

Research Article

Erysiphe diffusa, a New Record for Powdery Mildew Fungi from Pakistan Breeding

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Abstract | During mycological field surveys in 2021, infected leaves of *Lespedeza tomentosa* were gathered from District Swat and Battagram, Khyber Pakhtunkhwa, Pakistan. The infection symptoms seemed to be white powder on foliage with numerous tiny blackened to brownish round ascomata. The causal agent was recognized as *Erysiphe diffusa* based on molecular and morpho-anatomical analyses. This is the first report of *Erysiphe diffusa* on *Lespedeza tomentosa* from Pakistan.

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Keywords | Ascomycota, *Erysiphales*, ITS, Pathogenic fungi, Phylogeny



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Introduction

A group of infective fungi belonging to *Erysiphaceae*, commonly known as powdery mildews, are causing a serious disorder on approximately 10,000 angiosperm plants species (Bradshaw and Tobin, 2020; Amano, 1986). From Pakistan, only 50 species in 10 genera of *Erysiphaceae* are confirmed. *Erysiphe* R. Hedw. is the biggest genus of *Erysiphaceae* with twenty-two species in Pakistan, (Afshan et al., 2021; 2022, 2023). Previously, two species of *Erysiphe* i.e., *E. communis* (Wallr.) Schltdl. and *E. glycines* var. *Lespedezae* (Braun and Zheng, 1985) were observed on the plant genus *Lespedeza* Mich., worldwide (Manjunatha et al., 2020; Far and Rossman, 2012). Genus *Lespedeza* comprises about 40 species, found

in East and Tropical Asia, Tropical Australia and Temperate North America. Five species of *Lespedeza* occur in Pakistan. During field scrutiny in Khyber Pakhtunkhwa, plants of *Lespedeza* with a white powdery mildew fungus on the leaves were found in Swat, and Battagram districts. They were collected, dried and labelled.

Microscopic and molecular phylogenetic investigation of parasitized plants indicate that the causing agent of the disease is associated with *Erysiphe*. A thorough explanation is provided including illustrations of morpho-anatomical structures and a phylogram to demonstrate its accurate taxonomic position with the genus *Erysiphe*.

Materials and Methods

Study area

During field visits of district Swat and Battagram (Khyber Pakhtunkhwa) of Pakistan in 2021, samples of *Lespedeza tomentosa* (Thunb.) Siebold ex Maxim. infected with powdery mildews were gathered. Swat is located in 34.80°N; 72.36°E and 980 meters above sea level. Biari is a small town sited at 34.38°N, 73.24°E at height of 1904 m. a.s.l. in district Battagram. The climatic zone varies from moist temperate to sub-tropical regions (Haq et al., 2010; Haq, 2012). A study of powdery mildew fungi on foliage and inflorescence of *Lespedeza tomentosa* was carried out.

Collection and preservation

The samples of diseased plant were shadow dehydrated on blotting sheets. They were preserved by pressing and placing them in airtight polyethylene envelopes, in brownish paper wrappers with collection notes. The samples were placed at the herbarium of Department of Botany, Hazara University, Mansehra (HUP) and at the Institute of Botany, University of the Punjab, Lahore (LAH).

Morphological description

Photographing infected plant samples was conducted in the field later with EMZ-TR stereomicroscope (Meiji Techno, Japan). The fungi on plants were mounted with lactic acid and examined with LABOMED light microscope. Measurements of microscopic structures i.e., chasmothecia, appendages, asci, ascospores, conidiophores, conidia and appressoria were captured with Scope Image camera software.

Molecular analysis

Molecular phylogenetic analysis was executed. GeneJET Plant Genomic DNA Purification Mini Kit #K0791 (Thermo Fisher Scientific, Waltham, USA) was used to isolate the genomic DNA according to the manufacturer's guidelines. The rDNA Polymerase Chain Reaction was used to amplify PMITS1 and PMITS2 for internal transcribed spacer regions as forward and backward primers (Cunnington et al., 2003). The sequencing were done by TsingKe, China. BioEdit software v.7.2.5 was used for consensus sequences from both primers reads (Hall, 1999). Online NCBI BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) was intended for seeking and gathering similar sequences of ITS regions from GenBank

database (NCBI, 2022). The maximum percentage of uniqueness and query analysis of sequences by linked taxa was recorded. MAFFT (Multiple sequence alignment tool) were used to align all sequences together with the original sequences (Kattoh et al., 2019). The software MEGA 7.0 was used to develop a phylogenetic tree via Maximum Likelihood with 1000 rapid bootstrap copies, using Kimura 2-parameter (Tamura et al., 2013).

