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Septoria malagutii as an endophytic fungus of *Achillea millefolium* from Iran

S. Hatamzadeh

K. Rahnama ✉

S. Nasrollahnejad

Department of Plant Protection, Faculty of Crop Production, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

Kh.-Br. Fotouhifar

Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

K. Hemmati

Department of Horticulture, Faculty of Plant production, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

J. F. White

Department of Plant Biology, Rutgers University, New Brunswick, New Jersey, USA

From May until September 2015, a survey was conducted to collect endophytic fungi from healthy medicinal plants in the natural ecosystem of Golestan province. Endophytic fungi were isolated from *Achillea millefolium* plants. Different samples were provided from roots, leaves and stems of the healthy and mature plants. The plants were rinsed gently under running water and samples were cut into the 0.5–1cmpieces. The surface sterilization was performed by sodium hypochlorite (NaOCl) and 75% ethanol. The surface sterilized samples were placed on PDA plates supplemented with 50 mg/L tetracycline to suppress the bacterial growth and incubated at 28 ± 2°C up to 14 days. Morphological identification was performed usingfungal identif ication keys (Cline & Rossman 2006; Sutton 1980; Priest 2006, Verkley et al. 2013). Subsequently, the nucleic acid was extracted using the cetyl trimethyl ammonium bromide (CTAB) method (Doyle 1990). The strains were sequenced partially for four genomic regions including internal transcribed spacer regions (ITS) of nrDNA, 28S nrDNA gene (LSU), translation elongation factor 1-α*(tef1-α)* and β-*tubulin* (*tub2*). All the sequences were deposited in NCBI's GenBank Database.

A basic alignment of the obtained sequence data was done using MAFFT v. 7 and Mr Modeltest 2.3 was used to determine the best substitution models for each locus. Bayesian analyses were performed on the concatenated loci using MrBayes v. 3.2.1 (Huelsenbeck & Ronquist 2001).

Based on the four-regions tree,the examined strain was grouped in the same clade with *S. malagutii* (CBS 106.80) and identified as *Septoria malagutii*.

The genus *Septoria*, is one of the largest genera of plant pathogens, causing a range of disease symptoms including leaf and fruit spots (Verkley et al 2013). However, the endophytic species of this genus had not yet been reported. This is the first report of *S. malagutii* as an endophytic fungus from Iran (Ershad 2009).

Septoria malagutii E.T. Cline, Mycotaxon 98: 132 (2006).

*Septoria malagutii*have been isolated as an endophyte from *A. millefolium* in the Golestan province with (36 °36′10″N 54°29′55″E) geographical coordinates. Morphology on PDA after 15 days of culture at 23°C: colony 22 mm in diameter, aerial mycelium white, underlying color grey to black, surface floccose, slightly raised and reverse faintly ringed. Conidiomata pycnidial, slightly paler, epigenous, scattered, globose to subglobose, solitary, smooth, 97µmin diameter, formed mostly on the agar surface, ostiole was not observed, immature pycnidia black, 43–70 µmin diameter. Conidiophores were not observed. Conidiogenous cells were ampulliform, rarely doliform, discrete, determinate, hyaline,4–10 × 2–7 µm. Conidia holoblastic, hyaline, filiform, strongly curved, occasionally straight, sharply pointed at both ends, 4–6 septate, sometimes slightly rounded at base, 60–125 × 1.7–2 µmin diameter.The fungal isolate was deposited in the Iranian Fungal Culture Collection (IRAN C) of the Iranian Research Institute of Plant Protection, Tehran, Iran with voucher code: IRAN 3261C. Accession numbers in the NCBI's GenBank Database: (ITS: MH259175, LSU: MH255545, *tef1*-α:MK952153), *tub2*:MK952152).

