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*Full Length Research Paper*

# Morphological, pathogenic and genetic variability in *Colletotrichum capsici* causing fruit rot of chilli in Tamil Nadu, India

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**Chilli (*Capsicum annuum* L.) fruit rot disease caused by *Colletotrichum capsici* under tropical and subtropical conditions, results in qualitative and quantitative yield losses. Twenty (20) isolates of *C. capsici* were collected from conventional chilli growing areas of Tamil Nadu. In culture, most of the isolates produced cottony, fluffy or suppressed colonies. However, no significant differences were noticed in shape and size of conidia. The reaction of the 20 isolates on an indigenously developed differential set of *Capsicum* cultivars indicated the existence of different virulences in Tamil Nadu chilli populations. The genetic relationship between 20 morphological groups recognized within *C. capsici* was investigated using random amplified polymorphic DNA (RAPD) analysis. Molecular polymorphism generated by RAPD confirmed the variation in virulences of *C. capsici* and different isolates were grouped into two large clusters. The pathological and RAPD grouping of isolates suggested no correlation among the test isolates.**

**Key words:** Chilli*, Colletotrichum capsici,* variability, RAPD.

## INTRODUCTION

Chilli (*Capsicum annuum* L.) is an important spice crop of India. Chilli originated in the Latin American regions of New Mexico as a wild crop in and around 7500 BC. It is affected by several fungal, bacterial and viral diseases, of which chilli anthracnose causes considerable damage, inflicting severe quantitative and qualitative losses. The estimated loss due to this disease ranged from 8 to 60% in different parts of India (Suthin Raj et al., 2009). The fungus *Colletotrichum capsici* infects both unripe (green) and ripe (red) chilli fruits, and survives on seed as acervuli and microsclerotia (Suthin Raj et al., 2009). Infection of *C. capsici* will be higher in mature stage than in the early stage of chilli plant (Suthin Raj et al., 2013). The objective of this study was to investigate the variability in *C. capsici* populations infecting chillies by using morphological, pathological and molecular approaches.

## MATERIALS AND METHODS

### Isolation and identification of *C. capsici* isolates

Diseased chilli fruits showing typical symptom of fruit rot disease were collected fresh from 20 conventional chilli growing areas of

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Tamilnadu. The pathogens isolated from each of these localities formed one isolate of *C. capsici.* The pathogens were isolated on potato dextrose agar (PDA) medium from the diseased specimen showing typical symptoms. The infected portion of the fruit was cut into small bits, surface sterilized in 0.1% mercuric chloride solution for 30 s, washed in repeated changes of sterile distilled water and plated onto PDA medium. The plates were incubated at room temperature (28 ± 2°C) for five days and were observed for fungal growth. The fungus was purified by single spore isolation technique (Rangaswami, 1958). Identification of the isolate was confirmed by comparing it with the culture obtained from ITCC, IARI, New Delhi and the purified isolates were maintained on PDA slants.

### Morphological characteristic of *C. capsici* isolates in Tamil Nadu

Measurements of 100 spores were taken under the microscope (Magnification 45 × 10x) by using ocular and stage micrometers. The mean values and the range were determined. Fine sliced pieces of potato tuber, ripe chilli fruit and green chilli fruit were boiled for 10 min and the extracts were filtered. To the extract, other ingredients of the medium were added and the volume was made up to 1000 ml with distilled water and autoclaved at 1.04 kg $cm^{-2}$ for 15 min. The sterilized and warm medium was poured into sterilized Petri plates (90 mm) in 20 ml quantities and allowed to solidify. The isolates were inoculated at the centre of the plate by placing a seven-day-old 8 mm PDA culture disc of the pathogen. The plates were incubated at room temperature (28 ± 2°C) with four replications. The radial growth of the mycelium was measured eight days after inoculation. The colony colour and the growth pattern on the culture media were also recorded.

