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RESEARCH ARTICLE

Sugarcane Wilt: Pathogen Recovery from Different Tissues and Variation in Cultural Characters

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Abstract The fungal pathogen *Fusarium sacchari* causing wilt in sugarcane exhibits enormous variation in cultural characters. Detailed studies were conducted on recovery of the pathogen from wilt infected canes and to characterize the pathogen for its cultural characters. From 346 samples collected from 15 states, 263 *Fusarium* isolates were recovered and no other fungal genera were recovered. Overall, a higher pathogen recovery was recorded in nodal tissues as compared to internodal tissues of wilt infected stalk tissues. Cultural characterization of 117 isolates divided the *Fusarium* isolates into three groups based on radial growth as slow, moderate and fast. Based on mycelial colour, the isolates were grouped into seven groups viz., white, orange, pinkish orange, pink, dark pink, pinkish violet and reddish brown. The isolates were further divided into 21 groups based on mycelia colour on the top and agar pigmentation at the reverse side of the culture plate and three groups viz., submissive, raised and fluffy based on topology of the mycelium. Based on conidial frequency, the isolates were grouped as those produce microconidia with low, moderate, higher frequencies, micro and macroconidia at lower frequencies and micro and macroconidia production at higher frequencies. The results of this study revealed extensive variation in *F. sacchari* isolates recovered for their cultural characters from wilt infected stalks and sick soils.

Keywords Sugarcane · Wilt · *Fusarium sacchari* · Isolates · Cultural characteristics · Variation

Introduction

In India, wilt is an important fungal disease which causes significant crop losses in sugarcane. Like red rot, the disease also affects sugarcane stalks which make them unfit for juice extraction and milling. Wilt epidemics in earlier decades of the last century resulted in serious losses to cane production in many commercial varieties (Kirtikar et al. 1972; Agnihotri and Rao 2002). Several commercial varieties like Co 245, Co 321, Co 419, Co 449, Co 453, Co 527, Co 951, Co 1107, Co 1122, Co 1223, Co 89003, etc. were withdrawn from cultivation in North and southern parts of India due to wilt. Although the disease occurs in all the parts of the country, Viswanathan et al. (2006) reported its epidemic in sub-tropical plains, South Gujarat and east coastal regions. Wilt epidemic is very common in delta regions where, conducive environment prevails and susceptible host varieties are available. The disease adversely affects germination. Besides yield reduction, wilt also causes 15–30 % reduction in juice extraction and up to 20 % reduction in sugar recovery. Also partial infection drastically reduces juice extraction and juice quality as in red rot infections. Combined infection of red rot and wilt causes more loss to the crop than their infection alone. Similarly, infestations of sugarcane by borer pests aggravate wilt and cause more damage to the crop (Viswanathan 2012; Viswanathan and Rao 2011).

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Butler and Khan (1913) studied sugarcane wilt in detail and described *Cephalosporium sacchari* as the associated pathogen. Subsequently, several workers reported *Fusarium moniliformae* var *subglutinans* as the pathogen. Gams (1971) coined a new species *Fusarium sacchari* (Butler) W.Gams to which both *C. sacchari* and *F. moniliformae* var *subglutinans* were made synonym. Later Nirenberg (1976) distinguished two varieties of *F. sacchari* namely, *F. sacchari* var *sacchari* and *F. sacchari* var *subglutinans*, the former having mostly unseptate conidia in the aerial mycelium, no sporodochia, while the latter with 1–3 septate conidia, macroconidia more commonly formed often in sporodochia. Besides *F. sacchari*, *Acremonium implicatum* and *Acremonium furcatum* were isolated from wilt infected samples in subtropical India by Singh and Singh (1974). Although there are several reports on the incidence of wilt and associated pathogens in India (Agnihotri and Rao 2002) only scattered information was available on disease situation across the country, affected varieties, pathogen variation and associated factors. Hence we made a detailed survey in different sugarcane growing states in the country and assessed occurrence of wilt in major sugarcane growing states, factors influencing disease development and isolation of fungi causing wilt (Viswanathan et al. 2006). Further studies were continued to characterize the isolates collected from different regions using different mycological techniques and on the variability in cultural characters of 117 *F. sacchari* isolates associated with wilt.

Materials and Methods

Isolation of Wilt Pathogen(s) from Infected Stalks

The infected canes were split open longitudinally and recorded internal symptoms. Later, pathogen isolation was done by tissue segment method, in which about 15–20 nodal and internodal tissues of 8 mm thickness were removed using a cork borer and washed in sterile distilled water followed by 70 % ethanol. The bits were then surface sterilized in 0.1 % $HgCl_2$ for 10 s and then given a few changes of sterile distilled water to remove $HgCl_2$. The bits were transferred to Petri plates containing solidified oat meal agar (OMA) and incubated at room temperature (25–30 °C) for a week and observed for the fungal growth. The fungal isolates were purified subsequently and preserved. Details of 249 isolates recovered from 345 wilt infected sugarcane stalks are given in the Table 1.

Isolation of Wilt Pathogen from Soil

One gram soil adhering rhizosphere of infected canes was taken in a conical flask containing 100 ml of sterile distilled water to give a dilution of 10^{-2} and mixed well the contents of the flask by placing it on a rotary shaker at 75 rpm for 5 min. Serial dilutions of up to 10^{-6} and 10^{-8} were made in sterile distilled water. One ml of the soil suspension from each dilution was transferred with a pipette and spread evenly across the agar surface. Even distribution of the soil suspension was accomplished by carefully manipulating the plate with gentle agitation to move a wetting front across the entire agar surface or by using a glass "hockey stick" applicator to spread the suspension. Among OMA and Coon's media used initially, the latter was found as the best selective medium for the isolation of *Fusarium* sp. from rhizosphere soil as it eliminated all other contaminants present in the soil. The inoculated plates were incubated at room temperature for a week. Fourteen isolates obtained from 29 different rhizosphere soil samples collected from 10 different states were subsequently purified by repeated subculturing on plates containing OMA or Coon's agar. List of isolates recovered from rhizosphere soil samples is given in Table 2.

Cultural Characters

Potato dextrose agar (PDA) was used to assess cultural characters of wilt associated fungal isolates. Mycelial plugs of 0.4 cm from 5 days old mother culture were placed at the center of PDA plates and incubated at 25 °C for 10 days. Three replicates were maintained for each isolate. Later the parameters namely, growth rate, mycelial colour, reverse pigmentation at bottom of the plates and colony topology were observed. Radial growth was recorded by measuring the colony diameter on the reverse side of the culture plate. The isolates that showed growth below 5.5 cm were categorized as slow and that reached more than 7.5 cm in 10 days were categorized as fast growing. The other isolates with growth of 5.5–7.5 cm were rated as medium growth. Mycelial colour of the isolates both at top and reverse pigmentation at bottom of the culture plates were recorded as pink, white or varying shades of pink or white and categorized in one of the following combinations as listed in Table 3. Colony texture of the isolates with cottony hyphal growth above the agar surface (aerial mycelium) was marked as fluffy and the other isolates with powdery mycelia growing along with agar and without any aerial mycelia were noted as submissive.

To record types of conidia produced in the plate, six PDA plugs of uniform diameter of 0.5 cm were taken from the 3 replica plates randomly and spore suspension was prepared by agitating six uniform PDA plugs in 20 ml sterile distilled water for 15 min at 120 rpm. Ten microliters of conidial suspension was pipetted out on to a haemocytometer and was observed under a Leica DMLB2 light microscope. Number of conidia in a small square was counted under 45× objective and average count of 5 such

Table 1 List of *Fusarium* isolates isolated from the wilt infected sugarcane stalks from different states in India

