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Molecular identification of some anamorphic powdery mildews (Erysiphales) in Guilan province, north of Iran

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Abstract: In this study, ITS–rDNA region was used to identify some anamorphic powdery mildews in Guilan province. According to the results, *Erysiphe* species on *Vicia faba* and *Sesbania punicea* showed 100% similarity to each other, however, without ITS sequence of holotype of *E. sesbaniae* it is impossible to make conclusion whether *Vicia faba* powdery mildew fungus actually belongs to *E. sesbaniae* or *E. trifoliorum* complex. ITS sequence from isolate of *Lagerstroemia indica* powdery mildew showed 100% similarity to *E. australiana. Podosphaera* on *Vigna* fells into phylogenetic group containing *P. xanthii* on cucurbitaceous hosts. ITS sequence of chamomile (*Matricaria chamomilla*) powdery mildew fungus showed 100% similarity to *P. xanthii* on *Xanthium strumarium.* Molecular characteristics and morpho–logical examination of conidia and conidia germin–ation clearly showed that *Dahlia* powdery mildew in Guilan province is conspecific with *G. spadiceus*. Anamorph morphology and ITS sequence of *Podosphaera* on *Epilobium* and *Erysiphe* on *Platanus orientalis* showed that these species belong to *P. epilobii* and *Erysiphe platani* respectively.

Key words: Erysiphaceae, *Erysiphe*, *Oidium*, *Podosphaera*, *Pseudoidium*

INTRODUCTION

Powdery mildews are a group of obligate fungi that are well–characterized by their appearance on plant surface as white powdery spots on the leaves, stems, flowers and fruits. Powdery growth may cover whole plant leaves, stems, so that sometimes bushes become completely white. In the early growth stage, fungal structures on plant surface include mycelia, conidiophores and large numbers of asexual spores (conidia). As the disease progresses, sexual structures (chasmothecia) begin to appear as small black structures on the mycelium tomentum. All structures produced in both asexual and sexual stages along with host range information are usually critical and are often required for exact taxonomic treatment and identification of powdery mildew species (Braun 1987, Braun & Cook 2012, Shin 2009). However, there are many species that produce only asexual or sometimes sexual morphs. There are circumstances in which sexual stage in powdery mildew being absent. Researchers have shown that some species are heterothallic and two mating types are necessary for chasmothecia production (White 1970, Coyier 1973). In Australia the sexual stage of powdery mildew fungi has been recorded for only 20 out of 100 species known which attributed to the lack of appropriate mating strain (Cunnington et al. 2003). Identification being more complicated when sexual stages are unavailable to researchers, because currently nearly all identification keys have basically been provided according to sexual morph (Braun & Cook 2012). Although some mycologists have tried to develop identification keys for asexual powdery mildew fungi (Boesewinkel 1980, Cook et al.1997) but such taxonomic experience was not generally accepted and no longer being useful for species identification purposes. Moreover, some characters of powdery mildews are not accessible when dried herbarium materials are used such as conidium germination, exact measurement and description of conidia, conidiophores and appressoria. Hence, identification of some species remains usually difficult or problematic based solely on asexual stage morphology.

Host range and distribution of powdery mildew fungi in Iran has previously been treated by Khodaparast & Abbasi (2009). According to this paper, a total of 90 species of the *Erysiphales* have been identified in Iran, for some species no sexual stage is known. In Guilan province about 50 species occurs on several plant species, however, some of them have only been identified based on asexual stage (Khodaparast 2007).

The ribosomal DNA internal transcribed spacer (ITS) regions are useful for identifying powdery mildew fungi at species level (Hirata & Takamatsu 1996, Hirata et al. 2000, Ito & Takamatsu 2010, Khodaparast et al. 2001, 2007, 2012, 2016). It could

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represent a useful region for linking anamorphic specimens with their respective teleomorphs. In this study we used ITS region to identify some anamorphic powdery mildews in Guilan province, Iran.

MATERIALS AND METHODS

Morphological examination

To observe the hyphae, conidiophores and conidia, clear adhesive tapes was used to strip off these structures from the leaf surface. A solution consisting of equal amounts of glycerol and lactic acid was used for mounting the fungal structures (Heidari et al. 2015). An Olympus light microscope (BH–2, Japan) equipped with a Sony digital camera was used for microscopic observations. All measurements were based on at least 20 to 30 observations. Morphological features of asexual states of the species were compared to the description of related taxa available in Braun & Cook (2012).

