Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Sclerotium rolfsii* Causing Collar Rot on Berseem (*Trifolium alexandrinum*) in India

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Berseem (*Trifolium alexandrinum*) is a winter season legume fodder crop widely cultivated in the central and northern parts of India. It is considered the “king of fodder” for its high-quality green fodder, which is a rich source of protein and low in fiber. Symptoms similar to collar rot were observed in experimental sites at the ICAR-Indian Grassland and Fodder Research Institute, Jhansi (N25° 52' 749.20", E78° 55' 452.70"), Uttar Pradesh, India, in March 2019. The incidence of disease ranged from 18 to 22% during 2019. Symptoms included dark-colored water-soaked lesions at the base of stems, stem thinning (resembles wire stem), and eventually wilting of the whole plant. A white mycelial mat was observed on the stem and collar region, and light brown to tan colored sclerotial bodies formed as the disease progressed. To determine the etiology of the infection, 30 diseased plants with typical symptoms were collected from the three different fields and used for the isolation of causal agent. Infected stem portions were cut into small pieces (5 mm), surface sterilized with 2% sodium hypochlorite (NaOCl) for 2 min, washed three times with sterile distilled water, and air dried. The sterilized infected tissues were cultured on potato dextrose agar amended with streptomycin sulfate at 50 µg/ml and incubated at 28 ± 1°C for 3 days. After 4 days, hyaline septate mycelia ranging 2 to 3 µm in diameter grew radially over the whole plate (90 mm). Sclerotia formation started 6 days after incubation. Sclerotia were initially white and later turned brownish to tan as they matured. The number of sclerotia per plate ranged from 55 to 120 (*n* = 5) at 12 days after inoculation. The diameter of matured sclerotial bodies ranged from 0.1 to 1.35 mm (*n* = 25). Genomic DNA was extracted from mycelium using the

CTAB method (Murray and Thompson 1980). Three regions of rDNA, namely, internal transcribed spacer (ITS), large subunit (LSU), and small subunit (SSU), were used to identify the etiology of the disease. The isolate was amplified with ITS1 (5'-CGGATCTCTTGGTTCTGGGA-3')/ITS4 (5'-GACGCTCGAACATGCC-3') described by White et al. (1990) and sequenced. The ITS sequence (NCBI GenBank accession no. MT026581) showed 98.21% similarity to *Athelia rolfsii* (MH514001.1). The isolate also amplified with primers LSU (LROR, ACCCGCTGAACCTAAGC/LR5, TCCTGAGGGAACTTCG) and SSU (NS1, GTAGTCATATGCTTGTCTC/NS4, CTTCCGTC AATTCCCTTTAAG). The LSU (MT225781) and SSU (MT225782) sequences showed 99.90 and 100% similarity to *A. rolfsii* (AY635773.1) and *A. rolfsii* (AY635773.1), respectively. Based on the morphological and molecular characteristics, the pathogen responsible for collar rot in berseem was identified as *A. rolfsii* (anamorph: *Sclerotium rolfsii*) (Mordue 1974). To confirm pathogenicity, inoculum was prepared by inoculating mycelial plugs of pathogen into autoclaved corn/sand meal (5:95) and incubating at 28 ± 1°C for 12 days. The inoculum (30 g) was placed on the stem portion of 15-day-old seedlings (*n* = 30) of berseem (cv. Wardan) raised in pots filled with sterilized soil. Seedlings (*n* = 25) inoculated with sterilized corn/sand meal (30 g) served as the control. The pots were placed inside of a plant growth chamber (26 ± 2°C, 65% relative humidity) for 15 days. Water-soaked spots with white mycelium were observed on the collar region after 3 days. After 7 days, stems were completely covered by mycelia, and death of seedlings was observed 14 days after inoculation. The pathogen was recovered from the artificially inoculated berseem seedlings (*n* = 15). No symptoms were observed in control plants. Based on morphological and molecular characterization, the present isolate was confirmed as *S. rolfsii*. To the best of our knowledge, this is the first report of *S. rolfsii* causing collar rot of berseem in India.

References:

- Mordue, J. E. M. 1974. Descriptions of Pathogenic Fungi and Bacteria, No. 410. CMI, Kew, U.K.
- Murray, M. G., and Thompson, W. F. 1980. Nucleic Acids Res. 8:4321.
- White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.

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