S. no.	Isolate designation	Variety/clone	Place of collection	District	State
1	Fs 003 P1L1	Co 89003	Taggadabadala	Hoshiarpur	Punjab
2	Fs 003 P2L1	Co 89003	Taggadabadala	Hoshiarpur	Punjab
3	Fs 003 P3L1	Co 89003	Taggadabadala	Hoshiarpur	Punjab
4	Fs 003 P4L1	Co 89003	Taggadabadala	Hoshiarpur	Punjab
5	Fs 003 P5L1	Co 89003	Taggadabadala	Hoshiarpur	Punjab
6	Fs 003 P6L2	Co 89003	Mukerian	Gurdaspur	Punjab
7	Fs 003 P7L2	Co 89003	Mukerian	Gurdaspur	Punjab
8	Fs 003 P8L2	Co 89003	Mukerian	Gurdaspur	Punjab
9	Fs 003 P9L2	Co 89003	Mukerian	Gurdaspur	Punjab
10	Fs 003 P10L2	Co 89003	Mukerian	Gurdaspur	Punjab
11	Fs 003 P11L2	Co 89003	Mukerian	Gurdaspur	Punjab
12	FsJ 085 P1	CoJ 85	Mukerian	Gurdaspur	Punjab
13	FsJ 085 P2	CoJ 85	Mukerian	Gurdaspur	Punjab
14	FsJ 085 P3	CoJ 85	Mukerian	Gurdaspur	Punjab
15	FsJ 085 P4	CoJ 85	Mukerian	Gurdaspur	Punjab
16	FsJ 085 P5	CoJ 85	Mukerian	Gurdaspur	Punjab
17	FsJ 085 P6	CoJ 85	Mukerian	Gurdaspur	Punjab
18	FsJ 085 P7	CoJ 85	Mukerian	Gurdaspur	Punjab
19	FsJ 085 P8	CoJ 85	Mukerian	Gurdaspur	Punjab
20	FsJ 085 P9	CoJ 85	Mukerian	Gurdaspur	Punjab
21	FsJ 085 P10	CoJ 85	Mukerian	Gurdaspur	Punjab
22	FsJ 085 P11	CoJ 85	Mukerian	Gurdaspur	Punjab
23	FsJ 085 P12	CoJ 85	Mukerian	Gurdaspur	Punjab
24	Fs 120 P1	Co 0120	Panwan	Hoshiarpur	Punjab
25	Fs 120 P2	Co 0120	Panwan	Hoshiarpur	Punjab
26	Fs 120 P3	Co 0120	Panwan	Hoshiarpur	Punjab
27	Fs 120 P4	Co 0120	Panwan	Hoshiarpur	Punjab
28	Fs 120 P5	Co 0120	Panwan	Hoshiarpur	Punjab
29	Fs 003 H1	Co 89003	Karnal	Karnal	Haryana
30	FsBln 173 B1	CoBln 03173	Motipur	Muzaffarpur	Bihar
31	FsBln 173 B2	CoBln 03173	Motipur	Muzaffarpur	Bihar
32	FsBln 173 B3	CoBln 03173	Motipur	Muzaffarpur	Bihar
33	FsBln 173 B4	CoBln 03173	Motipur	Muzaffarpur	Bihar
34	FsBln 173 B5	CoBln 03173	Motipur	Muzaffarpur	Bihar
35	FsBln 175 B1	CoBln 03175	Motipur	Muzaffarpur	Bihar
36	FsBln 175 B2	CoBln 03175	Motipur	Muzaffarpur	Bihar
37	FsBln 175 B3	CoBln 03175	Motipur	Muzaffarpur	Bihar
38	FsBln 175 B4	CoBln 03175	Motipur	Muzaffarpur	Bihar
39	FsBln 176 B1	CoBln 03176	Motipur	Muzaffarpur	Bihar
40	FsBln 176 B2	CoBln 03176	Motipur	Muzaffarpur	Bihar
41	FsBln 176 B3	CoBln 03176	Motipur	Muzaffarpur	Bihar
42	FsSe 231 B	CoSe 2231	Motipur	Muzaffarpur	Bihar
43	Fs 121 UP	Co 0121	Simbhaoli	Ghaziabad	UP
44	Fs 123 UP	Co 0123	Simbhaoli	Ghaziabad	UP
45	Fs 240 UP	Co 0240	Simbhaoli	Ghaziabad	UP
46	FsJ 64 UP	CoJ 64	Simbhaoli	Ghaziabad	UP
47	FsS 767 UP	CoS 767	Simbhaoli	Ghaziabad	UP
48	FsLG 022 UP[a]	LG 96022	Lucknow	Lucknow	UP

Table 1 continued

S. no.	Isolate designation	Variety/clone	Place of collection	District	State
49	FsJn 964 MP1	CoJn 964	Powarkheda	Hosangabad	MP
50	Fs 805 O1	Co 7805	Chikinia	Cuttack	Orissa
51	FsA 085 O1	CoA 89085	Chikinia	Cuttack	Orissa
52	FsA 085 O2	CoA 89085	Chikinia	Cuttack	Orissa
53	FsA 085 O3	CoA 89085	Chikinia	Cuttack	Orissa
54	FsA 085 O4	CoA 89085	Chikinia	Cuttack	Orissa
55	FsA 085 O5	CoA 89085	Chikinia	Cuttack	Orissa
56	FsA 085 O6	CoA 89085	Chikinia	Cuttack	Orissa
57	FsA 085 O7	CoA 89085	Chikinia	Cuttack	Orissa
58	Fs 032 M1L1	Co 86032	Someshwar Nagar	Pune	MS
59	Fs 032 M2L2	Co 86032	Kopargaon Bolki	Ahmednagar	MS
60	Fs 012 M1	Co 94012	Pravaranagar	Ahmednagar	MS
61	Fs 012 M2	Co 94012	Pravaranagar	Ahmednagar	MS
62	Fs 036 G1	Co 85036	Chalthan	Surat	Gujarat
63	Fs 036 G2	Co 85036	Chalthan	Surat	Gujarat
64	Fs 002 G1	Co 86002	Sayan	Surat	Gujarat
65	Fs 002 G2	Co 86002	Sayan	Surat	Gujarat
66	Fs 032 G1	Co 86032	Kosamadi Farm	Surat	Gujarat
67	Fs 032 G2	Co 86032	Kosamadi Farm	Surat	Gujarat
68	Fs 025 G	Co 88025	Sayan	Surat	Gujarat
69	Fs 012 G	Co 94012	Bardoli	Surat	Gujarat
70	Fs 005 G	Co 95005	K.Farm	Surat	Gujarat
71	Fs 006 G1	Co 95006	Navsari	Navsari	Gujarat
72	Fs 006 G2	Co 95006	Navsari	Navsari	Gujarat
73	Fs 006 G3	Co 95006	Navsari	Navsari	Gujarat
74	Fs 016 G	Co 95016	Sayan	Surat	Gujarat
75	Fs 020 G	Co 95020	Chalthan	Surat	Gujarat
76	Fs 010 G	Co 98010	Chalthan	Surat	Gujarat
77	Fs 110 G	Co 2001-10	Navsari	Navsari	Gujarat
78	FsC 671 G1	CoC 671	Navsari	Navsari	Gujarat
79	FsC 671 G2	CoC 671	Navsari	Navsari	Gujarat
80	FsSi 071 G	CoSi 95071	Chalthan	Surat	Gujarat
81	FsV 102 G	CoV 94102	Chalthan	Surat	Gujarat
82	FsVi 337 G	CoVSi 9337	Navsari	Navsari	Gujarat
83	Fs 810 G1	99N 810	Sayan	Surat	Gujarat
84	Fs 810 G2	99N 810	Sayan	Surat	Gujarat
85	Fs 810 G3	99N 810	Sayan	Surat	Gujarat
86	Fs 193 G	2001N 193	Navsari	Navsari	Gujarat
87	Fs 032 G	Co 86032 (underground stem)	Bardoli	Surat	Gujarat
88	Fs 805 AP1L1	Co 7805	Chinnathadepalli	West Godavari	AP
89	Fs 805 AP2L1	Co 7805	Chinnathadepalli	West Godavari	AP
90	Fs 805 AP3L2	Co 7805	Vodlamurru	East Godavari	AP
91	Fs 032 AP1L1	Co 86032	Mortha	West Godavari	AP
92	Fs 032 AP2L2	Co 86032 (TR)	Rudrur	Nizamabad	AP
93	Fs 009 AP	Co 2002-09	Rudrur	Nizamabad	AP
94	FsV 048 AP1	81V 48	Tirumali	East Godavari	AP
95	FsV 048 AP2	81V 48	Tirumali	East Godavari	AP
96	FsV 048 AP3	81V 48	Tirumali	East Godavari	AP

