DOI: 10.22092/bot.j.iran.2025.367941.1409

Parastagonospora poae and *P. minima,* new species for the funga of Iran

Received: 10.03.2025 ================== Revised: 07.04.2025 ================== Accepted: 19.04.2025

Fariba Ghaderi⊠: Associate Prof., Department of Plant Protection, College of Agriculture, Yasouj University, Yasouj Iran (f.ghaderi@yu.ac.ir)

Azadeh Habibi: Assistant Prof., Department of Biodiversity, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran

Abstract

Parastagonospora species are common plant pathogens that infect wheat, barley, and a wide range of wild grasses of the Poaceae. In a survey on fungal species associated with leaf spot diseases of wild grasses growing in wheat fields in Kohgiluyeh & Boyer-Ahmad and Fars Provinces (Iran). *Parastagonospora* isolates were recovered from necrotic lesions on leaves, ears, and stems of *Aegilops tauschii* growing within and near wheat fields. Based on morphological characterization coupled with LSU and ITS molecular data, the species were identified as *P. poae* and *P. minima*. This is the first report of these species on *A. tauschii* worldwide and the first report of these species in Iran. The obtained *Parastagonospora* species caused necrosis lesions on the 'Chamran' cultivar of wheat in greenhouse. The presence of *Parastagonospora* spp. on wild grass species growing within or near fields of cultivated wheat likely represents a source of emerging pathogens from these regions in the future.

Keywords: Fars, Kohgiluyeh & Boyer-Ahmad, *Phaeosphaeria*, phylogenetic analysis, Poaceae

Parastagonospora poae **و** ***P. minima*****، گونه‌های جدیدی برای قارچ‌های ایران**

دریافت: ۱۴۰۳/۱۲/۲۰ ================== بازنگری: ۱۴۰۴/۰۱/۱۸ ================== پذیرش: ۱۴۰۴/۰۱/۳۰

فریبا قادری⊠: دانشیار گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه یاسوج، یاسوج، ایران (f.ghaderi@yu.ac.ir)

آزاده حبیبی: استادیار گروه تنوع‌زیستی، پژوهشگاه علوم و تکنولوژی پیشرفته و علوم محیطی، دانشگاه تحصیلات تکمیلی صنعتی و فناوری پیشرفته، کرمان، ایران

خلاصه

گونه‌های *Parastagonospora* از بیمارگرهای رایج گیاهی هستند که گندم، جو و دامنه وسیعی از علف‌های هرز گندمیان را آلوده می‌کنند. طی نمونه‌برداری‌هایی که با هدف شناسایی گونه‌های قارچی مرتبط با لکه‌برگی‌ها روی علف‌های هرز در مزارع گندم از استان‌های کهگیلویه و بویراحمد و فارس انجام شد، جدایه‌هایی از گونه‌های *Parastagonospora* از برگ‌ها، خوشه‌ها و ساقه‌های *Aegilops tauschii* جداسازی شدند. براساس تلفیق داده‌های ریخت‌شناختی و مولکولی نواحی ITS و LSU، دو گونه *P. poae* و *P. minima* شناسایی شدند. براساس اطلاعات موجود، این نخستین گزارش از گونه‌های مذکور برای قارچ‌های ایران و نخستین گزارش از این گونه‌ها روی میزبان *A. tauschii* در دنیا است. هر دو گونه *P. poae* و *P. minima* لکه‌های نکروز روی گندم (رقم چمران) در شرایط گلخانه ایجاد نمودند. شناسایی این گونه‌ها از علف‌های هرز در مزارع گندم، بیانگر احتمال وجود منبعی برای بیمارگرهای نوظهور از این مناطق در آینده است.

واژه‌های کلیدی: فارس، کهگیلویه و بویراحمد، گندمیان، واکاوی فیلوژنتیکی، *Phaeosphaeria*

Introduction

Parastagonospora species are important pathogens of wheat, barley, and a wide range of wild grasses with global distribution (Croll *et al*. 2021). The origin of *Parastagonospora* species and their hosts is in the Fertile Crescent (McDonald *et al*. 2012, Salamini *et al.* 2002). *Parastagonospora nodorum* (Berk.) Quaedvl., Verkley & Crous and *P. avenae* (A.B. Frank) Quaedvl., Verkley & Crous cause leaf and glume blotch on wheat, barley and a wide range of wild grasses (Solomon *et al*. 2006, Goonasekara *et al*. 2019). *Parastagonospora avenae* f. sp. *avenae* (Weber 1922) Eriksson is the major leaf pathogen of oats (Ghaderi *et al.* 2022). *Parastagonospora avenaria* f. sp. *tritici* (Pat) is pathogenic on wheat and other cereals, but cannot infect oats (McDonald *et al.* 2012, Croll *et al.* 2021) and is split into Pat1 to Pat6 based on host specialization and genetic differences between host-specialized forms (Ueng & Chen 1994, Ueng *et al*. 1998, Malkus *et al.* 2005, McDonald *et al.* 2012). Other *Parastagonospora* species associated with wild grasses around the world include *P. stipae* B.A. McDonald, P.C. Brunner, Croll, D. Pereira, Mordecai & Crous from *Stipa pulchra* (Croll *et al.* 2021), *P. allouniseptata* W.J. Li, Camporesi, D.J. Bhat & K.D. Hyde, *P. dactylidis* W.J. Li, Camporesi, D.J. Bhat & K.D. Hyde, *P. minima* W.J. Li, Camporesi, D.J. Bhat & K.D. Hyde, *P. italica* W.J. Li, Camporesi, D.J. Bhat & K.D. Hyde and *P. uniseptata* W.J. Li, Camporesi, D.J. Bhat & K.D. Hyde from *Dactylis* spp. (Li *et al.* 2015), *P. elymi* Goonas., Bulgakov & McKenzie from *Elymus repens*, *P. macrouniseptata* Goonas., Camporesi & McKenzie from *Dactylis glomerata* (Goonasekara *et al.* 2019), and *P. poae* Quaedvlieg, Verkley & Crous from *Poa* sp. (Quaedvlieg *et al.* 2013).

Aegilops tauschii Coss., [syn. *Triticum tauschii* (Coss.) Schmal. (Triticeae, Poaceae)], commonly known as Tausch's goatgrass or rough-spike hard grass, is an annual weed in wheat fields. This species is a valuable source of desirable genes for wheat breeding (Murphy *et al.* 2001, Friesen *et al*. 2008, Ma *et al.* 2023). The genome of *A. tauschii* contains many biotic and abiotic stress-resistance genes, many of which have been successfully introgressed into bread wheat to improve wheat traits (May & Lagudah 1992, Murphy *et al.* 2001, Hasanpour *et al.* 2023).

During the spring of 2021–22, necrotic lesions on leaves, ears, and stems of *A. tauschii* accompanied by black and brown pycnidia in the center of the necrotic lesions were seen in wheat fields in Kohgiluyeh & Boyer-Ahmad and Fars Provinces (Iran). The species richness of *Parastagonospora* in Iran is consistent with the hypothesis that, the Fertile Crescent is the center of origin for the species within this fungal genus (Ghaderi *et al.* 2020) and wheat (Salamini *et al.* 2002). Studying *Parastagonospora* spp. associated with *A. tauschii* provides some information to survey the hypotheses about how these pathogens might have emerged. The objectives of this study were: a) to characterize and identify *Parastagonospora* species from the wild grass, *A. tauschii*, in Kohgiluyeh & Boyer-Ahmad and Fars Provinces in Iran; and b) to assess the pathogenicity of the isolates in wheat to test the hypothesis that, *Parastagonospora* species from the wild grasses pose a threat to wheat.

