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**Tamin**

Department of Plant Pathology,  
IGKV, Raipur, Chhattisgarh,  
India

**AS Kotasthane**

Department of Plant Pathology,  
IGKV, Raipur, Chhattisgarh,  
India

**Vivekanand Uraiha**

Department of Plant Pathology,  
IGKV, Raipur, Chhattisgarh,  
India

## Morphological variability in host specific isolates of *Colletotrichum* spp.

**Tamin, AS Kotasthane and Vivekanand Uraiha**

**Abstract**

The genus *Colletotrichum* includes a many number of plant pathogens, causing diseases of a wide range, variety of woody and herbaceous plants. On the host reddish brown spot appears and later they turn black. On infected pods, acervilli (fruiting bodies) resemble small pin cushions surrounded by setae. Single spore isolation of 10 different selected sample *i.e.* anthracnose of pigeon pea, leaf spot of turmeric, bean anthracnose, anthracnose of dragon fruit, chilli anthracnose, anthracnose of mungbean, anthracnose of bellpeper, anthracnose of cowpea, fruit anthracnose of brinjal and pod blight of soybean. Morphological variability studies of 10 different host specific isolates of *Colletotrichum* spp., conidial length and breadth of *Colletotrichum* spp. ranged between 15.95 - 27.00 X 2.50-4.84 µm and setae size varied from 140.75 -249.21 X 4.31-5.96 µm. Out of 10 isolates, isolate C3 (bean) are self induce perithecia. Premature and mature perithecia embedded on surface of PDA agar media plate, generally formed in cluster and sometimes individual. After maturation, outer layer cells of cell wall of perithecia become tough and dark in colour.

**Keywords:** *Colletotrichum*, single spore, morphological and cultural characteristics, perithecia, *Glomerella*

**Introduction**

*Colletotrichum* are fungal pathogens that devastate crop plants. There are many high-profile species, that affect tropical, subtropical and temperate crops. The symptoms mainly appear on pods, leaves, cotyledon and stem. The disease causes highly damage by reducing plant stand, growth, yield, seed quality and seed germination. On infected pods, acervilli (fruiting bodies) resemble small pin cushions surrounded by setae.

Only a small number of *Colletotrichum* species have been associated with the teleomorph (*Glomerella*) and generally these teleomorphs are rarely observed in nature. In the present investigation single spore Sutton, 1992 isolates were derived from different naturally infected hosts, which allowed us to demonstrate morphological variability in the fungus. Attempts were made to induce the teleomorph and to determine the fungal mating system.

Mating study suggests on *Colletotrichum* species comprise homothallic as well as heterothallic isolates, but also indicates that the mating systems do not always fit the typical system of homo- and heterothallic ascomycete fungi.

**Materials and Methods****Collection of naturally infected host**

Based on general typical different symptoms, the disease was identified as anthracnose of pigeon pea, leaf spot of turmeric, bean anthracnose, anthracnose of dragon fruit, chilli anthracnose, anthracnose of mungbean, anthracnose of bellpepper, anthracnose of cowpea, anthracnose of brinjal and soybean pod blight (Table 1).

Many studies on *Colletotrichum* are restricted to strains affecting single crop species (*e.g.* González *et al.*, 2006<sup>[6, 7]</sup>; Mackenzie *et al.*, 2007<sup>[14]</sup>; Gazis *et al.*, 2011)<sup>[5]</sup>, significantly reducing the extent of the gene pool being sampled. The ability of many *Colletotrichum* species to exist as endophytes adds extra complication to our understanding of host specificity (Lu *et al.* 2004, Liu *et al.* 2007)<sup>[13, 12]</sup>. Thon *et al.* (2000)<sup>[22]</sup> reported single gene effects on pathogenicity are documented interaction between *C. graminicola* and maize. O'Connell *et al.* (2012)<sup>[16]</sup> research the molecular basis of host-parasite interactions in *Colletotrichum* is highly active.

