**Geliş(Recevied)** :12.10.2022 ***Research Article***
**Kabul(Accepted)** :19.12.2022 **Doi: 10.30708.mantar.1187178**

# Phenotypic and Molecular Identification of Green Pea Powdery Mildew Pathogen from Alborz, Iran

Simin Tahery ARDESTANI[1*], Seyed Akbar KHODAPARAST[2]
Ahmad Abbasi MOGHADAM[3], Mehrzad AHMADI[4]

**Corresponding author: simintahery@ gmail.com*

[1*] Seed and Plant Improvement Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran
Orcid ID: 0000-0002-9273-7901 simintahery@ gmail.com
[2] Department of Plant Protection, College of Agricultural Sciences, University of Guilan, Rasht, Iran.
Orcid ID: 0000-0002-7480-4170/ khodaparast@guilan.ac.ir
[3] Seed and Plant Improvement Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran
Orcid ID: 0000-0001-5984-6455/ abasimoghadam@ gmail.com
[4] Seed and Plant Improvement Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran
Orcid ID: 0000-0001-7333-9660/ ahmadimehrzad@ymail.com

**Abstract:** Green pea (*Pisum sativum.*) is an important consumable vegetable crop worldwide rich in protein, vitamins, and nutrition. It is cultivated in many parts of Iran, particularly during the summer season, and recently under protected greenhouse. Powdery mildew is an important disease of green pea, especially during the pod fill and maturation stage and decreases the photosynthetic area of leaves which causes crop loss. This experiment was conducted in order to identify fungi associated with powdery mildews on some accessions under regeneration of green pea collection at the genetic department and national plant gene bank of Iran, seed, and plant improvement institute during the year 2021. Powdery mildew became epidemic on the plants at the pod stage from the end of July to August. The formation of white, floury patches initially on the leaf progressing towards tendrils, pods, and stems observed that on susceptible accessions covered the most aerial part of the plants. The *Erysiphe pisi*, *Erysiphe trifolii* and *Erysiphe baeumleri* were reported causing disease on pea. The infected plant samples were collected and transferred to the lab for morphological studies, then, the genomic DNA was isolated and used for amplification and sequencing using the internal transcribed spacers (ITS) regions. Based on morphological characteristics of asexual and sexual stages as well as rDNA-ITS sequence analyses, the fungus was identified as *Erysiphe pisi.* Due to the presence of accessions with high levels of resistance to this disease, further investigation is recommended to breed resistant cultivars for cultivation in this province.

**Key words:** Pisum sativum, Erysiphe pisi, rDNA, ITS sequence analyses

# Alborz, İran'dan Yeşil Bezelye Külleme Patojeninin Fenotipik ve Moleküler Tanımlanması

**Öz:** Bezelye (*Pisum sativum*.), dünya çapında protein, vitamin ve besin açısından zengin, önemli bir tüketilebilir sebze ürünüdür. İran'ın birçok yerinde özellikle yaz mevsiminde ve son zamanlarda örtülü seralarda yetiştirilmektedir. Külleme, yeşil bezelyenin özellikle bakla doldurma ve olgunlaşma aşamasında önemli bir hastalığıdır ve yaprakların fotosentetik alanını azaltarak ürün kaybına neden olur. Bu deney, 2021 yılında İran genetik departmanı ve ulusal bitki gen bankası, tohum ve bitki ıslah enstitüsünde yeşil bezelye koleksiyonunun rejenerasyonu altındaki bazı çeşitlerde külleme ile ilişkili mantarları belirlemek için yapılmıştır. Temmuz sonundan ağustos ayına kadar bitkiler bakla aşamasındadır. Başlangıçta yaprak üzerinde dallara, baklalara ve gövdelere doğru ilerleyen beyaz, unlu yamaların oluşumu, duyarlı erişimlerde bitkilerin en hava kısmını kapsadığı gözlendi. *Erysiphe pisi*, *Erysiphe trifolii* ve *Erysiphe baeumleri*'nin bezelyede

hastalığa neden olduğu bildirilmiştir. Enfekte bitki örnekleri toplandı ve morfolojik çalışmalar için laboratuvara aktarıldı, ardından genomik DNA izole edildi ve dahili transkripsiyonlu ayırıcılar (ITS) bölgeleri kullanılarak amplifikasyon ve sekanslama için kullanıldı. Eşeysiz ve eşeyli evrelerin morfolojik özelliklerine ve ayrıca rDNA-ITS sekans analizlerine dayanarak, mantar *Erysiphe pisi* olarak tanımlandı. Bu hastalığa karşı yüksek düzeyde dirençli çeşitlerin varlığından dolayı, bu ilde dayanıklı çeşitler üretmek için daha fazla araştırma yapılması önerilir.

