Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Alternaria alternata* Causing Leaf Blight on Little Millet (*Panicum sumatrense*) in India

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Plant Dis. 105:1202, 2021; published online as <https://doi.org/10.1094/PDIS-06-20-1373-PDN>. Accepted for publication 13 October 2020.

Little millet (LM) is a minor cereal crop grown in the Indian subcontinent. During October 2018, dark brown, circular to oval necrotic spots surrounded by concentric rings were observed on the upper leaf surface of the LM (cv. VS-13) grown in the fields of the University of Agricultural Sciences, Bengaluru, India (13.0784°N, 77.5793°E). As the disease progressed, infected leaves became blighted. Disease incidence up to 53% was recorded in three fields of 0.4-ha area each. Thirty symptomatic leaves were collected to isolate the associated causal organism. The margins of diseased tissue were cut into 5 × 5-mm pieces, surface sterilized in 75% ethanol for 45 s followed by 1% sodium hypochlorite for 1 min, rinsed in sterile distilled water five times, and placed on potato dextrose agar (PDA). After 7 days of incubation at 25°C, greyish fungal colonies appeared on PDA. Single-spore isolations were performed to obtain 10 isolates. Pure cultures of the fungus initially produced light gray aerial mycelia that later turned to dark gray. All isolates formed obclavate to pyriform conidia that measured 22.66 to 48.97 µm long and 6.55 to 13.79 µm wide with one to three longitudinal and two to seven transverse septa with a short beak (2.55 to 13.26 µm) (*n* = 50). Based on the conidial morphology, the fungus was identified as *Alternaria* sp. Further, the taxonomic identity of all 10 isolates was confirmed as *Alternaria alternata* using species-specific primers (AAF2/AAR3, Konstantinova et al. 2002) in a PCR assay. Later, one of the isolates (UASB1) was selected, and its internal transcribed spacer (ITS) region, glyceraldehyde-3-phosphate dehydrogenase (*gapdh*), major allergen *Alt a 1* (*Alt a 1*), major endo-polygalacturonase (*endoPG*), OPA10-2, and

KOG1058 genes were amplified in PCR (Berbee et al. 1999; White et al. 1990; Woudenberg et al. 2015), and the resultant products were sequenced and deposited in NCBI GenBank (ITS, MN919390; *gapdh*, MT637185; *Alt a 1*, MT882339; *endoPG*, MT882340; OPA10-2, MT882341; KOG1058, MT882342). BLASTn analysis of ITS, *gapdh*, *Alt a 1*, *endoPG*, OPA10-2, and KOG1058 gene sequences showed 99.62% (with AF347031), 97.36% (with AY278808), 99.58% (with AY563301), 99.10% (with JQ811978), 99.05% (with KP124632), and 99.23% (with KP125233), respectively, identity with reference strain CBS916.96 of *A. alternata*, confirming UASB1 isolate to be *A. alternata*. For the pathogenicity assay, conidial suspension of UASB1 isolate was spray inoculated to 10 healthy LM (cv. VS-13) plants (45 days old) maintained under protected conditions. The spore suspension was sprayed until runoff on healthy leaves, and 10 healthy plants sprayed with sterile water served as controls. Later, all inoculated and control plants were covered with transparent polyethylene bags and were maintained in a greenhouse at 28 ± 2°C and 90% relative humidity. The pathogenicity test was repeated three times. After 8 days post-inoculation, inoculated plants showed leaf blight symptoms as observed in the field, whereas no disease symptoms were observed on noninoculated plants. Reisolations were performed from inoculated plants, and the reisolated pathogen was confirmed as *A. alternata* based on morphological and PCR assay (Konstantinova et al. 2002). No pathogens were isolated from control plants. There is an increasing acreage of LM crop in India, and this first report indicates the need for further studies on leaf blight management and the disease impacts on crop yields.

References:

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The author(s) declare no conflict of interest.

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Keywords: little millet, *Alternaria alternata*, leaf blight, India

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