isolate only. Of the different CoJ 64 pathotypes, Cf64-I exhibited higher virulence and showed susceptible to intermediate reaction on different host differentials. This study suggests continuous evolution of red rot pathogen and its adaptation to the new host cultivars in sub-tropical India.

Keywords

Colletotrichum falcatum; Host differential; Pathogenic behaviour pattern; Virulence

Introduction

Red rot caused by *Colletotrichum falcatum* Went *Glomerella tucumanensis* Speg. Arx & Muller [1] the most dreaded disease of sugarcane. The disease has been widely distributed and reported from 68 sugarcane growing countries. In India, the first documented epidemic in 1901 and subsequently several epiphytotics of the disease had occurred resulting in failure of many popular varieties [2-4]. Over the years, the disease has been observed as most destructive in subtropical India; however, since last five decades the disease has drastically affected tropical areas also [4]. Variability in red rot pathogen has been main cause of varietal break down of sugarcane varieties, considered resistant at the time of their release. Thus, appearance of new pathotype of *C. falcatum* is an important factor in the red rot epidemics and in the failure of popular varieties [4]. In *C. falcatum* pathogenic variation was observed for the first time [5]. Since then, the existence of pathogen variability has been recognized by various workers. In India, various workers have studied the variability among C. *falcatum* isolates based on morphological, pathological and molecular characterization and host resistance from both tropical and sub-tropical regions of the country periodically [6-10]. Efforts were made to identify the pathotypes based on differential host interaction also in tropical and sub-tropical regions [11,12]. In subtropical India after the break down of the popular variety Co 1148 in the previous decades many new pathotypes have emerged during the last three decades especially on the popular cultivars Co 7717, CoJ64, CoJ 85 and CoS 767 and about 11 pathotypes were designated as CF1 to CF11 [3,8]. Recently Viswanathan [13] identified a new pathotype CF12 of the pathogen, isolated from the cv Co 94012 from tropical region. This pathotype exhibited greater virulence than the reference pathotype CF06 of the tropical region. The popular variety CoS 8436 in the subtropical region remained disease free for two decades, recently succumbed to C. *falcatum*. Similarly red rot was recorded on the other cvs CoSe 95422, BO 130 and CoJ 64 in the recent years. Hence a detailed study was undertaken to assess the variability of the pathogen on a set of host differentials. The variability exhibited by the isolates was categorized based on their reaction on the differentials and comparative virulence with the reference pathotypes.

Materials and Methods

C. *falcatum* isolates

Pathogenic variability of *C. falcatum* isolates was studied on 16 host differentials viz. Khakai (*Saccharum sinense*), SES 594 (*S. spontaneum*), Baragua (*S. officinarum*), Co 419, Co 975, Co 997, Co 1148, Co 7717, Co 62399, Co 89003, BO 91, CoJ 64, CoJ 83, CoS 767, CoS 8436 and CoC 671. Seven established *C. falcatum* (Cf) pathotypes *viz.* CF01, CF02, CF03, CF07, CF08, CF09, CF11 along with 11 Cf isolates isolated from different varieties namely CoJ 64 (Cf64-I, Cf64-II, Cf64-III, Cf64-IV, CfUP1, CfUP2, CfUP3), one each from CoS 8436-Karnal (Cf8436), BO 138 (CfBO138), CoSe 95422 (CfSe95422) and CoBLN 05221 (CfBLN 05521) were included in the present studies (Table 1). These isolates have been maintained at Plant Pathology Laboratory, ICAR- Sugarcane Breeding Institute, Coimbatore and evaluated on the host differentials for the eight consecutive seasons (2007-2015) at ICAR- Sugarcane Breeding Institute- Regional Centre, Karnal, Haryana.

Pathogen inoculation and evaluation

Six months old healthy canes were inoculated during the month of August by standardized plug method of inoculation [14]. A hole was made using red rot inoculator into the 2nd or 3rd internode above the ground at a 45° inclination and 2-3 drops of *C. falcatum* conidial suspension (1×10^6 conidia/ml) were placed and the bore hole was sealed with activated clay. Further *C. falcatum* inoculation was done

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Table 1: *C. falcatum* pathotypes / isolates collected from sub-tropical India.