Table 1 continued

S. no.	Isolate designation	Variety/clone	Place of collection	District	State
97	FsNG 159 K1	57NG 159 yellow	Kannur	Kannur	Kerala
98	FsNG 159 K2	57NG 159 yellow	Kannur	Kannur	Kerala
99	FsNG 159 K3	57NG 159 yellow	Kannur	Kannur	Kerala
100	FsNG 159 K4	57NG 159 yellow	Kannur	Kannur	Kerala
101	FsNG 219 K1	57NG 219	Kannur	Kannur	Kerala
102	FsNG 219 K2	57NG 219	Kannur	Kannur	Kerala
103	FsNG 219 K3	57NG 219	Kannur	Kannur	Kerala
104	FsNG 219 K4	57NG 219	Kannur	Kannur	Kerala
105	FsNG 219 K5	57NG 219	Kannur	Kannur	Kerala
106	FsNG 226 K	57NG 226	Kannur	Kannur	Kerala
107	Fs BT K1	Black Tanna	Kannur	Kannur	Kerala
108	Fs BT K2	Black Tanna	Kannur	Kannur	Kerala
109	Fs BT K3	Black Tanna	Kannur	Kannur	Kerala
110	Fs BT K4	Black Tanna	Kannur	Kannur	Kerala
111	Fs BT K5	Black Tanna	Kannur	Kannur	Kerala
112	Fs BT K6	Black Tanna	Kannur	Kannur	Kerala
113	Fs BT K7	Black Tanna	Kannur	Kannur	Kerala
114	Fs Lajai K1	Lajai	Kannur	Kannur	Kerala
115	Fs Lajai K2	Lajai	Kannur	Kannur	Kerala
116	Fs LC A1	Local cultivar	Nagaon	Nagaon	Assam
117	Fs LC A2	Local cultivar	Jorhat	Jorhat	Assam
118	FsBln 173 A1[a]	CoBln 02173	Buralikshan	Jorhat	Assam
119	FsBln 173 A2[a]	CoBln 02173	Buralikshan	Jorhat	Assam
120	Fs LC ArP1	Local cultivar	–	–	Ar.P
121	Fs LC ArP2	Local cultivar	–	–	Ar.P
122	Fs M clone TN1	M Clone	Appakudal	Erode	TN
123	Fs M clone TN2	M Clone	Appakudal	Erode	TN
124	Fs M clone TN3	M Clone	Appakudal	Erode	TN
125	Fs M clone TN4	M Clone	Appakudal	Erode	TN
126	Fs M clone TN5	M Clone	Appakudal	Erode	TN
127	Fs M clone TN6	M Clone	Appakudal	Erode	TN
128	Fs M clone TN7	M Clone	Appakudal	Erode	TN
129	Fs M clone TN8	M Clone	Appakudal	Erode	TN
130	Fs M clone TN9	M Clone	Appakudal	Erode	TN
131	Fs 032 TN1L1	Co 86032	Polur	Tiruvannamalai	TN
132	Fs 032 TN2L1	Co 86032	Polur	Tiruvannamalai	TN
133	Fs 032 TN3L1	Co 86032	Polur	Tiruvannamalai	TN
134	FsS 255 TN	CoS 95255	SBI main farm	Coimbatore	TN
135	FsBln 172 TN1	CoBln 03172	SBI main farm	Coimbatore	TN
136	FsBln 172 TN2	CoBln 03172	SBI main farm	Coimbatore	TN
137	FsSe 436 TN	CoSe 96436	SBI main farm	Coimbatore	TN
138	FsS 268 TN1	CoS 96268	SBI main farm	Coimbatore	TN
139	FsS 268 TN2	CoS 96268	SBI main farm	Coimbatore	TN
140	Fs 032 TN4L2	Co 86032	Vedapatti	Coimbatore	TN
141	FsS 739 TN	CoS 92739	SBI main farm	Coimbatore	TN
142	FsN 956 TN1	57N 9-56	SBI main farm	Coimbatore	TN
143	FsN 956 TN2	57N 9-56	SBI main farm	Coimbatore	TN
144	FsPIR 325 TN1	PIR 96-325	SBI main farm	Coimbatore	TN

Table 1 continued

S. no.	Isolate designation	Variety/clone	Place of collection	District	State
145	FsPIR 325 TN2	PIR 96-325	SBI main farm	Coimbatore	TN
146	FsPIR 325 TN3	PIR 96-325	SBI main farm	Coimbatore	TN
147	Fs 032 TN5L3	Co 86032	Keelkavaraipattu	Cuddalore	TN
148	Fs 032 TN6L3	Co 86032	Keelkavaraipattu	Cuddalore	TN
149	Fs 032 TN7L3	Co 86032	Keelkavaraipattu	Cuddalore	TN
150	FsV 101 TN1L1	CoV 94101	Thiruvadigai	Cuddalore	TN
151	FsV 101 TN2L2	CoV 94101	Karamani Kuppam	Cuddalore	TN
152	FsAVT 153 TN1	AVT 21153	Cuddalore	Cuddalore	TN
153	FsAVT 153 TN2	AVT 21153	Cuddalore	Cuddalore	TN
154	FsAVT 153 TN3	AVT 21153	Cuddalore	Cuddalore	TN
155	FsC 061 TN1	CoC 99061	Cuddalore	Cuddalore	TN
156	FsC 061 TN2	CoC 99061	Cuddalore	Cuddalore	TN
157	FsC 063 TN1	CoC 90063 (TR)	Vaidipakkam	Cuddalore	TN
158	FsC 063 TN2	CoC 90063 (TR)	Vaidipakkam	Cuddalore	TN
159	FsC 063 TN3	CoC 90063 (TR)	Vaidipakkam	Cuddalore	TN
160	FsC 063 TN4	CoC 90063 (TR)	Vaidipakkam	Cuddalore	TN
161	Fs 004 TN1	Co 85004	Vedapatti	Coimbatore	TN
162	Fs 004 TN2	Co 85004	Vedapatti	Coimbatore	TN
163	Fs 004 TN3	Co 85004	Vedapatti	Coimbatore	TN
164	Fs 004 TN4	Co 85004	Vedapatti	Coimbatore	TN
165	Fs 004 TN5	Co 85004	Vedapatti	Coimbatore	TN
166	Fs 007 TN1	2002-07	Vedapatti	Coimbatore	TN
167	Fs 007 TN2	2002-07	Vedapatti	Coimbatore	TN
168	Fs 219 TN1	Co 7219	Vedapatti	Coimbatore	TN
169	Fs 219 TN2	Co 7219	Vedapatti	Coimbatore	TN
170	Fs 287 TN1L1	Co 1287	Vedapatti	Coimbatore	TN
171	Fs 287 TN2L1	Co 1287	Vedapatti	Coimbatore	TN
172	Fs 107 TN	2002-107	Vedapatti	Coimbatore	TN
173	Fs 108 TN1	2002-108	Vedapatti	Coimbatore	TN
174	Fs 108TN2	2002-108	Vedapatti	Coimbatore	TN
175	Fs 004 TN1	Co 6304	Vedapatti	Coimbatore	TN
176	Fs 004 TN2	Co 6304	Vedapatti	Coimbatore	TN
177	Fs 004 TN3	Co 6304	Vedapatti	Coimbatore	TN
178	Fs 004 TN4	Co 6304	Vedapatti	Coimbatore	TN
179	Fs 004 TN5	Co 6304	Vedapatti	Coimbatore	TN
180	Fs 022 TN1	2002-22	Vedapatti	Coimbatore	TN
181	Fs 022 TN2	2002-22	Vedapatti	Coimbatore	TN
182	Fs 022 TN3	2002-22	Vedapatti	Coimbatore	TN
183	Fs 022 TN4	2002-22	Vedapatti	Coimbatore	TN
184	Fs 022 TN5	2002-22	Vedapatti	Coimbatore	TN
185	Fs 030 TN	2002-30	Vedapatti	Coimbatore	TN
186	Fs 007 TN	2002-07	Vedapatti	Coimbatore	TN
187	Fs 022 TN6	2002-22	Vedapatti	Coimbatore	TN
188	Fs 022 TN7	2002-22	Vedapatti	Coimbatore	TN
189	Fs 219 TN1	Co 7219	Vedapatti	Coimbatore	TN
190	Fs 219 TN2	Co 7219	Vedapatti	Coimbatore	TN
191	Fs 219 TN3	Co 7219	Vedapatti	Coimbatore	TN
192	Fs 036 TN	2002-36	Vedapatti	Coimbatore	TN