DNA sequencing and data analysis

Total DNA was isolated from fungal specimens by the Chelex method that had previously been used by several researchers (Walsh et al. 1991; Hirata and Takamatsu 1996; Khodaparast et al. 2001, 2007, 2012, 2016a, 2016b). Universal primers ITS1 (5'–TCCGTA GGTGAACCTGCGG–3') and ITS4 (5'–TCCTCCGC TTATTGATATGC–3') were used for amplification and sequencing of the fungal internal transcribed spacers (White et al. 1990).

The nucleotide sequences of the polymerase chain reaction (PCR) products were obtained using direct sequencing in an ABI 3730xl sequencer (Applied Biosystems, USA). Sequences were analyzed and edited using MEGA 7.0 (Kumar et al. 2016). Sequences were compared with the sequences available in the NCBI GenBank nucleotide database using a BLASTN search method. Several sequences from GenBank were selected for phylogenetic analyses. Sequences alignment was performed using muscle plug–in of MEGA 7.0 with the default settings (Edgar 2004). Phylogenetic trees were obtained using the minimum–evolution (Rzhetsky & Nei 1992) method in MEGA 7.0 (Kumar et al. 2016). In the ME method, the evolutionary distances were computed using the Kimura 2–parameter method (Kimura 1980). The ME tree was searched using the close–neighbour–interchange (CNI) algorithm at a search level of 1 (Nei & Kumar 2000). All ambiguous positions were removed for each sequence pair. All nucleotide substitutions were equally weighted and unordered. The neighbor–joining algorithm was used to generate the initial tree (Saitou & Nei 1987). The strength of the internal branches from the resulting trees was statistically tested by bootstrap analysis with 1000 replicates (Felsenstein 1985). All ITS sequences generated in this study are deposited in GenBank under accession numbers MF663773–MF663781.

RESULTS and DISCUSSION

Powdery mildew fungi collected in this study belong to different genera including *Erysiphe* section *Erysiphe*, *E.* sect. *Microsphaera*, *E.* sect. *Uncinula*, *Podosphaera* and *Golovinomyces*. ITS sequences were used in phylogenetic analysis. Due to the presence of two different phylogenetic groups of species belonging to euoidium and pseudoidium types, two different data matrices were used for analysis and resulting trees were shown in Fig. 1–2. Further results are presented here for each species.

Erysiphe on *Sesbania* and *Vicia*

According to Braun et al. (2010) and Braun & Cook (2012) three powdery mildew species including *E. sesbaniae* Wolcan & U. Braun 2010, *Pseudoidium fabacearum* (Hosag.) U. Braun & R.T.A. Cook, *Microidium agatidis* (É.E. Foëx) U. Braun 2012 have been recorded on *Sesbania* spp. *M. agatidis* is well–characterized by having small and catenate conidia. Two remaining species are morphologically very similar. However, based on description available in Braun & Cook (2012) *E. sesbaniae* differs in having two types of conidia and amphigenous mycelium. Mycelium of the examined specimens was amphigenous, usually covering most part of leaves. Conidia were more or less of two types. Such characteristics resemble those of *E. sesbaniae*.

Another fungus was collected on heavily infected leaves of *Vicia faba* in greenhouse which possess anamorph characteristics similar to *Sesbania*. Moreover, ITS sequence of this taxon showed 100% similarity to the Sesbania powdery mildew fungus. Four *Erysiphe* species have been recorded on *Vicia* spp. *viz*. *E. baeumleri* (Magnus) U. Braun & S. Takam., *E. ludens* (E.S. Salmon) U. Braun & S. Takam., *E. pisi* DC. and *E. viciae–unijugae* (Homma) U. Braun . These species are mainly distinguished based on teleomorph morphology. Characteristics of anamorphic state are limited to allow their identification. ITS sequences for *E. baeumleri*, *E. pisi* and *E.viciae–unijugae* are available in GenBank and are well–distinguished from *Vicia*/*Sesbania* powdery mildew sequences obtained in this study. We could not find ITS sequence for *E. ludens* but anamorph for this species was not recognized and the fungus is endemic to Canada. We found three substitutions between *E. trifoliorum* (from Iran) and *Vicia*/*Sesbania* powdery mildew fungus. However, several ITS sequences under the name of *E. trifoliorum* were found to be 100% similar to *Vicia*/*Sesbania* powdery mildew. A group of species including *E. trifoliorum*, *E. sesbaniae*, *E. robiniae*, *E. sophorae*, *E. crispula*, all on Fabaceae, are

