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First Report of *Sclerotinia sclerotiorum* Causing Stem Rot on Soybean (*Glycine max*) in Ethiopia

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Sclerotinia sclerotiorum causes a devastating disease on soybean (Sclerotinia stem rot) and the attacks over 500 other hosts (Grau and Hartman 2015). In October 2018, research plots at Jimma Agricultural Research Center, Ethiopia were evaluated for soybean diseases. A sample of 100 arbitrarily selected plants of soybean line T44-15-T110-16SH1 were evaluated for Sclerotinia stem rot in a research plot that was 4 m by 2.4 m with 60 cm between the four rows and 5 cm between plants within a row. All but 16 stems had stem rot symptoms with an average plant severity rating of 3.5 (SE = 0.18) based on an adapted 1 (1 to 10% of stems affected) to 5 (91 to 100% of the stem affected) rating scale (Little and Hills 1978). Five infected stems were selected and sent to the USDA-ARS Soybean Disease and Pest Research Laboratory, Urbana, IL. Three sclerotia removed from each of three infected stems ranged from 4 to 18 mm long and 1 to 2 mm wide. Sclerotia were placed on potato dextrose agar (PDA) in 9.8 cm diameter Petri plates and incubated at 24°C with a 16 hr photoperiod for 4 days. The white, appressed mycelia grew from the sclerotia and covered the entire plate after 48 hours with sclerotia forming on the edge of the plate by 4 days. To confirm pathogenicity, a mycelial plug was removed from the margin of a 2 day-old-colony of one of the isolates was obtained by pressing the large end of a 200 µl pipette tip into the culture and placed on top of a cut stem above the second trifoliolate of four 3-week-old plants of soybean cultivar Williams 82. Plants were then incubated in a moist chamber for 48 hours prior to being placed in a greenhouse held at 22°C with a 16-hour photoperiod. Necrotic lesions and white mycelia appeared on the stems four days post-inoculation. The pathogen was re-isolated and cultured on PDA. After 4 days the re-isolated cultures with sclerotia appeared morphologically to be *S. sclerotiorum*. For further confirmation, 8 mm diameter mycelial plugs were excised from the three representative cultures. DNA

was released from the mycelia by disruption in Lysing Matrix A and CLS-Y solution, as provided by the FastDNA Spin Kit (MP Biomedicals, Solon-OH). Disruption was accomplished in a FastPrep-24 lemniscate homogenizer (MP Biomedicals) for 40 s at a speed of 6 m/s. DNA was extracted as instructed by the manufacturer. The resulting eluates were diluted ten-fold with 5 mM tris, pH 8, containing 1 mM NaCl. Five microliter subsamples were subjected to PCR using ITS (ITS4: 5'-TCCTCCGCTTATTGATATGC-3'; ITS1: 5'- TCCGTAGGTGAACCTGCGG-3') primers which produced 477 bp amplicons that were purified using the Qiaquick PCR Cleanup Kit (Qiagen, Germantown-MD) and delivered to a core facility for Sanger sequencing (Roy J. Carver, Biotechnology Center, University of Illinois), using the same primers. The top BLAST hit for the ITS region (GenBank accession MK882510 had 99.79% identity to *S. sclerotiorum* (MF776031.1; Alaska). In Africa, *S. sclerotiorum* has been found on soybean in Nigeria (Akem and Dashiell 1992) and South Africa and on common bean in Ethiopia (Allen 1995). To our knowledge this is the first report of Sclerotinia stem rot on soybean in Ethiopia. Sclerotina stem rot could become a major limiting factor as soybean production in Ethiopia has increased from 3,807 ha in 1997 to 39,021 ha in 2017 (FAO 2019).

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