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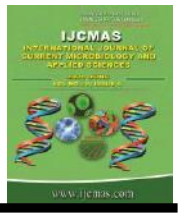

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Molecular Identification of *Colletotrichum capsici* Isolates Causing Chilli Anthracnose and Fruit Rot

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A B S T R A C T

A total of forty isolates of *Colletotrichum capsici* were isolated from diseased samples collected from different districts of Andhra Pradesh and Telangana and identified based on conidial morphology, size, colour, acervuli and setae. Isolates were designated as Cc 1 to Cc 40. All 40 *C. capsici* isolates were also identified by molecular means using species specific primers i.e., CcapF (50 -GTA GGC GTC CCC TAA AAA GG-30) and CcapR (50 -CCC AAT GCG AGA CGA AAT G-30). A specific band of about 394 bp was obtained in the DNA of all the *C. capsici* isolates whereas no amplified product was obtained in case of DNA of negative controls of isolates of *C. gloeosporioides, Pyricularia oryzae* and *Ustilaginoidea virens*.

Introduction

Chilli is the most important spice crop in the world. Indian chilli is considered to be world famous for two important commercial qualities such as colour (due to pigment Capsanthin) and pungency levels (due to Capsaicin). In India, chilli is cultivated in an area of 8.40 lakh hectares producing 20.96 lakh tonnes (FAOSTAT, 2016). Telangana stands second in India with an area of 1.2 lakh hectares producing 3.37 lakh tonnes accounting for 14.47 % of area and 18.00 % of total production of dry chillies in India. This yield is very low as compared to other developed countries of the world and this low production is attributed to several biotic and abiotic factors. Among the biotic factors, anthracnose and fruit rot disease is the most important factor which results in crop losses 10 % to 80 % in different parts of (Poonpolgul and Kumphai, 2007).

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. (Sharma *et al.,* 2005; Madhavan *et al.,* 2010; Garg *et al.,* 2014). Existence of cultural, morphological and pathological variability in the *C. capsici* is reported in various countries. Hence, the present study is undertaken for molecular confirmation of *Colletotrichum capsici* isolates associated with the chilli anthracnose and fruit rot disease.

Materials and Methods

Isolation and collection of *Colletotrichum capsici* isolates

Pathogen isolates were isolated from the fruits showing typical symptoms of chilli fruit rot disease by tissue segmentation method (Rangaswamy and Mahadevan, 1999) on PDA medium. Small bits measuring about three mm size were cut from the leaves showing lesions in such a way that it contains both infected and healthy portions. Such bits were surface sterilized in 1 per cent sodium hypochlorite (NaOCl) solution for one minute followed by three washings in sterile distilled water for 30 seconds each.

These bits were further transferred to sterile discs of blotting paper for removing excess water. The bits were subsequently transferred to sterile PDA medium in petri plates under aseptic conditions. The petri plates were incubated at 27 ± 2°C and observed periodically for growth of the fungus.

All the isolates of the pathogen were purified by single spore isolation method (Rangaswamy and Mahadevan, 1999). A diluted spore suspension (approximately 3000 conidia/ml) of each isolate was prepared in sterile distilled water. Using sterilized pipette one ml of spore suspension was poured in each water agar plate, the plate was tilted slowly for uniform spread of the spore suspension and kept in an incubator. The plates were observed under microscope for single germinated spores using 40x objective lens. Well germinated spores were located and circled round the spore on the bottom of the plate with the help of a glass marking pen. Individual spores along with the medium were cut with sterilized needle and then transferred to PDA slants. The slants were incubated at 27 ± 2 °C for growth of the fungus and observed for pure cultures (Dhingra and Sinclair, 1985) (Plate 1).

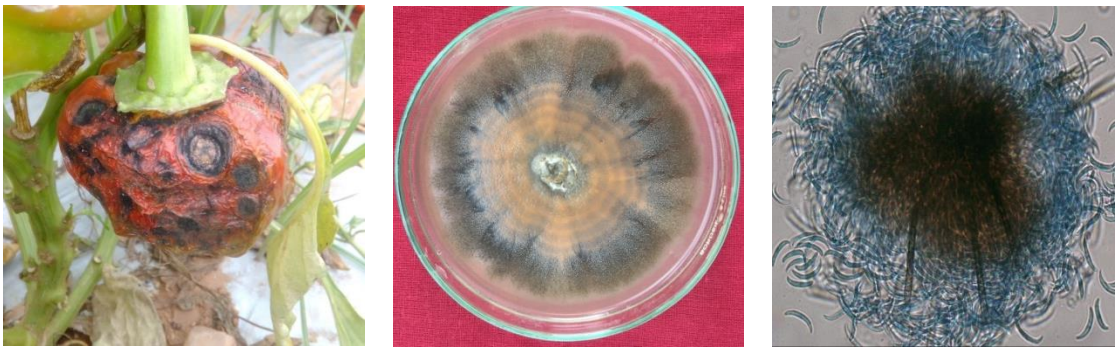


Plate.1 Symptoms on chilli fruits, mycelial growth, sporulation, setae and conidia of *Colletotrichum capsici*

A total of forty isolates were isolated from the diseased samples collected from different chilli growing districts of Andhra Pradesh and Telangana. These isolates were designated as Cc 1 to Cc 40 (Cc = *Colletotrichum capsici*). (Table 1).

Molecular Identification of *C. capsici* Isolates Using Species Specific Primers

The identity of the 40 *C. capsici* isolates was reconfirmed by molecular means using two species specific primers CcapF and CcapR with an expected amplicon size of 394 bp, by following the procedure described by Torres-Calzada *et al*. (2011). Negative controls of DNA from *C. gloeosporioides*, *Pyricularia oryzae* and *Ustilaginoidea virens*.