**Anahtar kelimeler:** *Pisum sativum*, *Erysiphe pisi*, rDNA, ITS dizi analizleri

**Introduction**

So far, the research on green pea production focuses on the breeding high yield cultivars adopted to different climatic condition, but vulnerability against diseases was a constraint for obtaining maximum yield.

Green pea production is practiced in some area of Alborz province of Iran according to economical and nutritional values including protein, minerals, dietary fiber, vitamins and its ability to improve soil fertility by nitrogen assimilation.

Powdery mildews on pea found responsible for the withering of foliage, poor pod quality with 25%–80% yield loss (Nisar et al., 2006; Warkentin et al., 1996).

It is a prevalent disease in Alborz province of Iran which causes significant yield losses of green pea especially in summer.

Powdery mildew appears on whole aerial parts of green pea plants. The initial symptoms are slight, sparse mycelia and spores on the upper part of leaves, especially on the lowest part of plants. In favorable conditions, the patches cover the entire surface of the plant (Kraft & Pfleger, 2001).

Up to now, the causal agent of powdery mildew on peas reported as *Erysiphe pisi*, *Erysiphe baeumleri*, and *Erysiphe trifoliorum* (Seethapathy et al., 2022; Fondevilla & Rubiales, 2012; Attanayake et al., 2010; Glawe, 2008).

The *Erysiphe pisi* DC causal agent of Pea powdery mildew reported as an air-borne disease that is the obligate biotrophic fungus with worldwide distribution. It is specialy described important in climate condition with warm dry days and cool nights (Smith et al., 1996). Seethapathy et al., (2022) found that pathogen has evolved the ability to adapt to both macro- and micro-environments to which it is exposed during pea cultivation.

Glawe (2008) denoted that *Erysiphe* species rDNA sequences analyses could be more closely correlated lineages with anamorphic features such as conidial ontogeny than teleomorphic features. Detection and proper identification of this disease causal agent illustrated the necessary action for sustainable and environmentally friendly production of green peas with integrated disease management (Maharjan et al., 2015).

To precise identification of the powdery mildew pathogen on green peas in the Alborz province of Iran, morphological studies carried out then the molecular analyses were performed on fungal isolates using rDNA internal transcribed spacer (ITS) sequences.

**Materials and Methods**

Powdery mildews disease on some under regeneration green pea accessions appeared during 2021 and 2022. These accessions were cultivated at the experimental field of the genetic department and national plant gene bank of Iran, seed and plant improvement institute in Alborz province. The disease symptoms were observed during all stages of the green pea plants' development.

A phenotypic and genotypic analysis of pea powdery mildew pathogen was performed on diseased green pea leaves collected from the fields. The phenotypic fungal structures including anamorphic structure: mycelium, hyphal branching, Appressoria, conidiophors and conidia teleomorphic stracture including Chasmothecium and asci were observed by light microscope (BH2, Olympus, Tokyo, Japan). At least 50 measurements were made for each character. In order to study genetic characteristics, DNA was extracted from fungal isolates using the Chelex method proposed by Hirata & Takamatsu (1996). The rDNA-ITS region (ITS1-5.8S rDNA-ITS2) was amplified by polymerase chain reaction (preheating at 95°C for 2 min and 30 cycles of denaturing at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min, followed by a final extension cycle of 2 min at 72°C) with the fungal specific primers ITS-1a 5′-TCCGTAGGTGAACCTGCGG-3′ and ITS-4a 5′-TCCTCCGCTTATTGATATGC-3′ (Matsuda, et al., 2005). The sequences were obtained using direct sequencing in a 3500 Genetic Analyzer (Applied Biosystems, USA) in Codon Genetic Group (Iran,

Tehran). The obtained sequences were compared with closely related sequences in genebank using the BLASTN search method. The sequences with high query coverage and identity (97-100%) were selected and used for phylogenetic analyses. According to Kimura's two-parameter method, evolutionary distances were calculated. Pairwise deletion was used for each sequence pair to remove all ambiguous positions. The branches made by using 1000 bootstrap replications. Evolutionary analyses were conducted in MEGA11(Tamura et al., 2021). Two species including *Phyllactinia moricola* and *Leveillula taurica* were selected as outgroup taxa.

