



## First report of a '*Candidatus Phytoplasma aurantifolia*'-related phytoplasma strain associated with yellowing symptoms on pineapple palm in Iran

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Pineapple palm, *Phoenix canariensis*, is one of the most widely grown and appreciated ornamental palms, including in the south of Iran. During the last three years, symptoms resembling nutrient deficiency were observed on pineapple palms in urban landscapes in Ahvaz in southwestern Iran. The symptoms included leaf yellowing in the lower fronds which began from the leaf tips and extended towards the blade and the rachis (Figs. 1, 2, 3). During January 2016, *P. canariensis* palms were surveyed for phytoplasma. Leaf samples from three diseased and one symptomless palm were collected. The midribs were freeze-dried and then powdered in liquid nitrogen. Total DNA was extracted using a CTAB-based method (Maixner *et al.*, 1995). Total DNA was analysed by nested PCR assay using generic primers P1/P7-R16F2n/R16R2 (Deng & Hiruki, 1991; Gundersen & Lee, 1996). Amplicons of the expected size (c. 1.2 kb) were obtained from all diseased palms, and were purified (GF-1 AmbiClean Kit, Malaysia) and directly sequenced. The partial consensus 16S rDNA sequence was deposited in GenBank (Accession No. KX088466) and compared to those of reference phytoplasmas by BLAST analysis. The consensus 16S rDNA sequence showed 99% sequence identity to members of group 16SrII '*Candidatus Phytoplasma aurantifolia*'. A phylogenetic tree (Fig. 4) based on the 16S rDNA sequences of *P. canariensis* phytoplasma and those of reference phytoplasmas was constructed using the maximum likelihood algorithm under the TN93+G+I model of the MEGA 6 software (Tamura *et al.* 2013). The *P. canariensis* phytoplasma (Ahvaz-5 isolate) clustered within the 16SrII phytoplasma group. Restriction mapping of the partial 16SrRNA sequence (1161bp) with typical endonucleases *AluI*, *BamHI*, *BfaI*, *BstUI*, *DraI*, *EcoRI*, *HaeIII*, *HhaI*, *HinfI*, *HpaI*, *HpaII*, *KpnI*, *Sau3AI*, *MseI*, *RsaI*, *SspI* and *TaqI* in a virtual digest using Restriction-Mapper V.3 online software (<http://www.restrictionmapper.org>) showed that the virtual

RFLP pattern of the *P. canariensis* phytoplasma was identical (similarity coefficient 1.00) to the reference pattern of subgroup 16SrII-D (Y10097). Both, the virtual RFLP and phylogenetic analyses confirmed that the *P. canariensis* phytoplasma is a member of the 16Sr II group, subgroup D.

*Phoenix canariensis* is affected by the Texas phoenix palm decline phytoplasma, a member of the 16SrIV group, in Florida and Texas, USA. To our knowledge, this is the first report of a phytoplasma disease on palm in Iran. The *P. canariensis* phytoplasma (Ahvaz-5 isolate) is affecting approximately 20% of the pineapple palms in Ahvaz. The identification of this phytoplasma will contribute to improving the future management of the disease.

### References

Deng S, Hiruki C, 1991. Amplification of 16S rRNA genes from culturable and non-culturable mollicutes. *Journal of Microbiological Methods* **14**, 53–61. [http://dx.doi.org/10.1016/0167-7012\(91\)90007-D](http://dx.doi.org/10.1016/0167-7012(91)90007-D)

Gundersen DE, Lee IM, 1996. Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer sets. *Phytopathologia Mediterranea* **35**, 144-151.

Maixner M, Ahrens U, Seemuller E, 1995. Detection of the German grapevine yellows (Vergilbungskrankheit) MLO in grapevine, alternative hosts and a vector by a specific PCR procedure. *European Journal of Plant Pathology* **101**, 241-250. <http://dx.doi.org/10.1007/BF01874780>

Tamura K, Stecher G, Peterson D, Filipiński A, Kumar S, 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* **30**, 2725-2729. <http://dx.doi.org/10.1093/molbev/mst197>



Figure 1



Figure 2



Figure 3

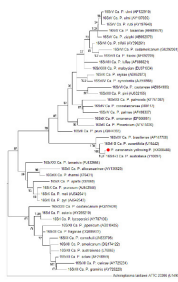


Figure 4

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