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RESEARCH ARTICLE

A new foliar disease of *Ficus religiosa* caused by *Diaporthe acutispora,* identified using molecular phylogeny based multigene DNA sequence analyses

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Abstract: *Ficus religiosa* is a large tree, native to India, and considered to have religious significance and found invariably in Buddhist shrines. The manuscript reports a new foliar disease, named as '*Diaporthe* leaf disease' (DLD) in *F. religiosa*. DLD was first observed in a Buddhist temple in Colombo, Sri Lanka, in 2012 and now spread over some other parts of the island. Causal organism was identified as *Diaporthe acutispora*, based on combined ITS, TUB and TEF phylogenetic analysis. Koch's postulates were fulfilled, confirming that *D. acutispora* was the pathogen, producing symptoms originally observed in *F. religiosa* leaves. Initial infections occur in younger but fully expanded leaves, producing darkened, 1.0-1.5 cm segments of the mid-rib or primary/secondary veins. Peripheral tissues around the darkened mid-rib/vein became necrotic with large, circular and chlorotic zones. The most striking symptom was the appearance of clusters of numerous, shiny, blackish and spherical to irregular conidiomata (pycnidial) over the leaf surface and along the affected mid-rib/veins of senescing leaves. Infected leaves, younger or mature, tended to roll upwards showing desiccation probably due to blockage of water movement through infected mid-rib/veins. Considering the potentially devastative nature of the DLD, and that the disease has already spread over to parts of the country, findings in the present work will be significant in terms of disease diagnosis and management. Accurate identification of the pathogen is a prerequisite to determine the epidemiology and, effective disease management strategies. This is the first report of DLD in *F. religiosa* anywhere in the world.

Keywords: β-tubulin, *Diaporthe*, sacred fig, translation elongation factor.

INTRODUCTION

Ficus religiosa Linn. (Family Moraceae) is a large tree native to India that is thought to have religious significance and is always found in Buddhist shrines, hence the names Sacred Fig or Sacred 'Bo' in Nepal, Sri Lanka, South-eastern China, and Indochina (Corner, 1981). *Ficus religiosa* is a dry season-deciduous or semi-evergreen tree growing up to 30 meters tall without aerial roots from the branches. The tree is considered to have a religious significance in three major religions that originated in the Indian subcontinent, Hinduism, Buddhism and Jainism. *Ficus religiosa* is known to have a very long lifespan. A sprig of the tree, under which the Gautama Buddha attained enlightenment, was brought to Sri Lanka in the year 288 B.C. It has survived up to the present day by means of its ability to supplant the ageing trunk with new shoots and this tree, in Anuradhapura (North Central Province), is acclaimed as one of the oldest in the historical record (Corner, 1981).

Leaves are cordate shaped and spirally arranged; the new leaves are pink in colour. The leaf lamina has

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a truncate base, caudate-acuminate with a distinctive extended drip tip, 25–90 mm long. The petiole is 35–130 cm, mostly longer than leaf lamina (Corner, 1981). The tree is also known to have been used in traditional medicine to treat various health complications (Kumar *et al*., 2018).

Ficus religiosa is affected by several fungal diseases of which the leaf spot disease caused by *Glomerella cingulata* is the most common in Sri Lanka (Abeygunawardhane, 1969). A leaf blotch caused by a *Botryosphaeria* species was also reported (Maharachchikumbura & Adikaram, 2009).

A leaf blight disease was reported in *F. religiosa* from Jaipur, India, and the causal agent was identified as *Phyllosticta* sp. (Sharma *et al*., 2011). Another leaf spot disease was reported from Lahore, Pakistan, caused by *Curvularia aeria* (Nayab & Akhtar, 2016). Brown root disease caused by *Phellinus noxius* is responsible for the mysterious death of individual trees (Abeygunawardhane, 1969).

