Mycologia Iranica 7(2): 253 – 257, 2020

Short Article

DOI: 10.22092/MI.2021.124510

Morphological and molecular characterization of *Podosphaera lini* on *Linum usitatissimum* in IRAN

S. Taheri Ardestani
Seed and Plant Improvement Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran

S. A. Khodaparast ✉
Department of Plant Protection, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran

A. Abbasi Moghaddam
F. Ghanavati
Seed and Plant Improvement Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran

H. Darsaraei
Department of Plant Protection, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran

Abstract: *Linum usitatissimum*, known as flax, is grown all around the world. Powdery mildew is a common disease on this crop so that this disease has been described from most flax-growing areas of the world. Clear symptoms of powdery mildew were observed on the field of different flax cultivars of Seed and Plant Improvement Institute of Iran in 2020 growing season. The causal pathogen of flax powdery mildew was identified as *Podosphaera lini* based on the morphological features and the nuclear ribosomal DNA internal transcribed spacer sequence. This is the first documented report of *P. lini* from flax in Iran.

Keywords: *Erysiphaceae*, morphology, phylogeny, plant disease

INTRODUCTION

Origin of flax (*Linum usitatissimum* L.), is the Near East and Mediterranean region, somewhere in Turkey, Iran and Syria (Cullis, 2007). Linseed cultivars have been planted to get fibers and oils. Powdery mildew diseases are widely distributed all around the world. They are economically important diseases on wild as well as cultivated plants. Powdery mildew disease of flax has been described from most flax-growing areas of the world (Reddy et al. 2013). Up to 38%, substantial yield loss has been reported as a result of its incidence (Pandey & Misra, 1992). In order to identify the powdery mildew collection on flax from Iran, morphological studies have been carried out and supplemented by phylogenetic analysis using the nuclear ribosomal DNA internal transcribed spacer (ITS) sequences.

MATERIALS AND METHODS

In the winter of 2019, germplasms of flax cultivars were planted for the regeneration of seeds on research fields of Seed and Plant Improvement Institute, Karaj, Alborz province, Iran. During spring and summer of 2020, the flax plants have been observed with powdery mildew symptoms on all aerial parts of plants, including stem, leaves, pods and fruits (Fig. 1).

The fungal structures were studied by means of a standard light microscope. Total DNA was extracted from fungal specimens by the Chelex method (Hirata & Takamatsu 1996).

The ITS region of the nuclear rDNA (ITS1-5.8 S-ITS2) was amplified by polymerase chain reaction (PCR) with the powdery mildew specific primers PMITS1 (5'-TCGGACTGGCC(T/C)AGGGAGA-3') (Cunnington et al. 2003) and PM11 (5'-TACCGCTTC ACTCG CCGTTA-3') (Bradshaw & Tobin, 2020) for the first, and PM10 (5'-GGCCGGAAAGTTGTCCAA AC-3') (Bradshaw & Tobin 2020) and PM11 for the second PCR (semi-nested PCR). The nucleotide sequence was obtained using direct sequencing in a 3500 Genetic Analyzer (Applied Biosystems, USA) in Codon Genetic Group (Iran, Tehran). Sequences were analyzed and edited using MEGA 7.0 (Kumar et al. 2016). The obtained sequence was submitted to NCBI to find closely related sequences using a BLASTN search method. Several sequences with high query coverage and identity (97-100%) were selected for

---

Submitted 7 July 2020, accepted for publication 18 July 2020
✉ Corresponding Author E-mail: khodaparast@guilan.ac.ir

https://mij.areeo.ac.ir

comparison and phylogenetic analyses. The phylogenetic tree was obtained using the Minimum Evolution method in MEGA 7.0 (Kumar et al. 2016). *Sawadaea koelreuteriae* (MT008194) was selected as the outgroup.