Materials and Methods

- Sample collection, isolation, and morphological characterization

During early spring and late summer 2021–22, surveys were conducted across 10 wheat fields of Kohgiluyeh & Boyer-Ahmad Province as well as 15 wheat fields of Fars Province (Iran). Rough-spike hard grass plants (*Aegilops tauschii*) were observed with necrotic spots on the leaves, stems, and ears surrounded by a chlorotic halo with the presence of pycnidia and taken to the laboratory in polyethylene bags. Symptomatic tissues were cut into segments of 5–7 mm, disinfected for 1 min in 1% sodium hypochlorite solution, rinsed in sterile water, placed on glass slides with tape, and kept in moist chambers to enhance sporulation until the pycnidia produced cirri containing pycnidiospores. Single-conidial colonies were established. Isolates were grown on Yeast Sucrose Agar (YSA, 10g/L Yeast Extract, 10g/L sucrose, 5g sterilized poaceous straws extract, 1.2% agar) and incubated at 18–20 °C and a 12h photoperiod under near-ultraviolet with light for one month to promote pycnidia formation and sporulation (Halama & Lacoste 1992). Colony color and growth rate, conidial and conidiomatal morphology, and pigmentation, were used for morphological identifications (Quaedvlieg *et al.* 2013).

- DNA extraction, PCR amplification, and sequencing

Isolates were grown on YSA plates in darkness at 25 °C for five days. Pycnidiospores were transferred to 50 ml yeast sucrose broth (YSB; 10 g/L yeast extract, 10 g/L sucrose) and incubated on a shaker at 120 rpm for seven days at 120 rpm at 20 °C. Mycelia were harvested and crushed using liquid nitrogen, and total DNA was extracted using a CTAB extraction procedure according to Murray and Thompson (1980). The ITS rDNA and large subunit rDNA (LSU) regions were amplified using primers ITS1/ITS4 (White *et al.* 1990) and LROR/LR5 (Vilgalys & Hester 1990), respectively. The PCR reaction mixtures were prepared in a final volume of 20 µl, comprising 0.04 µM of each primer (Microsynth, Switzerland), 0.4 µM dNTPs (MBI Fermentas, Germany), 1× Dream Taq Buffer (MBI Fermentas), and 0.4 U Dream Taq DNA polymerase (MBI Fermentas). The cycling conditions consisted of initial denaturation at 96 °C for 6 min, followed by 35 cycles of 96 °C for 30 s, annealing at 49 °C for LSU and at 52 °C for ITS-rDNA for 45 s, and extension at 72 °C for 90 s. A final extension step was applied at 72 °C for 10 min (Quaedvlieg *et al.* 2013, Li *et al*. 2015).

Sequencing was performed in both directions by the DNA Sequencing Service of Macrogen (Macrogen Inc., South Korea). DNA sequences were checked and manually edited with Geneious software (Biomatters Inc., USA). A BLAST search was used to compare the obtained sequences with those in NCBI/Genebank database to find the closest matching taxa. The obtained ITS and LSU sequences were submitted to NCBI's GenBank (https://www.ncbi.nlm.nih.gov/genbank) under the accession numbers PQ097847-PQ097859 for ITS and PQ107786- PQ107798 for LSU (Table 1).

- Phylogenetic analysis and molecular identification

The newly generated sequences of LSU and ITS-rDNA in this study, along with sequences of representative taxa retrieved from GenBank, database were used in phylogenetic analyses to determine the taxonomic status of the isolated *Parastagonospora* species (Table 1). The sequences were aligned using the ClustalW alignment tool under Geneious. Phylogenetic analyses were performed using heuristic searches in PAUP* 4.0a133 (Swofford 2002) for parsimony analysis with bootstrap analysis of 1,000 replicates to test the support of the branches.

- Mating type idiomorphs and fertility

Mating type idiomorphs, *MAT1-1* and *MAT1-2*, were amplified for all isolates. Amplifications were carried out using a multiplex PCR with primers designed by Bennett *et al.* (2003) according to the method described in Sommerhalder *et al.* (2006). To induce the production of the sexual morph (pseudothecia), genetic crosses were carried out between isolates of opposite mating types, following the method of Halama & Lacoste (1992).

- Pathogenicity assay

Pathogenicity tests were conducted using *Triticum aestivum* cv. 'Chamran' under greenhouse conditions. Isolates Pp-Iran1 to Pp-Iran8 and Pm-Iran1 to Pm-Iran5 (Table 1) were used in the assays. The 'Chamran' cultivar was selected for the pathogenicity tests for the following reasons: (a) *Parastagonospora* isolates were collected from wheat fields with the 'Chamran' cultivar. Furthermore; (b) Ghaderi *et al.* (2016) showed that, the 'Chamran' cultivar was the most susceptible to *Phaeosphaeria nodorum*; and (c) the 'Chamran' cultivar was one of the most dominant cultivars in Kohgiluyeh & Boyer-Ahmad and Fars Provinces.

For inoculum preparation, 4-day-old fungal mycelia of 13 isolates grown on YSA were used. Mycelial discs (5 mm) were transferred to 250 ml of YSB and placed in a shaker at 120 rpm and at 18 °C for seven days. The resulting suspension was diluted to 4×10^6 spores ml^{-1}.

Conidial suspensions were applied to runoff onto 8-week-old seedlings with a hand sprayer. Inoculated plants were covered with plastic bags for two days at 20 °C and then transferred to the greenhouse at 20–28 °C under natural daylight conditions. In addition, control seedlings were sprayed with sterile water. Three weeks after inoculation, necrosis lesions and the formation of pycnidia on these lesions were investigated in the aerial parts of in wheat Chamaran cultivar.

Table 1. Isolates included in phylogenetic analysis of *Parastagonospora* species. The newly generated sequences are indicated in bold