Diaporthe (syn. *Phomopsis*) species are well-known as pathogens, endophytes or saprobes on a range of economically important crops, ornamentals and forest trees (Udayanga *et al*., 2011). Species of *Diaporthe* cause various diseases on a range of plant hosts including some economically important hosts worldwide (Gomes *et al*., 2013). The genus is placed in the family Diaporthaceae, order Diaporthales, in the class Sordariomycetes (Maharachchikumbura *et al*., 2016). Morphological characters are not always suitable for species definition because of their plasticity and overlap between different species (Santos & Phillips, 2009).

Literature surveys have established no previous records of a leaf disease from *F. religiosa* with symptoms and causal agents similar to those of the disease reported in the present study, in Sri Lanka or elsewhere. Therefore, the leaf disease that the present study reports was taken as a new and unknown disease. The objectives of the study were to study and record the symptomatology, isolate and identify the causal agent of the disease using morphological characteristics and multigene DNA sequence analyses and determine phylogeny. Koch's postulates were performed to test if the fungus isolated could be consistently isolated from all symptomatic leaves, the pathogenicity of the fungus and, its ability to produce symptoms that were originally shown by the disease, on re-inoculation.

METHODOLOGY

Diseased specimens

Leaves of *F. religiosa* showing DLD symptoms were collected from trees located in several places from Colombo (Western Province) in 2012, when the disease was first encountered. Since then, diseased leaves were sampled from Danthure, Peradeniya and Hantana (Kandy District, Central Province). The diseased leaves were first examined and photographed in the field in every sampling, and the observations were recorded. Leaves at different stages of symptom development were collected in separate polythene bags and delivered to the Plant Pathology laboratory at the Department of Botany, the University of Peradeniya for further examination.

Examination of diseased specimens and isolation of the pathogen

Leaves showing DLD symptoms were first examined visually for external symptoms. Surface scrapings were taken from lesions and transverse sections cut through diseased tissues were examined under the light microscope (Olympus BX53 with Olympus DP72 digital camera), and the observations were recorded. The fungus was isolated from diseased tissues on leaves at different stages of symptom development. Small (0.5 × 0.5 cm^2) segments cut from the advancing margin of necrotic lesions on leaf lamina, necrotic areas of the mid-rib, lateral veins, and petioles were surface sterilized by immersing in 2% sodium hypochlorite (Clorox© USA) for 2 min (Indrakeerthi & Adikaram, 1995). After placing on a sterilized filter paper to remove excess liquid the segments were transferred aseptically onto Potato Dextrose Agar (PDA) medium supplemented with tetracycline (10 mg/L) to suppress bacterial growth. The plates were incubated at 25±3°C for one week, and the colonies that emerged from diseased tissue were examined under a light microscope and sub-cultured on a fresh PDA medium.

Preparation of mono-conidial cultures

Mono-conidial cultures of the isolate were prepared using the procedure described by Johnston & Booth (1983). The plates were incubated for seven days at 28–30°C, and the isolates were sub-cultured on PDA to be used in morphological and molecular studies.

Morphological studies

The colony morphology viz. colony colour, texture, pigmentation underneath, the presence or absence of concentric rings and sectoring etc., was studied using two weeks-old cultures. Conidial morphology was also studied taking 4–5 wks ancient cultures. A drop (20 µL) of a conidia suspension, prepared as described previously, was mounted on a clean slide. The conidial characteristics, the shape and colour of conidia, presence of septa etc., were observed under the microscope and photographed and recorded. The dimensions of 50 randomly selected conidia were measured in µm with a graticule connected to an eyepiece of a light microscope. The average length and breadth were calculated and presented with their range.

For morphological studies, the conidiomata obtained from 4–5 wks old cultures and diseased tissues on senescing or fallen leaves were mounted on slides and after crushing by gently tapping over the coverslip, the structures were examined under the light microscope. The conidiomata, conidiogenous cells and conidia were photographed, the dimensions of conidia were measured and averaged.

DNA extraction, PCR amplification and sequencing

DNA was extracted using a Wizard® genomic DNA purification kit (Promega Corporation, USA) and a modified protocol (Doyle & Doyle, 1987; Lee & Taylor, 1990).