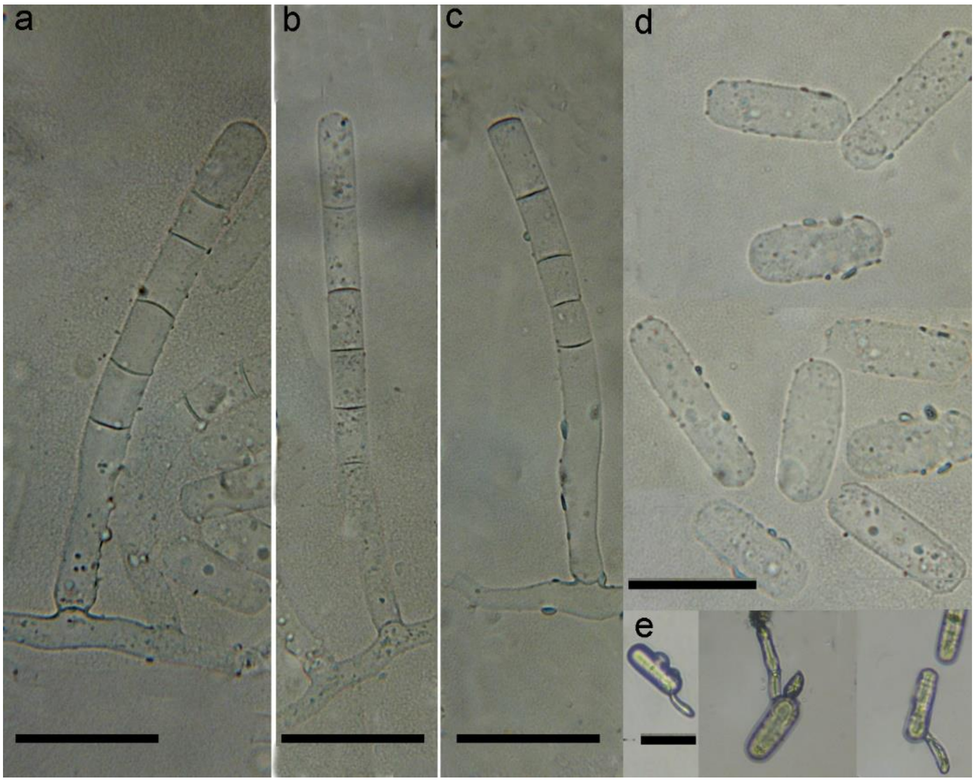
Fig. 1. Symptoms of powdery mildew on flax plant. a. a general view of cultivated plants; b-g. symptoms on different parts of the plant.

The ITS sequence generated in this study was deposited in the GenBank under accession number MZ540779.

RESULTS AND DISCUSSION

Flax plants typically showed disease symptoms with different severity. In the early stages, the upper side of leaves covered with effuse, thin, white mycelium. The mycelium then developed to both sides, which often results in covering the entire surface of the leaves, stems and pods, along with quick covering the whole areal, especially the upper part of the plants. There were two types of mycelia. Primary mycelium was tender, hyaline, and thin-walled, while secondary mycelium was persistent, slightly thick-walled, white to yellowish, straight to sometimes flexuous, septate and branched. Hyphal appressoria were rare, solitary and nipple-shaped, sometimes with the crenulate margin occasionally indistinct. Conidiophores were solitary, straight, rarely curved, arising from top and lateral end of the hyphal mother cells with 70–150 (–220) µm length and 7.5–11 (–14) µm width. Foot cells were cylindrical or subcylindrical, mostly straight, occasionally curved, 22–53 (–130) µm long, sometimes widening at the base or apical part, followed by 1–3 (−4) shorter cells. Conidia were colorless, one-celled, thin-walled, not very swollen, bearing 3–6 in chain (Euoidium-type), usually ellipsoid to cylindrical, 25–30 (–36) × 8–16 (−18) µm, with a length/width ratio (L/W) 1.7-4.5. Conidia were typically containing fibrosin bodies. Conidial germination was observed almost perihilar, and sometimes lateral (Figs. 2).

Fig. 2. *Podosphaera lini*. a-c. conidiophores; d. conidium; e. conidium germination (on plant leaves). — Scale bars: a-c =50 µm, d-e = 30 µm.



Chasmothecium was not observed. The specimen was deposited in Fungarium of University of Guilan (accession number GUM 1785).

Four powdery mildew species viz., *Erysiphe lini* Tanda, *Golovinomyces orontii* (Castagne) V.P. Heluta, *Leveillula taurica* (Lév.) G. Arnaud, and *Podosphaera lini* (Zvetkov) U. Braun & S. Takam have been recorded on *Linum* species (Braun & Cook 2012). *Erysiphe lini*, and *L. taurica* are well differentiated by their conidium state from *P. lini* (Zvetkov) U. Braun & S. Takam so that, conidia in *E. lini* are single (not catenescent), while in *L. taurica* are dimorphic. *Golovinomyces orontii*, another powdery mildew species with catenate conidia, may confuse with *P. lini*. However, fibrosin bodies are conspicuous in *P. lini* in fresh material. Moreover, ITS sequencing is a good complementary option to distinguish *P. lini* from *G. orontii* and some closely related *Podosphaera* species.

