RESEARCH ARTICLE

Sugar Tech 8(1) (2006) : 54-58

Specific Adaptation of *Colletotrichum falcatum* Pathotypes to Sugarcane Cultivars

P. MALATHI, R. VISWANATHAN* and R. JOTHI

Division of Crop Protection
Sugarcane Breeding Institute, Coimbatore-641007, Tamil Nadu, India

ABSTRACT

Colletotrichum falcatum pathotypes exhibit distinct differential host interaction where certain pathotypes specifically infect their adapted host cultivars. This adaptation phenomenon was tested with two distinct pathotypes, *viz.* Cf1148 and Cf7717 isolated from cultivars Co 1148 and Co 7717, respectively. The results revealed that pathogenicity of both the pathotypes was influenced by their respective/host specific parental cultivars and not vice versa. Cane juice from the host cultivars influenced cultural characters of the pathogen including mycelial growth and conidial germination and they were also varied depending on juice from position of cane stalk. Adaptability of Cf1148 and Cf7717 was tested by cross inoculation on their incompatible hosts viz. Co 7717 and Co 1148, respectively. On cross inoculation, restricted symptoms were produced. Reisolation from such canes yielded fungal activity with changes in growth pattern as compared to original cultures was noticed. After repeated inoculations the dark isolates at initial phases become light with increased sporulation on their adapted hosts. Development of light isolates and reduced latent period for symptom expression by repeated inoculations on incompatible hosts indicated the increased virulence or pathogenicity of that pathotype for adaptation on a particular cultivar.

Key words : Sugarcane, *Colletotrichum falcatum*, adaptation

INTRODUCTION

The red rot disease is a major constraint for sugarcane production in India and the subcontinent faced many epidemics in the past resulting in elimination of many popular varieties from cultivation (Viswanathan and Samiyappan, 2000). It is because the pathogen has gained virulence in recent years (Alexander and Viswanathan, 2002). Prevalence of variation in *Colletotrichum falcatum* Went (Perfect state: *Glomerella tucumanensis* (Speg.) Arx and Muller) pathotypes is well known. The pathotypes exhibit distinct differential host interaction where certain pathotypes specifically infect their adapted host cultivars. Species specificity of *C. falcatum* has already been proved by cross inoculating *C. falcatum* and *C. graminicola* in sorghum and sugarcane respectively (Abbott and Hughes, 1961). However, the development of new pathotypes by adapting to the incompatible hosts has not been proved so far. When a pathogen develops the capacity to carry out a biochemical process that it could not perform originally, it is said that the fungus has adapted and this phenomenon is called as adaptation. It may be due to adaptation of new types to cytoplasm or the pathogen acquiring tolerance to toxic materials and change in virulence to the host plant. Besides it may also be due to evolution of physiologic races through mutation or hybridization. Histological examinations of Neil and Bauchan (2000) in 14 of the most anthracnose-resistant accessions revealed that *C. trifolii* spores germinated and produced typical appressoria, but failed to penetrate and produce the primary and secondary hyphae characteristic of susceptible interactions. They also reported that resistant reactions were similar to those found in incompatible interactions with *C. trifolii* and alfalfa, which have been associated with specific genes leading to the production of isoflavonoid phytoalexins.

In the present study, two distinct pathotypes of *C. falcatum viz.* Cf1148 and Cf7717 designated as *C. falcatum* races CF01 and CF02 respectively were selected from the type culture collection in the plant pathology section. These pathotypes were well differentiated earlier by pathogenicity, serological and molecular studies (Viswanathan *et al.*, 2000; Mohanraj *et al.*, 2002). Although variation in pathogenic, serologic, molecular and cultural characters are known (Viswanathan *et al.*, 2003) origin of new pathotypes or adaptation of *C. falcatum* to a sugarcane cultivar which was hitherto resistant is not clearly understood. Hence detailed

*Author for Correspondence : R. Viswanathan
e-mail : rasaviswanathan@yahoo.co.in

studies are required to identify the mechanism(s) by which the pathogen variant overcomes the host resistance.

