Molecular detection and characterization of a 16SrII-D phytoplasma associated with streak yellows of date palm in Oman

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Abstract

Date palm is the most important fruit crop in Oman, covering about 50% of the agricultural area in the country. During a survey in 2018–2020, symptoms resembling phytoplasma diseases were observed in date palms in Muscat, Oman. Date palms showed streak yellows and dryness of young and mature leaflets and leaves. Polymerase chain reaction (PCR) assays were conducted using the universal primer pairs P1/P7 followed by R16F2n/R16r2n. In addition, the phytoplasma genes *imp*, *secA*, and *tuf* were also targeted in semi-nested PCR assays by using specific primers for each gene. Symptomatic trees were positive for phytoplasma presence in nested and semi-direct PCR, however, no amplification was observed from the symptomless and negative control. Sequence analysis of the rRNA, *imp*, *secA*, and *tuf* genes confirmed that the phytoplasma associated with streak yellow date palm (SYDP) is a strain of the 16SrII-D subgroup. This is the first record of association of a phytoplasmas of the 16SrII-D subgroup with date palms in Oman. This finding increased our knowledge about the distribution of phytoplasma diseases in date palms in the world.

Keywords *Phoenix dactylifera* · 16SrII-D · Multilocus · Immunodominant · Translocase · Translation elongation

Date palm (*Phoenix dactylifera* L.) is the most important fruit crop in the arid regions of the Middle East. Based on FAO, date palm production totaled 8.5 Mt in 2018 (FAO 2019). In Oman, date palm occupies approximately 50% of the agricultural area, with a total production of 369,000 tons in 2018, placing Oman the 8th producer of dates in the world (FAO 2019).

Date palm production is affected by several diseases. Phytoplasmas are known to cause major diseases in palms, including the lethal yellowing disease (Gurr et al. 2016). Several reports are available on the association between diverse phytoplasma groups and date palms, including the 16SrI in Egypt (Alkhazindar 2014), 16SrII in Saudi Arabia (Alhudaib et al. 2007), 16SrIV in the USA (Ntushelo et al.

2012), 16SrVI and VII in Iran (Zamharir and Eslahi 2019) and 16Sr XIV in Sudan (Cronjé et al. 2000). The most known symptoms included yellowing and streak yellows in leaves, while in some regions lethal yellowing symptoms have been observed.

During a survey in 2018–2020, date palms showing streak yellows and dryness of young and mature leaflets and leaves were observed in Muscat, Oman. The objective of the study was to determine the association of phytoplasmas with the symptoms.

A total of 25 date palm samples showing streak leaf yellowing and leaflet/leaf dryness (Fig. 1) were collected from Muscat during 2018–2020. The symptoms were apparent on trees, 5–20-year-old over all seasons. Ten symptomless date palms, seemingly healthy, were included for comparative purposes. Samples were obtained from leaves, trunk, and roots. Wood from basal trunk was sampled by using a portable electric drill and roots near the soil surface were collected by shovel. Samples were put in a plastic bag, labeled, and transferred to Sultan Qaboos University for further processing.

Total nucleic acid was extracted from leaf tissue, woods and roots by using a procedure described by Doyle and Doyle (1990) for the leaves and Harrison et al. (2008) for the trunk and root samples. A nested-PCR assay primed by P1/P7, followed by R16F2n/R16R2n was conducted to

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Fig. 1 Symptoms of phytoplasma infection in date palms: yellow streaking (A, B), premature dryness of leaflets (B)

amplify the 16S rRNA region (Deng and Hiruki 1991; Gundersen and Lee 1996). In addition, distilled water (DW) and DNA from symptomless trees were used as negative control, while alfalfa and lime witches' broom phytoplasmas were used as positive control (Al-Subhi et al. 2018).