Three gene regions, internal transcribed spacer (ITS), partial β-tubulin (TUB) and partial translation elongation factor 1-alpha (TEF) were amplified using primer pair ITS1 and ITS4, BT2a and BT2b, and EF1-728F and EF1-986R, respectively (White *et al*., 1990; Glass & Donaldson, 1995; Carbone & Kohn, 1999). PCR reactions were performed using Applied Biosystems Veriti 96-well thermal cycler (9902, Singapore). Amplification reactions had a total reaction volume of 40 µL, which was composed of 1× PCR buffer (Promega Corporation, USA), 5.6% DMSO (v/v), 40 µM dNTPs (Promega Corporation, USA), 0.2 µM of each forward and reverse primers, 0.25 U of GoTaq™ *Taq* DNA polymerase (Promega Corporation, USA), sterilized water and 10 ng of genomic DNA. For ITS and TUB regions, 1.5 mM of $MgCl_2$ was used, and for the TEF region, two mM $MgCl_2$ was used. PCR cycling conditions were; an initial denaturation for 3 min at 94 °C, followed by 40 cycles of denaturation at 94 °C for 30 s; annealing for 1 min at 58 °C for ITS, 55 °C for TEF and TUB, and elongation at 72 °C for 1 min; and final extension step at 72 °C for 3 min. Amplicons were sequenced at Macrogen Inc. (Seoul, South Korea) and the Department of Molecular Biology, Faculty of Science, University of Peradeniya, Sri Lanka. DNASTAR Lasergene SeqMan Pro v. 8.1.3 was used to gain consensus sequences from sequences generated from forward and reverse primers, and these were deposited in GenBank (Table 1).

Phylogenetic analysis

A preliminary species identification of the isolate was made through a BLASTn search conducted with each locus at the NCBI (*http://blast.ncbi.nlm.nih.gov*). The sequences produced in this study with more than 98% similarity with reference sequences for *Diaporthe* were chosen for phylogenetic analysis (Table 1). Multiple sequences were aligned with MEGA v.7.0.26 (Kumar *et al*., 2016) and edited manually. The maximum likelihood (ML) analysis was conducted using RAxML v8.2.10 (Stamatakis, 2014) performed on the CIPRES Science Gateway server (Miller *et al*., 2012) and using the GTRGAMMA model. Branch support values were determined using the rapid bootstrapping algorithm with 1000 replicates. The phylogenetic tree was visualised in FigTree v. 1.4.0 (Rambaut, 2014) and edited Adobe Illustrator.

Koch's postulates

Diseased *Ficus* leaves were examined and symptoms were recorded. The pathogen isolated was grown in pure culture. Uniform-sized saplings of *F. religiosa* devoid of blemishes or any disease symptoms were used for artificial inoculation. Drops (20 µL) of conidia from a suspension (1×10^6 conidia/mL) were applied onto the mid-rib and two lateral veins on the upper surface of the leaf lamina. Six replicate leaves in three plants were used for inoculation and another three leaves were treated with drops of sterile distilled water as controls. Inoculated and control leaves were kept covered with perforated polythene bags at 28–30°C, and after 24 h, the polythene bags were taken off. Inoculated leaves were examined regularly. Symptoms, when appeared, were described and compared with those of the original diseased leaves. The pathogen was re-isolated from the symptomatic leaves on PDA. The isolate's colony and asexual reproductive morphology were compared with those of the original isolate used for inoculation.

RESULTS AND DISCUSSION

This disease was first encountered in June 2012 in a *F. religiosa* tree, at a temple in Kalubowila area, Colombo (Western Province), Sri Lanka and a few months later in another temple in Danthure, Kandy District (Central Province). Following this, *F. religiosa* trees showing similar leaf symptoms were found in temples, wayside trees, etc. in villages in Kandy area and within the premises of the University of Peradeniya. *Diaporthe* species generally produce alpha- and beta-conidia in pycnidia in large numbers, which can also persist under adverse conditions. Splash dispersal of conidia during rain is an effective method for the spread of the fungus (Linders *et al*., 1996) serving as a source of inoculum for new infections. The fungus also survives in plant debris (Panwar *et al*., 1970). *Diaporthe* species have been introduced into new areas as endophytes or latent pathogens together with plant produce (Torres *et al*., 2016). Production of conidia in large numbers and splash dispersal during rain (Linders *et al*., 1996) coupled with warm and humid conditions conducive to infection facilitate the spread of the disease posing a major threat to *F. religiosa* trees in the country.

