



# Molecular identification of *Kalanchoe* powdery mildew, *Erysiphe sedi* from Türkiye

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## Abstract

In February 2025, intense powdery mildew disease was observed on leaves and branches of commercial *Kalanchoe blossfeldiana* grown in Erzurum, Türkiye. The infectious agent was identified both microscopically and molecularly. The results showed that infected *K. blossfeldiana* plants with *Erysiphe sedi*. Scanning electron microscopy studies showed that the dimensions of cylinder-shaped conidia were approximately  $37.1 \times 14.5 \mu\text{m}$ . As a result of blast nucleotide analysis performed according to Internal transcribed spacer rRNA (ITS) partial region, it was determined that it showed 100% identity with *Erysiphe sedi* (accession No. MW168996) isolated from *Sedum aizoon* in China, while it showed 99.83% nucleotide similarity with the previously reported isolate from Türkiye (MT568642). In the phylogenetic analysis performed according to Maximum Likelihood, they clustered together with the Chinese isolate, while they clustered quite far from the other Turkish isolate. In the Kimura-2 parameter analysis performed for advanced molecular analyses, the transition/transversion distance between all *E. sedi* isolates was determined as 0.000. Sequence demarcation tool (SDTv 1.3) analysis also provided similarity values supporting both the phylogenetic tree results and the Kimura-2 parameter analysis. In this study, detailed morphological and molecular characterization of *E. sedi*, which was found to infect commercial *K. blossfeldiana* plants intensively in Türkiye, was performed. This study will shed light on the studies that need to be done to biocontrol powdery mildew caused by *E. sedi*.

**Keywords** Plant pathogenic fungi · Greenhouse pathology · Powdery mildew disease · Molecular identification · Ornamental plant disease

## Introduction

Flaming Katy, *Kalanchoe blossfeldiana*, is a popular ornamental plant of African origin, belonging to the family Crassulaceae. It flowers multiple times throughout the year (Milad et al. 2014; Schwabe 2019). In addition to its commercial value, it plays a significant role in reducing the thickness of biofilms formed by bacteria such as *Pseudomonas aeruginosa*, owing to its production of cardioactive glycosides and phenolic compounds, including phenolic acids, flavonoids, and tannins (Sarkar et al. 2015; Assis de Andrade et al. 2023). In addition, a study was conducted for the first time in Türkiye, which reported the use of *K.*

*blossfeldiana* leaves, locally known as woundwort, in the treatment of skin diseases such as boils, abscesses, wounds, and acne (Gürbüz et al. 2019).

Despite its resilience and value, *K. blossfeldiana* is susceptible to several diseases, among which powdery mildew, caused by the obligate biotrophic fungus *Erysiphe sedi*, is of particular concern. *E. sedi* is an unculturable plant pathogen that causes powdery mildew disease, leading to premature leaf drop and reduced plant quality (Ramos et al. 2024). Initially identified on *Sedum* species, *E. sedi* was reclassified in 2019 to affect *K. blossfeldiana* in Germany (Götz et al. 2019). The disease was briefly reported for the first time in Istanbul, Türkiye, in 2021 (Soylu et al. 2021). The disease caused by *E. sedi* is characterised by the formation of white colonies on the leaves and stems of infected plants. Symptoms typically appear approximately seven days after contact between diseased and healthy leaves of *Kalanchoe* plants (Cho et al. 2012). Therefore, the risk of disease

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spread in mass cultivation and retail environments must be carefully considered.

An obligate biotroph, *E. sedi* cannot be cultured in vitro, making it difficult to study in laboratory conditions and precluding traditional diagnostic and screening approaches. Transmission occurs primarily through direct contact between infected and healthy tissues, with the onset of symptoms typically occurring within seven days (Cho et al. 2012). This rapid infection cycle and ease of spread pose a significant risk in intensive production environments, such as greenhouses and nurseries, where plants are frequently in close contact and handled regularly.

There is very little information in the literature about *E. sedi* that has been isolated from *K. blossfeldiana*. This study aimed to characterise *E. sedi* morphologically and molecularly, as it was found to cause severe infections in the leaves and branches of *K. blossfeldiana* cultivated and sold in the eastern region of Türkiye (Erzurum). The study also aimed to contribute to the existing literature on this topic. Such detailed identification studies are crucial for informing future biological control efforts.