Sequences designated as *Podosphaera spiraeae* and *Podosphaera euphorbiae-helioscopiae* in GenBank are very similar to *P. lini*, with one substitution. It seems that ITS region has not enough discriminatory power for identification of these species from *P. lini* (Fig. 3). However, according to Braun & Cook 2012, these species are well differentiated from *P. lini* by having longer appendages on chasmothecia. Based on these morphological and molecular characteristics, powdery mildew on flax was identified as *P. lini*.

Saeidi and Sharifnabi (2004) published a paper on "effect of powdery mildew on some agronomic traits of flax in Iran". In this paper, they reported a causal agent of flax powdery mildew as *Oidium lini*. However, they did not describe their species. We could not find any documented source other than this article. There are at least two names related to *Oidium* on flax *viz*. *Oidium lini* Bondartsev and *Oidium lini* Škoric. Identity of *O. lini* reported by Saeidi and Sharifnabi (2004) is quite unclear due to the occurring of at least three powdery mildew species on flax with *Oidium* anamorphic state and they have not provided any description or illustration. Recently, *Oidium lini* Škoric complex was reviewed by Braun et al. (2019) and this name was regarded as a synonym of *Podosphaera lini*. Moreover, they reported *O. lini* Bondartsev being invalid according to Art 38.1 (a) of the Code (ICN).