Taxon	Isolate No.	Host	Country	LSU	ITS	Reference
Parastagonospora nodorum	CBS 110109[T]	*Lolium perenne*	Denmark	KF251681	KF251177	Quaedvlieg *et al.* 2013
P. avenae	CBS 289.69	*L. perenne*	Germany	KF251678	KF251174	Quaedvlieg *et al.* 2013
P. avenae	CBS 290.69	*L. perenne*	Germany	KF251679	KF251175	Quaedvlieg *et al.* 2013
P.nodorum	CBS 259.49	*Triticum* sp.	Canada	KF251688	KF251185	Quaedvlieg *et al.* 2013
P. poae	CBS 135089 [T]	*Poa* sp.	Netherlands	KF251682	KF251178	Quaedvlieg *et al.* 2013
P. poae	CBS 135091	*Poa* sp.	Netherlands	KF251683	KF251179	Quaedvlieg *et al.* 2013
P. poagena	CBS 136776 [T]	*Poa* sp.	Netherlands	KJ869174	KJ869116	Li *et al.* 2015
P. caricis	CBS 135671	*Carex acutiformis*	Netherlands	KF251680	KF251176	Quaedvlieg *et al.* 2013
P. nigrans	CBS 307.79	*Zea mays*	Switzerland	KF251687	KF251184	Quaedvlieg *et al.* 2013
P. dactylidis	IRAN-1	*Phalaris arundinacea*	Iran	MK078104	MK032162	Ghaderi & Razavi 2018
P. dactylidis	MFLUCC 13-0375[T]	*Dactylis* sp.	Italy	KU058722	KU058712	Li *et al.* 2015
P. uniseptata	MFLUCC 13-0387	*Daucus* sp.	Italy	KU058725	KU058715	Li *et al.* 2015
P. minima	MFLUCC 13-0376[T]	*Dactylis* sp.	Italy	KU058723	KU058713	Li *et al.* 2015
P. italica	MFLUCC 13-0377[T]	*Dactylis* sp.	Italy	KU058724	KU058714	Li *et al.* 2015
P. fusiformis	MFLUCC 13-0215	*D. glomerata*	Italy	KX910088	KX926418	Thambugala *et al.* 2017
P. forlicesenica	MFLUCC 13-0557	*D. glomerata*	Italy	KY769661	KY769660	Thambugala *et al.* 2017
Zymoseptoria passerinii	CBS 120384	*Hordeum vulgare*	USA	JQ739844	JF700879	Quaedvlieg *et al.* 2013
Parastagonospora poae	Pp-Iran1	*Aegilops tauschii*	Iran	**PQ097847**	**PQ107786**	In this study
P. poae	Pp-Iran2	*A. tauschii*	Iran	**PQ097848**	**PQ107787**	In this study
P. poae	Pp-Iran3	*A. tauschii*	Iran	**PQ097849**	**PQ107788**	In this study
P. poae	Pp-Iran4	*A. tauschii*	Iran	**PQ097850**	**PQ107789**	In this study
P. poae	Pp-Iran5	*A. tauschii*	Iran	**PQ097851**	**PQ107790**	In this study
P. poae	Pp-Iran6	*A. tauschii*	Iran	**PQ097852**	**PQ107791**	In this study
P. poae	Pp-Iran7	*A. tauschii*	Iran	**PQ097853**	**PQ107792**	In this study
P. poae	Pp-Iran8	*A. tauschii*	Iran	**PQ097854**	**PQ107793**	In this study
P. minima	Pm-Iran1	*A. tauschii*	Iran	**PQ097855**	**PQ107794**	In this study
P. minima	Pm-Iran2	*A. tauschii*	Iran	**PQ097856**	**PQ107795**	In this study
P. minima	Pm-Iran3	*A. tauschii*	Iran	**PQ097857**	**PQ107796**	In this study
P. minima	Pm-Iran4	*A. tauschii*	Iran	**PQ097858**	**PQ107797**	In this study
P. minima	Pm-Iran5	*A. tauschii*	Iran	**PQ097859**	**PQ107798**	In this study

Results

- Fungal isolation and identification

Dark brown lesions containing black pycnidia at the center, often surrounded by a yellow halo were observed on ears, leaves, and stems of *A. tauschii* in wheat fields (Fig. 1). In total, 13 *Parastagonospora* isolates were obtained from symptomatic tissues. Nearly 16 plants were taken from each wheat field to the laboratory and eight tissue pieces of each plant were placed on culture medium. Based on morphological characterization and molecular criteria, the isolates were identified as *P. poae* and *P. minima*. Eight *P. poae* isolates were isolated from the ears, and five *P. minima* isolates were obtained from the stems of *A. tauschii*.

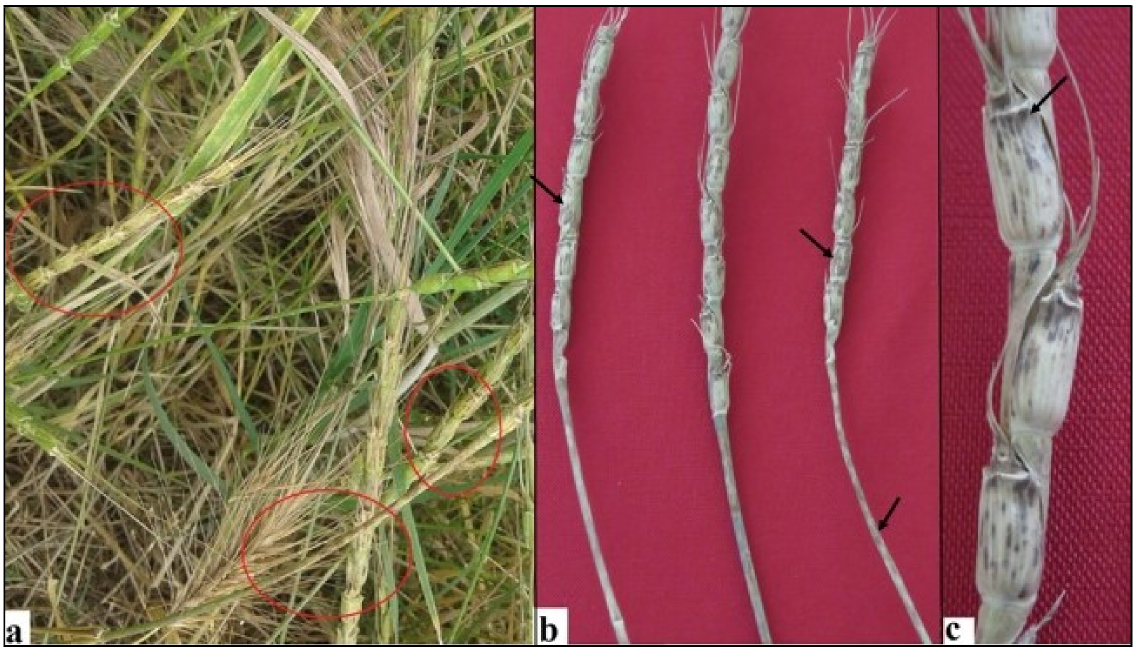

Fig. 1. Tausch's goatgrass plants showing necrotic lesions with abundant pycnidia on ears and stems: a-c. *Parastagonospora poae* species isolated from pycnidia on the ears of *Aegilops tauschii*, b. *P. minima* species isolated from pycnidia on the stems of *A. tauschii*.

Parastagonospora poae Quaedvlieg, Verkley & Crous

Colonies on YSA medium reaching 5 cm diam. after seven days at 25 °C in darkness, with dense, white aerial mycelium to olive green, with rounded, smooth, margins, reverse black (Fig. 2a-b). Conidiomata pycnidial, up to 220 µm diam., brown to black, globose with central ostiole, immersed, formed on WA medium containing sterilized *Aegilops* straws extract, exuding black conidial cirrhus, (Fig. 2c-d). Conidiogenous cells phialidic, smooth, hyaline, subcylindrical, 6.50–9 × 4–5 µm. Conidia hyaline, smooth, thin-walled, cylindrical with obtuse apex and truncate base, mostly 1-septate, 22–29 × 2.1–2.4 µm (Fig. 2e-i).

None of the *P. poae* isolates formed pseudothecia (sexual morph) when grown alone or in any of the crosses in laboratory conditions.

