First report of powdery mildew caused by *Podosphaera* sp. on *Cynanchum auriculatum*

Narayan Chandra Paul^{1,2} · Sang-Keun Oh³ · Young-Guk Kim¹ · Seon-Woo Cha¹ · Tae-Jin An¹

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Abstract Powdery mildew symptoms were observed on the upper surface of leaves of *Cynanchum auriculatum* in Eumseong, Korea in October 2015. Microscopic observations and molecular characteristics determined this fungus as *Podosphaera* sp., which is reported for the first time on *Cynanchum auriculatum*.

Keywords *Cynanchum auriculatum* · *Podosphaera* sp. · ITS · Morphology · Powdery mildew

Cynanchum auriculatum is a climbing vine, which is a species of swallow-worts under the family *Apocynaceae*. The plant is native to Asian temperate and tropical regions and is found in China, Korea, Bhutan, Nepal, northern parts of Pakistan and India. The habitat of this plant is mountainous shrubland terrain. The plant leaf extracts have been used traditionally as medicine.

Typical symptoms of powdery mildew were observed on the leaves of *Cynanchum auriculatum* in National Institute of Horticultural and Herbal Science (NIHHS) glasshouse, Eumseong, Korea during October–November, 2015. The

upper surface of the leaves of *Cynanchum auriculatum* were covered with superficial, white and dense mycelia with conidiophores and conidia (Fig. 1). Around 20 % of leaves exhibited powdery mildew symptoms. Diseased plant leaf samples were collected and preserved in ‘Plant Disease and Pest Lab.’, NIHHS, Eumseong, Korea (ID: HCRD15501).

The disease occurrence was observed only on the *Cynanchum auriculatum* plant leaves. *Cynanchum wilfordii*, another plant species in the *Apocynaceae* family, was grown in the same glasshouse, but did not display any powdery mildew symptoms. The disease might be host specific.

Microscopic observations of the fungal hyphae revealed upright chains of ellipsoidal to cylindrical conidia (2 to 6 conidia/chain) and the length and width of conidia were 35.3 (28.2–42.0) × 20.8 (16.2–25.0) μm ($n = 26$) with a length-to-width ratio of 1.4 to 2.2. Conidiophores were mostly erect to curved, 198.5 to 262.6 μm long. The foot cells in conidiophores were cylindrical, 50.0 to 80.5 μm long and constricted at the base (Fig. 2). Indistinct appressorium were observed and no chamosthecia were found. Based on the morphological characteristics, the fungus was identified as *Podosphaera* sp. (Braun et al. 2002; Chen et al. 2015). Morphologically, *Podosphaera* sp. from this study was similar to *P. xanthii*, *P. fusca* and *P. fuliginea*. Conidial shapes were more similar to *P. xanthii* than *P. fusca* and *P. fuliginea* (Table 1). But Cho et al. (2013) described *P. xanthii* producing a higher number of conidia (up to 12) on a single chain, whereas the present fungus produced a maximum of 6 conidia.

To confirm the identity *Podosphaera* sp. obtained in the present study, genomic DNA was extracted using the SolgTM Genomic DNA prep. Kit (Solgent Co. Ltd., Daejeon, South Korea) and the internal transcribed spacer (ITS) region of rDNA was amplified using the primer sets ITS5 and ITS4 (White et al. 1990). Amplification reactions were performed in a total volume of 50 μL, containing 0.5 pmol of each

✉ Tae-Jin An
atj0083@korea.kr

¹ Department of Herbal Crop Research, National Institute of Horticultural and Herbal Science (NIHHS), Eumseong, Chungbuk 27709, Republic of Korea

² Bioenergy Crop Research Institute, National Institute of Crop Science, Rural Development Administration, Muan 58545, Republic of Korea

³ Department of Applied Biology, College of Agriculture & Life Sciences, Chungnam National University, Daejeon 34134, Republic of Korea

