

## Short Communication

# Morphological and Molecular Characterization of *Erysiphe necator* var. *necator*, A Fungal Pathogen of Grapevines in Pakistan

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**Abstract** | The presence of *Erysiphe necator* var. *necator* causing powdery mildew on grapevines is being reported in Pakistan. Infected plants were collected from districts Mansehra and Abbottabad of Khyber Pakhtunkhwa, and district Lahore of Punjab, Pakistan, during phytopathogenic surveys in 2015–2020. The causal fungus was observed and identified on the basis of morpho-anatomical and molecular analyses. This is the first report of powdery mildew infection caused by *Erysiphe necator* var. *necator* from Punjab and Khyber Pakhtunkhwa provinces of Pakistan.

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**Keywords** | Cystothecaceae, District Mansehra, Erysiphales, Grapevines, Powdery mildew



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## Introduction

Grapevines (*Vitis* L.) are one of the oldest and most significant perennial crops worldwide. They represent a major source of phenolic compounds among fruits. *Vitis* is a genus of 60–70 species, distributed mostly in the temperate regions of the Northern Hemisphere as well as in the tropics and subtropics. It is represented here in Pakistan by three species i.e., *V. jacquemontii* Parker, *V. parvifolia* Roxb. and *V. vinifera* L. (Stewart, 1982). More than 70 percent of the grapes in Pakistan are produced in southwestern Balochistan province and the rest in Khyber Pakhtunkhwa and Punjab provinces. All varieties of grape seeds planted in Pakistan are imported (Saeed, 2020). Among these, *V. vinifera*, a most famous source plant for grapes and

wine, is cultivated worldwide since ancient times. In Pakistan, this important plant is being severely affected by powdery mildew fungi. For their analysis, infected specimens were collected from the districts Abbottabad and Mansehra, Khyber Pakhtunkhwa and from district Lahore, Punjab, Pakistan during 2015–2020. The infected leaf surfaces were fully covered with white powdery mycelial masses with yellowish brown to dark brown chasmothecia indicating infection and disease severity.

After careful morpho-anatomical and molecular analysis, the fungal pathogen was identified as *Erysiphe necator* var. *necator*, which is the first report of this powdery mildew spread in Pakistan. This newly reported powdery mildew infection poses a potential

threat to the health and beauty of grapevines in Pakistan and needs serious attention for developing control strategies.

## Materials and Methods

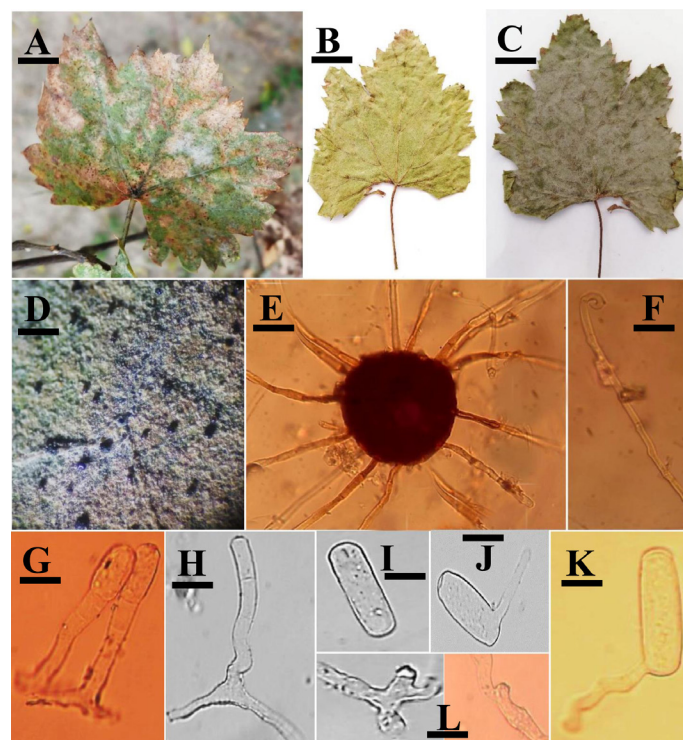
During field surveys of regions of KP and Punjab, Pakistan, leaves of *Vitis vinifera* were found to be infected with powdery mildews. The infected specimens were preserved and photographed both in field and under Meiji EMZ Japan stereomicroscope. For the study of morphological characters, slides were made in lactic acid and then observed under a light microscope (Swift M4000-D Japan). Morphological characters including mycelium on the host; appressoria; size and shape of conidia, conidiophores and chasmothecia; shape, size and number of ascospores; and number of asci per chasmothecium were determined. Thermo scientific gene JET plant genomic DNA purification mini kit was used for DNA extraction and genetic analysis of the sample JP#3 (LAH36139) was carried out. Two primers PMITS1 (5'-TCGGACTGGCC [T/C] AGGGAGA-3') and PMITS2 (5'-TCACTCGCCGTTACTGAGGT-3') were used for amplification of Internal Transcribed Spacer (ITS) region of nrDNA (Cunnington *et al.*, 2003). Amplicons were sent to BGI-Hong Kong (Tsingki) China for sequencing. Following Miller *et al.* (2010), a phylogenetic tree based on maximum likelihood method was constructed using CIPRES portal (Figure 3).

