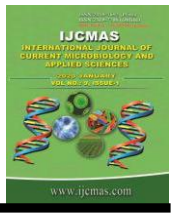
Int.J.Curr.Microbiol.App.Sci (2020) *9*(4): 3272-3288

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 4 (2020)**
Journal homepage: http://www.ijcmas.com



Original Research Article https://doi.org/10.20546/ijcmas.2020.904.381

Cultural and Morphological Variability of *Colletotrichum capsici* Isolates from Andhra Pradesh and Telangana Causing Fruit Rot of Chilli

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Keywords

Colletotrichum capsici, Cultural and morphological variability, AP, Telangana

Article Info

Accepted:
02 April 2020
Available Online:
10 April 2020

A B S T R A C T

Chilli is an important cash crop and India is the largest grower, consumer and exporter of dry chillies and other products to over 90 countries around the world. This crop suffers heavy losses in yield due to many diseases especially dieback and fruit rot diseases. The present study was undertaken to know the behaviour of the disease and biology of the pathogen so as to devise better management practices of the diseases to avoid losses. Cultural variations varied and colonies appeared from fluffy to suppressed with regular to irregular margins. Colour of colonies ranged between whitish grey to black. Growth rate of isolates was between 5.97 – 9.00 mm day-1. Morphological studies of isolates revealed average conidial length varied from 18.88 µm to 24.59 µm and mean conidial width ranged between 2.86 µm (Cc 40) to 5.94 µm (Cc 37). The average setae size varied from 78 -146 µm. Cluster analysis of the data on cultural and morphological characterization of C. capsici isolates revealed two major groups (I and II) based on distance matrices at the distance of 4.0. The group I and II included 27 and 13 isolates, respectively.

Introduction

Chilli is the most widely used spice in the world and is named as 'wonder spice' because of its innumerable and precious beneficial effects. Fungi, bacteria and viruses causing in diseases are the major constraints to chilli production. Among the fungal diseases, anthracnose and fruit rot of chilli caused by *Colletotrichum* species is one of the most important disease causing severe yield losses from 10% to 80% in different parts of the world (Poonpolgul and Kumphai, 2007). It has been reported that a part of postharvest losses of fruit quality deterioration of chilli is due to anthracnose ranges from 21 - 47% (Rajapakse *et al*., 2007). The symptoms of anthracnose invasion are sunken necrotic

lesions on fruits (Waller *et al*., 2002). The anthracnose lesions on chilli fruit reduced their marketable value (Manandhar *et al*., 1995). *Colletotrichum* was the eighth most important group of plant pathogenic fungi in the world (Dean *et al*., 2012). Several species of *Colletotrichum* etiologically associated with anthracnose diseases in chilli include *C. acutatum, C. coccodes, C. dematium, and C. gloeosporioides, C. capsici* etc. Anthracnose or ripe fruit rot caused by *Colletotrichum capsici* (Sydow) Butler and Bisby is the most important problem limiting the profitable cultivation in India. (Garg *et al.,* 2014).When any of the progeny exhibits a characteristic that is different from those present in the ancestral individuals, this individual is called a variant (Agrios, 2005). Compatibility of plant-pathogen interactions is often governed by the gene-for-gene model in many pathosystems. Existence of cultural, morphological and pathological variability in the *C. capsici* is reported in various countries. Hence the present study is undertaken to know the variability among the isolates of pathogen collected from major chilli growing areas of Andhra Pradesh and Telangna, India which will provide useful information for devising research strategies for successful management.

Materials and Methods

Anthracnose infected chilli twigs and fruits were collected from different fields situated in Guntur, Krishna, Kurnool, Prakasam and Chittoor districts of Andhra Pradesh and Warangal, Karimnagar, Khammam and Rangareddy districts of Telangana in India. The diseased part of the fruits was cut at advanced margin of lesions into small pieces (5mm diameter). The pieces were surface sterilized in aqueous solution of mercuric chloride (0.1:100w/v), streptocyclin and transferred in PDA after washout. The PDA plates seized with paraffin film and transferred in incubator at room temperature (27±2°C) for 7 10 days in dark condition. After 2 days, margin of mycelial growth was transferred to another Petri plate in aseptic condition. After 3-4 days the culture was revised for the purification of *Colletotrichum capsici* in aseptic condition, for culture growth. *Colletotrichum capsici* identification was based on morphological characters such as size and shape of conidia and appressoria, existence of setae or presence of a telomorph, and cultural characters such as colony colour, growth rate and texture (Smith and Black, 1990). A total of forty *Colletotrichum capsici* isolates (Table.1) were isolated from the samples and further studied.

The isolates of *Colletotrichum capsici* were grown in PDA medium for the cultural and morphological study. The colony growth starts in 1 2 days at 27±2°C under darkness on PDA. The cultural and morphological character (colony radial growth, colony colour, colony reverse, pigmentation, zonation and nature of growing margin) were recorded after 10 days of inoculation in each replicate (Talhinhas *et al*., 2005). Slides were prepared from 10 days old culture and number of spores, presence of conidial masses and seate were measured with hemocytometer.

Cluster analysis of the data on cultural and morphological characterization of *C. capsici* isolates were evaluated in all the 40 isolates using R (Version 3.4.0) statistical tool. Details of the characters and their attributes used for cluster analysis were presented in the Table 2.

Results and Discussion

Morphological Variability

Forty isolates of *C. capsici*differs among themselves in respect of morphological characteristics. Observations were made on

the size and shape of the conidia, size of the setae, number of setae per acervulus and formation of acervuli.

Conidial characteristics

Isolates of *C. capsici* differed with regard to the conidial length, width and shape. Mean conidial length in micrometers (µm) ranged between 18.88 (Cc 4) and 24.59 (Cc 28). Isolates Cc 4 (18.88 µm), Cc 2 (19.36 µm) and Cc 19 (19.89 µm) produced conidia with less than 20 µm while isolates Cc 7 (24.05 µm), Cc 34 (24.42 µm) and Cc 28 (24.59 µm) produced conidia with greater than 24 µm. Eight isolates, Cc 1, Cc 3, Cc 6, Cc 13, Cc 17, Cc 22, Cc 29 and Cc 30 were observed with conidia between 20-22 µm. Majority of the isolates under study produced conidia with length between 22-24 µm. Mean conidial width in micrometers (µm) of isolates ranged between 2.86 (Cc 40) and 5.94 (Cc 37). Among the isolates, 13 isolates (Cc 1, Cc 2, Cc 4, Cc 5, Cc 6, Cc 12, Cc 17, Cc 18, Cc 19, Cc 20, Cc 21, Cc 22, Cc 40) produced conidia with width less than 4 µm, 9 isolates (Cc 3, Cc 7, Cc 8, Cc 9, Cc 14, Cc 15, Cc 16, Cc 35, Cc 36) produced conidia with width between 4.0-4.5 µm and the remaining 18 isolates produced conidia with width of more than 4.5 µm. All the isolates produced conidia with either only falcate shaped (21 isolates; Cc 2, Cc 3, Cc 4, Cc 5, Cc 8, Cc 10, Cc 13, Cc 15, Cc 18, Cc 21, Cc 22, Cc 23, Cc 26, Cc 27, Cc 28, Cc 29, Cc 30, Cc 32, Cc 36, Cc 38, Cc 40) or falcate and spindle shaped (19 isolates; Cc 1, Cc 6, Cc 7, Cc 9, Cc 11, Cc 12, Cc 14, Cc 16, Cc 17, Cc 19, Cc 20, Cc 24, Cc 25, Cc 31, Cc 33, Cc 34, Cc 35, Cc 37, Cc 39) conidia. Even in isolates producing falcate and spindle shaped conidia, majority of the spores (more than 95 %) were in falcate shape only (Table 3).

