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RESEARCH ARTICLE

Varietal Breakdown to Red Rot in Sugarcane Revealed by Comparing Two *Colletotrichum falcatum* Inoculation Methods

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Abstract *Colletotrichum falcatum* Went causing red rot is a major constraint to cane production and productivity across sugarcane growing countries in Asia. The fungal pathogen exhibits enormous variation under field conditions; the pathogenic variants emerge regularly in tune with deployment of new host varieties for cultivation making the resistant to susceptible referred as 'varietal breakdown'. Although such phenomena occurred regularly, how the varieties succumb to the *C. falcatum* pathotypes is not clearly established, and hence, longevity of red rot-resistant varieties become unpredictable under field conditions. The soil-borne inoculum surviving as crop debris is the known source of *C. falcatum* (*Cf*) inoculum to cause infection in the field, probably after repeated attempts in a variety which was hitherto resistant. Hence, a detailed study was conducted with a set of *Cf* pathotypes varying in their virulence and ten varieties with their disease reactions vary from moderately resistant (MR) to highly susceptible (HS) by inoculating the pathogen by the plug method on standing canes and applying *Cf* inoculum to the soil, under field conditions. The three seasons study clearly indicated that disease reactions in sugarcane varieties to different *Cf* pathotypes in the plug method did not vary among the seasons, whereas the sugarcane varieties behaved differently for disease development from soil-borne inoculum. When disease development from the two inoculation methods was compared with respect to their known disease reaction, MR and HS varieties showed a similar pattern. However, four of the five moderately susceptible (MS) varieties showed a deviation for higher disease development from soil inoculum to certain pathotypes. Such disease development in the trial from soil inoculum is reflected by a similar behaviour in disease endemic locations, where they succumbed to the pathogen. Overall, the study explains susceptibility of sugarcane varieties for *Cf* infection from the inoculum surviving in the soil in due course in the field, although their host reactions are MR or MS. Also these findings provide an evidence for varietal breakdown to *C. falcatum* in sugarcane from soil-borne inoculum under field conditions, for the first time.

Keywords *Colletotrichum falcatum* · Sugarcane · Pathogen variation · Differential reaction · Host resistance · Varietal breakdown

Introduction

Red rot of sugarcane caused by *Colletotrichum falcatum* Went is a major constraint affecting sugarcane production and productivity for more than a century in India and many other Asian countries (Viswanathan et al. 2018). Earliest red rot epidemics in the tropical and subtropical regions were recorded during 1895 to 1899 on *Saccharum officinarum* clones such as Namalu, Keli, Striped Mauritius and Bourbon (Barber 1901, Butler 1906). Subsequently, interspecific hybrids were developed and elite clones among them were deployed to harness higher cane and sugar yield in the country (Chona 1980, Viswanathan 2010). Many such hybrid clones starting from Co 205 released in 1918 made a sugar revolution in the country, especially in the subtropical belt. However, red rot epidemics, especially during 1930s and 1940s, caused a catastrophic impact on sugar industry and many varieties like Co 213, Co 290, Co

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301, Co 312, Co 313, Co 357, Co 370, Co 385, Co 393, Co 421, Co 453, BO 3, BO 10, BO 14, BO 17, CoS 5, etc. succumbed to the disease. Similarly, many elite varieties have succumbed to the disease in the subsequent epidemics, till the recent decades in both tropical and subtropical regions (Duttamajumder 2002; Singh and Singh 1989; Viswanathan 2010, 2018). Although the varieties intended to replace the susceptible varieties were resistant to red rot at the time of their release for commercial cultivation, they also became susceptible over time. This is attributed to the development of new pathogenic variants adapted to a particular variety referred to as 'varietal breakdown' in sugarcane (Malathi et al. 2006; Srinivasan 1965; Viswanathan et al. 2003). Existence of pathogenic variation in *C. falcatum* and the emergence of new virulent pathotypes were documented over the decades (Chona 1980; Srinivasan 1962; Viswanathan 2010).

To asses red rot resistance in sugarcane, the standard plug method of inoculation is followed by inoculating 6- to 8-month-old standing canes with reference pathotypes (Chona 1954; Mohanraj et al. 2012). A disease rating scale of 0–9 developed by Srinivasan and Bhat (1961) is used to categorize disease reactions into resistant (R) to highly susceptible (HS). Sugarcane varieties with disease reactions of R and moderately resistant (MR) are recommended for their release and commercial cultivation in the country. The resistance reaction in the plug method of inoculation denotes physiological resistance or biochemical resistance which is dynamic (Srinivasan 1965; Viswanathan 2010). This method of evaluation for red rot resistance is followed in different countries where the disease is affecting sugarcane stalks. There were also apprehensions on the injury made on rind tissue to inoculate the pathogen in this method and concerned on the breach of barrier of defence in rind. Notwithstanding those arguments, the plug method of inoculation is being followed in the country to assess disease resistance under All India Coordinated Research Project (AICRP) on sugarcane at all the research centres due to its consistency in disease ratings without disease escapes (Viswanathan 2010). Recent studies of Viswanathan et al. (2017) revealed extensive variation among *C. falcatum* pathotypes from tropical and subtropical regions. These new pathotypes often infect sugarcane varieties that were resistant to other pathotype-resistant hosts and cause disease outbreak (Viswanathan et al. 2020a).

In the field, *C. falcatum* infects from the primary sources of inoculum such as infected setts/crop debris in the soil or through secondary source of inoculum through irrigation/rain water, rain splash and to some extent through airborne conidia (Agnihotri 1983, Singh and Singh 1989). It is believed that this inoculum serves as the source to infect the host varieties and slowly adapt to the new varieties. However, we have no information on how the primary and secondary sources inoculum make infections on sugarcane varieties that are resistant to red rot under field conditions. Further, we have limited information on the interaction between field inoculum of *C. falcatum* pathotypes and disease reactions by the plug method, commonly used to screen sugarcane varieties for red rot resistance. Our previous studies revealed a differential response of soil-borne inoculum of *C. falcatum* pathotypes against different sugarcane varieties varying in disease resistance (Viswanathan et al. 2020b). In the present study, we have inoculated *C. falcatum* by two methods, viz. the plug method with injury on internodes and soil inoculation at the time of planting which simulates natural way of infection on sugarcane varieties vary in disease resistance. We assessed how the sugarcane varieties respond to pathogen infection from two sources of inocula and how the pathotypes showed their differential ability to challenge the host resistance.

Materials and Methods

Sugarcane Varieties and *C. falcatum* Pathotypes Used

Field experiments were conducted during 2017–2018, 2018–2019 and 2019–2020 seasons at Plant Pathology Farm, ICAR-Sugarcane Breeding Institute, Coimbatore (11.99° N, 76.91° E). The experiments comprised of ten sugarcane varieties varying in red rot resistance that include Co 94012 and CoC 671 (Highly susceptible; 8.1–9.0 in 0–9 scale), Co 86032, Co 06022, Co 06027, Co 06030 and CoV 09356 (moderately susceptible; 4.1–6.0 in 0–9 scale), Co 0212, Co 0238 and Co 0403 (moderately resistant; 2.1–4.0 in 0–9 scale). The nine *C. falcatum* pathotypes collected from different locations in Tamil Nadu State were used in the study. The pathotypes included two designated pathotypes CF06 (*Cf*671) and CF12 (*Cf*94012) used for red rot screening in the tropical region to assess varietal resistance and other pathotypes were collected in the recent years (Table 1). All these pathotypes were collected from red rot-affected areas in Tamil Nadu State during different years and maintained at the Plant Pathology laboratory of the institute as part of *C. falcatum* culture collections. The cultures were maintained on oatmeal agar subcultured every year, multiplied on the same medium for inoculation as reported earlier (Viswanathan 2017). The pathotypes were named after the respective varieties from which they were isolated (Table 2). Two budded setts of these sugarcane varieties were planted in the field during February every year, and the crop was grown following standard cultural practices required for sugarcane (Sundara 1998).

