



First report of a '*Candidatus Phytoplasma aurantifolia*'-related strain associated with witches'-broom disease of limequat in Iran

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Witches'-broom disease of lime (WBDL) caused by '*Candidatus Phytoplasma aurantifolia*' was found first in Mexican lime (*Citrus aurantifolia*) in a small area in southern Iran in 1997 (Bové *et al.*, 2000). Since then WBDL has spread to many areas devastating plantations in the major lime-growing provinces of Fars, Hormozgan, Kerman and Sistan-Baluchista.

The prevalence of WBDL in southern Iran has necessitated regular monitoring of this phytoplasma disease in citrus orchards. Following these monitoring programmes in 2009, we found typical symptoms of phytoplasma disease including witches' broom, little leaf and internode shortening on limequat trees (*Citrus aurantifolia* × *Fortunella* sp.) in private gardens in the region of Hasht Bandi of Minab (Hormozgan province). Four leaf samples were collected from one symptomless and three symptom-bearing trees (Fig. 1). Total DNA was extracted from the midrib tissue using a modified CTAB method. Samples were tested for phytoplasma DNA by direct-PCR using the phytoplasma universal primer pair P1/P7 (Deng & Hiruki, 1991; Schneider *et al.*, 1995) and nested-PCR using primer pairs P1/P7 and R16F2n/R16R2 (Gundersen & Lee, 1996). Amplicons of c. 1.8 and 1.25 kb were amplified from all symptom-bearing trees with direct and nested-PCR, respectively, but not from symptomless plants or sterile distilled water included as negative controls.

Amplified PCR products (1.25 kb) from symptom-bearing limequat trees were digested with endonucleases *AluI*, *TaqI*, *HpaII*, *HhaI*, *MseI* and *RsaI*, and showed identical patterns to '*Ca. Phytoplasma aurantifolia*'. The 1.25 kb amplicon was cloned using the commercial kit #K1214 (Thermo Fisher Scientific Inc., USA), sequenced, and deposited in GenBank (Accession No. KY171947). BLAST analysis revealed that the partial 16S rDNA sequence of the phytoplasma associated with witches' broom disease of limequat shared 99% identity with strains of group 16SrII (peanut witches' broom group), including *Hishimonus phycitis* phytoplasma (KX828609, Iran) as well as several phytoplasmas associated with witches' broom disease on Mexican lime (LN873017 and LN872963 from Oman and Iran, respectively), grapefruit and citron (JF518980 and JN967029, Iran). Phylogenetic analysis using the neighbour-joining method (MEGA 5 software) based on the 16S rRNA gene sequence of the phytoplasma isolated from limequat and other phytoplasmas demonstrated that the limequat witches'-broom phytoplasma is a member of the 16SrII

phytoplasma clade (Fig. 2).

Natural occurrence of witches' broom disease related to 16SrII group of phytoplasma has been reported on Mexican lime, bakraee (a citrus hybrid), citron (*C. medica*) and grapefruit (*C. paradisi*) in Iran (Djavaheri & Rahimian, 2004; Bagheri *et al.*, 2010). To our knowledge, this is the first report of witches' broom disease of limequat associated with '*Ca. P. aurantifolia*' in Iran and probably globally. Our findings suggest that limequat may act as a natural host for the '*Ca. P. aurantifolia*' and may be a potential reservoir for the pathogen in southern Iran.

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Figure 1

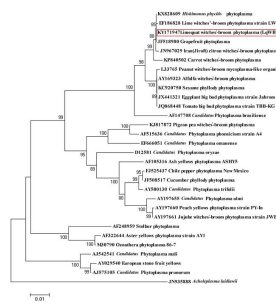


Figure 2

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