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## Biochemical studies on electrophoretic banding pattern among the different isolates of *Colletotrichum capsici* causing fruit rot in chilli

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**Abstract**

Ten isolates of *Colletotrichum capsici* collected from different regions of Vidarbha and Marathwada (Maharashtra), Bidar district (Karnataka) and Guntur district (Andhra Pradesh) showing varied type of pathogenic ability against chilli variety Jayanti and designated as strongly, moderately and weakly pathogenic on the basis of per cent leaf area covered by the disease. Electrophoresis pattern of polyphenol oxidase, esterase and protein have been used to estimate the polymorphism in different fungal populations or species. Therefore, studies were undertaken to know the biochemical variation among the isolates of *C. capsici* in the form of appearance of isozyme and protein banding pattern on polyacrylamide gel. Electrophoretic protein banding pattern revealed that, Rm values ranged between 0.208 to 0.725 possessing 1 to 4 bands. Isolates among weakly to highly pathogenic, 5 isolates exhibited one band each, while two bands each were existed in 3 isolates. Three bands of Cc<sub>6</sub> (Rm value 0.208, 0.350 and 0.725) relates to strongly pathogenic. Polyphenol oxidase isozyme was estimated and Rm values were observed from 0.057 to 0.192, possessing 2 to 3 bands. Eight isolates showed presence of 3 bands each whereas, 2 isolates exhibited 2 bands with Rm values 0.142 and 0.192. Esterase isozyme pattern was observed in all the test isolates of *C. capsici* and Rm values ranged from 0.048 to 0.544 possessing 3 to 4 bands.

**Keywords:** *Colletotrichum, capsici*, protein, isozyme, esterase, chilli

**Introduction**

Chilli (*Capsicum annum* L.) belongs to the family Solanaceae is one of the important spice cum vegetable crop in India. Most widely cultivated species all over the world for its pungent and non-pungent fruits. Many chilli constituents are important for nutritional value, flavor, aroma, texture and colour. Chillies are low in sodium and cholesterol free, rich in vitamin A, vitamin C, vitamin E, a good source of potassium and folic acid (Chandrakala and Vidyasagar, 2018) [3]. Chilli production suffers from many biotic factor i.e. diseases caused by fungi, bacteria, viruses, nematodes and also by abiotic factor. The Chilli anthracnose pathogen *C. capsici* infects diverse host with a high degree of pathogenic variability (Akhtar and Singh, 2007) [1]. The genus *Colletotrichum* causes anthracnose on wide range of fruits, vegetables, cereals, grasses and ornamental plants (Dean *et al.* 2012) [4]. The seed borne nature of *C. capsici* may be transmitted from mother plant, which were present throughout the storage period, which cause severe seed rot, seedling decay, twig blight, fruit rot and affect the seed germination of chilli and *C. capsici* able to survive up to the next crop season in the infected seeds (Patil and Moniz, 1973) [17]. Anthracnose caused the healthy green fruits lost 31 per cent and red ripe fruits lost 46 per cent ascorbic acid after 14 days of pathogenesis (Patil and Moniz, 1973) [17], 25 per cent loss of capsaicin content (Singh, 1995) [22]. Electrophoresis pattern of polyphenol oxidase, esterase and protein have been used to estimate the polymorphism in different fungal populations or species. Therefore, studies were undertaken to know the biochemical variation among the isolates of *C. capsici* in the form of appearance of isozyme and protein banding pattern on polyacrylamide gel.

## Materials and Methods

### Collection of disease samples

The disease samples of fruit rot of chilli (plant parts) were collected from different geographical areas of Vidarbha and Marathwada region of Maharashtra (M.S.). Some isolates of *Colletotrichum* causing diseases in chilli were collected from Bidar district of Karnataka and Guntur district of Andhra Pradesh.