**Corresponding Author:****Tamin**

Department of Plant Pathology,  
IGKV, Raipur, Chhattisgarh,  
India

**Table 1:** *Colletotrichum* isolates collected from different host

S. No.	Isolate ID	List of naturally infected host	Disease symptoms observed in nature
1	C1	Pigeon pea	Pod blight/anthracnose
2	C2	Turmeric	Leaf spot
3	C3	Bean	Anthracnose
4	C4	Dragon fruit	Anthracnose
5	C5	Chilli	Anthracnose
6	C6	Mungbean	Pod blight
7	C7	Bellpepper	Anthracnose
8	C8	Cowpea	Anthracnose
9	C9	Brinjal	Anthracnose
10	C10	Soybean	Pod blight

### Isolation of single spore progenies

Spores were picked using capillary needle under the stereobinocular microscope. Using a light bent fine capillary glass needle the spores were separated on the surface of 4% water agar plate and incubated at 27 °C for 4-6 hr. to allow the spores to germination. The germtube proliferated and penetrated the water agar. Isolated germinating spores were observed and agar surrounding the germinating spore was cut down using a sterilized fine pointed inoculation needle (pony No. 12). A single germinating spore containing agar block was transferred to Potato agar slants tube, maintained and incubated at 27 °C.

Tagging, mapping and all genetic analysis process isolating the gene are specialized approaches and essentially required for isolating microorganisms in or genetically pure form. It is possible to genetically pure isolate for different microorganisms by enrichment technique but require an inordinate amount of time, labour, money, medium selection, dilutions, incubation and sample handling, determine the number and types of microorganisms from soil, water and plant (Hildebrand, 1938; Collins *et al.* 1977; Choi *et al.* 1999; Gupta *et al.* 2011) [10, 4, 2, 8].

### Morphological variability in different host specific isolates of *Colletotrichum* spp.

On PDA plates the *Colletotrichum* isolates were inoculated separately. Seven days old single spore culture of fungal mycelial disc of the respective isolates were cut down with the help of sterilized (flame sterilization) cork borer (5 mm) and the discs of each specific isolates were transferred to centre of plate. Their plates were incubated at 26±1°C and maintained for nine days and recorded colony characters, texture and growth of *Colletotrichum* spp.

### Measurement of radial mycelial growth

Evaluation of radial mycelial growth of the *Colletotrichum* and another fungus was similar, mycelia growth recorded on two to eighth days respectively. The colony diameter was measured in (mm) two marked directions at right or left angles to each other, passing through the centre of colony (culture plate).

### Measurement of size of conidia and setae

Dimensions of each isolates conidia (100X) and setae (20X) were measured by using Leica fluorescent microscope (Nikon Eclipse 50i attached Nikon Digital sight screen). The observations were based on mean size calculate of twenty conidia and setae of per isolates.

### Micrometry

First an ocular micrometer is set inside the eyepiece, it should simultaneously enable to properly see a sample and micrometer scale when you look pass through a microscope. Determining single pitch length of micrometer scale, measure the actual sample size.

$$\text{Formula: Pitch Length of Ocular Micrometer} = \frac{\text{(Actual Pitch Length)}}{\text{(Objective Lens Magnification)}}$$

### Morphology of conidia, conidiophores and Apperessoria

Observed the shape (100X) of all isolates conidia, conidiophores and apperessoria by Nikon Eclipse 50i attached with Nikon Digital sight screen to capture the perfect image.

### Pairing

All the field isolates were crossed using dual culture technique in culture tube, in every possible combination using a crossing block prepared in tubular form.

### Results and Discussion

#### Morphological variation in different isolates of *Colletotrichum* spp.

##### Cultural morphology

morphological variaion for host specific isolates derived from naturally infected host. All isolates show different types of colony morphology were evident among the isolates with significant variation in production and colour of conidial masses on PDA plates (Table 2). Among all isolates, colony of *Colletotrichum* spp. except isolate C3, C4 and C5 initially showed black colour but later showed dull whitish puffy growth at the centre of plate. Colony growth of isolate C3 and C5 was subdued to flat where other isolates expressed slightly raised.