Specimens examined: IRAN: Fars Province, NoorAbad, eight *P. poae* isolates (Pp-Iran1 to Pp-Iran8) isolated from ears of *Aegilops tauschii*, 23.07.2022, F. Ghaderi.

Notes: *Parastagonospora poae* is morphologically similar to the *P. nodorum* (Quaedvlieg *et al.* 2013). Nonetheless, these two species can be distinguished based on conidia width and septation, of which *P. poae* conidia are mostly 1-septate, and 19–21 × 1.8–2 µm wide vs. 1–3-septate and 14–27 × 2.9–4.5 µm wide in *P. nodorum* (Quaedvlieg *et al.* 2013).

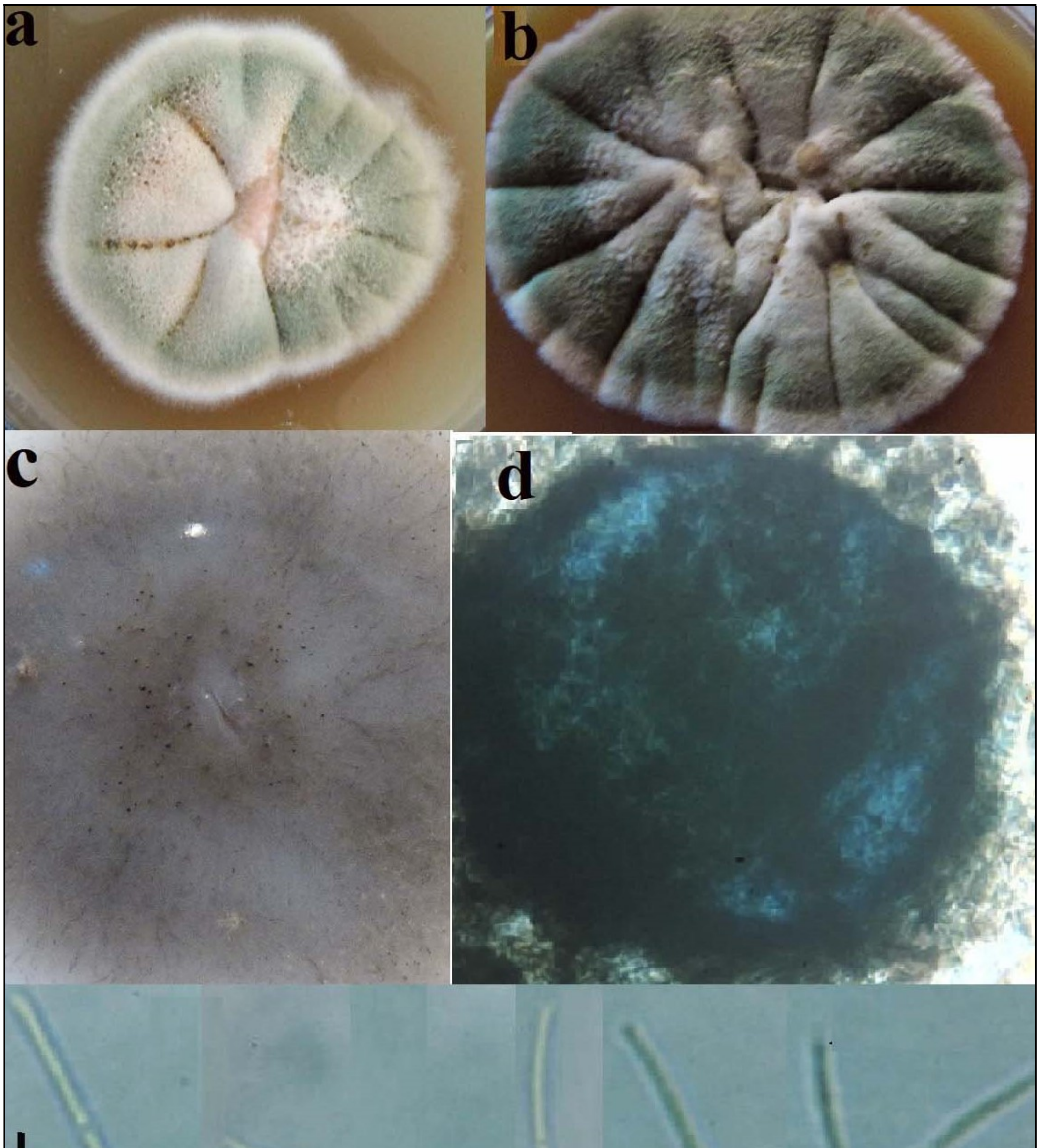

Fig. 2. *Parastagonospora poae*: a. Colony on YSA, b. PDA after seven days at 25 °C in darkness, b-d. Pycnidia formed in culture medium, e. Pycnidiospores (Bar = 10 µm).

Parastagonospora minima W.J. Li, Camporesi, D.J. Bhat & K.D. Hyde

Colonies on YSA medium reaching 5 cm diam. after seven days at 25 °C in darkness, flat, dense, margin entire, pink at the center and white at the margin, sparse aerial mycelium at the center (Fig. 3a). Conidiomata pycnidial, up to 120 µm diam., brown to black, almost immersed, globose with central ostiole formed on YSA medium containing sterilized *Aegilops* straws extract (Fig. 3b-d). Conidiogenous cells phialidic, smooth, hyaline, 3–6 × 4–6.5 µm. Conidia hyaline, subcylindrical, slightly curved, smooth-walled, 3-septate, 23–25 × 3.6–4.1 µm (Fig. 3e-n).

None of the *P. minima* isolates formed pseudothecia (sexual morph) when grown alone or in any of the crosses in laboratory conditions.

Specimens examined: IRAN: Kohgiluyeh & Boyer-Ahmad Province, Dehdasht, five *P. minima* isolates (Pm-Iran1 to Pm-Iran5) obtained from *Aegilops tauschii*, 05.05.2022, F. Ghaderi.

Notes: *Parastagonospora minima* is morphologically similar to *P. nodorum* and *P. dactylidis*, however, they can be distinguished based on differences in conidum shape and septation. *Parastagonospora minima* regularly has 3-septate conidia, whereas *P. nodorum* has 1–3-septate conidia (Quaedvlieg *et al.* 2013).