## Results and Discussion

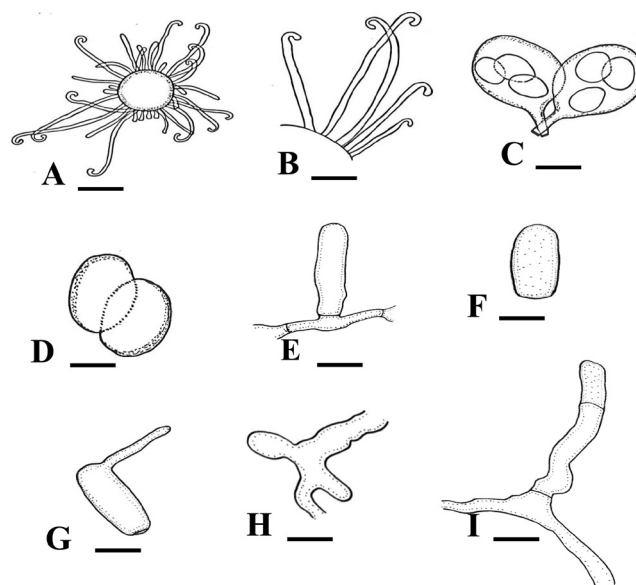
*Erysiphe necator* Schwein., Trans. Am. phil. Soc., New Series 4(2): 270 (1832) [1834] var. *necator* (Figure 1 and 2).

*Mycelium amphigenous*, mostly on leaves, sometimes on fruits, dense, irregular patches as thick mat, persistent. Hyphae thin-walled, septate, hyaline, 2–3 µm in diameter. Hyphal Appressoria solitary or in pairs, slightly lobed to multilobed, up to 7 µm in size. Conidiophores rising from top of hyphal cells, erect or slightly curved, length variable, ranging from 60–200 µm long. Foot cells cylindrical, somewhat straight to twisted at base, mostly from basal septum, followed by 1–3 shorter cells, 35–95 µm long, forming conidia singly. Conidia ellipsoid to obovoid, size variable, 32–43×12–22 µm, germ tubes terminal. Chasmothecia brown to blackish brown, scattered or gregari-

ous, 100–135 µm diam, peridial cells 12–20 µm diam, rounded to polygonal in shape.



**Figure 1:** *Erysiphe necator* var. *necator* A, B, C: Infected leaves of *Vitis vinifera* (JP#3, SA01, SK17), D: Infection under stereomicroscope, E: A Chasmothecium, F: An uncinuate appendage, G: Conidiophores, H: Foot cell along with shorter cells, I: A Conidium, J–K: Germinating conidia, L: Appressoria. Scale bars: A–C = 5 cm, D = 3 mm, E–F = 48 µm, G = 10 µm, H = 7 µm, I = 17 µm, J–K = 19 µm, L = 4 µm.