### DNA extraction

The total genomic DNA of *C. capsici* was isolated from mycelia. Isolates were incubated at 28°C for four days in tubes containing 20 ml of potato dextrose broth, agitated at 180 rpm. Mycelia were harvested by filtration through filter paper, dried between two layers of filter paper and stored at -80°C for further use. Dried mycelium was ground to fine powder with pestle and mortar using liquid nitrogen and transferred to 1.5 ml Eppendorf tube. 600 µl cetyltrimetylammonium bromide (CTAB) was added and incubated at 65°C for 30 min, tubes were vortexed every 10 min. After cooling at room temperature, equal volume (600 µl) of chloroform: isoamyl alcohol (24:1, v/v) was added in fume hood cabinet, gently mixed for 20 to 30 min and centrifuged at 7000 rpm for 5 min at 4°C. The aqueous phase was transferred to new tubes and repeat CIA extraction. After the second CIA wash, the DNA was precipitated by adding 300 µl isopropanol, tubes were gently mixed and incubated at room temperature for 30 min. Tubes were centrifuged at 12000 rpm for 10 min and supernatant was decanted. The DNA pellet was dissolved in 50 µl of $ddH_2O$.

### RAPD

Random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) procedure described by Williams et al. (1990) was used by the following of a reaction mixture of 25 µl volume which consisting of 2.5 µl of 10X PCR buffer, 2.5 µl of 25 mM $MgCl_2$, 0.5 µl dNTPs mix (10 mM each of dATP, dCTP, dGTP, dtp), 0.4 µl Taq DNA polymerase, 2.0 µl of primer, 1.0 µl of genomic DNA and 16.1 µl of sterilized double-distilled water. The reaction mixture was vortexed and centrifuged at 12000 rpm for 2 min. Amplification was carried out in a thermal cycle by using three temperature profiles, programmed for initial DNA denaturation at 94°C for 3 min, followed by 35 cycles consisting of DNA denaturation for 30 s at 94°C, primer annealing at 35°C for 30 s and polymerization for 1 min at 72°C with a final extension period of 10 min at 72°C. Amplification products were separated on 1.5% agarose gel in 1X TAE buffer at 110 V for about 3 h; 100 bp + 1 kb DNA ladder mix (Fermentas) (0.5 µg/µl) was run for weight size comparison. Gels were stained with ethidium bromide for 30 min; they were visualized with UV light and photographed.

### Analysis of DNA fingerprints

Differences in fingerprinting patterns between isolates were assessed visually. Polymorphisms including faint bands that could be scored unequivocally were included in the analyses. Presumed homologous bands were scored as present (1) or absent (0) to create a binary matrix. A similarity matrix was generated from the binary data using DICE similarities coefficient in SIMQUAL program of NTSYS-PC Package (Rohlf, 2000). Cluster analysis was done with the Unweighed Pair Group Method with Arithmetic Mean (UPGMA) in the SAHN program of NTSYS-PC Package and a dendrogram was constructed based on genetic distances.

## RESULTS

Chilli fruits showing typical fruit rot and leaf spots symptoms were collected from 20 conventional chilli growing areas of Tamil Nadu with a view to find out the pathogenic fungi involved in leaves and fruit rot of chilli in the different places (Suthin Raj and Christopher, 2009; Suthin Raj et al., 2012). From the samples of fruit and leaves, the pathogen was isolated and purified. Identification of the isolates were confirmed by comparing with the culture obtained from ITCC, IARI, New Delhi and the purified isolates named as Cc1 to Cc20 were furnished. Similar results on pathogenic and cultural properties were identified by Singh et al. (2010).