Table 1 Details of *Colletotrichum falcatum* pathotypes used in the study

Sl no	Isolates	Host variety	Location of collection	Year	Latitude and longitude
1	*Cf*671 (CF06)[a]	CoC 671	Chidamparam, Cuddalore Dt	1997	11.3923; 79.7030
2	*Cf*94012 (CF12)[a]	Co 94012	Palakudi, Thanjavur Dt	2009	10.7535; 79.5179
3	*Cf*99006-MPM	Co 99006	Mundiampakkam, Villupuram Dt	2015	11.9297; 79.5289
4	*Cf*V09356-ENGR	CoV 09356	Ellanganur, Ariyalur Dt	2012	11.1521; 79.0694
5	*Cf*86027-NKU	Co 86027	Nathakadu, Namakkal Dt	2016	11.2187; 78.1679
6	*Cf*2001-13-PPM	Co 2001-13	Perambakkam, Tiruvallur Dt	2016	13.1419; 79.8957
7	*Cf*06022-KUT	Co 06022	Kuthalam, Nagapattinam Dt	2016	11.0699; 79.5572
8	*Cf*86032-SKPM	Co 86032	Srikanthapuram, Thanjavur Dt	2015	10.7836; 79.1336
9	*Cf*0323-TNV	Co 0323	Ambasamudram, Tirunelveli Dt	2015	8.7078; 77.4387

[a]Designated pathotypes of *C. falcatum* from the tropical region used in screening for red rot resistance

Table 2 Sugarcane varieties used in the trial, their parentage and red rot reaction

Sl no	Varieties	Parents	Year of varietal identification	Red rot reaction
1	Co 86032	Co 62198 × CoC 671	1986	MS
2	Co 94012	CoC 671 Somaclone	1994	HS
3	Co 0212	Co 7201 × ISH 106	2002	MR
4	Co 0238	CoLk 8102 × Co 775	2002	MR
5	Co 0403	Co 8371 × Co 86011	2004	MR
6	Co 06022	GU 92275 × Co 86249	2006	MS
7	Co 06027	CoC 671 × IG 91-1100	2006	MS
8	Co 06030	CoC 671 × IG 91-1100	2006	MS
9	CoC 671	Q 63 × Co 775	1975	HS
10	CoV 09356	CoA 92082 × CoV 92102	2009	MS

Plug Method of Inoculation

Colletotrichum falcatum pathotypes were grown on oat meal agar at 28 °C with 12 h/12 h of light and dark cycles in the microbiological incubator. After 10 days, they were inoculated on 6–8-month canes by making an incision at the third internode from the bottom using red rot inoculator (Viswanathan, 2010). About 8 mm of tissue was taken out to place, and 0.5 mL of conidial suspension (1×10^6 conidia per ml) was placed onto the bore hole, after which tissues core was placed into the hole and sealed with plasticine modelling clay. A minimum of five canes free from borer pest infestation, cracks and other damages were inoculated with each pathotype in each season. During the first two seasons, all the nine pathotypes were evaluated and in 2019–2020, only four of them were evaluated in the trial plots. Two months after inoculation, the inoculated canes were cut at the base, split open vertically along the plug hole to assess the pathogen progress, and the disease severity was assessed on a 0–9 rating scale. Four major parameters that decide red rot reaction, viz. nodal transgression, lesion width, white spots and top yellowing/drying, were scored from each of the split-open canes, and these parameters were given maximum score weightage of 3, 3, 2 and 1, respectively, to compute disease reaction. Finally, the disease reactions were rated as resistant to highly susceptible using the 0–9 rating scale of Srinivasan and Bhat (1961) (Mohanraj et al. 2012).

Soil Inoculation

Two budded setts of sugarcane varieties were planted in 3-m rows as mentioned above; *C. falcatum* multiplied on sorghum grain was applied in the rows at the rate of 100 g per row at the time of planting. To have uniform distribution of the fungal inoculum in the rows, the sorghum grain inoculum was diluted in field soil before application (Mohanraj et al. 2012; Viswanathan et al. 2020b). About 20 two budded setts from healthy sugarcane free from red rot or other stalk diseases were used for planting in each row. After inoculum application, the setts were covered with a thin layer of soil from both sides of ridges and irrigated.

Healthy control plot of all the varieties was maintained without *C. falcatum* inoculum application. The disease development in the form of yellowing or orange discolouration of sprouts/shoots, death of stalks/tillers, was recorded in the inoculated plots at monthly intervals, and the final rating was expressed as per cent disease incidence at the time of harvest (12 months). The cumulative number of disease-affected clumps was compared with the total number of the clumps in a plot, and per cent disease incidence for each pathotype/variety was computed.

The pathogenic reactions categorized into HS to R in the plug method were converted into highly virulent (HV) (8.1–9.0), virulent (V) (6.1–8.0), moderately virulent (MV) (4.1–6.0), less virulent (LV) (2.1–4.0) and least virulent (LeV) (0.0–2.0) to denote pathogenic virulence on different varieties as reported earlier (Viswanathan et al. 2017). The pathogenic virulence in the plug method was compared with disease development from soil-borne inoculum applied at the time of planting.

Data Analyses

To assess relationship between disease development in the plug and soil inoculation methods by the respective pathotypes was made in scattered plots using XLStat2020 software. For plotting the graph, the disease reaction values in the plug method and disease incidence in the soil inoculation method were taken in x- and y-axes, respectively. The R, MR, MS, S and HS values were converted as 1, 3, 5, 7 and 9, respectively. The per cent red rot incidence in the soil application method was scaled down to 1 to 10 scale to achieve proportionate scattered plot. To have clear interpretation, the scattered plot was divided into four quarters manually.

Results

Behaviour of Sugarcane Varieties to *C. falcatum* Pathotypes: Pathogenic reactions of ten varieties to nine *C. falcatum* pathotypes were assessed by inoculating them by the plug method. On the susceptible cv CoC 671, the pathogenic reactions were V or HV except for MV during 2017–2018 season to the pathotypes *Cf*2001-13 and *Cf*06022. On the other susceptible cv Co 94012, the pathotypes behaved as V/HV during the three seasons except LV reaction of *Cf*2001-13 during 2017–2018. In the plots where soil inoculum was applied, the disease development in these varieties was noticed from germination phase onwards till harvest as drying/death of canes. In both the susceptible varieties, as expected, the soil inoculum of all the *C. falcatum* pathotypes caused red rot in the three seasons to varying intensities. However, there was V or HV behaviour of the pathotypes in the plug method against no disease development for the respective pathotypes in case of soil inoculum and vice versa, where MV and LV behaviour in the plug method against disease development from soil inoculum was observed. During 2017–2018, V/HV behaviour of the pathotypes *Cf*94012, *Cf*99006 and *Cf*0323 did not match with the disease development from soil inoculum in the cv CoC 671. During 2018–2019, virulence behaviour in the plug method had a matching disease development in all the pathotypes. In case of the cv Co 94012, during 2017–2018, two pathotypes *Cf*671 and *Cf*86027 did not have matching behaviour between the two methods of inoculation. Although the pathogenic virulence was between V and HV in the pathotypes during the three seasons, disease intensity recorded as per cent disease incidence varied from 3.4 to 100% for soil-borne inoculum. Among the three seasons, the disease intensities were very high in the ranges of 71.4–93.3% and 12.5–90% during 2018–2019 and 2019–2020, respectively, in the cv CoC 671, whereas in the 2017–2018 season it was 4.2–13.3% showing a poor disease development from the soil inoculum. The respective figures for the cv Co 94012 were 75–100%, 14.3–85.7% and 3.4–20.0%. Although the disease development from the soil inoculum during 2019–2020 was higher than 2017–2018 season on the susceptible varieties, it was less severe as compared to 2018–2019. Further, during the season, the sugarcane varieties recorded low disease intensities against the pathotypes *Cf*V09356, *Cf*2001-13 and *Cf*0323.

