Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Athelia rolfsii* (= *Sclerotium rolfsii*) Associated with Southern Blight Disease of *Macrotyloma uniflorum* in India

S. Mahadevakumar,¹ Josna Joy,² L. S. Mamatha Bhanu,³ K. A. Sharvani,⁴ S. Niranjana Raj,¹ and S. Chandranayaka^{5,†}

¹ Molecular Phytodiagnostics Laboratory, Department of Studies in Microbiology, Karnataka State Open University, Mukthagangotri, Mysuru-570006, Karnataka, India

² Department of Studies in Microbiology, University of Mysore, Manasagangotri, Mysuru-570006, Karnataka, India

³ Department of Biotechnology, Yuvaraja's College (Autonomous), University of Mysore, Mysuru-570005, Karnataka, India

⁴ Department of Botany, Yuvaraja's College (Autonomous), University of Mysore, Mysuru-570005, Karnataka, India

⁵ Phytopathology Laboratory, Institution of Excellence, Department of Studies in Biotechnology, University of Mysore, Manasagangotri, Mysuru-570006, Karnataka, India

Funding: J. Joy acknowledges the OBC Cell of University of Mysore for the award of a Junior Research Fellowship. Plant Dis. 106:2533, 2022; published online as <https://doi.org/10.1094/PDIS-12-21-2835-PDN>. Accepted for publication 5 February 2022.

Horse gram (*Macrotyloma uniflorum* (Lam.) Verdc., Fabaceae) is an important legume crop cultivated widely in arid and semiarid regions. During a survey in the Mysore district (Karnataka, India, October 2021), horse gram plants showed symptoms of southern blight disease. Disease incidence ranged from 20 to 27% in the approximate 52 ha of horse gram fields evaluated. The symptoms initiated as tan lesions and the developing mycelial threads colonized the infected root–stem interface, causing girdling; lesions on leaves enlarged and developed into distinct spots. Infected parts (leaves and stem) ($n = 30$) were collected in plastic bags and incubated in a moist chamber overnight, followed by surface sterilization of small segments of stem and leaf with 2% NaOCl, rinsing with sterile water (SW), and placing onto potato dextrose agar (PDA, HiMedia Lab, Mumbai) supplemented with chloramphenicol (40 mg/liter). The plates were incubated at room temperature ($28 \pm 2^\circ\text{C}$) for 5 to 7 days. The fungal colonies that developed from the diseased segments were subcultured through hyphal tipping to fresh PDA

plates and pure cultures were obtained. Fungal colonies with dense, aerial whitish-cottony mycelia with uniformly globoid sclerotia (0.5 to 2.9 mm) were observed after 10 to 12 days of incubation. Sclerotia were white in the beginning and turned brown later. The average number of sclerotia produced per plate ranged from 112 to 320 ($n = 20$). To determine the identity of the isolated fungal pathogen, ITS-rDNA was amplified and sequenced using ITS1/ITS4 (White et al. 1990) primers. The amplified PCR product was purified and sequenced directly. The ITS sequences (OM037658 and OM037659) shared 100% (630/643 bp) sequence similarity to *Athelia rolfsii* (KY640622.1, AB075298). The phylogenetic tree (neighbor-joining method) constructed based on the ITS-rDNA region confirmed that the sequences shared a common clade with a reference sequence of *A. rolfsii*. Thus, the identity was confirmed based on micromorphology and phylogenetic analysis (Mordue 1974). Pathogenicity tests were conducted on 20 plants (5 to 6 weeks old) in greenhouse conditions ($28 \pm 2^\circ\text{C}$ and 70% relative humidity) by inoculating with sclerotia from 15-day-old cultures on stem and leaves, and 14 plants were found infected 5 days postinoculation, while uninoculated control plants remained healthy. Similarly, a detached leaf assay (Mahadevakumar et al. 2016, 2018) was performed under in vitro conditions at $28 \pm 2^\circ\text{C}$ in a moist chamber, and 28 out of 30 leaves showed leaf spot symptoms after 3 to 5 days of inoculation. Uninoculated control leaves remained healthy. The identity of the fungus was confirmed by morphology and molecular analysis after reisolation. The occurrence as a pathogen on horse gram has not been previously reported elsewhere. This is the first report of southern blight disease caused by *A. rolfsii* on horse gram from India. Early diagnosis of this leaf spot disease will help farmers to adopt suitable management practices to avoid losses in production.

References:

- Mahadevakumar, S., et al. 2016. Eur. J. Plant Pathol. 145:215.
- Mahadevakumar, S., et al. 2018. Eur. J. Plant Pathol. 151:1081.
- Mordue, J. E. M. 1974. CMI Descriptions of Pathogenic Fungi and Bacteria. No. 410. Commonwealth Mycological Institute, Kew, UK.
- White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: cereals and grains, fungi, pathogen detection

[†]Indicates the corresponding author.
S. Chandranayaka; moonnayak@gmail.com