The isolates of *C. capsici* exhibited variation in respect of colony colour and growth pattern. The isolates of Cc1, Cc2, Cc3 and Cc11, which produced acervuli in a scattered manner, had fluffy and raised colonies. The isolate Cc1 produced various colours in different media. The colony colours of the different isolates of *C. capsici* in the various media are furnished in Table 1. Udhayakumar and Usha rani (2010) attributed the growth of fungus to nutritional factors. Minnatallah and Kumar (2005) also reported similar results. Twenty (20) isolates of *C. capsici* were isolated from chilli plants in different parts of Tamil Nadu. Initially, 20 random 10-mer primers (Genei, Bangalore) were screened to select primer exhibiting maximum polymorphism, of these six primers (Table 2) which produced easily scorable and consistent banding patterns were used for RAPD analysis of test isolates and consistent producing 4 to 8 bands of 0.3 to 2.5 Kb and the dendrogram drawn from the RAPD patterns using unweighed pair group method with arithmetic mean (UPGMA). The RAPD pattern obtained with primer code S1027 base sequence ACGAGCATGG is shown in Figure 1. Of the 20 races that displayed intra-race variability for RAPD phenotype, only four isolates (Cc-5, Cc-31

**Table 1.** Characteristics of twenty isolates of *C. capsici* in Tamil Nadu, India.

| Isolate | Colony colour | | | | |
|---|---|---|---|---|---|
| | Potato dextrose agar | Czapek's dox agar | Richard's agar | Green chilli extract agar | Ripe chilli fruit extract agar |
| Cc1 | White | Greyish white | Greyish white | Blackish white | White |
| Cc2 | Black | Black | Greyish white | Black | Black |
| Cc3 | White | White | White | White | Black |
| Cc4 | White | Greyish white | Greyish white | Black | Black |
| Cc5 | Black | White | Whitish black | Whitish black | White |
| Cc6 | Black | White | Greyish white | Black | White |
| Cc7 | Black | Black | White | Greyish black | Brownish black |
| Cc8 | Black | White | Whitish black | Greyish white | White |
| Cc9 | White | White | White | White | White |
| Cc10 | White | White | White | Black | Brownish black |
| Cc11 | White | Black | Black | Black | White |
| Cc12 | Greyish black | Whitish black | Greyish black | White | White |
| Cc13 | Whitish black | Black | Whitish black | White | Black |
| Cc14 | Whitish black | White | White | White | Greyish white |
| Cc15 | White | White | Greyish black | Black | White |
| Cc16 | White | Black | White | Whitish black | Black |
| Cc17 | Greyish black | Black | Greyish black | Greyish white | Black |
| Cc18 | White | Black | Greyish black | Black | White |
| Cc19 | White | White | White | White | White |
| Cc20 | Black | White | White | White | White |