In general, the pathotypes exhibited LV/LeV behaviour on the resistant cvs Co 0212, Co 0238 and Co 0403 during the three seasons, with few MV/V/HV behaviour. In the cv Co 0238, the pathotype *Cf*V09356 behaved as 'V' and 'MV' during 2017–2018 and 2018–2019 seasons, respectively. Also the pathotype *Cf*86027 exhibited behaviour of 'V' on this variety during 2017–2018, and during 2018–2019 the behaviour was 'LeV', indicating variable exhibition of pathogenic virulence in two different seasons on this variety. Furthermore, of the nine pathotypes, only *Cf*V09356 and *Cf*86027 could exhibit virulent behaviour in 2017–2018 on this variety, whereas no disease development was observed from soil inoculum in all the three seasons except for trace incidences during 2019–2020 against *Cf*94012 and *Cf*V09356 pathotypes. However, other two cvs Co 0212 and Co 0403 succumbed to the soil-borne inoculum of *C. falcatum* pathotypes sporadically. The pathotype *Cf*V09356 exhibited virulence on Co 0212 as like Co 0238 by 'V' behaviour during the first two seasons. The cv Co 0403 behaved as susceptible to the pathotype during 2018–2019 in the plug method. The pathotypes *Cf*671, *Cf*99006, *Cf*86027 (2017–2018) and *Cf*06022 behaved as MV on the cv Co 0212 during the first two seasons. However, the pathotypes *Cf*86032 behaved as

'V' and 'MV' during 2017–2018 and 2018–2019 seasons, respectively. During 2019–2020, four test pathotypes exhibited LV or LeV behaviour on this variety. In spite of the pathotypes behaviour as virulent in two to three occasions, the respective pathotypes could not cause disease in the variety from the soil-borne inoculum. However, the pathotype *Cf*86027 could cause a limited infection of 4.0% during 2017–2018, even though the behaviour in the plug method was MV. In the variety, the soil inoculum of the pathotype *Cf*V09356 caused 11.1% disease against LeV in the plug method and similarly the pathotype *Cf*86032 caused 9.1% infection against LV during the 2019–2020 season. In case of the cv Co 0403, the four pathotypes *Cf*99006, *Cf*V09356, *Cf*06022 and *Cf*86032 exhibited virulence in one or two seasons. The pathotype *Cf*06022 behaved as 'HV' on this variety, destroying entire canes after plug inoculation in 2018–2019 season; however all these virulent behaviour of the pathotypes in the plug method did not match with its ability to cause the disease in this variety when the inoculum was applied in the soil except for 'V' behaviour in the plug matched with 50% disease during 2019–2020 season in case of *Cf*86032 pathotype. In contrast, during three occasions, the pathotypes *Cf*671, *Cf*86027 and *Cf*2000-13 caused the disease from soil inoculum, though their respective behaviour was 'LV', 'MV' and 'MV' in the plug method during 2018–2019 season. During 2019–2020, the variety exhibited 72.7% disease against the pathotype *Cf*2001-13 followed by 50, 31.8 and 4.8% against the pathotypes *Cf*86032, *Cf*86027 and *Cf*06022, respectively, from the soil inoculum (Table 3). As in the case of susceptible varieties, the pathogenic behaviour clearly evidenced a clear differential reaction in both the methods of pathogen inoculation on the resistant varieties with no relation between the methods for exhibition of virulence with few exceptions.

The pathogenic behaviour of different pathotypes ranged from 'LeV' to 'HV' in the MS cvs Co 86032, Co 06022, Co 06027, Co 06030 and CoV 09356; however, most of the behaviour were between 'MV' and 'LV'. HV behaviour was observed in the cv Co 86032 to its matching pathotype *Cf*86032 during 2017–2018 and in the cv Co 06030 to the pathotype *Cf*06022 during 2018–2019. Among these five MS varieties, the cv Co 86032 behaved differently for disease development from soil inocula of different *C. falcatum* pathotypes. Except the pathotypes *Cf*671, *Cf*V09356 and *Cf*94012, other pathotypes exhibited 'V' behaviour at least in one season and 'HV' to its matching pathotype *Cf*86032 in the plug method in this variety. However, no disease development from soil inoculum was found against 'V'/'HV' behaviour in the plug method, except for *Cf*86027 in 2017–2018 where ~ 4.3% disease was recorded. During 2018–2019 and 2019–2020 seasons, ~ 7.1% disease from soil inoculum of *Cf*671 was found, although behaviour in the same season in the plug method was 'LV' and 'MV', respectively. On the contrary, other MS cvs Co 06022, Co 06027, Co 06030 and CoV 09356 with a clear differential behaviour in the plug method recorded very high disease intensity from soil inoculum of different pathotypes. The pathotype *Cf*86027 caused maximum of 100% disease in its host cv Co 06027 and Co 06030 during 2018–2019 and during the same season the pathotype *Cf*2001-13 caused a maximum disease of 80% in the cv Co 06022, and the pathotype *Cf*06022 caused 94.4% disease on the cv CoV 09356. Another interesting observation was that in 30 interactions of the pathogenic behaviour of 'MV', 'LV' or 'LeV' in plug method, we did not find any disease development, whereas in 32 such interactions we recorded moderate to severe disease from soil inoculum, indicating a different pathogenicity pattern of soil inoculum in infecting these four varieties during 2017–2018 and 2019–2020 seasons (Table 3). Only in six interactions, pathogenic behaviour in the plug method and soil inoculation matched for pathogen virulence, whereas in the three interactions, pathogenic virulence in plug method was not reflected in disease development from soil inoculum in these two seasons. As in the case of susceptible varieties, here also disease development from soil inoculum was very high during 2018–2019 as compared to the previous season. However, pathogenic behaviour in the cane after plug inoculation did not show much variation between seasons except a few.