Results and Discussion

Morphological study

Erysiphe diffusa (Cooke and Peck) U. Braun and S. Takam. = *Erysiphe lespedezae* (Zheng and Braun, 1983) = *Erysiphe glycines* var. *lespedezae* (Zheng and Braun, 1985), Figures 1 and 2.

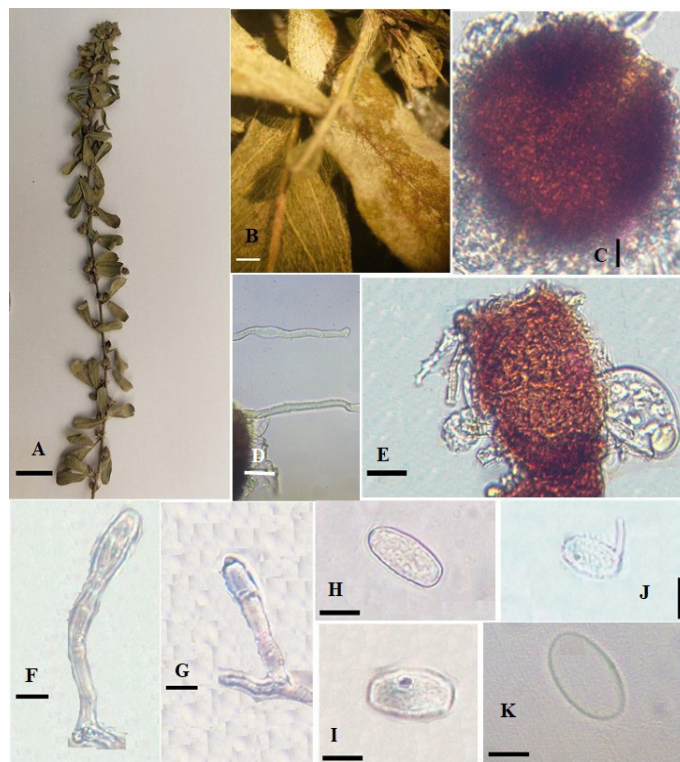


Figure 1: *Erysiphe diffusa* on *Lespedeza tomentosa* A: Infected leaves of *Lespedeza tomentosa* (AFE022,) B: Infection under stereomicroscope C: Chasmothecia D: Chasmothecial appendages E: An Ascus F and G: Conidiophores H and I: Conidia J: Germinating conidium K: An Ascospore. Scale bars: A= 5cm, B= 5mm, C–G= 50µm, H= 20µm, I–K= 10µm.

Mycelium amphigenous, effuse, seldom in spots, transient to persistent; Conidiophores erect, straight to curved, sometimes flexuous, foot cells cylindrical, 25–38 × 8–10 µm, having 1–2 shorter cells that form conidia singly—Conidia were ellipsoid–cylindrical

(-doliiform), 25–35 × 11–17 µm. Chasmothecia sporadic to gregarious, (65) 111–119 µm in diam., sometimes larger; Peridium cells unevenly polygonal, 10–22(–30) µm in diam.; appendages 7–30, arising equatorially, short appendages stiff, about 6–11 µm wide, ellipsoid-obovoid, saccate–Asci 4–10, 40–71 × 26–48(–50) µm, short to long stalked, 3–6 spored–Ascospores ellipsoid-ovoid, 15–21 × 9–15 µm, colourless.