Disease symptoms

Disease symptoms appear mainly in the foliage of *F. religiosa*. Leaf symptoms were slightly variable among trees, or with the location where the *F. religiosa* is grown. Disease symptoms first appeared in younger but fully expanded leaves as browning of 1–1.5 cm long segments on the mid-rib, secondary or tertiary vein, which were surrounded by circular to oval-shaped chlorotic areas of about 1–2 cm diameter in the leaf lamina which gradually enlarged in size (Figure 1).

A small area of leaf lamina immediately outside the browned mid-rib or vein became necrotic but most of the peripheral tissue remained chlorotic. In all affected leaves a portion of 2–4 cm of the mid-rib/vein was infected and turned blackish brown colour. The necrotic area originated around from the vein gradually expanded laterally towards one or both sides of the mid-rib/vein, covering an area of 2–3 cm diameter on either side of the leaf lamina to form a prominent necrotic zone with an irregular margin. A necrotic zone on one side of the affected mid-rib/vein was the most common (Figure 1). Occasionally, two or more infected areas in closer proximity coalesced to form larger lesions. The younger leaves and those with badly infected mid-rib/veins tended to roll upwards often during the daytime showing desiccation probably due to blockage of water transport along affected mid-rib/vein. In addition, the petiole is often angled downwards giving an appearance of epinasty. This could result in rolling leaves upwards, a symptom commonly shown by certain infected leaves.

As the disease progressed, widespread necrosis could be observed in the leaf lamina. In the senescing leaves, numerous black, small, raised, round or oval-shaped clusters of scattered conidiomata were seen crowded along the mid-rib, lateral secondary and tertiary veins. Small, circular to oval-shaped isolated necrotic patches were also observed along the petiole. The segments of the mid-rib and secondary veins in infected leaves become necrotic leading to disfunction of vascular tissues within leaves, which could cause devastative effects on the entire tree.

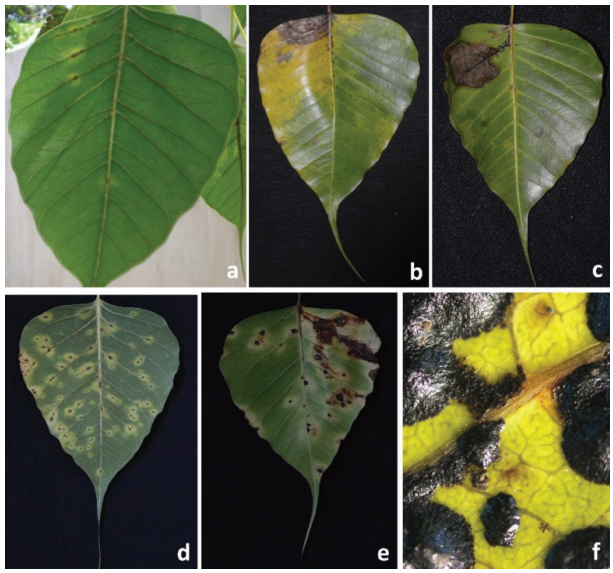

Figure 1: *Diaporthe* leaf disease in *F. religiosa*: (a) initial symptoms on fully-expanded leaf showing browning of smaller discrete segments of the vein surrounded by chlorotic area with a diffused margin; (b) a mature leaf with a large necrotic lesion bordered between two primary veins, developed towards one half from the infected mid-rib; (c) darkened, necrotic lesion at an advanced stage with black conidiomata formed on infected secondary and tertiary veins; (d) slightly different symptoms appeared in leaves in a *F. religiosa* tree with tiny necrotic lesion in the centre surrounded by somewhat larger, diffused chlorotic zone; (e) an advanced stage of the disease with irregular, dark brown and extensive necrotic lesions formed by coalescence of adjacent individual lesions.