**Table 2:** Significant variation in colour of conidial masses was observed

S. No.	Colour of conidial masses	Isolates
1	White	C1, C6 and C8
2	Yellow	C2, C3, C4 and C9
3	Pink	C5, C7 and C10

All specific isolates expressed variation in growth rate on PDA media (Below described in Table No.3). Isolate C3 derived from bean anthracnose was fastest growing, isolate

expressed 40.47 mm radial diameter after 8 days inoculation, C8, C2, C7 and C9 which expressed between 30.82mm to 30.32mm, radial diameter after 8 days of inoculation

respectively. Rest of the isolates C10, C4, C1, C5 and C6 expressed relatively slow growth rate, varied between 20.92mm to 20.52mm radial growth. The daily measurement of mycelial growth was basically used in the taxonomic analysis of genus *Colletotrichum* (Sutton 1992) [21].

Sharma and Kaushal (2011) [19] reported growth difference between different isolates of *C. truncatum* collected from different locations of Himachal Pradesh. Kee Don Han *et al.* (2004) also gave report about morphological and cultural features of *Colletotrichum dematium* causing ginseng pod blight and reported colony colour varied from dark in center and grey at margin on PDA. Bernstein *et al.* (1995) [1], Nagaraj and Jahagiridhar (2014) [15] reported that the morphological variation in soybean (*Colletotrichum truncatum*) isolates and their colony colour variation from dull cottony white to dark gray.

### Morphology of conidia

All host specific isolates of *Colletotrichum* spp. produced hyaline, aseptate, smooth wall, straight, cylindrical to clavate shaped and both ends slightly acute type conidia on PDA media. All isolated of *Colletotrichum* spp. conidia were hyaline, non-septate and falcate with oil droplets within cytoplasm, except C3 isolate conidia was rod shape. Dimensions of conidia of each isolate are presented in Table No.3. Maximum conidial length is 27.05  $\mu\text{m}$  was noticed in isolate C6 and minimum in the isolate C3 (15.95  $\mu\text{m}$ ). Isolate C3 (4.84  $\mu\text{m}$ ) showed maximum and C5 exhibited (2.50  $\mu\text{m}$ ) minimum conidial width.

Sharma and Kaushal (2001) [19] they reported size variation in conidia of different isolates of *C. truncatum* causing urdbean pod blight. Jagtap and Sontakke (2009) [11] reported distinct morphological variation of conidia and also setae of *C. truncatum* isolates from pod blight of soybean. Nagaraj and Jahagiridhar (2014) [15] studied on the conidia size varying with the different isolates of *C. truncatum* that causing pod blight in soybean.

### Morphology of setae

Setae of all isolated of *Colletotrichum* spp. are showed light to deep brown colour, 1-4 septa, verruculose, conical sometimes inflated, base cylindrical and rounded tip. The length and width of setae in different isolates of *Colletotrichum* showed variation on 10X and 20X magnifications, except C4 isolate (setae absent). Measurements of setae size of 9 isolates are presented in below Table No.3. Isolate C5 exhibited higher setal length (249.21  $\mu\text{m}$ ) and lowest was noticed in C2 (140.75  $\mu\text{m}$ ). Other isolate setae length varied between 165.15 - 224.56  $\mu\text{m}$ . Similarly setae width differed in different isolates of *Colletotrichum* spp. maximum width (5.96  $\mu\text{m}$ ) was induced in isolate C2 and minimum (4.31  $\mu\text{m}$ ) in isolate C1.

Similar study conducted by Sinclair and Backman (1989) [20] also reported variation in setae size. Variation in the conidial and setae size varying with isolates are in accordance, with findings of Gorawar *et al.* (2006) [6] they recorded size and shape of setae (50 to 140  $\mu\text{m}$ ) of turmeric leaf spot disease caused by *Colletotrichum capsici*.

