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First report of the *Colletotrichum gigasporum* causing anthracnose on *Solanum torvum* in Sri Lanka

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Abstract*: Solanum torvum is an important plant with higher nutritional and medicinal value. Anthracnose is a major plant disease cause by different pathogens. Colletotrichum is one of the most reported pathogens causing anthracnose to Sri Lankan cash crops, fruits and vegetables. This study reporting the Anthracnose of Solanum torvum caused by Colletotrichum gigasporum for the first time in Sri Lanka with molecular and morphological confirmation.*

Keywords—*Solanum torvum*, Colletotrichum, Anthracnose

1. INTRODUCTION

Solanum torvum (Turky berry) is an important one out of nearly 3000 species belongs to the family Solanaceae (1). It originated in South America and its berries available in many tropical countries as a cultivated vegetable (2). These berries are rich in nutrients such as minerals, vitamins and antioxidants (3). It is a native plant to Sri Lanka and has higher Ayurveda medicinal value (4). Berries, leaves, roots and seeds are used to treat nasopharyngeal infections, coughs, diarrhea, dysentery, kidney and urinary tract diseases and skin diseases in Sri Lankan ayurvedic medicine. Further, the antidiabetic and antihyperglycemic characters of berries are well known (5).

Anthracnose is one of the major diseases recently identified that attacks to young leaves, flowers and berries. *Colletotrichum acutatum* and *Curvularia pallescens* were identified as causal agents of anthracnose disease in *S. torvum* (6). Colletotrichum is the major genera reported in Sri Lanka as the causal agent of rubber anthracnose (7). Further it was identified as a major postharvest pathogen in many fruits and vegetables.

2. METHODOLOGY

2.1 Sample collection

S. torvum leaf sample with necrotic brown to black sunken lesions was collected from a medicinal plant garden at Awissawella, Sri Lanka. Sterile perforated polythene bag was used to transport the sample to the laboratory.

2.2 Isolation and culture of the pathogen

After surface sterilization of the infected leaves using 70% ethanol, blot dried lesions were cut opened and small pieces were placed on potato dextrose agar (PDA). The arising mycelium was sub cultured and prepared single conidia derived cultures for use in further analysis.

2.3 Morphological characterization

Colony color, color of the conidial masses, shape and size of appressoria, shape and size of conidia and redial growth rate was measured as morphological characters. Each character was measured with 10 replicates of seven days old single conidia derived cultures. Conidia and appressoria characters measured using seven days old slide cultures.

2.4 Molecular Identification

Genomic DNA was extracted from 2 days old mycelia grown on PDA, using an optimized protocol (8). Internally transcribed spacer (ITS) region of the isolate was PCR amplified using ITS1Ext and ITS4Ext primers (9).

PCR reactions were performed using a thermo cycler (Eppendorf master cycler, USA) with 5 min of denaturation at 95°C followed by 35 cycles of 30 s at 95°C, 30 s at 60°C and 90 s at 72°C (10) and 5 min of the final extension at 72°C. PCR product was used to perform bidirectional sequencing. The sequence was analyzed using Bio Edit version 7.2.0 (11). A BLASTN search in GenBank (http://www.ncbi.nlm.nih.gov/ genbank/) with ITS sequence was conducted for initial identification of the isolate. Resulted sequence was deposited in NCBI GenBank (KT582198).

Phylogenetic tree constructed based on Maximum Likelihood method with the reference ITS sequences of *C. gigasporum* species complex using MEGA 6.0 (12)

2.5 Pathogenicity testing

Pathogenicity of the isolate was tested by wound inoculation of healthy *S. torvum* leaves with 10µl of conidia suspension ($1x10^6$conidia/ml). Inoculated samples were incubated at 28˚C under 90% relative humidity.

3. RESULTS AND DISCUSSION

The upper side of seven days old culture on PDA was white with gray middle and the reverse side was greenish gray in

color. Pale orange conidial masses were observed in ten days old culture. Complying with the characters reported by Liu et al. (13), hyaline, oblong, straight conidia (26 – 30 µm x 5 – 8 µm) characteristic to *C. gigasporum* and brown colored lobed appressoria with dark walls (15 – 20 µm x 8 – 10 µm) were observed in seven days old slide cultures (Fig. 1). Average radial growth rate of the culture on PDA was 12.2 mm/24 h.

