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ERYSIPHE AUSTRALIANA: THE CAUSE OF POWDERY MILDEW ON CRAPE MYRTLE TREE IN PAKISTAN

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Abstract

A powdery mildew belonging to the genus *Erysiphe* was collected on the leaves of *Lagerstroemia indica* at the two sites in Azad Jammu & Kashmir, Pakis on the leaves of *Lagerstroemia indica* tan. Molecular phylogenetic analyses were carried out based on sequence data of rDNA ITS. After morphological and molecular examinations, the causal agent of this powdery mildew was identified as *Erysiphe australiana*, which is a new record for Pakistan. This contribution to the taxonomic knowledge of powdery mildews may serve as a help to create awareness amongst the growers of ornamentals to adopt control measures.

Key words: Azad Jammu & Kashmir, *Erysiphaceae*, Molecular phylogeny.

Introduction

Lagerstroemia indica L. (Crape myrtle), also commonly known as "Pride of India", hails from the family Lythraceae and has been extensively used as ornamental tree in public parks, gardens and parking lots. It is commonly grown in temperate regions (Kim, 2021).

A common plant disease affecting leaves of Crape myrtle is the powdery mildew *Erysiphe australiana* (McAlpine) U. Braun & S. Takam. (Lee *et al*., 2017). Powdery mildews are common widespread detrimental plant pathogenic fungi globally. About 900 species from 19 genera have been described worldwide (Takamatsu *et al*., 2016; Braun *et al*., 2000; Jin *et al*., 2021). From Pakistan, 52 powdery mildew taxa belonging to 10 genera have been reported. Approximately 80 host plants infected by powdery mildews in Pakistan are so far known, including cereals, ornamentals, fruit trees, nuts, etc. (Afshan *et al*., 2021, 2022a, 2022b, 2022c, 2023; Zafar *et al*., 2022, 2023a, 2023b; Afzal *et al*., 2023; Riaz *et al*., 2024).

In the course of fungal surveys in October to December 2020 and 2021 in different regions of Azad Jammu & Kashmir, this landscape ornamental tree was found to be infected with *Erysiphe* species. Previously, four powdery mildew species have been reported on *L. indica* worldwide (Braun & Cook, 2012; Farr & Rossman, 2022). This species is described morpho-anatomically along with molecular analyses. After careful investigations, the above said fungus was identified as *Erysiphe australiana*, a new report for Pakistan.

Material and Method

Study area, collection & preservation: Plants infected with powdery mildews were collected from District Bagh and Muzaffarabad of Azad Jammu & Kashmir during October and December of the years 2020 and 2021. This area is located in the north-eastern part of Pakistan having an undulating terrain with very rich vegetation, which is predominated by coniferous forests (Dar *et al*., 2012). For preservation the infected plants were dried, pressed, placed in an air tight polythene bag, kept in envelopes, and deposited at the Herbarium of Institute of Botany, University of the Punjab, Lahore, Pakistan (LAH).

Macro-morphological characterization: Morphologically, infected samples were observed using a stereomicroscope (Meiji Techno, EMZ-5TR, Japan). Slides were prepared in lactic acid. Hyphae on the host, shape and size of asci and ascospores were examined by a compound microscope (SWIFT M4000-D) having a 9MP camera system. At least twenty (20) measurements were taken for each diagnostic feature.

Extraction of DNA and PCR amplification: Fructifications were taken from fresh fungal specimens with the help of a razor blade. These were ground in liquid nitrogen and stored in Eppendorf tubes at –18°C. Extraction of DNA was done via Thermo Scientific Gene JET Plant Genomic DNA Purification Mini Kit #K0791. Using PMITS1 as forward primer and PMITS2 as reverse primer and the ITS region was amplified (Cunnington *et al*., 2003). Through a gel documentation system (Sambrook & Russel, 2001), visualization of products of PCR was done with agarose gel having 1% concentration. PCR products were sent for sequencing to Tsingke, China. Raw sequenced data were edited on BioEdit (Hall, 1999) and sequences were searched on BLAST against the database of GenBank (www.ncbi.nlm.nih.gov). Maximum query coverage and percent identity of sequences with related species were noted. All sequences were aligned along with the new sequences through MAFFT (multiple sequence alignment tool). Alignment and trimming of sequences were done at conserved sites from both 5′ and 3′ ends. The phylogenetics tree was executed within MEGA 6.0 (Tamura *et al*., 2013), using ML (Maximum Likelihood Method) based on Kimura 2-parameter with 1000 rapid bootstrap replicates. The selection of the model of evolution was done by searching for the best model of DNA for ML analysis in MEGA 6.0 (Tamura *et al*., 2013). *Golovinomyces ambrosiae* (Schwein.) U. Braun & R.T.A. Cook served as out-group in the analysis of *E. australiana* (Lee *et al*., 2017).