During 2019–2020, though we did not have disease reactions to five of the pathotypes, disease development from soil inoculum from all the nine pathotypes revealed that the pathotypes could cause infections in 3–4 varieties except the pathotype *Cf*94012 which caused disease in only one MS variety. The disease severity ranges in the MS varieties by other pathotypes were 5.6–50% (*Cf*86032) followed by 5.6–46.2% (*Cf*671), 13.3–42.9% (*Cf*06022) and the severity to other pathotypes was very less. However, the pathotype Cf2001-13 selectively caused severe incidence of 80% disease in the cv Co 06027. The cv Co 06030 recorded disease incidences of 46.2% (*Cf*671), 42.9% (*Cf*06022 and *Cf*86032), 35.7% (*Cf*86027) and 27.8% (*Cf*94012). Other pathotypes caused moderate incidences of red rot, and it is the only variety in the MS category which succumbed to all the pathotypes although the pathogenic virulence remained in the range of MV to LV in the plug method. In addition, the cv Co 06022 showed disease development against five pathotypes with maximum disease of 44.4% (*Cf*86027). In case of the cv Co 06027, six pathotypes caused disease development with more than 50% were recorded against *Cf*86032 (50%) and Cf2001-13 (80%). The cv CoV 09356 also picked up disease from six pathotypes with maximum against its own pathotype *Cf*V09356 (38.5%) followed by *Cf*06022

Table 3 Behaviour of sugarcane varieties to *Colletotrichum falcatum* pathotypes challenged by two methods of inoculation under field conditions

Sl no	Varieties	Pathogenic reactions of *C. falcatum* pathotypes and per cent disease incidence from soil inoculum																
		*Cf*671						*Cf*94012						*Cf*99006-MPM				
		Plug method[a]			Soil inoculum[b]			Plug method[a]			Soil inoculum[b]			Plug method[a]		Soil inoculum[b]		
		2017–2018	2018–2019	2019–2020	2017–2018	2018–2019	2019–2020	2017–2018	2018–2019	2019–2020	2017–2018	2018–2019	2019–2020	2017–2018	2015–2016	2017–2018	2018–2019	2019–2020
1	Co 0212	MV	LV	LV	0.0	0.0	0.0	LV	LV	LV	0.0	0.0	0.0	MV	LV	0.0	0.0	0.0
2	Co 0238	LV	LeV	LeV	0.0	0.0	0.0	LeV	LV	LV	0.0	0.0	3.1	LV	LV	0.0	0.0	0.0
3	Co 0403	LV	LV	LeV	0.0	2.5	0.0	LeV	LV	LV	0.0	0.0	0.0	V	V	0.0	0.0	0.0
4	Co 86032	MV	LV	MV	0.0	7.1	7.1	LV	MV	LV	0.0	0.0	0.0	MV	V	0.0	0.0	15.4
5	Co 06022	MV	LV	LV	0.0	14.3	20.0	LV	LV	MV	0.0	0.0	0.0	MV	V	0.0	10.3	5.6
6	Co 06027	LV	LV	LV	0.0	0.0	5.6	LV	LeV	LV	0.0	0.0	0.0	MV	V	17.4	0.0	0.0
7	Co 06030	MV	LV	LV	0.0	57.9	46.2	MV	LV	MV	0.0	82.6	27.8	MV	V	0.0	56.3	14.3
8	CoV 09356	LV	LV	LV	8.0	0.0	9.1	LV	MV	MV	0.0	0.0	0.0	LV	LV	0.0	9.1	7.7
9	Co 94012	HV	HV	HV	0.0	75.0	85.7	HV	HV	HV	4.3	85.7	58.3	V	HV	4.2	92.3	81.3
10	CoC 671	V	HV	HV	4.2	75.0	83.3	HV	HV	HV	0.0	73.3	66.7	V	V	0.0	87.5	90.0

Sl no	Varieties	Pathogenic reactions of *C. falcatum* pathotypes and per cent disease incidence from soil inoculum																
		*Cf*V09356-ENGR						*Cf*86027-NKU						*Cf*2001-13-PPM				
		Plug method[a]			Soil inoculum[b]			Plug method[a]		Soil inoculum[b]				Plug method[a]		Soil inoculum[b]		
		2017–2018	2018–2019	2019–2020	2017–2018	2018–2019	2019–2020	2017–2018	2018–2019	2017–2018	2018–2019	2019–2020		2017–2018	2018–2019	2017–2018	2018–2019	2019–2020
1	Co 0212	V	V	LeV	0.0	0.0	11.1	MV	LeV	4.0	0.0	0.0		LV	LV	0.0	0.0	0.0
2	Co 0238	V	MV	LV	0.0	0.0	5.0	V	LeV	0.0	0.0	0.0		LeV	LV	0.0	0.0	0.0
3	Co 0403	LV	V	LeV	0.0	0.0	0.0	LV	MV	0.0	23.2	31.8		LV	MV	0.0	12.1	72.7
4	Co 86032	LV	MV	LV	0.0	0.0	7.1	V	MV	4.3	0.0	0.0		LeV	V	0.0	0.0	4.5
5	Co 06022	MV	MV	LV	0.0	15.0	0.0	MV	LeV	4.3	50.0	44.4		LV	V	0.0	80.0	0.0
6	Co 06027	V	LV	LV	0.0	83.3	20.0	LeV	MV	0.0	100.0	0.0		MV	LV	0.0	44.0	80.0
7	Co 06030	MV	MV	LV	5.6	75.0	6.7	MV	LeV	9.1	100	35.7		LV	LV	0.0	77.8	5.0
8	CoV 09356	LV	V	LV	0.0	0.0	38.5	LV	LeV	60.0	2.0	4.8		LeV	LeV	0.0	70.0	4.0
9	Co 94012	V	HV	V	8.3	75.0	14.3	V	HV	0.0	94.4	36.8		LV	V	0.0	100	21.7
10	CoC 671	V	V	V	4.3	71.4	16.7	V	HV	7.1	93.3	43.8		MV	HV	0.0	81.8	12.5

Table 3 continued

Sl no	Varieties	Pathogenic reactions of *C. falcatum* pathotypes and per cent disease incidence from soil inoculum															
		*Cf*06022-KUT					*Cf*86032-SKPM						*Cf*0323-TNV				
		Plug method[a]		Soil inoculum[b]			Plug method[a]			Soil inoculum[b]			Plug method[a]		Soil inoculum[b]		
		2017–2018	2018–2019	2017–2018	2018–2019	2019–2020	2017–2018	2018–2019	2019–2020	2017–2018	2018–2019	2019–2020	2016–2017[c]	2017–2018	2017–2018	2018–2019	2019–2020
1	Co 0212	MV	MV	0.0	0.0	0.0	V	MV	LV	0.0	0.0	9.1	LV	LV	0.0	0.0	0.0
2	Co 0238	LV	LV	0.0	0.0	0.0	MV	LV	LV	0.0	0.0	0.0	LV	MV	0.0	0.0	0.0
3	Co 0403	LV	HV	0.0	0.0	4.8	V	LV	V	0.0	0.0	50.0	LV	LV	0.0	0.0	0.0
4	Co 86032	V	V	0.0	0.0	0.0	HV	MV	V	0.0	0.0	23.1	MV	V	0.0	0.0	11.1
5	Co 06022	MV	LV	26.3	68.8	15.8	MV	V	LV	10.0	0.0	28.6	MV	LV	0.0	20.0	0.0
6	Co 06027	MV	LV	0.0	75.0	13.3	V	LV	V	0.0	68.4	50.0	LV	MV	0.0	11.1	0.0
7	Co 06030	MV	HV	12.5	75.0	42.9	V	LV	MV	8.3	0.0	42.9	V	V	4.3	84.6	16.7
8	CoV 09356	LV	MV	5.0	94.4	36.4	LV	LeV	LeV	0.0	75.0	5.6	LV	LV	20.8	0.0	0.0
9	Co 94012	V	HV	20.0	100	62.5	V	V	V	15.4	90.0	71.4	HV	V	3.4	93.3	19.0
10	CoC 671	MV	HV	13.3	80.0	30.0	V	HV	V	11.1	80.0	33.3	HV	V	0.0	90.0	13.3

[a]Disease reactions in 0–9 scale were converted to pathogenic reactions as LeV-Least virulent (0–2.0), LV-Less virulent (2.1–4.0), MV- Moderately virulent (4.1–6.0), V-Virulent (6.1–8.0) and HV—Highly virulent (8.1-–9.0).