## Material and method

### Disease detection in *Kalanchoe blossfeldiana*

Powdery mildew disease was detected in a total of six *K. blossfeldiana* plants grown together and compared macroscopically with healthy *K. blossfeldiana* in a commercial greenhouse where kalanchoe plants were sold in Erzurum, Türkiye, under conditions of 18 °C air temperature and 50% relative humidity. The host plant was identified as *K. blossfeldiana* based on morphological characteristics using standard taxonomic keys (The Plant List 2013). The number of leaves on each infected plant was counted, and the severity of the disease was determined using the powdery mildew disease evaluation scale (Savas et al. 2020). The Townsend–Heuberger formula was applied to the obtained scale values, and the percentage disease severity was calculated (Townsend and Heuberger 1943). Additionally, samples were taken from the leaf surface, and fungus counts were performed on a Thoma slide using a light microscope (Leica, DM750). The average amount of conidia per milliliter was then calculated.

### Microscopic identification of fungi

To examine the conidia and conidiophore structures of the fungus covering the leaves of *K. blossfeldiana* in detail, a slide was prepared and viewed under a light microscope. The fungus, collected from the leaf with a sterile loop, was

placed on a microscope slide and treated with 2.5 µl of lactophenol cotton blue dye. The conidiophore and conidial structures of the fungus were examined under a light microscope (Leica DM750) at 1000x magnification. At the same time, the fungus was removed from the leaf surface with a sterile scalpel, mixed with 20 µL of dH<sub>2</sub>O, and spread onto the stab. After drying overnight, the sample was examined under a scanning electron microscope (FEI Quanta FEG 250) at 2000× magnification. Additionally, the dimensions of the fungal structures, including conidia, conidiophores, foot cells, and hyphae, were measured using scanning electron microscopy.

### Molecular identification of fungi

Fungal samples from diseased *K. blossfeldiana* leaves were collected with a sterile loop, and fungal DNA extraction was performed according to the procedure of the Qiagen DNeasy plant mini kit (Cat. No. 69104). For the identification of the fungus, the Internal transcribed spacer regions (of rDNA) were amplified using primers ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990). The amplification reaction was prepared in a volume of 25 µL [3 µL 35 ng DNA, 1 µL forward and 1 µL reverse primers (10 µM), 12.5 µL master mix, and up to 25 µL dH<sub>2</sub>O]. According to the manufacturer's instructions (EcoTech Biotechnology, Türkiye), the reaction programme was set as follows: pre-denaturation at 98 °C for 30 s, 35 cycles at 94 °C for 10 s, 53 °C for 15 s, 72 °C for 60 s, and a final extension at 72 °C for 60 s. After the reaction, the sample was subjected to agarose gel electrophoresis at 70 V for 50 min. The sample showing a single and thick band under the UV gel documentation device was sent to a bioinformatics company (Sentebiolab, Ankara-Türkiye) for Sanger-dideoxy sequence analysis. The obtained sequence was trimmed at the beginning and end to remove low-quality reads, and then compared with samples in the blastn database to determine the species name. To determine the taxonomic position of our isolate's ITS region sequence compared to other isolates in the database, a phylogenetic tree was constructed using the Maximum Likelihood method with the Tamura-Nei model in MEGA11 (Tamura et al. 2021). Bootstrap analysis with 1000 replicates was performed to assess the reliability of the inferred branches. Only bootstrap analysis was conducted because it provides a straightforward estimate of branch support, indicating the confidence in each clade; bootstrap values above 70% are generally considered to reflect strong support. Phylogenetic analysis, Kimura-2 parameter analysis, and SDT analysis were performed for the ITS partial sequences of 1 neotype of *E. sedi* (*Pseudoidium kalanchoes*), 13 *E. sedi*, 4 *Erysiphe neolycopersici*, 3 *Erysiphe*

*aquilegia*, 2 *Erysiphe tortilis*, and 2 *Erysiphe passiflorae*. *Golovinomyces sordidus*, a member of the *Golovinomyces* genus, was used as the outgroup. Kimura-2 parameter analysis was performed in MEGA11 to determine the transition-transversion rate between the nucleotide sequences of the samples. Additionally, the nucleotide sequences of all samples were aligned using ClustalW and the sequence demarcation tool (SDT v1.3). The sequences were visualised using a colour-coded % similarity diagram to determine the pairwise identity matrix.