Table 1 continued

S. no.	Isolate designation	Variety/clone	Place of collection	District	State
193	Fs 287 TN3L2	1287B	Vedapatti	Coimbatore	TN
194	Fs 287 TN4L2	1287B	Vedapatti	Coimbatore	TN
195	Fs 287 TN5L2	1287B	Vedapatti	Coimbatore	TN
196	Fs 287 TN6L2	1287B	Vedapatti	Coimbatore	TN
197	Fs 272 TN1	2002-72	Vedapatti	Coimbatore	TN
198	Fs 272 TN2	2002-72	Vedapatti	Coimbatore	TN
199	Fs 272 TN3	2002-72	Vedapatti	Coimbatore	TN
200	Fs 272 TN4	2002-72	Vedapatti	Coimbatore	TN
201	Fs 272 TN5	2002-72	Vedapatti	Coimbatore	TN
202	Fs 346 TN	2003-46	Vedapatti	Coimbatore	TN
203	Fs 322 TN	2003-22	Vedapatti	Coimbatore	TN
204	Fs 3116 TN	2003-116	Vedapatti	Coimbatore	TN
205	FsSi 071 TN1L1	CoSi 95071	Vedapatti	Coimbatore	TN
206	FsSi 071 TN2L1	CoSi 95071	Vedapatti	Coimbatore	TN
207	Fs 806 TN	Co 6806	Vedapatti	Coimbatore	TN
208	Fs 052 TN	2003-52	Vedapatti	Coimbatore	TN
209	Fs 050 TN	2003-50	Vedapatti	Coimbatore	TN
210	FsSi 071 TN3L1	CoSi 95071	Vedapatti	Coimbatore	TN
211	FsSi 071 TN4L1	CoSi 95071	Vedapatti	Coimbatore	TN
212	Fs 076 TN	2003-76	Vedapatti	Coimbatore	TN
213	Fs 076 TN	2003-76	Vedapatti	Coimbatore	TN
214	Fs 740 TN1	Co 740	Vedapatti	Coimbatore	TN
215	Fs 740 TN2	Co 740	Vedapatti	Coimbatore	TN
216	Fs 740 TN3	Co 740	Vedapatti	Coimbatore	TN
217	Fs 085 TN	2003-85	Vedapatti	Coimbatore	TN
218	Fs 083 TN	2003-83	Vedapatti	Coimbatore	TN
219	Fs 093 TN	2003-93	Vedapatti	Coimbatore	TN
220	Fs 111 TN1	2003-111	Vedapatti	Coimbatore	TN
221	Fs 111 TN2	2003-111	Vedapatti	Coimbatore	TN
222	Fs 287 TN7L3	Co 1287	Vedapatti	Coimbatore	TN
223	FsV 101 TN3L3	CoV 94101	Thuhili	Thanjavur	TN
224	TN Cross1 TN1	Co 2000-10 × Co 94008 (90)	Vedapatti	Coimbatore	TN
225	TN Cross1 TN2	Co 2000-10 × Co 94008 (90)	Vedapatti	Coimbatore	TN
226	TN Cross1 TN3	Co 2000-10 × Co 94008 (99)	Vedapatti	Coimbatore	TN
227	TN Cross1 TN4	Co 2000-10 × Co 94008 (99)	Vedapatti	Coimbatore	TN
228	TN Cross1 TN5	Co 2000-10 × Co 94008 (99)	Vedapatti	Coimbatore	TN
229	TN Cross1 TN6	Co 2000-10 × Co 94008 (228)	Vedapatti	Coimbatore	TN
230	TN Cross1 TN7	Co 2000-10 × Co 94008 (229)	Vedapatti	Coimbatore	TN
231	TN Cross1 TN8	Co 2000-10 × Co 94008 (229)	Vedapatti	Coimbatore	TN
232	TN Cross2 TN1	Co 8371 × Co 93009 (256)	Vedapatti	Coimbatore	TN
233	TN Cross2 TN2	Co 8371 × Co 93009 (256)	Vedapatti	Coimbatore	TN
234	FsS 510 TN	CoS 510	Coimbatore	Coimbatore	TN
235	Fs 249 TN	Co 86249	Kodangudi	Cuddalore	TN
236	Fs 032 TN8L4	Co 86032	Sakthinagar	Erode	TN
237	Fs 012 TN	Co 92012	Dakshinapallam	Thanjavur	TN
238	Fs 032 TN9 L5	Co 86032	Enanallur	Thanjavur	TN
239	Fs 032 TN10 L6	Co 86032	Dakshinapallam	Thanjavur	TN
240	Fs 003 TN	Co 94003	Palakudi	Thanjavur	TN

Table 1 continued

S. no.	Isolate designation	Variety/clone	Place of collection	District	State
241	Fs 032 TN11 L7	Co 86032	Paccheri	Sivaganga	TN
242	FsSi 071 TN1	CoSi 86071	Vayalur	Kancheepuram	TN
243	FsSi 071 TN2	CoSi 86071	Vayalur	Kancheepuram	TN
244	FsSi 071 TN3	CoSi 86071	Vayalur	Kancheepuram	TN
245	Fs 032 TN12 L8	Co 86032	Perumalpudur	Tiruppur	TN
246	Fs 032 TN13 L8	Co 86032	Perumalpudur	Tiruppur	TN
247	TN SF TN1	Sugarcane fluff	Coimbatore	Coimbatore	TN
248	FsV 101 TN4L4	CoV 94101	Padamathur	Sivaganga	TN
249	FsC 671 TN	CoC 671	Padamathur	Sivaganga	TN

AP Andhra Pradesh, *Ar.P* Arunachal Pradesh, *MP* Madhya Pradesh, *MS* Maharashtra, *TN* Tamil Nadu, *UP* Uttar Pradesh

[a] Pathogen isolated from the quarantine samples received from the respective centres

Table 2 Details of *Fusarium* isolates isolated from wilt sick rhizosphere soil

S. no.	Isolate designation	Varieties/clones	Place of collection	District	State
1	Fs 076 TN3	2003-76	Vedapatti	Coimbatore	Tamil Nadu
2	Fs M Clone TN10 TN	M Clone	Appakudal	Erode	Tamil Nadu
3	Fs 032 TN5L2	Co 86032	Vedapatti	Coimbatore	Tamil Nadu
4	Fs 069 TN	2003-69	Vedapatti	Coimbatore	Tamil Nadu
5	Fs 096 TN	2003-96	Vedapatti	Coimbatore	Tamil Nadu
6	Fs 098 TN1	2003-98	Vedapatti	Coimbatore	Tamil Nadu
7	Fs 098 TN2	2003-98	Vedapatti	Coimbatore	Tamil Nadu
8	Fs 052 TN2	2003-52	Vedapatti	Coimbatore	Tamil Nadu
9	Fs 047 TN	2003-47	Vedapatti	Coimbatore	Tamil Nadu
10	Fs 119 TN	2003-119	Vedapatti	Coimbatore	Tamil Nadu
11	Fs 287 TN8L4	1287 B	Vedapatti	Coimbatore	Tamil Nadu
12	Fs 304 TN	Co 6304	Vedapatti	Coimbatore	Tamil Nadu
13	Fs 036 TN2	2003-36	Vedapatti	Coimbatore	Tamil Nadu
14	Fs 012 M3	Co 94012	Pravaranagar	Ahmednagar	Maharashtra

small squares was taken to estimate sporulation. Since one small square has an area of 0.0025 mm^2, the number of cells per mm^2 was computed with the formula

$$\text{Number of cells per mm}^2 = (\text{Number of cells in small square}/0.0025) \times \text{dilution factor}$$

Types of conidia produced (microconidia, macroconidia or both) were also recorded under 45× magnification. Based on the intensity of sporulation and type of conidia produced the isolates were grouped into 8 classes (Table 5).

Results

Recovery of Wilt Pathogens from Infected Sugarcane Stalks and Rhizosphere Soil

Initially four different media namely OMA, Coon's medium, PDA and Czapek's medium were compared for the effective recovery of the pathogen. Isolation by tissue segment method in OMA gave comparatively higher recovery of the pathogen, with few other organisms and PDA gave higher recovery of both *Fusarium* and other microbes. However, recovery of *Fusarium* on Czapek's medium was low compared to Coon's medium which is specific for *Fusarium* (results not shown). All our isolates recovered in the study showed typical characters of *Fusarium* and no other fungi were isolated in the infected samples.

Wilt pathogen was isolated from cane samples collected from 15 different states. Totally 346 wilt infected stalks and 24 rhizosphere soil samples were subjected for isolation. Of them, 249 isolates were recovered from cane samples and 14 isolates from rhizosphere soil samples (Tables 1, 2, 4).

Comparison on the recovery of wilt fungus from nodal and internodal tissues revealed that wilt fungi are recovered both from nodal and internodal tissues in 31 of the 125

Table 3 Patterns in mycelial colour and pigmentation in the culture plates of *Fusarium* isolates recovered from wilt infected cane stalk and soils

S. no.	Pigmentation	
	Top	Bottom
1	White	White
2	White	Orange
3	White	Pinkish orange
4	Orange	Orange
5	Orange	Pinkish orange
6	Pinkish orange	Orange
7	Pinkish orange	Reddish brown
8	Pink	White
9	Pink	Orange
10	Pink	Pinkish orange
11	Pink	Pink
12	Pink	Dark pink
13	Pink	Pinkish violet
14	Pink	Reddish brown
15	Dark pink	Dark pink
16	Dark pink	Reddish brown
17	Dark pink	Orange
18	Pinkish violet	Pinkish orange
19	Pinkish violet	Pinkish violet
20	Pinkish violet	Reddish brown
21	Reddish brown	Reddish brown

cane samples subjected for pathogen isolation. Of the remaining 94 samples, 54 samples yielded wilt fungus only from nodal tissues and 40 samples yielded the fungus only from internodal tissues. Overall, higher recovery of wilt fungus was obtained from samples collected from Uttar Pradesh, Orissa, Bihar, Gujarat, Punjab and Kerala (Table 4). Observation on cultivars indicated that Co 0121 from Uttar Pradesh recorded the highest recovery of 100 and 75 % for nodal and internodal tissues, respectively. However in other cultivars the fungal recovery was between 5 and 100 %. Based on the percentage of recovery, the samples were categorized into three groups viz., high (>50 %), medium (25–50 %) and low (<25 %) recovery. Three samples viz., Co 0121 of Uttar Pradesh, Co 6304 of Tamil Nadu, Black Tanna of Kerala gave higher recoveries of 87.5, 60 and 52.9 % respectively. Nine samples gave medium recovery viz., Co 89003 of Punjab, CoJ 85 of Punjab, CoBln 03176 of Bihar, CoA 89085 of Orissa, Co 85004 of Tamil Nadu, 57NG159 yellow of Kerala, genotype PIR 96-325 of Tamil Nadu and progeny Co 2000-10 × Co 94008 of Tamil Nadu. Overall, higher recovery of the pathogen was made from nodal tissues than internodes (Fig. 1). Coon's media was proved to be the best medium for isolation of *Fusarium* from wilt sick soil at dilutions of 10^{-2} and 10^{-4}. Fourteen wilt fungi were recovered from rhizosphere samples collected from Maharashtra and Tamil Nadu (Table 2).

