Diseases Caused by Bacteria and Phytoplasmas

First Report of *Klebsiella aerogenes* Inciting Stem Rot of Pearl Millet in Haryana, India

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Pearl millet [*Pennisetum glaucum* (L.) R. Br. Syn. *P. americanum* (L.) Leeke] is the oldest and most widely cultivated millet in Asian and African countries, mostly grown over low fertile soils in more than 40 countries covering an area of 31.2 million hectares (FAOSTAT 2017). In Haryana, India, crop was grown over an area of 430,000 hectares during fall 2019. During 2018 to 2020, a new devastating disease exhibiting stem rot like symptoms was observed in pearl millet in Haryana. The causal agent was a bacterium, where 16S rDNA-based nucleotide sequence deposited in GenBank (MZ433194.1) confirmed its nearness to *Klebsiella aerogenes* (Hormaeche and Edwards 1960; Tindall et al. 2017). Further, DNA gyrase genomic sequence (MZ707528.1) also had high homology to *K. aerogenes*. Genus *Klebsiella* is known to cause diseases in humans and animals, and also has been found inciting rots in different plantations viz. top rot in maize (Huang et al. 2016). Pearl millet is susceptible to minor bacterial diseases viz. bacterial leaf streak (*Xanthomonas campestris*), bacterial leaf spot (*Pseudomonas syringae*), and leaf stripe (*P. avenae*). Among all plant pathogenic bacteria, only *Erwinia chrysanthemi* is known to cause stem rot diseases in sorghum (Saxena et al. 1991). Extensive disease survey of pearl millet-growing regions (Hisar, Bhiwani, Rewari, Mohindergarh, and Bawal districts of Haryana) in the rainy seasons of 2019 and 2020 revealed the prevalence of typical stem rot disease, with up to 70% disease incidence in the infected fields. Symptomatic stem pieces of different plants were collected from two locations (Hisar and Bhiwani) and the associated organism was isolated following the techniques of Janse (2005). The resulting growth of bacterial cultures were further purified on nutrient agar (NA) using streak plate technique. The resulting bacteria were gram-negative and rod-shaped. Colonies were round and creamish white on NA. Isolated morphotypes were positive for indole production, methyl red, Voges Proskauer's test, citrate utilization, arabinose, mannitol, rhamnose, and sucrose, and negative for glucose, adonitol, lactose, and sorbitol tests. Biochemical tests were performed following standard methods (Holt et al. 1994). Molecular analysis of both isolates was performed using two sets of primers (universal 16S rRNA gene and genus-specific *gyrA* gene). The *gyrA* fragment (F: 5'-CGCGTACTATACGCCATGAACGTA-3'; R: 5'-ACCGTTGATCACTTCGGTCAGG-3') has been adopted as a *Klebsiella* genus-specific gene (Brise and Verhoef 2001). The quality and quantity of the isolated genomic DNA were analyzed using NanoDrop-2000 (Thermo Fisher Scientific) and resolved in 1% (w/v) agarose gel. The fragment 16S rDNA was amplified using 27F and 1492R primers, where a single discrete PCR amplicon of 1,500 bp was observed in 1% (w/v) agarose gel. Similarly,

the *gyrA* gene was amplified using 09510F and 09510R primers that conferred a single discrete band of 400 bp. The forward and reverse DNA sequencing reaction of purified PCR amplicons (16S rDNA and *gyrA*) was carried out using BDT v3.1 cycle sequencing kit on a genetic analyzer to generate gene sequences. The consensus sequences of both genes were generated from forward and reverse sequence data using aligner software. The obtained sequences of both genes were compared with the available nucleotide sequences in NCBI using BLAST. The sequenced PCR amplicons showed up to 100% similarity with *K. aerogenes* 16S RNA nucleotide sequences (NR102493.2, MT373521.1, MF682950.1, MF462979.1, etc.). The bacterium also showed high nucleotide homology to *K. aerogenes* *gyrA* gene sequences (LR607333.1, CP035466.1, CP049600.1, etc.). The molecular phylogenetic analysis was done by the maximum likelihood method based on the Tamura-Nei model, and 1,000 replicates for bootstrap testing in MEGA 7.0. The analysis involved 16 nucleotide sequences and evolutionary distances were computed. The 16S RNA based phylogenetic tree raised using MEGA7 (Kumar et al. 2016) shows that *K. aerogenes* Hisar formed a cluster with three *K. aerogenes* strains (MZ577128.1, MT373521.1, and MT 373520.1), whereas *K. aerogenes* Bhiwani displayed higher homology to NCBI sequences MF682950.1, MT355368.1, MW331687.1, and LC515412.1. Bacterial suspension was prepared by suspending bacterial cells into sterile water and cell density was adjusted to 1×10^7 CFU/ml. For pathogenicity, leaf whorl inoculation (10 ml suspension/whorl) was done on 15-day-old seedlings of pearl millet genotype 7042S raised under controlled conditions ($35 \pm 2^\circ\text{C}$, >80% RH). The pathogenicity was proved under field conditions as well. Initial symptoms were observed 4 to 5 days after inoculation as long streaks on leaves. Soon, a spike in the number of these leaf streaks was observed. Thereafter, water-soaked lesions appeared on the stem 20 to 25 days after inoculation that later turned brown to black. Severely diseased plants were dead, exhibiting hollowing of the stem and drying of leaves. The infected stem pith disintegrated and showed slimy rot symptoms and the pearl millet clumps toppled down. The rotten stems of both inoculations were again cut into small pieces and the reisolated bacterium showed exactly the same morphological, biochemical, and molecular characteristics. To our knowledge, this is the first report of stem rot of pearl millet incited by *K. aerogenes* in southwestern regions of Haryana, India. Because the stem rot caused by *K. aerogenes* poses a significant threat to pearl millet cultivation, further research on biology, epidemiology, and management choices is needed.

References:

- Brise, S., and Verhoef, J. 2001. Int. J. Syst. Evol. Microbiol. 51:915.
- FAOSTAT. 2017. <http://www.fao.org/faostat/en/#data/QC>
- Holt, J. G., et al. 1994. Bergey's Manual of Determinative Bacteriology, 9th Ed. Williams and Wilkins, Baltimore, MD.
- Hormaeche, E., and Edwards, P. R. 1960. Int. Bull. Bacteriol. Nomencl. Taxon. 10:71.
- Huang, M., et al. 2016. J. Integr. Agric. 15:1510.
- Janse, J. D. 2005. Page 366 in: Phytopathology: Principles and Practices. CABI Publishing, Wallingford, U.K.
- Kumar, S., et al. 2016. Mol. Biol. Evol. 33:1870.
- Saxena, S. C., et al. 1991. Indian J. Microbiol. 31:435.
- Tindall, B. J., et al. 2017. Int. J. Syst. Evol. Microbiol. 67:502.

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