In some trees, the initial symptoms appeared as bright yellow, up to 2 cm diameter circular areas surrounding a small circular necrotic area, always covering a segment of the mid-rib or the lateral vein. In older lesions, a greyish, irregular patch developed by expansion of the central necrotic area covering the mid-rib/lateral vein. In some leaves only a single lesion appeared and in others a few or numerous isolated, infected areas could be seen scattered over the leaf lamina. Although the disease's symptomatology is slightly variable among trees, the darkening of the mid-rib or the vein, secondary or tertiary, is a common symptom constantly associated with the disease. This is a striking symptom that could be conveniently used to diagnose the disease (Figure 1).

Pathogen morphology

Three isolates of the pathogen were obtained in the study. The isolate D2 used for morphological and molecular studies was slow growing on PDA. It produced a whitish colony initially and after about ten days, took a characteristic yellow colour with conidiomata appearing black scattered and mostly at the margins (Figure 2a) of concentric rings. Pigmentation began with the edges of the concentric rings which eventually spread all over the plate. The mycelium appeared sparse with concentric rings having irregular peripheral margins. The reverse was white colour at initially and turned orange brownish later (Figure 2b).

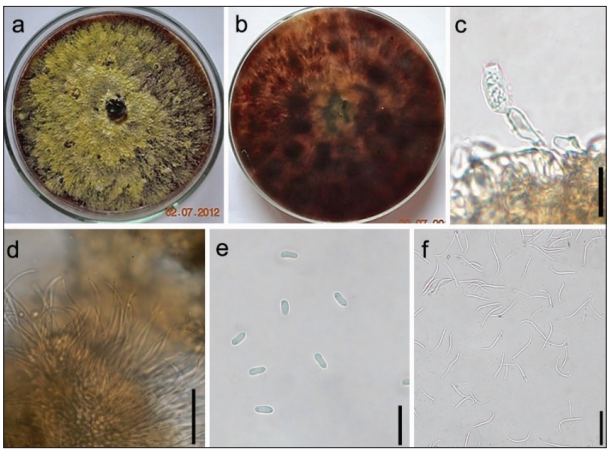

Figure 2: *Diaporthe acutispora* (isolate D2). Growth on PDA (a) from above; (b) from below; (c) alpha conidiogenous cells; (d) beta conidiogenous cells; (e) alpha and beta conidia, and (f) beta conidia (scale bars c–f = 20 µM)

Conidiomata were pycnidial, globose to irregular, black colour, thick-walled and shiny, varying in size, abundant, isolated or grouped, opening by apical ostiole, isolated or merged into smaller or larger clusters, 100–550 μM in diameter; conidiophores cylindrical to obclavate, hyaline, simple or branched, 9–35 × 2–3 μM. Two types of conidia were observed, alpha and beta conidia. Alpha conidia were hyaline, unicellular and fusiform to ellipsoid in shape (Figure 2e), biguttulate, 7–12 × 2–3 µM. Beta conidia were single-celled, hyaline, elongated, filiform and thin with a slight curvature at the centre (Figure 2f), 8–23 × 1–2 μM.

Molecular identification of the pathogen - phylogenetic analyses

The alignment contained 47 (Table 1) isolates and the tree was rooted to *Diaporthella corylina* (CBS 121124). The final alignment contained a total of 1,316 characters (ITS: 1-509, TUB: 510-951, TEF:952-1,316) used for the phylogenetic analyses, including alignment gaps. The RAxML analysis of the combined dataset yielded a best scoring tree with a final ML optimization likelihood value of -12417.118871 (Figure 3). The phylogenetic tree that resulted from the ML analysis is given in Figure 3. The bootstrap support values above 50% are given at the nodes. The species name is followed by the strain accession numbers, and the newly introduced strain is in red.