**Figure 2:** Line drawings of *Erysiphe necator* var. *necator* A: A Chasmothecium, B: Hooked appendages, C: Asci, D: Ascospores, E: A foot cell, F: A Conidium, G: A germinating conidium, H: Appressoria, I: Foot cell along with a shorter cell. Scale Bars: A–D = 40 µm, E = 10 µm, F = 17 µm, G = 20 µm, H = 4 µm, I = 7 µm.

Appendages long, outline somewhat irregular, variable in length, colorless at apex and center, pale yellow or

brown at lower part, thin-walled, smooth or faintly rough, septate, 4–9×183–297µm, apices loosely uncinuate to circinate. Asci ovate to globular or ellipsoid, with short stalk, 4–8 in number, hyaline, having 4–6 ascospores, 35–45 × 50–75 µm. Ascospores ellipsoid to obovoid in shape, hyaline, 13–18×18–25 µm.

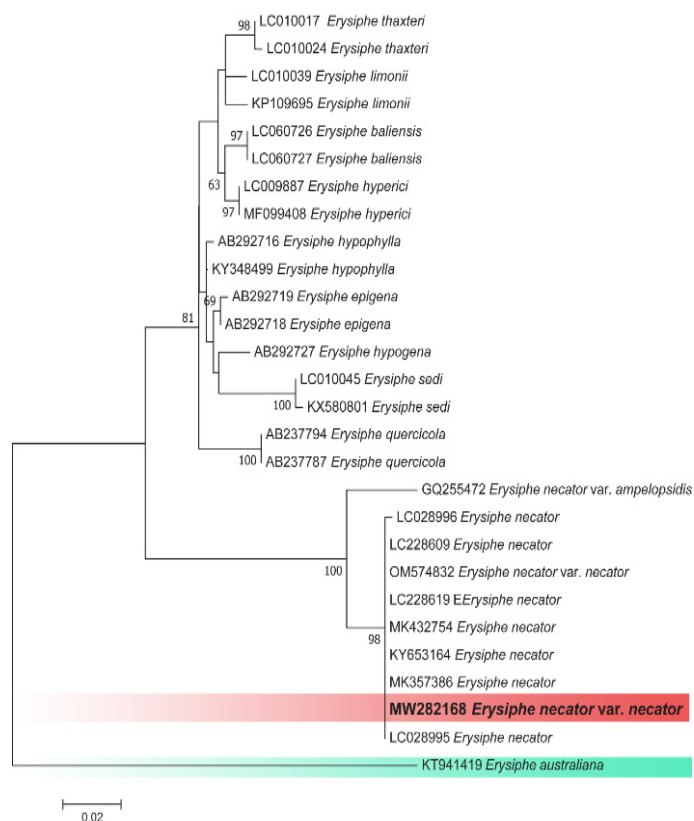
*Material examined*

On *Vitis vinifera* L., asexual stage, PAKISTAN, KHYBER PAKHTUNKHWA, Abbottabad, Mukshpuri 2800 m a. s. l., 12<sup>th</sup> August 2018, Najam-ul-Sehar Afshan, Abdul Rehman Niazi and Javeria Majeed, JP#3 (LAH 36139); On *Vitis vinifera* L., with sexual and asexual stages, Pakistan, Punjab, University of the Punjab, Lahore, at 217 m a. s. l., November 2020, Abdul Rehman Niazi and Shaila Anjum, # SA01(LAH 37452); On abaxial surface of *Vitis vinifera* L., with sexual and asexual stages, Pakistan, Khyber Pakhtunkhwa, Mansehra district, Lasaan Nawab, at 3569 m a. s. l., December 2015, M. Faiz, K. Habib, S. Khanam, # SK17 (HUP Herbarium No. SK-12017).

