Tip blight disease of *Dracaena fragrans* caused by *Lasiodiplodia theobromae (Botryosphaeriaceae),* from India

ARGHYA BANERJEE
BIRENDRANATH PANJA
JAYANTA SAHA
Department of Plant Pathology
Bidhan Chandra Krishi Viswavidyalaya
Mohanpur-741252
Nadia, West Bengal, India
Email: arghyabanerjee18@gmail.com

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Abstract: *Dracaena fragrans* (cornstalk plant) is an ornamental house plant of *Asparagaceae* family and native to tropical Africa. Typical tip and marginal blighting of leaves along with the production of numerous concentric black dot-like structures was observed on the upper surface of dead leaf for the first time at Darbhanga Tropical Garden Plant House of the Agri-Horticultural Society of India, Alipore road, Kolkata, India, during 2014 to 2016. The blighted portion was surrounded by a dark brown zonate margin followed by yellow halos. The leaf dried up basipetally**.** The colour of the leaf changed from brown to grey or straw in later stage. The length and breadth of straw coloured regions varied from 1.5–6.5 cm and 0.4–4.6 cm. Numerous erumpent pycnidia were formed sub-epidermally on straw coloured dead tissues. Conidia were initially double-layered, hyaline and unicellular but on maturity became light to dark-brown, equally 2-celled, oblong, bilamellate with 16.5–28.3 µm (av. 22.1 µm) × 11.6–24.7 µm (av. 14.1 µm) in size. Based on pycnidial and conidial morphology, and pathogenicity, the pathogen was identified as *Lasiodiplodia theobromae*. This is the first report of *Lasiodiplodia theobromae* causing tip blight disease of *Dracaena fragrans* from India.

Zusammenfassung: *Dracaena fragrans* (Duftender Drachenbaum) ist eine dekorative Zimmerpflanze der *Asparagaceae* und stammt aus dem tropischen Afrika. Die typischen Ausbleichungen an den Blattspitzen und Blatträndern zusammen mit der Entstehung zahlreicher konzentrischer schwarzer punktartiger Strukturen wurde auf der Oberseite abgestorbener Blätter wurde zum ersten Mal im Darbhanga Tropical Garden Plant House der Agri-Horticultural Society of India, Alipore Road, Kolkata, Indien, von 2014 bis 2016 beobachtet. Die ausgebleichten Stellen wurden von einem dunkelbraunen Rand umgeben, gefolgt von gelben Halos. Das Blatt vertrocknete basipetal. Die Farbe des Blattes wechselte in späteren Stadien von braun zu grau oder zu strohfarben. Die Länge und Breite der strohfarbenen Bereiche variierte von 1,5–6,5 cm und 0,4–4,6 cm. Zahlreiche hervorbrechende Pyknidien wurden subepidermal an strohfarbenen toten Geweben gebildet. Die Konidien waren zunächst zweischichtig, hyalin und einzellig, wurden aber bei Reife hell bis dunkelbraun, sowie 2-zellig, länglich, bilamellat und waren 16,5–28,3 µm (durchschnittlich 22,1 µm) × 11,6–24,7 µm (durchschn. 14,1 µm) groß. Basierend auf pyknidialer und konidialer Morphologie und Pathogenität wurde der Erreger als *Lasiodiplodia theobromae* identifiziert. Dies ist der erste Bericht aus Indien von *L. theobromae*, der die Krautfäule von *Dracaena fragrans* verursacht.

Dracaena fragrans (L.) KER. GAWL., known as corn or cornstalk plant, a native of tropical Africa, is an important slow growing ornamental house plant belonging to the

family *Asparagaceae*. It is widely grown as a hedge plant in Africa, helps to remove indoor pollutants and exhibits significant antimicrobial activity against *Pseudomonas aeruginosa, Staphylococcus aureus* and *Fusarium oxysporum* (MINH & al. 2009). In India the plant has been introduced in different states including West Bengal. Since 2014, the present investigator group noticed that during May, foliar infections were observed but reached peak in severe form during September to October when temperature ranges from 25–35 ˚C and thereafter declined and continued up to the end of January (BANERJEE 2016). For the last 2 years, a detailed study on the disease along with its causal agent has been conducted during present investigation.

Tab. 1. Conidial size and septation for *Lasiodiplodia theobromae* described under different synonyms.