Based on combined ITS, TUB and TEF phylogenetic analysis, the isolate D2 clustered with the isolates of *Diaporthe acutispora* (including the type CGMCC 3.18285). The causative agent was identified as *Diaporthe acutispora* Y.H. Gao & L. Cai. *Diaporthe acutispora* was first isolated from *Coffea* sp. as an endophyte and described in 2017 (Gao *et al*., 2017). Only the asexual morph of *D. acutispora* is known and the sexual morph is not described. The species has also been isolated from *Camellia sasanqua* as an endophyte (Dissanayake *et al*., 2017; Gao *et al*., 2017). *Diaporthe acutispora*, being a relatively new species (Gao *et al*., 2017), not much is known about the species as a plant pathogen or its host range. However, *Diaporthe* species are widely known as important plant pathogens (Dissanayake *et al*., 2017).

Table 1: Sequence data used for phylogenetic analysis. The newly introduced strain is in red.

Taxon	Isolate	GenBank accessions		
		ITS	TUB	TEF
Diaporthe acutispora	LC6160	KX986763	KX999194	KX999154
Diaporthe acutispora	CGMCC 3.18285	NR_152466	KX999195	KX999155
Diaporthe acutispora	LC6142	KX986762	KX999193	KX999153
Diaporthe acutispora	D2	MW255638	MW276072	MW276073
Diaporthe anacardia	CBS 720.97	NR_111841	KC343992	KC343750
Diaporthe aseana	MFLUCC 12-0299	NR_154920	KT459432	KT459448
Diaporthe aspalathi	CBS 117169	NR_165951	KC344004	KC343762
Diaporthe baccae	CBS 136972	NR_152458	MF418509	KJ160597
Diaporthe biconispora	ZJUD62	KJ490597	KJ490418	KJ490476
Diaporthe canthi	CBS 132533	NR_111758	KC843230	KC843120
Diaporthe caulivora	CBS 127268	NG_064239	KC344013	KC343771
Diaporthe chamaeropis	CBS 454.81	KC343048	KC344016	KC343774
Diaporthe cinerascens	CBS 719.96	KC343050	KC344018	KC343776
Diaporthe crotalariae	CBS 162.33	MH855395	KC344024	KC343782
Diaporthe cytosporella	FAU461	MN899309	KC843221	-
Diaporthe dorycnii	MFLUCC 17-1015	NR_152505	KY964099	KY964171
Diaporthe elaeagni	CBS 504.72	KC343064	KC344032	KC343790
Diaporthe elaeagni-glabrae	LC4802	KX986779	KX999212	KX999171
Diaporthe eugeniae	CBS 444.82	KC343098	KC344066	KC343824
Diaporthe foeniculacea	CBS 111553	NR_145303	KC344069	KC343827
Diaporthe hickoriae	CBS 145.26	MH854869	KC344086	KC343844
Diaporthe hongkongensis	CBS 115448	NR_111848	KC344087	KC343845
Diaporthe incomplete	LC6754	KX986794	KX999226	KX999186
Diaporthe inconspicua	CBS 133813	NR_111849	KC344091	KC343849
Diaporthe isoberliniae	CPC 22549	KJ869133	KJ869245	-
Diaporthe litchicola	BRIP 54900	NR_147521	KF170925	JX862539
Diaporthe lithocarpus	CGMCC 3.15175	NR_147524	KF576311	KC153095
Diaporthe macintoshii	BRIP 55064	NR_147539	KJ197269	KJ197251
Diaporthe maytenicola	CPC 21896	NR_137826	KF777250	-
Diaporthe multigutullata	ZJUD98	NR_158389	KJ490454	KJ490512
Diaporthe oncostoma	CBS 589.78	KC343162	KC344130	KC343888
Diaporthe osmanthusis	GUCC9165	MK303388	MK502091	MK480610
Diaporthe parapterocarpi	CPC 22729	KJ869138	KJ869248	-
Diaporthe pseudomangiferae	CBS 101339	NR_111858	KC344149	KC343907
Diaporthe psoraleae	CPC 21634	KF777158	KF777251	KF777245
Diaporthe psoraleae-pinnatae	CPC 21638	NR_137827	KF777252	-
Diaporthe ravennica	MFLUCC 15-0479	KU900335	KX432254	KX365197
Diaporthe rhoina	CBS 146.27	KC343189	KC344157	KC343915
Diaporthe saccarata	CBS 116311	NR_120260	KC344158	KC343916
Diaporthe stictica	CBS 370.54	KC343212	KC344180	KC343938
Diaporthe undulata	LC6624	KX986798	KX999230	KX999190
Diaporthe vawdreyi	BRIP 57887	NG_059129	KR936128	KR936129