morphologically closely related and make a complex species with strongly sinuous–subgeniculate, thick–walled chasmothecial appendages (see Braun & Cook 2012). *E. sesbaniae* is recently described (Braun et al. 2010). Before description of *E. sesbaniae*, one record of *E. trifoliorum* on *Sesbania* belongs to Ukraine **(**Dudka et al. 2004).

Without ITS or other gene sequences of the holotype of *E. sesbaniae*, it is impossible to make conclusion whether our new ITS sequence on *Vicia faba* actually belongs to *E. sesbaniae* or *E. trifoliorum* complex. However, this fungus on *Sesbania punicea* has already been recorded under the name of *E. sesbaniae* on *Sesbania punicea* from Iran (Abbasi et al. 2013, Sharifi et al. 2013).

This is the first record of *E. trifoliorum* complex on *Vicia faba*. Moreover, this is the first time that rDNA ITS sequences is recorded for *Erysiphe* on *Sesbania*.

Erysiphe* on *Lagerstroemia indica

Two powdery mildews belonging to the genus *Erysiphe* viz. *Erysiphe australiana* (McAlpine) U. Braun & S. Takam. and *Pseudoidium yenii* (U. Braun) U. Braun & R.T.A. Cook have been recorded on *Lagerstroemia* spp. Both species have previously been recorded from Iran (Abbasi et al. 2013, Sharifi et al. 2013). Both reports are based on anamorphic state from Guilan province and teleomorph has not been recognized. Braun & Cook (2012) stated that relation of *Ps. yenii* to *E. australiana* is not clear, however, *Ps. yenii* differs by having narrower hyphae, conidiophores and type of conidial germination (long germination tube without lobed appressoria). This collection in Guilan province possesses wide hyphae and short germination tube with lobed appressoria and belongs to *E. australiana*. Sequence of ITS regions from this fungus showed 100% similarity to *E. australiana*. We could not demonstrate presence of *Ps. yenii* by molecular or morphological data. This is the first record of ITS sequence for *E. australiana* from Iran.

Erysiphe* on *Platanus orientalis

Erysiphe platani (Howe) U. Braun & S. Takam. is a well–known fungus occurring on *Platanus orientalis* worldwide. This species restricted to species of *Platanus*, despite the fact that the anamorph state