Species	Host	Origin	Conidial size (µm)	Septum	Reference
Diplodia gossypina COOKE	*Gossypium* sp.	India	22×12	0	COOKE (1879)
Botryodiplodia theobromae PAT.	*Theobroma cacao*	Ecuador	25–35×12–15	1	PATOUILLARD & LAGERHEIM (1892)
Lasiodiplodia tubericola ELLIS & EVERH.	*Ipomoea batatas*	Java	18–22×11–14	1	CLENDENIN (1896)
L. nigra K. R. APPEL & LAUBERT	*T. cacao, Carica papaya*	Samoa	28–32×18–21	1	APPEL & LAUBERT (1907)
L. theobromae (PAT.) GRIFFON & MAUBL.	*T. cacao*	Equatorial America	20–30×11–15	1	GRIFFON & MAUBLANC (1909)
L. triflorae B. B. HIGGINS	*Prunus* sp.	USA	22–25× 3–16.5	1	HIGGINS (1916)
Diplodia theobromae (PAT.) W. NOWELL	*T. cacao*	unknown	25–30×12–15	1	NOWELL (1923)

Materials and methods

Collection of diseased samples: Diseased leaves were collected from Darbhanga Tropical Garden Plant House of the Agri-Horticultural Society of India, Alipore Road (22° 53' N latitude and 88° 33' E), Kolkata, West Bengal. The diseased specimen was deposited at *Herbarium Cryptogamae Indiae Orientalis* (HCIO No. 52023), IARI, New Delhi.

Pure culture isolation: Diseased leaves were cut into small pieces, surface sterilized in 1% sodium hypochlorite for 45–60 sec, rinsed three times in sterile distilled water, plated on water agar (Fig. 1C) and then incubated at 28 ˚C for 5 days. Hyphal tips from the margin of developing colony were picked up and transferred to potato dextrose agar (PDA) for pure culture (Fig. 1D). Further subculturing was done on peptone salt agar (PSA), oat meal agar (OMA) and V8 media to induce sporulation. A culture of the isolate was deposited at Indian Type Culture Collection (ITCC No.7905), IARI, New Delhi.

Morphological observations: After isolation of fungal pathogen in purified form, it was grown on culture medium, and kept for 8–10 days for sporulation. Sometimes the composition of the medium had to be modified for induction of sporulation. Series of slides were prepared from cultures or infected parts for morphometric studies of fungal spores, spore bearing and other structures. Microphotograph of all fungal structures was taken with a Leica Binocular Microscope and or Zeiss Phase Contrast Microscope (under 10×, 20×, 40× and 100 ×) and by using a Canon Powers Shot A640 camera. Dimensions (e.g. length and breadth) of conidia, conidiophore, pycnidia and hyphae were measured

using AxioVision (Rel. 4.8) software. Based on the morphometric characteristic, fungi were identified to genus or species level (SUTTON 1980, BOEREMA & al. 2004; Tab. 1).

Establishment of pathogenicity: A pathogenicity test was performed by inoculating healthy leaves with six-day-old purified active fungal culture under controlled laboratory condition. Mycelial and conidia plugs (5 mm diam.) were taken from potato dextrose agar (PDA) and placed on detached leaves. The inoculated leaves were kept in moistened plastic bags in the dark for two days and then in natural light at 20 °C. Although the method of inoculation was somewhat artificial, the symptoms were similar to those observed in the field and the fungus was re-isolated from infected parts. The control leaves, inoculated with PDA plugs, remained healthy.