### Isolation and maintenance of cultures

The samples showing characteristic symptoms of fruit rot, dieback and anthracnose were collected from different localities and cut along with healthy tissues. The infected bits were washed with sterilized water and surface sterilized in 0.1 per cent mercuric chloride solution for one minute in the Petriplates and subsequently three changes of water was given to remove the traces of mercuric chloride. The bits were dried around the flame of spirit lamp, then transferred to solidified sterile potato dextrose agar (PDA) in Petriplates and were incubated at room temperature ( $27 \pm 2^\circ\text{C}$ ) for seven days. All the operations were carried aseptically. The fungus growth of *Colletotrichum capsici* was then transferred on PDA slants. The cultures thus obtained were further purified by single spore isolation technique.

The single spore isolates, thus obtained were further used in various experiments. The cultures were abbreviated as Cc. (*Colletotrichum capsici*) and reviewed by periodic transfer and maintained on PDA.

### Pathogenicity test

Monosporous cultures of isolates were obtained from the fungal cultures. The epidermal layer of fruits, leaves of the susceptible variety of chilli were injured by carborandom powder before inoculation. The spore suspension of each isolate of *Colletotrichum capsici* was used for inoculating the plants in pots by using sterilized cotton swab. The seedlings were kept for predisposition for 24 hour prior to inoculation by irrigating and covered the moist hessian cloth. Inoculation by cotton swab was made in the evening hour and covered with hessian cloth to provide 100 per cent humidity for spore germination and infection up to 48 hour. Similarly the fruits were also inoculated by smearing the inoculums on the upper surface and incubated in the humid chamber. After inoculating the plants with *Colletotrichum capsici*, the host plants were examined periodically for development of symptoms. Re-isolations were made and the fungus obtained was compared with the original one.

### Grouping and selection of isolates

Thirteen isolates of *Colletotrichum capsici* were tested by applying suspension on the foliage for their virulence on susceptible host variety. The per cent disease intensity was recorded and the isolates of *Colletotrichum capsici* were tentatively divided into three groups based on their virulence. Ten isolates were selected on the basis of aggressiveness for further studies.

## Biochemical studies on electrophoretic banding pattern Protein and isozyme extraction

All the test isolates of *C. capsici* were mass multiplied on PDA medium and seven days old growth was scrapped with the help of scrapper. Mycelium (100 mg) was homogenized in 3 ml sodium phosphate buffer by motorized homogenizer for 8 minutes. The mycelial extract was transferred to 1.5 ml centrifuge tubes. Samples in the tubes were centrifuged at 10000 rpm for 18 minutes at  $4^\circ\text{C}$  using Remi (C-24). The clear supernatant was collected and used as protein and enzyme source for electrophoresis. Entire extraction procedure was done under cold condition.

Electrophoretic studies of protein and isozyme from pure culture of *C. capsici* isolates was carried out by using SDS PAGE and Native PAGE respectively, by following user's protocol of Hoefer Scientific Instruments described by Laemmli (1970) [12] and Dunn (1993) [5]. The isozyme of polyphenol oxidase was localized on polyacrylamide gel as per the procedure suggested by Park *et al.* (1980) [16].

Analysis of protein and isozyme gel was done by gel documentation system (Bio-Rad) and the Rm value of protein and isozyme bands were obtained by using Gel-Doc EQ4-5-0 software.

## Results and discussion

**Collection, isolation, purification and identification of pathogen:** Fruit rot infected plant parts were collected from different geographical areas of Vidarbha and Marathwada region of Maharashtra and some samples were collected from Bidar (Karnataka) and Guntur (Andhra Pradesh). The usual tissue isolation technique was followed to isolate the pathogen from infected plant parts showing fruit rot, anthracnose symptoms. Potato dextrose agar was used as basal medium for isolation of the fungus. The pure culture was obtained using single spore method. The culture thus obtained was identified as *Colletotrichum capsici* on the basis of pathogenic ability and morphological characters as per the CMI publications. Purified cultures of the fungus were maintained on PDA slants for further studies and abbreviated as Cc.