[b]Disease incidence was recorded from germination phase onwards, and cumulative disease was scored as per cent incidence by 360 days

[c]The pathotype *Cf*0323-TNV was evaluated during 2016–2017 and 2017–2018 in plug method

(36.4%). The cv Co 86032 exhibited disease from soil inoculum in the range of 4.5–23.1% to the pathotypes *Cf*671, *Cf*99006, *Cf*V09356, *Cf*2001-13, *Cf*86032 and *Cf*0323 during the 2019-20 season, the highest incidence to its matching pathotype *Cf*86032 (Table 3).

Overall, the finding of the study indicated that the MS cv Co 86032 remained more or less inaccessible for the pathogen infection when the inoculum was applied in the soil. Although six of the eight pathotypes exhibited 'V' or 'HV' behaviour by the plug method, the variety did not succumb to the respective soil inoculum. The other MS cvs Co 06022 and Co 06027 had such a behaviour from three pathotypes each, four in Co 06030 and one in CoV 09356 in the plug method. The cvs Co 06027 and CoV 09356 exhibited disease development from soil inoculum in case of six pathotypes each, seven in the cv Co 06022 and eight in the cv Co 06030. As in the case of susceptible varieties, here also disease intensities were in the ranges of 4.3–80.0%, 11.1–100%, 5.6–100% and 2–94.4% in the cvs Co 06022, Co 06027, Co 06030 and CoV 09356, respectively, indicating that the disease severity in these MS varieties was almost equal to the susceptible varieties CoC 671 and Co 94012 to certain *C. falcatum* pathotypes.

Varietal Behaviour to *C. falcatum* Pathotypes in Scattered Plot

From the plot, it is obvious that the MR cvs Co 0212 and Co 0238 occupied the lower left quarter indicating pathogen virulence of LV or LeV and limited or no disease development from soil inoculum, whereas the known susceptible cvs Co 94012 and CoC 671 occupied the right upper quarter which is for the varieties exhibiting the highest pathogenic virulence and severe disease development from soil inoculum. However, the cv Co 0212 had a tendency to drift to lower right quarter due to higher virulence of the pathotype *Cf*V09356 and this analysis also depicted exhibition of higher virulence by the pathotypes *Cf*06022 and *Cf*86032 on this variety. Another MR cv Co 0403 was unstable and moved to lower right quarter frequently indicating its vulnerability to some of the pathotypes that show specific virulence on this variety (Fig. 1). Among the MS varieties, the cv Co 06030 was highly unpredictable in its reaction to *C. falcatum* pathotypes in both the methods. It moved to upper left quarter thrice indicating its susceptibility to soil inoculum and upper right along with the susceptible varieties for two pathotypes showing its weakness to both kinds of inocula of the specific pathotypes. Similarly, other MS cvs Co 06022, Co 06027 and CoV 09356 were inconsistent in their behaviour to both kinds of inoculum by scattering in all the four quarters. This has also revealed a clear differential interaction of the sugarcane varieties against the pathotypes in both the inoculation methods. However, the cv Co 86032 remained in the bottom left or right quarters indicating its innate moderate susceptibility to the inoculum delivered through the plug method of inoculation with tolerance to the same pathotypes inoculated in the soil. Such phenomenon can be seen against the pathotypes *Cf*86027, *Cf*2001-13, *Cf*06022, *Cf*V09356 and *Cf*86032 in the plot, whereas the other MS varieties are seen in the upper quarters with some of them recording 100% disease incidences for the soil inoculum.

Discussion

In sugarcane, varietal resistance to red rot is considered as one of the criteria along with cane yield and sucrose content to release varieties for commercial cultivation (Viswanathan 2018). The plug method of inoculation was adopted to assess host resistance in sugarcane genotypes to *C. falcatum* (Chona 1954) on a 0–9 rating scale (Srinivasan and Bhat 1961). There were several attempts to standardize natural way of inoculating sugarcane on nodal tissues without injury to the rind as in the plug method such as the Indian Institute of Sugarcane Research (IISR) method, nodal injury method and leaf sheath inoculation (Agnihotri 1983). To screen sugarcane varieties for red rot resistance, two methods, viz. 'plug method' and 'nodal method', are followed in all the sugarcane research centres in India. The latter is considered to simulate natural way of *C. falcatum* infection, in which conidial suspension was delivered inside leaf sheath of 2–3 leaves in the whorl to facilitate the pathogen to reach its portals of entry on the node, viz. bud, root eyes and cambial region. The method was expected to be ideal to simulate natural infection process of the pathogen; however, enormous disease escapes were found under field conditions. The disease escapes were attributed to the environmental factors prevail during incubation and the first author also experienced the same while inoculating the susceptible check cv CoJ 64, in which he found infection only in one out of five seasons at Karnal during 2007–2011 indicating the critical role played by weather factors. To circumvent the concerns, the nodal swabbing method was developed at this institute, in which a cotton pad dipped in conidial suspension is swabbed around two partially matured nodes after removing the leaf sheath in 6–8-month old canes (Viswanathan 2013; Viswanathan et al. 2018). The pads are covered with parafilm® to prevent drying of cotton and to favour pathogen infection. This method has ensured ideal disease development from nodal tissues, and disease development is seen in most of the genotypes that are susceptible in the plug method, except a few that exhibits a clear nodal resistance every season. This information suggests that by directly placing