Cultural Characterization

Of the 263 isolates recovered from wilt infected sugarcane stalks and rhizosphere soil, 117 isolates that differed in their place and source of collection were subjected to cultural characterization. The isolates were grouped into three groups based on the growth rate. Eighteen of 117 isolates studied, were found to be slow in growth (below 5.5 cm), 80 moderate (5.6–7.5 cm) and 19 fast growing (more than 7.5 cm) (Supplementary Table 1). Most of the Kerala isolates (8 of 13) were categorized under slow growth group and on the other hand half the isolates population from Orissa (4 of 8) studied were categorized to be fast growing. However, majority of the isolates studied from other states were of moderate growth. Seven Andhra Pradesh isolates, 17 of Punjab, 12 of Bihar, 3 of Maharashtra, 14 of Gujarat, 15 of Tamil Nadu, one each from Uttar Pradesh and Haryana and two each from Assam and Arunachal Pradesh were of moderate nature (Figs. 2, 3).

Characteristic pigmentation of the fungal cultures varied widely among the isolates. Based on the mycelial colour, the isolates were categorized into 7 groups viz., white, orange, pinkish orange, pink, dark pink, pinkish violet and reddish brown. Nineteen of 117 isolates were found to be white, 2 were orange, 3 pinkish orange, 76 pink, 7 dark pink, 8 pinkish violet and 2 reddish brown. More than 75 % of the isolates showed typical pinkish pigmentation and other cultures exhibited varying shades of pinkish pigmentation. However, when reverse pigmentation was compared, the isolates were further categorized into 21 groups (Supplementary Table 2).

The isolates Fs BT K1 and Fs BT K7 were white and 11 other isolates from Kerala had pink mycelia. Similarly 4 Punjab isolates viz., Fs 003 P2L1, Fs 003 P5L1, Fs 003 P10L2 and Fs 120 P4, FsJn 964 MP1 from Madhya Pradesh, FsA 085 O1 and FsA 085 O5 from Orissa, FsC 671 G2, Fs 193 G and Fs 032 G1 from Gujarat, Fs 012 M3 from Maharashtra, Fs 805 AP1L1, FsV 048 AP2 and FsV 048 AP3 from Andhra Pradesh and FsC 063 TN1 and Fs 012 TN from Tamil Nadu had white mycelia. The other isolates from these states exhibited a pinkish mycelium in plates.

When reverse pigmentation was considered, Fs 003 P2L1, Fs 003 P5L1, Fs 003 P10L2, Fs 120 P4 and Fs 120 P5 from Punjab, FsJn 964 MP1 from Madhya Pradesh, FsA 085 O1, FsA 085 O5, FsA 085 O6 and FsA 085 O7 from Orissa, FsC 671 G2 and Fs 193 G from Gujarat, Fs 805 AP1L1 and FsV 048 AP2 from Andhra Pradesh, Fs LC A1 from Assam, Fs 012 M3 from Maharashtra, Fs 121 UP1

Table 4 Recovery of wilt pathogen from nodal and internodal tissue samples of wilt infected canes from different regions

S. no.	State	Variety	Location	% Recovery		
				Internode	Node	Mean
1	Punjab	Co 89003	Taggadabadala	0.00	70.00	35.00
			Mukerian	20.00	20.00	20.00
		CoJ 85	AB Sugars	0.00	5.00	2.50
			Mukerian[a]	40.00	25.00	32.50
		Co 0120	AB Sugars	10.00	0.00	5.00
2	Haryana	Co 89003	Karnal	0.00	25.00	12.50
3	Uttar Pradesh	Co 0121	Simbhaoli	75.00	100.00	87.50
4	Bihar	CoBln 03173	Motipur	29.16	16.66	22.91
		CoBln 03175	Motipur	16.66	10.00	13.33
		CoBln 03176	Motipur	45.00	29.16	37.08
		CoSe 02231	Motipur	5.00	0.00	2.50
5	Orissa	Co 7805	Chikinia	10.00	0.00	5.00
		CoA 89085	Chikinia	20.83	45.83	33.33
6	Madhya Pradesh	CoJn 964	Powarkheda	0.00	10.00	5.00
7	Gujarat	Co 85036 (1)	Chalthan	0.00	0.00	2.50
		Co 85036 (2)	Chalthan	5.00	0.00	2.50
		Co 86002	Sayan	5.00	5.00	5.00
		Co 86032	Chalthan	0.00	5.00	2.50
			K. Farm	33.33	5.00	19.17
		Co 88025	Sayan	10.00	0.00	5.00
		Co 94012	Bardoli	5.00	0.00	2.50
		Co 95005	Chalthan	0.00	5.00	2.50
			K. Farm	0.00	5.00	2.50
		Co 95006	Navsari	0.00	29.16	14.58
		Co 95016	Sayan	10.00	0.00	5.00
		Co 95020	Chalthan	10.00	0.00	5.00
		Co 98010	Chalthan	10.00	0.00	5.00
		Co 2001-10	Navsari	10.00	0.00	5.00
		CoC 671	Navsari	10.00	0.00	5.00
		CoSi 95071	Chalthan	0.00	10.00	5.00
		CoV 94102	Chalthan	0.00	0.00	2.50
		CoVSi 9337	Navsari	5.00	0.00	2.50
		99N 810	Sayan	10.00	0.00	5.00
		2001N 193	Navsari	10.00	0.00	5.00
		No: 3 underground stem	Bardoli	5.00	0.00	2.50
8	Maharashtra	Co 86032	Someshwar Nera	0.00	10.00	5.00
			Kopargaon Bolki	20.00	0.00	10.00
		Co 94012	Pravaranagar	15.00	0.00	7.50
9	Andhra Pradesh	Co 7805	Chinnathadepalli	0.00	20.83	10.42
			Vodlamurru	5.00	0.00	2.50
		Co 86032	Mortha	10.00	0.00	5.00
			Rudrur	0.00	15.00	7.50[a]
		Co 2002-09	Rudrur	0.00	15.00	7.50
		81V48	Tirumali	5.00	5.00	5.00

Table 4 continued

S. no.	State	Variety	Location	% Recovery		
				Internode	Node	Mean
10	Tamil Nadu	Co 740	SBI	5.00	10.00	7.50
		Co 1287	SBI	0.00	5.00	2.50
		Co 1287B	SBI (L1)	0.00	10.00	5.00
		Co 1287B	SBI (L2)	10.00	10.00	10.00
		Co 6304	SBI	60.00	60.00	60.00
		Co 6806	SBI	0.00	5.00	2.50
		Co 7219 (1)	SBI	0.00	10.00	5.00
		Co 7219 (2)	SBI	0.00	10.00	5.00
		Co 85004	SBI	5.00	60.00	32.50
		Co 86032	Polur	0.00	5.00	2.50
			Polur	0.00	30.00	15.00
			KK pattu	10.00	0.00	5.00
			Sakthinagar	20.00	10.00	15.00
			Paccheri	5.00	0.00	2.50
			SBI	0.00	20.00	10.00
			Enanallur	0.00	10.00	5.00
			Dakshinapallam	20.00	0.00	10.00
			Sivagangai	0.00	5.00	2.50
			Perumalpudur	5.00	5.00	5.00
		Co 86249	Kodangudi	5.00	0.00	2.50
		Co 92012	Dakshinapallam	0.00	10.00	5.00
		Co 94003	Palakudi	0.00	20.00	10.00
		2002-07	SBI (L1)	5.00	5.00	5.00
		2002-07	SBI (L2)	0.00	10.00	5.00
		2002-22	SBI (L1)	20.00	0.00	10.00
		2002-22	SBI (L2)	0.00	10.00	5.00
		2002-30	SBI	5.00	0.00	2.50
		2002-36	SBI	0.00	5.00	2.50
		2002-72	SBI	20.00	40.00	30.00
		2002-107	SBI	0.00	20.00	10.00
		2002-108	SBI	0.00	20.00	10.00
		2003-22	SBI	0.00	5.00	2.50
		2003-46	SBI	0.00	16.66	8.33
		2003-50	SBI	0.00	10.00	5.00
		2003-52	SBI	20.00	0.00	10.00
		2003-76	SBI	15.00	5.00	10.00
		2003-83	SBI	0.00	10.00	5.00
		2003-85	SBI	0.00	5.00	2.50
		2003-93	SBI	0.00	5.00	2.50
		2003-111	SBI	0.00	10.00	5.00
		2003-116	SBI	20.00	0.00	10.00
		CoBln 02173 (1)	SBI	0.00	6.66	3.33
		CoBln 02173 (2)	SBI	0.00	6.66	3.33
		CoBln 03172	SBI	4.00	20.00	12.00
		CoC 671	Sivagangai	13.33	0.00	6.67

