Stemphylium vesicarium causing *Sansevieria trifasciata* (viper's bowstring hemp) leaf blight in Iran

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Abstract

Leaves of *Sansevieria trifasciata* with leaf blight symptoms were collected from greenhouses of Miyandoab, West Azerbaijan province, Iran, during the summer of 2016. Leaf blight symptoms were large, elongated and gray to pale brown with brown margins. Three single spore isolates were obtained from diseased leaves and the pathogenicity of each isolate to plants was confirmed by inoculation tests based on Koch's postulates. The pathogen was identified as *Stemphylium vesicarium*, based on both morphological characteristics and sequencing of the *Glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) gene. This is the first report of *Stemphylium vesicarium* causing leaf blight on *Sansevieria trifasciata* in Iran and the world.

Keywords Pathogen identification · *GAPDH* gene · Morphology · Pathogenicity

Sansevieria trifasciata (viper's bowstring hemp, Agavaceae) is commonly known as snake plant and/or mother-in-law's tongue because of its leaf shape and margin sharpness. *Sansevieria trifasciata* is native to tropical Africa and was introduced to America, Asia, Australia, and the Pacific Islands as an ornamental and fiber crop (USDA-ARS 2017). In Iran, *S. trifasciata* is usually used as an ornamental plant in homes and offices. So far, several fungal species have been reported on *Sansevieria* spp. and some of them cause disease on leaves and roots (Farr and Rossman 2017).

During the summer of 2016, some leaves of *S. trifasciata* with leaf blight symptoms were collected from greenhouses of Miyandoab, West Azerbaijan province, Iran. Leaf blight symptoms were large, elongated and gray to pale brown with brown margins (Fig. 1a). A single spore produced on the surface-sterilized leaf was transferred to water-agar and then fungal colonies were cultured from hyphal tips on potato dextrose agar (PDA) culture medium. A subculture was transferred to Potato Carrot Agar (PCA) and incubated

at 22–25 °C under cool/white fluorescent with 10/14 h light/dark photoperiod for 5–7 days (Simmons 1969). Macro- and micro-morphological features were recorded and compared with available literature. Three single-spore isolates of *Stemphylium* sp. made from lesions on symptomatic leaves were deposited in the fungal culture collection of Urmia University (CCUU) as CCUU1362, CCUU1363, and CCUU1364. Measurements and microphotographs were taken from slide mounts in lactophenol using an Olympus light microscope (model BH2). Based on morphological characteristics, the isolates were further identified as *Stemphylium vesicarium* (Simmons 1969). Colonies on PCA after seven days were ca 60 mm diam., olivaceous green to light brown with concentric zones of growth and sporulation. Sporulation abundant mostly from superficial hyphae and to a lesser extent from submerged hyphae (Fig. 2a). Hyphae pale brown, septate, branched and 5–7 µm wide. Conidiophores straight or curved, pale brown, septate, short to moderate, 20–75 × 5–7 µm in size. Conidiogenous cells swollen at the apex, dark brown, 6–8 µm wide and occasionally with 1–4 apical proliferation. Matured conidia dark brown, spherical to oblong, cylindrical to rectangular, rounded at the base and conical to angular at the apex, with a verrucose conidial wall, 6–7 transverse septa, 1–5 longitudinal septa and 1–4 oblique septa, 24–48 × 11–20 µm (Fig. 2b).

Genomic DNA was extracted from the three isolates using the method of Liu et al. (2000). The *Glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) gene was amplified for

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Fig. 1 Symptoms of leaf blight caused by *Stemphylium vesicarium* on *Sansevieria trifasciata*. **a** leaf blight symptoms on naturally infected *S. trifasciata* in greenhouse. **b** leaf blight symptoms on inoculated *S. trifasciata* under greenhouse conditions 10 days after inoculation

each isolate with PCR using the primer pair gpd1/gpd2 (Berbee et al. 1999). Each PCR mixture contained 0.4 pM of each primer, 8 μ L of a ready master mix (Taq 2X Master Mix Red 1.5 Mm, Amplicon Company, Denmark) and about 10 ng of template DNA in a final volume of 25 μ L. A touchdown (TD) PCR method was used (Korbie and Mattick 2008) with an initial denaturation for 90 s at 95 $^{\circ}$ C and then, a cycle of 60 s denaturation at 95 $^{\circ}$ C, 60 s annealing at 62 $^{\circ}$ C and 60 s extension at 72 $^{\circ}$ C, followed by 10 cycles with a 62–57 $^{\circ}$ C annealing temperature (annealing temperature decreased 0.5 $^{\circ}$ C per cycle) and 25 cycles with a 57 $^{\circ}$ C annealing temperature and a final extension for 5 min at 72 $^{\circ}$ C. The PCR

products were purified and sequenced by Macrogen Corporation (South Korea). The newly generated sequences of *GAPDH* in this study were deposited in GenBank (MG668997, MG914073 and MG914074).