### Pathogenicity and symptoms

Pathogenic ability of ten isolates of *Colletotrichum capsici* was tested on a susceptible chilli variety (Jayanti). Observations were recorded on per cent disease intensity after 12 DAI and the results are presented in Table 1. Symptoms under field condition on chilli causing fruit rot appeared in the form of black circular spots on skin spreading along the fruit length and turning to elliptic, heavily infected fruits turned straw coloured and numerous dots like acervuli was observed. The characteristic symptoms on leaves are in the form of irregular to circular areas with brown margins. On fruits grayish black colour elongated spots forming acervuli in advanced stage arranged in elliptical manner was examined. The *Colletotrichum capsici* causing chilli fruit rot was isolated and pathogenicity was proved. These results confirm the findings of Sydow (1913) [24] who reported infection of *Colletotrichum capsici* for the first time in chilli, Gotmare (1981) [8] who observed the symptoms of *Colletotrichum capsici* on chilli as dark brown stripes on twigs and petioles. Similarly, Ramakrishnan (1954) [18] reported the leaf spot disease of chilli and turmeric (*Curcuma longa*) caused by *C. capsici* and Roy *et al.* (1997) [19] confirms the pathogenicity of *C. capsici* on bell pepper by tooth prick inoculation. Similar

Sr. No.	Category	Group	Per cent disease intensity
1	Weakly pathogenic	WP	11-25%
2	Moderately pathogenic	MP	26-50
3	Strongly pathogenic	SP	>50%

reports were made earlier by Gupta *et al.* (2017)<sup>[9]</sup> and Sunil Kumar (2017)<sup>[23]</sup>. Karthik Pandi *et al.* (2018)<sup>[11]</sup> isolated the pathogen from the symptomatic chilli fruits showing small black circular spots on the skin of the fruits that in the direction of the long axis. The spots were sunken and light grey coloured with black margin. The spots enlarged into larger lesions and on the surface of the lesions acervuli, the fruiting body of the fungus appeared as minute black dots. All the isolates of pathogen was identified as *Colletotrichum* species.

#### Grouping of different *Colletotrichum capsici* isolates

The isolates of *Colletotrichum capsici* were grouped according to the pathogenic ability on susceptible variety and tentatively divided into three groups.

Two isolates Cc<sub>6</sub> and Cc<sub>9</sub> were categorized as strongly pathogenic, four Cc<sub>1</sub>, Cc<sub>5</sub>, Cc<sub>8</sub> and Cc<sub>10</sub> as moderately pathogenic and rest of the four Cc<sub>2</sub>, Cc<sub>3</sub>, Cc<sub>4</sub> and Cc<sub>7</sub> as weakly pathogenic (Table 1). The basis for categorization of different isolates was adopted by considering the published data of Palarpawar and Ghurde (1994)<sup>[15]</sup> categorized the isolates as aggressive and less aggressive and Beynon *et al.* (1995)<sup>[2]</sup> in coffee berry disease caused by *C. kahawe*. Giri (2002)<sup>[6]</sup> classified four group of *Fusarium udum* as highly pathogenic, weakly pathogenic, moderately pathogenic and strongly pathogenic. Similar observations were also mentioned by Sangdee *et al.* (2011)<sup>[21]</sup> and Lubna Massodi *et al.* (2013)<sup>[13]</sup>.

Sr. No.	Reaction	Per cent disease intensity	Number of isolates
1	Weakly pathogenic (WP)	11-25%	4
2	Moderately pathogenic (MP)	26-50%	4
3	Strongly pathogenic (SP)	>50%	2

#### Electrophoretic banding pattern in different isolates of *C. capsici*

Electrophoretic protein and isozyme pattern in ten different isolates of *C. capsici* was studied to assess the variability among the pathogen.