Results

Disease detection in *Kalanchoe blossfeldiana*

It was observed that all the branches and leaves (on the front and back of the pots) were heavily infected with powdery mildew. A macroscopic comparison was made with a healthy plant (see Fig. 1). It was also observed that some of the leaves had wilted and that the loss had started with the smaller leaves. All the leaves were then counted, and according to the powdery mildew disease evaluation scale, a scale value of 4 was determined, i.e., more than 60% of the leaf surface was covered in powdery mildew. The

Townsend–Heuberger formula was applied to the obtained scale values, and the percentage disease severity was defined as 100% (Townsend and Heuberger 1943). Additionally, samples were taken from the leaf surface with mixed dH<sub>2</sub>O, and the number of fungi was counted on a Thoma slide using a light microscope (Leica DM750). We calculated that there were 1.3 × 10<sup>9</sup> conidia per millilitre within a leaf measuring 2.5 × 4 cm.

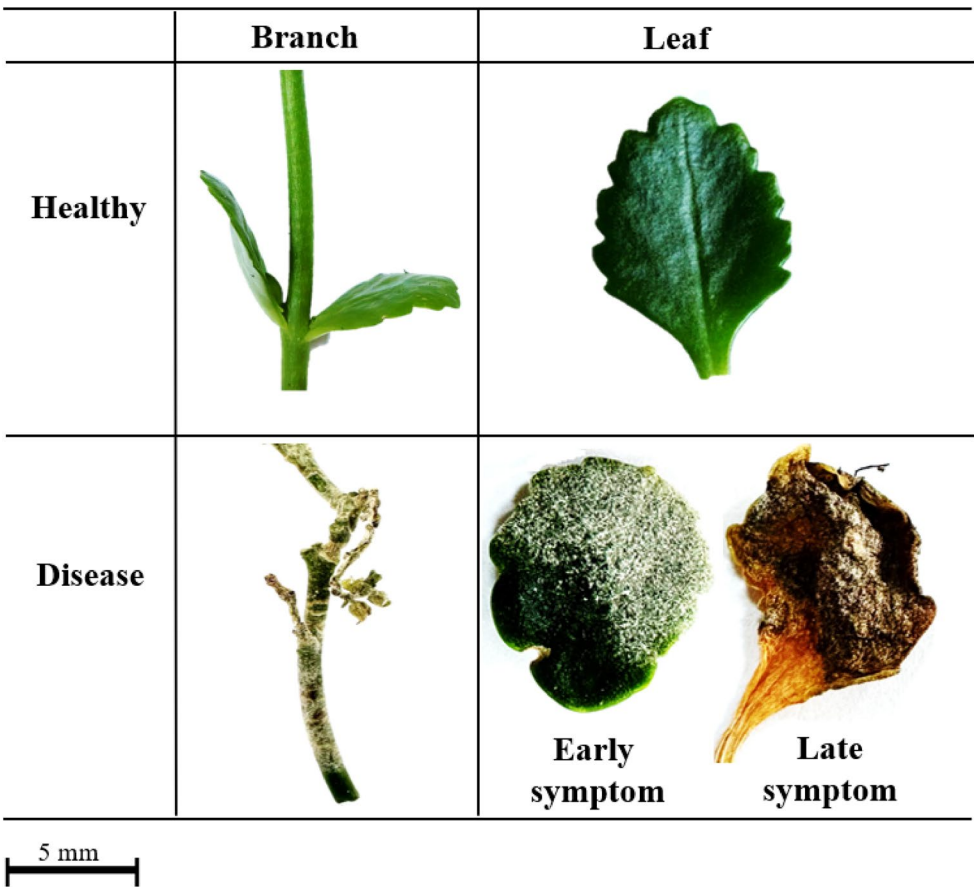
Microscopic identification of fungi

*E. sedi* was stained with lactophenol cotton blue and examined under a light microscope. It was determined that the conidia and conidiophores had a typical cylindrical morphology (Fig. 2a). Scanning electron microscopy revealed that the cylinder-shaped conidia measured 37.1 × 14.5 µm, were cylindrical in shape, and were borne on conidiophores measuring 72–138 × 6.8–13.2 µm; foot cells were 34 µm long, hyphae had a width of 5.5 µm, and no chasmothecia were observed (Fig. 2b).