The *tuf*, *imp* and *secA* genes were amplified using primer pairs specific for phytoplasmas in the 16SrII group. The TUF-II-F1 and TUF-II-R1 were utilized to prime the elongation factor TU targeting 1490 bp, followed by using the semi-nested PCR primers TUF-II-F2 and TUF-II-R1, which target 1094 bp of the *tuf* gene. The *imp* gene was amplified by using IMP-II-F1 and IMP-II-R1 primer pairs (targeting 786 bp) in the direct PCR followed by IMP-II-F1/IMP-II-R1 (targeting 717 bp) in the semi-nested PCR assay (Al-Subhi et al. 2018). A partial of translocase protein A gene was amplified in semi-direct PCR assays by using SecA-II-F1/SecA-II-R1 in the first reaction followed SecA-II-F1/SecAII-R4 in the second reaction. The PCR reaction mix and conditions of the 16S rRNA, *tuf*, *imp* and *secA* were as described by Al-Subhi et al. (2018).

Three positive products of each gene were sent to Macrogen (South Korea) and directly sequenced by the primer pairs used in the nested and semi-nested assays. Genious Prime (version 2020.1.1) (Kearse et al. 2012) and ClustalX were utilized to assemble and align the obtained sequences. A neighbor-joining method using Kimura-parameter models with 1000 replicates for bootstrap analysis were used to construct phylogenetic tree by MEGA 6 software (Tamura et al. 2013). To root the phylogenetic tree of 16S rRNA, *tuf*, and *secA*, *B. subtilis* reference sequences AB042061, D10279, and GCA_000789275 were used, respectively. Reference phytoplasma sequences of the four genes from NCBI

GenBank were included in phylogenetic tree construction (Supplementary Tables 1, 2). A combined tree based on the four genes was created with MEGA 6 using the model and method described in single gene tree.

Symptomatic date palms showed longitudinal streak yellows in the midribs and leaflets, which developed on other parts of the leaves. This was followed by dryness of the yellow leaflets from the top to the bottom. In addition, some palms displayed dryness of the older leaves and some young leaves.

In total, 12 leaf samples showing streak yellows and leaf dryness were positive in PCR assays of the 16S rRNA but no amplification was observed from the trunk and root samples. PCR amplification of the symptomatic plants and positive control showed the expected size products in semi-nested PCR assays, which were 1250, 726, 1320, and 998 bp for the 16S rRNA, *imp*, *secA*, and *tuf* genes, respectively. No fragments were amplified in DW and healthy plants.

Sequence analysis showed that the three sequences of the 16S rRNA had 100% identity to each other and 100% sequence identity with those of *Cycas revoluta* lethal yellowing phytoplasma CLY clones (MT293343–6) from Oman, *Roystonea regia* yellowing phytoplasma clones (MT232233–6) from Oman, and Alfalfa phytoplasma clone AP2 (KY449416) from Sudan. Neighbor-joining phylogenetic tree analyses of the partial 16S rRNA sequences (1152 bp) indicated that date palm streak yellow (DPSY) phytoplasma clustered in a subclade with phytoplasma group II-D with 100% bootstrap support. They were closely related to Alfalfa witches' broom (KX35856), and "*Candidatus* Phytoplasma australasia" (Y10097), which are from the 16SrII-D subgroup phytoplasma (Fig. 2A).

Fig. 2 Phylogenetic tree based on the 16S ribosomal RNA gene sequences (A) and the combined 16S ribosomal RNA, *tuf*, *secA* and *imp* gene sequences (B) of date palm phytoplasmas (black circles) with 41 published phytoplasma sequences from GenBank. The tree was rooted

using *Bacillus subtilis* (AB04261, GCA000789275). The phylogenetic tree was constructed by the neighbor-joining method and units are the number of base substitutions per site. Bootstrap values are expressed as percentage of 1000 replicates

The *imp* gene sequence analysis using BLASTn search showed 100% identity with *Vicia faba* phyllody (KX358606), *Cucurbita pepo* phytoplasma (KX358601), and *Medicago sativa* phytoplasma isolate (KX358600), which are from Oman. The three sequences clustered with tomato big bud (JQ745285) and alfalfa witches' broom phytoplasmas, which are strains of the 16SrII-D subgroup (Supplementary Fig. 1A).