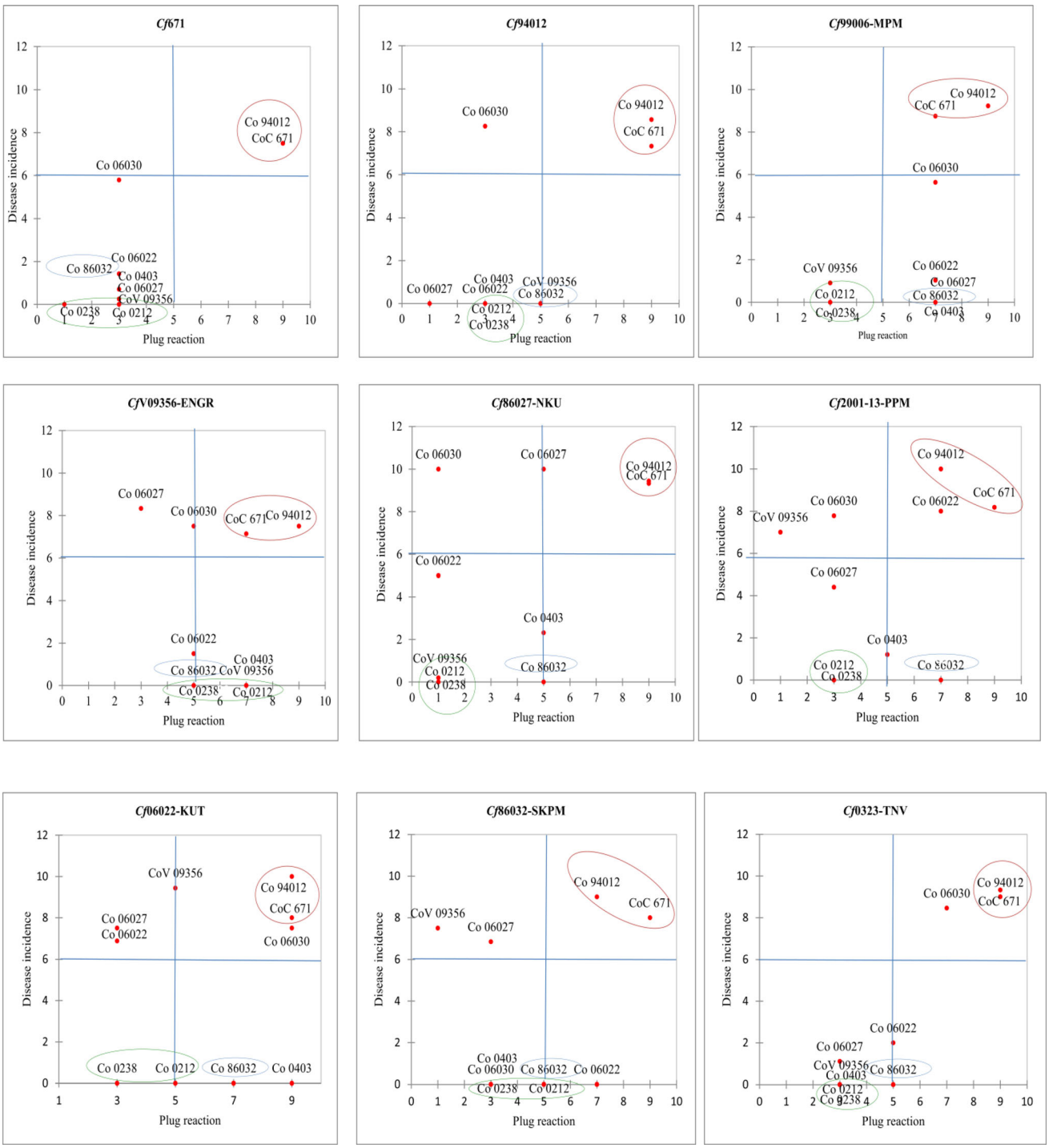

Fig. 1 Responses of different sugarcane varieties to two inoculation methods of *Colletotrichum falcatum* pathotypes are depicted in scattered plot diagrams. Pathogenic reaction in 0–9 scale is plotted in 'x'-axis, and disease incidence from soil-borne inoculum is plotted in 'y'-axis after scaling down the per cent values to 0–10. The susceptible cvs Co 94012 and CoC 671, the resistant cvs Co 0212 and Co 0238 and the field tolerant cv Co 86032 are encircled in different colours

pathogen inoculum inside the internode tissue, it easily causes infection leading to susceptible reaction, whereas when the inoculum is placed on the nodes, the pathogen faces resistance and unable to cause the disease in certain genotypes. In a disease screening method, we always look for a resistant genotype; hence, such rare occurrences are ignored. Currently, the nodal swabbing method is adopted in All India Coordinated Research Project (AICRP) on

sugarcane trials along with the plug method to evaluate red rot resistance (Viswanathan et al. 2018). Phenotyping by nodal swabbing is preferred to ascertain whether the resistant phenotype by plug is vulnerable to the pathogen by nodal inoculation. However, we found resistance reaction in the plug method always coincide with the nodal method with exceptions of nodal resistance with plug susceptible in few rare cases.

While investigating the severe red rot epiphytotics in 1940s, Chona and Padwick (1942) identified emergence of a different *C. falcatum* phenotype with profuse sporulation and higher virulence named as 'light race'. This and subsequent studies laid a foundation on physiological variation in *C. falcatum* and documented enormous variation in the pathogen in different states (Beniwal and Virk 1989; Chona 1980, Rafay and Singh 1957; Padmanaban et al. 1996; Viswanathan et al. 2003). Recently, huge variation for pathogenicity exhibited by *C. falcatum* was demonstrated by testing ~ 117 pathotypes of tropical and subtropical origin simultaneously at Coimbatore and Karnal, representing tropical and subtropical regions, respectively. The variable behaviour of *C. falcatum* pathotypes is attributed to their innate virulence, host variety of origin and prevailing environment at the time of testing (Viswanathan et al. 2017). Pathogenic variation in *C. falcatum* is continuously assessed on a set of host differentials every year under AICRP on sugarcane at 12 centres. Based on distinct variation and stability in pathogenic virulence, the newly characterized pathotype becomes 'designated pathotype'. Such designated pathotypes are used to screen sugarcane genotypes for red rot resistance in different agroclimatic zones categorized for sugarcane such as North West, North Central, North East, East Coast and Peninsular zones. Recently, a new pathotype from tropical region *Cf*94012 (CF12) has been designated after detailed characterization for its virulence and its stability (Viswanathan 2017). Subsequently, studies were conducted to assess the pathogenic behaviour in a set of sugarcane varieties varying in host resistance. This host–pathogen interaction involving studies 20 varieties and 10 *C. falcatum* pathotypes for 10 seasons clearly revealed that apart from pathogenic variation, they tend to break host resistance more frequently in the MS varieties but also to a limited extent in the resistant types. This study concluded that *C. falcatum* continuously evolves new variants to match to the new host varietal background (Viswanathan et al. 2020a).

At this juncture, we need to understand how the soil-borne inoculum of *C. falcatum* survives and causes disease in the field since this is the one challenging the host resistance under field conditions causing varietal breakdown. In general, the pathogen inoculum as conidial suspension is short-lived, whereas infected crop residues survive for several months in the soil. Chona and Nariani (1952) reported that *C. falcatum* is capable of growing in soil and produces acervuli abundantly. However, they found that the fungus does not survive in the soil beyond 3–4 months. Later, Singh et al. (1986) reported that *C. falcatum* survives for 8 to 9 months when infected nodal/internodal pieces of diseased cane stalks are placed on soil surface. But, it was found that the pathogen survival is considerably reduced if the pieces are buried in the soil. Soil-borne inoculum either in the soil or in debris plays a major role as primary source of inoculum in initiating infection as well as in build-up of red rot epidemics (Viswanathan 2010). Once an infection focus is created by death of a stool, secondary spread takes place by spread of inocula through irrigation or rain water. Such displaced inoculum causes fresh infections in the field depending on the prevailing weather conditions after surviving in the soil for varying periods.

We found *C. falcatum* pathotypes reaction as V or HV on the susceptible varieties throughout the seasons in the plug method. Further, the pathotypes *Cf*671 and *Cf*94012 recorded 'HV' behaviour on the respective host varieties except 'V' behaviour of the pathotype *Cf*671 during 2017-18. Both these pathotypes caused severe disease intensity of more than 73.3% from soil inoculum during 2018–2019 season, whereas the pathotype *Cf*671 did not infect the cv Co 94012 during 2017–2018, similarly the pathotype *Cf*94012 did not infect the cv CoC 671 during the same season. However, during 2018–2019 and 2019–2020, both the susceptible varieties recorded very high levels of red rot to both the pathotypes giving a clear relation between HV behaviour and severe disease from soil inoculum. Poor disease development during 2017–2018 season may be due to extended drought occurred during the early growth phase of the crop. Higher disease incidences during the last two seasons may be due to rainfall received continuously for 8–9 weeks during April–June which would have favoured inoculum survival in the soil, initiation of infection and disease development (Table 4). These results clearly established that pathogenic virulence is not affected in both the methods of inoculation in the susceptible varieties although in the case of soil inoculum the disease development is influenced by rainfall to a certain extent. Earlier also we found a positive relation between rainfall and disease incidence from soil inoculum of *C. falcatum* pathotypes (Viswanathan et al. 2020b). However, we need to establish how the weather factors influence survival and infectivity of the soil-borne inoculum of the pathogen. Perhaps, under endemic locations, both the host and the pathogen inoculum are available throughout the season; hence, we expect continuous attempts from both the primary and secondary sources of the pathogen inoculum to infect sugarcane. Probably, such interactions may be influenced by the environment and we