#### Protein banding pattern in different isolates of *C. capsici*

Electrophoretic protein banding pattern was assessed for the ten isolates of *C. capsici*. The Rm values ranged between 0.208 to 0.725 possessing 1 to 4 bands. Isolates among weakly to highly pathogenic, Cc<sub>2</sub>, Cc<sub>3</sub>, Cc<sub>4</sub>, Cc<sub>8</sub> and Cc<sub>9</sub> exhibited one band each, while two bands each were existed in Cc<sub>1</sub>, Cc<sub>7</sub> and Cc<sub>10</sub>. Isolate Cc<sub>5</sub> and Cc<sub>6</sub> exhibited three bands each (Table 2). Three bands of Cc<sub>6</sub> (Rm value 0.208, 0.350 and 0.725) relates to strongly pathogenic, however in isolate Cc<sub>9</sub> one band (Rm value 0.208) was observed, which belongs to strongly pathogenic. In weakly pathogenic isolates Cc<sub>2</sub>, Cc<sub>3</sub> and Cc<sub>4</sub> one band each was present (Rm value 0.725), whereas in Cc<sub>7</sub> two bands each were present. In Cc<sub>8</sub> one band was observed at Rm value 0.725, while in Cc<sub>1</sub> and Cc<sub>10</sub> two bands each were present, whereas in Cc<sub>5</sub> three bands were present and are recognized under moderately pathogenic group. However, there is no consistency in correlation with appearance of bands and pathogenic ability of the isolates. The present findings confirms the results of Uma Devi *et al.* (2001)<sup>[25]</sup> observed the identical protein profile for all the isolates with respect to major bands were observed for total proteins and Gopalakrishnan and Prakasam (2007)<sup>[7]</sup> reported protein profile of five isolates, showed marked variations in their pattern detected by polyacrylamide gel electrophoresis

(PAGE). French bean and lablab isolates showed a few similarities in their protein profile. Similar reports was recorded by Madhvan *et al.* (2011)<sup>[14]</sup> with isolates of *Burkholderia* sp.

#### Isozyme pattern (Polyphenol oxidase, Esterase isozyme) of different isolates of *C. capsici*

Polyphenol oxidase isozyme was estimated and Rm values were observed from 0.057 to 0.192, possessing 2 to 3 bands. Eight isolates viz., Cc<sub>1</sub>, Cc<sub>2</sub>, Cc<sub>3</sub>, Cc<sub>4</sub>, Cc<sub>5</sub>, Cc<sub>7</sub>, Cc<sub>8</sub> and Cc<sub>10</sub> showed presence of 3 bands each, whereas Cc<sub>6</sub> and Cc<sub>9</sub> exhibited 2 bands with Rm values 0.142 and 0.192 (Table 3). Isolate Cc<sub>1</sub>, Cc<sub>2</sub>, Cc<sub>3</sub>, Cc<sub>4</sub>, Cc<sub>5</sub>, Cc<sub>7</sub>, Cc<sub>8</sub> and Cc<sub>10</sub> showed close similarity, which belongs to weakly and moderately pathogenic group, while Cc<sub>6</sub> and Cc<sub>9</sub> categorized as strongly pathogenic and exhibited close similarity with absence of one band out of 3 bands at Rm value 0.057. Similar reports was recorded by Madhvan *et al.* (2011)<sup>[14]</sup> with isolates of *Burkholderia* sp.

Esterase isozyme pattern was observed in all the test isolates of *C. capsici* and Rm values ranged from 0.048 to 0.544 possessing 3 to 4 bands. Except isolate Cc<sub>2</sub> and Cc<sub>3</sub> all other isolates of *C. capsici* exhibited all 4 bands, whereas Cc<sub>2</sub> and Cc<sub>3</sub> exhibited 3 bands each with Rm values 0.048, 0.160 and 0.464 and are grouped under weakly pathogenic. Isolate Cc<sub>6</sub> and Cc<sub>9</sub> showed presence of all 4 bands and categorized under strongly pathogenic group (Table 4). Similar reports was recorded by Madhvan *et al.* (2011)<sup>[14]</sup> with isolates of *Burkholderia* sp.

