First report of a 'Candidatus Phytoplasma asteris'-related strain (16SrI-B) associated with *Morus alba* (white mulberry) witches' broom in Iran

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White mulberry (*Morus alba*), belongs to the family Moraceae and is grown domestically and commercially in Iran for use as fresh fruit or dried nuts. During 2018-19, white mulberry plants showing witches' broom (WMWB) symptoms (Fig. 1) were observed sporadically in the Marvast district of Yazd province, Iran. The maximum incidence of disease was 4%. In affected trees, the witches' broom was restricted to some branches and did not affect the whole canopy.

Total DNA was extracted from 0.2g of midrib tissue of fresh leaves from a total of 14 diseased plants from five different orchards, and three asymptomatic plants. DNA samples were tested for the presence of phytoplasmas by nested PCR, using primers P1/P7 (Deng & Hiruki, 1991; Schneider et al., 1995) in the first round, and primers R16F2n/R16R2 (Gundersen & Lee, 1996) in the nested round. PCR amplicons of 1.8 and 1.25 kb (respectively) were obtained from all diseased plants but not from asymptomatic plants. Restriction fragment length polymorphism (RFLP) analysis of R16F2n/R16R2 amplicons using *Hae* III, *Hpa* II, *Hha* I, *Mse* I, *Alu* I, *Mbo* I, *Hind* III, *Ava* II, *Sst* I and *Tha* I restriction endonucleases showed RFLP patterns similar to the 16SrI phytoplasma group.

The PCR products from three randomly selected WMWB plants were sequenced and showed 100% sequence identity with each other and 99.6% identity with *Ca. P. asteris* (M30790). One R16F2n/R16R2 16S rDNA sequence from the Marvast WMWB strain was submitted to GenBank (Accession No. MT075612). BLAST searches showed that the WMWB phytoplasma shared more than 99% sequence identity with those of *Ca. P. asteris* and related strains. Phylogenetic analysis using Mega7 software confirmed that the phytoplasma strain associated with WMWB clustered within the 16SrI phytoplasma clades closest to *Ca. P. asteris* (M30790) (Fig. 2).

Many phytoplasma strains related to 'Candidatus Phytoplasma aurantifolia' (from carrot and sunflower), 'Ca. Phytoplasma australasia' (from alfalfa, almond, beetroots, marigold, sesame, squash and tamarisk), 'Ca. Phytoplasma trifolii' (from alfalfa, aubergine, cucumber and tomato), 'Ca. Phytoplasma solani' (from bindweed and grapevine) and 'Ca. Phytoplasma oritaniensis' (from bindweed and peach) have been reported previously in Yazd province. A 16SrI-B related phytoplasma has been reported associated with *Euca sativa* (rocket) phyllophy in Yazd province (Esmaizladeh Hosseini et al., 2017). To our knowledge, this is the first report of a 'Ca. Phytoplasma asteris' (16SrI-B) strain associated with witches' broom of *Morus alba* in Iran.

References


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