**Results**

Symptoms of powdery mildew were observed on all aerial parts of plants during summers 2021-2022, including stems, leaves, pods, and fruits of green pea cultivars planted for seeds regeneration. Green pea accessions typically showed disease symptoms with 0 to 100 % severity on different accessions. Early symptoms appeared on the lower leaves of plants, followed by effuse, thin, white mycelium covering the upper leaves. After the mycelium has developed on both sides, it often covers the entire surface of the leaves, stems, and pods, as well as covering the entire area of the plant, particularly the upper portion.

Powdery mildew became epidemic on the plants at the pod stage at the end of July to start of August. The formation of white, floury patches initially on the leaf progressing towards tendrils, pods, and stems observed that on susceptible accessions covered the most aerial part of the plants (Fig. 1).

Figure 1. Powdery mildews symptom on green pea plant in the field

The morphological fungal characteristics were observed as hyaline and flexuous, hyphae which were branched at a right angle. Appressoria is moderately lobed with a crenated surface. Conidiophores (65 µm - 110 µm in length and 7.7 µm-10.2 µm in width) are hyaline, straight, cylindrical, simple, and thin-walled with internal septa which produced 2-4 cells. The foot cell is usually longer than the second and third cells measuring 13–34 × 8–10 µm in size.

Conidia (24.6 µm-56.4 µm in length and 10 µm-19.2 µm width, length/width ratio 1.5–2.9, on average 2.2) are ellipsoid-cylindrical (immature conidia often ellipsoid and mature cindia often cylindrical), thin-walled, smooth to verrucose surface. No well-developed fibrosin bodies are present.

Germtubes are simple, usually at end of the conidium but sometimes subapical. Chasmothecium were measured as 90.4 µm-112.8 µm. Short myceliod chasmothecial appendages. The asci length and width were 48.2 µm-70.3 µm and 25 µm-48.3 µm, respectively (Fig. 2).

Specimen was deposited in Fungarium of University of Guilan under the accession number GUM 1809.

Full rDNA ITS sequence (including 5.8S) gene amplified and sequenced. The amplified fragment length was 696 bp

According to BLAST search, the full rDNA ITS sequence (including 5.8S) gene showed 99% similarity to accession No. LC009890, KY653208, and KY653209 and 100% identity to *Erysiphe pisi* species (Fig. 3).)

