TAXONOMY AND MORPHOLOGY OF *COLLETOTRICHUM TRUNCATUM* ISOLATES PATHOGENIC TO SOYBEAN

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The morphology of both conidia and setae of *Colletotrichum truncatum* (Schw.) Andrus and Moore isolates from soybean were compared and found to be distinct. Conidial shape was more useful than size in isolate determination. Average conidial length was maximum (26.46 µm) in isolate Ct-1 followed by Ct-2 (23.75 µm), Ct- 4 (22.25 µm), Ct-3 (21.72 µm), Ct-5 (20.72µm) and Ct-6 (18.81 µm), whereas the width was maximum (4.63 µm) in isolate Ct-3 followed by Ct-2 (4.12 µm), Ct-4 (3.92 µm), Ct -5 (3.86 µm), Ct-1 (3.67 µm) and Ct-6 (3.56 µm). Average setal length was maximum (166.65 µm) in isolate Ct-1 followed by Ct-2 (122.16 µm), Ct -3 (61.73 µm), Ct-4 (52.58 µm), Ct-5 (47.83 µm) and Ct -6 (44.36 µm) whereas the width was maximum (5.50 µm) in isolate Ct-1 followed by Ct-6 (5.39 µm), Ct-4 (5.37 µm), Ct-5 (5.22 µm), Ct-2(5.07 µm) and Ct-3 (4.92 µm). Total number of setae per fascile was maximum in isolate Ct-1 (158) followed by Ct-3 (125), Ct-4 (115), Ct-5(105), Ct-2 (95) and Ct-6 (48).

Key words: *Colletotrichum truncatum*, soybean, conidial, setal.

INTRODUCTION

Colletotrichum truncatum (Schwein.) Andrus and W.D. Moore which is considered the major cause of anthracnose (Pod Blight) of soybean is the specie that is most geographically widespread on soybean and most frequently isolated from soybean plant parts. It is an important disease under warm (20-25°C) humid conditions especially in the tropics. (Arx, 1957, 1970; Athow, 1987; Bryant and Walters, 1980; Hepperly et al., 1983: Lenne, 1992: Miller and Roy, 1982: Sinclair and Backman, 1989; Tiffany and Gilman, 1954). Traditionally, the specie has been identified by conidial morphology, presence or absence of setae, presence or absence of the teleomorph *Glomerella singulata* and colony color. Preliminary studies on *C. truncatum* isolates from soybean and herbarium material showed that the criteria used in species identification were not reliable. Conidia of the six isolates were different in size and ie was that conidia produced on standard media can exhibit considerable variation in morphology. This study was initiated to define criteria that could be used to accurately identify *C. truncatum* isolates.

MATERIALS AND METHODS

Infected roots, stems, leaves and pods exhibiting different types of typical symptoms of anthracnose (Pod Blight) disease were collected separately from the field grown plant of different soybean cultivars from the crop research center, GBPUA&T, Pantnagar and nearby farmer's field. The diseased samples were brought to the laboratory for microscopic examination and isolation.

Isolation of the fungus

The diseased samples of the various soybean cultivar showing different types of typical symptoms were thoroughly washed in tap water and separately cut into small pieces at about half a centimeter in size, showing half healthy and half diseased tissue, with the help of previously sterilized blade. These pieces were surface sterilized with aqueous mercuric chloride ($HgCl_2$) solution (1:1000) for 40 to 60 s, followed by 3 - 4 changes with washings with sterilized distilled water. The surface sterilized diseased pieces were then aseptically transferred separately to the slants containing potato dextrose agar (PDA) medium and then incubated at 24 ± 2 °C. After 48-72 h of incubation, the growing mycelium from the margin of apparently distinct colonies was sub-cultured on fresh PDA slants. In this way, the cultures of different isolates were obtained. The cultures of the fungus thus obtained were purified by single spore isolation and maintained on PDA medium to keep the cultures viable, sub-culturing was done at an interval of 15 days and preserved at low temperature (5 ± 1°C) before use.

