Occurrence of target spot of tomato caused by *Corynespora cassiicola* in Sri Lanka

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Highlights

- Cottony, grayish fungal colony on PDA and cylindrical, straight, multi-septate conidia
- *Corynespora cassiicola* was identified based on morphological, molecular and pathological data
- Sri Lankan *C. cassiicola* strain TS-1 (GenBank Accession No. MT071510.1)
- 97 % sequence identity with *C. cassiicola* in GenBank Accession No. MH780759

SHORT COMMUNICATION

Occurrence of target spot of tomato caused by *Corynespora cassiicola* in Sri Lanka

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Abstract: In February 2019, a new destructive leaf and stem spot disease was reported from commercial tomato fields in Kimbissa, Central Province, Sri Lanka. This disease resulted in significant loss of foliage where the disease severity ranged from 50-70% in the infected tomato fields. The objectives of the study were to isolate and characterize the pathogen causing the disease and confirm its pathogenicity. A fungal pathogen with cottony, grayish mycelium and cylindrical, straight, multi-septate conidia was isolated from the leaf and stem lesions on Potato Dextrose Agar PDA. Based on morphological, molecular and pathological data, the fungus was identified as *Corynespora cassiicola*. Re-inoculation of leaves and stems of healthy tomato plants under green house conditions produced symptoms similar to those observed in the field. Sequence was deposited in GenBank for Sri Lankan *C. cassiicola* strain TS-1 (Accession No.MT071510.1). A

BLAST search revealed 97% sequence identity to *Corynespora cassiicola* in GenBank Accession No. MH780759.1 (Soybean, Brazil).

Keywords: Molecular identification, Target spot, Tomato.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.), belonging to the family Solanaceae, is one of the most popular vegetable crops grown in Sri Lanka. Many constraints affect productivity and quality of tomato among which diseases caused by fungi, bacteria, viruses and phytoplasma play a major role. In February 2019, a destructive leaf and stem spot disease was reported from farmer fields of tomato in Kimbissa, Central Province of Sri Lanka, which has

Figure 1: (a) Severely infected tomato plants showing target spot symptoms, (b) in leaves, (c) stem, and (d) fruits at farmers' field in Kimbissa.

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resulted a significant loss of foliage. The disease severity ranged 50-70 % in the affected tomato fields. The infected tomato plants exhibited brown spotting on leaves and stems, spreading on to all leaflets (Figure 1a).

Affected plants exhibited symptoms, initially as small brown lesions with a yellow halo on leaves and later spread to the petiole, stems (Figure 1b & c) and fruits. These lesions become larger, showing “target spot” appearance and coalesced lesions on the stems were longer and thinner whereas brown to dark brown sunken lesions occur on fruits (Figure 1d), resulting reduced fruit quality and yield. These symptoms were similar to those of target spot disease of tomato caused by *C. cassiicola* as described by Schlub *et al.* (2007).

Early stage of brown spot symptoms on leaves were quite similar to that of early blight of tomato caused by *Alternaria solani* (Adam *et al.*, 2018). Hence, the confirmation of the pathogen of this destructive disease is important for formulating management strategies against it. The objectives of the present study were to identify the pathogen of target spot disease in tomato in Sri Lanka, using morphological and molecular techniques and to confirm its pathogenicity.

MATERIALS AND METHODS

Isolation and characterization of the pathogen

Samples of infected leaf, stem and petiole were collected from exotic tomato variety ‘Platinum’ from two locations at Kimbissa area in February 2019. Isolation and molecular identification studies were conducted at the Horticultural Crop Research and Development Institute (HORDI), Gannoruwa, Sri Lanka. Isolations were made from affected leaves (TS1), stems (TS2) and petioles (TS3) on Potato Dextrose Agar (PDA) medium.

Small pieces of tissues, excised from symptomatic areas, were surface sterilized in 70% ethanol for 2-3 min (Jayasuriya and Thennakoon, 2007) and transferred on to PDA plates supplemented with streptomycin 0.1% W/V. The plates were incubated at room temperature, 26±2 °C. After 1-2 days of incubation, the fungal mycelium grown out of the diseased tissues were sub-cultured and the plates were incubated for 7-14 days at 26±2 °C. Single spore isolates were raised from all cultures which were maintained as axenic cultures on PDA at 4 °C for further use. Morphological characteristics of colonies, grown on PDA, were described and the morphology of fungal structures were observed under light microscope and recorded.