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✉Corresponding Author E-mail: rahnama@gau.ac.ir

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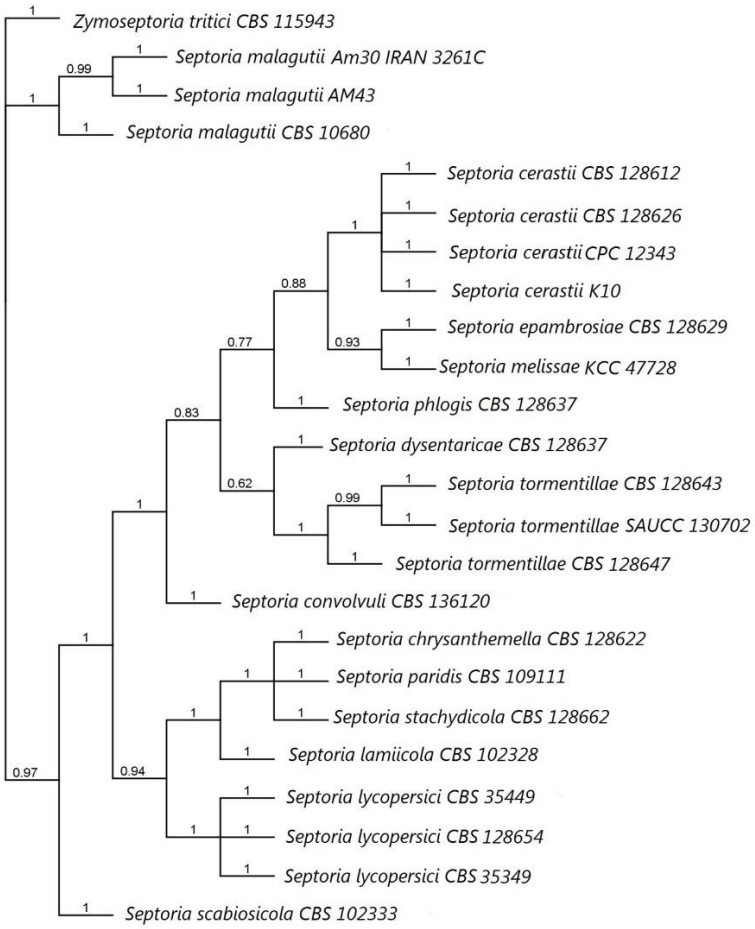

Fig. 1. Bayesian tree from a concatenated of ITS, LSU, *tef1* and *tub2*data sets. Numbers on the branches are Bayesian posterior probabilities (PP). The tree was rooted with *Zymoseptoria tritici* as outgroup taxa. IRAN 3261C is related to the *S. malagutii* in this study.

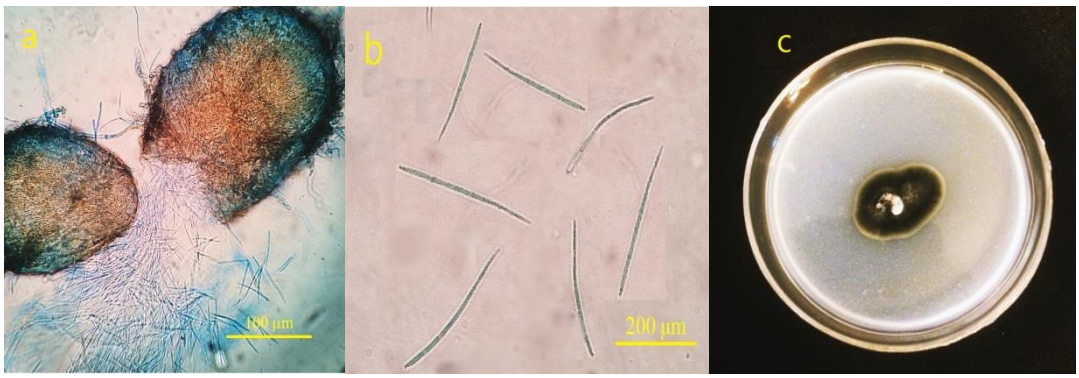

Fig. 2. *Septoria malagutii:* a. pycnidia, b.conidia, c.Colony on PDA (potato dextrose agar) after 14days of culture.

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