Fig. 1 Symptoms of powdery mildew on leaves of *Cynanchum auriculatum*

Fig. 2 Conidiophores and conidia of *Podosphaera* sp. from *Cynanchum auriculatum* leaves. **a–b**: conidiophores with chain of conidia **c**: Conidia. Scale bars (**a**; 100 μ m and **b–c**; 50 μ m)

primer, dNTPs at 0.2 mM, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 2.5 U of Taq polymerase, and 15 ng of template DNA. PCR was carried out in iCycler® Flexibility (BIO-RAD, USA) under the following conditions: initial denaturation 5 min at 94 °C followed by 30 cycles of denaturation 1 min (94 °C), annealing 1 min (55 °C), extension 1.30 min

(72 °C) and final extension 10 min at 72 °C (Paul et al. 2012). PCR products were purified using a Wizard® PCR prep kit (Promega Corporation, Madison, WI, USA) and sequenced using a commercial sequencing service provider (Macrogen, Seoul, South Korea). Sequencing was done using forward ITS5 primer. The resultant sequence was deposited in

Table 1 Morphological comparison of the present fungus (*Podosphaera* sp. HCRD15501) with related species

Species	Conidial size (μ m)	No. of conidia in chains	Length-width ratio	Foot-cells (μ m) with shorter cells	Reference
<i>Podosphaera</i> sp. (HCRD15501)	Ellipsoidal to ovate, sometime cylindrical 28.2–42.0 $\times$ 16.2–25.0	2–6	1.4–2.2	50.0–80.5 $\times$ 10.1–13.0, 2–3	Present study
<i>P. fusca</i>	ellipsoid to ovoid 17–33 $\times$ 13–18	4–6	1.4–2.1	– 1–3	Xu et al. 2015
<i>P. fuliginea</i>	ellipsoid-ovoid to doliform 24–35 $\times$ 15–19	– ^a	1.2–2.0	45–95 $\times$ 9.5–12 2–3	Braun 1987
<i>P. xanthii</i>	ellipsoid to ovate 32–48 $\times$ 17.5–25	2–12	1.4–2.3	50 $\times$ 95 2–3	Cho et al. 2013
<i>P. balsaminae</i>	27–45 $\times$ 15–21	–	1.87	35.5–97.5 $\times$ 11–15	Wolcan 2004

^a Not described

Fig. 3 One of the parsimonious trees inferred from the sequence of ITS region. Numbers above the branches are bootstrap values in 1000 bootstrap replicates. The scale bar indicates the number of nucleotide substitutions. GenBank accession numbers are represented in the parentheses

GenBank, (accession number KU711923) and compared with the sequences of related species available in the GenBank database, by using BLAST search. The sequence generated from the materials used in the present study and the sequence retrieved from GenBank was initially aligned using the CLUSTAL X program (Thompson et al. 1997). Phylogenetic relationships were estimated by a maximum parsimony analysis using the MEGA 5 program. Bootstrap analysis using 1000 replications was performed to assess the relative stability of the branches (Tamura et al. 2011). The tree was rooted with the sequence of *P. leucotricha* (HM579841).

BLAST search analysis showed 100 % sequence similarity with *Podosphaera xanthii* (KM260741), *P. fusca* (KP329589), *P. fuliginea* (JN627140) and *P. balsaminae* (FJ625796). The maximum parsimony analysis revealed that the fungus obtained in this study clustered together with the species (*Podosphaera xanthii*, *P. fusca*, *P. fuliginea* and *P. balsaminae*) mentioned earlier (Fig. 3.). Based on the results, it is clear that the powdery mildew pathogen on *Cynanchum auriculatum* belongs to the genus *Podosphaera*. These four *Podosphaera* species, belong to the fusca-xanthii group (Braun and Cook 2012) and are different species. However, recently, many researchers have merged *Podosphaera* species together as *P. xanthii* because of their molecular single cluster formation and morphological similarities (Eg. *P. balsaminae*, *P. fusca* to *P. xanthii*) (Briellmaier-

Liebetanz et al. 2015). To the best of our knowledge, this is the first record of the powdery mildew *Podosphaera* sp. on *Cynanchum auriculatum*.

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