



First report of a 16SrVI group related phytoplasma associated with cucumber phyllody in a greenhouse in Iran

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Received: 03 Jul 2015. Published: 03 Aug 2015. Keywords: Yazd province, 16SrVI phytoplasma

Greenhouse cucumber (*Cucumis sativus*) is one of the most economically important vegetable crops in Iran. In September 2014, an outbreak of phyllody disease was observed in up to 82% of greenhouse cucumber in cultivars Keyhan and Negin in Nasr Abad (Taft, Yazd province). Symptoms were characterised by flower virescence, phyllody and sterility (Fig.1). Total DNA was extracted from 0.2 g of fresh phyllody-affected flowers and from symptomless cucumber plants (Zhang *et al.*, 1998). DNA samples were tested for phytoplasma by direct and nested PCR using the phytoplasma primers P1/P7 (Deng & Hiruki, 1991; Schneider *et al.*, 1995) followed by R16F2n/R16R2 (Gundersen & Lee, 1996). Primer pair P1/P7 amplifies an 1800 bp fragment of the phytoplasma ribosomal operon which includes the 16SrRNA gene, the 16S-23S intergenic spacer region (SR) and a portion of the 5' region of the 23S rRNA gene. R16F2n/R16R2 primer pair amplifies about 1250 bp of the phytoplasma 16S rRNA gene. PCR amplicons of ~1.8 and ~1.25 kbp were obtained from symptom-bearing, but not from symptomless greenhouse cucumber plants.

Seven R16F2n/R16R2 amplicons from infected areas in Nasr Abad were directly sequenced. The R16F2n/R16R2 sequences shared 100% sequence identity. A consensus sequence corresponding to the Saleh Abad (Nasr Abad, Taft, Yazd province) greenhouse cucumber phyllody (SGCP) was deposited in GenBank (Accession No. KR633068). BLAST search showed that the SGCP phytoplasma had the highest sequence identity with those of the 16SrVI (*Candidatus* Phytoplasma trifolii) phytoplasma group. Phylogenetic analysis (MEGA software version 6.0) confirmed that the SGCP phytoplasma clustered within the 16SrVI phytoplasma clade (Fig. 2). R16F2n/R16R2 SGCP phytoplasma sequence computer-simulated restriction analysis (Fig.3) with restriction endonucleases *AluI*, *BamHI*, *BfaI*, *BsuI*, *DraI*, *EcoRI*, *HaeIII*, *HhaI*, *HinfI*, *HpaI*, *HpaII*, *KpnI*, *Sau3AI*, *MseI*, *RsaI*, *SspI* and *TaqI* using iPhyClassifier (Zhao *et al.*, 2009) revealed that virtual RFLP patterns of the SGCP phytoplasma differ from those previously recorded 16SrVI subgroups (A, B, C, D, E, F, H, I). Although the SGCP phytoplasma RFLP profiles were more similar to those of subgroup A (AY390261), it showed a similarity coefficient of 0.93; which suggests that the SGCP phytoplasma may represent a new subgroup within the 16SrVI phytoplasma group. This is the first report of the association of

a 16SrVI phytoplasma with cucumber phyllody disease in a greenhouse in Iran. A 16SrII phytoplasma (*Ca. P. aurantifolia*) was associated with cucumber phyllody in a greenhouse of the Fars province (Dehghan *et al.*, 2014). Therefore the SGCP phytoplasma may represent a new epidemiological constraint for the development of the phyllody disease in greenhouses in Iran.

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Figure 1

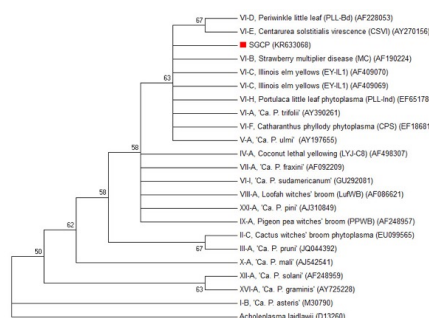


Figure 2

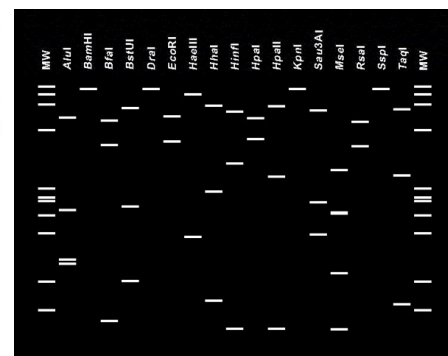


Figure 3

To cite this report: Esmailzadeh Hosseini SA, Salehi M, Mirchenari SM, Tarizeh D, Gholampoor H, 2015. First report of a 16SrVI group related phytoplasma associated with cucumber phyllody in a greenhouse in Iran. *New Disease Reports* **32**, 5.

<http://dx.doi.org/10.5197/j.2044-0588.2015.032.005>

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