Results

Taxonomy

***Erysiphe australiana*:** (McAlpine) U. Braun & S. Takam., *Schlechtendalia* 4: 17 (2000) (Fig. 1).

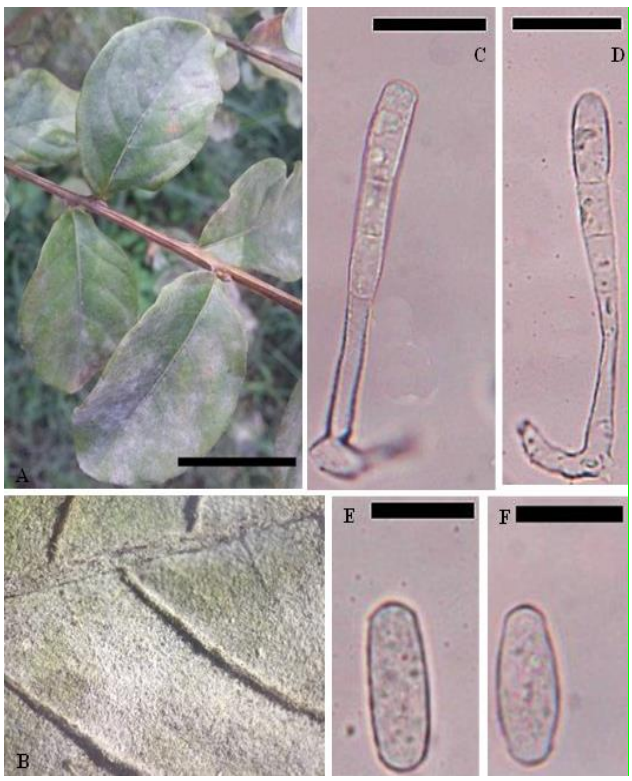

Fig. 1. *Erysiphe australiana*; A = Infected leaves of host plant; B = Infection under stereomicroscope; C–D = Conidiophores; E–F = Conidia; Scale Bars: A = 2 cm, C–D = 30 µm, G–H = 20 µm.

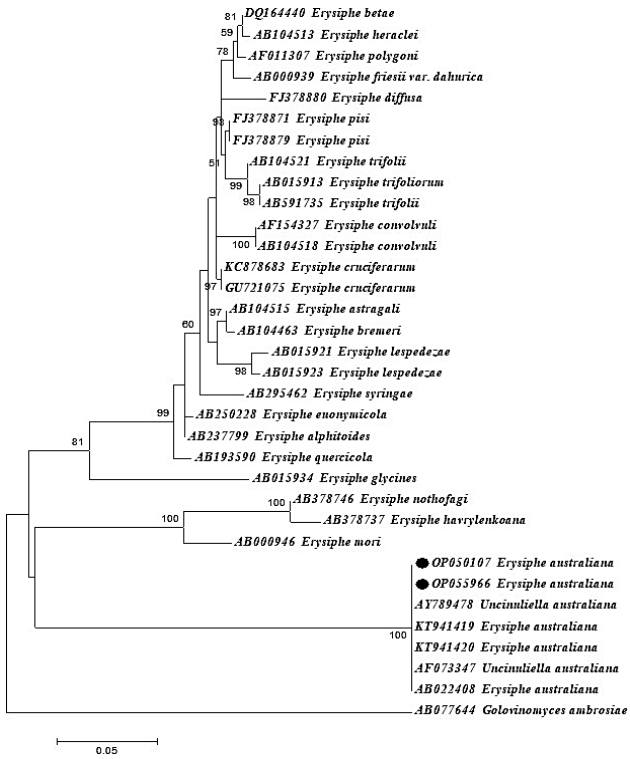

Fig. 2. Maximum Likelihood phylogenetic analysis of *Erysiphe* drawn from dataset of 34 ITS sequences, including one sequence of *Golovinomyces ambrosiae* as outgroup. The amplified sequences are highlighted by a black circle.

Mycelium on leaves and stalks, evanescent to persistent, dense, white or sometimes turning greyish in color, effused or in patches, amphigenous, sometimes conspicuous, covering the whole leaf. **Hyphae** thin, smooth, 2–6 µm wide. **Conidiophores** erect, straight, arising centrally from the adaxial surface of the hyphal mother cell, up to 95 µm long. **Foot cells** straight, 27–45 × 5–8 µm, followed by 1–2 shorter cells developing single conidia. **Conidia** ellipsoid to ovoid and cylindrical, 22–34 × 8–11 µm. **Hyphal Appressoria** multilobed, present on opposite position occasionally, 2–5 µm diam.

Specimens examined-Pakistan. Azad Jammu & Kashmir, Muzaffarabad District: On *Lagerstroemia indica* L., with anamorphic stage, 737 m.a.s.l., Oct. 3rd, 2020, Irsa Zafar and Najam-ul-Sehar Afshan, (MZ-04) (LAH #37467), GenBank accession number (OP050107) (ITS); AZAD JAMMU & KASHMIR, Bagh District: On Lagerstroemia indica L., with anamorphic stage, 1676 m. a.s.l., Dec. 8th, 2021, Irsa Zafar and Najam-ul-Sehar Afshan, (MZ-05) (LAH #37468), GenBank accession number (OP055966) (ITS).