Table 4 continued

S. no.	State	Variety	Location	% Recovery		
				Internode	Node	Mean
		CoC 99062	Cuddalore	10.00	5.00	7.50
		CoC 90063	Vaidipakkam	13.33	0.00	6.67
		CoC 90063	Vaidipakkam	5.00	15.00	10.00[b]
		CoS 510	SBI	0.00	5.00	2.50
		CoS 92739	SBI	0.00	10.00	5.00
		CoS 95255	SBI	0.00	30.00	15.00
		CoS 96268	SBI	25.00	0.00	12.50
		CoSe 96436	SBI	10.00	0.00	5.00
		CoSi 86071	Vayalur (L1)	5.00	0.00	2.50
		CoSi 86071	Vayalur (L2)	0.00	5.00	2.50
		CoSi 86071	Vayalur (L3)	0.00	5.00	2.50
		CoSi 95071	SBI (L1)	5.00	0.00	2.50
		CoSi 95071	SBI (L2)	0.00	10.00	5.00
		CoSi 95071	SBI (L3)	0.00	25.00	12.50
		CoV 94101	Thiruvadigai	0.00	10.00	5.00
			Tuhili	0.00	20.00	10.00
			Sivagangai	15.00	0.00	7.50
			KKuppam	10.00	0.00	5.00
		AVT 21153	Cuddalore	0.00	45.83	22.92
		LG 96022	SBI	10.00	0.00	5.00
		M clone	Appakudal	25.00	15.00	20.00
		PIR 96-325	SBI	25.00	30.00	27.50
		57N 9-56	SBI	20.00	0.00	10.00
		Co 2000-10 × Co 94008 (1)	SBI	5.00	15.00	10.00
		Co 2000-10 × Co 94008 (2)	SBI	10.00	40.00	25.00
		Co 2000-10 × Co 94008 (3)	SBI	0.00	5.00	2.50
		Co 2000-10 × Co 94008 (4)	SBI	5.00	25.00	15.00
		Co 8371 × Co 93009	SBI	5.00	20.00	12.50
11	Kerala	57NG159 yellow	Kannur	33.33	45.83	39.58
		57NG219	Kannur	0.00	10.00	5.00
		57NG226	Kannur	0.00	16.66	8.33
		Black Tanna	Kannur	60.00	45.83	52.92
		Lajai	Kannur	0.00	20.83	10.42
12	Assam	Local cultivar	–	5.00	5.00	5.00
13	Arunachal Pradesh	Local cultivar	–	10.00	0.00	5.00
		Local cultivar	–	10.00	0.00	5.00
Mean				8.47	11.81	9.98

SBI Sugarcane Breeding Institute, Coimbatore

A total of 20–24 tissue bits were used for isolation. The number of pathogen recovered bits varied from 1 to 20 giving a recovery of 5–100 % for a total of 20 bits kept for isolation and 4.17–100 % for a total of 24 bits used for isolation. Partial information in this table was published earlier in Sugar Cane International, 24 (4)-7

[a] The wilted canes were found associated with top rot (pokkah boeng) caused by *Fusarium verticillioides* (*Gibberella fujikuroi*); L-numbers after represents different locations of sample collection; (1)-numbers within parenthesis represents different samples which yielded different percentage of recovery

from Uttar Pradesh FsC 063 TN1 and Fs 012 TN from Tamil Nadu and Fs BT K1 from Kerala produced no pigments at all. All the other isolates produced varying intensities of different pigments so that bottom of the culture plates was orange, pinkish orange, pink, dark pink, pinkish violet and reddish brown in colour (Figs. 2, 3).

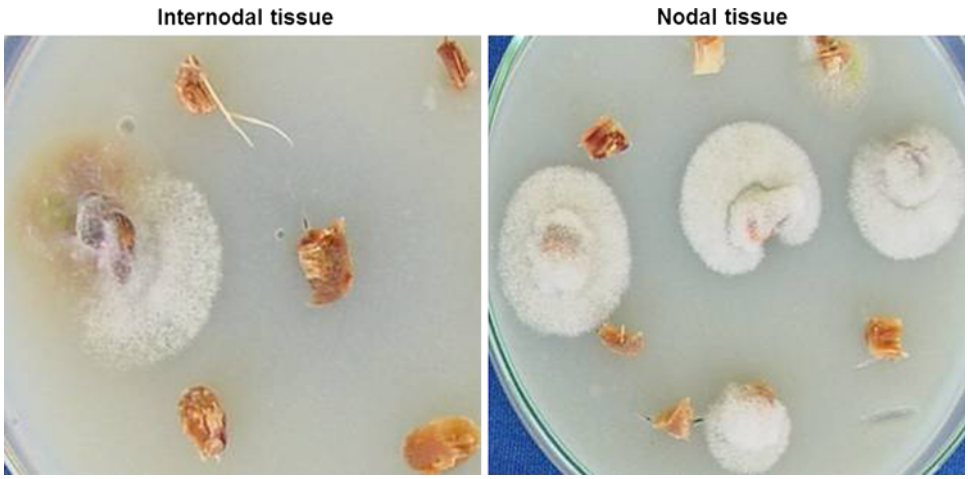

Fig. 1 Isolation of wilt pathogen from nodal and internodal tissues of cv CoA 89085 from Orissa

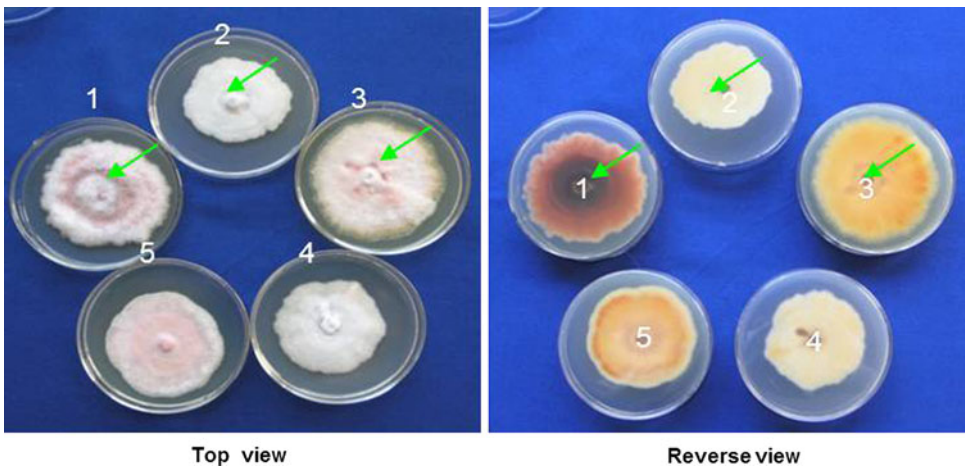

Fig. 2 Cultural characterization of *Fusarium* isolates associated with sugarcane wilt in cv Co 89003 from Punjab. Growth of *Fusarium* isolates on PDA after 10 days incubation at 28 °C (*top* and *reverse* view of the culture plates); *1–5*: isolates Fs 003 P1L1, Fs 003 P2L1, Fs 003 P3L1, Fs 003 P4L1, Fs 003 P5L1 from Punjab; *arrows* point out the variation in growth and pigmentation of the pathogen isolated from the same variety Co 89003

Texture

The 117 isolates were categorized into three groups based on the topology of the culture observed on PDA plates incubated at 25 °C for 10 days. Thirty of 117 isolates studied were found submissive (without aerial mycelium), 68 raised (with aerial mycelium) and 19 fluffy (with abundant cottony mycelia) (Supplementary Table 3). Except the isolate FsV 048 AP3 (fluffy), all the 8 isolates from Andhra Pradesh had raised topology on the agar surface. Six of Punjab isolates were submissive and 17 had raised topology. Four isolates from Bihar, one from Madhya Pradesh, 4 from Gujarat, 3 from Orissa, one each from Uttar Pradesh and Andhra Pradesh, 2 from Maharashtra, 4 each from Tamil Nadu and Kerala were submissive without any aerial mycelium and the remaining isolates from these states produced aerial mycelium. Abundant aerial mycelia resulted in fluffy growth of the isolates FsSe 231 B from Bihar, Fs 036 G2, FsSi 071 G, FsV 102 G, Fs 002 G1, Fs 002 G2, Fs 010 G, Fs 006 G1, Fs 006 G2, Fs 006 G3 (Gujarat), FsV 048 AP3 (Andhra Pradesh), Fs 012 M3 (Maharashtra), Fs LC ArP1 (Arunachal Pradesh), Fs Mclone TN9, FsSi 071 TN1, FsSi 071 TN2, FsV 101 TN3L3, Fs 032 TN8L4 and Fs 032 TN4L2 (Tamil Nadu) (Figs. 2, 3).

