



## First report of a 16SrII group related phytoplasma associated with witches' broom of *Cupressus sempervirens* var. *horizontalis* in Iran

A. Bahari<sup>1</sup>, S.M. Alavi<sup>2\*</sup>, M. Shams-Bakhsh<sup>1</sup> and E. Saberi<sup>1</sup>

<sup>1</sup> Plant Pathology Department, Faculty of Agriculture, Tarbiat Modares University, PO Box 14115, Tehran, Iran; <sup>2</sup> Department of Plant Biotechnology, National Institute of Genetic Engineering and Biotechnology, PO Box 14965/161, Tehran, Iran

\*E-mail: mealavi@nigeb.ac.ir

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*Cupressus sempervirens* var. *horizontalis* (Mediterranean cypress) is highly valued because of its forestry and ornamental use, and its importance for religious purposes (Caudullo & Rigo, 2016). In 2014-2015, cypress trees with witches' broom, abnormal shoot proliferation and dieback on one side of the trees, were observed in Tehran Province (Fig. 1). Diseased shoots were collected from four affected trees at two locations. Shoots from two symptomless trees were also collected and used as negative controls. Total DNA was extracted from one gramme of leaves of each sample using a modified CTAB method (Doyle & Doyle, 1990). The samples were tested for the presence of phytoplasmas using nested PCR with primer pair P1/P7 for the first PCR and the R16F2/R16R2 primer pair for the nested PCR (Deng & Hiruki, 1991; Schneider *et al.*, 1995; Gundersen & Lee, 1996). A nested PCR product of the expected size (1,246 bp) was amplified from all symptom-bearing samples but not from symptomless samples, suggesting an association between phytoplasma and diseased cypress. The nested PCR products of two samples were cloned into a pTZ57R/T vector (Fermentas, Vilnius, Lithuania) and sequenced. BLAST analysis of the 16SrDNA sequences showed that the phytoplasma detected in both samples were identical and had highest sequence identity (99%) with phytoplasmas belonging to the 16SrII ribosomal group. Phylogenetic analysis using MEGA software (version 6.0) confirmed that the *Cupressus sempervirens* var. *horizontalis* witches' broom phytoplasma (GenBank Accession No. KU647632) was clustered within the 16SrII phytoplasma clade (Fig. 2) and was most closely related to the 16SrII-B group ('*Candidatus* Phytoplasma aurantifolia'). However, *in silico* RFLP patterns of the 16S rDNA sequence with restriction endonucleases *AluI*, *Bam*HI, *Bfa*I, *Bst*UI, *Dra*I, *Eco*RI, *Hae*III, *Hha*I, *Hinf*I, *Hpa*I, *Hpa*II, *Kpn*I, *Sau*3AI, *Mse*I, *Rsa*I, *Ssp*I and *Taq*I using iPhyClassifier (Zhao *et al.*, 2009) revealed that the virtual RFLP patterns of the phytoplasma differs from all previously reported 16Sr groups and subgroups. Although the *Cupressus sempervirens* var. *horizontalis* witches' broom phytoplasma RFLP pattern has greatest similarity with the reference pattern of the 16SrII-D type phytoplasma ('*Ca.* P. australasia'; Y10097), it has a similarity coefficient of 0.90, suggesting

that this phytoplasma represents a new 16SrII subgroup. To our knowledge this is the first report of the natural occurrence of a phytoplasma associated with a witches' broom disease of *C. sempervirens* var. *horizontalis* in Iran.

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Figure 1



Figure 2

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