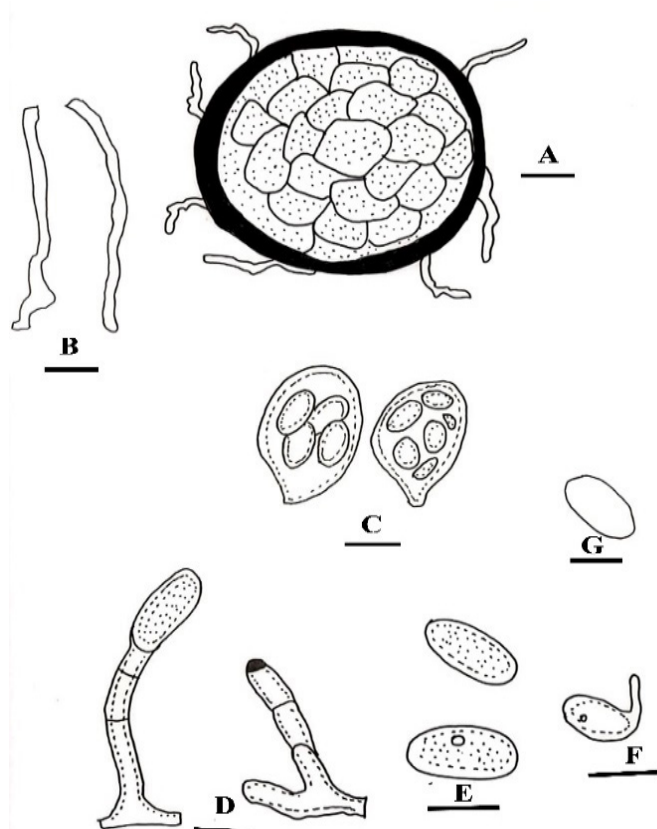


Figure 2: A: Chasmothecium B: Chasmothecial appendages C: Asci D: Conidiophores E: Conidia F: Germinating conidium G: An ascospore. Scale bars: A=5cm, B=5mm, C=50µm, E–F=20µm, G=10µm.

Material examined

On *Lespedeza tomentosa* (Papilionaceae), with anamorphic and teleomorphic stages, Pakistan, Khyber Pakhtunkhwa, District Swat, 980 m a.s.l., 17 August, 2021, (Afzal et al., 2023)(PM-74) (LAH-38040); On *L. tomentosa*, with teleomorph stage, Pakistan, Khyber Pakhtunkhwa, Hazara Division, Battagram District, Biari, 1604 m. a. s. l., 13 October, 2021, (AFE022) (HUP voucher no. 15645).

Molecular phylogenetic analyses

The ITS locus was amplified for all specimens. For reconstruction of frontward and backward sequences, the BioEdit sequence alignment editor was used (Hall,

1999). For a subsequent phylogenetic evaluation, the closest matching sequences were copied from GenBank. Multiple sequence alignment was using MAFFT v 7.0 with all restrictions fixed to default standards (Katoh et al., 2019).