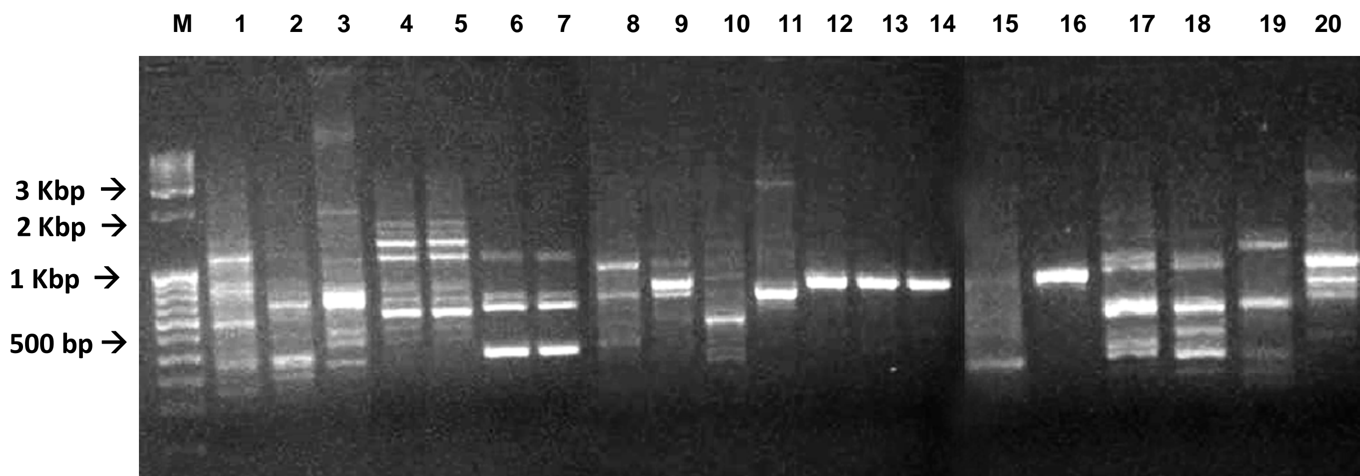


**Figure 1.** Random amplified polymorphic DNA profile observed in *C. capsici* isolates using S1027 primer from fruit rot of chilli in Tamil Nadu, India. Lane M: 100 bp to 1 Kbp DNA Ladder. Lanes 1- 20: *C. capsici* isolates.

and Cc-27, Cc-35) showed 89% similarity.

According to the dendrogram (Figure 2), *C. capsici* isolates in Tamil Nadu can be divided into two main groups. The first group includes one isolate which was isolated from Sivapuri. The second group contains other three isolates of *C. capsici* which were isolated from Virurdhunagar, Naduthittu and Sattur respectively. There was no congruence between the RAPD and virulence pattern of test isolates.

## DISCUSSION

Different species of *Colletotrichum* were reported to possess a high degree of molecular variability when evaluated by RAPD analysis. RAPD markers were used

