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Candidatus phytoplasma fraxini related (16S rRNAVII) strain associated with date yellows disease in Iran

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Abstract Phytoplasma was detected in date palm trees (*Phoenix dactylifera*) displaying symptoms of yellowing disease by nested polymerase chain reaction using 16 SrRNA gene universal primers of phytoplasmas. Sequence identities of 98 % were found to several isolates of '*Candidatus* Phytoplasma fraxini' (Ash yellows group, 16SrVII group).

Keywords Phytoplasma · Date palm · Ash yellows group · Yellows

Iran is one of the leading date producing and exporting countries in the world. The main production areas in Iran are Hormozgan (21.6 %), Khusistan (17.6 %), Bushehr (13.4 %), Beluchistan (12.2 %), Fars (12.3 %) and Kerman (21.1 %) provinces. Various pathogens such as fungi, viruses and phytoplasma severely affect palm tree cultivation worldwide (Harries 1977; Howard 1983, Abdullah Samir et al. 2010). Natural occurrence of leaf yellows disease on several date (*Phoenix dactylifera*) plants were observed in gardens at Khusistan province, Iran, during winter of 2013–15. The symptoms were leaf yellows, tiny narrow leaves and leaf drying (Fig. 1). Due to date economic importance for the country and Khusistan province, the identification of the causal agent of the leaf yellows disease of date was necessary. To investigate the possibility of a phytoplasma causal agent, the total DNA was isolated from leaf tissue of 25 symptomatic and 10 healthy date plants following the protocol of Doyle and Doyle (1990). The initial PCR was performed using P1/P7 universal primers specific to the 16S rRNA Gene (Schneider et al. 1995). Further, nested PCR was carried out with primers R16F2n/R16R2 (Gundersen and Lee 1996) employing the initial PCR product as the template.

Agarose gel electrophoresis of nested PCR products obtained with the 16S rRNA-gene-specific primers resulted in the expected size DNA fragments of 1.2 kb, from infected plant samples but not from healthy samples. The 1.2-kb amplicons obtained from the nested PCR was cloned, sequenced and the data deposited in GenBank (Accession number KX347967). BLAST search analysis of KX347967 revealed 98 % sequence

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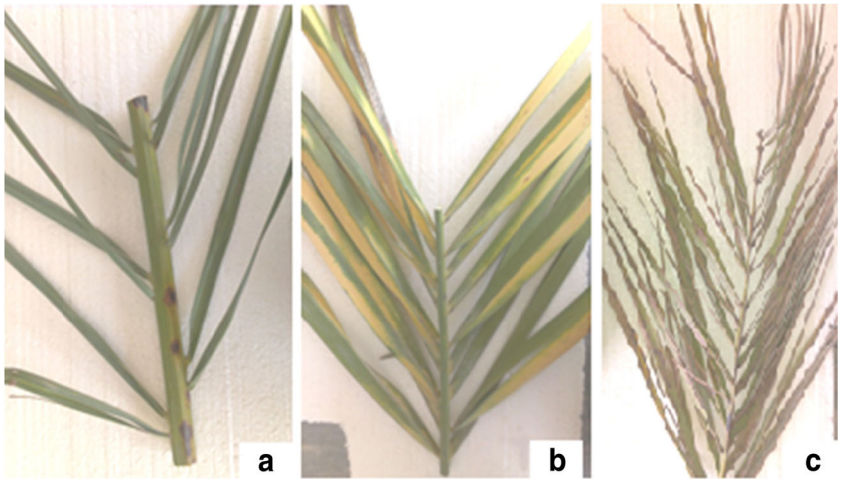

Fig. 1 Plant showing elongated internodes and abnormal branches (**a**). Plant leaves with yellows (**b**) and date leaf drying (**c**)

similarity with the 16S rRNA gene of '*Candidatus* Phytoplasma fraxini' isolate L1–09, L3–09, Cajica Straw CR1, '*Magnolia grandiflora*' phytoplasma strain Magn1 (Accession numbers KP324846, KP324845, KJ910297, KJ093444) respectively, belonging to the 16S rVII group.

Phylogenetic analysis of the phytoplasma isolates using molecular evolutionary genetics analysis (MEGA) 6.0 tool (Tamura et al. 2007) also showed a close relationship with isolates of ash yellows '*Candidatus* Phytoplasma fraxini' (16SrVII) group (Fig. 2).

Date trees are known to be affected by phytoplasmas including lethal yellowing (16SrIV-A), Texas phoenix palm decline (16SrIV-D) (Howard 1983; Schuiling et al. 1992) and Al-Wijam decline (16SrI) (El-Zayat et al. 2002), however to the best of our knowledge, the association of '*Candidatus* Phytoplasma fraxini' with yellows disease of date palm is the first report of this kind from Iran.

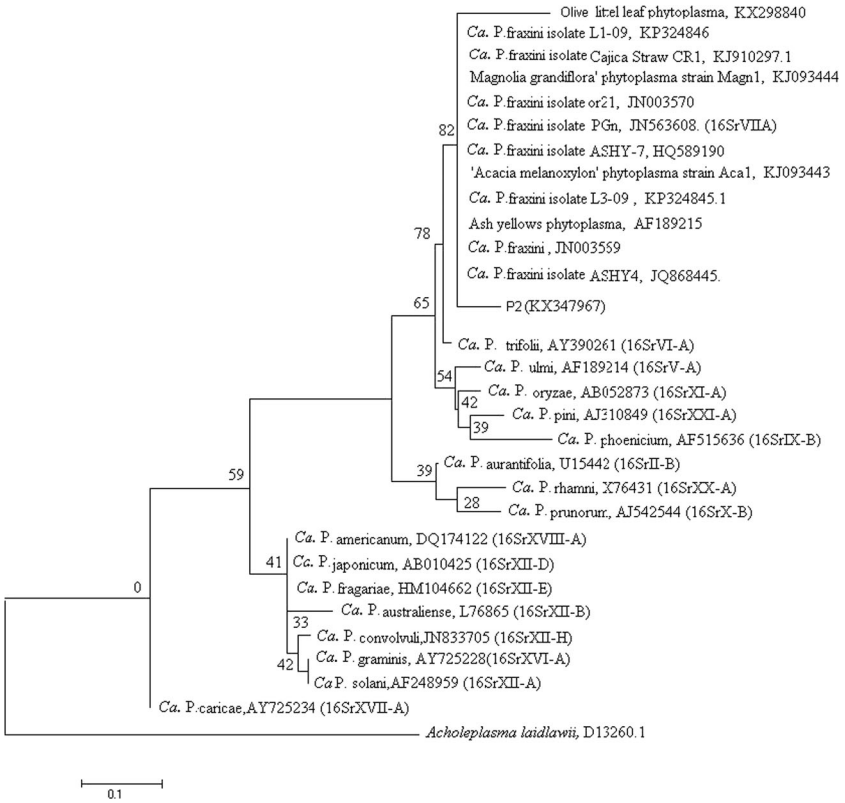

Fig. 2 Phylogenetic tree constructed by neighbour-joining method of 16S rRNA gene sequences from date palm phytoplasma strains (P2) and reference phytoplasmas from GenBank. 'Ca. P.' stands for '*Candidatus* Phytoplasma species'. The numbers on the branches are bootstrap (confidence) values of 100 replicates

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