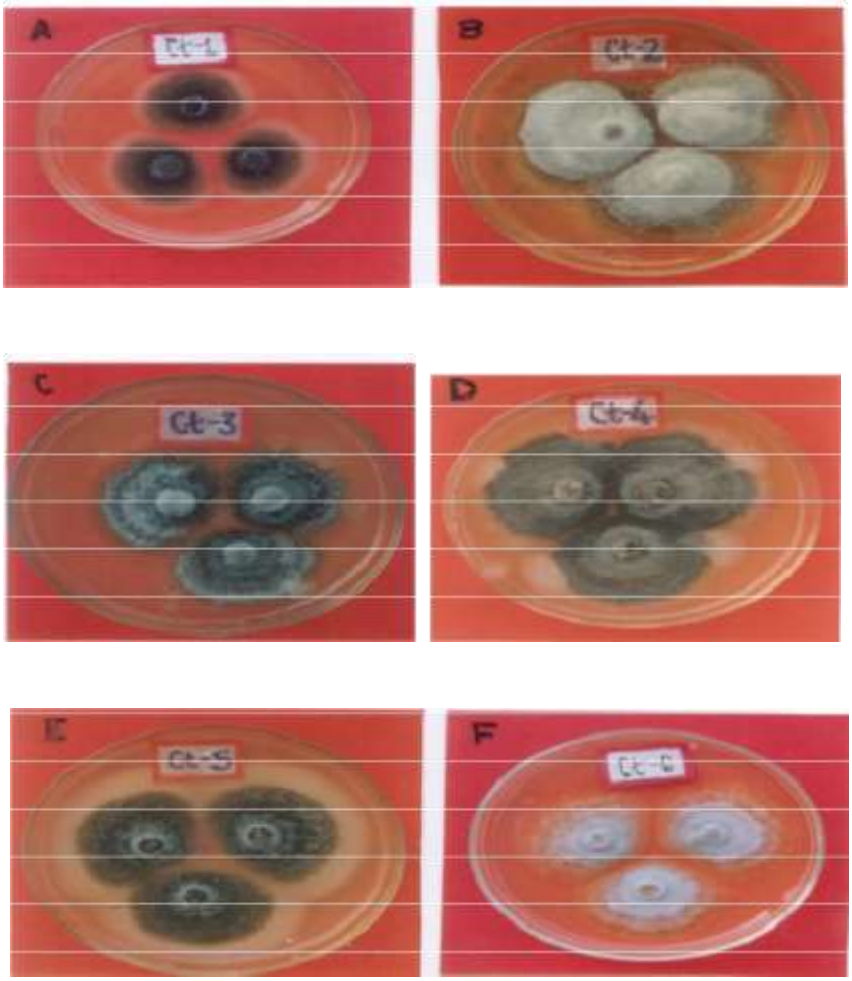

Figure 1. Close view of colony growth of six isolates of *C. truncatum* at the end of 10 days of incubation at 24 + 2^{o}C.

Morphological and cultural studies of the *C. truncatum*

isolates *In vitro growth and colony characters*

The growth pattern and colony colour was determined after 10 days growth on PDA at 25 ± 2°C (Figure 1).

Mycelial characters

The nature of growth and septation in hyphae was recorded.

Conidial morphology

For measurements and morphological observations of conidia each isolate was grown on a plate of Potato Dextrose Agar (PDA) at 25°C incubation temperature. Conidia were harvested after 14 days from acervuli produced on the PDA plate. Conidia were mounted in cotton blue in lacto phenol and measured at 100 magnification with the aid of ocular and stage micrometer in compound microscope (Figure 2). Tests to characterize conidia of all isolates on PDA were repeated twice. Thirty conidia of each isolate were measured each time.