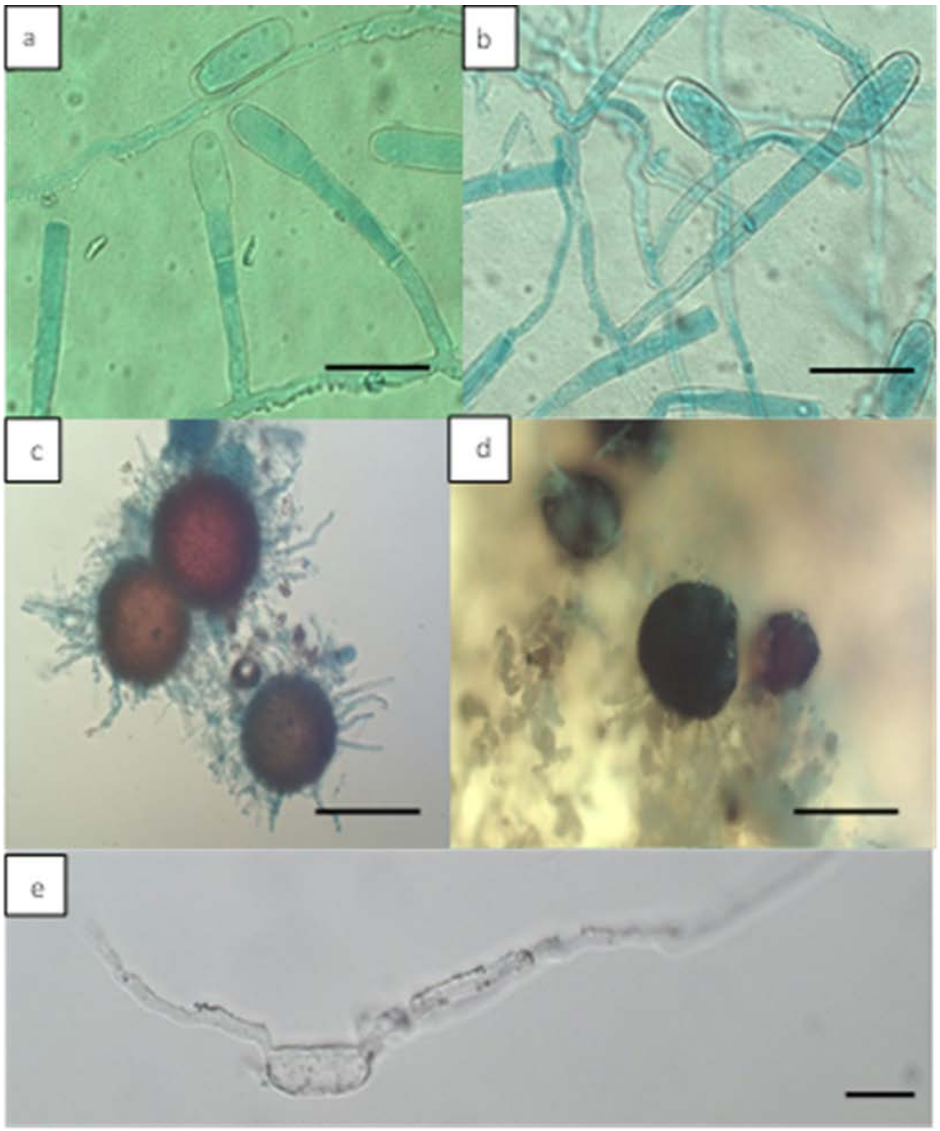


Figure 2. *Erysiphe pisi*, a and b: conidiophores and conidia; c and d: Chasmothecium with mycelioid type appendages; e: conidium germination (on plant leaves). Scale bar for a, b = 50 µm, c, d= 100 µm, e = 30 µm