Frequency of Sporulation

According to conidial frequency and type of spores produced, the isolates fell into five groups (Table 5; Fig. 4). Two of the Gujarat isolates viz., Fs 036 G1 and FsVi 337 G produced both micro and macroconidia at higher frequencies. Conversely, Fs 036 G2, Fs 032 G1, Fs 032 G2, Fs LC A1, Fs 003 P2L1, FsJ 085 P5, FsBln 175 B3, Fs Lajai K2 and Fs 012 M3 produced micro and macroconidia at lower frequencies. The remaining 106 isolates showed only microconidia and the frequency of sporulation differed among the isolates. In 57 of 117 isolates microconidia concentration was low, moderate concentration of 6–10 $\times$ 10^6/mm^2 in 34 and high in 15 isolates. Spore

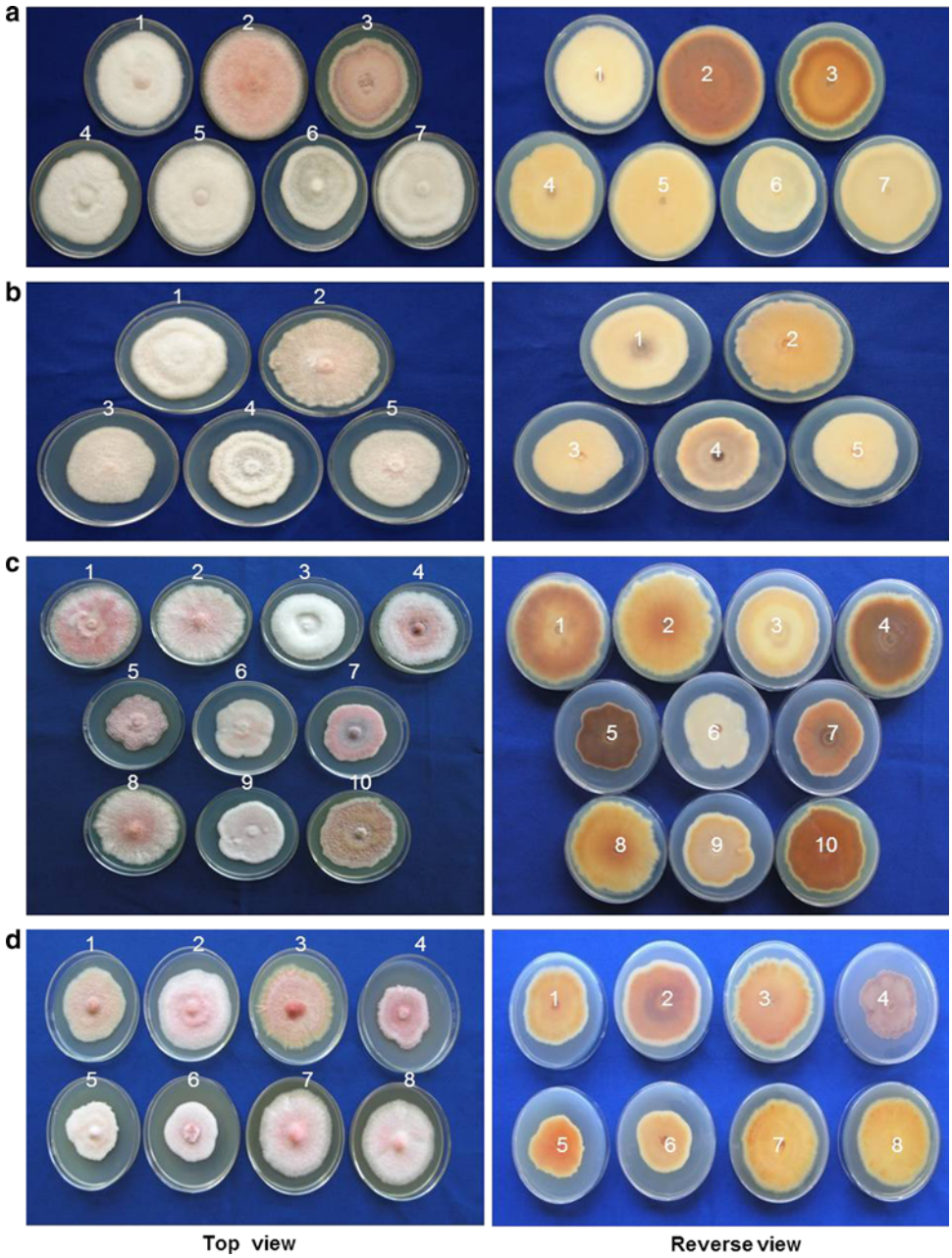

Fig. 3 Cultural characterization of *Fusarium* isolates associated with sugarcane wilt. Growth of *Fusarium* isolates on PDA after 10 days incubation at 28 °C; Plate **a**–**d** represent *top* and *reverse* view of the culture plates; **a** *1–7* isolates Fs 805 O1, FsA 085 O2, FsA 085 O3, FsA 085 O4, FsA 085 O5, FsA 085 O6, FsA 085 O7 from Orissa; **b** *1–5* isolates FsBln 176 B1, FsSe 231 B, FsBln 175 B4, FsBln 175 B3, FsBln 175 B2 from Bihar; **c** *1–10* Fs 036 G1, Fs 036 G2, FsSi 071 G, FsV 102 G, Fs 002 G1, Fs 002 G2, Fs 010 G, Fs 006 G1, Fs 006 G2, Fs 006 G3 from Gujarat; **d** *1–8* FsNG 159 K1, FsNG 159 K2, FsNG 159 K3, FsNG 159 K4, Fs Lajai K1, Fs Lajai K2, FsNG 219 K1, FsNG 219 K2 from Kerala

suspension of isolates recovered from wilt infected samples of Andhra Pradesh, Arunachal Pradesh, Uttar Pradesh, Haryana, Madhya Pradesh, Orissa and Tamil Nadu produced only microconidia at different frequencies.

Discussion

Fusarium produces woolly to cottony, flat, spreading colonies, may be white, cream, tan, salmon, cinnamon, yellow, red, violet, pink, or purple. On the reverse, it may be colourless, tan, red, dark purple or brown. Generally a purple reverse is indicative of species in section *Liseola* (Seifert 1996). The pigmentation of colonies grown on carbohydrate-rich media is variable in some species. Leslie and Summerell (2006) observed that the mycelia of *F. oxysporum* ranged from white to pale violet in colour and the mycelia floccose, sparse or abundant on PDA. They also reported that *F. oxysporum* usually produced pale to dark violet or dark magenta pigmentation in the agar but some isolates produced no pigment at all. Mycelia of *F. subglutinans* and *F. sacchari* are initially white but becomes violet as the culture ages and agar pigmentation ranged from colourless to dark purple.

The growth of *Fusarium* isolates was found to vary between 5.5–8.5 cm and most of the isolates, 80 of 117 recorded moderate growth (5.6–7.5 cm). Growth rate of

Table 5 Conidial frequency of *Fusarium* isolates associated with sugarcane wilt in potato dextrose agar plates

S. no	Conidial frequency ($\times 10^6$/mm^2)	Isolates	Number of isolates
1	Micro-low (below 5)	Fs 003 P1L1, Fs 003 P4L1, Fs 003 P5L1, Fs 003 P6L2, Fs 003 P8L2, Fs 003 P9L2, Fs 003 P10L2, FsJ 085 P11, Fs 120 P1, Fs 120 P2, Fs 120 P3, Fs 120 P4, Fs 120 P5, FsBln 173 B2, FsBln 173 B3, FsBln 176 B1, FsJn 964 MP1, Fs 121 UP1, Fs 805 O1, FsA 085 O1, FsA 085 O3, FsA 085 O5, FsA 085 O6, Fs 032 M1L1, Fs 012 M1, FsSi 071 G, FsV 102 G, Fs 006 G2, Fs 006 G3, Fs 005 G, Fs 025 G, Fs 193 G, G23, Fs 012 G, Fs 805 AP1L1, Fs 805 AP3L2, FsV 048 AP2, Fs 032 TN3L1, FsAVT 153 TN2, FsC 063 TN1, Fs 012 TN, FsSi 071 TN2, FsSi 071 TN3, FsNG 159 K1, FsNG 159 K2, FsNG 159 K3, FsNG 159 K4, Fs NG 226 K, Fs Lajai K1, Fs BT K1, Fs BT K2, Fs BT K3, Fs BT K7, Fs NG 219 K2, Fs LC A2, Fs LC ArP1, Fs LC ArP2	57
2	Micro-medium (6–10)	Fs 003 P3L1, Fs 003 P7L2, FsJ 085 P3, FsJ 085 P7, FsSe 231 B, FsBln 173 B1, FsBln 173 B4, FsBln 173 B5, FsBln 175 B1, FsBln 175 B4, FsBln 176 B3, FsA 085 O2, FsA 085 O4, FsA 085 O7, Fs 012 M2, Fs 032 M2 L2, Fs 003 H1, Fs 002 G2, FsC 671 G1, FsC 671 G2, Fs 010 G, Fs 006 G1, Fs 025 G, Fs 810 G1, Fs 016 G, Fs 006 G2, Fs 805 AP2L1, FsV 048 AP1, FsV 048 AP3, FS M Clone TN9, FsS 268 TN2, Fs 032 TN8L4, Fs 032 TN4 L2, Fs 003 TN, FsC 062 TN1	34
3	Micro-high (more than 10)	FsJ 085 P2, FsJ 085 P4, FsJ 085 P8, FsJ 085 P9, FsBln 175 B2, FsBln 176 B2, Fs 002 G1, Fs 810 G2, Fs 032 AP1L1, Fs 032 AP2L2, Fs 009 AP, FsV 101 TN3L3, FsSi 071 TN1, Fs SF TN1, Fs 047 TN	15
4	Micro and macro-low (below 5)	Fs 036 G2, Fs 032 G1, Fs 032 G2, Fs LC A1, Fs 012 M3, Fs 003 P2L1, FsJ 085 P5, FsBln 175 B3, Fs Lajai K2	9
5	Micro and macro-High (more than 10)	Fs 036 G1, FsVi 337 G	2