Homology searches were performed using the NCBI BLAST search. BLAST searches showed that the sequence data were identical to those of *S. vesicarium*, *S. herbarum*, and *S. alfalfae* from GenBank. The *GAPDH* sequence comparison showed 99% identity (565 out of 567 identical base pairs) to *S. vesicarium* type strain CBS 138138 (GenBank accession KU850735) (Woudenberg et al. 2017) and 100% identity (501 out of 501 identical base pairs) to EPS45 strain (JX075155) (Puig et al. 2015). Phylogenetic analysis based on the maximum likelihood (ML) method of selected *GAPDH* sequences of *Stemphylium* spp. from GenBank (Woudenberg et al. 2017) was conducted using MEGA6.0 software (Tamura et al. 2013). ML analysis based on *GAPDH* sequences indicated that the three isolates from the present study grouped in a *S. vesicarium* clade, supported by bootstrap value of 75% (Fig. 3). According to morphological and phylogenetic species hypothesis, the isolates were identified as *S. vesicarium*.

Sequence data of *GAPDH* in *S. vesicarium* isolates were identical to those of *S. alfalfae* (KU850735) and *S. herbarum* (AF443884). Systematic studies have shown that *S. alfalfae*, *S. brassicicola*, *S. cremanthodii*, *S. herbarum*, *S. mali*, *S. sedicola*, *S. tomatonis* and *S. vesicarium* were conspecific (Inderbitzin et al. 2009; Woudenberg et al. 2017). Woudenberg et al. (2017) revised species of *Stemphylium* based on three loci ITS, *GAPDH*, and *cmdA* and synonymized these seven species under *S. vesicarium*.

A pathogenicity test was conducted with *S. trifasciata* plants that were sown in plastic pots containing sterilized soil in the greenhouse. Three isolates of *S. vesicarium* were cultured on PDA at 25 $^{\circ}$ C for 10 days. The conidial suspensions of *S. vesicarium* were adjusted to 10^5 conidia/ml using a hemacytometer (Köhl et al. 2009). Healthy leaves were inoculated by spraying a conidial suspension (10^5 conidia/ml) of the

Fig. 2 *Stemphylium vesicarium*. **a** Colony on PCA; **b** Conidiophores and conidia. Bar (b) = 20 μ m

Fig. 3 Maximum likelihood (ML) tree based on of the *Glyceraldehyde-3-phosphate dehydrogenase* (GAPDH) sequences of *Stemphylium* spp. retrieved from GenBank. The numbers above the nodes are the bootstrap values obtained from 1000 replicates

fungus. At the same time, some leaves were scratched with a sterile needle and the conidial suspension sprayed onto these wounded parts. The pathogenicity tests were conducted using three replicates of each isolate and three plants for each replicate. Inoculated plants were placed in the greenhouse at 20–25 °C and 75–85% RH along with control plants (three plants) that had been sprayed with sterile, distilled water. Plants were

covered with plastic bags for 48 h to maintain high humidity. Inoculated plants were inspected daily for disease symptom development for 20 days after inoculation. Leaf lesions were visible on all healthy and wounded leaves of inoculated plants after 10 days (Fig. 1b), but symptoms were not observed on control plants. The spots were pale brown with brown margins (Fig. 1b). The fungus was re-isolated from leaf lesions on

inoculated plants and morphologically identified, but not from leaves of control plants, confirming Koch's postulates.

Stemphylium vesicarium was first described from onion plants by Simmons (1969). This species is known as a plant pathogenic fungus and causes leaf spot on a wide variety of plant species. According to Farr and Rossman (2017), it has been associated with more than 20 plant species worldwide. *Stemphylium vesicarium* is an economically important pathogen of agricultural crops. The fungus causes brown spot disease on leaves and fruits of pear (Köhl et al. 2009), leaf blight on onion or garlic (Simmons 1969) and leaf/purple spot on asparagus (Graf et al. 2016). *Stemphylium vesicarium* has previously been reported as leaf blight on broad bean (Sheikh et al. 2015), onion (Ershad 2009), *Helianthus annuus* (Arzanlou et al. 2012), and black (sooty) head mold of wheat and barley (Poursafar et al. 2016) in Iran. Recently, Kee et al. (2018) reported *S. lycopersici* as the cause of leaf spot on *S. trifasciata* in Malaysia and estimated disease severity and incidence at 25 and 30% (total of 30 plants), respectively. The lesions were circular brown spots that gradually enlarged, forming oval to irregular chlorotic lesions with reddish-brown borders on the leaves (Kee et al. 2018). Symptoms caused by *S. lycopersici* on *S. trifasciata* in Malaysia were similar to symptoms caused by *S. vesicarium* in the present study. However, morphological and molecular analysis showed that our isolates belonged to *S. vesicarium* (Fig. 3). To our knowledge, this is the first record of leaf blight disease on *S. trifasciata* caused by *S. vesicarium* in Iran and the world (Farr and Rossman 2017). Further studies are needed to determine the distribution and to assess the economic impacts of this disease in Iran.