S.No.	Pathotype / isolate	Source	Place of collection	State
1.	CF01	Co 1148	Yamuna nagar	Haryana
2	CF02	Co 7717	Karnal	Haryana
3	CF03	CoJ 64	-	Haryana
4	CF07	CoJ 64	Naraingarh	Haryana
5	CF08	CoJ 64	Bhatinda	Punjab
6	CF09	CoS 767	Kaithal	Haryana
7	CF11	CoJ 64	-	UP
8	CfBO138	BO 138	Pusa	Bihar
9	CfSe95422	CoSe 95422	Seorahi	Bihar
10	CfBLN 05521	CoBln 05521	Pusa	Bihar
11	Cf8436 (Karnal)	CoS 8436	Naraingarh	Haryana
12	Cf64-I	CoJ 64	Dhaurala	UP
13	Cf64 -II	CoJ 64	Dhaurala	UP
14	Cf64 -III	CoJ 64	Dhenuja	UP
15	Cf64 -IV	CoJ 64	Dhenuja	UP
16	CfUP1	CoJ 64	Khaiya	UP
17	CfUP2	CoJ 64	Razoolpur	UP
19	CfUP3	CoJ 64	Thir	UP

during first week of August each year. The inoculation and incubation period coincided with active south west monsoon season, which is considered ideal for *C. falcatum* inoculation in sugarcane. After inoculation, frequent irrigation was given as per requirements to maintain a relative humidity of >80% during incubation period.

For evaluation of red rot development, canes were split longitudinally 60 days after inoculation along the point of inoculation. Inoculated canes free from borer infestation and disease reaction was assessed on a 0-9 scale of Srinivasan and Bhat [15]. The disease reaction was categorized in to resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S) and highly susceptible (HS) based on the major pathogenicity parameters viz. Nodal transgression of the pathogen from the inoculated internode, lesion width in proportion to the cane width, presence and intensity of white spots and nature of foliage is green or yellow/dry. Pathogenic reaction of seven designated pathotypes of *C. falcatum* and 12 new pathogenic isolates were recorded on the differentials for eight seasons from 2007 onwards. The disease scores of the pathotype/isolate on differentials for eight seasons were pooled and average scores were computed and presented for comparison.

Results and Discussion

In the present investigation attempts have been made to document the comprehensive information generated during the last eight years on the pattern of pathogenic behaviour of *C. falcatum* isolates on a set of host differentials under sub-tropical conditions. Pathogenic reaction of the 19 pathotypes/isolates on a set of 16 host differentials revealed that none of the pathotypes/isolates resembled another pathotype/isolate in its pathogenic behaviour. Among the seven designated pathotypes, CF02 pathotype isolated from Co7717 exhibited higher virulence on the set of 16 host differentials followed by CF11, CF09, CF08 and CF07 pathotypes of sub-tropical India (Table 2). The pathotype CF02 was found to be highly virulent caused 4 S and 5 MS reactions on 16 host differentials evaluated for eight seasons followed by CF11 (2 S and 8 MS), CF09 (2 S and 5 MS), CF08 (2 S and 2 MS) and CF07 (2 s and 2 MS) (Table 2). The CF03 and CF01 pathotypes showed only one S and 2 or 3 MS reactions on 16 host differentials and found to be less virulent. CF01 showed S on the host cv Co 1148 and CF03 pathotype also showed S on the its host cv CoJ 64. But these pathotypes lost their virulence due to their maintenance in the slants. However , the pathotype CF02 exhibited a higher virulence on many differentials but its virulence was not high on host cv Co7717 (Table 2). Similarly CF11 and CF09 also showed only MS reactions on their host cvs CoJ 64 and CoS 767, respectively. But CF07 and CF08 caused S on their host cv CoJ 64. Among the four isolates of CoJ 64, Cf64- III found to be highly virulent (1 S and 1MS) and Cf64-II (3 MS) and Cf64-IV (2 MS) and Cf64-I (1 MS) showed less virulence on the different host differentials tested. Of the different Cf isolates studied. The new isolate from the popular cv CoS 8436 (Cf8436 Karnal) exhibited higher virulence (3 S and 1 MS), followed by CfBLN 05521 (1 S and 2 MS) and CfUP2 (1 S and 2 MS) isolates (Table 2). The differential CoS 8436 succumbed only to the Cf8436 Karnal isolate during the last four consecutive years. This isolate caused S reactions on cvs CoC 671 and Co 997 and MS reactions on Khakai and CoS 8436 (host cultivar). Three resistant differentials viz. BO 91, Baragua and SES 594 had shown consistently resistant reactions to all the test isolates (Data not shown).