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Taxon	Isolate	GenBank accessions		
		ITS	TUB	TEF
Diaporthe velutina	LC4421	KX986790	KX999223	KX999182
Diaporthe woodii	CBS 558.93	KC343244	KC344212	KC343970
Diaporthe xishuangbanica	LC6707	KX986783	KX999216	KX999175
Diaporthella corylina	CBS 121124	KC343004	KC343972	KC343730

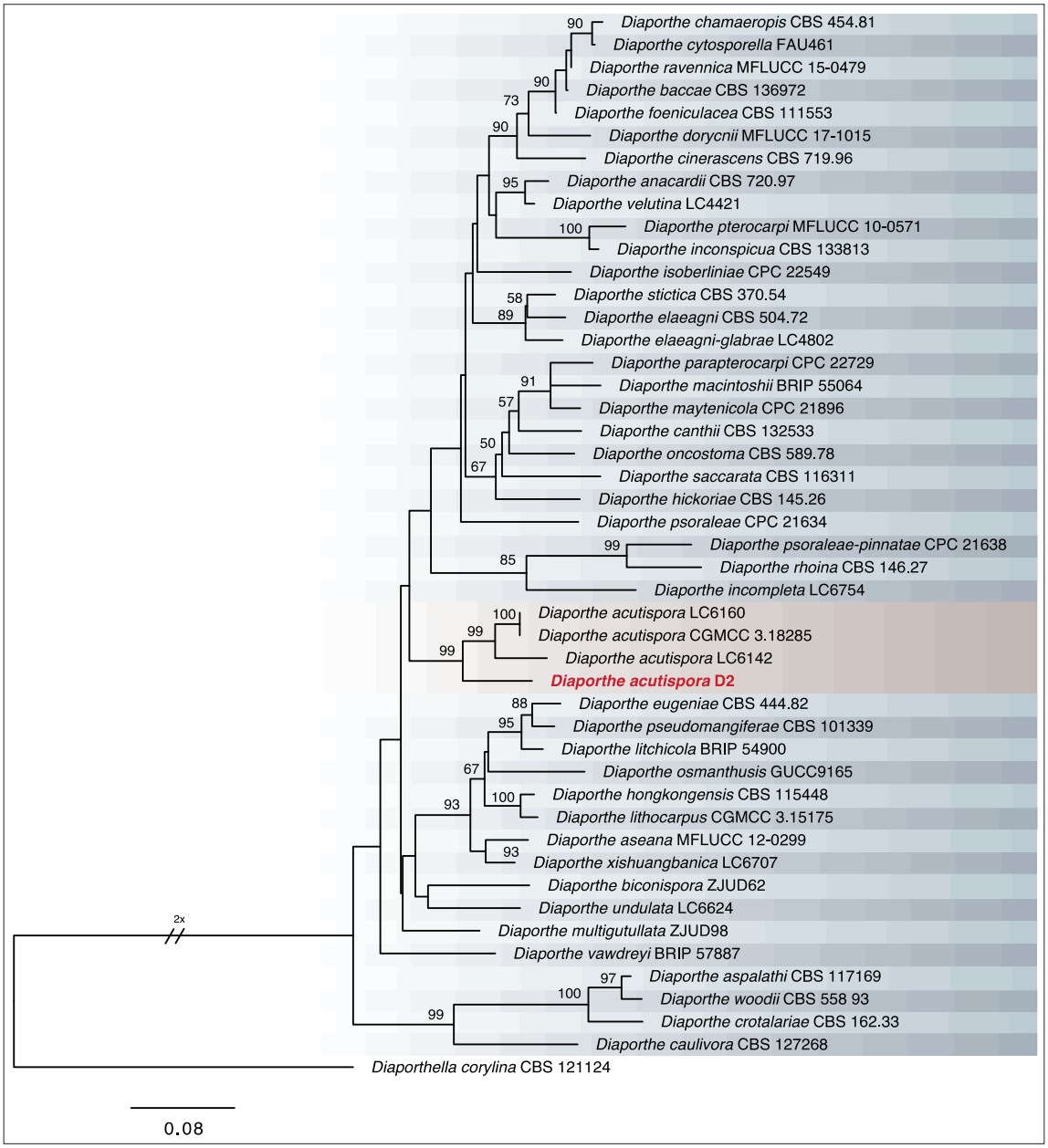

Figure 3: Maximum likelihood tree resulting from a RAxML analysis of the combined (ITS, TUB and TEF) alignment of the analysed *Diaporthe* species. The bootstrap support values above 50% are given at the nodes. The species name is followed by the strain accession numbers, and the newly introduced strain is in red.

Figure 4: Browned mid-rib and secondary veins with extended necrosis of the leaf lamina of *F. religiosa* following artificial inoculation with conidia of *D. acutispora*.

Koch's postulates

The fungus could be repeatedly isolated from diseased leaves and grown on pure culture, indicating its consistent presence. Healthy leaves of *F. religiosa* artificially inoculated with *D. acutispora* showed darkening of the mid-rib and lateral veins, the most consistent symptom observed in the disease (Figure 4). The inoculation also resulted in a dark brown extensive necrosis bordered by already darkened mid-rib and lateral secondary veins 7–14 days after inoculation. There were no symptoms observed in the control plants. Cultural and morphological characteristics of conidia of the fungus obtained from re-isolation were similar to those of the fungus used for inoculation.

Species of *Diaporthe* are known as important plant pathogens, endophytes, or saprobes (Udayanga *et al.,* 2011). They have host ranges, including cultivated crops, trees, and ornamentals (Rossman *et al*., 2007). Some *Diaporthe* species are responsible for severe die-back, cankers, leaf spots, blights, decay or wilts on different plant hosts of which some are economically important (Gomes *et al*., 2013).