MATERIALS AND METHODS

Influence of host cultivar on *C. falcatum*

C. falcatum pathotypes viz., Cf1148 and Cf7717, their host cultivars Co 1148 and Co 7717 have been used for the entire study. The pathotypes were maintained on oatmeal agar and their growth characters were studied on oatmeal agar and sucrose/ host extract amended Czapek's media. For host extract medium, host extract/ juice crushed from top, middle and bottom canes of eight months old were used @ 250g cane juice to supplement carbon source of one litre Czapek's medium. Growth characters of pathotypes were recorded in terms of mycelial growth and sporulation. Influence of host extract on conidial germination was recorded at 100% concentration of juice obtained from different positions of sugarcane cultivars viz. Co 1148 and Co 7717. In which, conidial suspension prepared in pure juice was placed in cavity slides which were then incubated for 24h in a moist chamber and observed for the germination by using light microscope.

Influence of cane position on cultural characters of *C. falcatum in vitro* was studies by extracting juice from three equal portions of the cane as bottom, middle and the top. Juice analysis for brix, purity, sucrose was carried out by using the standard Autopol (Meade, 1977). Total phenolic content of the cane tissue from top, middle and bottom of both the cultivars Co 1148 and Co 7717 was estimated as per standard protocol by Malik and Singh (1980).

Adaptability of C. *falcatum* pathotypes

Inoculation of pathotypes in host cultivars was carried out by standard plug method (Srinivasan and Bhat, 1961). The pathotypes Cf1148 and Cf7717 were self-inoculated on their host cultivars and also cross-inoculated on Co 7717 and Co 1148 respectively. Both the self and cross inoculated cultures were reisolated after the symptom development depending on the latent period. In self-inoculated cultures were reisolated within 25 days while it took 60 days to isolate cross-inoculated cultures, which were named as Cf1148g_1 from Co 7717 and Cf7717g_1 from Co 1148. These cultures were again reinoculated as above and second generation cultures were obtained as Cf1148g_2 and Cf7717g_2 after 45 days. During this process the cultural characters of the reisolated cultures were studied on growth pattern, mycelial growth rate, sporulation and spore germination.

RESULTS AND DISCUSSION

In general growth and sporulation of the pathotypes were influenced by sugarcane juice significantly and such influence was not observed with oatmeal agar/ sucrose amended synthetic media (Table 1). Also there was appreciable difference between cultures in growth and sporulation and the maximum was noticed with Cf7717. However, there was no significant interaction with media and cultures. It was also found that both the pathotypes were influenced by their

Table 1. *C. falcatum* variation in different media

Media	Mycelial growth (cm) (4 DAI)			Sporulation (8 DAI)	
	Cf1148	Cf7717	Mean	Cf1148	Cf7717
Oat meal agar	5.6	6.4	6.00	+	+
Czapek's agar	3.2	4.5	3.85	-	-
Host extract of parental cultivars	8.3	9.0	8.65	++	+++
Mean	5.70	6.63	6.17		

CD (P=0.05) Medium - 0.28; Mycelial growth - 0.23; Medium x Growth - N.S

Sporulation intensity : + - 9 to 10, + + - 22 to 25, + + + - 32 to 35 x 10^5 / 9mm disc

respective/host specific parental cultivars and not vice versa (Table 2). Further results revealed that the mycelial growth and sporulation of the cultures during incompatible interaction was not influenced by the cane position. Influence of host on the development of variants of virulent and avirulent types was reported by Srinivasan (1962). His results revealed that some varieties of sugarcane were shown to induce rapid development and dominance in infected tissues of the dark, avirulent type of varient, while others appear to encourage the dominance of the virulent parental clone. Our studies indicate that under *in vitro* conditions also compatibility/ incompatibility between *C. falcatum* pathotypes and sugarcane cultivars persists. Probably certain inhibitory components in the cane tissue may influence the growth restriction in incompatible interaction.