Acervulus and setae Characteristics

Variability among the isolates with regard to the acervuli and setae production was studied. Isolates exhibited less variability in setae length and width. Highest mean setae length (115.76 µm) was observed in isolate Cc 40 and lowest (104.70 µm) was observed in isolate Cc 15. Studies on the mean setae length of 40 isolates, revealed that 22 isolates were having less than 110 µm while the 18 isolates were having more than 110µm. Within the isolates lowest setae length observed was 78 µm (in Cc 23 and Cc 26) and the highest setae length (146 µm) observed was (Cc 22 and Cc 34). Highest mean setae width of 4.49 µm was observed in Cc 15 whereas isolate Cc 7 recorded lowest setae width of 3.15 µm. Four isolates (Cc 4, 6, 7, 8) recorded mean setae width of less than 3.5 µm. The number of isolates with mean setae width between 3.5 to 4.0 µm is 17 and the number of isolates with mean setae width between 4.0 to 4.5 µm is 19. With in the isolates lowest setae width of 2 µm was observed in Cc 2 and the highest setae width observed was 7.0 µm in Cc 3.

Studies on the nature of formation of acervuli in culture revealed that the isolates produced acervuli either as ‘raised’ or ‘submerged’ in culture medium. Further, they were ‘scattered’ or in ‘concentric rings’. In all three different formation of acervuli were observed, namely, ‘raised, scattered’ in 16 isolates, ‘submerged, scattered’ in 21 isolates and ‘submerged, concentric rings’ in 3 isolates. Isolates, Cc 3, Cc 8, Cc 29 produced acervuli in ‘submerged, concentric rings’ manner. Isolates also varied with regard to the number of setae per acervulus. The no. of setae per acervulus in isolates were categorized as either dense (> 15 per acervulus) or as sparse (< 15 per acervulus). In 7 isolates, Cc 1, Cc 6, Cc 10, Cc 17, Cc 23, Cc 25, Cc 26 setae appeared as sparse (< 15 per acervulus). In the remaining of the isolates (33), setae appeared as dense i.e. (> 15 per acervulus) (Table 4 & Plate 1).

Cultural Variability

Studies on cultural characteristics of 40 *C. capsici* isolates were studied by growing the cultures on PDA. Observations were made on colony colour, type of mycelial growth, colony margins, sectoring, colour of conidial mass, growth rate, mycelial dry weight, and sporulation. Isolates differed among themselves in respect of cultural characteristics (Plate 2)

Colony colour and mycelial growth

Isolates of *C. capsici* varied in their colour *i.e.,* greyish white, white, and black. Out of the 40 isolates 9 isolates (Cc 3, Cc 7, Cc 8, Cc 9, Cc 15, Cc 23, Cc 31, Cc 37, Cc 40) were in black colour. The remaining isolates were either white (16 isolates; Cc 4, Cc 5, Cc 11, Cc 12, Cc 13, Cc 14, Cc 16, Cc 17, Cc 21, Cc 25, Cc 27, Cc 28, Cc 30, Cc 32, Cc 35, Cc 39) or greyish white (15 isolates; Cc 1, Cc 2, Cc 6, Cc 8, Cc 10, Cc 18, Cc 19, Cc 20, Cc 22, Cc 24, Cc 26, Cc 29, Cc 33, Cc 34, Cc 36, Cc 38). In the present study, the isolates produced two types of mycelial growths, i.e., 'suppressed' and 'fluffy'. 'Suppressed' type of growth is observed in 9 isolates (Cc 3, Cc 7, Cc 9, Cc 15, Cc 23, Cc 29, Cc 31, Cc 37, Cc 40) where as the remaining 31 isolates produced 'fluffy' type of mycelial growth (Table 5).

Colony margins, sectoring and colour of conidial mass

Among the 40 isolates of *C. capsici*, in 8 isolates (Cc 5, Cc 12, Cc 14, Cc 23, Cc 29, Cc 31, Cc 37, Cc 38) produced mycelial growth with regular margins where as the remaining 32 isolates produced mycelial growth with irregular margins. Sectoring in culture plates is not observed in majority (35 isolates) of isolates and observed only in 5 isolates (Cc 3, 23, 29, 37, 38). Isolates produced conidial masses in grey and orange colour. In majority of the isolates (35 isolates), the colour of conidial mass is grey (Cc 1, Cc 2, Cc 4, Cc 5, Cc 6, Cc 7, Cc 9, Cc 10, Cc 11, Cc 12, Cc 13, Cc 14, Cc 15, Cc 16, Cc 17, Cc 18, Cc 19, Cc 21, Cc 22, Cc 23, Cc 24, Cc 25, Cc 26, Cc 27, Cc 28, Cc 29, Cc 30, Cc 31, Cc 32, Cc 33, Cc 35, Cc 36, Cc 37, Cc 39 and Cc 40) and only in 5 isolates (Cc 3, Cc 8, Cc 20, Cc 34, Cc 38) the conidial mass colour is orange (Table 5).

Radial growth and growth rate

Isolates of *C. capsici* differed significantly with regard to the radial growth in PDA after 10 days. Maximum radial growth was observed in isolates Cc 38 (90.00 mm), Cc 39 (89.67 mm) and Cc 17 (89.67 mm) which were on par with each other. Isolates Cc 1 (84.67 mm), Cc 5 (86.67 mm), Cc 10 (85.00 mm), Cc 20 (85.33 mm), Cc 21 (85.33 mm), Cc 29 (85.67 mm), Cc 32 (85.67 mm) produced next best radial growth among the isolates. Least radial growth of 59.67 mm was observed in isolate Cc 11. Average growth rate was found to be highest in isolates Cc 38 (9.00 mm day $^{-1}$), Cc 39 (8.97 mm day $^{-1}$) and Cc 17 (8.97 mm day $^{-1}$) which were on par with each other. Least growth rate of 5.97 mm day $^{-1}$ was recorded in isolate Cc 11 (Table 5).