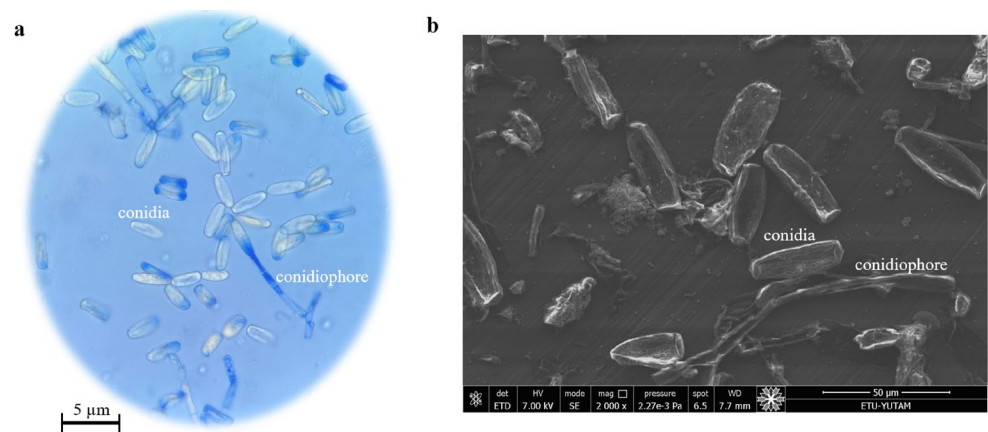
Molecular identification of fungi

As a result of PCR amplification, an approximately 614-nucleotide fragment from the ITS-rDNA and partial

**Fig. 1** Morphological comparison of healthy and diseased *Kalanchoe blossfeldiana* branches and leaves



**Fig. 2** Microscopic observation of *Erysiphe sedi*. **(a)** Light micrographs of conidia and conidiophore *Erysiphe sedi* (1000x), **(b)** Scanning electron micrographs of conidia and conidiophores of *Erysiphe sedi* (2000 x)



LSU regions was obtained, and sequence analysis was subsequently performed. In the sequence analysis, the bad sequences read from the beginning and end were trimmed, and the 614-nucleotide partial sequence was blasted, and it was determined that it showed 100% similarity to the *E. sedi* Chinese isolate (accession number: MW168996) isolated from *Sedum aizoon*. Accordingly, the sequence from *E. sedi* isolate E1 was deposited in GenBank (NCBI) under accession number PV596678. Its similarity to *E. sedi* (accession number: MT568642), previously isolated from the same plant in Türkiye, was determined as 99.83% in blastn. As a result of the phylogenetic tree drawn according to the ITS gene region sequences of *E. sedi* E1 Türkiye isolate and other *Erysiphe* species from the database, it was determined that *E. sedi* E1 clustered together with the neotype *Pseudoidium kalanchoes*, with a bootstrap support of 73% (Fig. 3). In the Kimura-2 parameter analysis, the transition/transversion distance between all *E. sedi* isolates and the neotype was determined as 0.0000 for the ITS nucleotide sequences. The transition/transversion distance of the isolates of *E. neolycopersici*, *E. aquilegia*, and *E. tortilis* isolates to the *E. sedi* Türkiye isolate in this study was 0.0017, while that of the isolates of *E. passiflorae* was 0.0035 (Suppl. 1). SDT analysis also provided similarity values supporting the phylogenetic tree results and Kimura-2 parameter analysis. In the dendrogram, samples showing 100% identity were represented in dark red, samples showing 99% identity were represented in light red, and samples showing 70% and above identity were represented in green (Fig. 4). Accordingly, in the identity matrix, *E. sedi* isolated in this study and all other *Erysiphe* isolates were shown to have over 99–100% identity (Fig. 4).