Table 4 Rainfall received during the early phases of crop growth at Coimbatore

Standard week		Rainfall (mm)			No. of rainy days		
		2017	2018	2019	2017	2018	2019
7	February II	0	0	0	0	0	0
8	February III	0	0	0	0	0	0
9	March I	0	0	0	0	0	0
10	March II	44.6	0	0	2	0	0
11	March III	11.8	18.2	0	1	2	0
12	March IV	3.6	9.2	0	1	1	0
13	April I	0	0	0	0	0	0
14	April II	0	16.2	0	0	1	0
15	April III	8.8	9.4	0	1	1	0
16	April IV	0	0	6.1	0	0	2
17	May I	0	18.6	18.0	0	1	1
18	May II	0	10.9	0	0	6	0
19	May III	0	94.2	21	0	4	1
20	May IV	17	12	33.8	2	1	2
21	June I	7.2	31.6	4.0	2	4	1
22	June II	0	0	27	0	0	2
23	June III	10.4	5.8	4.8	1	2	1
24	June IV	0	37.4	5.6	0	4	1
	Total	103.4	263.5	120.3	10	27	11

need to study further on differential interaction of the soil-borne inoculum of the pathotypes. Since there was no information available on the pathogenic variation in *C. falcatum* pathotypes surviving in the soil, we studied behaviour of different pathotypes in the soil. The results revealed the role of soil inoculum of *C. falcatum* in inciting damages to the growing plant, adaptation of the pathotypes to the host varieties and differential interaction of the pathotypes in sugarcane varieties as in the case of stalk inoculation (Viswanathan et al. 2020b).

The MS cvs Co 06022, Co 06027, Co 06030 and CoV 09356 behaved as MS/MR in the plug method succumbed to inocula from soil against different pathotypes, and the disease severity was equal to those in susceptible varieties to some of the *C. falcatum* pathotypes (Table 3). In these canes, although the host varieties exhibited resistance even after introducing inoculum inside the internode tissues, the pathogen could not colonize/damage host tissues probably due to protoplast resistance. This type of resistance also referred as 'biochemical resistance' is governed by induction of different pathogenesis-related (PR) proteins and 3-deoxyanthocyanidin phytoalexins (Ganesh Kumar et al. 2015; Viswanathan et al. 1996, 2005). Furthermore, molecular basis of red rot resistance has been established through genomic and proteomic approaches. These studies revealed an early induction of defence genes in resistant interactions as compared to susceptible ones, to restrict pathogen movement inside the host after initial infections (Sathyabhama et al. 2015, 2016; Viswanathan 2012; Viswanathan et al. 2016). Recent studies of Nandakumar et al. (2020) with GFP-tagged *C. falcatum* also clearly demonstrated that the pathogen could not establish in the stalk tissues after the plug method of inoculation in resistant varieties as in susceptible ones. However, the same variety which is exhibiting protoplast resistance succumbs to the pathotype when infection is initiated on the sprouting bud/growing shoot/nodal tissues in the bottom internodes and such infection makes a good progress and colonizes entire cane tissues, causing total death of the canes. Unlike the plug method, here infections start from bud sprouting onwards and go till harvest, with maximum disease infections in germination and tillering phases (Viswanathan et al. 2020b).

Unlike these four MS varieties, the cv Co 86032 exhibited a high level of tolerance to *C. falcatum* inoculum applied in the soil. It can be argued that this variety does have limited protoplasmic resistance as in the other MS varieties, but effectively protects itself from soil-borne infections. This unique behaviour of the variety may probably attribute to its general field tolerance against *C. falcatum* in different states. The variety survived under the abundant soil inocula left over from the affected cvs CoC

671, CoC 85061, CoC 92061, Co 6304, CoSi 96071, etc. after the historical red rot epiphytotics in the tropical region during 1998 to 2005 (Viswanathan 2010). The first author has documented its field tolerance against red rot in different places in Tamil Nadu, Puducherry, Andhra Pradesh, Gujarat and Odisha for the past two decades except few stray incidences of red rot in these states. In the same endemic areas of Cauvery delta and East Coast region in Tamil Nadu State, the cvs CoV 09356, Co 06022 and Co 06027 recorded moderate to severe incidences of red rot during 2014–2018, whereas the cv Co 86032 remained free from the disease during the period (Viswanathan et al. 2020b). This observation corroborates with the present findings on red rot in these varieties from soil-borne inoculum. This is a paradoxical situation where some sugarcane varieties exhibit resistance under artificial testing (plug method), but succumbs to the soil inoculum of the pathogen and vice versa.

Hence, resistance in the plug method of inoculation may symbolize for adult plant resistance and this needs further studies. Earlier, the first author has found weakness for *C. falcatum* infection in younger nodes in the susceptible sugarcane cv CoC 671 and matured nodes remain free from pathogen infections. This partly answers on vulnerabilities of host varieties to *C. falcatum* probably during their early growth phases. However, a variety behaved as disease free in a season as against 100% disease to the same pathotype in another season probably indicates influence of specific weather factors on the inoculum applied in the soil for its survival, infection and disease progress. Overall, season to season disease reaction in the plug method is not affected possibly due to placement of the inoculum inside the internode tissue. Here, once the pathogen reaches its active site of colonization inside the stalks, it makes rapid progress in linear spread depending on the host–pathogen interaction.

Conclusion

We found that the sugarcane varieties with MS/MR reactions behave differently to various *C. falcatum* pathotypes for pathogenicity. However, such pathogenic reactions do not coincide with disease infection from the soil inoculum. This variable infection potential of *C. falcatum* pathotypes for infection in two methods, one artificial inoculation with injury and the other, placing inoculum in the soil near to sprouting buds/growing canes which is closer to nature, reflects the interaction between the host varieties and the method of inoculation of the pathotypes. The present study also revealed that *C. falcatum* with its extensive variation continuously making attempts to invade the host varieties even though they possess resistance in sugarcane ecosystem. Probably, when favourable environment prevails in the ecosystem, the pathogen makes a 'detrimental effect' to break the host resistance and gradually causes 'varietal breakdown'. This is the first comprehensive study to demonstrate *C. falcatum* adaptation to sugarcane varieties under field conditions, and it attempted to partly answer the basis for 'varietal breakdown' to red rot in sugarcane.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and Animal Participants The present research did not involve human participants and/or animals.

Informed Consent It was obtained from all individual participants included in the study.

References

Agnihotri, V.P. 1983. *Diseases of Sugarcane*, 363. New Delhi: Oxford and IBH Publishing Co.

Barber, C.A. 1901. Sugarcane diseases in Godawari and Ganjam Districts. *Madras Department Land Records and Agricultural Bulletin* 512(43): 181–194.

Beniwal, M.S., and K.S. Virk. 1989. Pathogenic variability in *Colletotrichum falcatum* incitant of red rot of sugarcane. *Indian Phytopathology* 42: 95–98.

Butler, E.J. 1906. Fungal diseases of sugarcane in Bengal. *Memoirs of Department of Agriculture, India, Botany Series* 1(3): 2–24.

Chona, B.L. 1954. Relative resistance of sugarcane varieties to red rot. *Indian Journal of Agricultural Sciences* 24: 301–315.

Chona, B.L. 1980. Red rot of sugarcane and sugar industry—A review. *Indian Phytopathology* 33: 191–207.