Mycelial dry weight and sporulation

Significant variation in the production of dry mycelial weight among the isolates of *C. capsici* was observed. Among the isolates, highest mycelial dry weight production was observed in Cc 2 (651.67 mg), Cc 18 (651.33 mg), Cc 20 (644.67 mg), Cc 25 (657.67 mg), Cc 33 (661.33 mg), Cc 34 (651.67 mg) and Cc 35 (641.67 mg) which were on par with each other. Lowest dry mycelial weight of 503.67 mg was observed in isolate Cc 22 (Table 5).

Table.1 Details of *Colletotrichum capsici* isolates

S. No	District	Mandal	Village	Diseased Plant Part	Isolate Designate
1	Guntur	Prathipadu	Garikapadu	Twig	Cc 1
2	Guntur	Sattenapalli	Kondepadu	Fruit	Cc 2
3	Guntur	Bollapalli	Bhrugubanda	Fruit	Cc 3
4	Guntur	Dachepalli	Dachepalli	Fruit	Cc 4
5	Guntur	Macherla	Pullareddygudem	Fruit	Cc 5
6	Krishna	Penuganchiprolu	Penuganchiprolu	Fruit	Cc 6
7	Krishna	Vissannapeta	Putrela	Leaf	Cc 7
8	Krishna	Gampalagudem	Konijerla	Fruit	Cc 8
9	Krishna	Tiruvuru	Ganugapadu	Twig	Cc 9
10	Kurnool	Nandyal	Padurangapuram	Fruit	Cc 10
11	Kurnool	Pamulapadu	Pamulapadu	Fruit	Cc 11
12	Kurnool	Gadivemula	Gadivemula	Fruit	Cc 12
13	Kurnool	Gudur	Munagala	Leaf	Cc 13
14	Kurnool	Gonegandla	Peddanelatur	Fruit	Cc 14
15	Prakasam	Dornala	Dornala	Twig	Cc 15
16	Prakasam	Arthaveedu	Magutur	Leaf	Cc 16
17	Prakasam	Podili	Dondleru	Fruit	Cc 17
18	Prakasam	Donakonda	Indla cheruvu	Fruit	Cc 18
19	Chittoor	Vadamalapeta	Pudi	Twig	Cc 19
20	Chittoor	Ramachandrapuram	Gangireddypalli	Fruit	Cc 20
21	Chittoor	Gandara Nellore	Pachigunta	Twig	Cc 21
22	Chittoor	Somala	Somala	Leaf	Cc 22
23	Warangal	Mangapeta	Komatipalli	Fruit	Cc 23
24	Warangal	Govindraopeta	Dammakkapalle	Fruit	Cc 24
25	Warangal	Wardhannapeta	Dammannapet	Fruit	Cc 25
26	Warangal	Zaffernagar	Thimmampet	Leaf	Cc 26
27	Warangal	Eturunagaram	Teegalavai	Twig	Cc 27
28	Khammam	Khammam	Thirthala	Fruit	Cc 28
29	Khammam	Tallada	Pinapaka	Leaf	Cc 29
30	Khammam	Konijerla	Thummalapalli	Fruit	Cc 30
31	Khammam	Thirumalapalem	Mohammadapuram	Twig	Cc 31
32	Khammam	Bhadrachalam	Lingalapalle	Fruit	Cc 32
33	Karimnagar	Mutharam	Pegdapalle	Leaf	Cc 33
34	Karimnagar	Kamanpur	Kamanpur	Fruit	Cc 34
35	Karimnagar	Kataram	Morepalli	Twig	Cc 35
36	Karimnagar	Manthani	Mallepalle	Leaf	Cc 36
37	Rangareddy	Rajendranagar	Rajendranagar	Fruit	Cc 37
38	Rangareddy	Chevella	Chevella	Twig	Cc 38
39	Rangareddy	Shankarpally	Shankarpally	Leaf	Cc 39
40	Rangareddy	Shamshabad	Shamshabad	Fruit	Cc 40

Table.2 Cultural and morphological attributes used for grouping of *C. capsici* isolates from different areas of Andhra Pradesh and Telangana.

S.No.	Character	Attributes
1	Mycelium Colour	White - 0 Greyish white - 1 Black - 2
2	Mycelial growth pattern	Elevated - 0 Suppressed - 1
3	Conidial mass colour	Grey - 0 Orange - 1 Pink - 2
4	Margins	Regular - 0 Irregular - 1
5	Sectoring	No - 0 Yes - 1
6	Colour of conidial mass	Grey - 0 Orange - 1
7	Sporulation	Poor - 0 Moderate - 1 Good - 2 Excellent - 3
8	Radial growth	< 70 mm - 0 70-80 mm -1 >80 mm -2
9	Conidial length	< 23 µm - 0 > 23 µm - 1
10	Conidial width	< 4 µm - 0 > 4 µm - 1
11	Conidial shape	Falcate only - 0 Falcate and spindle - 1
12	Setae length	< 110 µm - 0 > 110 µm - 1
13	Setae width	< 4 µm - 0 >4 µm - 1
14	No. of setae per acervulus	< 15 Sparse - 0 > 15 Dense - 1
15	Acervulus formatiom	Submerged - 0 Raised - 1

Table.3 Conidial morphology of *Colletotrichum capsici* isolates collected from major chilli growing districts of Andhra Pradesh and Telangana