As in growth studies host specificity was noticed for conidial germination (Table 3). Among the cultivars Co 7717 influenced higher germination particularly juice from top and middle positions than Co 1148 which had less than 50% germination for both the pathotypes. Higher germination in Co 7717 juice can be correlated with higher brix, sucrose and purity values and lower phenolics as compared to Co 1148 (Table 4). Results of the study on juice quality clearly indicated that there was significant difference between cultivars for brix and sucrose values and they were reduced from top to bottom position of the cane stalk. Correspondingly total phenolics got reduced in Co 1148 from top, while it was increased at bottom position of Co 7717, which has negative correlation with conidial germination. Also there was inverse relationship between juice quality and phenolic content of the cultivars was noticed. However earlier studies of Srinivasan (1963) revealed that sporulation of the pathogen increased with

Table 2. Influence of host extract on original and adaptive cultures

Cultivars	Mycelial growth (cm)					Sporulation			
	Cf1148	Cf1148g-2 with sectors	Cf7717	Cf7717g-2 with sectors	Mean	Cf1148	Cf1148g-2	Cf7717	Cf7717g-2
Co 1148									
Top	9.0	9.0	7.6	9.0	8.65	++	++	++	+++
Middle	9.0	9.0	8.4	9.0	8.85	++	++	++	+++
Bottom	9.0	9.0	6.9	9.0	8.48	++	++	++	+++
Co 7717 Top	8.3	9.0	9.0	9.0	8.82	++	+++	+++	++
Middle	9.0	9.0	9.0	9.0	9.00	++	+++	+++	++
Bottom	7.5	9.0	9.0	9.0	8.63	++	++	+++	++
Mean	8.63	9.00	8.32	9.00	8.74				

CD (P=0.05)
[Sporulation intensity: + - 2.08 to 16.67, + + - 16.67 to 29.17, + + + - 29.17 to 41.67 x 10^5/9mm disc]
Cane position - NS; Cf cultures - 0. 34 [x 105 / 9 mm disc) ++ -]; Interaction - 0.83 [++ - 29.17 to 41.67]

varietal resistance and there was no relationship appeared between brix, sucrose and purity/ pH of the juice on the one hand and the sporulation on the other. These values were found to reduce from top to bottom in both the cultivars. Lower germination in the juice from Co 1148 top may be due to higher phenolic content of that cultivar (Table 4). The presence of phenolic substances in relatively greater proportions in varieties of sugarcane resistant to red rot than in those, which are susceptible, was indicated as early in 1938 by Abbott. Above results have clearly indicated that some pathotypes have specificity towards parental cultivars.

Investigation on adaptability of Cf1148 and Cf7717 after inoculation revealed that the latent period for original culture on self inoculation was only 25 days for expressing the symptom, while it took 60 days to isolate cross inoculated cultures which were named as Cf1148g_1 from Co 7717 and Cf7717g_1 from Co 1148. However the symptom production was localized during incompatible interaction as against susceptible reaction in the parental cultivars (Fig. 1). These cultures were again reinoculated as above and second generation cultures were obtained as Cf1148g_2 and Cf7717g_2 after 45 days. It indicates that the latent period has reduced