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References

- Arzanlou M, Khodaei S, Babai-Ahari A (2012) *Helianthus annuus* as a natural host for *Stemphylium vesicarium* in Iran. Australas Plant Dis Notes 7:167–170. <https://doi.org/10.1007/s13314-012-0076-4>
- Berbee ML, Pirseyedi M, Hubbard S (1999) *Cochliobolus* phylogenetics and the origin of known, highly virulent pathogens, inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. Mycologia 91:964–977. <https://doi.org/10.2307/3761627>
- Ershad D (2009) Fungi of Iran, 3rd edn. Iranian Research Institution of Plant Protection, Tehran
- Farr DF, Rossman AY (2017) Fungal Databases, U.S. National Fungus Collections, ARS, USDA. Retrieved November 24, 2017, from <https://nt.ars-grin.gov/fungaldatabases/>
- Graf S, Bohlen-Janssen H, Miessner S, Wichura A, Stämmler G (2016) Differentiation of *Stemphylium vesicarium* from *Stemphylium botryosum* as causal agent of the purple spot disease on asparagus in Germany. Eur J Plant Pathol 144:411–418. <https://doi.org/10.1007/s10658-015-0777-6>
- Inderbitzin P, Mehta YR, Berbee ML (2009) *Pleospora* species with *Stemphylium* anamorphs: a four locus phylogeny resolves new lineages yet does not distinguish among species in the *Pleospora herbarum* clade. Mycologia 101:329–339. <https://doi.org/10.3852/08-071>
- Kee YJ, Latiffah Z, Masratul Hawa M (2018) First Report of *Stemphylium lycopersici* causing leaf spot on *Sansevieria trifasciata* in Malaysia. Plant Dis 102:445–446. <https://doi.org/10.1094/PDIS-07-17-1049-PDN>
- Köhl J, Molhoek WML, Groenenboom-de Haas BH, Goossen-van de Geijn HM, Speksnijder A, Kastelein P, de Hoog S, Gerrits van der Ende AHA (2009) Pathogenicity of *Stemphylium vesicarium* from different hosts causing brown spot in pear. Eur J Plant Pathol 124: 151–162. <https://doi.org/10.1007/s10658-008-9402-2>
- Korbie DJ, Mattick JS (2008) Touchdown PCR for increased specificity and sensitivity in PCR amplification. Nat Protoc 3:1452–1456. <https://doi.org/10.1038/nprot.2008.133>
- Liu D, Coloe S, Baird R, Pederson J (2000) Rapid mini-preparation of fungal DNA for PCR. J Clin Microbiol 38:471
- Poursafar A, Ghosta Y, Javan-Nikkah M (2016) A taxonomic study on *Stemphylium* species associated with black (sooty) head mold of wheat and barley in Iran. Mycol Iran 3:99–109. <https://doi.org/10.22043/mi.2017.26183>
- Puig M, Ruz L, Montesinos E, Moragrega C, Llorente I (2015) Combined morphological and molecular approach for identification of *Stemphylium vesicarium* inoculum in pear orchards. Fungal Biol 119:136–144. <https://doi.org/10.1016/j.funbio.2014.11.006>
- Sheikh F, Dehghani H, Aghajani MA (2015) Screening faba bean (*Vicia faba* L.) genotypes for resistance to *Stemphylium* blight in Iran. Eur J Plant Pathol 143:677–689. <https://doi.org/10.1007/s10658-015-0718-4>
- Simmons EG (1969) Perfect states of *Stemphylium*. Mycologia 61:1–26. <https://doi.org/10.2307/3757341>
- Tamura K, Stecher G, Peterson D, Filipowski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol Biol Evol 30:2725–2729. <https://doi.org/10.1093/molbev/mst197>
- USDA-NRCS (2017) The plants database. National Plant Data Team, Greensboro. <http://plants.usda.gov/>
- Woudenberg JHC, Hanse B, van Leeuwen GCM, Crous PW, Groenewald JZ (2017) *Stemphylium* revisited. Stud Mycol 87:77–103. <https://doi.org/10.1016/j.simyco.2017.06.001>