Isolate	Length (µm)		Width (µm)		Shape of Conidia
	Range	Mean	Range	Mean	
Cc 1	14.21 - 29.27	20.38	2.24 - 5.32	3.75	Falcate & spindle
Cc 2	16.43 - 24.23	19.36	2.42 - 6.32	3.97	Falcate
Cc 3	14.27 - 25.84	20.87	2.43 - 5.38	4.13	Falcate
Cc 4	15.43 - 24.23	18.88	2.43 - 5.84	3.88	Falcate
Cc 5	19.71 - 25.31	22.71	2.09 - 3.74	2.95	Falcate
Cc 6	13.87 - 25.43	20.4	2.32 - 5.84	3.33	Falcate & spindle
Cc 7	19.71 - 25.37	24.05	2.32 - 5.84	4.14	Falcate & spindle
Cc 8	19.46 - 25.41	23.33	2.17 - 5.75	4.29	Falcate
Cc 9	19.43 - 25.31	23.37	2.17 - 5.75	4.22	Falcate & spindle
Cc 10	19.46 - 25.37	23.43	4.27 - 6.32	5.16	Falcate
Cc 11	18.23 - 25.37	22.51	3.16 - 6.32	5.09	Falcate & spindle
Cc 12	17.23 - 25.35	22.35	3.14 - 6.23	3.95	Falcate & spindle
Cc 13	15.43 - 25.45	20.93	3.14 - 6.39	4.51	Falcate
Cc 14	18.37 - 25.42	22.35	3.14 - 5.33	4.35	Falcate & spindle
Cc 15	19.12 - 25.42	22.85	2.18 - 6.39	4.48	Falcate
Cc 16	17.47 - 25.49	23.52	2.13 - 5.75	4.16	Falcate & spindle
Cc 17	14.32 - 25.84	21.08	2.13 - 5.75	3.78	Falcate & spindle
Cc 18	18.37 - 25.49	22.64	2.27 - 4.38	3.14	Falcate
Cc 19	15.43 - 25.49	19.89	2.37 - 5.45	3.76	Falcate & spindle
Cc 20	19.12 - 25.62	22.89	2.18 - 5.45	3.63	Falcate & spindle
Cc 21	19.61 - 25.63	22.88	2.11 - 5.32	3.39	Falcate
Cc 22	16.43 - 25.37	21.39	2.14 - 4.24	3.16	Falcate
Cc 23	19.61 - 25.62	23.79	2.14 - 6.39	5.05	Falcate
Cc 24	14.23 - 25.65	22.35	4.42 - 6.39	5.48	Falcate & spindle
Cc 25	14.23 - 25.62	22.28	2.14 - 6.29	4.94	Falcate & spindle
Cc 26	18.32 - 25.28	23.37	4.37 - 5.57	4.93	Falcate
Cc 27	19.29 - 25.24	23.22	2.14 - 5.57	4.7	Falcate
Cc 28	19.26 - 25.5	24.59	4.27 - 5.43	4.89	Falcate
Cc 29	16.43 - 25.28	21.2	4.36 - 5.75	5.15	Falcate
Cc 30	15.43 - 24.23	21.18	2.61 - 6.29	5.4	Falcate
Cc 31	19.29 - 25.28	22.88	4.14 - 5.78	5.36	Falcate & spindle
Cc 32	20.19 - 25.31	23.29	4.38 - 6.29	5.29	Falcate
Cc 33	17.32 - 25.28	22.86	2.61 - 6.29	5.06	Falcate & spindle
Cc 34	19.29 - 25.28	24.42	2.61 - 6.19	5.19	Falcate & spindle
Cc 35	18.46 - 25.24	23.03	3.27 - 6.19	4.39	Falcate & spindle
Cc 36	20.19 - 25.32	22.65	3.27 - 6.38	4.16	Falcate
Cc 37	20.19 - 26.34	23.85	4.39 - 6.38	5.94	Falcate & spindle
Cc 38	19.29 - 25.28	23.8	3.27 - 6.56	5.35	Falcate
Cc 39	18.46 - 25.87	23.65	4.39 - 6.38	5.37	Falcate & spindle
Cc 40	18.93 - 25.73	23.48	2.09 - 3.54	2.86	Falcate

Table 4. Morphological characteristics of acervuli and setae of *Collectotrichum capsici* isolates collected from major chilli growing areas of Andhra Pradesh and Telangana

Isolate	Setae Length (µm)		Setae width (µm)		No. of Setae in Acervulus	Formation of Acervuli
	Range	Mean	Range	Mean		
Cc 1	88.00 - 130.00	108.82	2.00 - 5.60	3.98	Sparse	Submerged, scattered
Cc 2	89.00 - 131.00	110.38	2.50 - 7.00	4.11	Dense	Raised, scattered
Cc 3	93.00 - 126.00	110.96	2.30 - 5.20	3.52	Dense	Submerged, concentric rings
Cc 4	95.00 - 137.00	109.82	2.50 - 4.50	3.35	Dense	Submerged, scattered
Cc 5	85.00 - 139.00	108.44	3.10 - 4.30	3.60	Dense	Submerged, scattered
Cc 6	84.00 - 137.00	109.58	2.70 - 4.50	3.28	Sparse	Submerged, scattered
Cc 7	89.00 - 137.00	105.48	2.30 - 4.30	3.15	Dense	Raised, scattered
Cc 8	89.00 -131.00	110.34	2.30 - 4.50	3.34	Dense	Submerged, concentric rings
Cc 9	92.00 - 141.00	112.98	2.80 - 4.60	3.82	Dense	Raised, scattered
Cc 10	92.00 - 137.00	107.66	2.30 - 4.80	3.66	Sparse	Submerged, scattered
Cc 11	93.00 - 131.00	107.50	2.30 - 4.90	3.74	Dense	Submerged, scattered
Cc 12	89.00 - 139.00	106.06	2.00 - 4.60	3.72	Dense	Submerged, scattered
Cc 13	93.00 - 126.00	109.68	2.50 - 5.00	3.98	Dense	Submerged, scattered
Cc 14	89.00 - 131.00	108.14	2.00 - 4.90	3.89	Dense	Raised, scattered
Cc 15	90.00 - 135.00	104.70	3.80 - 5.00	4.49	Dense	Raised, scattered
Cc 16	89.00 - 139.00	106.12	2.90 - 5.00	4.05	Dense	Submerged, scattered
Cc 17	86.00 - 139.00	108.28	2.90 - 5.00	4.03	Sparse	Submerged, scattered
Cc 18	92.00 -138.00	114.82	2.10 - 5.00	3.88	Dense	Raised, scattered
Cc 19	89.00 - 141.00	112.74	2.90 - 4.90	4.29	Dense	Raised, scattered
Cc 20	92.00 - 131.00	113.40	2.50 - 4.60	3.82	Dense	Raised, scattered
Cc 21	89.00 - 141.00	109.10	2.30 - 4.50	3.89	Dense	Submerged, scattered
Cc 22	85.00 - 146.00	106.94	2.30 - 4.90	4.10	Dense	Submerged, scattered
Cc 23	78.00 - 141.00	106.30	2.80 - 4.90	4.23	Sparse	Raised, scattered
Cc 24	89.00 - 145.00	108.84	2.30 - 4.90	4.05	Dense	Raised, scattered
Cc 25	85.00 - 145.00	107.68	2.30 - 4.80	4.06	Dense	Submerged, scattered
Cc 26	78.00 - 135.00	108.14	2.30 - 4.90	3.89	Sparse	Submerged, scattered
Cc 27	85.00 - 145.00	113.52	2.90 - 4.90	4.01	Dense	Submerged, scattered
Cc 28	85.00 - 138.00	106.60	2.70 - 4.60	4.14	Dense	Raised, scattered
Cc 29	92.00 - 145.00	108.08	2.30 - 4.60	4.11	Dense	Submerged, concentric rings
Cc 30	89.00 - 141.00	111.36	3.00 - 4.90	4.12	Dense	Submerged, scattered
Cc 31	89.00 - 145.00	114.66	3.00 - 5.00	3.92	Dense	Raised, scattered
Cc 32	84.00 - 140.00	109.10	3.00 - 4.80	4.08	Dense	Submerged, scattered
Cc 33	93.00 -142.00	112.52	2.30 -4.80	4.20	Dense	Raised, scattered
Cc 34	86.00 - 146.00	111.24	3.00 -4.90	4.10	Dense	Raised, scattered
Cc 35	94.00 - 141.00	110.62	3.40 - 4.80	4.08	Sparse	Submerged, scattered
Cc 36	89.00 -141.00	111.92	2.30 - 4.90	4.10	Dense	Submerged, scattered
Cc 37	82.00 - 137.00	112.52	3.00 - 5.10	4.15	Dense	Submerged, scattered
Cc 38	85.00 - 124.00	112.94	2.30 - 4.60	3.82	Dense	Raised, scattered
Cc 39	86.00 - 138.00	114.74	3.00 - 4.50	3.93	Dense	Submerged, scattered
Cc 40	98.00 -141.00	115.76	3.00 - 4.80	3.99	Dense	Raised, scattered