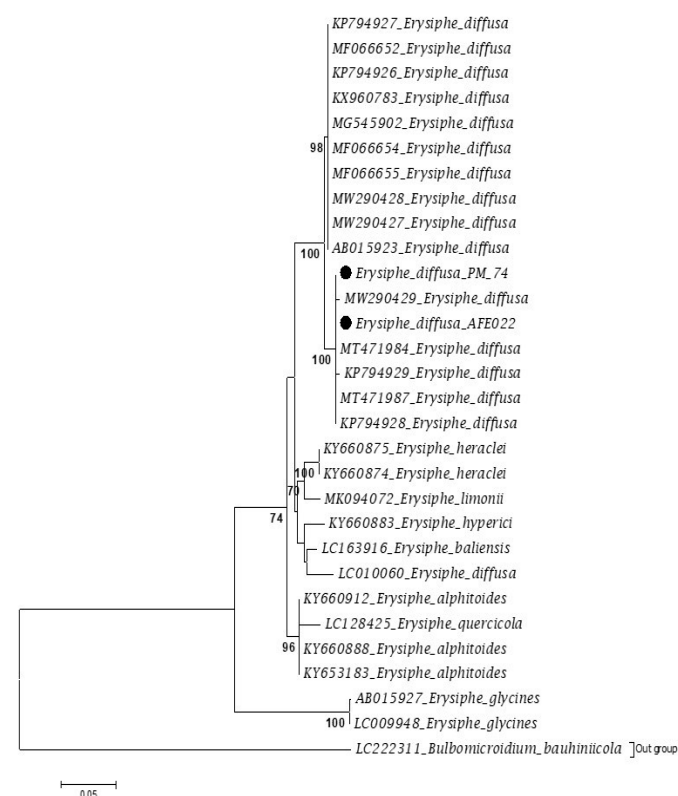


Figure 3: Molecular phylogenetic analysis of *Erysiphe diffusa*. The sequences made from recent collection are obvious with black circles.

Thirty ITS sequences were analysed, of which twenty-eight were already in GenBank (Xiao et al., 2020). A phylogenetic tree was developed from a data matrix of 700 related nucleotide sequences, which consisted for 397 conserved 196 variables, 97 parsimony-informative, and 98 singletons variants. *Bulbomicroidium bauhiniicola* (Braun and Cook, 2012) was picked as an outgroup. In phylogram (Figure 3), the sequences of fungi on *L. tomentosa*, grouped with *E. diffusa* with 100% similarity with other sequences (MT471987, MW290429, MT471984, KP794929 and KP794928) from Taiwan and China.

Lespedeza tomentosa is a shrub belonging to the family Papilionaceae that occur in some countries of East Asia such as China, Korea, Japan, and Pakistan. From August to October, 2021, sporadic white to cottony colonies were monitored on *Lespedeza* foliage that spread to stems producing a minor chlorosis in Swat and Battagram districts, Khyber Pakhtunkhwa,

Pakistan. Morphologically, the conidiophores and conidia of our specimens (PM-74, AFE022) were similar to those of *Erysiphe diffusa* (= *Erysiphe lespedezae*). The fungus was determined to be *E. diffusa* based on its morphological features and ITS rDNA sequences with 100% similarity with other sequences of *E. diffusa* from Taiwan and China (MT471987, MW290429, MT471984, KP794929 and KP794928) from Taiwan and China. *E. diffusa* as *E. lespedezae* on *Lespedeza tomentosa* has been reported from China and in Asia (Braun and Cook, 2012; Farr and Rosman, 2017), as well as in Japan. In Korea, *E. diffusa* (= *E. pisi* DC ex. Saint Amans), has been testified on *Lespedeza bicolor* Turcz. and *L. cyrtobotrya* Miq. (Shin, 2000). *E. diffusa* on *Bauhinia purpurea* L. was described from Thailand by Meeboon and Takamatsu (2017), on *Quercus* species in China and Russia (Lee, 2013). Hence, *Erysiphe diffusa* on *Lespedeza tomentosa* is new record from Pakistan.

Conclusions and Recommendations

Morphological observations and ITS rDNA sequences confirm the identity of powdery mildew isolate of *Lespedeza tomentosa* in Pakistan as *Erysiphe diffusa*. This study reports the first record of this fungus in Pakistan.

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Novelty Statement

This study presents the first documented occurrence of *Erysiphe diffusa*, a powdery mildew fungus, on *Lespedeza tomentosa*. This novel host-pathogen interaction expands our understanding of the fungal pathogen's host range and provides valuable insights into its ecological significance.

Key novelties

- *New host record*: *Erysiphe diffusa* has not been

previously reported to infect *Lespedeza tomentosa*.

- *Ecological implications*: This discovery contributes to a better understanding of the fungal pathogen's distribution, host specificity, and potential impact on the health and growth of *Lespedeza tomentosa*.
- *Disease management*: The identification of this new host-pathogen interaction may inform future disease management strategies for *Lespedeza tomentosa*, particularly in regions where *Erysiphe diffusa* is prevalent.

Author's Contribution

Afshan Akbar: Conceptualization, data curation, writing and review.

Saliha Afzal: Conceptualization, data curation, writing and review.

Najam Ul Sehar Afshan: Conceptualization, administration, review and writing.

Muhammad Fiaz: Administration. **Abdul Nasir Khalid**: Administration, review and writing.

Conflict of interest

The authors have declared no conflict of interest.

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