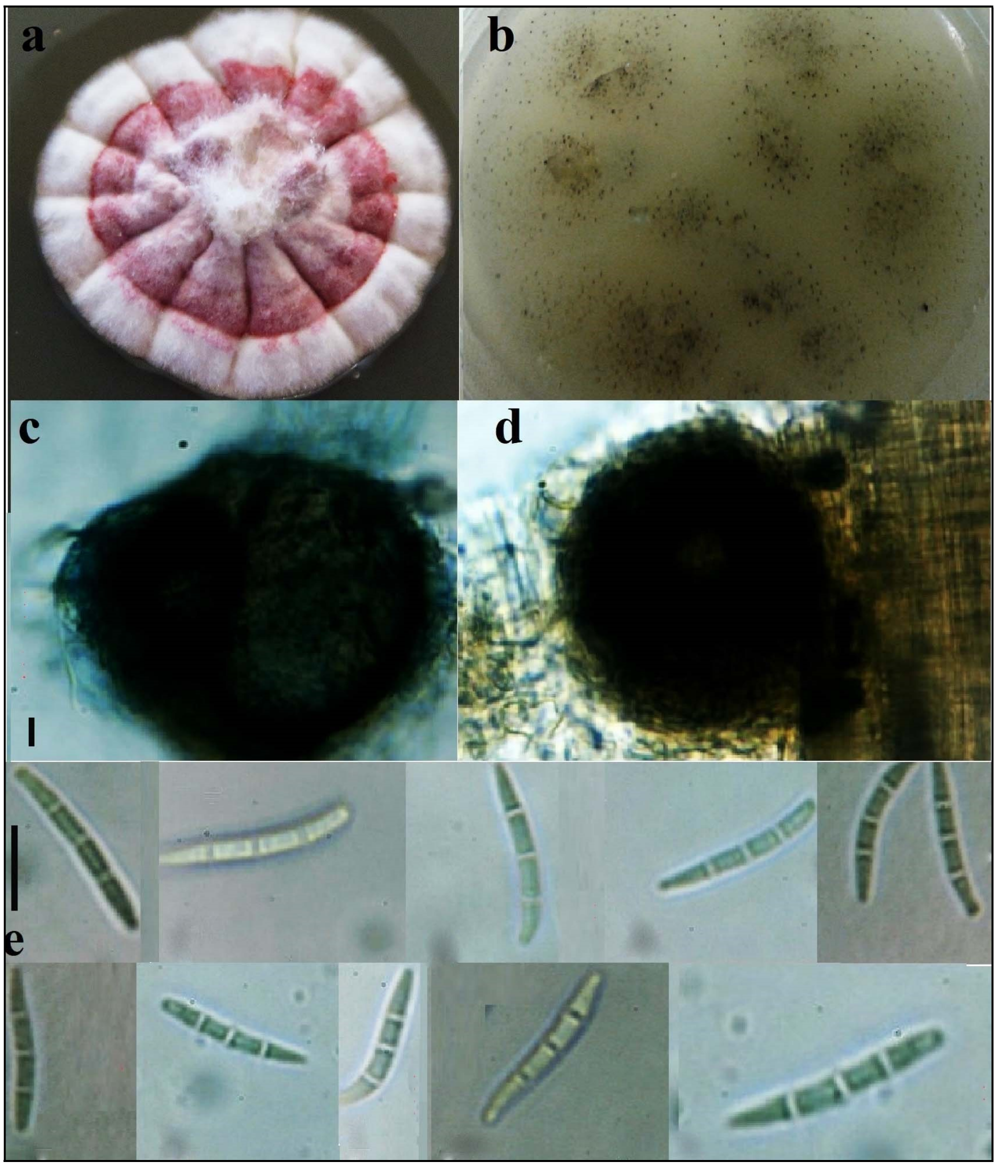

Fig. 3. *Parastagonospora minima*: a. Colony on YSA after seven days at 25 °C, b. Pycnidia formed in culture medium, c-d. Pycnidia, e. Pycnidiospores (Bar = 10 μm).

- Molecular identification

A combined analysis of the ITS and LSU sequences, consisted of 29 *Parastagonospora* spp. Sequences was used to confirm the phylogenetic placement of the present sudy isolates, with *Zymoseptoria passerinii* (CBS120385) as the outgroup taxon (Fig. 4). The maximum parsimony dataset consisted of 1399 characters, of which 1049 were constant, 303 were variable and parsimony-informative and 47 were parsimony-uninformative. The most parsimonious tree yielded the following metrics: CI = 0.91, RI = 0.83, RC = 0.76, and HI = 0.09. Pp-Iran1 to Pp-Iran8 isolates clustered with *P. poae* and Pm-Iran1 to Pm-Iran5 clustered with *P. minima*, with 72 and 89 bootstrap values, respectively.

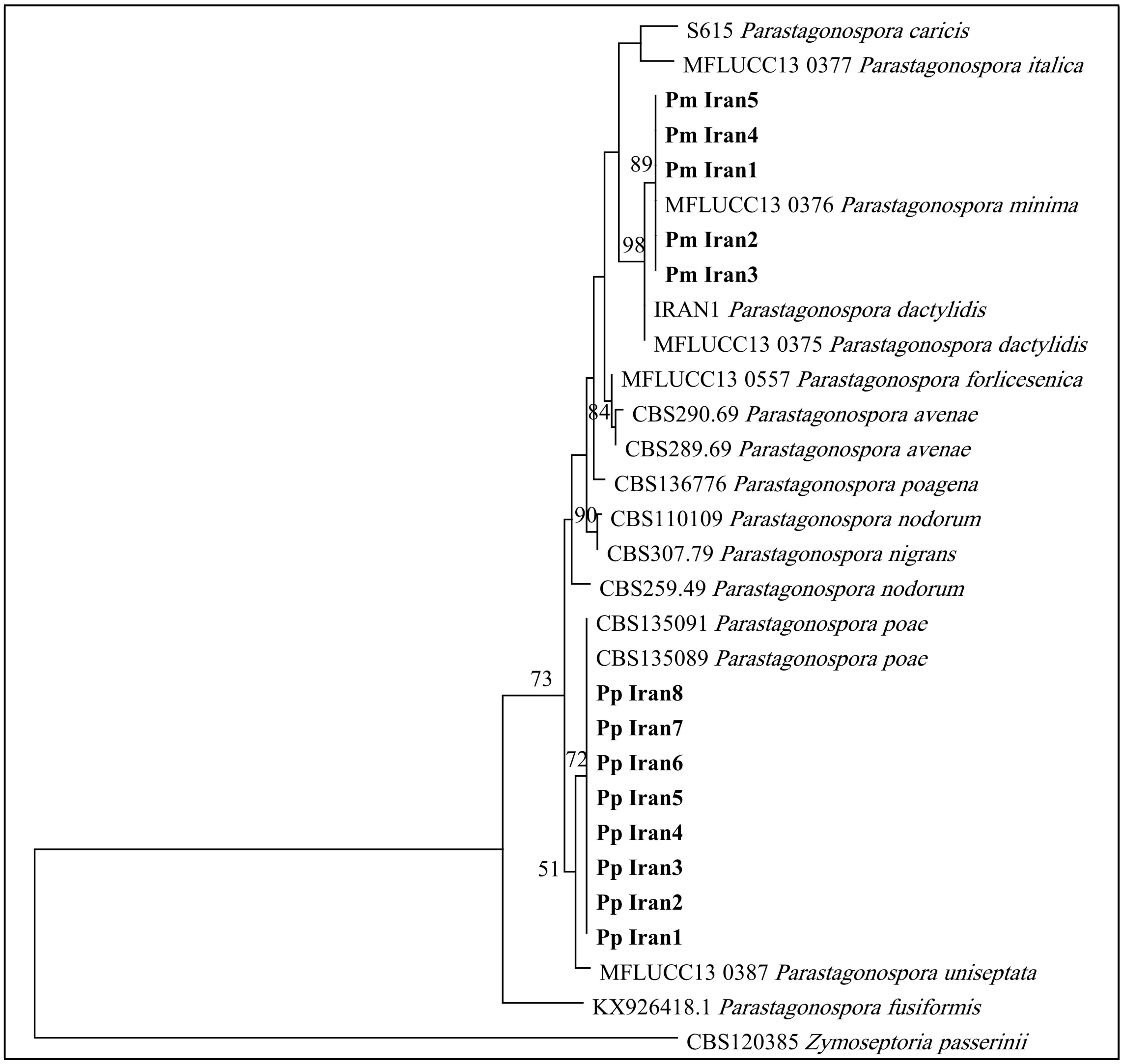

Fig. 4. Phylogram showing one of the most parsimonious trees inferred from maximum parsimony analysis of combined ITS and LSU sequence data for *Parastagonospora* spp. Numbers on the branches are bootstrap support values. Isolates identified in this study are in bold.