Table.5 Cultural characteristics of *Colletotrichum capsici* isolates collected from major chilli growing districts of Andhra Pradesh and Telangana

Isolate	Colony colour	Mycelial growth pattern	Margins	Secto ring	Conidial mass	Radial Growth (mm) after 10 days	Average growth rate/ day (mm)	Mycelial dry weight (mg)	Sporulation
Cc 1	Greyish White	Fluffy	Irregular	No	Grey	84.67	8.47	631.33	+++
Cc 2	Greyish White	Fluffy	Irregular	No	Grey	80.67	8.07	651.67	+++
Cc 3	Black	Suppressed	Irregular	Yes	Orange	82.33	8.23	532.00	++++
Cc 4	White	Fluffy	Irregular	No	Grey	79.67	7.97	639.67	++
Cc 5	White	Fluffy	Regular	No	Grey	86.67	8.67	611.00	++
Cc 6	Greyish White	Fluffy	Irregular	No	Grey	79.33	7.93	633.00	++
Cc 7	Black	Suppressed	Irregular	No	Grey	82.33	8.23	527.00	++++
Cc 8	Black	Fluffy	Irregular	No	Orange	79.67	7.97	555.33	++++
Cc 9	Black	Suppressed	Irregular	No	Grey	80.00	8.00	620.00	+++
Cc 10	Greyish White	Fluffy	Irregular	No	Grey	85.00	8.50	632.67	+++
Cc 11	White	Fluffy	Irregular	No	Grey	59.67	5.97	575.33	++
Cc 12	White	Fluffy	Regular	No	Grey	80.00	8.00	626.33	++
Cc 13	White	Fluffy	Irregular	No	Grey	70.33	7.03	603.67	+
Cc 14	White	Fluffy	Regular	No	Grey	75.33	7.53	633.67	++
Cc 15	Black	Suppressed	Irregular	No	Grey	70.67	7.07	533.67	++++
Cc 16	White	Fluffy	Irregular	No	Grey	80.33	8.03	624.33	++
Cc 17	White	Fluffy	Irregular	No	Grey	89.67	8.97	625.67	++
Cc 18	Greyish White	Fluffy	Irregular	No	Grey	75.33	7.53	651.33	+++
Cc 19	Greyish White	Fluffy	Irregular	No	Grey	80.33	8.03	621.33	+++
Cc 20	Greyish White	Fluffy	Irregular	No	Orange	85.33	8.53	644.67	+++
Cc 21	White	Fluffy	Irregular	No	Grey	85.33	8.53	627.00	++

Cc 22	Greyish White	Fluffy	Irregular	No	Grey	75.33	7.53	503.67	++
Cc 23	Black	Suppressed	Regular	Yes	Grey	78.67	7.87	533.00	++++
Cc 24	Greyish White	Fluffy	Irregular	No	Grey	81.00	8.10	607.33	+++
Cc 25	White	Fluffy	Irregular	No	Grey	69.00	6.90	657.67	++
Cc 26	Greyish White	Fluffy	Irregular	No	Grey	70.00	7.00	640.00	++
Cc 27	White	Fluffy	Irregular	No	Grey	78.67	7.87	633.33	++
Cc 28	White	Fluffy	Irregular	No	Grey	80.33	8.03	640.67	++
Cc 29	Greyish White	Suppressed	Regular	Yes	Grey	85.67	8.57	619.67	+++
Cc 30	White	Fluffy	Irregular	No	Grey	80.33	8.03	624.67	++
Cc 31	Black	Suppressed	Regular	No	Grey	80.33	8.03	609.00	+++
Cc 32	White	Fluffy	Irregular	No	Grey	85.67	8.57	613.67	++
Cc 33	Greyish White	Fluffy	Irregular	No	Grey	79.67	7.97	661.33	+
Cc 34	Greyish White	Fluffy	Irregular	No	Orange	84.33	8.43	651.67	++
Cc 35	White	Fluffy	Irregular	No	Grey	80.33	8.03	641.67	++
Cc 36	White	Fluffy	Irregular	No	Grey	69.33	6.93	568.67	++
Cc 37	Black	Suppressed	Regular	Yes	Grey	80.67	8.07	557.33	++++
Cc 38	Greyish White	Fluffy	Regular	Yes	Orange	90.00	9.00	662.00	+++
Cc 39	White	Fluffy	Irregular	No	Grey	89.67	8.97	638.67	++
Cc 40	Black	Suppressed	Irregular	No	Grey	69.33	6.93	527.00	++++
					CD (0.05)	2.06	0.21	20.61	
					SE(m)	0.73	0.07	7.31	
					CV	1.59	1.59	2.08	

++++ - Excellent (> 30 X 10^4 ml^{-1})
+++ - Good (21 - 30 X 10^4 ml^{-1})
++ - Moderate (10 – 20 X 10^4 ml^{-1})
+ - Poor (< 10 X 10^4 ml^{-1})