Colletotrichum capsici specific primers used:

Forward Primer : CcapF (50-GTA GGC GTC CCC TAA AAA GG-30)

Reverse Primer : CcapR (50-CCC AAT GCG AGA CGA AAT G-30)

The primers and other materials required for this experiment were obtained from Eurofins Genomics India Pvt. Ltd, Bangalore, India. The PCR reaction mixture contained 50 ng of DNA, 1X PCR buffer (10X: 20 mM Tris HCl, 500 mM KCl, pH 8.4), 0.20 mM of each dNTP, 2.5 mM $MgCl_2$, 1 µM Primers, 1.25 U Taq DNA polymerase in a volume of of 25 µl. DNA amplification was performed in a Thermal Cycler (Bio-Rad Laboratories).

Steps followed for PCR

Step		Details
Initial denaturation	:	95°C for 5 minutes
Annealing (25 cycles)	:	94°C for 30 seconds
	:	62°C for 30 seconds
	:	72°C for 2 minutes
Final extension	:	72°C for 5 minutes

PCR products were visualized by electrophoresis in 1.5 % (w/v) agarose gel run in 1X Tris–Borate-EDTA [TBE] buffer and stained with ethidium bromide. The procedure for DNA extraction, preparation of gels and electrophoresis was followed as per standard protocols.

Results and Discussion

The identity of the 40 *C. capsici* isolates was reconfirmed by molecular means using two *Colletotrichum capsici* species specific primers CcapF (50 -GTA GGC GTC CCC TAA AAA GG-30) and CcapR (50 -CCC AAT GCG AGA CGA AAT G-30). A specific band (Plate 2) of about 394 bp was obtained in the DNA of all the *C. capsici* isolates where as no amplified product was obtained in case of DNA of negative controls of isolates of *C. gloeosporioides, Pyricularia oryzae* and *Ustilaginoidea virens.* Thus all the isolates of *C. capsici* identified morphological and cultural characters were once again confirmed by molecular means using species specific primers.

Adhipathi *et al.* (2013) also made molecular based confirmation of all the 20 isolates of *C. capsici* was obtained by using forward and reverse primers ITS - 1 F (5'-G T C C T A A C A A G G T T T C C G T A - 3') and ITS-4 R (5'-T T C T C C G C T T A T T G A T A T G C - 3') which produced an amplified a PCR product of approximately 590 bp.

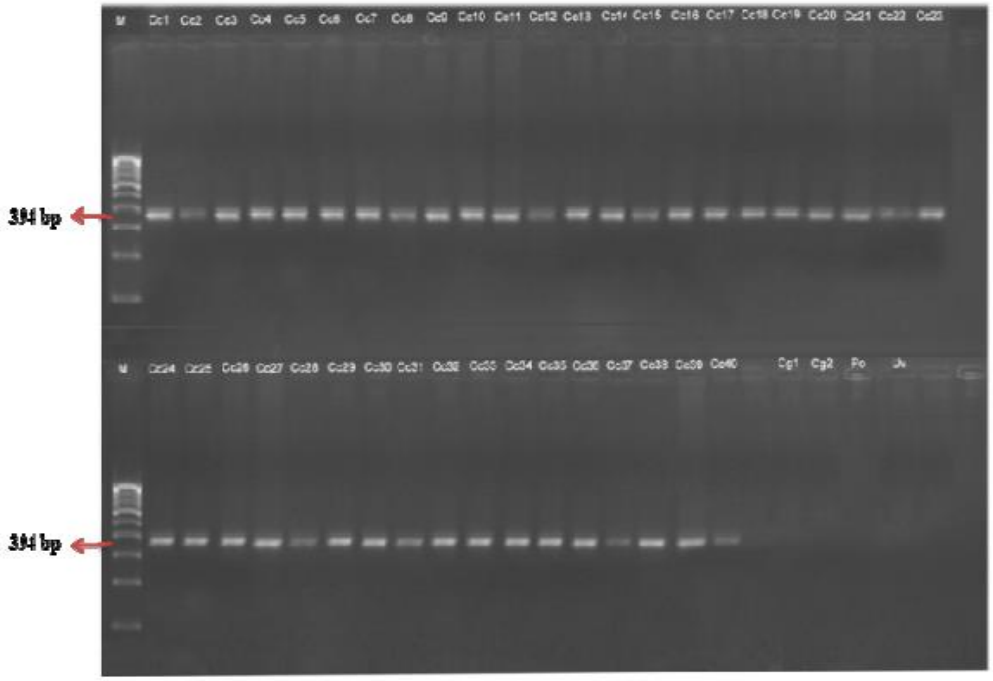
Similarly, Devi and Prakasam (2013) identified *C. capsici* isolates by using Forward (5'-TCC GTA GGT GAA CCT GCG G- 3') Reverse (5'-TCC TCC GCT TAT TGA TAT GC- 3') which produce an amplified product of approximately 560 bp in size. *C. capsici*. Molecular confirmation of *C. capsici* using species specific primers was also reported by Madhavan *et al.* (2014) and Machenahalli (2014). Similar identification

using specifically designed molecular markers for a particular species was reported by Raj *et al.* (2014b).

Table 1. Details of *Colletotrichum capsici* isolates collected from different villages of Andhra Pradesh and Telangana

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

Plate.2 Molecular identification of *C. capsici* isolates using CcapF and CcapR species specific primers



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