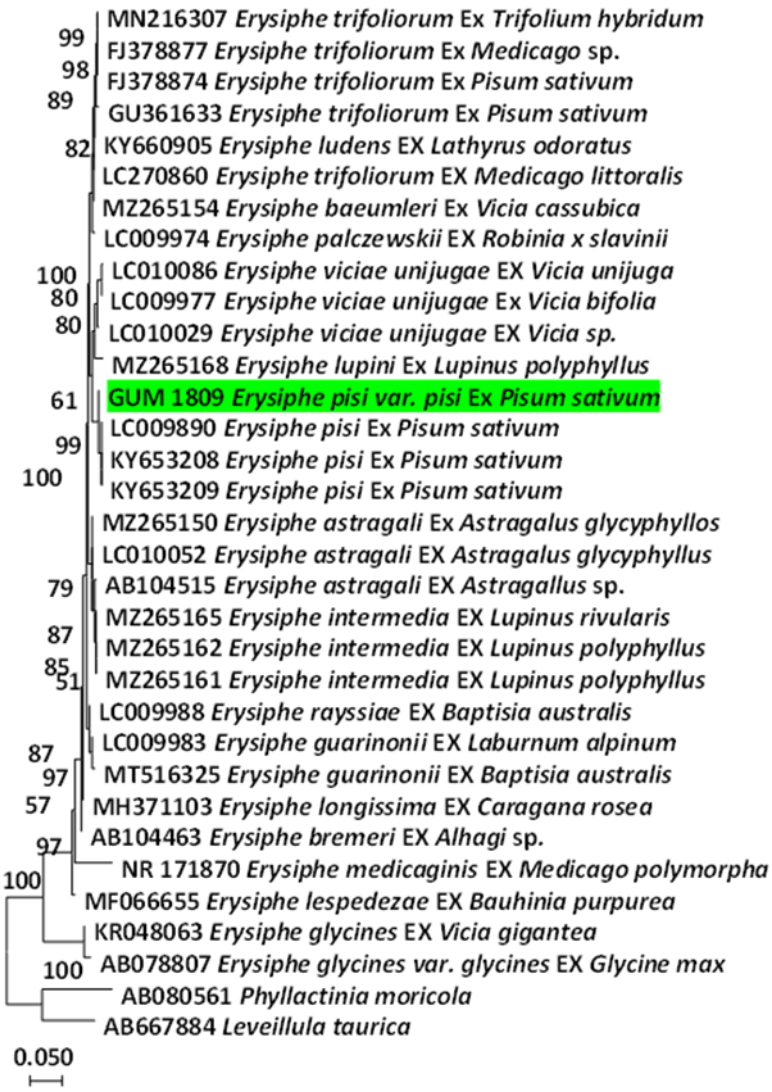


Figure 3: A minimum-evolution tree based on ITS data for 31 *Erysiphe* and two outgroup taxa. The evolutionary distances were computed using the Kimura 2-parameter method. All ambiguous positions were removed for each sequence pair (pairwise deletion option). The numbers above the branches represent branch support using 1000 bootstrap replications (Bootstrap values below 50% are not shown). Evolutionary analyses were conducted in MEGA11.

**Discussions**

Powdery mildew of pea is an important air born desease in all around the world spescialy in climate condition with warm day and cool night.

The *E. pisi* also reported as *Erysiphe communis* auct. p.p. or *Erysiphe polygoni* auct. p.p. up to now. Braun suggested the powdery mildew on the *Pisum*, *Astragalus*, *Lathyrus, Lens, Lotus, Lupinus, Medicago, Melilotus*, *Phaseolus, Trifolium* and *Vicia* caused by *E. pisi* var. *pisi* and on the *Lathyrus* and *Ononis* caused by *E. pisi* var. *cruchetiana* (Braun, 1987).

So far *Erysiphe baeumleri* and *Erysiphe trifoliorum* were reported causing disease in Czech Republic and US Pacific Northwest (Ondřej et al., 2005; Attanayake et al., 2010). These three species can be identified by use of rDNA ITS sequences combind with morphological characterstics.

Appendages of chasmothecium in *E. pisi* are mycellium type and in *E. trifolii* and *E. baeumleri* are dichotomously branch type. The *Erysiphe trifoliorum* and the *E. baeumleri* can be differentiated by appendages

spread and colour (Braun, 1987, Glawe, 2008; Fondevilla & Rubiales, 2012; Seethapathy et al., 2022).

Much of the results presented in this work can apply to identify powdery mildews species on green pea with high precision by morphological and molecular biology study. However, simillar to species characters determination that carried out by Braun (1985) and Schmidt & Braun (2020) the *Erysiphe pisi* is the main causing fungal species of green pea in Alborz province, Iran. Therefor the species of powdery mildewa on green pea of this region of Iran is similar to those reports showed that *Erysiphe pisi* is the causal agent of powdery mildews on pea in Canada, Mexico and Pakistan (Beltrán-Peña et al., 2022; Nisar et al., 2006; Warkentin et al., 1996).

For accurate molecular study, It is essential to choose clean, fresh powdery mildew specimens when obtaining DNA since powdery mildews cannot be grown in vitro, are often contaminated by other microscopic fungi, and DNA decays rapidly upon drying.

According to Yeh et al., (2021) and Meeboon & Takamatus (2020) the ITS is the single available barcode for species identification in *Erysiphales*. The ITS gene region of fungal DNA found critical for both species and intra-species molecular systematics determination by Seethapathy et al., (2022) and also with in this study. A sequence-based identification method is important for identifying powdery mildew species. But even in phylogenetic analyses for *E. pisi* there was high similaritied between ITS sequences of related species. Chen & Kirschner (2018) and Yeh et al., (2021) also described the difficulty of separation of the related species only by rDNA ITS sequence and suggest to add the traditional combination of host occurrence and fungal morphology analysis.

This study revealed the merit of rDNA ITS sequence analysis for the precise identification of *E. pisi* along with the mophological studies.

**Acknowledgement**

The authors wish to thanks the Seed and Plant Improvements Institue, Agrisultural research, education and extension organization of Iran and the University of Guilan that provides facility for the conducting the experiments and the mauscript reviwer for their valuable suggestion.

**References**

Attanayake, R. N., Glawe, D. A., McPhee, K. E., Dugan, F. M., & Chen, W. (2010). Erysiphe trifolii–a newly recognized powdery mildew pathogen of pea. *Plant pathology*, *59*(4), 712-720.

Beltrán-Peña, H., Solano-Báez, A. R., Leyva-Madrigal, K. Y., Tovar-Pedraza, J. M., Sauceda-Acosta, C. P., Lizárraga-Sánchez, G. J., & Mora-Romero, G. A. (2022). First report of powdery mildew caused by Erysiphe pisi on Parkinsonia aculeata in Mexico. *Journal of Plant Pathology*, 1-1.

