First report of *Peronospora saturejae-hortensis* in Iran

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Received: 11 June 2020 / Accepted: 20 December 2020 / Published online: 6 February 2021
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Abstract

In November 2018 severe downy mildew infection was observed on summer savory plants in Kermanshah Province, Iran. Leaves on infected plants displayed chlorosis-browning on the upper surfaces and abundant dark gray-brown sporulation of the asexual morph on the lower surfaces. Based on the morphological characteristics and molecular analyses using ITS-1 and ITS-4, the species was identified as *Peronospora saturejae-hortensis*. The nucleotide sequence of this pathogen had a very high (99.9%) identity with a previously reported sequence of *P. saturejae-hortensis* from Germany. The possibility of infection on other native *Satureja* species in Iran is discussed.

Keywords Aromatic plants · Lamiaceae · ITS · rDNA · Peronosporales

Summer savory (*Satureja hortensis*) is an important aromatic plant in the Lamiaceae family. It is being used as a fresh vegetable, food additive and in many medicinal applications (Hassanzadeh et al. 2016; Mozaffarian 2013).

In November 2018 severe downy mildew infection was observed on approximately 70% of summer savory plants growing in experimental plots inside a research greenhouse at the College of Agriculture, Razi University (Kermanshah, Iran). This greenhouse had high humidity conditions and the temperatures inside were between 18–25 °C. Leaves on infected plants were chlorotic-brown on the upper surface (Fig. 1a) and there was a dark gray-brown sporulation consisting of conidiophores bearing conidia which covered the entire lower surface (Fig. 1b). Later, most infected leaves died. A voucher specimen of diseased material (designated as Felfelak1) was deposited with the herbarium number RUHK-2903 in Razi University Herbarium, (Kermanshah, Iran).

The fungal mass (comprising the asexual morph) was scraped from the lower leaf surfaces and mounted in water or lactophenol-cotton blue on microscope slides. Slides were examined and photographed using an Olympus CH40 microscope equipped with a JVC color video camera (model TK-C1480E). Measurements of conidia and conidiophores

(30 for each) were done by BioloMICS software (Version 1.0.2, BioAware) and are reported as follows, (minimum-) y-z(-maximum), where y = mean-standard deviation and z = mean + standard deviation. All the values were rounded to the nearest whole number. The conidiophores were (190-)345–585(-610) µm in length and had four to five branches (Fig. 1c). The lengths of the longer and the shorter ultimate branchlets (Fig. 1d) were (7-)9–18(-22) µm and (4-)4–7(-8) µm, respectively. Obovoid, brown-olive conidia (17-)17–23(-25) µm × (12-)13–18(-21) µm, length/width ratio 1.1–1.7, developed singly at the tips of the ultimate branchlets (Fig. 1e). Conidia germinated by producing a single unbranched germ tube. No oospore was found in the examined samples.

The species had morphological characteristics very similar to those of *Peronospora saturejae-hortensis* (Gabler et al. 2012). The dimensions of conidia were similar to those reported by Gabler et al. (2012), i.e. (15-)16–25 (-26) × (9-)10–18(-19), but were larger than the dimensions published in the original description (Novotelnova and Pystina 1985), i.e. 14.5–18 × 12–15 µm. The reason for this difference with the latter could be due to the influence of the environment, differences in host matrix (Runge et al. 2012) and that the species author had not examined fresh, fully developed material (Gabler et al. 2012).

Pathogenicity testing was conducted as described by Gabler et al. (2012). The inoculum was prepared from the same infected samples that were used for morphological and molecular studies by stirring 150 g infected leaves in

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Fig. 1 Symptoms, signs and microscopic images of downy mildew caused by *Peronospora saturejae-hortensis* on summer savory; **a** browning of upper leaf surfaces on infected plants, **b** sporulation of the asexual morph on the lower leaf surfaces, **c** conidiophore (bar = 100 μ m), **d** ultimate branchlets (bar = 20 μ m), **e** conidia (bar = 10 μ m)

400 ml water with the addition of 30 μ l Tween 20. The suspension was adjusted to approximately 1×10^6 conidia/ml. Inoculation was done by submerging the eight weeks old summer savory plants in the aqueous suspension of conidia for about 30 s. The inoculated plants were then covered for the entire period of the experiment in a moistened plastic bag starting immediately after inoculation and incubated in a greenhouse at 18–24 °C. The first symptoms appeared after 12 days as small yellow spots on the upper leaf surfaces and after 20 days sporulation consisting of conidiophores and conidia identical to those of *P. saturejae-hortensis* occurred on the abaxial surfaces of the spots. Microscopic examination of the conidiophores and conidia on the inoculated plants confirmed that it was consistent with *P. saturejae-hortensis*.

