



Phytoplasmas associated with grapevine yellows disease in Iran: first report of a '*Candidatus Phytoplasma trifolii*'-related strain and further finding of a '*Ca. P. solani*'-related strain

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Over recent years, grapevines (*Vitis vinifera*) with symptoms similar to grapevine yellows disease (severe yellows and redness, inward curling of leaves and stunting) have been observed in vineyards from different regions of Iran, especially Zanjan province (Figs 1-2). Therefore, a survey was conducted in 2018 to identify possible phytoplasma agents. A total of 19 samples from symptomatic (16) and asymptomatic (3) plants (cvs. Keshmeshi Qermez and Askari) were collected from vineyards in the Darsajin, Khoramdareh and Zanjan areas in Zanjan province. Total DNA was extracted (Maixner *et al.*, 1995) from the main veins and petioles and nested-PCR with universal primers P1/P7 (Deng & Hiruki, 1991) in the first round followed by R16F2n/R2 (Gundersen & Lee, 1996) in the nested round was conducted. Nested PCR products were obtained from all symptomatic plants but not from asymptomatic plants. Direct sequencing of the partial 16S rRNA gene from six randomly selected plants confirmed that phytoplasmas associated with grapevine yellows were related to two different ribosomal groups.

In BLAST analysis, the sequences obtained (1220 bp) from samples 1, 3 and 5 (GenBank Accession Nos MK392483-MK392485) had 99-100% identity with strains affiliated with '*Ca. P. trifolii*' (MK15809, MK379605 and MK372596) and samples 6, 7 and 8 (MK392486-MK392488) had 99-100% identity with '*Ca. P. solani*' related strains (KF583785, MN398472 and MN398467). There was no sequence variation between the three isolates of the '*Ca. P. trifolii*'-related strain, or the isolates of the '*Ca. P. solani*'-related strain. The percentage sequence identity for the isolates in this study to the reference strain for each species was 99.83% for '*Ca. P. trifolii*' (AY390261) and 99.59% for '*Ca. P. solani*' (AF248959).

Phylogenetic analysis was conducted with the maximum likelihood method using MEGA7 software (Kumar *et al.*, 2016). According to the phylogenetic tree based upon the 16S rDNA sequences, grapevine yellows phytoplasma strains were clustered in '*Ca. P. solani*' (16SrXII-A) and '*Ca. P. trifolii*' (16SrVI-A) (Fig. 3). '*Ca. P. solani*' and '*Ca. P. trifolii*'-related strains have been reported from many plant species in different areas worldwide. Previous studies reported several '*Ca. Phytoplasma*' species

associated with grapevine yellows disease from Iran including '*Ca. P. solani*', '*Ca. P. aurantifolia*', '*Ca. P. fraxini*', and '*Ca. P. phoenicium*' (Mirchenari *et al.*, 2015; Zamharir *et al.*, 2017). To our knowledge, this is the first report of a '*Ca. P. trifolii*'-related strain associated with grapevine yellows disease in Iran.

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Figure 1



Figure 2

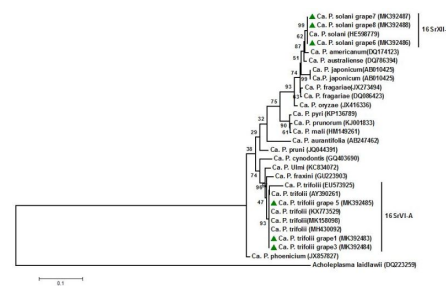


Figure 3

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