Bradshaw, M., & Tobin, P. C. (2020). Sequencing herbarium specimens of a common detrimental plant disease (powdery mildew). *Phytopathology*, *110*(7), 1248-1254.

Braun, U. (1987). A monograph of the Erysiphales (powdery mildews). *Beihefte zur Nova Hedwigia*, (89).

Chen, K. L., & Kirschner, R. (2018). Fungi from leaves of lotus (Nelumbo nucifera). *Mycological Progress*, 17(1), 275-293.

Cunnington, J. H., Takamatsu, S., Lawrie, A. C., & Pascoe, I. G. (2003). Molecular identification of anamorphic powdery mildews (Erysiphales). *Australasian Plant Pathology*, *32*(3), 421-428.

Fondevilla, S., & Rubiales, D. (2012). Powdery mildew control in pea. A review. *Agronomy for sustainable development*, *32*(2), 401-409.

Glawe, D. A. (2008). The powdery mildews: a review of the world's most familiar (yet poorly known) plant pathogens. *Annu. Rev. Phytopathol*., 46, 27-51.

Hirata, T., & Takamatsu, S. (1996). Nucleotide sequence diversity of rDNA internal transcribed spacers extracted from conidia and cleistothecia of several powdery mildew fungi. *Mycoscience*, *37*(3), 283-288.

Kraft, J. M., & Pfleger, F. L. (2001). *Compendium of pea diseases and pests* (No. Ed. 2). American Phytopathological Society (APS Press).

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular biology and evolution*, *33*(7), 1870-1874.

Maharjan, A., Bhatta, B., Acharya, R. P., GC, S., & Shrestha, S. (2015). Efficacy assessment of treatment methods against powdery mildew disease of pea (Pisum sativum L.) caused by Erysiphe pisi var. pisi. *World Journal of Agricultural Research*, *3*(6), 185-191.

Matsuda, Y., Sameshima, T., Moriura, N., Inoue, K., Nonomura, T., Kakutani, K., ... & Toyoda, H. (2005). Identification of individual powdery mildew fungi infecting leaves and direct detection of gene expression by single conidium polymerase chain reaction. *Phytopathology*, *95*(10), 1137-1143.

Meeboon, J., & Takamatsu, S. (2020). Hosts of asexual morph of Erysiphe quercicola from Thailand. *Tropical Plant Pathology*, *45*(2), 122-135.

Nisar, M. O. H. A. M. M. A. D., Ghafoor, A., Khan, M. R., & Qureshi, A. S. (2006). Screening of Pisum sativum L. germplasm against Erysiphe pisi Syd. *Acta Biol. Crac. Ser. Bot*, *48*(2), 33-37.

Ondrej, M., Dostalova, R. A. D. M. I. L. A., & Odstrcilová, L. (2005). Response of Pisum sativum germplasm resistant to Erysiphe pisi to inoculation with Erysiphe baeumleri, a new pathogen of pea. *PLANT PROTECTION SCIENCE-PRAGUE-*, *41*(3), 95.

Seethapathy, P., Sankaralingam, S., Pandita, D., Pandita, A., Loganathan, K., Wani, S. H., & Elansary, H. O. (2022). Genetic Diversity Analysis based on the Virulence, Physiology and Regional Variability in Different Isolates of Powdery Mildew in Pea. *Journal of Fungi*, 8(8), 798.

Schmidt, A., & Braun, U. (2020). Asexual morphs of powdery mildew species (Erysiphaceae)–new and supplementary morphological descriptions and illustrations. *Schlechtendalia*, *37*, 30-79.

Smith, P. H., Foster, E. M., Boyd, L. A., & Brown, J. K. M. (1996). The early development of Erysiphe pisi on Pisum sativum L. *Plant Pathology*, *45*(2), 302-309.

Warkentin, T. D., Rashid, K. Y., & Xue, A. G. (1996). Fungicidal control of powdery mildew in field pea. *Canadian journal of plant science*, *76*(4), 933-935.

Yeh, Y. W., Chou, P. Y., Hou, H. Y., & Kirschner, R. (2021). First records of powdery mildew fungi (Erysiphales) on medicinal plants in Taiwan. *Botanical studies*, *62*(1), 1-12.