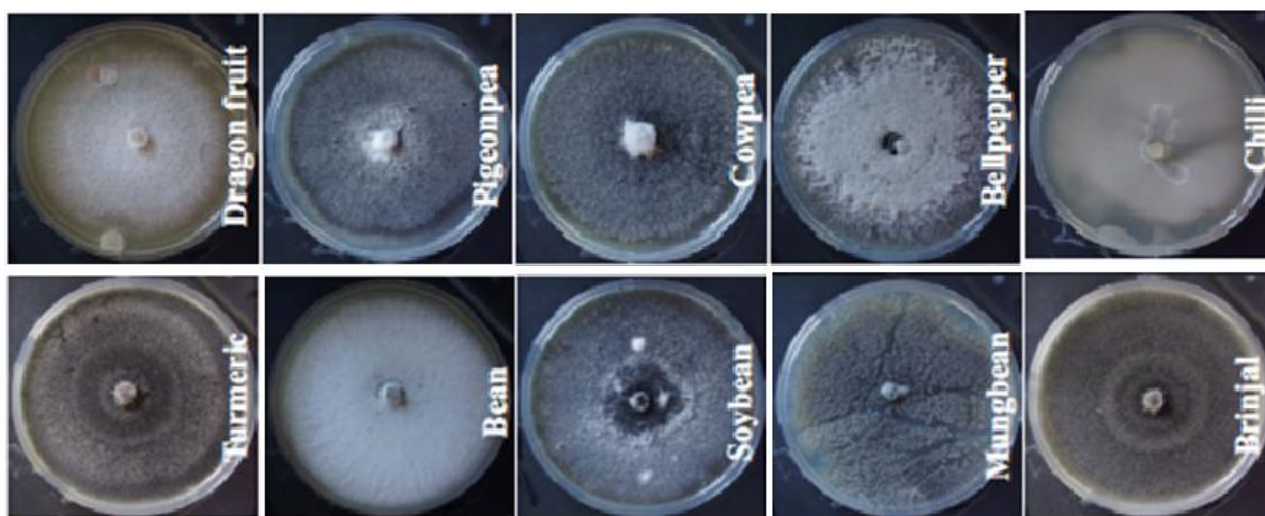


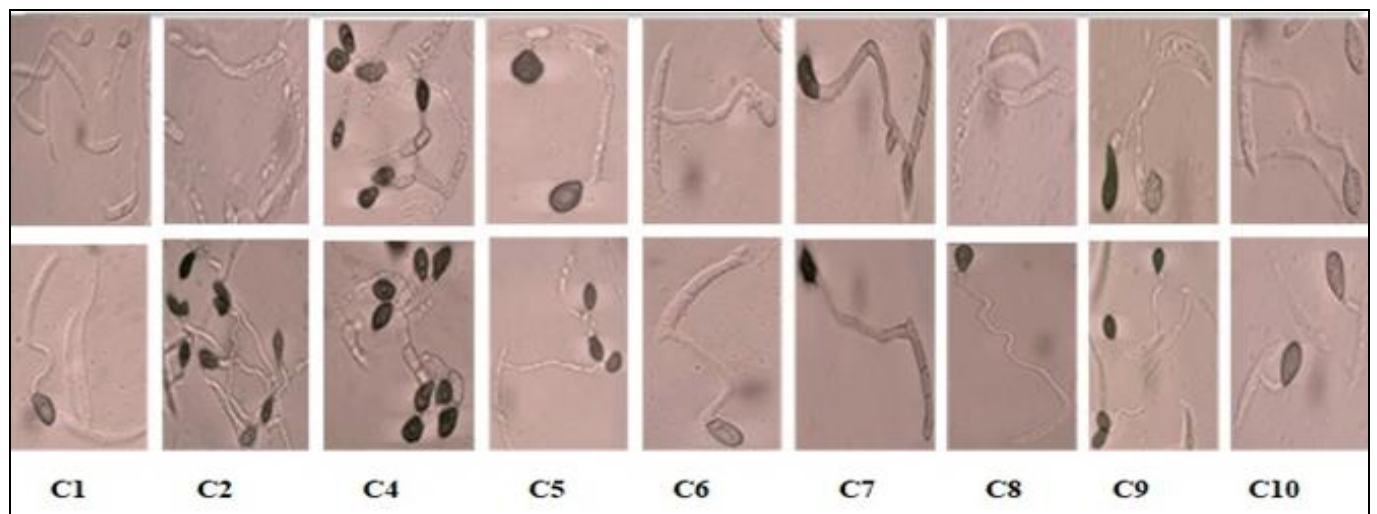