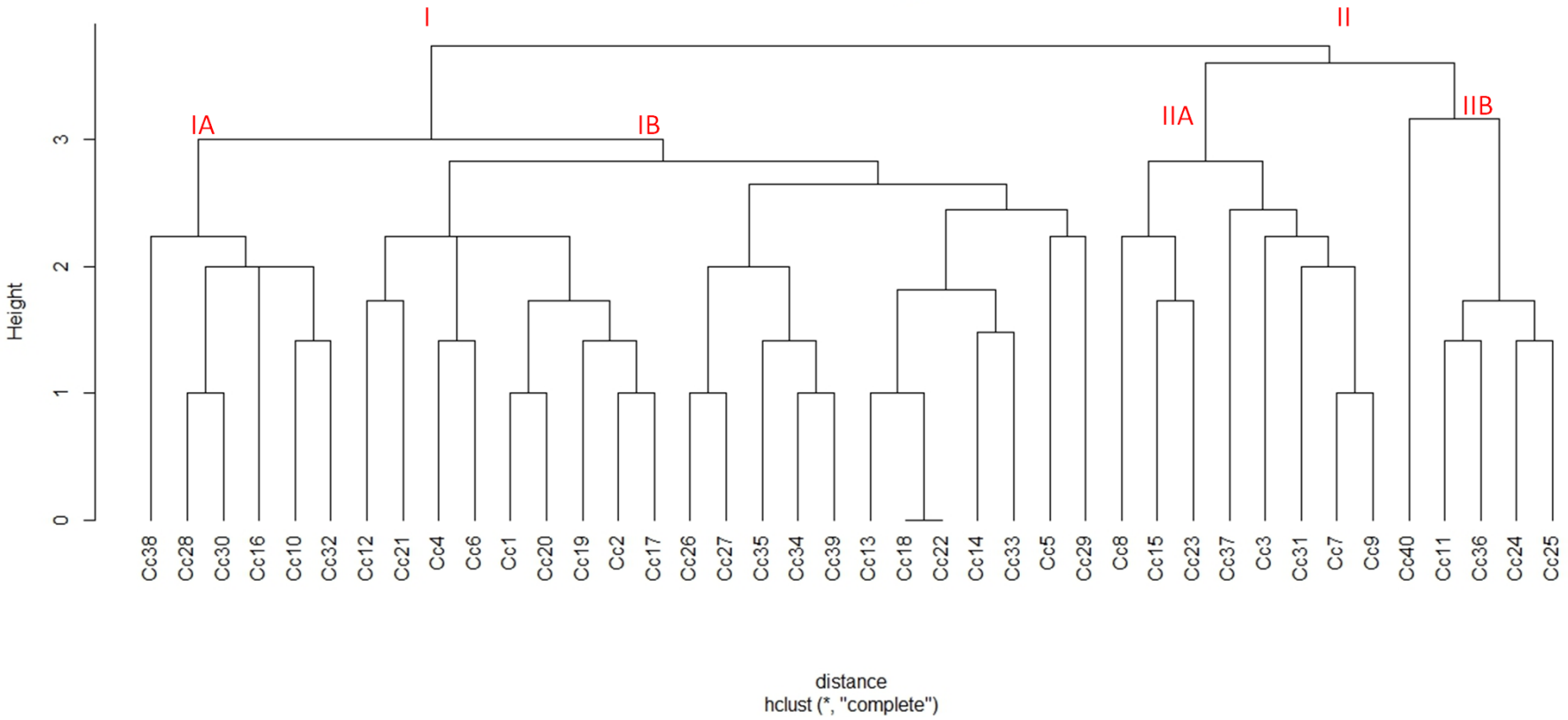

Figure.1 Cluster Dendrogram of *Colletotrichum capsici* isolates based on cultural and morphological characteristics

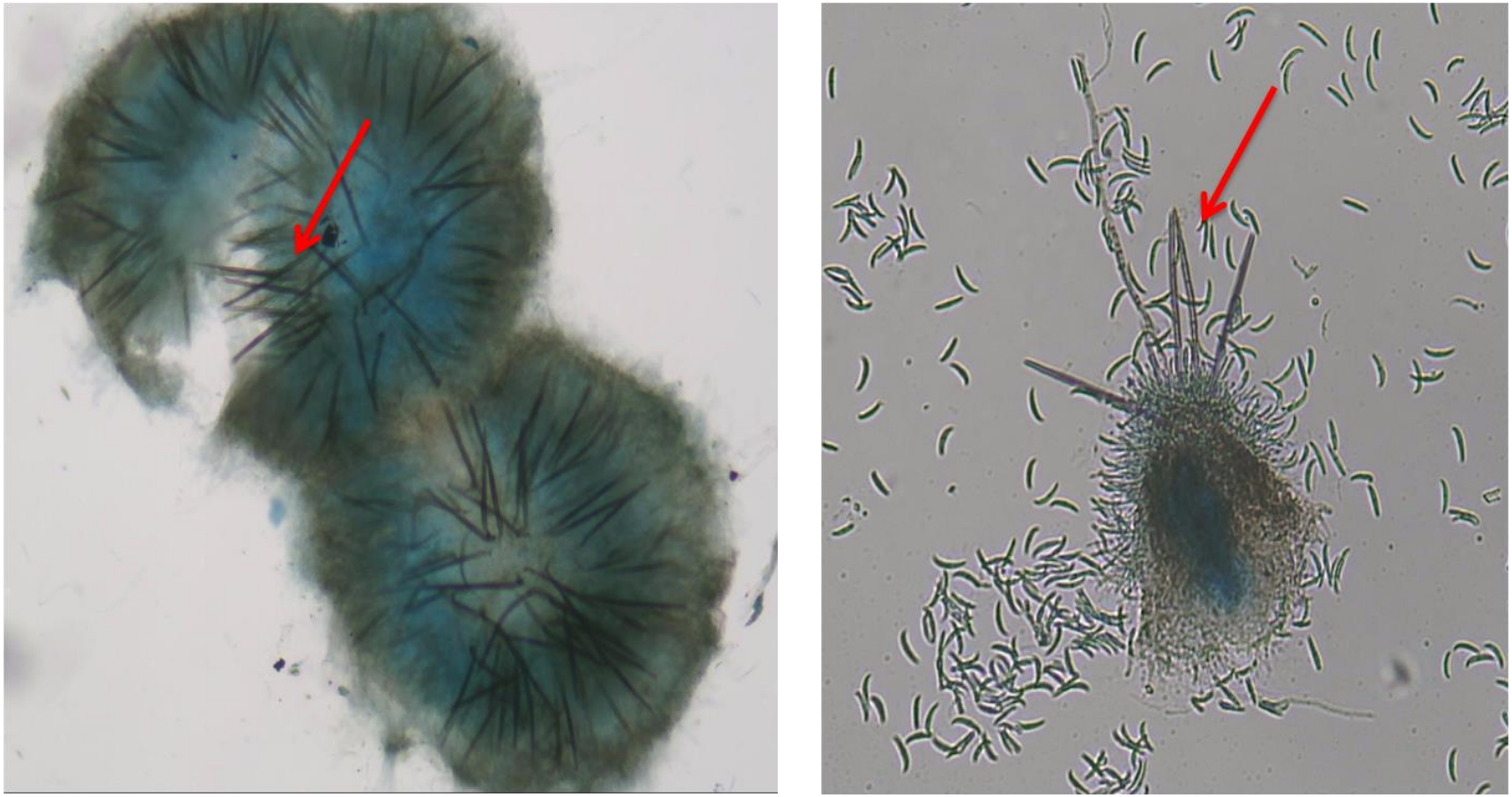

Plate.1 Acervuli with dense and sparse setae in *C. capsici* isolates
A: Dense (> 15/Acervulus); **B:** Sparse (< 15/Acervulus)