## Discussion

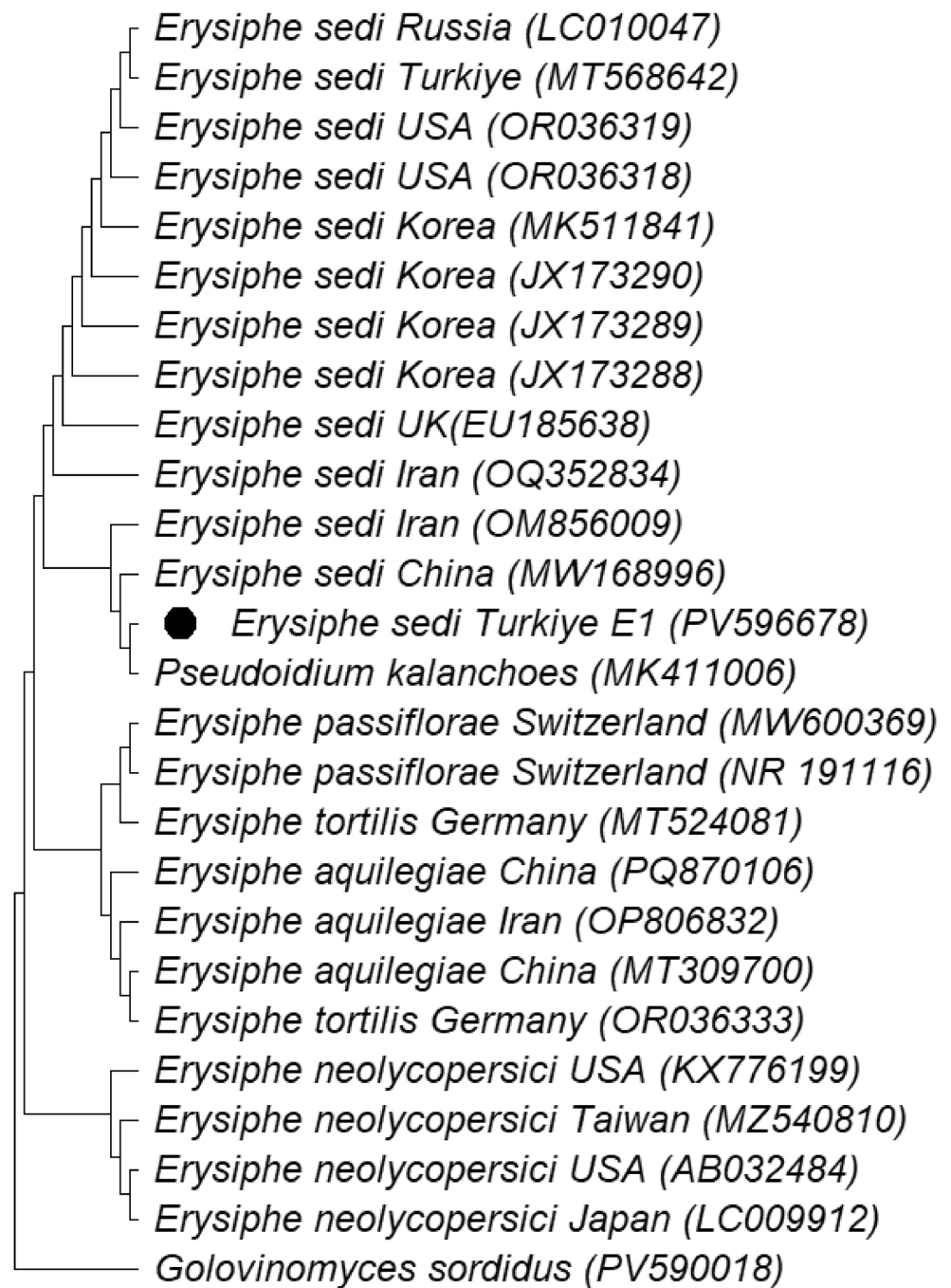
Our *Kalanchoe blossfeldiana* specimen aligns with the type description in terms of leaf size, shape, glabrous surface, and tubular, 4-lobed flowers, with minor variations likely due to environmental conditions (von Poellnitz 1934).

*E. sedi* was initially identified as a pathogen of *Sedum* species, but it has since been reported to infect other members of the Crassulaceae family, including *Hylotelephium*, *Phedimus*, *Rhodiola*, and *Kalanchoe* (Götz et al. 2019; Shi et al. 2020). *E. sedi* shows strong specialisation towards aquatic and semi-aquatic taxa within its host plant range, suggesting ecological and physiological adaptations. The expansion of its host plant range to include *K. blossfeldiana* was first documented in Germany in 2019 (Götz et al. 2019), indicating a significant epidemiological shift.

