Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot of Maize Caused by *Curvularia geniculata* in India

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In India, 25.90 million metric tons of maize (*Zea mays* L.) was produced on 9.63 million hectares, with an average yield of 2.69 metric tons per hectare (Singh and Singh 2018). In March 2017, leaf spot was observed on maize cv. Kanchan during a survey of the Ballia district, with disease severity ranging from 1 to 20% (26.08°N, 83.71°E) in Uttar Pradesh, India. The upper mid canopy of symptomatic plants showed elliptical light brown spots (0.25 to 2.5 mm diameter) surrounded by chlorotic halo lesions with dark margins. Five plants showing leaf spots were collected and the symptomatic tissue was cut into pieces (4 mm²), surface sterilized with 1% sodium hypochlorite for 1 min, then rinsed three times with distilled water, plated on potato dextrose agar medium amended with streptomycin sulfate (125 ppm), and incubated at 26°C with a 12-h light period. After 5 days of incubation, five pure cultures were obtained using the hyphal tip technique and incubated at 28°C for 15 days. The upper surface of the colony was dark grayish black and the reverse colony was dark brown. Conidiophores on the apex were geniculate, unbranched, and septate. Conidia were spindle to elliptical in shape, light brown, with 3 to 4 septa with an enlarged central cell. Conidial size ranged from 10.0 to 14.1 µm wide and 19.3 to 26.2 µm long ($n = 35$). Based on morphology, the fungus was tentatively identified as *Curvularia geniculata* (Tracy & Earle) Boedijn (Hosokawa et al. 2003). Later, genomic DNA was extracted from the E29 isolate using the nucleopore GDNA Fungus Kit (Genetix Brand, India). ITS (White et al. 1990), LSU (Vilgalys and Hester 1990), D1 and D2 region of LSU (O'Donnell 1993), and glyceraldehyde-3-phosphate dehydrogenase (*gpd*) (Berbee et al. 1999) loci were amplified for molecular identification (Manamgoda et al. 2012). The amplicons were bidirectionally sequenced using the Sanger sequencing method. BLASTn analysis of different gene sequences of isolate E29 showed 99 to 100% similarity with

sequences of *C. geniculata*. The gene sequences were submitted to GenBank, and accession numbers were assigned (ITS, MT524330; *gpd*, LC552684; LSU, MT516309; and D1 and D2 region of LSU, MT533847). The similarity index for ITS, *gpd*, LSU, and D1 and D2 region of LSU sequences of E29 showed high similarity with isolates of *C. geniculata*: 99.81% with MH856584 (CBS187.50), 99.52% with KM083609 (CBS187.50), 100% with MH868092 (CBS187.50), and 100% with MH868533 (CBS220.52), respectively. Pathogenicity of the isolate E29 was confirmed by inoculating 25-day-old maize cv. Kanchan ($n = 10$) with a spore suspension (10⁶ spores/ml) prepared from 15-day-old cultures. Using a hand atomizer, the spore suspension was sprayed onto leaves until runoff and the noninoculated plants ($n = 10$) were sprayed with sterile distilled water. The plants were maintained in a glasshouse at 26 ± 2°C and humidity ≥90%. After 10 days, elliptical spots with chlorotic halo were observed on inoculated plants similar to those observed in the field, but no symptoms developed on noninoculated plants. *C. geniculata* was reisolated from symptomatic leaves, and its identity was confirmed by morphological observations. The pathogenicity tests were repeated twice. The disease appears not to be yield limiting; however, it has caused several stakeholder concerns, and anecdotal evidence suggests that maize hybrids may differ in susceptibility. To our knowledge, this is the first report of leaf spot of maize caused by *C. geniculata* in India, which extends the known agents of maize leaf spot.

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