Setal morphology

For measurements and morphological observations of setae, each isolate was grown on a plate of PDA at 24±2°C incubation temperature. Setae were harvested after 21-30 days from acervuli produced on the PDA plate. Setae were mounted in cotton blue in lacto phenol and measured at 40X with the aid of ocular and stage micrometer in compound microscope. Tests to characterize setae of all isolates on PDA were repeated twice. The number of setae measured per isolate per replication varied from 7- 50 because setae production was not uniform. Colony color was determined after 7 days on PDA at 250°C under conditions of temperature and light described previously. The colony diameter of isolates Ct-1, Ct- 2, Ct-3, Ct-4, Ct- 5 and Ct-6 was recorded after 5 days growth in darkness on PDA at 25 ± 20°C.

RESULTS

Conidial morphology

C. truncatum isolates produced abundant conidia on PDA which were consistent in morphology. Dimension of the

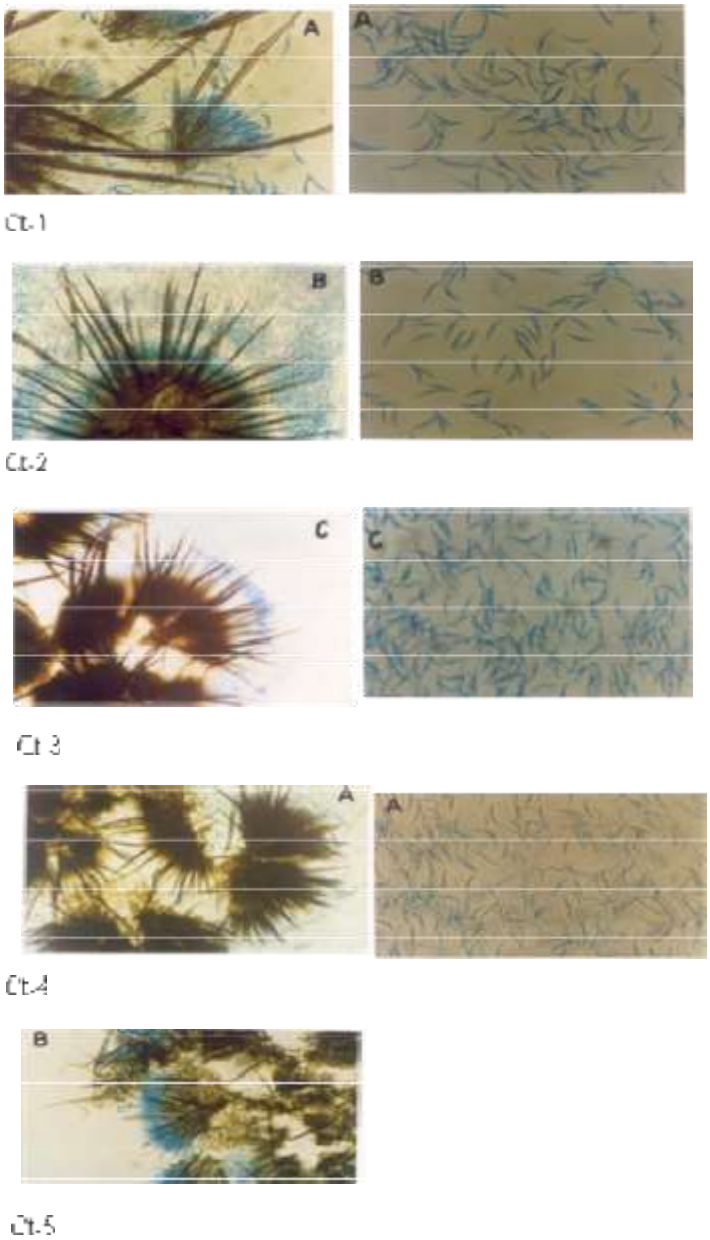

Figure 2. Close view of acervulus and conidia of five isolates of *C. truncatum.*

conidia for each isolate from PDA are presented in Table 2. Average conidial length was maximum (26.46 µm) in isolate Ct-1 followed by isolate Ct-2 (23.75 µm), Ct-4 (22.25 µm), Ct-3 (21.72 µm), Ct-5 (20.70 µm) and Ct-6 (18.81 µm) whereas the measurement of conidial width was maximum (4.63 µm) in isolate Ct-3 followed by isolate Ct-2 (4.12 µm), Ct-4 (3.92 µm), Ct-5 (3.86 µm), Ct- 1 (3.67 µm) and Ct-6 (3.56 µm) (Table 1). Both ends of conidia of isolate Ct-1 was more acute than the rest of the isolates. The conidia of isolate Ct-3 was wider than the rest of the isolates with swelling on one end of the conidia. Conidia in all the six isolates were non septate,

hyaline, falcate, truncate, uninucleate with oil droplets in the cytoplasm.