The existence of variation in *C. falcatum* has been reported in India and abroad for several decades. In the beginning, two distinct dark and light type races were recognized [16]. Many isolates were intermediate to these two races. The light race produced abundant conidia and proved more virulent than the dark one. The failure of variety PoJ 213 in USA during 1930-31 was due to a change from previous existing dark type race to the more virulent light coloured race [17]. Chona and Padwick [18] observed an exact parallel in the failure of Co 213 in north India during epidemic years of 1938-41 due to appearance of light coloured, highly sporulating *C. falcatum* isolates. All the previous isolates from India were dark types with sparse sporulation and the light type isolates were reported to be more virulent than the old dark type isolates. Studies in *C. falcatum* from 1957 onwards indicated the existence of variability and appearance of several strains of the fungus [19]. Only in few studies efforts have been made to identify specific pathotypes based on differential reactions on host differentials. Satyanarayana and Satyanarayana [20] reported that the two isolates of red rot pathogen from the varieties Co 419 and Co 997 from coastal Andhra Pradesh constituted two distinct races. In sub-tropical India Khirbat et al. [7] reported the existence of three races in Haryana on the basis of differential reactions. Further, Beniwal et al. [8] also reported prevalence of three pathotypes based on differential reaction from varieties Co 7717, Co 1148 and CoJ 64. Detailed studies conducted by Padmanaban et al. [11] revealed existence of three more pathotypes viz Cf419, Cf997 and Cf671 later designated as CF04, CF05 and CF06 isolated from Co 419, Co 997 and CoC 671, respectively from tropical region. Subsequently, with emergence of new variants, five new pathotypes CF07, CF08, CF09, CF10 and CF11 with distinct pathogenic behaviour on the host differentials were designated [4]. Among these pathotypes, CF09 maintained its virulence for many years and used for red rot screening in sub-tropical India along with CF08. After the removal of the popular varieties CoS 767 and CoJ 64 from the cultivation due to their high susceptibility to the red rot pathogen, the pathotypes CF09 and CF08, respectively showed a decline in their virulence pattern. Even though the pathotype CF08 remained virulent on its host cultivar CoJ 64 (CF09 showed only MS reaction on its host cv CoS 767), it could not cause the same type of virulence on the recently developed cultivars. Further this study revealed that a pathotype no longer maintains its

Table 2: Pathogenic behaviour pattern of *C. falcatum* isolates on host differentials during 2007-2015.