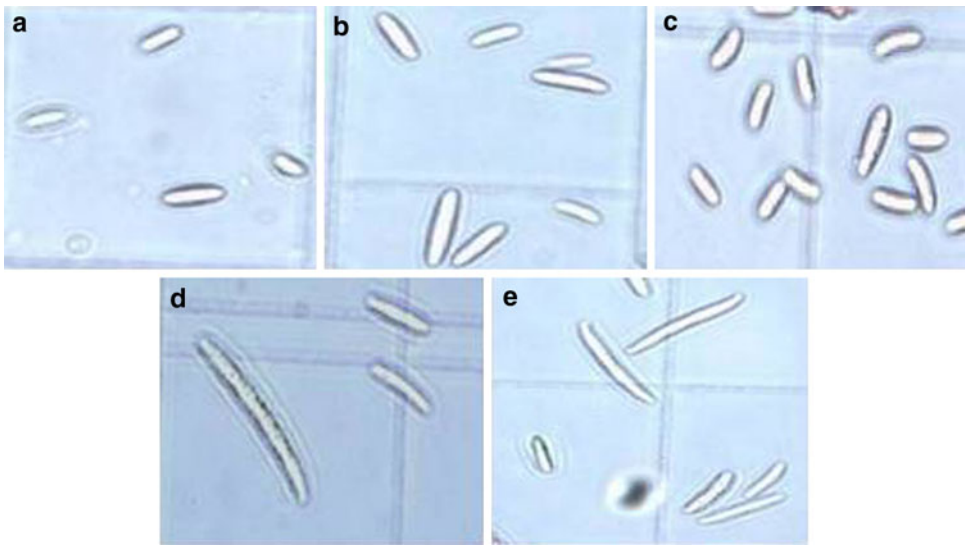

Fig. 4 Variation of conidial frequencies in the characterized *Fusarium* isolates. Conidial frequency of the *Fusarium* isolates observed on a haemocytometer: **a** Fs 003 P1L1 with low (<5 $\times$ 10^6/mm^2) microconidia production; **b** Fs 003 P3L1 with medium (6–10 $\times$ 10^6/mm^2) production of microconidia; **c** FsJ 085 P2 showing high (>10 $\times$ 10^6/mm^2) concentration of microconidia; **d** Fs 003 P2L1 with both micro and macro production at low (<5 $\times$ 10^6/mm^2) frequency; **e** Fs 036 G1 with micro and macro production at high (>10 $\times$ 10^6/mm^2) frequency

our isolates emphasized that they do not belong to *Acremonium* as they are faster than *Acremonium* in their growth rate which is found to be 3 cm in 7 days (http://www.mycology.net/). Campbell et al. (1996) reported growth of 50 mm in diameter in 1 week and the texture was found to be smooth to floccose with orange or pink pigmentation. It is noteworthy that most of the slow growing isolates viz., Fs 003 P2L1, Fs 003 P6L2, Fs 120 P4, FsSe 231 B1, FsJn 964 MP1, Fs 032 M1L1, FsVi 337 G, Fs 032 G1, FsV 048 AP2, Fs Lajai K2, Fs BT K1, Fs BT K2, Fs BT K3, Fs BT K7 were white or orange in colour and the other isolates showed varying shades of pink colour. Gerlach and Nirenberg (1982) and Nelson et al. (1983) also observed pale to violet pigments in different isolates of *Fusarium* as the culture ages. Most of the isolates were found to have aerial mycelium with raised topography as described by Gams (1971), which clearly concludes that they belong to *Fusarium.* However, 30 of 117 isolates were submissive in growth, growing along with agar surface. Pink pigmentation and submissive texture of isolates confuse the identity of the pathogen as *Acremonium* or *Fusarium* as Butler and Khan (1913) misinterpreted

Fusarium as *Cephalosporium.* However, growth rate of isolates clearly demarcated that our isolates belong to *Fusarium* but growth rate is not always reliable as it tends to change with environmental conditions. Although several workers characterized sugarcane wilt pathogens from the period of Butler and Khan (1913), currently *Fusarium* taxonomy is based on the work of Gerlach and Nirenberg (1982) and Nelson et al. (1983) after several reviews in taxonomy. Our results were similar to the observation of Leslie and Summerell (2006) who also observed that the mycelia of *F. subglutinans* and *F. sacchari* were white which then turned to violet upon ageing of the culture and pigmentation of the agar ranged from colourless to dark purple observed on the reverse side of the culture plate. On the other hand they also observed mycelia of *F. oxysporum* which ranged from white to pale violet in colour and the mycelia texture was floccose, sparse or abundant on PDA. They also reported that *F. oxysporum* usually produced pale to dark violet or dark magenta pigmentation in the agar but some isolates produced no pigment at all.

Growth rate of the fungus is a commonly used secondary character. There can be some variation in this trait (Leslie and Summerell 2006). The linear growth rate of the fungus under controlled conditions was used as a taxonomic characteristic by Booth (1971) and others (Burgess et al. 1988) but must also be used with caution. Isolates within a species may vary considerably with respect to the secondary characters. The degree of variation shown by a particular secondary character may differ between species. Bourne (1953) discussed the identity of the white and purple strains of *Fusarium* occurring in association with cane stalk rots and pokkah boeng disease in Florida. The fungus was found to be identical with *F. verticilloides* (*F. moniliforme* (Sheld.) Snyd. et Hans). Subsequent to publishing these data from Florida, the purple strain of *F. verticilloides* (*F. moniliforme*) was maintained in pure culture for 4 years. After this period it still proved highly pathogenic to cane cuttings and growing stalks. However, it was discovered that, after a period of approximately only 2 years in artificial culture stored at 23 °C, this strain completely lost the ability to produce chromogenic substances in nutrient PDA, or to produce septate macroconidia when transferred frequently on this medium. This phenomenon confirms the observations made by Wineland (1924) that certain strains that produced macroconidia in abundance at first, lost this character after a time and afterwards produced nothing but mycelium and microconidia. Loss of colour accompanied this change and in a few instances, cultures never produced pseudo-pionnotes or sporodochia and showed very little colour. This inconsistency in pigment production also agrees well with that recorded by Leonian (1929).

Results of the cultural study imply that there is a good variation among the 117 isolates studied and based on each character they are divided into 3 or more groups. However, most of the isolates fall in a single group. This predicts that amidst cultural variation in some cultures, majority of cultures show uniformity in their characters for cultural observation and these characters may not be adequately distinguish the variation in the fungi. The cultural characters were inconsistent and they tend to change with culture conditions. The same cultures after a month showed a difference in pigmentation and growth rate under different climatic conditions. This change in cultural characters with respect to time and environment was also observed by Butler as mentioned in his personal communication to Bourne in 1939 (Agnihotri and Rao 2002). Although majority of the isolates produced only microconidia in nutrient rich PDA further studies on carnation leaf agar (CLA) clearly established production of macroconidia by the isolates (Viswanathan, Unpublished). This may be the reason that earlier workers have assumed *F. verticillioides* (*F. moniliforme*) as the causal organism of the disease.

Singh et al. (1975) reported that frequency of isolation of *F. sacchari* is more in roots and internodal tissues while of *A. implicatum* in the nodal tissues. Hence they suggested that the former is a pathogen of parenchymatous tissue and the latter is of vascular tissues. They suggested that higher frequency of *A. implicatum* in the nodal tissues, but does not prove that this pathogen gets trapped in the nodal tissues because of anastomosis of vascular strands. However, our previous studies indicated that no distinction could be made in isolation of *F. sacchari* based on nodal and internodal tissues. Cultural characteristics of majority of isolates from different regions revealed that *F. sacchari* is the most commonly isolated wilt fungi in sugarcane (Viswanathan et al. 2006). Our studies clearly indicated lack of any *Acremonium* sp. among the 117 isolates which represent the pathogen spread throughout the country. Since cultural characters alone are unreliable for characterizing the isolates further studies were conducted on morphological and molecular characterization. These studies evidenced that *F. sacchari* is the causative fungus (Poongothai 2010; Viswanathan et al. 2011, 2012). Probably this is the first systematic pathogenic isolation of wilt associated pathogen in sugarcane from different locations and their characterization in India.

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