- Pathogenicity tests

The obtained *Parastagonospora* species caused necrosis lesions on the 'Chamran' cultivar of wheat in greenhouse. Pycnida developed three weeks after inoculations (Fig. 5). No symptoms were observed in the control plants. *Parastagonospora* spp. were re-isolated from the inoculated plants, fulfilling Koch's postulates.

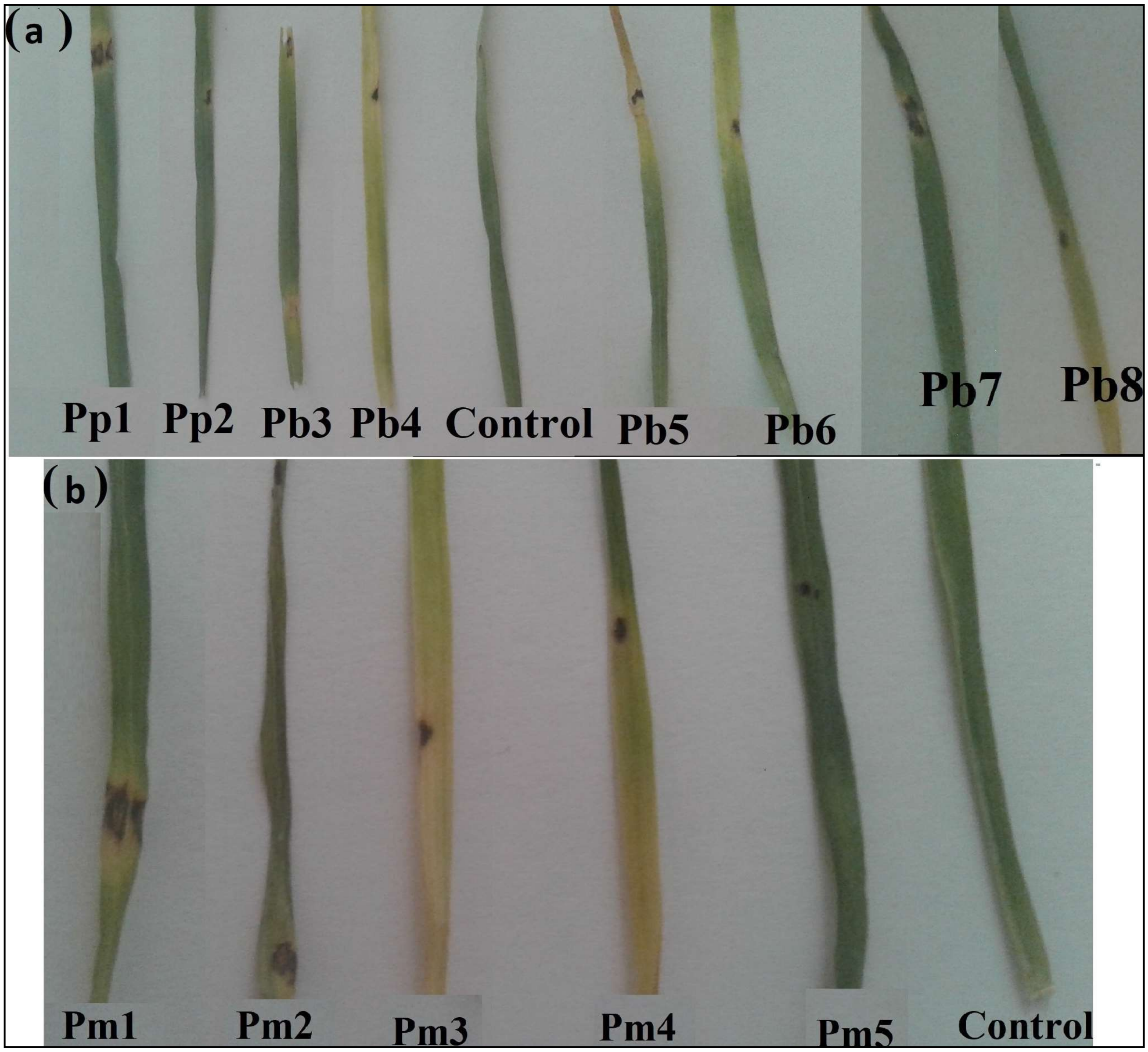

Fig. 5. Necrotic lesions and the formation of pycnidia in the Chamaran cultivar artificially inoculated with (a) *Parastagonospora poae* isolates (Pp-Iran1 to Pp-Iran8), (b) *Parastagonospora minima* isolates (Pm-Iran1 to Pm-Iran5). Control = non-inoculated control.

- Mating type idiomorphs and fertility

Amplification of mating-type idiomorphs were successfully carried out for all obtained isolates. All five *P. minima* isolates carried only the *MAT1-2* allele and amplified a specific 510 bp PCR product, while the eight *P. poae* isolates carried only the *MAT1-1* allele and amplified a specific 360 bp PCR product. This precluded making crosses between isolates of the two species. Pseudothecia development was not observed in any of the crosses between *MAT1-1* isolates of the *P. poae* and *MAT1-2* isolates of *P. minima*.

Discussion

Isolation of fungi from symptomatic ears and stems of the wild grass, *A. tauschii*, yielded coelomycetous fungi with hyaline, cylindrical, transversely euseptate conidia, similar to *Parastagonospora* spp. Phylogenetic analysis using sequence data from ITS and LSU regions revealed that, the obtained isolates clustered with representative isolates of *P. poae* and *P. minima*. Inoculation of the 'Chamran' cultivar widely cultivated in Iran, showed that, the isolates obtained from *A. tauschii* were pathogenic on wheat. *Parastagonospora poae* and *P. minima* were identified and reported as new records for the funga of Iran in this study. To the author's knowledge, this is the first report on association of *P. poae* and *P. minima* with *A. tauschii* worldwide and *Triticum aestivum* as the potential host of the species.

The taxonomy of *Parastagonospora* spp. is based on morphological characteristics of their asexual morph such as conidia shape and septation, coupled with molecular data (Phookamsak *et al.* 2017). LSU and ITS sequence data have been successfully used to distinguish many of the presently known genera within the Phaeosphaeriaceae (Bakhshi *et al.* 2018). However, there are cases that have reported protein-coding genes are needed to distinguish species within the Phaeosphaeriaceae (Goonasekara *et al.* 2019). In the present study, ITS and LSU regions were suitable for differentiating *P. poae* and *P. minima* from related taxa. The overall topology of the obtained tree was in agreement with previously published phylogenies (Croll *et al*. 2021, Li *et al.* 2015).