Ramos et al. (2024) reported the presence of *E. sedi* in autumn. However, in this study, the fungus was found during winter in a commercial greenhouse where *Kalanchoe* plants were sold in Erzurum, Türkiye, under conditions of 18 °C air temperature and 50% relative humidity. Although this plant needs little water because it is adapted to dry environments, excessive irrigation in some greenhouses may also support fungal growth. Irrigation and high humidity create very favorable conditions for other fungi, such as phylloplane fungi on leaf surfaces and saprotrophic fungi in soil and potting medium (Li and LaMondia 2010).

Macroscopic observations indicated intense powdery mildew disease on both the front and back sides of branches and leaves. Detailed microscopic and molecular analyses were performed to detect this fungus. The morphology of *E. sedi* has been described as hyaline, cylindrical conidiophores and cylindrical or ellipsoidal conidia arranged in small chains (Ramos et al. 2024). In this study, conidiophores and conidia were examined using light and scanning electron microscopes. In this study, the cylinder-shaped conidia measured  $37.1 \times 14.5 \mu\text{m}$ , conidiophores were  $72\text{--}138 \times 6.8\text{--}13.2 \mu\text{m}$ , foot cells  $34 \mu\text{m}$  long, and hyphae  $5.5 \mu\text{m}$  wide. Previous

**Fig. 3** Phylogenetic tree of *Erysiphe sedi* isolated from *Kalanchoe blossfeldiana* as per the partial ITS gene. The phylogenetic tree was constructed with MEGA11 software (1000 replicates) using the Maximum Likelihood method and the Tamura-Nei model. The marked branch represents the sample obtained from the study



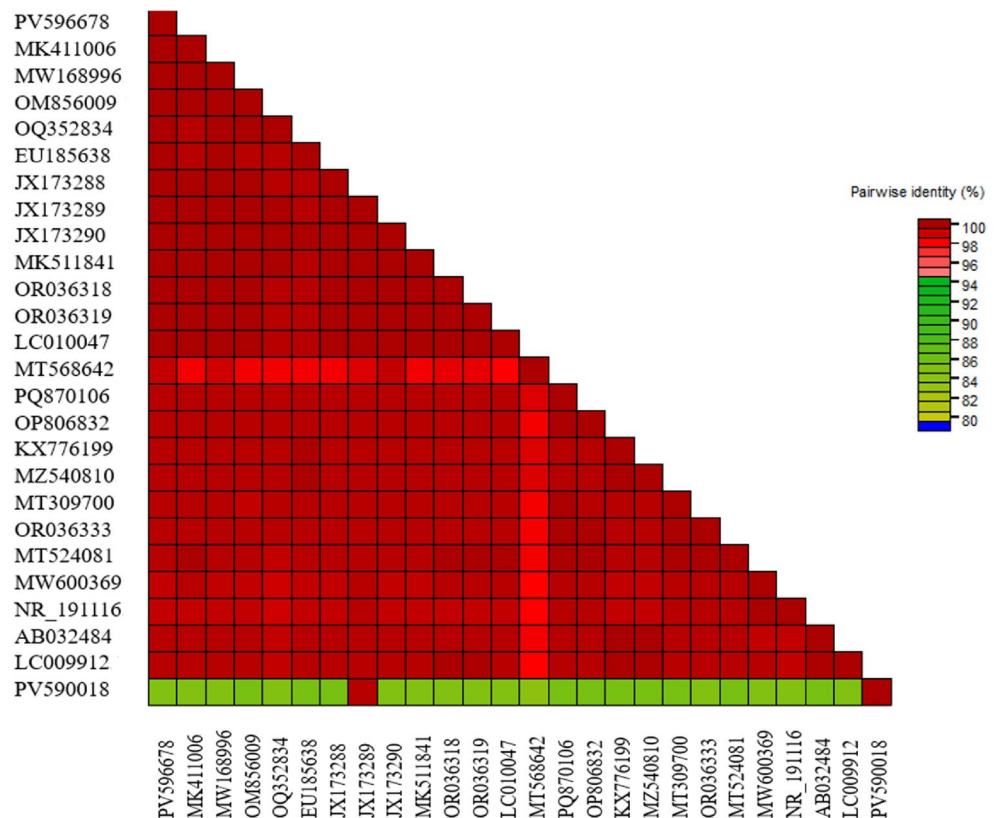
studies reported similar dimensions: Cho et al. (2012) – conidia 30–55 × 14–24 µm, conidiophores 70–145 × 7–11.5 µm, foot cells 28–48 µm, hyphae 3–6 µm; Fang et al. (2017) – conidia 28–50 × 12–20 µm, conidiophores 58–126 µm, foot cells 20–46 × 6–12 µm, hyphae 3.1–6.6 µm; Ramos et al. (2024) – conidia 26–55.5 × 14–20.5 µm, conidiophores 48.5–78.5 (–93) µm.

