



# First report of a '*Candidatus Phytoplasma aurantifolia*'-related strain associated with leaf roll symptoms on eucalyptus in Iran

M. Azimi, R. Farokhi-Nejad and M. Mehrabi-Koushki\*

Department of Plant Protection, College of Agriculture, Shahid Chamran University of Ahvaz, Iran

\*E-mail: mhdmrhb@gmail.com

Received: 12 Nov 2016. Published: 19 Jan 2017. Keywords: *Eucalyptus camaldulensis*, nested PCR

The eucalyptus (*Eucalyptus camaldulensis*) is a common tree in many parts of the world including Iran. During 2015-16, symptoms of leaf roll, yellowing, little leaf and short internodes (Figs. 1-2) were observed on eucalyptus trees in Ahvaz, southwestern Iran. Leaf samples from three diseased trees growing in the downtown regions and eastern and northern suburbs, and one symptomless tree from each region were tested for phytoplasmas. The leaf midribs were separated and freeze-dried, then powdered in liquid nitrogen. Total DNA was extracted using CTAB (Maixner *et al.*, 1995) and tested for the presence of phytoplasmas using a nested PCR assay that amplifies part of the 16S rDNA using the universal primers P1/P7 (Deng & Hiruki, 1991) followed by R16F2n/R16R2 (Gundersen & Lee, 1996). The amplicons of c. 1.2 kb produced from all symptomatic samples were directly sequenced, and one (Ahvaz-3) was deposited in GenBank (Accession No. KX088462). Using a BLAST search the consensus sequence showed 99% identity to members of group 16SrII '*Candidatus Phytoplasma aurantifolia*'. A maximum likelihood phylogenetic tree based on the 16S rDNA sequences, constructed with MEGA 6.0 software (Tamura *et al.*, 2013) showed that the *E. camaldulensis* leaf roll phytoplasma isolate, Ahvaz-3, clustered with the 16SrII phytoplasma group (Fig. 3). A virtual RFLP with 17 typical endonucleases (Lee *et al.*, 1998) using Restriction-Mapper V.3 software (<http://www.restrictionmapper.org>), and confirmed by iPhyClassifier (Zhao *et al.*, 2009) revealed that this phytoplasma is closely related to the reference strain of the 16SrII-D subgroup (Y10097).

The association of '*Candidatus Phytoplasma aurantifolia*' strains with diverse symptoms in herbaceous and woody plants has been reported from different climates of Iran in recent years. To our knowledge, this is the first report of a 16SrII phytoplasma infecting eucalyptus trees in Iran. The *E. camaldulensis* leaf roll phytoplasma is affecting approximately 5% of

eucalyptus trees in Ahvaz and its detection will contribute to improving 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.
- Lee IM, Gundersen-Rindal DE, Davis RE, Bartoszyk IM, 1998. Revised classification scheme of phytoplasmas based on RFLP analysis of 16S rRNA and ribosomal protein gene sequences. *International Journal of Systematic Bacteriology* **48**, 1153–1169. <http://dx.doi.org/10.1099/00207713-48-4-1153>
- 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>
- Zhao Y, Wei W, Lee IM, Shao J, Suo X, Davis RE, 2009. Construction of an interactive online phytoplasma classification tool, iPhyClassifier, and its application in analysis of the peach X-disease phytoplasma group (16SrIII). *International Journal of Systematic and Evolutionary Microbiology* **59**, 2582–2593. <http://dx.doi.org/10.1099/ijs.0.010249-0>



Figure 1



Figure 2

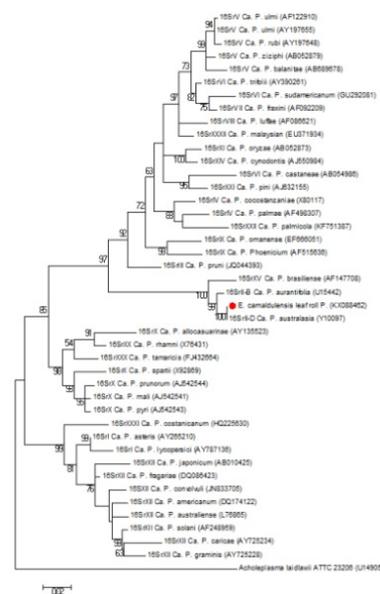


Figure 3

**To cite this report:** Azimi M, Farokhi-Nejad R, Mehrabi-Koushki M, 2017. First report of a '*Candidatus Phytoplasma aurantifolia*'-related strain associated with leaf roll symptoms on eucalyptus in Iran. *New Disease Reports* **35**, 4.

<http://dx.doi.org/10.5197/j.2044-0588.2017.035.004>

©2017 The Authors

This report was published on-line at [www.ndrs.org.uk](http://www.ndrs.org.uk) where high quality versions of the figures can be found.