Zuber and Manibhusanrao (1982)<sup>[26]</sup> stated that, there was no correlation with the enzyme pattern and virulence of the *Rhizoctonia solani*. They also estimated the polyphenol oxidase, peroxidases and protein banding pattern. No correlation was established with the enzyme pattern and virulence of the isolates. The data in respect of virulence or aggressiveness does not correlate with the presence or absence of any isozyme bands. Polyphenol oxidase and esterase bands existence did not correlate with the virulence of the isolates but it could reflect the existence of varied number of bands with different Rm values. These results are on the similar findings reported by different workers. Uma Devi *et al.* (2001)<sup>[25]</sup> reported the variability on the basis of isozyme and protein profile in twelve isolates of *Ascochyta rabae*. Ibrahim *et al.* (2003)<sup>[10]</sup> reported the esterase, peroxidase and malate dehydrogenase activity in *Fusarium* spp. with low correlation in diversity also corroborates the present results. Sailaja Rani and Kumar (2007)<sup>[20]</sup> studied the variability of six isolates of *Pythium aphanidermatum* from three districts of Royalseema and significant variation was observed among the six isolates with respect to protein and esterase pattern.

**Table 1:** Pathogenic ability of *Colletotrichum capsici* isolates showing disease reaction against susceptible variety (Jayanti)

Isolate	Location	PDI (%)	Reaction
Cc <sub>1</sub>	Chinchola	43.33	MP
Cc <sub>2</sub>	Parbhani	19.50	WP
Cc <sub>3</sub>	Bidar	17.67	WP
Cc <sub>4</sub>	Divatana	24.33	WP
Cc <sub>5</sub>	Neemkhed	37.17	MP
Cc <sub>6</sub>	Guntur	51.50	SP
Cc <sub>7</sub>	Karla	18.67	WP
Cc <sub>8</sub>	Washim	33.83	MP
Cc <sub>9</sub>	Akola	51.83	SP
Cc <sub>10</sub>	Jalna	38.83	MP

**Table 2:** Protein banding pattern in different isolates of *Colletotrichum capsici*.

Isolates	Rm values				Total number of bands
	0.208	0.350	0.466	0.725	
Cc <sub>1</sub>	-	+	+	-	2
Cc <sub>2</sub>	-	-	-	+	1
Cc <sub>3</sub>	-	-	-	+	1
Cc <sub>4</sub>	-	-	-	+	1
Cc <sub>5</sub>	-	+	+	+	3
Cc <sub>6</sub>	+	+	-	+	3
Cc <sub>7</sub>	-	-	+	+	2
Cc <sub>8</sub>	-	-	-	+	1
Cc <sub>9</sub>	+	-	-	-	1
Cc <sub>10</sub>	+	-	-	+	2

+ = Presence of band

- = Absence of band

**Table 3:** Polyphenol oxidase banding pattern in different isolates of *Colletotrichum capsici*

Isolates	Rm values			Total number of bands
	0.057	0.142	0.192	
Cc <sub>1</sub>	+	+	+	3
Cc <sub>2</sub>	+	+	+	3
Cc <sub>3</sub>	+	+	+	3
Cc <sub>4</sub>	+	+	+	3
Cc <sub>5</sub>	+	+	+	3
Cc <sub>6</sub>	-	+	+	2
Cc <sub>7</sub>	+	+	+	3
Cc <sub>8</sub>	+	+	+	3
Cc <sub>9</sub>	-	+	+	2
Cc <sub>10</sub>	+	+	+	3

+ = Presence of band,

- = Absence of band

**Table 4:** Esterase banding pattern in different *C. capsici* isolates

Isolates	Rm values				Total number of bands
	0.048	0.160	0.464	0.544	
Cc <sub>1</sub>	+	+	+	+	4
Cc <sub>2</sub>	+	+	+	-	3
Cc <sub>3</sub>	+	+	+	-	3
Cc <sub>4</sub>	+	+	+	+	4
Cc <sub>5</sub>	+	+	+	+	4
Cc <sub>6</sub>	+	+	+	+	4
Cc <sub>7</sub>	+	+	+	+	4
Cc <sub>8</sub>	+	+	+	+	4
Cc <sub>9</sub>	+	+	+	+	4
Cc <sub>10</sub>	+	+	+	+	4

+ = Presence of band

- = Absence of band

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