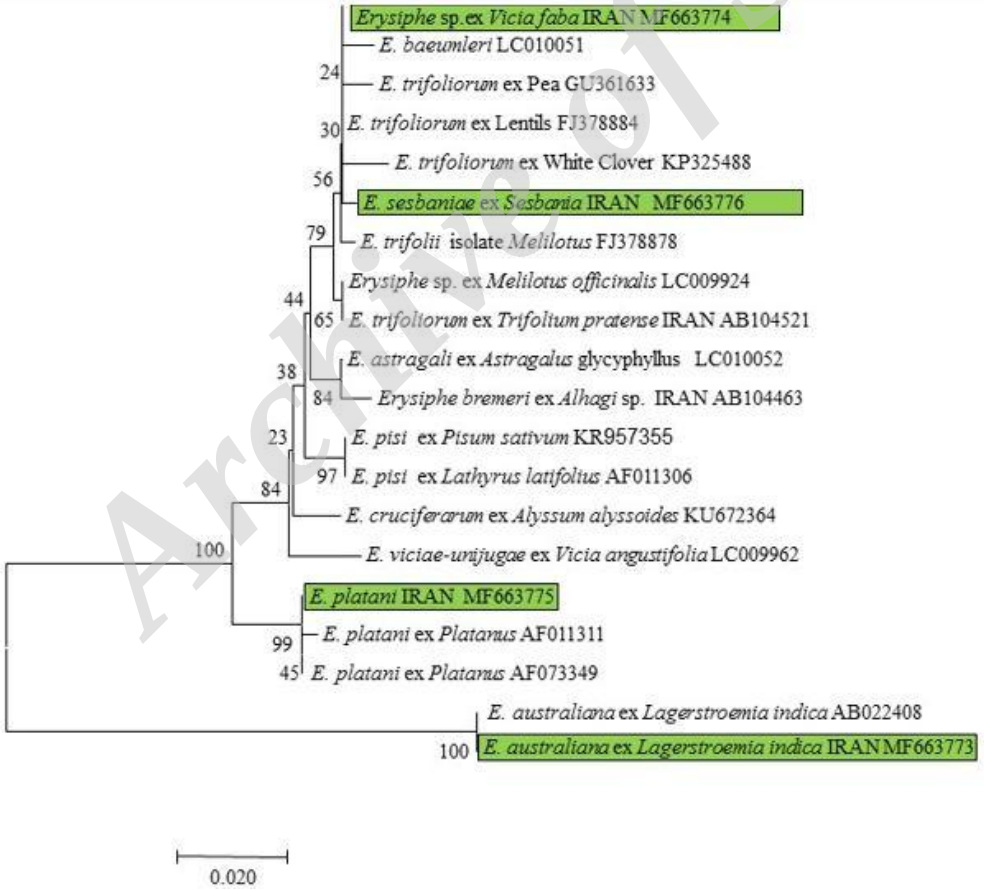

Fig. 1. A Minimum Evolution tree based on ITS sequences for 20 taxa of *Erysiphe* species. The optimal tree with the sum of branch length = 0.32298257 is shown. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. All positions containing gaps and missing data were eliminated. There were a total of 585 positions in the final dataset. Evolutionary analyses were conducted in MEGA 7.

