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Research Article:

Morphological and cultural variability in Fusarium oxysporum f. sp. cubense causing vascular wilt of banana in Kerala

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SUMMARY: Fusarium wilt disease caused by Fusarium oxysporum f. sp. cubense (Foc) is one of the most important diseases that affects the banana production which leads to huge economic loss. Thus, to avoid yield loss and manage the disease on time, morphological and cultural characterization of the pathogen is essential as a preliminary identification step. Thirty isolates were collected from different banana growing regions of Kerala. The isolates were grown on half strength PDA medium. The isolates showed extreme variations in their cultural and morphological characters. Most of the isolates produced white coloured aerial mycelium and few of them were greyish white with white to pink pigmentation. Colony diameter ranged from 59.6 to 90.0 mm at seven days after incubation at 25°C. The mycelial growth rate varied from 0.83 cm/day to 2.40 cm/day. Length and breadth of macroconidia ranged from 15.01 to 20.20 μ m and 2.14 to 5.07 μ m whereas, it ranged from 4.49 to 7.42 μ m and 1.35 to 3.13 μ m for microconidia. The diameter of chlamydospores varied from 5.68 to 9.58 µm. Whereas, the inter septal length and breadth of hyphae varied from 16.14 µm to 22.94 µm and 4.22 µm to 6.57 µm. The cluster analysis based on the quantitative parameters classified the isolates based on variety and genome of the host plants. All the isolates from Rasthali/Poovan (AAB) and Njalipoovan (AB) belonged to the cluster A1 whereas the isolates from Kadali and Chenkadali varieties of banana formed the cluster A2. While, the clustering based on qualitative parameters was irrespective of the variety or genome of the host plant.

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BACKGROUND AND OBJECTIVES

Banana (Musa acuminata) is one of the most important fruit crops in India as well in different parts of the world. Its year-round availability, affordability, varietal range, nutritive and medicinal value makes it the favourite fruit across the globe. However, its

cultivation is constrained by several incidence of pests and diseases. Among the diseases, Fusarium wilt of banana caused by the soilborne fungal pathogen, Fusarium oxysporum f. sp. cubense (Snyder and Hansen, 1940) is the most destructive one (Parham, 1935; Correll, 1991; Ploetz and Pegg, 2000 and Visser et al., 2010).

Fusarium wilt of banana and plantains was first explained by Bancroft (1876) in Australia, who was unknown that this disease would be a most dangerous disease in the history of banana cultivation worldwide. This was first reported from Southeast Asia and then, it disseminated rapidly worldwide including Panama region of Tropical America (Stover, 1962). It is reported to be one of the major limiting factors for the cultivation of banana varieties like Rasthali (AAB), Njalipoovan (AB) and Kadali (AA) in Kerala.

The pathogen enters plants and colonises inside vascular system of pseudostem and rhizome (Wardlaw, 1961; Ploetz and Churchill, 2011). It induces characteristic wilting symptoms accompanied by necrosis and rotting of roots, rhizome and pseudostem vessels and plant dies finally (Kumar and Saxena, 2015).

Purposive sampling surveys were conducted to study the characters and variability among Foc populations (Sivamani and Gnanamanickam, 1987; Somu et al., 2013 and Mostert et al., 2017). Pathogen was isolated from infected strands (Das et al., 2012) and maintained on quarter strength PDA (Ainsworth, 1971). Morphological and cultural characters such as radial growth, pigmentation, dimensions of spores and hyphae of the isolates from were studied (Honnareddy and Dubey, 2007 and Dubey et al., 2010).

Studies on pathogenic variability are very important to develop integrated management practices. Though internationally significant progress has been made to understand the biology of this pathogen, no attempts have been made to study the variability and diversity of the pathogenic isolates from Kerala. Hence, the present study was aimed to isolate and characterize the Foc isolates collected from various locations and to observe the variation among them.