Parastagonospora species are heterothallic loculoascomycetes and sexual reproduction requires the mating of two distinct isolates carrying *MAT1-1* and *MAT1-2* idiomorphs (Sommerhalder 2006). Knowledge of the extent of sexual reproduction predicted by frequencies of both mating-type idiomorphs in the population is a key factor in evaluating the evolutionary potential of a pathogen (Solomon *et al.* 2004). The results of this study showed that, all five *P. minima* isolates from Kohgiluyeh & Boyer-Ahmad Province had the 'Chamran' cultivar *MAT1-2* idiomorph, while the eight *P. poae* isolates from Fars Province had the *MAT1-1* idiomorph. The absence of both mating-type idiomorphs in the sampled populations supports the hypothesis that, the asexual stage is the dominant part of life cycle in the sampled areas in this two regions. Sexual structures and ascospores were not found in the collected samples, which also suggests that, they do not play a role in the epidemiology of *P. minima* and *P. poae* in Kohgiluyeh & Boyer-Ahmad and Fars. There is the possibility that, the two populations have a skewed distribution of mating types, considering the small sample size in the two sampling regions. Obtaining a precise estimation of the mating-type distribution needs a large number of isolates and extensive samplings (Solomon *et al.* 2004). Further comprehensive studies with more isolates are required. Another possible explanation is that, a pycnidial clone has been sampled. The pycnidia used in this study were from lesions that were collected from different locations. The present results showed that, there is a possibility of skewed mating-type ratios of *P. minima* and *P. poae* in Kohgiluyeh & Boyer-Ahmad and Fars Provinces which needs further sampling. Skewed mating-type frequencies in *Stagonospora* spp. have been reported by other researchers (Halama 2002, Solomon *et al*. 2004, Ghaderi *et al.* 2022).

In this study, *P. poae* and *P. minima* were isolated from the wild grass i.e., *A. tauschii*, growing within and next to fields of cultivated wheat cv. 'Chamran' in Kohgiluyeh & Boyer-Ahmad and Fars Provinces (Iran). *P. poae* and *P. minima* isolates could infect the 'Chamran' cultivar, the dominant wheat cultivar grown in Kohgiluyeh & Boyer-Ahmad and Fars Provinces. Wild grass species growing within or next to fields of cultivated wheat likely represent a source of new pathogens (Stukenbrock & McDonald 2008). Plant pathogens will continue to emerge in agricultural ecosystems via several mechanisms, including host-tracking, host jumps, and spill-back (Stukenbrock & McDonald 2008, Kelly *et al.* 2009, Habibi *et al.* 2023). Croll *et al.* (2021) showed that, *P. nodorum* originated as a pathogen of wild grasses in the Fertile Crescent and then emerged as a wheat pathogen via host-tracking during the domestication of wheat in the same region. Kelly *et al*. (2009) hypothesized that, *Parastagonospora* species infecting the wild grass, *Stipa pulchra*, in California possibly emerged through a 'spill-back' process from *P. nodorum*.

The discovery of two species of *Parastagonospora* spp. infecting wild grass species in Kohgiluyeh & Boyer-Ahmad and Fars Provinces suggests that, new *Parastagonospora* species could emerge from this region in the future. The collected isolates of *Parastagonospora* spp. from wild grasses in Iran provide the opportunity to study the evolutionary history of *Parastagonospora*. However, genome sequences from various *Parastagonospora* spp. found in Iran will be needed to perform further studies.

Acknowledgment

The authors would like to appreciate the financial support of the Yasouj University (Yasouj, Iran), under grant number 1403d/89/947.

References

Arseniuk, E., Góral, T. & Scharen, A.L. 1998. Seasonal patterns of spore dispersal of *Phaeosphaeria* spp. and *Stagonospora* spp. Plant Disease 82(2): 187–194.

Bakhshi, M., Arzanlou, M., Groenewald, J.Z., Quaedvlieg, W. & Crous, P.W. 2019. *Parastagonosporella fallopiae* gen. et sp. nov. (Phaeosphaeriaceae) on *Fallopia convolvulus* from Iran. Mycological Progress 18: 203–214.

Bathgate, J.A. & Loughman, R. 2001. Ascospores are a source of inoculum of *Phaeosphaeria nodorum*, *P. avenaria* f. sp. *avenaria* and *Mycosphaerella graminicola* in Western Australia. Australasian Plant Pathology 30(4): 317–322.

Bennett, R.S., Yun, S.H., Lee, T.Y., Turgeon, B.G., Arseniuk, E., Cunfer, B.M. & Bergstrom, G.C. 2003. Identity and conservation of mating type genes in geographically diverse isolates of *Phaeosphaeria nodorum*. Fungal Genetics and Biology 40: 25–37.

Cowger, C. & Silva-Rojas, H.V. 2006. Frequency of *Phaeosphaeria nodorum*, the sexual stage of *Stagonospora nodorum*, on winter wheat in North Carolina. Phytopathology 96: 860–866. DOI: 10.1094/PHYTO-96-0860.

Croll, D., Crous, P.W., Pereira, D., Mordecai, E.A., McDonald, B.A. & Brunner, P.C. 2021. Genome-scale phylogenies reveal relationships among *Parastagonospora* species infecting domesticated and wild grasses. Persoonia 46: 116–128. DOI: 10.3767/persoonia.2021.46.04.

Friesen, T.L., Xu, S.S. & Harris, M.O. 2008. Stem rust, tan spot, *Stagonospora nodorum* blotch, and Hessian fly resistance in Langdon durum *Aegilops tauschii* synthetic hexaploid wheat lines. Crop Science 48(3): 1062–1070. DOI: 10.2135/cropsci2007.08.0463.

Ghaderi, F., Habibi, A. & Sharifnabi, B. 2022. Phylogenetic analysis of *Phaeosphaeria* species sing mating type genes and distribution of mating types in Iran. Plant Pathology Journal 38(2): 78–89. DOI: 10.5423/PPJ.OA.10.2021.0154.

Ghaderi, F. & Razavi, M. 2018. Identification of the species *Parastagonospora dactylidis* on poaceous plants in Iran. Mycologia Iranica 5: 35–41. DOI: 10.22043/mi.2019.118075.

Ghaderi, F., Sharifnabi, B. & Javan-Nikkhah, M. 2016. Assessment of partial resistance of wheat cultivars to *Phaeosphaeria nodorum* in Iran. Journal of Applied Entomology and Phytopathology 84: 55–66. DOI: 10.22092/jaep.2016.106668.

Ghaderi, F., Sharifnabi, B., Javan-Nikkhah, M., Brunner, P.C. & McDonald, B.A. 2020. SnToxA, SnTox1, and SnTox3 originated in *Parastagonospora nodorum* in the Fertile Crescent. Plant Pathology 69: 1482–1491. DOI: 10.1111/ppa.13233.

Goonasekara, I.D., Campores, E., Bulgakov, T.S., Phookamsak, R., Jayawardena, R.S. & Saichana, N. 2019. Two novel species of *Parastagonospora* (Phaeosphaeriaceae, Pleosporales) on grasses from Italy and Russia. Asian Journal of Mycology 2(1): 170–182. DOI: 10.5943/ajom/2/1/8.

