Diseases Caused by Bacteria and Phytoplasmas

First Report of Pearl Millet Bacterial Leaf Blight Caused by *Pantoea* stewartii Subspecies indologenes in India

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Funding: Funding was provided by Indian Council of Agricultural Research (Professional Attachment Training [ICAR-IISS, Mau]). Plant Dis. 105:3736, 2021; published online as https://doi.org/10.1094/PDIS-03-21-0669-PDN. Accepted for publication 7 June 2021.

Pearl millet (Cenchrus americanus L.) field-grown plants of cultivar 7042S showed unusual water-soaked lesions on leaf tips spreading toward the leaf base from Manasagangothri region (12.31°N, 76.61°E), Karnataka, a southern Indian state, during March 2020. Later those infected plants showed extensive necrosis and typical leaf blight symptoms with 70% disease incidence and 59% severity. Surface-sterilized $(3 \times 3 \text{ mm})$ infected leaf tissues were crushed in 1 ml of sterile distilled water and streaked onto nutrient agar medium. Bright-yellowish, circular, mucoid single bacterial colonies (PPi-M1) with a regular margin were recovered after 24 h of incubation at 28°C, and the same bacterial colonies were used for further biochemical and molecular characterization. The isolate, PPi-M1, was found as gram-negative rods, gelatin and starch hydrolysis negative, and catalase and indole production positive. The partial sequence of 16S rRNA gene (primers: 27F/1492R) of the isolate PPi-M1 was amplified and sequenced, and the curated sequence was submitted to NCBI GenBank (accession no. MN808555). In nucleotide BLAST search for homologous sequences, 99.5% nucleotide matching similarity (1,410 bp) was observed with other Pantoea stewartii subspecies indologenes strains (MF163274; NR_104928) in the NCBI database, indicating that our isolate PPi-M1 belongs to this species. In phylogenetic analysis using the maximum likelihood method and Tamura-Nei model (Tamura and Nei 1993), PPi-M1 formed a distinct cluster with other P. stewartii strains with bootstrap value >95, and it was distant from P. allii, P. ananatis, P. agglomerans, and P. dispersa. Besides, the subspecies-specific PCR assay and subsequent sequencing of galE and recA genes (primers: 3614galE/ 3614galEc; 3614recA/3614recAc; 372 and 223 bp) also confirmed the identity of the isolate as P. stewartii subspecies indologenes. Further, the pathogenicity test was performed in planta on 21-day-old seedlings of pearl millet cultivar CO-10. The bacterial suspension of isolate PPi-M1 (1×10^8 CFU/ ml) was used for inoculation by the leaf clipping method (Ke et al. 2017). All the inoculated plants (n = 4 leaves per plant; 15 plants) maintained under greenhouse conditions (27 to 29°C; 80 to 85% relative humidity) except the mock (sterile water) inoculation showed similar water-soaked lesions from the cut end of the leaf, with a definite spreading margin and a typical leaf blight symptom as those observed in the field at 8 days postinoculation. Reisolated bacterial colonies from infected leaves shared similar morphological characters and molecular identity with inoculated culture, thus proving Koch's postulates. This pearl millet leaf blight causing bacterial strain PPi-M1 was deposited in the National Agriculturally Important Microbial Culture Collection, Mau, India (accession no. NAIMCC-B-02508). Previously, P. stewartii was reported to cause leaf blight and rot diseases on rice and maize (Kini et al. 2017; Roper 2011); also, the International Seed Federation has instigated phytosanitary measures highlighting its true seed transmission ability (Pataky et al. 2003). This study will supplement future pearl millet breeding programs, and to our knowledge, this is the first report of P. stewartii subsp. indologenes inciting pearl millet leaf blight disease in India.

References:

Ke, Y., et al. 2017. Bio Protoc. 7:e2568.

Kini, K., et al. 2017. Plant Dis. 101:242

Pataky, J., et al. 2003. Pest risk analysis. International Seed Federation, Nyon, Switzerland.

Roper, M. C. 2011. Mol. Plant Pathol. 12:628.

Tamura, K., and Nei, M. 1993. Mol. Biol. Evol. 10:512.

The author(s) declare no conflict of interest.

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Keywords: pearl millet, leaf blight, 16S rRNA, Pantoea

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