Phylogenetic analysis: Two powdery mildew samples MZ-04 (LAH37467) and MZ-05 (LAH37468) were genetically examined. The sequences of the nrDNA ITS region of these specimens were achieved using both forward and reverse primers. In this study, 34 sequences (Table 1) were used with 2 of them (ITS) being newly generated. BLASTn analysis of the NCBI revealed that these ITS sequences (OP050107) (OP055966) showed 100.00% similarity with *E. australiana* (KY611159, AB022408, MT892941) containing 87% query coverage. The final aligned dataset of the ITS region consisted of 629 characters with gaps; out of these 370 were conserved, 250 were variable, 188 were parsimony informative, and 62 were singleton sites. Entire aligned gaps which are ambiguous in the final aligned dataset were treated as 'missing'. The Maximum Likelihood analysis assembled the sequences of the Pakistani *Erysiphe* collections (OP050107, OP055966) with numerous sequences of *E. australiana* (AY78947, KT941419, KT941420, AF073347, AB022408) showing 100% bootstrap support (Fig. 2). Morpho-anatomically, our species agrees well with *E. australiana* on *Lagerstroemia indica* (Braun & Cook, 2012). On the basis of these results, we identified this powdery mildew fungus as *E. australiana* on *L. indica* in Pakistan. This is a new report for Pakistan.

Discussion

Based on morphological and phylogenetic analyses, the powdery mildew fungus on *Lagerstroemia indica* was identified as *Erysiphe australiana*. This fungal species has been formerly recorded on *Lagerstroemia* spp. causing powdery mildew in Argentina (Delhey *et al*., 2003), Australia, Brazil (Fonesca *et al*., 2015), China, India (Baiswar *et al*., 2009), Italy, Japan, Korea, New Zealand, Portugal, Russia, South Africa, Spain, Switzerland, Turkey (Göre, 2009), Taiwan, USA, United Kingdom, and Ukraine (Braun & Cook, 2012). The morphological features of our anamorphic collections agreed well with the published description of *Erysiphe australiana* (Braun & Cook, 2012), except for the shorter and narrower conidia (22–34 × 8–11 µm vs. 28–40 (–47) × (12–) 13–18 µm). Based on ITS barcoding data, this is a new report of *E. australiana* for Pakistan.

Table 1. Taxa used to construct phylogram with their accession numbers and locality.

Species	Accession number	Host plant	Country
Erysiphe betae	DQ164440	*Beta vulgaris* subsp. *vulgaris*	USA
E. heraclei	AB104513	*Pimpinella affinis*	Iran
E. polygoni	KJ703014	*Polygonum arenastrum*	USA
E. friesii var. *dahurica*	AB000939	*Rhamnus japonica*	Japan
E. diffusa	FJ378880	*Pisum sativum*	USA
E. pisi	FJ378871	*Pisum sativum*	USA
E. pisi	FJ378879	*Pisum sativum*	USA
E.trifolii	AB104521	*Trifolium pratense*	Iran
E. trifoliorum	AB015913	*Trifolium vulgaris*	Japan
E. trifolii	AB591735	*Trifolium pratense*	Japan
E. convolvuli	AF154327	*Convolvulus* sp.	Australia
E. convolvuli	AB104518	*Convolvulus arvensis*	Iran
E. cruciferarum	KC878683	*Brassica rapa* subsp. *pekinensis*	China
E. cruciferarum	GU721075	*Brassica oleracea* var. *acephala*	South Korea
E. astragali	AB104515	*Astragallus* sp.	Iran
E. bremeri	AB104463	*Alhagi* sp.	Iran
E. lespedezae	AB015921	*Lespedeza cuneata*	Japan
E. lespedezae	AB015923	*Lespedeza thunbergii*	Japan
E. syringae	AB295462	*Syringa* sp.	Japan
E. euonymicola	AB250228	*Euonymus japonicus*	Japan
E. alphitoides	AB237799	*Mangifera indica*	Japan
E. quercicola	AB193590	*Quercus phillyraeoides*	Japan
E. glycines	AB015934	*Amphicarpaea edgeworthii* var. *japonica*	Japan
E. nothofagi	AB378746	*Nothofagus pumilio*	Japan
E. havrylenkoana	AB378737	*Nothofagus alpina*	Argentina
E. mori	AB000946	*Morus bombycis*	Japan
E. australiana	**OP050107**	***Lagerstroemia indica***	**Pakistan**
E. australiana	**OP055966**	***Lagerstroemia indica***	**Pakistan**
Uncinuliella australiana	AY789478	*Lagerstroemia indica*	USA
E. australiana	KT941419	*Lagerstroemia speciosa*	Brazil
E. australiana	KT941420	*Lagerstroemia indica*	Brazil
Uncinuliella australiana	AF073347	*Lagerstroemia indica*	Australia
E. australiana	AB022408	*Lagerstroemia indica*	Japan
Golovinomyces ambrosiae	AB077644	*Xanthium strumarium*	Japan

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