Ficus religiosa in Sri Lanka is highly susceptible to *D. acutispora* infection, and once infected, often producing disease symptoms in the entire foliage. *Diaporthe* Leaf Disease could incur considerable damage to the foliage of *F. religiosa*, including the mid-rib and secondary veins interfering with water movement within the leaf leading to potentially devastative consequences. The outcome of the present study could contribute significantly towards diagnosis of the disease, including identification of the causal organism accurately and management which are crucial to understand epidemiology and, prerequisites for developing effective disease management strategies.

Cultural practices such as burning of plant debris (i.e. fallen leaves) or burying them by deep ploughing (Singh, 1987) may reduce disease incidence. Tebuconazole-based fungicide (250 g/L) diluted (5 mL in 10 L) and tested *in vitro* showed 100% inhibition of mycelial growth (*Komala Vithanage, Unpublished data*).

CONCLUSIONS AND RECOMMENDATIONS

The disease, named as *Diaporthe* Leaf Disease (DLD), was new to *F. religiosa.*

The disease is caused by a fungus *Diaporthe acutispora*, identified using cultural and reproductive morphology, DNA sequence analyses, and phylogeny.

The symptoms varied slightly among *F. religiosa* trees, but the darkening of the mid-rib or the vein within the lesion remained a constant characteristic of the disease.

The symptoms described with colour images in the article would conveniently assist diagnosis of the disease.

This is also the first record of *D. acutispora* in Sri Lanka.

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Conflict of interest statement

All the authors disclose that there is no conflict of interest.

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