Resources and Methods

Survey and collection of samples:

Purposive sampling surveys were conducted in selected banana growing districts of Kerala viz., Thiruvananthapuram, Ernakulam, Thrissur, Palakkad, Kozhikode and Wayanad to collect infected samples for further studies. Samples were collected from pseudostem of the infected banana plants. Vascular strands were separated from the infected pseudostem and placed in between tissue papers. These were brought to the laboratory for further studies. Since the plastic bags are

known to cause sweating and bacterial infection, the paper bags were used for collection of samples.

Isolation of the pathogen:

The pathogen was isolated from discoloured pseudostem strands of infected plants collected from all the locations surveyed. Culture media used for isolation and maintenance of the fungal isolates was half strength potato dextrose agar (PDA). Isolation of the pathogen was done after the strands kept in between tissue papers become dry. Strands of size 0.5-1.0 cm were sterilized in 70 per cent alcohol and placed in sterile Petri plates with half strength PDA and incubated at 25°C. Fungus grown from these strands were purified by single hyphal tip method and sub cultured. The pure cultures were maintained on PDA slants at 4°C.

Characterization of isolates:

All isolates of the pathogen isolated from collected samples from various locations were identified by its cultural, morphological and molecular characterisation.

Cultural characterization of isolates:

Cultural characters such as colour, texture and colony diameter, rate of mycelial growth, pigmentation and development of spores for all isolates were studied as a part of preliminary identification of the pathogen. This was done by inoculating the fungal disc of size 5 mm on half strength PDA medium plated in sterile Petri plates. Mycelial fragments were taken on a microscopic slide and stained with cotton blue and observed under the light microscope (Olympus) with 40 x magnification.

Morphological characterization of isolates:

The morphological characters viz., septation and size of macroconidia, microconidia and hypha as well as the diameter of chlamydospores. Mycelial fragments were taken on a microscopic slide containing cotton blue stain and observed under a Leica Image analyser. The dimensions of hypha and spores were measured.

Cluster analysis of isolates based on cultural and morphological characters:

Cluster analysis of all 30 isolates was done using cultural and morphological characters as similarity coefficient by Minitab 19 software. The data was classified into quantitative and qualitative data and the dendrogram



was constructed based on these variables. Quantitative parameters included colony diameter, rate of mycelial growth, length and breadth of macroconidia, microconidia and hypha and the diameter of chlamydospores. Qualitative parameters used for cluster analysis were colour of mycelium, the texture of mycelium and pigmentation.

OBSERVATIONS AND ANALYSIS

The findings of the present study as well as relevant discussion have been summerized under following heads:

Isolation of the pathogen

Fusarium wilt pathogen of banana was isolated according to the method explained by Carlier *et al.* (2002). Purification of the fungus was done by hyphal tip method described by Brown (1924). Infected vascular strands and the growth of mycelium in half strength PDA is shown in the Fig. 1. The isolation of the pathogen from collected samples was carried out on half-strength potato dextrose agar (PDA) medium. Ainsworth (1971) also used the half-strength PDA for the isolation of Foc from banana samples. A total of 30 Foc isolates were collected from various locations surveyed. Among these, four

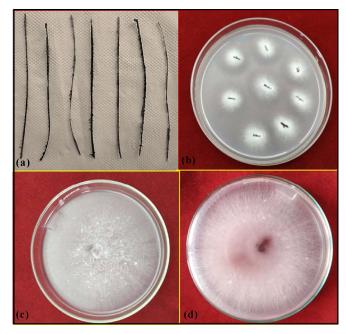


Fig. 1: (a) Infected vascular strands separated from pseudostem; (b) Fungal mycelium growing from vascular strands; (c) White coloured mycelial growth of Foc (d) Pinkish pigmentation of Foc colony

isolates were from the variety Kadali, two from Njalipoovan, one from Chenkadali and twenty-three from Poovan/Rasthali. The pathogen was isolated from pseudostem strands of infected plants. Das *et al.* (2012) isolated Foc from pseudostem strands of infected banana and maintained on PDA.

Characterization of isolates:

The pathogen associated with the disease was identified based on cultural, morphological and molecular characters of the collected isolates.