The morphology of the asexual and sexual morphs of the powdery mildew sample found on *Vitis vinifera* in Pakistan agrees well with the concept and description of *Erysiphe necator* var. *necator* in Braun and Cook (2012) and Darsaraei et al. (2021). It is reported on numerous species of the genus *Vitis*, wherever grapevine is grown (Africa, North and South America, Asia, Australia, New Zealand, Europe) (Braun and Cook, 2012; Farr and Rossman, 2020). Previously, *Uncinula necator* (Schwein.) Burrill (≡ *Erysiphe necator* var. *necator* Schwein.) was reported on *Vitis sylvestris* W. Bartram and *V. vinifera* from Quetta (Malik and Virk, 1968; Ahmad et al., 1997; Afshan et al., 2021). This is the first confirmed report of *Erysiphe necator* var. *necator* from district Lahore, Punjab and Khyber Pakhtunkhwa, Pakistan, as well as the first molecular evidence of this species as *E. necator* var. *necator*.

The initial BLAST search led to consensus sequence of 569 base pairs. To find out comparable sequences, the new sequence was BLAST searched in NCBI database. The initial BLAST revealed that *Erysiphe necator* var. *necator* (MW282168) presented 100.00% identity with *E. necator* (KY653164, MK357386) having 100% query coverage and 0.0 E. value. The phylogram of *E. necator* var. *necator* was represented by three clades (Figure 3). *E. australiana* (KT941419) was chosen as outgroup taxon. *E. necator* var. *necator* clustered in clade 2 along with *E. necator* (LC028996,

LC228619, MK432754, KY653164, MK357386, LC228609 and LC028995) and *E. necator* var. *necator* (OM574832) with the bootstrap value of 100 ML. Only two varieties of *E. necator* are described to date (Braun and Cook, 2012). To further confirm the identity of our species as *E. necator* var. *necator*, a sequence of *E. necator* var. *ampelopsidis* (Peck) U. Braun and S. Takam. has also been included in phylogram which makes separate lineage from *E. necator* var. *necator*.



**Figure 3:** Molecular and phylogenetic analysis of ITS region of *Erysiphe necator* var. *necator* performed by maximum likelihood method in RAxML-HPC2 using CIPRES portal. Amplified sequence is in bold.

**Conclusions and Recommendations**

In the present study, a new powdery mildew fungus that is infectious on grapevine has been collected, characterized and identified as *Erysiphe necator* var. *necator* from Khyber Pakhtunkhwa and Punjab, Pakistan. This is the first confirmed report of *E. necator* var. *necator* from Pakistan, as well as the first molecular evidence of this species as *E. necator* var. *necator*. Grapevine powdery mildew is one of the most important fungal diseases worldwide. All of the green tissues of the grapevine, such as the leaves, stems, buds and berries, can be infected by *E. necator* var. *necator*. Our field observations suggest that grapevines

are being severely affected by this powdery mildew fungus in Pakistan and it would be a potential threat to the health, beauty and cultivation of grapevines in Pakistan. It needs serious attention for developing control strategies against this fungal taxon.

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### Novelty Statement

*Erysiphe necator* var. *necator* causing powdery mildew on grapevines is being reported as an addition to the fungal flora of Pakistan using morpho-anatomical and molecular tools. This is the first molecular evidence of this species as *E. necator* var. *necator* from Pakistan.

### Author Contribution

**Najam-ul-Sehar Afshan:** Corresponding author, preparation of manuscript, supervision of research work.

**Javeria Majeed, Saima Khanum, Maria Riaz and Shaila Anjum:** Morpho-anatomical and molecular characterization of casual fungus.

**Abdul Rehman Niazi and Muhammad Fiaz:** Collection and identification of infected plants and fungus.

### Conflict of interest

The authors have declared no conflict of interest.

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