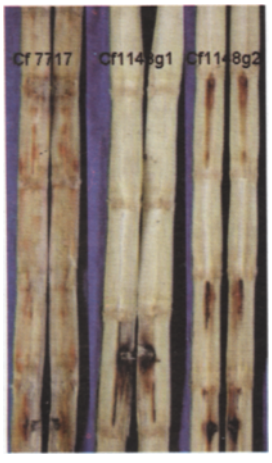

Fig. 1. Disease symptom in Co 7717 by self and cross inoculation

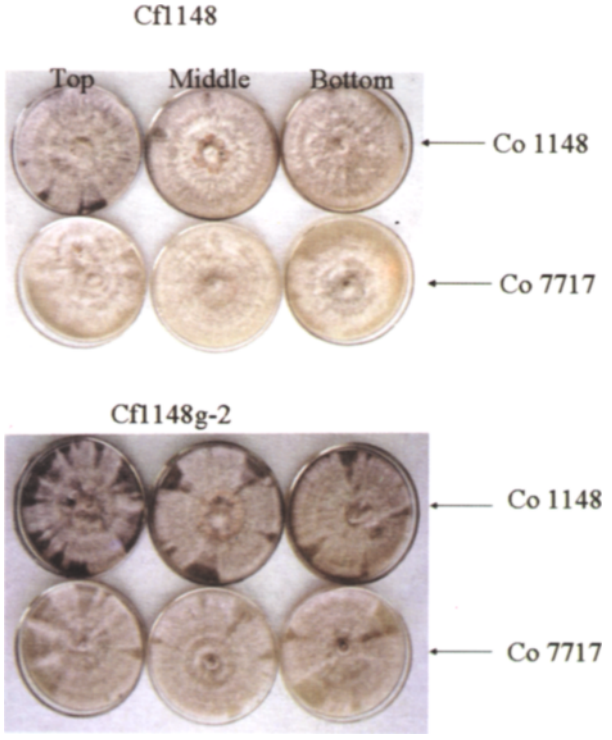

Fig. 2. Cultural variation in Cf1148 developed from incompatible interaction

Table 3. Influence of sugarcane host on conidial germination of *C. falcatum*

Host cultivars	Per cent conidial germination				
	Cf1148	Cf1148g_2	Cf7717	Cf7717g_2	Mean
Co 1148 – Top	7	8	5	15	8.75
	(15.33)	(16.69)	(13.56)	(58.28)	(25.97)
middle	15	15	30	45	26.25
	(22.77)	(22.83)	(33.29)	(43.57)	(30.61)
bottom	5	2	10	30	11.75
	(13.87)	(9.25)	(20.85)	(33.20)	(19.29)
Co 7717 – Top	20	50	100	30	50.00
	(25.82)	(45.00)	(90.00)	(35.36)	(49.05)
middle	8	100	80	85	68.25
	(16.60)	(90.00)	(63.61)	(67.50)	(59.43)
bottom	12	80	60	60	53.00
	(20.20)	(65.32)	(51.71)	(51.06)	(47.08)
Mean	11.2	42.5	47.5	44.2	36.3
	(19.10)	(41.52)	(45.50)	(48.16)	(38.57)

(CD=0.05) Cane position - 1.21; Cf cultures - 8.33; Interaction - 20.41

Table 4. Juice quality parameters of cane tissues of two sugarcane cultivars at different cane positions

Host cultivar	Brix	Sucrose %	Total phenolics (µg/g of tissue)
		Co 1148	
Top	19.10	16.96	126.3
Middle	19.00	16.43	120.0
Bottom	17.50	14.83	110.7
		Co 7717	
Top	21.30	19.58	105.6
Middle	21.30	19.18	102.6
Bottom	19.90	16.81	125.1
CD (P=0.05)	1.28	1.32	4.45