Cultural characterization of isolates:

Among the 30 isolates collected, twenty five isolates produced white coloured aerial mycelium, four of them were greyish white and one was grey. White or pinkish white $(S_1, S_2, S_6, S_8, S_{11}, S_{19}, S_{21}, S_{22}, S_{23}$ and $S_{26})$ pigmentation was common in most of the isolates while dull-white $(S_5, S_{16} \text{ and } S_{27})$, pink $(S_{12}, S_{13}, S_{15}, S_{17}, S_{18}, S_{24} \text{ and } S_{25})$ and violet $(S_4 \text{ and } S_{14})$ pigmentations were also noticed. Similar results were reported by Stover (1962) and Ploetz (1990) while working on Foc in banana. Honnareddy and Dubey (2007) also reported the similar results of pigmentation of F. oxysporum f. sp. ciceris isolates causing wilt in chickpea. Difference in the colony texture was also noticed among the isolates. Isolates were also variable with respect to colony texture. Cottony and fluffy mycelial mat was the commonly found texture but thin and sparse growth were also seen. Colony diameter ranged from 59.6 mm to 90.0 mm at seven days after incubation at 25°C. Based on the colony diameter after seven days of inoculation, isolates were grouped into 3 categories. Out of the thirty isolates, twenty-three included in the first category which produced more than 80.00 mm colony diameter. Among the remaining isolates, three were classified under the second group coming in between 70.00 to 80.00 mm. Whereas, four isolates belonged to the third category *i.e.*, the mycelial growth was less than 70.00 mm. The rate of mycelial growth ranged from 0.83 cm/day to 2.40 cm/day. The highest mycelial growth rate (2.40 cm/day) was recorded in the isolate S_{26} followed by the isolate S_{10} (2.30 cm/ day) and S₃ (2.23 cm/day). The lowest mycelial growth rate was recorded in the isolate S_{25} (0.83 cm/day). Similar results were reported by Joshi et al. (2013) in F. oxysporum causing wilt disease in tomato. The results are presented in Table 1.

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Sr. No.	Colour and texture	Pigmentation	Radial growth at 7 DAI (cm)	Rate of mycelial growth (cm/day)
S ₁	Greyish white, cottony, thin	Whitish pink	90.0	2.20
S_2	White, thick, fluffy	Whitish p ink	90.0	1.98
S ₃	Greyish white, cottony	Purplish pink	89.3	2.23
S_4	White, cottony	Violet	70.3	1.4
S ₅	White, cottony	Dull white	90.9	2.10
S_6	White, cottony, sparse	Pinkish white	65.3	0.93
S_7	White, cottony	White	84.7	1.21
S_8	Greyish white, thin	Pinkish white	89.0	1.28
S ₉	White, thin with zonation	White	82.3	1.18
S ₁₀	Grey, cottony	White	90.0	2.30
S ₁₁	White, fluffy	Whitish pink	90.0	2.26
S ₁₂	White, moderate fluffy	Bright pink	87.3	1.25
S ₁₃	White, net like and sparse	White	62.3	0.88
S ₁₄	White, very thin	Violet	75.7	1.09
S ₁₅	White, thick fluffy	Pink	90.0	2.09
S ₁₆	White, moderate fluffy	Dull white	81.0	1.16
S ₁₇	White, thin and sparse	Pink	83.3	1.20
S_{18}	White, cottony	Pink	90.0	1.99
S ₁₉	Greyish white, profuse fluffy	Pinkish white	90.0	2.14
S ₂₀	White, sparse	White	67.0	0.97
S ₂₁	White, thin	Pinkish white	73.7	1.07
S ₂₂	White, cottony	Pinkish white	90.0	2.20
S ₂₃	White, cottony	Pinkish white	84.6	1.22
S ₂₄	White, thick fluffy	Pink	90.0	2.05
S ₂₅	White, very thin	Pink	59.6	0.83
S ₂₆	White, thick	Pinkish white	90.0	2.40
S ₂₇	White, cottony	Dull white	90.0	2.10
S_{28}	White, moderate fluffy	White	80.7	1.13
S ₂₉	White, cottony	White	90.0	2.24
S ₃₀	White, cottony	White	88.3	1.26