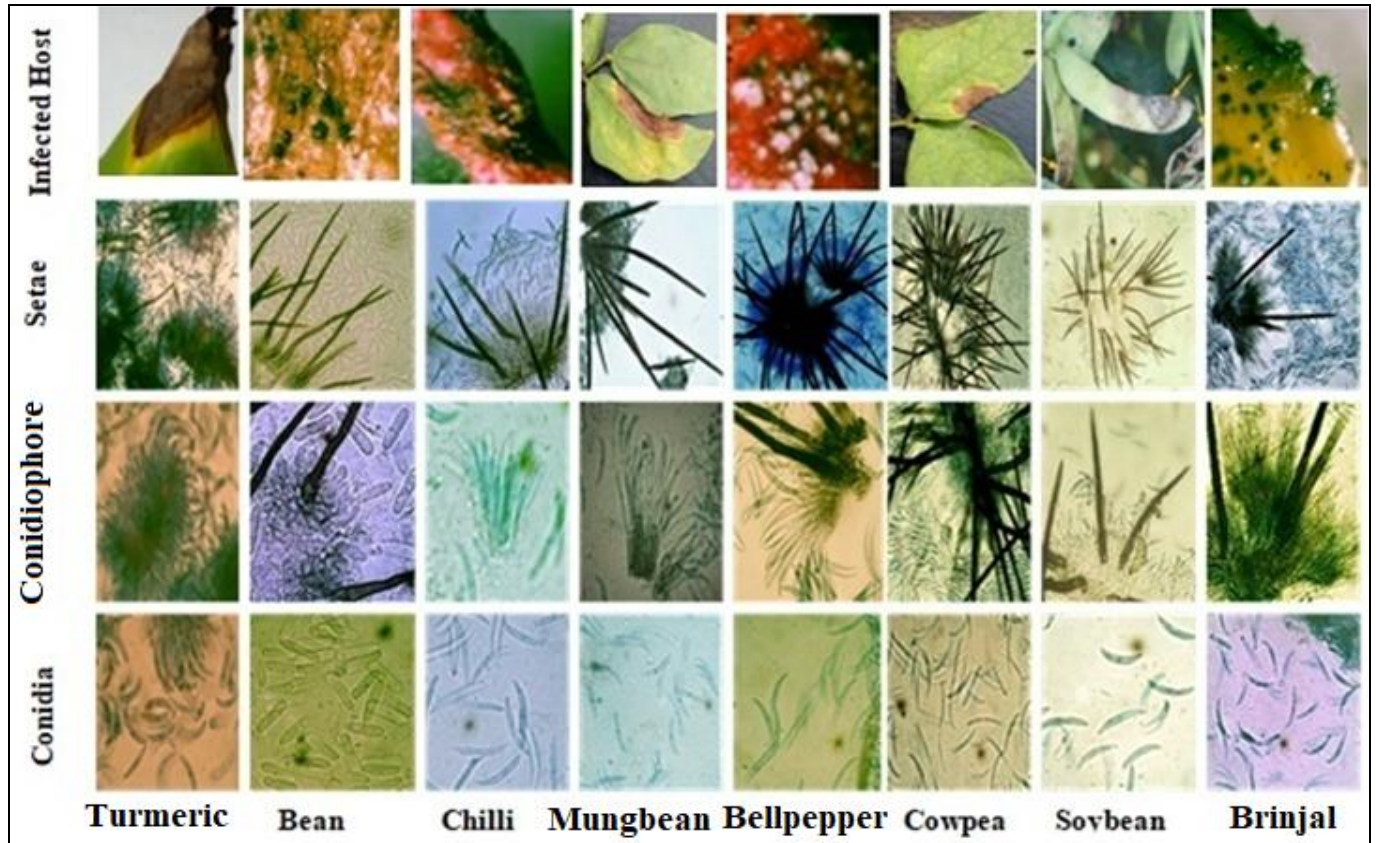
Fig 1: Variation in cultural characteristics of different isolates