Habibi, A. Ghaderi, F. & Banihashemi, Z. 2023. Coevolution of *Polystigma amygdalinum* through a process of host tracking. Plant Pathology Science 12(1): 36–45. DOI: 10.61186/pps.12.1.36.

Halama, P. 2002. Mating relationships between isolates of *Phaeosphaeria nodorum* (anamorph: *Stagonospora nodorum*) from geographical locations. European Journal of Plant Pathology 108: 593–596.

Halama, P. & Lacoste L. 1992. Etude des conditions optimales ermettantlapycniog´en`esede *Phaeosphaeria* (Leptosphaeria) *nodorum*, agent de la septoriose du bl´e. Agronomie 12: 705–710.

Hasanpour, K., Aalami, A., Ghanbari Moheb Seraj, R., Hosseini, R., Naeimi, S. & Esmaeilzadeh-Salestani, K. 2023. Identification of drought-tolerant hub genes in Iranian KC-2226 genotype of *Aegilops tauschii* using transcriptomic analysis. Scientific Reports 13(1): 9499. DOI: 10.1038/s41598-023-36133-0.

Kelly, D.W., Paterson, R.A., Townsend C.R. *et al.* 2009. Parasite spillback: a neglected concept in invasion ecology? Ecology 90: 2047–2056. DOI: 10.1890/08-1085.1.

Li, W.J., Bhat, D.J., Camporesi, E., Tian, Q., Wijayawardene, N.N., Dai, D.Q., Phookamsak, R., Chomnunti, P., Bahkali, A.H. & Hyde, K.D. 2015. New asexual morph taxa in Phaeosphaeriaceae. Mycosphere 6: 681–708. DOI: 10.5943/mycosphere/6/6/5.

Ma, F., Li, R., Guo, G., Nie, F., Zhu, L., Liu, W. & Song, C.P. 2023. Introgression of QTL from *Aegilops tauschii* enhances yield-related traits in common wheat. The Crop Journal 11(5): 1521–1532. DOI: 10.1016/j.cj.2023.05.001.

Malkus, A., Reszka, E., Chang, C.J., Arseniuk, E., Chang, P.F.L. & Ueng, P.P. 2005. Sequence diversity of β-tubulin (tubA) gene in *Phaeosphaeria nodorum* and *P. avenaria.* FEMS Microbiology Letters 249: 49–56. DOI: 10.1016/j.femsle.2005.05.049.

May, C.E. & Lagudah, E.S. 1992. Inheritance in hexaploid wheat of *Septoria tritici* blotch resistance and other characteristics from *Triticum tauschii*. Australian Journal of Agricultural Research 43: 433–442.

McDonald, B.A., Miles, J., Nelson, L.R. & Pettway, R.E. 1994. Genetic variability in nuclear DNA in field populations of *Stagonospora nodorum*. Phytopathology 84: 250–255.

McDonald, M.C., Razavi, M., Friesen, T.L., Brunner, P.C. & McDonald, B.A. 2012. Phylogenetic and population genetic analysis of *Phaeosphaeria nodorum* and its close relatives indicate cryptic species and an origin in the Fertile Crescent. Fungal Genetics and Biology 49: 882–895. DOI: 10.1016/j.fgb.2012.08.001.

Murphy, N.E.A., Loughman, R., Wilson, R.E., Lagudah, E.S., Appels, R. & Jones, M.G.K. 2001. A single gene controls resistance to *septoria nodorum* blotch in the *Aegilops tauschii* accession AUS21712. Australian Journal of Agricultural Research 52(12): 1403–1407.

Murray, M. & Thompson, W.F. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research 8(19): 4321–4325.

Phookamsak, R., Wanasinghe, D.N., Hongsanan, S., Phukhamsakda, C. *et al.* 2017. Towards a natural classification of Ophiobolus and ophiobolus-like taxa; introducing three novel genera *Ophiobolopsis*, *Paraophiobolus* and *Pseudoophiobolus* in Phaeosphaeriaceae (Pleosporales). Fungal Diversity 87: 299–339. DOI: 10.1007/s13225-017-0393-1.

Quaedvlieg, W., Verkley, G.J.M., Shin, H.D., Barreto, R.W., Alfenas, A.C., Swart, W.J., Groenewald, J.Z. & Crous, P.W. 2013. Sizing up *Septoria*. Studies in Mycology 75: 307–390. DOI: 10.3114/sim0017.

Salamini, F., Ozkan. H., Brandolini, A. *et al*. 2002. Genetics and geography of wild cereal domestication in the Near East. Nature Reviews Genetics 3: 429–441.

Shaw, M.W. 1999. Epidemiology of *Mycosphaerella graminicola* and *Phaeosphaeria nodorum*: An Overview. Pp. 93–97. *In*: *Septoria* and *Stagonospora* Diseases of Cereals: A Compilation of Global Research (van Ginkel, M., McNab, A. & Krupinsky, J.; eds). International Maize and

Wheat Improvement Center, Mexico D.F., Mexico.

Solomon, P.S., Lowe, R.G.T., Tan, K.C., Waters, O.D.C. & Oliver, R.P. 2006. *Stagonospora nodorum*: cause of *Stagonospora nodorum* blotch of wheat. Molecular Plant Pathology 7: 147–156. DOI: 10.1111/j.1364-3703.2006.00326.x.

Solomon, P.S., Parker, K., Loughman, R. & Oliver, R.P. 2004. Both mating types of *Phaeosphaeria* (anamorph *Stagonospora nodorum*) are present in Western Australia. European Journal of Plant Pathology 110: 763–766.

Sommerhalder, R.J., McDonald, B.A. & Zhan, J. 2006. The frequencies and spatial distribution of mating types in *Stagonospora nodorum* are consistent with recurring sexual reproduction. Phytopathology 96: 234–239. DOI: 10.1094/PHYTO-96-0234.

Swofford, D.L. 2002. PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods). Sinauer Associates, Sunderland, Massachusetts.

Stukenbrock, E.H. & McDonald, B.A. 2008. The origins of plant pathogens in agroecosystems. Annual Review of Phytopathology 46: 75–100. DOI: 10.1146/annurev.phyto.010708.154114.

Thambugala, K.M., Daranagama, D.A., Phillips, A.J., Bulgakov, T.S. *et al.* 2017. Microfungi on Tamarix. Fungal Diversity 82: 239–306. DOI: 10.1007/s13225-016-0371-z.

Ueng, P.P. & Chen, W. 1994. Genetic differentiation between *Phaeosphaeria nodorum* and *P. avenaria* using restriction fragment length polymorphisms. Phytopathology 84: 800–806.

Ueng, P., Subramaniam, K., Chen, W., Arseniuk, E., Wang, Lm., Cheung, A., Hoffmann, G. & Bergstrom, G. 1998. Intraspecific genetic variation of *Stagonospora avenae* and its differentiation from *S. nodorum*. Mycological Research 102: 607–614.

Vilgalys, R. & Hester, M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172: 4238–4246.

Weber, G.F. 1922. *Septoria* diseases of cereals. I. Speckled blotch of oats caused by *Leptosphaeria*. Phytopathology 12: 449–470.

White, T.J., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. Pp. 315–322. *In*: PCR Protocols: a guide to methods and applications (Innis, M.A., Gelfand, D.H., Sninsky J.J. & White, T.J.; eds). Academic Press, San Diego, California.