Approximately 10 years ago, Takamatsu et al. (2015) created the *Erysiphe aquilegiae* complex for the phylogeny of *Erysiphe* species. However, Shin et al. (2019) argued that this classification was inadequate as it was based solely on the

internal transcribed spacer (ITS) region of the nuclear ribosomal DNA, and recommended using the phylogenetic classification by Götz et al. (2019) as a basis, with *E. sedi* considered separately. In this study, 13 *E. sedi* isolates clustered together with the neotype *Pseudoidium kalanchoes* based on ITS partial gene sequences. This joint clustering indicates the close genetic relationship of the isolates with the neotype, supporting their identification as *E. sedi* and confirming previous phylogenetic observations within the genus *Erysiphe* (Götz et al. 2019).

To our knowledge, this is the first study to examine *Erysiphe* from *Kalanchoe* using Kimura-2 and SDT analyses. These two

**Fig. 4** A colour-coded pairwise identity matrix was generated using the Sequence Demarcation Tool (SDT v1.3) for 24 *Erysiphe* isolates and 1 outgroup taxon based on rDNA ITS regions. Each coloured cell in the matrix shows the percentage identity score between two sequences (one shown horizontally to the left and one shown vertically below). A colour key shows the level of agreement between the colours in the matrix and the pairwise identities



analyses are important because they are based on different parameters that corroborate the phylogenetic tree data (Kimura 1980; Muhire et al. 2025). The phylogenetic tree previously isolated from *Crassula capitella* for *E. sedi* was drawn according to the Kimura-2 parameter model, and the extra Kimura-2 parameter compute pairwise distance analysis was not performed (He et al. 2020). This study is also the first to use SDT analysis for *Erysiphe*, thereby demonstrating its importance in species demarcation and corroborating the findings of the other two analyses. As a result of Kimura-2 parameter analysis of *E. sedi* isolates that clustered together in the phylogenetic analysis, the transition/transversion distance was determined as 0.0000 for ITS nucleotide sequences. Similarly, it was determined that they showed 99–100% identity in SDT analysis.

Although *E. sedi* was reported to cause powdery mildew on *K. blossfeldiana* in Türkiye, detailed morphological and advanced molecular analyses have not been performed (Soylu et al. 2021). In this study, it was determined that *E. sedi* caused severe powdery mildew on *K. blossfeldiana*, and its molecular status with other *Erysiphe* isolates was analyzed. Thus, the necessary studies for the control of this fungus will be initiated.

## Conclusion

Historically, powdery mildew diseases have been managed using chemical fungicides. However, concerns about fungicide resistance, phytotoxicity, environmental impact, and chemical residues have led to a shift towards biological control strategies (Lahlali et al. 2022). Therefore, there is an urgent need to develop biological control agents for the control of *E. sedi*. Transmission of the fungus to other ornamental plants in the same family will increase the disease potential, leading to economic loss and reduced product quality. This is important for all species in the Crassulaceae family in greenhouses. In this study, detailed morphological and molecular characterization of *E. sedi*, which was found to infect commercial *K. blossfeldiana* plants intensively in Türkiye, was performed. This study will shed light on the studies that need to be done to biocontrol powdery mildew caused by *E. sedi*.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s42161-026-02189-w>.

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**Author Contributions** Merve Almula Bakırdöğen Bakar: Conceptualization, methodology, and data curation, özde Büşra Eroğlu: Investigation, writing original draft, and supervision.

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**Data availability** The datasets generated or analyzed during this study are included in this published article.

## Declarations

**Ethics approval and consent to participate** Not applicable.

**Consent for publication** All authors are agreeing for publication.

**Conflict of interest** The authors have no competing interests.

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