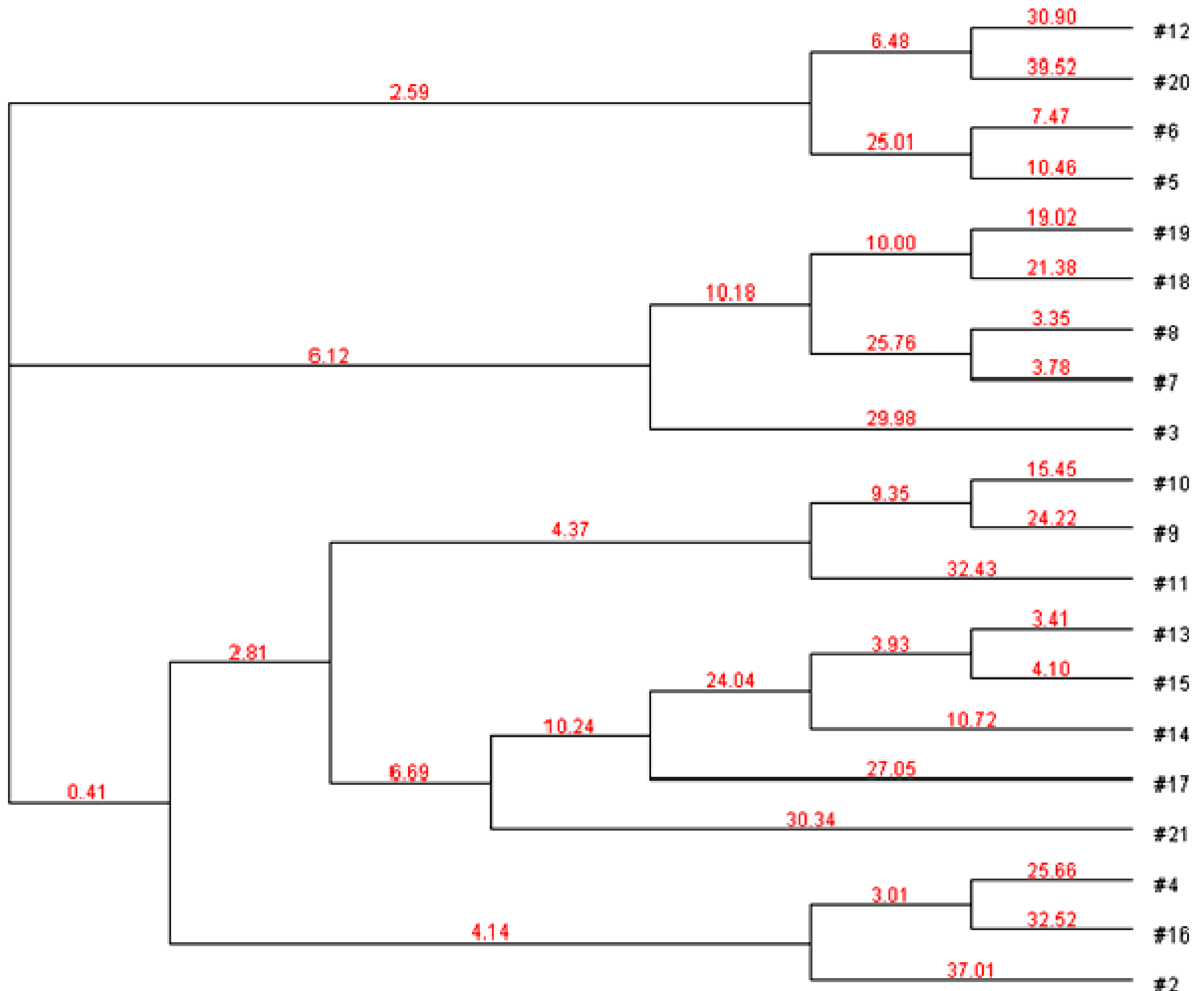


**Figure 2.** UPGMA Dendrogram of twenty (20) *C. capsici* isolates from chilli fruit rot in Tamil Nadu, India.

**Table 2.** Nucleotide sequence of primers generating amplification of *C. capsici.*

| Primer code | Base sequence (5'-3') |
|---|---|
| S1027 | ACGAGCATGG |
| S1063 | GGTCCTACCA |
| S1089 | CAGCGAGTAG |
| S1136 | GTGTCGAGTC |
| S1155 | GAAGGCTCCC |
| S1181 | GACGGCTATC |

for the intraspecific characterization of a number of pathogens (Balardin et al., 1997). This approach was found useful for proper identification of races of *Colletotrichum lindemuthianum* as it yielded race-specific amplified DNA profiles (Balardin et al., 1997). Singh et al. (2010) categorized 51 isolates of *Colletotrichum gloeosporioides* into four groups following RAPD analysis, which were previously categorized on the basis of morphology and virulence. They did not find any correlation between classification of different isolates by RAPD and rate of growth of isolates in culture or their geographic origin. On the basis of polymorphism, Sharma et al. (2010) reported similar results on rhizospheric *Trichoderma* isolates.

The colony colours of the different isolates of *C. capsici* in the various media are furnished. Udhayakumar and Usha rani (2010) attributed the growth of fungus to nutriational factors. Minnatallah and Kumar (2005) also reported similar results. In each RAPD group, isolates from different locations were present indicating high genotypic diversity. Variability among the isolates of *Ustilago*

*segetum* revealed a high genetic variability by RAPD as shown by Padmaja et al. (2006). Isolates that were identical for virulence were most often dissimilar for RAPD markers. Isolates classified in race group CCP-I was distributed across different areas. Similar variation in response of tomato types were also reported by Hema et al. (2007).

## Conclusion

It is concluded that *C. capsici* causing fruit rot or anthracnose of chillies in Tamil Nadu possessed variable populations as is evident from differential inoculation and RAPD analysis. The phylogenetic grouping based on RAPD data did not appear to be congruent with morphological and virulence pattern. RAPD-based DNA fingerprinting could be one of the methods of studying genetic diversity in *C. capsici* in the absence of a definite differential set. The use of a large number of random primers or use of other DNA markers may yield race-specific DNA marker for the detection of particular race.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

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