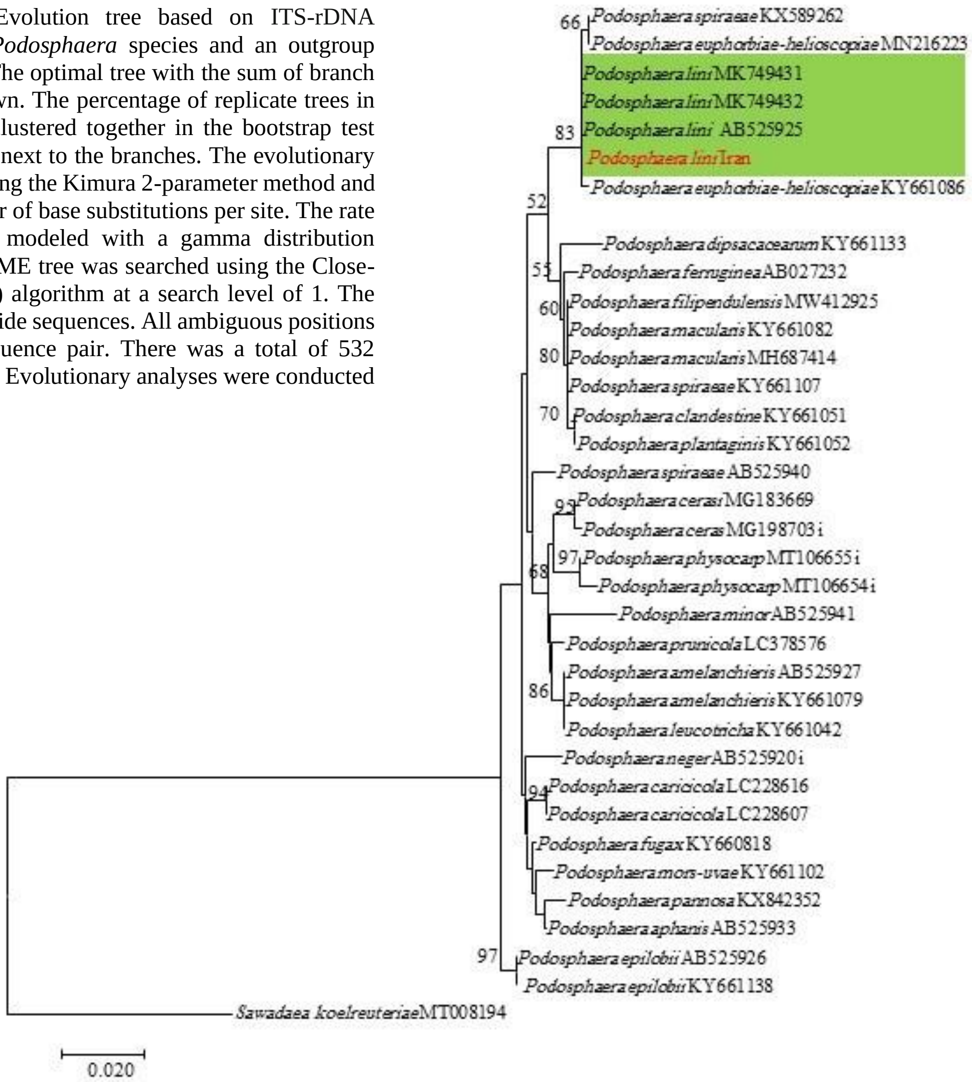


Fig 3. A Minimum Evolution tree based on ITS-rDNA sequences for 34 taxa of *Podosphaera* species and an outgroup (*Sawadaea koelreuteriae*). The optimal tree with the sum of branch length = 0.31516663 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). The ME tree was searched using the Close-Neighbor-Interchange (CNI) algorithm at a search level of 1. The analysis involved 35 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There was a total of 532 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

According to our knowledge, this is the first documented report of *Podosphaera lini* from flax in Iran.

ACKNOWLEDGEMENTS

This work has been supported by the Deputy of Research and Technology of the University of Guilan, Iran and Seed and Plant Improvement Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.

REFERENCES

Bradshaw M, Tobin PC. 2020. Sequencing herbarium specimens of a common detrimental plant disease (powdery mildew). Phytopathology 110: 1248-1254.

Braun U, Cook R. 2012. Taxonomic manual of the Erysiphales. CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherland.

Braun U, Preston C, Cook R, Götz M, Takamatsu S. 2019. Podosphaera lini (Ascomycota, Erysiphales) revisited and reunited with Oidium lini. Plant Pathology & Quarantine 9: 128-138.

Cullis CA. 2007. Flax. In: Oilseeds. (Kole C. ed): 275-295. Springer, Berlin, Heidelberg, Germany.

Cunnington JH, Takamatsu S, Lawrie AC, Pascoe IG. 2003. Molecular identification of anamorphic powdery mildews (Erysiphales). Australasian Plant Pathology 32: 421-428.

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: 283-288.

Kumar S, Stecher G., Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33:1870-1874.

Pandey R, Misra D. 1992. Assessment of yield loss due to powdery mildew of linseed. Indian Bot. Reptr 11: 62-64.

Reddy M, Arsul B, Shaik N, Maheshwari J. 2013. Estimation of heterosis for some traits in linseed (Linum usitatissimum L.). J. Agri and Vet. Sci 2: 11-17.

Saeidi Gh. and Sharifnabi B. 2004. Effect of powdery mildew on some agronomic traits of flax. Agricultural Science and Technology 20 :14-20.

ویژگی‌های ریخت شناسی و مولکولی *Podosphaera lini* روی گیاه *Linum usitatissimum* از ایران

سیمین طاهری اردستانی[۱]، سیداکبر خداپرست[۲]✉، احمد عباسی مقدم[۱]، فرنگیس قنواتی[۱]، حمیده دارسرائی[۲]

۱- موسسه تحقیقات اصلاح و تهیه نهال و بذر، سازمان تحقیقات، آموزش و ترویج کشاورزی، کرج، ایران
۲- گروه گیاه‌پزشکی، دانشکده علوم کشاورزی، دانشگاه گیلان، رشت، ایران

چکیده: گیاه کتان با نام علمی *Linum usitatissimum* در سراسر جهان کشت می شود. یکی از بیماری‌های متداول روی این گیاه سفیدک پودری می باشد. در فصل رویشی ۱۳۹۹ در مزارع موسسه تحقیقات اصلاح و تهیه نهال و بذر علائم مشخص از بیماری سفیدک پودری روی ارقام مختلف این گیاه مشاهده گردید. پس از بررسی‌های ریخت‌شناختی و مولکولی مبتنی بر توالی rDNA-ITS ، عامل سفیدک پودری روی این گیاه با نام *Podosphaera lini* شناسایی شد. این اولین گزارش مستند از *P. lini* روی گیاه کتان از ایران می باشد.

کلمات کلیدی: اریزیفاسه، ریخت شناسی، تبارشناسی، بیماری گیاهی

مکاتبه کننده: سیداکبر خداپرست Email: khodaparast@guilan.ac.ir

تاریخ دریافت: ۱۳۹۹/۰۴/۱۷ تاریخ پذیرش: ۱۳۹۹/۰۴/۲۶