First report of leaf spot of *Sarcococca saligna* caused by *Paraconiothyrium brasiliense* in Pakistan

Najam ul Sehar Afshan¹ · Umaima Mujahid¹ · Aamna Ishaq² · Abdul Nasir Khalid¹

Received: 15 April 2019 / Accepted: 5 November 2019 / Published online: 14 November 2019
© Società Italiana di Patologia Vegetale (S.I.Pa.V.) 2019

Keywords Khanspur · Leaf spot · Pakistan · *Sarcococca*

Sarcococca saligna (D. Don) Müll. Arg. is a common shrub, traditionally used in pharmaceuticals in Pakistan. Leaf, bark and root of plant are used in country because of its cardio-suppressant, vasodilator and tracheal relaxant properties (Ghayur and Gilani 2006). In October 2018, infected leaves of *S. saligna* plants were collected from fields of Khanspur (Pakistan). Approximately 15 to 35% of plants exhibited symptoms of varying shades of brown spots on leaves in oblong streaks with necrotic lesions. Infected leaves (5 mm in length) were surface sterilized with 2% sodium hypochlorite, rinsed with sterile distilled water, air dried and cultivated on Malt Extract Agar (MEA) at 27 °C, 4–5 days after inoculation. Colonies on MEA were white with pointed, filamentous margins, oblong with aerial mycelium; reverse light orange-yellow, but with tawny zone around centre; frequent complex conidiomata developing from 7 to 11 days onwards, were visible as scattered black dots on both sides of plate, later converting into sub-marginal ring. Hyphae were dark walled, conidia prominent, ellipsoid or cylindrical, colourless to olive ($3.5\text{--}4.5 \times 2\text{--}2.3 \mu\text{m}$). The pathogen was identified as *Paraconiothyrium brasiliense* Verkley, based on morphological characteristics. The nrDNA of internal transcribed spacer (ITS1–5.8S–ITS2) region was amplified using primers ITS1F (forward) /ITS4 (reverse) (White et al. 1990). The obtained sequence (MK779925) has 99% similarity

with *P. brasiliense* (JF439492). Studied taxon was found conspecific with *P. brasiliense* in phylogenetic tree clustering with specimens collected from Indian Ocean (MH183258), South Korea (KM520128) and China (MH305508) with strong bootstrap support. To prove pathogenicity, 10 seedlings of healthy *S. saligna* plants were sprayed with 50 μl conidial suspension of the fungus (10^7 conidia/ml, 10 ml/plant). Five control plants were treated with sterile distilled water. All plants were maintained at 15 ± 5 °C, with 90% humidity. Characteristic leaf spots were evident 4 weeks post-inoculation on leaves, and *P. brasiliense* was consistently reisolated from all inoculated plants. No symptoms were observed on control plants. *P. brasiliense* has previously been reported from China on *Acer truncatum*; from Brazil on *Coffea arabica*, *Phoradendron perrottettii* and *Tapirira guianensis*; from Italy on *Magnolia* sp.; from South Africa on *Prunus persica*, *P. salicina* and from Spain on *Vitis vinifera*, respectively (Farr and Rossman 2019). To the best of our knowledge, this is the new record in Pakistan.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s42161-019-00449-6>) contains supplementary material, which is available to authorized users.

✉ Najam ul Sehar Afshan
pakrust@gmail.com; najamulsehar.hons@pu.edu.pk

¹ Department of Botany, Faculty of Life Sciences, Quaid-e-Azam Campus, University of the Punjab, Lahore, Pakistan

² Department of Botany, University of Veterinary and Animal Sciences, Pattoki, Pakistan

References

- Farr DF, Rossman AY (2019) Fungal Databases, U.S. National Fungus Collections, ARS, USDA. Retrieved April 4, 2019, from <https://nt.ars-grin.gov/fungaldatabases/>
- Ghayur MN, Gilani AH (2006) Studies on cardio-suppressant, vasodilator and tracheal relaxant effects of *Sarcococca saligna*. Arch Pharm Res 29(11):990–997
- White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic Press, San Diego, pp 315–332

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.