S. No.	Pathotype / isolate	Source variety	Red rot reaction on host differentials											
			Co 419	Co 975	Co 997	Co 1148	Co 7717	Co 62399	Co 89003	CoC 671	CoJ 64	CoJ 83	CoS 767	CoS 8436	Khakai
1.	CF01	Co1148	MS (5.4)	R (1.4)	MS (5.5)	S (6.4)	R (1.4)	MR (3.0)	R (1.7)	MR (3.6)	MR (3.3)	MR (2.2)	MR (2.4)	R (1.3)	R (1.3)
2	CF02	Co 7717	S (7.0)	MS (6)	S (6.4)	MR (3.8)	MS (5)	S (6.1)	MS (5)	S (6.3)	MS (4.4)	MR (3.4)	R (1.3)	MR (3.6)	MS (4.1)
3	CF03	CoJ 64	MS (5.7)	R (1.2)	MS (4.6)	MR (4.0)	R (1.4)	MR (3.0)	MR (2.3)	MS (5.6)	S (6.4)	MR (2.6)	R (2.0)	MR (3.0)	MR (3.7)
4	CF07	CoJ 64	MS (4.6)	MR (2.1)	MS (5.5)	MR (2.3)	R (1.3)	MR (3.0)	MR (3.5)	S (7.2)	S (7.0)	MR (2.6)	MR (2.7)	MR (2.4)	MR (3.6)
5	CF08	CoJ 64	MS (5.5)	MR (2.3)	MS (6.0)	MR (2.1)	R (2.0)	MS (5.0)	MR (2.3)	S (6.6)	S (6.3)	MR (3.4)	MR (3.0)	R (2.0)	MS (4.3)
6	CF09	CoS 767	MS (5)	MR (3.3)	M((5.6)	MR (3.3)	MR (2.1)	MR (3.6)	MR (3.3)	S (7.4)	S (6.3)	MS (4.4)	MS (5)	MS (4.4)	MR (4)
7	CF11	CoJ 64	MS (4.7)	MS (4.3)	S (7.4)	MS (5.3)	MS (4.5)	MS (4.3)	MR (3.6)	S (6.3)	MS (4.8)	MR (3.6)	MS (4.4)	R (1.3)	MS (4.2)
8	CfBO138	BO 138	MR (4.0)	MR (2.5)	MR (3.1)	MS (5.0)	MR (3.5)	R (2.0)	R (1.0)	S (7.0)	MR (3.0)	MR (3.0)	R (1.3)	R (2.0)	MR (3.5)
9	CfSe 95422	CoSe 95422	MR(3.0)	R (1.0)	MR (2.5)	MR (3.5)	MR (2.3)	MS (5.0)	R (1.0)	MS (5.5)	MR (2.8)	R (1.0)	R (1.0)	R (2.0)	MR (2.3)
10	CfBLN 05521	CoBln 05521	R (2.0)	R (1.0)	MR (2.3)	R (2.0)	MR (2.5)	MR (2.5)	R (1.0)	S (7.0)	MS (6.0)	R (1.0)	R (1.0)	MR (2.5)	MS (4.3)
11	Cf8436 (K)	CoS 8436	MR (2.3)	R (1.0)	S (7.0)	MR (3.0)	R (1.0)	MR (3.0)	R (1.0)	S (7.0)	MR (2.3)	R (1.0)	R (1.0)	S (7.0)	MS (5.0)
12	Cf64-I	CoJ 64	MR (3.4)	MR (2.4)	MR (4)	MR (4.0)	MR (2.5)	MR (3.4)	R (1.0)	MS (4.6)	MR (4.0)	MR (3.0)	R (1.0)	R (1.8)	MR (4.0)
13	Cf64-II	CoJ 64	MR (3.8)	R (1.0)	MR (2.8)	R (1.0)	R (1.0)	R (1.8)	R (1.0)	MS (5.5)	MS (5.8)	MR (2.3)	R (1.0)	R (1.0)	MR (3.4)
14	Cf64-III	CoJ 64	MR (3.7)	R (1.0)	MR (3.0)	R (1.8)	MR (3.5)	MR (3.0)	R (1.0)	MS (5.7)	S (7.0)	MR (2.5)	R (2.0)	R (1.7)	R (1.0)
15	Cf64-IV	CoJ 64	MS (5.2)	R (2.0)	MR (4.0)	R (2.0)	MR (3.4)	MR (2.7)	R (1.0)	MS (6.0)	MR (4.0)	R (1.0)	R (1.7)	R (1.7)	MR (2.7)
16	CfUP1	CoJ 64	MS (5.0)	MR (2.3)	MS (5.3)	R (1.7)	MR (3.3)	MS (4.6)	R (2.0)	MS (4.5)	MS (5.3)	R (1.5)	R (2.0)	R (1.3)	MR (4)
17	CfUP2	CoJ 64	MR (3.0)	R (1.4)	MS (5.0)	MR (2.6)	MR (3.0)	MR (2.6)	R (2.0)	MS (4.6)	S (7.4)	MR (4.0)	R (1.8)	R (1.4)	MR (3.2)
19	CfUP3	CoJ 64	MR (2.2)	R (1.0)	MS (4.5)	MR (2.6)	R (1.5)	MR (2.6)	R (2.0)	MS (4.2)	MS (4.6)	MR (2.0)	R (1.7)	R (1.3)	MR (3.5)