Morphological characterization of isolates:

Significant changes were noticed with the size of macroconidia, microconidia, chlamydospores and hyphae among the isolates (Table 2). The length and breadth of macroconidia ranged from 15.01 to 20.20 μ m and 2.14 to 5.07 μ m, respectively whereas, it ranged from 4.49 to 7.42 μ m and 1.35 to 3.13 μ m, respectively in the case of microconidia. The diameter of chlamydospores varied from 5.68 to 9.58 μ m. The results were in accordance with the earlier findings by Fourie *et al.* (2011). The macroconidia were 3 to 5 septate and the microconidia were aseptate or single septate. Similar results were

reported by Smith (2006) in Foc of banana. All the isolates were found to be sporodochial in nature and the hyphae were septate. The length and breadth of hyphae ranged from 16.14 μ m to 22.94 μ m and 4.22 μ m to 6.57 μ m.

Based on the cultural and morphological characters the pathogen associated with the collected isolates were identified as *Fusarium oxysporum* f. sp. *cubense* (Foc).

Cluster analysis of Foc isolates based on cultural and morphological characters:

Cluster analysis of all 30 isolates was done using cultural and morphological characters as similarity co-

		ical characters of isolates Macroconidia (μm) Microconidia (μm)			Chlam ydospo res	Hyphae (µm)	
Sr. No.	Length	Breadth	Length	Breadth	(μm)	Inter septal length	Breadth
S ₁	20.20	5.01	7.31	2.24	9.58	22.94	6.57
S_2	16.79	3.49	5.01	1.35	6.66	18.64	6.09
S_3	16.24	3.01	5.75	2.01	5.81	17.89	4.91
S_4	15.01	2.76	5.69	2.19	6.43	17.63	4.88
S ₅	18.31	3.18	7.24	1.95	8.49	21.63	5.11
S ₆	18.29	3.45	6.98	2.40	6.93	18.11	5.80
S ₇	18.22	3.45	6.03	2.15	8.25	19.25	5.02
S_8	16.44	2.92	7.19	2.42	7.32	18.45	4.46
S ₉	19.13	4.37	5.06	2.84	6.58	17.22	5.01
S ₁₀	17.25	3.24	4.89	2.93	6.18	16.86	4.64
S ₁₁	17.29	2.91	5.19	2.17	6.07	18.78	4.92
S ₁₂	16.65	2.63	4.72	1.84	8.92	19.13	5.28
S ₁₃	18.45	3.24	5.66	2.04	6.19	19.63	4.89
S ₁₄	16.16	2.72	6.16	1.84	6.62	19.66	6.24
S ₁₅	18.28	3.55	5.97	3.13	5.99	17.10	5.23
S ₁₆	17.46	2.69	4.29	2.18	7.26	18.57	5.58
S ₁₇	19.20	5.07	7.42	2.13	9.34	19.88	6.93
S ₁₈	15.16	2.41	5.64	2.40	5.68	16.29	4.35
S ₁₉	14.91	2.15	5.03	1.87	7.55	16.14	4.57
S ₂₀	18.40	3.40	7.35	2.62	6.36	17.96	5.86
S ₂₁	18.09	3.67	4.91	1.65	6.38	18.25	5.34
S ₂₂	16.58	2.37	6.11	1.87	8.43	17.32	4.22
S ₂₃	15.91	2.24	4.97	2.34	6.82	18.43	4.76
S ₂₄	18.36	4.21	5.42	2.12	7.36	19.01	5.53
S ₂₅	19.04	4.49	5.88	1.41	9.98	19.21	6.28
S ₂₆	17.69	3.99	5.72	2.16	6.54	17.56	4.96
S ₂₇	16.33	2.22	5.36	2.19	5.87	17.13	5.08
S ₂₈	16.70	2.33	6.28	1.47	8.04	16.47	4.33
S ₂₉	17.49	3.19	4.49	2.21	6.46	18.03	4.59
S ₃₀	16.40	2.14	6.18	1.75	9.29	17.16	5.42

efficient by Minitab 19 software. The data was classified into quantitative and qualitative data and the dendrogram was constructed based on these variables.