Setal morphology

Setae of *C. truncatum* isolates were morphologically distinct. Measurements of setae of the six isolates from PDA are presented in Table 3. Average setal length was maximum (166.65 µm) in isolates Ct-1, followed by Ct-2 (122.16 µm), Ct-3 (61.73 µm), Ct-4 (52.58 µm), Ct-5
(47.83 µm) and Ct-6 (44.36 µm) whereas, the

Table 1. Comparative morphological characters of *C. truncatum* isolates on PDA.

Characters	*Colletotrichum truncatum*					
	Ct-1	**Ct-2**	**Ct-3**	**Ct-4**	**Ct-5**	**Ct-6**
Colony characters						
Growth	Slow	Fast	Medium	Fast	Medium	Medium
Shape	Circular	Circular	Circular	Circular	Circular	Circular
Margins	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Colour	Black	Off-white	White-grey	Grey	Black-white	White-cottony
Texture	Thick thin	Thick	Thick-Fluffy	Thick-Fluffy	Thick	Thick-fluffy
Zonations	Absent	Distinct	Distinct	Poor	Poor	Absent
Mycelium						
Septation	Septate	Septate	Septate	Septate	Septate	Septate
Conidia						
Length	26.46 □m	23.75 □m	21.72 □m	22.25 □m	20.70 □m	18.81 □m
Width	3.67 □m	4.12 □m	4.63 □m	3.92 □m	3.86 □m	3.56 □m
Shape	Falcate	Falcate	Falcate (Swelling at one end)	Falcate	Falcate	Falcate
Setae						
Length	166.65 □m	122.16 □m	61.73 □m	52.78 □m	47.83 □m	44.39 □m
Width	5.50 □m	5.07 □m	4.92 □m	5.37 □m	5.22	5.39 □m
Total no of setae/ fascicles	648	619	512	520	382	78
Sporulation	158	95	125	115	105	48

Table 2. Conidial dimensions (µm) of *C. truncatum* isolates grown on PDA after 14 days of inoculation at 24 ± 2°C.

Isolate	Origin (cultivar and part)	Conidial dimensions (µm)[a]			
		Length		Width	
		Mean	Range	Mean	Range
Ct-1	Leaves and pods (JS-72-44)	26.46	23.50-29-42	3.67	3.12-4.23
Ct-2	Stems (PK-564)	23.75	21.23-26.27	4.12	3.52-4.72
Ct-3	Pods (VLS-2)	21.72	18.24-25.21	4.63	3.62-5.64
Ct-4	Leaves (PK-1024)	22.25	20.25-24.25	3.92	3.32-4.52
Ct-5	Seeds (PK-1042)	20.70	18.20-23.21	3.86	3.31-4.42
Ct-6	Stems (PK-262)	18.81	17.50-20.12	3.56	3.00-4.12

[a] Mean size based on 50 conidia per isolates of *C. truncatum* from soybean after growth on PDA for 10d at 24 ± 2 °C. Range measurements as : minimum, maximum.

measurement of the setal width was maximum (95.59 µm) in isolate Ct-1 followed by Ct-6 (5.39 µm), Ct-4 (5.37 µm) Ct-5 (5.22 µm), CT-2 (5.07 µm) and Ct-3 (4.92 µm).

Colony colour

Colony colour was determined after 7 days on PDA at 25°C under conditions of temperature and light described previously.