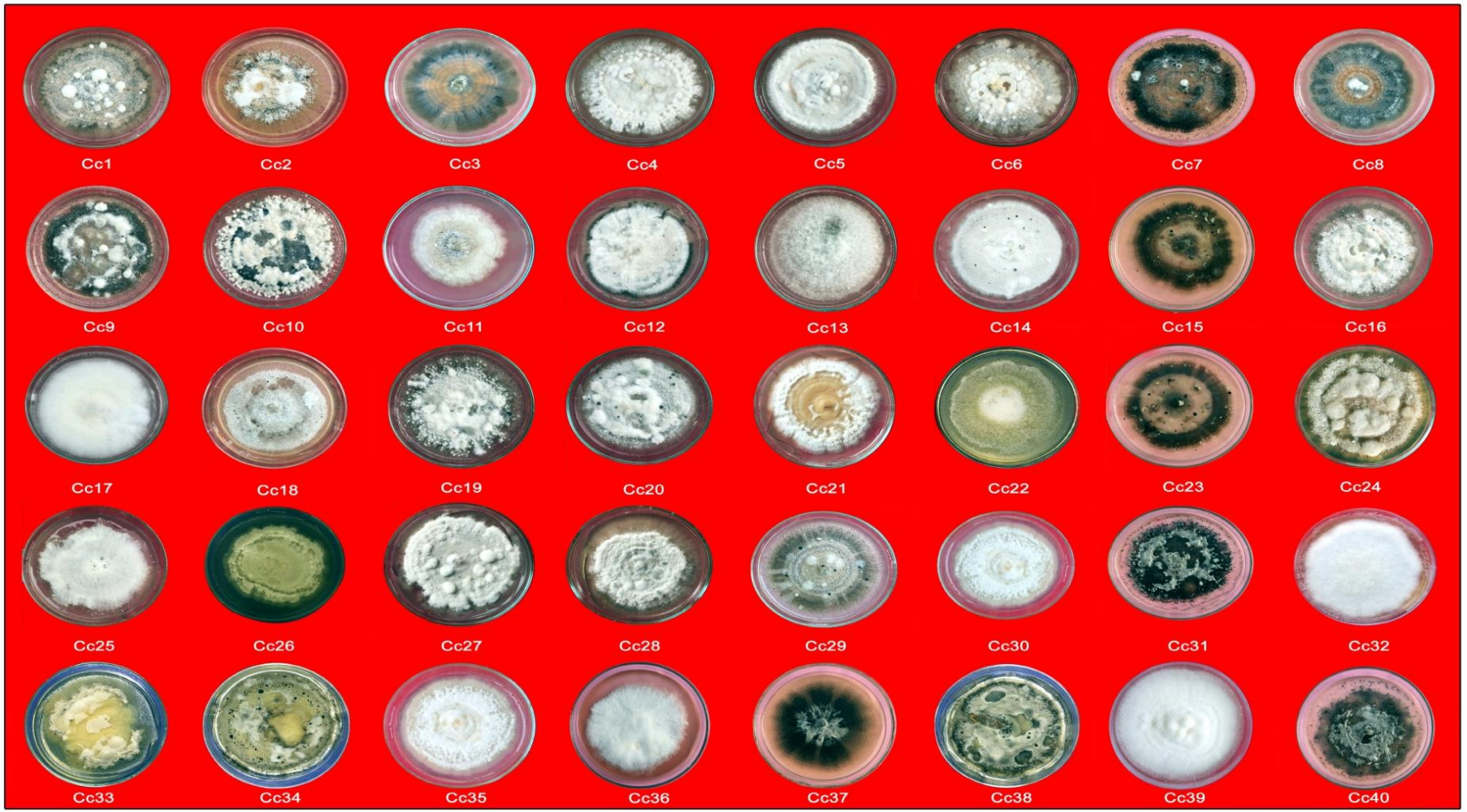

Plate.2 Cultural variability of 40 isolates of *C. capsici*

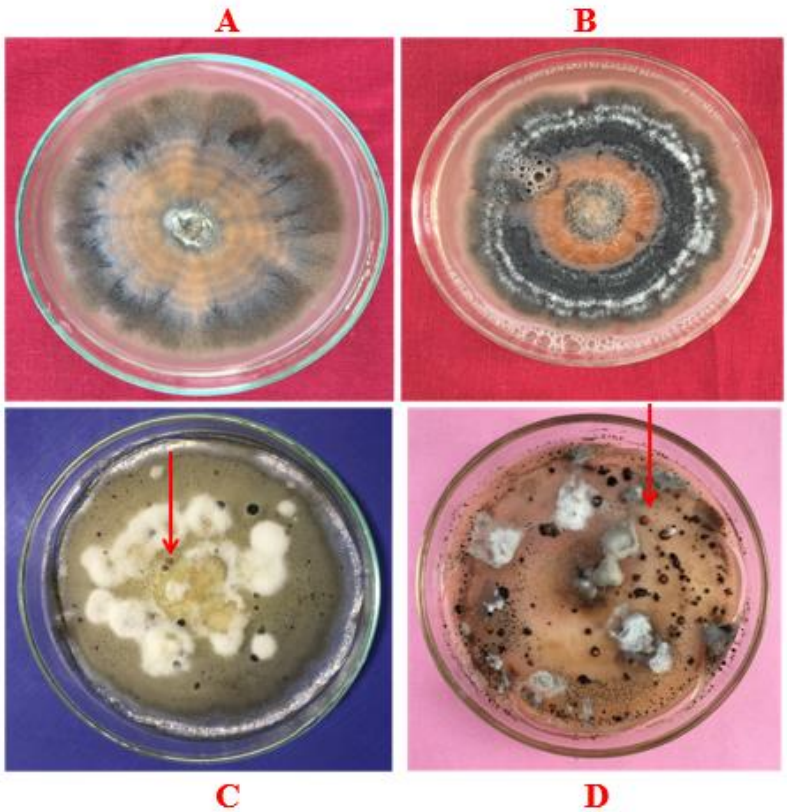

Plate.3 Different growth characteristics of *C. capsici*
A & B : Acervuli production in concentric circles
C : Greyish coloured conidial mass
D : Pink coloured conidial mass

The isolates of *C. capsici* differed in their sporulation ability. Sporulation ability of the isolates was found to be poor (< 10 x 10^4 conidia ml^{-1}) in isolates Cc 13 and Cc 33. Moderate (10-20 x 10^4 conidia ml^{-1}) sporulation was observed in 20 isolates (Cc 4, Cc 5, Cc 6, Cc 11, Cc 12, Cc 14, Cc 16, Cc 17, Cc 21, Cc 22, Cc 25, Cc 26, Cc 27, Cc 28, Cc 30, Cc 32, Cc 34, Cc 35, Cc 36, Cc 39). Good sporulation (21-30 x 10^4 conidia ml^{-1}) was observed in 11 isolates (Cc 1, Cc 2, Cc 9, Cc 10, Cc 18, Cc 19, Cc 20, Cc 24, Cc 29, Cc 31, Cc 38). Excellent sporulation (>30 x 10^4 conidia ml^{-1}) was observed in 7 isolates (Cc 3, Cc 7, Cc 8, Cc 15, Cc 23, Cc 37, Cc 40) (Table 5 & Plate 3).