by repeated inoculation on incompatible hosts. During cross inoculation process, changes in mycelial growth rate, growth pattern of fungal cultures (sectoring) were noticed (Fig. 2). Mycelial growth of incompatible strains was varied depending on juice from position of cane and maximum was noticed in the juice from middle portion (Table 2). It was interesting to note that the reduced mycelial growth and sporulation on medium with incompatible host juice become equal with compatible host when it was passed two times in the incompatible hosts. Also the dark isolates have become light with increased sporulation on their newly adapted hosts. It is also very apparent that the conidial germination of original cultures was highly influenced by their parental cultivars and not by incompatible hosts (Table 3). However the germination was influenced significantly more than 100 per cent when it was passed two generations through incompatible hosts. Development of light isolates coupled with increased sporulation and spore germination by repeated inoculation on incompatible hosts indicates the increased virulence on pathogenicity of that pathotype for adaptation on particular cultivar. Increased virulence of light types over dark types has been reported even in 1935 by Abbott. From his extensive studies, he concluded that the isolates obtained from red rot affected canes fell into two types, those which in culture has light coloured aerial mycelium and were relatively highly sporulating and those which had a dark coloured mycelium and were poor sporulators. The large scale epidemics in North Indian sugar belt were attributed to the appearance of new light strains with abundant sporulation and highly pathogenic to particular variety (Chona and Padwick, 1942). Subsequently successive failure of many varieties were attributed to the appearance of new strains (Rafay and Singh, 1957) which might be due to the adaptation of older strains. Results of the present investigation clearly indicates that the changes in pathotypes upon infection on new cultivar may be due to tolerance of pathogen to toxic materials in the cytoplasm and hence changes its pathogenicity accordingly.

REFERENCES

Abbott, E. V. (1938). Red rot of sugarcane. Tech. Bull USDA, 641, p96.

Abbott, E. V. and Hughes, C. G. (1961). Red rot. In: Sugarcane Diseases of the World Vol., (Eds) J. P. Martin, E. V. Abbott and C. G. Hughes, Elsevier, Amsterdam, pp 262-287.

Alexander, K. C. and Viswanathan, R. (2002). Diseases of sugarcane in India and its rapid diagnosis. In: **Sugarcane Crop Management**, (Eds. S. B. Singh, G. P. Rao, S. Eswaramoorthy), SCI TECH Publishing LLC, Houston, Texas, USA, pp 10-51.

Chona, B. L. and Padwick, W. G. (1942). More light on the red rot epidemic. *Indian Farming*, 3 70-3.

Meade, C. (1977). Cane sugar handbook. A Wiley Interscience

Publication ial stage of *Colletotrichum falcatum* Went in India. Indial Phytopath., **5:** 158-160., John Wiley and Sons., New York. 947p.

Malik, C. P and Singh, M. B. (1980). In: Plant Enzymology and Host Enzymology, Kalyani Publishers, New Delhi, P. 286.

Mohanraj, D., Kumaresan, S. and Sreenivasan, T. V. (2002). Molecular characterization of isolates of the sugarcane red rot pathogen *Colletotrichum falcatum* Went. *Indian Phytopath.*, **55:** 147-151.

Neill, O. N. R. and Bauchan, G. R. (2000). Sources of resistance to anthracnose in the annual*Medicago* core collection. *Plant Dis.* **84:** 261-267.

Rafay, S. A. and Singh, V. B. (1957). A new strain of Glomerella tucumanensis. *Curr. Sci.*, **10:** 25-26.

Srinivasan, K. V. (1962). Some observations on variation in the red rot pathogen, Glomerella tucumanensis (Speg.) Arx and Muller. Proc. ISSCT, **11:** 795-802.

Srinivasan, K. V. (1963). Sporulation of the sugarcane red rot pathogen on the juice of varieties of varying resistance. *Science and Culture*, **29:** 87-88.

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

Viswanathan, R. and Samiyappan, R. (2000). in sugarcane: Challenges and prospects. *Madras Agric. J.*, **87:** 549-559.

Viswanathan, R., Padmanaban, P., Mohanraj, D. and Jothi, R. (2000). Indirect –ELISA technique for the detection of the red rot pathogen in sugarcane (*Saccharum* spp hybrid) and resistance screening. *Indian J. Agric Sci.*, **70:** 308-311.

Viswanathan, R., Malathi, P. and Padmanaban, P. (2003). Variation in sugarcane red rot pathogen *Colletotrichum falcatum* Went. In: **Frontiers of Fungal Diversity in India**. (Eds G. P. Rao, C. Manoharachari, D. J. Bhat, R. C. Rajak and T. N. Lakhanpal), International Book Distributing Co, Lucknow, pp639-667.

Received March 12, 2006; Accepted April 10, 2006