Value in parentheses is average score values of red rot reactions during eight years

virulence, once a popular cultivar removed from the cultivation as in the case of CoJ64 in subtropical India. The higher aggressiveness exhibited by the pathotype CF02 over the resistant (Co1148, Co 975 and Khakai) and susceptible differentials (Co 997, Co 62399, CoC 671, and CoJ 64) and new cultivar (Co 89003) clearly revealed higher virulence of CF02 pathotype. This study established that the virulence of the pathotype CF02 was not high on its host cultivar Co 7717, but it exhibited high virulence on the cultivars which are previously found to be resistant (Co1148, Co 975 and Khakai). Adaptation of red rot pathogen to different sugarcane cultivars is very well established [1] and the previous studies on red rot pathogen indicated that the pathogenic organism in the ecosystem slowly tries to adapt a new cytoplasm and develop tolerance to the host and lastly change the virulence against the host cultivar [4]. Studies conducted from ICAR-SBI, Coimbatore revealed that the repeated inoculation of the same *C. falcatum* pathotype in incompatible interaction of host and pathogen led to higher virulence of less virulent pathotype [21,22] and further studies conducted by the same group revealed that pathotypes vary in their potential to produce hydrolytic enzymes both cellulolytic and pectinolytic enzymes and melanin during their host pathogen interaction and pathogen virulence was found to be associated with the virulence in *C. falcatum* [23,24]. Production of these enzymes/ metabolites by the pathotype CF02 on incompatible host cultivars may be responsible for its increased virulence over the period of time on some of the resistant cultivars. This study also indicated that the new isolate Cf8436 exhibited higher virulence compared to 10 other *C. falcatum* isolates tested. Hence, breakdown of resistance in the variety CoS 8436 noticed in Haryana in recent years might be due to appearance of a new strain of *C. falcatum*. Agnihotri [25] has cited that for emergence of a new race in *C. falcatum* hybridization could not be ruled out beside mutations. Under favourable conditions, the disease spreads quickly in the susceptible varieties. It might be possible that existence of new strain of isolate Cf8436 will be more harmful for the newly developed varieties; therefore, the spectra of different *C. falcatum* pathotypes present in subtropics need to be studied in detail using different functional genomics approaches to identify the emergence of new pathotypes.

The information generated during the last eight years on pathogenic variability of *C. falcatum* isolates illustrated that the virulence pattern of the predominant sub-tropical pathotypes CF09 and CF08 used for red rot screening in sugarcane showed less virulence and the pathotype CF02 exhibited higher virulence on 16 tested host differentials. The pathotype CF02 distinctly different in virulence and it may be due to stability for many years as compared to the recently emerged pathtotypes. Among the different *C. falcatum* isolates tested, the new isolate Cf8436 was found to be highly virulent and the differential CoS 8436 showed susceptibility to this isolate only. During the past couple of years breakdown of resistance in the popular variety CoS 8436 has been noticed in Haryana and Uttar Pradesh, might be due to appearance of a new strain of red rot pathogen. Therefore, spectra of prevailing pathotypes in *C. falcatum* need to be studied in detail using different functional genomics approaches. Also there is a need to study emergence of new isolates by adapting to the new varieties deployed for commercial cultivation.

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