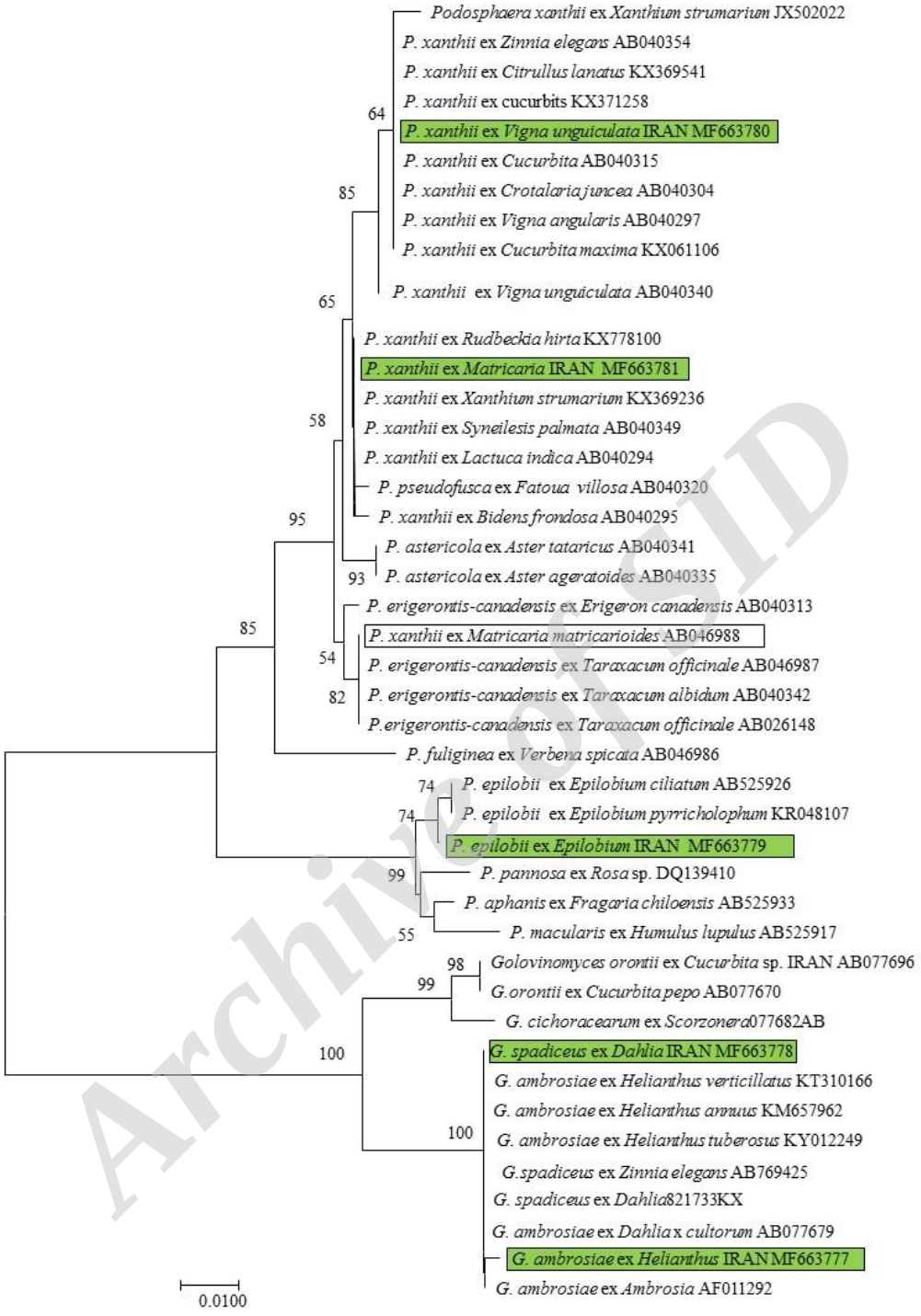

Fig. 2. A Minimum Evolution tree based on ITS sequences for 43 taxa of powdery mildews including *Podosphaera* and *Golovinomyces* species. The optimal tree with the sum of branch length = 0.29079068 is shown. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. All positions containing gaps and missing data were eliminated. There were a total of 413 positions in the final dataset. Evolutionary analyses were conducted in MEGA 7.

of *E. platani* occurs all over Guilan province and some other regions of Iran such as Tehran, Gorgan, Mazandaran provinces (Ershad 1995, Khodaparast & Abbasi 2009), however, there is no report for teleomorph of the fungus. All reports are based on anamorphic state. We sequenced full ITS region

except for first 11 nucleotides that were removed due to ambiguous reading. This sequence is 100% similar to more than 10 sequences available in GenBank (NCBI). This is the first attempt for the identification of *E. platani* using rDNA sequence analysis in Iran.

Podosphaera* on *Vigna

Heavily infected plants were observed in the greenhouse. Conidium morphology of the fungus more or less agrees with *P. xanthii* (Castagne) U. Braun & Shishkoff, however, accurate detection of *P. xanthii* from closely related species such as *P. fusca* based solely on conidium morphology is not possible. Sequence analysis showed that Vigna powdery mildew fungus in Iran is clustered with taxa belonging to *P. xanthii* complex. Anamorphic state of *P. xanthii* largely agrees with those of some related species such as *P. fusca* (Fr.) U. Braun & Shishkoff and *P. erigerontis–canadensis* (Lév.) U. Braun & T.Z. Liu, however, teleomorph is distinguished by having larger ascomata and larger terminal oculi on asci (Braun & Cook 2012). Hirata et al. (2000), used two ITS sequences of *Podosphaera* from *Vigna angularis* (AB040297) and *Vigna unguiculata* (AB040340). According to this study Vigna powdery mildew isolates clustered in two haplotypes. The two haplotypes differ only at one position (442). Iranian isolate showed 100% similarity to sequence obtained from *Podosphaera* on *Vigna angularis* (AB04029) except for one non–identified position at base number 333 in this isolate. Recently, Ito & Takamatsu (2010) reconstructed a new phylogenetic tree of *Podosphaera* subsection *Magnicellulatae* based on 28S and ITS rDNA. They showed that Magnicellulatae taxa often infect the same plant genus or species. The most important host plants for *P. xanthii* complex in Iran include *Cucumis sativa*, *Cucumis melo*, *Cucurbita* spp. and *Citrullus vulgaris*. According to the previous phylogenetic studies (Hirata et al. 2000, Ito & Takamatsu 2010) and this study, Vigna isolates fell into phylogenetic group containing Cucurbitaceae host plants.