Molecular identification

Total genomic DNA was extracted from pure cultures of three pathogenic isolates (TS1, TS2 and TS3) using Cetrinide Tetradecyl Trimethyl Ammonium Bromide (CTAB) extraction procedure (ICGEB, 2003). Polymerase Chain Reaction (PCR) amplification with universal primers, ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') which anneals at the end of 18S rDNA and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') which anneals at the beginning of 28S rDNA (White *et al.*, 1990). PCR

amplifications were performed in a total volume of 10 µl by mixing 5µl of Taq PCR master mixture (Promega- 25 units Taq DNA polymerase, 200 µM of each dNTP and 1X PCR buffer and 1.5 mM MgCl₂), 0.8 µl of each primer (10 mM), 0.5 µl of diluted (1:10) DNA template and 2.9 µl of sterile distilled water. PCR program included an initial denaturation of 5 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 55 °C and 1 min at 72 °C with a final extension at 72 °C for 10 min. using a thermo cycler (Labnet Gradient, USA). PCR products were visualized in 1.4% agarose gel for 01 h at 80 V and using a gel documentation system (ENDURO™ GDS). Promega- G571A 1 kb ladder was used as a marker.

PCR products were sequenced at the Department of Molecular Biology and Biotechnology, Faculty of Science, University of Peradeniya, Sri Lanka and were subjected to DNA homology search with BLAST (<http://www.ncbi.nlm.gov/BLAST/>).

Confirmation of pathogenicity

Pathogenicity of three fungal isolates was tested on 3-week old, healthy tomato (var. Thilina) plants, transplanted in plastic pots (8 cm diameter), containing sterilized soil mixture under greenhouse conditions at HORDI. A set of three tomato plants per isolate was inoculated by spraying a conidial suspension (1×10^5 ml⁻¹) of each fungal isolate and another set of plants was sprayed with sterile distilled water to serve as controls. All inoculated plants were subsequently kept covered with polyethylene bags, moistened with tap water, for 48 h to provide high humid conditions. The pathogen was re-isolated from symptomatic tissues as described previously to confirm the pathogenicity.

RESULTS AND DISCUSSION

Morphological characteristics of the isolated pathogen

The colonies of three fungal isolates on PDA were morphologically similar. They were cottony and initially white colour and later turned dark gray and the reverse was light to dark brown (Figure 2a; b) as the cultures aged. The conidia were hyaline to brown, cylindrical, straight, some slightly curved and 3 -13 septate often consisting of a protruding peg (hilum) at their base (Figure 2c). Conidial lengths and widths ranged from 40.16 to 279.90 µm and 6.87 to 17.3 µm, respectively. The conidia were taxonomically similar to original description of *C. cassiicola* (Caetano *et al.*, 2018).

Molecular identification

Amplification of DNA extracts from the 3 isolates of *Corynespora* sp. with universal primer pair, ITS1 and ITS4, generated approximately 530 base pair rDNA fragment and no band was produced with water controls in any of the reactions (Figure 3). The BLASTn search revealed 97% sequence identity to *Corynespora cassiicola* isolate Cc318-1 in GenBank Accession No. MH780759.1 (*Soybean, Brazil*). The sequence of *C. cassiicola* strain TS1 (Tomato, Sri Lanka) was deposited in GenBank Accession No.MT071510.1.

Figure 2: a) Upper and, b) lower surface view of *C. cassiicola* isolate TS1 grown on PDA after 10 days, and c) conidia (x 400).

Figure 3: Electrophoresis in agarose gel of PCR amplification products with ITS1 and ITS4 primers for *Corynespora cassiicola*. Lanes L: DNA ladder, 1: Isolate TS1, 2: Isolate TS2, 3: Isolate TS3, 4: negative control

Figure 4: Target spot symptoms development on a) leaves and b) stem inoculated with *C. cassiicola* isolate TS1 and c) control plant

Confirmation of Pathogenicity

Five days after inoculation, all three *C. cassiicola* isolates initiated spot symptoms on leaves and stems of tomato plants (Figure 4a; b). The symptoms were identical to those observed in the field. The control plants remained healthy (Figure 4c). The pathogen was re-isolated from inoculated plants and the colonies recovered were similar to those isolates originally obtained from diseased plants.

The fungus has been reported to infect 380 genera and 530 species of plants including monocots, dicots and ferns (Smith *et al.*, 2006). *Corynespora cassiicola* is a serious pathogen on winter tomato production areas of Florida, USA and causes heavy losses up to 11,800 kg/ha when the disease is not managed (Pernezny *et al.*, 2002). Target spot has been identified as one of the major threats to tomato production in India and disease severity ranged between 35% and 58% in different locations of West Bengal which ultimately causes tremendous loss of tomato foliage (Adam *et al.*, 2018).

In Sri Lanka, the fungus has been reported in rubber, cowpea, eggplant, winged bean, tomato, cocoa, papaya, sweet potato and manioc (Silva *et al.*, 2000). This is the first report of molecular identification of the pathogen causing target spot disease in tomato grown in Sri Lanka.

CONCLUSIONS

The pathogen causing the leaf and stem spot disease of tomato was identified as *C. cassiicola*. The BLASTn search revealed 97% sequence identity to *C. cassiicola* isolate Cc318-1 in GenBank Accession Nos. MH780759.1. Sequence was deposited in GenBank for Sri Lankan *C. cassiicola* strain TS-1 (Accession No. MT071510.1).

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interests.

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