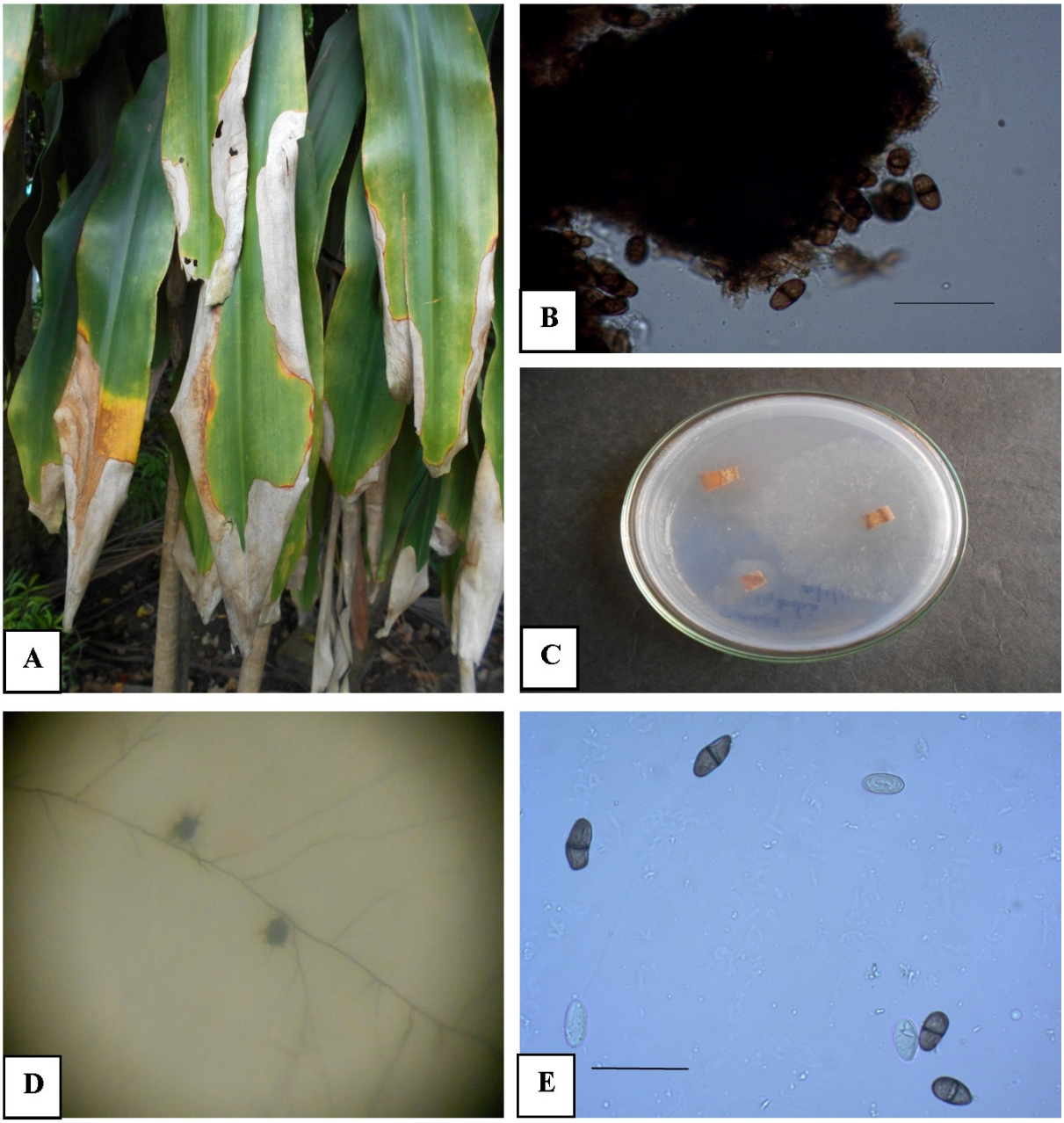

Fig. 1. *Lasiodiplodia theobromae*. *A* Tip blight disease symptoms of *Dracaena fragrans, B* mature conidia produced on host tissue , *C* isolation of pathogen on water agar medium, *D* purification of culture on Potato dextrose agar (PDA) medium and artificial production of pycnidia, *E* conidia produced on potato dextrose agar medium. Bars 50 µm.

Results and discussion

Typical tip and marginal blighting of leaves along with numerous productions of concentric black dot-like structures was observed on the upper surface of dead leaves. The blighted portion was surrounded by dark brown zonate margin followed by yellow halos. The leaf dried up basipetally (Fig. 1A). Colour of the leaf changed from brown to grey or straw in later stage. The length and width of the straw coloured regions varied from 1.5–6.5 cm and 0.4–4.6 cm. Numerous ostiolate erumpent pycnidia were formed sub-epidermally on the straw coloured dead tissues.

Tab. 2. Morphometric descriptions of various structures of the pathogen obtained from both host tissue and media.

Parameters recorded on	Leaf tissue	PDA
Mycelial characteristics	hyaline, thin septate hyphae 6.2–17.2 (av. 10.4) µm wide	white coloured, fluffy mycelial growth, hyphae hyaline, thin septate and 5.8–15.7 (av. 10.2) µm wide
Conidiomata	numerous, erumpent pycnidia formed sub-epidermally on straw coloured dead tissues of the leaf tips; solitary or confluent, glabrous, subglobose to globose, brown to black with pseudo-parenchymatous wall, 2–3 wall layers with composite wall thickness 13.4–29.9 µm (av. 20.8 µm), with dimension of 97.3–139.3 µm (av. 118.3 µm) × 149.5–226.6 µm (av. 188.1 µm)	pycnidia 354.4–496.9 µm (av. 436.4 µm) × 507.8–544.6 µm (av. 524.3 µm) in diam., black and dot-like; huge variation in pycnidial size, pycnidia aggregated to irregular structures embedded by several mycelia or hyphal strands
Ostiolar characteristics	ostiolate pycnidia, ostiole 24.3–24.6 µm (av.24.5 µm) in diam.	ostiolar region not particularly seen in the media
Conidiophore	not clearly visible	not seen
Conidia	developing from inner basal cells of the pycnidium; initially double layered, hyaline and unicellular, mature light to dark-brown, equally 2-celled, oblong, bilamellate, 16.5–28.3 (av. 22.1) × 11.6–24.7 (av. 14.1) µm (Fig. 1B).	double layered, initially hyaline and unicellular, mature light to dark-brown, equally 2-celled, oblong, bilamellate, 17.0–25.7 (av. 22.4) × 11.8–13 (av. 12.5) µm (Fig. 1E).
Chlamydospores	not found	terminal and intercalary, chain like, brown to dark brown

Lasiodiplodia theobromae infects monocot and dicot plants causing an array of symptoms including shoot blight and dieback (DURGADEVI & SANKARLINGAM 2012). There was a previous report of *L. theobromae* on *Dracaena fragrans* in China (XI & al. 2002). The presently described fungus was identified based on pycnidial and spore characteristics (Tab. 2). Identification was confirmed by the Indian Type Culture Collection Centre of the Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi. Occurrence of leaf tip blight caused by *Lasiodiplodia theobromae* on *Dracaena fragrans* is a new record for India.

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