New Disease Reports

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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Received: *29 Feb 2020.* ***Published:*** *06 May 2020.* ***Keywords:*** *Yazd province*

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 *Rsa*I, *Kpn*I, *Hpa*II, *Hae*III, *Alu*I, *Mse*I, *Hha*I, *Taq*I, *Bfa*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, beetroot, marigold, sesame, squash and tamarisk), '*Ca*. Phytoplasma trifolii' (from alfalfa, aubergine, cucumber and tomato), '*Ca*. Phytoplasma solani' (from bindweed and grapevine) and '*Ca*. Phytoplasma omanensis' (from bindweed and peach) have been reported previously in Yazd province. A 16SrI-B related phytoplasma has been reported associated with *Eruca sativa* (rocket) phyllody in Yazd province (Esmailzadeh 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.

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Figure 1

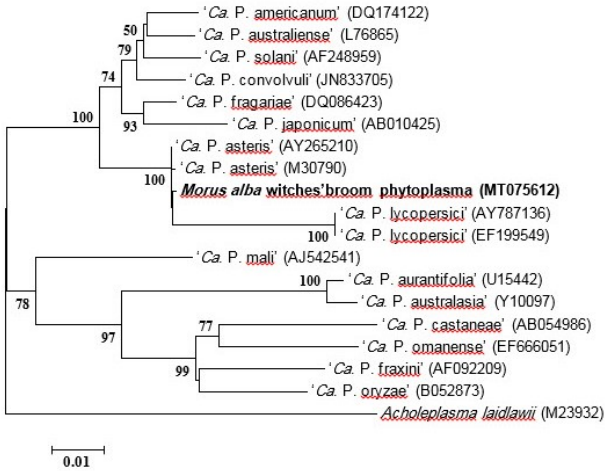

Figure 2

To cite this report: Salehi M, Salehi E, Esmaeilzadeh-Hosseini SA, 2020. First report of a '*Candidatus* Phytoplasma asteris'-related strain (16SrI-B) associated with *Morus alba* (white mulberry) witches' broom in Iran. *New Disease Reports* **41**, 25. http://dx.doi.org/10.5197/j.2044-0588.2020.041.025

 This report was published on-line at www.ndrs.org.uk where high quality versions of the figures can be found.