Diseases Caused by Bacteria and Phytoplasmas

First Report of *Candidatus* Phytoplasma cynodontis (16SrXIV-A Subgroup) Associated with Cauliflower Phyllody and Flat Stem in India

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Symptoms of suspected phytoplasma infection were observed in cauliflower (*Brassica oleracea* var. *botrytis*) (cultivar NS60N) at the Integrated Farming System Research Station, Trivandrum, Kerala, India (08°28'28" N, 76°57'47" E) in April 2021. The disease incidence was recorded up to 10% in different fields. The disease manifested as stunting, phyllody, floral malformation, and flattening of stem. Ten symptomatic and five asymptomatic plants were assayed for the presence of phytoplasma using nested PCR assays performed with P1/P7 and R16F2n/R16R2 primer pairs for 16S rRNA gene and SecAfor1/SecArev3 and SecAfor2/SecArev3 for secA gene (Deng and Hiruki 1991; Gundersen and Lee 1996; Hodgetts et al. 2008). The expected amplicons of ~1.25 kb and ~480 bp were consistently amplified in all the symptomatic cauliflower samples with the phytoplasma specific universal 16S rRNA and secA gene specific primers. Nested PCR products (~1.2 kb and 480 bp) amplified from cauliflower was cloned in *EcoRI* restriction sites of pGEM-T Easy vector (Promega, Madison, WI). The cloned nested PCR products were directly sequenced (16S rRNA gene: accession nos. MZ196223, MZ196224; secA gene: MZ215721, MZ215722) in both forward and reverse directions, which showed 99.77% sequence identity with

Candidatus Phytoplasma cynodontis reference strain (accession no. AJ550984). Further analyses of the 16S rRNA and secA gene-based phylogenetic tree and the iPhyClassifier-based virtual RFLP analysis of 16Sr RNA gene study demonstrated that the phytoplasma associated with cauliflower phyllody and flat stem disease (CaPP) belonged to 16SrXIV-A subgroup with a similarity coefficient of 1.0. No amplicon was observed from any of the asymptomatic cauliflower plants with the specific tested primers of both the genes. Earlier association of 16SrXV-A subgroup (*Ca. P. brasiliense*) and 16Sr III-J subgroup in Brazil (Canale and Bedendo 2013; Rappussi et al. 2012), 16SrII-A (*Ca. P. aurantifolia*) subgroup in China (Cai et al. 2016), and 16SrVI-A (*Ca. P. trifolii*) subgroup in Iran (Salehi et al. 2007), were reported in cauliflower. Another species of cabbage, *B. oleracea* var. *capitata* L., was reported as host of *Ca. P. trifolii* (16Sr VI-D subgroup) from north India (Gopala et al. 2018). To our knowledge, this is the first report of a '*Ca. P. cynodontis*', 16SrXIV-A subgroup related phytoplasma strain associated with cauliflower phyllody and flat stem in the world. The results described in this report confirm that the 16SrXIV-A phytoplasma, a widely distributed strain associated with sugarcane, wheat, grasses, sapota, and many ornamentals in India (Rao 2021), has also infected cauliflower. This is not only the first instance of cauliflower phyllody disease found in India, but also the first instance of CaPP disease caused by 16SrXIV-A subgroup phytoplasma worldwide. This report has epidemiological significance and needs immediate attention, as cauliflower is the one of the most common vegetable crops grown all over India.

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