### Morphology of appressoria and conidiophore

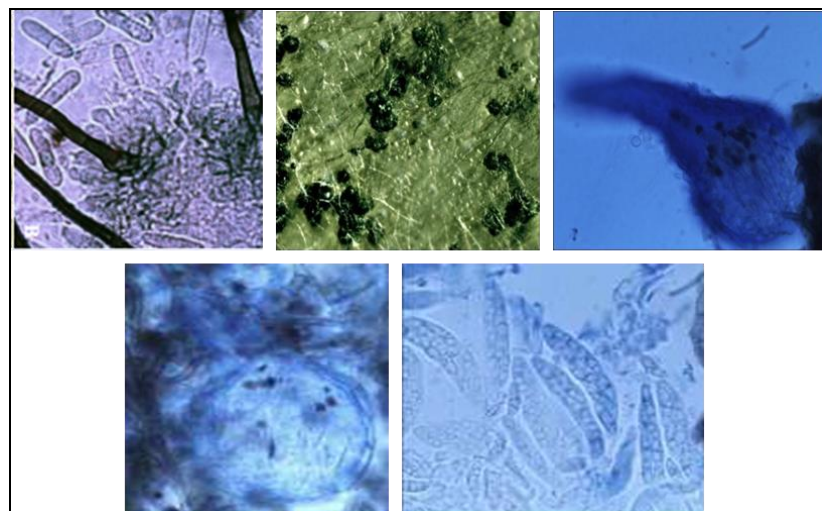
Appressoria appeared single or in loose groups on surface of PDA media plate, deep brown in colour, smooth wall, ovate, pear and clavate shaped, the entire edge lobate or sometimes undulate with irregular outline. Conidiophores hyaline to light brown, septate, smooth wall and branched. Conidiogenous cells hyaline to light brown, smooth wall, cylindrical and ampulliform, sometimes intercalary.

### Morphology of perithecia

Out of 10 isolates, isolate C3 (bean) are self induced perithecia. Premature and mature perithecia embedded on surface of PDA agar media plate, generally formed in cluster and sometimes individual. After maturation outer cells layer of cell wall of perithecia became tough and dark colour, non stromatic, often gregarious, partial immersed in media with spherical to subspherical base.



**Fig 3:** Morphology of appressoria in different host specific isolates of *Colletotrichum* spp



**Table 3:** Morphological characters of different host specific isolates of *Colletotrichum* spp

S. No.	Isolate	Colony Characteristic				Radial growth (mm)	Conidia 100X (in µm)		Setae 20X (in µm)	
		Growth of colony	Texture	Colony colour	Sporulation colour		L	W	L	W
1	C1	Circular	Raised	Black with whitish growth	White	20.70	25.79	3.55	177.97	4.31
2	C2	Circular	Raised	Black	Yellow	30.70	25.13	3.03	140.75	5.96
3	C3	Circular	Flat	White	Yellow	40.47	15.95	4.84	177.48	5.54
4	C4	Circular	Raised	White	Yellow	20.77	21.34	3.30	-	-
5	C5	Circular	Flat	White	Pink	20.65	26.73	2.50	249.21	5.29
6	C6	Circular	Raised	Black	White	20.52	27.05	2.98	202.62	4.65
7	C7	Circular	leathery	Black in colour later turned whitish growth mass	Pink	30.67	21.72	2.85	224.56	4.78
8	C8	Circular	Raised	Black	White	30.82	25.30	3.48	165.15	4.60
9	C9	Circular	Raised	Black	Yellow	30.32	22.71	3.23	220.61	5.07
10	C10	Circular	Raised	Black in colour later turned whitish growth mass	Pink	20.92	22.33	3.46	206.81	5.12
CD						0.828				
SE (m)±						0.293				

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