Cluster analysis based on various cultural and morphological characters of isolates

Cluster analysis of the data on cultural and morphological characterization of *C. capsici* isolates, *viz.*, colony colour, growth pattern, colony margins, sectors formation, color of conidial mass, radial growth, conidial morphology (length, width and shape) and setae morphology (length, width and number)were evaluated in all the 40 isolates using R (Version 3.4.0) statistical tool Two major groups (I and II) were formed based on distance matrices at the distance of 4.0. The group I included 27 isolates, which were further divided into two subgroups. The sub group IA included 6 isolates (Cc10, Cc16, Cc28, Cc 30, Cc32 and Cc38) and sub group IB included 21 isolates (Cc 1, Cc 2, Cc 4, Cc 5, Cc 6, Cc 12, Cc 13, Cc 14, Cc 17, Cc 18, Cc 19, Cc 20, Cc 21, Cc 22, Cc 26, Cc 27, Cc 29 Cc 33, Cc 34, Cc 35 and Cc 39). Similarly, the group II included a total of 13 isolates, which were further divided into two subgroups, subgroup IIA included 8 isolates (Cc 3, Cc 7, Cc 8, Cc 9, Cc 15, Cc 23, Cc 31 and Cc 37), subgroup IIb included five isolates (Cc 11, Cc 24, Cc 25, Cc 36 and Cc 40) (Figure 1).

Accurate identification of *Colletotrichum* species along with the knowledge of populations responsible for causing epidemics were essential for developing and implementing the effective disease management strategies (Freeman *et al.*, 1998). Traditionally, identification and characterization of *Colletotrichum* species have been based on morphological and cultural characters. In our present investigation, 40 isolates of *C. capsici*were isolated from different districts of Andhra Pradesh and Telangana and the exhibited variations in morphological and cultural characters.

Colonies of *C. capsici* isolates exhibited wide variability in their colour and appearance. Different isolates exhibited colours like white, greyish white, and black. Mycelial growth is either fluffy or suppressed, majority of the isolates produced irregular margins and few of them produced regular margins. The variation with regard to the sectoring in culture is little, only 4 of the 40 isolates produced sectors in culture where as the remaining isolates appeared without any sectors. Colour of the conidial masses is grey in majority of the isolates while it is orange in only 5 isolates.

When the data was studied together with the geographical origins of the isolates (Table 1) the following observations can be made. Although significant variation existed among different isolates in colony colour and appearance of mycelial growth, there is no relation with regard to those characters and the geographical distribution of the isolates. In majority of the districts surveyed, the isolates with all the three colours (greyish white, white, black) were observed. The only exceptions were Krishna district (greyish white, Cc 6 and black Cc 7, Cc 8, Cc 9 isolates), Chittoor and Karimnagar districts (only greyish white and white isolates), Kurnool district (all the isolates, Cc 10 to Cc 14 were white in colour). With regard to the appearance of the colony colour also, in majority of the districts both ‘fluffy’ and ‘suppressed’ type of colony growth was observed except in Kurnool (Cc 10 to Cc 14), Chittoor (Cc 19 to Cc 23) and Karimnagar (Cc 33 to Cc 36) district in which only fluffy growth is observed. There is no much variation with regard to the colony margins as in majority of isolates ‘irregular’ type of margins is observed and the isolates with ‘regular’ margins (Cc 5, Cc 12, Cc 14, Cc 23, Cc 29, Cc 31, Cc 37, Cc 38) were also distributed across Guntur, Kurnool, Warangal, Khammam and Ranga Reddy districts. There is no relation to the between sectoring in isolates and the colour of conidial mass, as the isolates with those characters were distributed in all the districts.

The isolates (Cc 38, Cc 39, Cc 17) with highest growth rate were observed in Ranga Reddy and Prakasam districts, but the same districts also have isolates with moderate or low mycelial growth. This indicates the variation present in the isolates of a district regarding the growth rates. The isolates of Kurnool (Cc 10 to Cc 14) district with similar colony colour, mycelial growth, sectoring and conidial mass colour varied with reference to the mycelial growth. Even in case of dry mycelial weight, isolates varied among themselves but distributed in all the districts from which they were isolated. When the sporulation levels and their colony colour is considered, it was observed that the isolates that were black in colour (Cc 3, Cc 7, Cc 8, Cc 15, Cc 23, Cc 37, Cc 40) produced excellent sporulation and in majority of them, the type of colony growth is suppressed.

The conidial characters also varied among different isolates. There is no relation between the conidial characters of different isolates and and their geographical distribution, as the isolates with conidia of different sizes and shapes existed with in a geographical areas (districts). Mean conidial length in micrometers (µm) ranged between 18.88 (Cc 4) and 24.59 (Cc 28) where as the

mean conidial width in micrometers (µm) of isolates ranged between 2.86 (Cc 40) and 5.94 (Cc 37). Conidia of all the isolates were single celled, hyaline with either falcate or spindle shaped. Highest mean setae length (115.76 µm) was observed in isolate Cc 40 (Ranga Reddy district) and lowest (104.70 µm) was observed in isolate Cc 15 (Prakasam district) but both districts had isolates with conidia of different measurements. Highest mean setae width of 4.49 µm was observed in Cc 15 (Prakasam district) whereas isolate Cc 7 (Krishna district) isolate recorded lowest setae width of 3.15 µm. The number of setae per acervulus and the type of formation of acervulus also varied within the isolates but no relation was found to any particular geographical location.

The findings of the morphological and cultural characters observed in diffferent isolates in this study were in agreement with the findings of earlier workers (Rajput, 2011; Sangdee *et al.*, 2011; Christopher *et al.*, 2013; Machenahalli, 2014).

Variation in morphological and cultural characteristics observed among the isolates in our study is supported by the work of various other workers who reported similar variations in the populations of *C. capsici* from different parts of the world. Variability with respect to the colony morphology, conidial shape, presence of setae, radial growth, colony colour, mycelial growth pattern, sporulation and pigmentation was reported by Kommula *et. al.* (2017), Serawit and Tesfaye (2017) and Pandi *et. al.* (2018).

In conclusion, this distribution of isolates with different characters across the districts separated geographically and the variation present in the isolates within the districts might be due to the fact that the pathogen is seed borne and the movement of pathogen along with seed from one area to the other is quite common for decades. As a result, the isolates with different characters were present in the same geographical area. Otherwise, the pathogen might be constantly producing variability within the geographical area by making changes in its genetic makeup based on the selection pressure operating on them.

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How to cite this article:

Venkata Ramesh, K., R. Sarada Jayalakshmi Devi, K. Gopal, B.K.M. Lakshmi, T. Murali Krishna. 2020. Cultural and Morphological Variability of *Colletotrichum capsici* Isolates from Andhra Pradesh and Telangana Causing Fruit Rot of Chilli. *Int.J.Curr.Microbiol.App.Sci.* 9(04): 3272-3288.
doi: https://doi.org/10.20546/ijcmas.2020.904.381