Growth response

From the result gotten, cultures and examination of authentic material showed that *C. truncatum* isolates can be separated on the basis of both conidial and setal morphology. In *Colletotrichum* taxonomy, the value of setal morphology has been greatly ignored in favour of conidial morphology, but our findings demonstrate that it is an important criterion in distinguishing among *C. truncatum* isolates. It was found that setae of

Table 3. Setal dimensions (µm)[a] of *Colletotrichum truncatum* isolates grown on PDA after 10 days of incubation at 24 ± 2℃.

Isolate	Origin (cultivar and part)	Setal dimensions (µm)[a]				Total no. of setae measured
		Length		Width		
		Mean	Range	Mean	Range	
Ct-1	Leaves and pods (JS-72-44)	166.65	51.1 - 282.2	5.5	4.5 - 6.25	648
Ct-2	Stems (PK-564)	122.16	42.23 - 202.1	5.07	4.32 - 5.82	619
Ct-3	Pods (VLS-2)	61.73	35.23 - 88.23	4.92	4.12 - 5.72	512
Ct-4	Leaves(PK-1024)	52.78	30.33 - 75.23	5.37	4.62 - 6.12	520
Ct-5	Seeds (PK-1042)	47.83	25.33 - 70.33	5.22	4.52 - 5.92	382
Ct-6	Stems (PK-262)	44.36	23.32 - 65.41	5.39	4.64 - 6.14	78

[a] Range measurements as: minimum, maximum.

C. truncatum isolates differ from one another and from setae of other described *Colletotrichum* species. Setae of *Colletotrichum* resemble those described for some other *Colletotrichum* species but could be useful in identifica- tion when used in conjunction with other taxonomic criteria, such as conidial morphology, colony color, pro- duction of teleomorph, production of sclerotia among others (Sutton, 1980). Although the scope of this paper was limited to *Colletotrichum* species pathogenic to soybean, we believe that setal morphology should be emphasized in the taxonomy of the genus as a whole.

DISCUSSION

Our findings based on studies of cultures and examina- tion of authentic material showed that *Colletotrichum gloeosporioides* and *Colletotrichum acutatum* are distributed worldwide on a number of hosts (Dyko and Mordue, 1979*). Colletotrichum fragariae* is not synony- mous with *Colletotrichum gloeosporioides* as proposed by Von Arx (1957) and the two species can be seperated on the basis of conidial and setal morphology. The original description of *C. fragariae* (Brooks 1931), however is in sufficient to delineate it from *C. gloeosporioides*. Brooks (1931) described the conidia of *C. fragariae* as "spindle to boat shaped" and depicted the conidia as cylindrical with obtuse ends, except for one, which was depicted as narrowly obovate. Brooks (1931) produced conidia of *C. fragariae* on oatmeal and lima bean agar which probably obscured the true shape of the conidia. The obovate nature of conidia was also noted by Welty (1984). Brooks (1931) noted that the setae of *C. fragariae* were somewhat sinuous, lighter towards the apex and were produced in groups, but stated that the setae were one to two septate, sometimes had a small, constricted apical cell and he did not report that the setae produced conidia. Sutton (1980) used the morphology of approssoria produced in slide culture as a species level character in the genus *Colletotrichum.* Baxter et al. (1983) noted that the formation of a red pigment by *C. acutatum* of fungi that conform to *C. acutatum* has been reported in the literature several time. Hindorf (1970) described wine red cultures of *C. acutatum* isolated from coffee. Dingley and Gilmour (1972) described *C. acutatum* f. sp. *pinea* as producing a carmine pigment on PDA. Andes and Keitt (1950) and Ramsey et al. (1951) were probably working with *C. acutatum* when they described aberrant strains of *Golmerella cingulata* from apple and peach respectively that formed pointed conidia, no teleomroph and a red pigment. Gorter (1962) named isolates from olive fruits that were identical to red strain of

G. cingulata studied by Andes and Keitt (1950) *Gloeosporium fructigenum* f *chromogenum* Setae are generally regarded as sterile element although this is not indicated in the formal definition (Hawksworth et al., 1983; Dyko and Mordue, 1979).

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