The *secA* gene of DPSY phytoplasma showed 100% identity with *Cicer arietinum* phyllody (KX358585), *Solanum melongena* big bud (KX358584), and *Pisum sativum* phyllody phytoplasma (KX358583), which are from Oman. A phylogenetic tree constructed using 536 bp from the *secA* sequences and 32 other phytoplasmas showed that the three sequences clustered with tomato big bud (KJ462021) and alfalfa witches' broom (KX358576) phytoplasmas, which are strains of the 16SrII-D (Supplementary Fig. 1B).

The BLASTn algorithms against the GenBank for the *tuf* gene showed the highest identity 99.8% (995 identical bases) with *Solanum melongena* big bud (KX358596), *Pisum sativum* phyllody (KX358595), and *Vicia faba* phyllody (KX358594) from Oman. The phylogenetic tree based on 385 bp of *tuf* sequences of DPSY and 41 other phytoplasma from GenBank is shown in Supplementary Fig. 1C. The *tuf* tree placed three *tuf* sequences of SYDP phytoplasma within one clade of the 16SrII-D phytoplasmas with alfalfa witches' broom (KX358588) and sweet potato little leaf (JQ824250) (16SrII-D), with 100% bootstrap support.

Three sequences for each gene were deposited in GenBank database under the accession numbers (MT387154, MT387155, MT387156) for the 16S rRNA, (MT361012, MT361013, MT361014) for the *imp*, (MT361006, MT361007, MT361008) for the *secA*, and (MT361009, MT361010, MT361011) for the *tuf* genes.

The combined tree placed the DPSY phytoplasma with the 16SrII-D clade, with a high bootstrap support (88%) (Fig. 2B).

In this study, the associated phytoplasma with streak yellow date palms was well characterized using multiple gene sequencing. Diverse phytoplasma groups have been reported associated with different symptoms in date palms. For example, Harrison et al. (2008) reported the association between *P. dactylifera* and 16SrIV-D subgroups and a mix infection of 16SrIV-A and IV-F showing severe symptoms like falling of premature fruits, yellowing and browning the leaves, dryness of the new leaves and finally death of the trees in the USA. Similar symptoms have also been observed in date palms associated with 16SrIV-A in Kuwait (Al-Awadhi et al. 2002) and Tanzania (Tymon et al. 1998). Al-Wijam disease showing reduction in fruits and stalk size, streak yellows in leaves and fruits, and leaf stunting has been reported in association with 16SrI and 16SrII-D subgroup phytoplasmas (Alhudaib et al. 2007; Alhudaib et al. 2008). White tip die-back displaying severe chlorosis of the new leaves, white longitudinal streaks of the midribs, and drying/whiteness of the crown has been reported to be associated with 16SrXIV in Sudan (Cronjé et al. 2000). However, the characteristic streak yellowing of leaves has been reported in Iran (Zamharir and Eslahi 2019). This is the first report of association of 16SrII-D with date palm in Oman. Strains of 16Sr II-D phytoplasma subgroups have been reported in association with diverse plant species in Oman (Al-Subhi et al. 2018). Our results obtained from phylogenetic tree of the three genes was in accordance with 16S rRNA analysis, and the combined phylogenetic tree confirmed the association between DPSY and a phytoplasma of the 16SrII-D subgroup.

The present study reports the first observation of 16SrII-D phytoplasmas associated with streak yellow of date palm in Oman. In addition, this study increased our knowledge of the geographical distribution of phytoplasma diseases of date palms in the world. 16SrII-D phytoplasma strains are the most dominant phytoplasmas types and have widespread host species in Oman. Further studies are needed to investigate the insect vectors of this phytoplasma in Oman.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest. All authors fully agree for submission of the manuscript.

Ethical approval The study does not contain any studies with human participants or animals performed by any of the authors.

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