Podosphaera* on *Matricaria chamomilla

ITS sequence of chamomile (*M. chamomilla*) powdery mildew fungus showed 100% similarity to *P. xanthii* on *Xanthium strumarium* (accession number: KX369236). In phylogenetic analysis this taxon fall on a clade containing ITS sequences from different host plant species including *Xanthium*, neotype genus for *P. xanthii*. According to Braun & Cook (2012) *M. chamomilla* is reported as a host plant for *P. erigerontis–canadensis*, but in our phylogenetic analysis this fungus showed close relationship to *P. xanthii*. There is one ITS sequence from *Podosphaera* on *M. matricarioides* (accession number: AB046988) in GenBank, but this sequence clustered with *P. erigerontis–canadensis*. As a result we conclude that *M. chamomilla* is infected with *P. xanthii* rather than *P. erigerontis–canadensis* in Guilan province.

Golovinomyces* on *Dahlia

ITS sequence from Dahlia powdery mildew in Iran showed high similarity to *Golovinomyces spadiceus* (Berk. & M.A. Curtis) U. Braun from *Zinnia elegans* (100%, AB769425), *Dahlia pinnata* (100%, KX821733), *Dahlia* x *cultorum* (100%, AB077679), *G. ambrosiae* (Schwein.) U. Braun & R.T.A. Cook isolates from *Ambrosia trifida* (100%, AF011292), *Helianthus tuberosus* (100%, KY012249), *Helianthus verticillatus* (100%, KT310166), *Helianthus annuus* (100%, KM657962).

Hence, ITS sequence might not possess enough variation to differentiate these two closely related species. *G. spadiceus* recently raised by Braun & Cook (2012). They pointed out some differences between the two species. According to these authors *G. spadiceus* differs by having narrower conidia and euoidium type of conidial germination. Morphological characterization of conidia and conidia germinations clearly showed that *Dahlia* powdery mildew is conspecific with *G. spadiceus* in Guilan province.

Both species of *Golovinomyces* (*G. ambrosiae* and *G. spadiceus*) have been reported on *Dahlia* (Braun and Cook 2012).

Podosphaera* on *Epilobium

ITS sequence of Epilobium powdery mildew showed 99 % similarity (one base substitution) to *Podosphaera epilobii* (AB525926). Morphological characterization showed that this fungus could belong to *P. epilobii.*

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شناسایی مولکولی تعدادی از آنامورف های سفیدک‌های پودری (Erysiphales) در استان گیلان

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چکیده: در این مطالعه ناحیه ITS–rDNA برای تشخیص تعدادی از قارچهای آنامورفیک مولد سفیدکی پودری در استان گیلان استفاده شد. بر اساس نتایج به دست آمده گونه *Erysiphe* روی *Vicia faba* و *Sesbania punicea* ۱۰۰ درصد شباهت نشان دادند . با وجود این، بدون دسترسی به توالی هولوتیپ گونه *E. sesbaniae* غیر ممکن است معلوم شود قارچ روی *Vicia faba* به *E. sesbaniae* تعلق دارد یا به مجموعه گونه‌ای *E. trifoliorum*. توالی ITS سفیدک پودری *Lagerstroemia indica* با توالی گونه *E. australiana* در بانک ژن ۱۰۰ درصد شباهت نشان داد. گونه *Podosphaera* روی *Vigna* داخل گروه فیلوژنتیکی قرار گرفت که سفیدک پودری تیره کدوئیان را در بر می گیرد. توالی ناحیه ITS سفیدک پودری بابونه (*Matricaria chamomilla*) ۱۰۰ درصد شباهت با *P. xanthii* روی *Xanthium strumarium* نشان داد. ویژگیهای مولکولی و ریخت شناسی قارچ عامل سفیدک پودری *Dahlia* نشان داد که این قارچ به گونه *G. spadiceus* تعلق دارد. مورفولوژی آنامورف و توالی ITS *Podosphaera* روی *Epilobium* و *Erysiphe* روی *Platanus orientalis* نشان داد که این گونه‌ها به ترتیب به *P. epilobii* و *E. platani* تعلق دارند.

کلمات کلیدی: Erysiphaceae، *Erysiphe*، *Oidium*، *Podosphaera*، *Pseudoidium*

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