Quantitative parameters included colony diameter, length and breadth of macroconidia, microconidia and hyphae and the diameter of chlamydospores. The dendrogram constructed with quantitative data of cultural and morphological characters is given in Fig. 2. The maximum variation among the Foc isolates was observed at zero per cent similarity where two clusters were formed namely A1 and A2. Only five isolates were included in the A2 cluster and the remaining 25 isolates were included in the cluster A1. All the Foc isolates collected from Rasthali/Poovan (AAB) and Njalipoovan (AB) varieties of banana belonged to the cluster A1. Whereas, all the Foc isolates with AA genome collected from Kadali and Chenkadali varieties of banana belonged to the cluster A2. Three subclusters were formed at 33.33 per cent similarity namely B1, B2 and B3. The cluster A1 branched into two subclusters (B1 and B2) whereas, the cluster A2 has only one sub-cluster (B3). All the isolates included in the sub-cluster B1 namely 3, 27, 18, 4, 14, 19, 23, 8, 12, 22, 30 and 28 were isolated from Rasthali/Poovan and was again branched into three at

66.67 per cent similarity. Whereas, in the sub-cluster B2, the isolates 6 and 20 were isolated from the variety Njalipoovan having a similarity of nearly 90 per cent and the remaining isolates 2, 10, 11, 29, 16, 9, 15, 24, 26, 13 and 21 were isolated from the Rasthali/Poovan variety. The isolates in the subcluster B3 *viz.*, 1, 17, 5 and 25 were isolated from Kadali variety and the isolate number 7 was from Chenkadali variety.

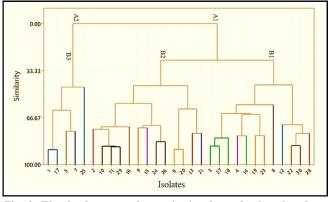


Fig. 2: The dendrogram of quantitative data of cultural and morphological characters

Qualitative parameters used for cluster analysis were colour of mycelium, the texture of mycelium and pigmentation. The dendrogram constructed with qualitative data of cultural and morphological characters is given in the Fig. 3. The maximum variability with respect to qualitative data was observed at 100 per cent dissimilarity. All the isolates were classified into two clusters namely A1 and A2. Nine isolates were included in the cluster A1 which consists of only one sub-cluster (B1). Whereas the remaining 22 isolates included in the A2 cluster which is again divided into two sub-clusters

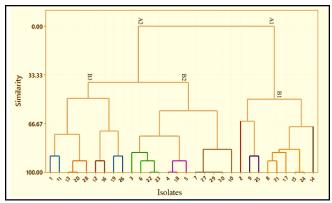


Fig. 3: The dendrogram of quantitative data of cultural and morphological characters

(B2 and B3). Among the isolates belonged to B1 subcluster, the isolates 2, 9, 8, 21, 15, 24 and 14 were isolated from Rasthali/Poovan and 17 and 25 were isolated from Kadali variety. In the B2 sub-cluster, the isolates 3, 22, 23, 4, 18, 7, 27, 29, 30 and 10 were isolated from Rasthali/ Poovan, isolate 6 from Njalipoovan and isolate 5 from Kadali variety whereas, in B1 sub-cluster, the isolates 1, 11, 13, 20, 28, 12, 16, 19 and 26 were isolated from Rasthali/Poovan, isolate 1 from Kadali and the isolate 20 from Njalipoovan.

Conclusion:

The present study revealed the information about morphological and cultural variability *F. oxysporum* f. sp. *cubense*, which could be used for identification of the pathogen and to adopt proper management strategies.

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