A spore suspension was collected by spraying the lower leaf surfaces of a downy mildew infected summer savory sample by a hand-held water sprayer and after centrifugation was stored at -20 °C for DNA extraction. DNA was extracted using a commercial kit (Zagros Bioidea Co., Razi University Incubator, Kermanshah, Iran). PCR was done by using primers

ITS1 and ITS4 (White et al. 1990). The PCR product was sequenced by a commercial service (Pishgam Biotech Co., Tehran, Iran) and the sequence was deposited in GenBank under accession number MT510002. BLAST analysis revealed a very high (99.9%) nucleotide identity (the only difference was an ambiguous nucleotide) with the ITS and ribosomal RNA sequence of *P. saturejae-hortensis* previously reported from Germany under the GenBank accession number JN882274 (Gabler et al. 2012). The phylogenetic tree (not shown) that was reconstructed by the same method and the same set of sequences used by Gabler et al. (2012), was similar to that report and the Iranian isolate was in the same clade as the the German isolate that was characterised by Gabler et al. (2012). Except for these two accession numbers (JN882274 and MT510002), there is currently no other available sequences from the ITS and ribosomal RNA genes of this species. The almost identical nucleotide sequences from these two accession numbers reveal a very high genetic conservation in this region and a potentially identical source for both occurrences. Based on both morphological and

molecular characterisation, the Iranian isolate RUHK-2903 can be considered to be *P. satorejae-hortensis*.

Although many *Peronospora* species show a high degree of host specialization (Spring et al. 2019; Thines and Choi 2016), there are also some species including *Peronospora belbahrii* (Ben-Naim et al. 2019; Cohen et al. 2017) and *Peronospora tabacina* (Hall 1989; Kröber and Massfeller 1961) that infect multiple plant species and genera. More than 16 species of *Satureja* such as *Satureja kermanshahensis* and *Satureja khuzistanica* (Jamzad 2010) grow naturally as annual or perennial plants in Iran (Mozaffarian 2013) and there is a possibility that *P. satorejae-hortensis* also causes a similar disease on one or more of these species which may act as inoculum sources and overwintering hosts. Another possible source of inoculum for the infection of summer savory plants that needs to be studied in future is infected seed which has been documented in the case of *P. belbahrii*, the causal agent of basil downy mildew (Garibaldi et al. 2004; Wyenandt et al. 2015).

P. satorejae-hortensis has been reported as the causal agent of downy mildew on summer savory from Germany and Armenia (Gabler et al. 2012; Novotelnova and Pystina 1985), whilst the causal agent of downy mildew on summer savory in Bulgaria and Lithuania has been recorded as *P. calamitinae* (Gabler et al. 2012). The only published report of *Peronospora* on summer savory in Iran was that of *Peronospora lamii* from Khuzestan Province in the south-west of the country (Ershad 1977). Except for this report and others on the occurrence of downy mildew on basil in the south-west (Ebrahimi and Minassian 1973), north (Khateri et al. 2007) and west (Khateri et al. 2019) of Iran, there was no information about the downy mildews on plants in the Lamiaceae in other parts of the country. Previous studies have identified many *Peronospora* collections from different Lamiaceae plants as *P. lamii* which is due to the application of a morphologically-based broad species concept (Gabler et al. 2012). This assumption may have been the reason for neglecting the possible preventive measures such as seed tests (Thines and Choi 2016). The molecular analysis by nucleotide sequences helps to override such problems and differentiate the nearly related but different species such as *P. lamii*, *P. belbahrii* and *P. satorejae-hortensis* (Gabler et al. 2012).

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