Chona, B.L., and T.K. Naraini. 1952. Investigations on the survival of *Colletotrichum falcatum* in soil. *Indian Phytopathology* 5: 152–157.

Chona, B.L., and W.G. Padwick. 1942. More light on the red rot of epidemic. *Indian Farming* 3: 70–73.

Duttamajumder, S.K. 2002. A century of red rot disease of sugarcane in India. In *Sugarcane Crop Management*, ed. S.B. Singh, G.P. Rao, and S. Eswaramoorthy, 52–108. Houston: SCI TECH Publishing LLC.

Ganesh Kumar, V., R. Viswanathan, P. Malathi, M. Nandakumar, and A.R. Sundar. 2015. Differential induction of 3-deoxyanthocyanidin phytoalexins in relation to *Colletotrichum falcatum* resistance in sugarcane. *Sugar Tech* 17: 314–321.

Malathi, P., R. Viswanathan, and R. Jothi. 2006. Specific adaptation of *Colletotrichum falcatum* pathotypes to sugarcane cultivars. *Sugar Tech* 8: 54–58.

Mohanraj, D., P. Padmanaban, and R. Viswanathan. 2012. Screening for red rot resistance in sugarcane. In *Functional Plant Science and Biotechnology 6 (Special Issue 2)*, ed. R. Viswanathan and A.R. Sundar, 51–62. Ikenobe: Global Science Books.

Nandakumar, M., P. Malathi, A.R. Sundar, and R. Viswanathan. 2020. Use of green fluorescent protein expressing *Colletotrichum falcatum* for precise host-pathogen interaction studies in sugarcane. *Sugar Tech* 22: 112-121 https://doi.org/10.1007/s12355-019-00751-8.

Padmanaban, P., D. Mohanraj, R. Viswanathan, M.M. Rao, N. Prakasam, R. Jothi, and K.C. Alexander. 1996. Differential interaction of sugarcane clones to pathotypes of *Colletotrichum falcatum* Went. *Sugar Cane* 4: 16–20.

Rafay, S.A., and V.B. Singh. 1957. A new strain of *Glomerella tucumanensis*. *Current Science* 26: 19–20.

Sathyabhama, M., R. Viswanathan, P. Malathi, and A.R. Sundar. 2016. Identification of differentially expressed genes in sugarcane during pathogenesis of *Colletotrichum falcatum* by suppression subtractive hybridization (SSH). *Sugar Tech* 18: 176–183.

Sathyabhama, M., R. Viswanathan, M. Nandakumar, P. Malathi, and A.R. Sundar. 2015. Understanding sugarcane defense responses during the initial phase of *Colletotrichum falcatum* pathogenesis by suppression subtractive hybridization (SSH). *Physiological and Molecular Plant Pathology* 91: 131–140.

Singh, N., S. Lal, and K. Singh. 1986. Survival of red rot pathogen in sugarcane infected debris. *Indian Journal of Sugarcane Technology* 3: 125–128.

Singh, K., and R.P. Singh. 1989. Red rot. In *Sugarcane Diseases of the World: Major Diseases*, ed. C.G. Ricaud, B.T. Egan, A.G. Gillaspie, and C.G. Hughes, 169–188. Amsterdam: Elsevier.

Srinivasan, K.V. 1962. Some observations on variation in the red rot pathogen, *Glomerella tucumanensis* (Speg.) Arx & Muller. *Proceedings of International Society of Sugar Cane Technologists* 11: 795–802.

Srinivasan, K.V. 1965. Towards the ideals of red rot resistance—Ends and means. *Proceedings of International Society of Sugar Cane Technologists* 12: 1108–1117.

Srinivasan, K.V., and N.R. Bhat. 1961. Red rot of sugarcane—Criteria for grading resistance. *Journal of Indian Botanical Society* 40: 566–577.

Sundara, B. 1998. *Sugarcane Cultivation*. New Delhi: Vikas Publishing House Pvt Ltd.

Viswanathan, R. 2010. *Plant Disease: Red rot of Sugarcane*. New Delhi: Anmol Publications Pvt Ltd.

Viswanathan, R. 2012. Molecular basis of red rot resistance in sugarcane. In *Functional Plant Science and Biotechnology 6 (Special Issue 2)*, ed. R. Viswanathan and A.R. Sundar, 40–50. Ikenobe: Global Science Books.

Viswanathan, R. 2013. Screening for diseases: Red rot. *Annual Report of Sugarcane Breeding Institute 2012–13*, Coimbatore, India.

Viswanathan, R. 2017. Pathogen virulence in sugarcane red rot pathogen versus varieties in cultivation: Classical case of loss in virulence in the pathotype CF06 (Cf671). *Sugar Tech* 19: 293–299.

Viswanathan, R. 2018. Changing scenario of sugarcane diseases in India since introduction of hybrid cane varieties: path travelled for a century. *Journal of Sugarcane Research* 8(1): 1–35.

Viswanathan, R., P. Malathi, and P. Padmanaban. 2003. Variation in sugarcane red rot pathogen *Colletotrichum falcatum* Went. In *Frontiers of Fungal Diversity in India*, ed. G.P. Rao, C. Manoharachari, D.J. Bhat, R.C. Rajak, and T.N. Lakhanpal, 639–667. Lucknow: International Book Distributing Co.

Viswanathan, R., P. Malathi, A. Ramesh Sundar, S. Aarthi, S.M. Premkumari, and P. Padmanaban. 2005. Differential induction of chitinases and thaumatin-like proteins in sugarcane in response to infection by *Colletotrichum falcatum* causing red rot disease. *Journal of Plant Diseases and Protection* 112: 537–542.

Viswanathan, R., P. Malathi, A.R. Sundar, K. Kaverinathan, M.L. Chhabra, B. Parameswari, and R. Jothi. 2017. Diversity of *Colletotrichum falcatum* population in India: Comparative virulence at two different agro-climatic regions. *International Sugar Journal* 119: 966–977.

Viswanathan, R., D. Mohanraj, P. Padmanaban, and K.C. Alexander. 1996. Synthesis of phytoalexins in sugarcane in response to infection by *Colletotrichum falcatum* Went. *Acta Phytopathologica et Entomologica Hungarica* 31: 229–237.

Viswanathan, R., M. Sathyabhama, P. Malathi, and A.R. Sundar. 2016. Transcriptome analysis of host-pathogen interaction between sugarcane and *Colletotrichum falcatum* by Suppression Subtractive Hybridization and Illumina sequencing. *Proceedings of the International Society of Sugar Cane Technologists* 29: 1639–1644.

Viswanathan, R., A.R. Sundar, R. Selvakumar, and P. Malathi. 2018. Progress in understanding fungal diseases affecting sugarcane: red rot. In *Achieving Sustainable Cultivation of Sugarcane, Volume 2: Breeding, Pests and Diseases*, ed. P. Rott, 201–220. Cambridge: Burleigh Dodds Science Publishing.

Viswanathan, R., P. Padmanaban, and R. Selvakumar. 2020a. Emergence of new pathogenic variants in *Colletotrichum falcatum*, stalk infecting ascomycete in sugarcane: Role of host varieties. *Sugar Tech* 22: 473–484. https://doi.org/10.1007/s12355-019-00780-3.

Viswanathan, R., R. Selvakumar, K. Manivannan, R. Nithyanandam, and K. Kaverinathan. 2020b. Pathogenic behaviour of soil borne inoculum of *Colletotrichum falcatum* in causing red rot in sugarcane varieties with varying disease resistance. *Sugar Tech* 22: 485–497. https://doi.org/10.1007/s12355-020-00800-7.