Phylogenetic tree constructed with obtained sequence and other reference sequences confirmed the identity of *C. gigasporum* isolate with 100% bootstrap support (Fig. 2).

After 6 days of inoculation by conidia suspension, typical anthracnose lesions were observed on *S. torvum* leaves. By that the Koch's postulate were fulfilled.

Though the studies conducted about *Colletotrichum* species causing anthracnose to *S. torvum* are rare, plenty of studies have been published about the inhibitory effects of *S. torvum* leaf extract against *Colletotrichum* species such as *C. musae* (14) and *C. capsici* (15). To the best of knowledge, this is the first report of *C. gigasporum* causing anthracnose on *Solanum torvum* in Sri Lanka.

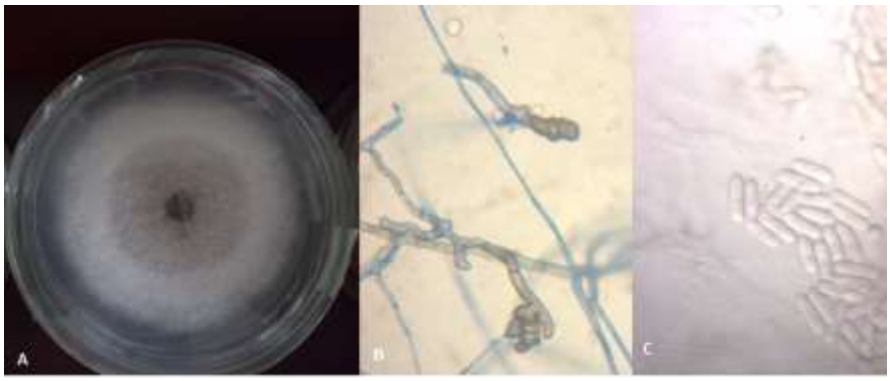

Fig 1 *Colony morphology (A), appressoria (B) and conidia (C) of the seven days old Colletotrichum gigasporum culture on potato dextrose agar medium.*

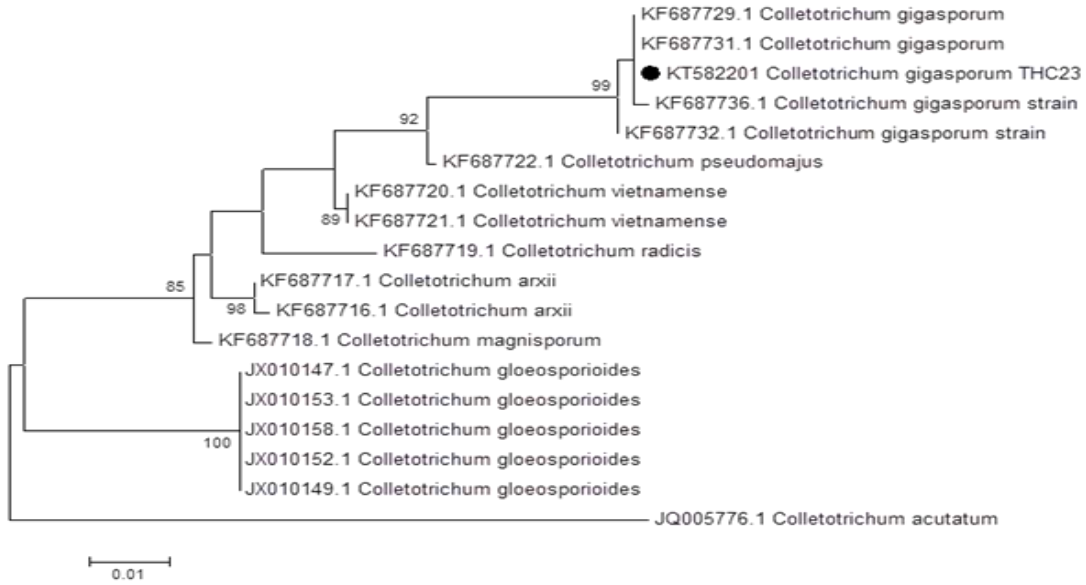

Fig 2 *Phylogenetic tree constructed with sequences of internal transcribed spacer region from Colletotrichum gigasporum identified in this study and reference species of the C. gigasporum species complex.*

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