Australasian Plant Disease Notes (2018) 13: 39
https://doi.org/10.1007/s13314-018-0326-1

16SrII phytoplasma associated with date palm and Mexican fan palm in Saudi Arabia

Ayman F. Omar[1,2] · Abdullah Alsohim[1] · Medhat R. Rehan[1,3] · Khaled A. Al-Jamhan[1] · Edel Pérez-López[4]

Received: 21 August 2018 / Accepted: 19 October 2018 / Published online: 26 October 2018
© Australasian Plant Pathology Society Inc. 2018

Abstract

Date palm and Mexican fan palm trees showing symptoms previously associated with phytoplasmas were observed in the Al-Qassim region, Saudi Arabia in 2017. DNA amplification, sequencing, and phylogenetic analysis revealed the presence of a '*Candidatus* Phytoplasma australasia'- related strain in eighteen of the eighty-three symptomatic plants collected that were positive using 16S-based assays. This study confirmed the presence of phytoplasma affecting date palms in Saudi Arabia and it is the first report of several date palm cultivars associated with '*Ca. P. australasia*'-related strains. This is also the first report worldwide of Mexican fan palm trees affected by 16SrII phytoplasma strains and the first report of this plant host affected by phytoplasmas in Saudi Arabia. The implications of these findings are vital to implement management strategies and avoid economic losses in Saudi Arabia and the Middle East, which are the main producers of dates in the world.

Keywords 16SrII · Middle East · Bacteriology · Phytopathology · Palm trees

Date palm (*Phoenix dactylifera*) is a member of the family Arecaceae, and one of the most economically important crops in Saudi Arabia, with 107,281 ha cultivated, more than 25 million of trees and an annual production of 1.132.887 t of dates (Statistical year book 2016). After the Riyadh region, the Qassim region is the second bigger producer of dates in Saudi Arabia with 39.301 ha cultivated and a production of almost 190.000 t per year (Ministry of Agriculture 2011). Around 29 cultivars of date palms are used by the farmers in the Qassim region (Aleid et al. 2015), but also in the area there are very common ornamental palms such as the Mexican fan palm (*Washingtonia robusta*), which are native to northwestern Mexico but are used worldwide as ornamental plants. The economic importance of date palms for Saudi Arabia is the reason why strict phytological surveillance is extremely important, not only to detect diseases affecting date palm trees, but also alternative plant hosts. Palm trees are one of the many susceptible plant hosts for phytoplasmas (Pérez-López et al. 2016), and the detection of date palms affected by phytoplasmas in Sudan (Cronjé et al. 2000), Egypt (AlKhazindar 2014), and Iran (Zamharir et al. 2016), countries surrounding Saudi Arabia (Fig. 1), have alarmed farmers and scientists in the country.

Phytoplasmas ('*Candidatus* Phytoplasma') are unculturable, cell wall-less bacteria that are taxonomically classified as class Mollicutes (Bertaccini 2007), and plant pathogens that have been associated with economically and ecologically important plant hosts worldwide (Jarzembowski et al. 2018). Phytoplasmas are classified into groups and subgroups based on restriction fragment length polymorphism (RFLP) analysis of their 16S rRNA-encoding loci with a set of seventeen endonucleases (Lee et al. 1998, 2000). In Saudi Arabia the number of plant hosts affected by phytoplasmas have increased drastically, which might be associated with the increasing number of studies focused on the identification of the pathogen (Omar 2016, 2017; Omar et al. 2017; Pérez-López et al. 2018). Symptomatic palm trees showing yellowing followed by dryness were observed the Qassim region of Saudi Arabia.

Seventy-four symptomatic date palm trees belonging to three different varieties and male date palm trees were sampled (Table 1) in date palm farms located though the Qassim region

✉ Edel Pérez-López
epl733@mail.usask.ca

[1] Department of Crop Production and Protection, College of Agriculture and Veterinary Medicine, Qassim University, Buraydah, Saudi Arabia

[2] Department of plant pathology, Plant Pathology and Biotechnology Lab., Faculty of Agriculture, Kafrelsheikh University, Kafrelsheikh 33516, Egypt

[3] Department of Genetics, Faculty of Agriculture, Kafrelsheikh University, Kafrelsheikh 33516, Egypt

[4] Department of Biology, University of Saskatchewan, Saskatoon S7N 5E2, Canada

Fig. 1 Geographic location of the Middle East countries where date palms have been reported affected by phytoplasmas

during 2017. The main symptom observed in the plants was discoloration of the foliage, which developed from the tip to the base of the leaves. The leaves showed yellowing followed by dryness (Fig. 2a) and most fruits fell early, particularly in the Sukkari cultivar. Seven Mexican fan palm trees (*Washingtonia robusta*) showing similar symptoms to those described for date palms were also sampled in the Qassim region (Table 1, Fig. 2b). The final stage of the symptoms is the death of the palm trees. One asymptomatic plant per cultivar and/or plant species was collected and used as negative controls.

Total DNA was extracted from palm leaves using a CTAB method (Maixner et al. 1995), and stored at −20 °C until the analyses were performed. DNA extracts were used as template in PCR with primer pair P1/P7 (Deng and Hiruki 1991; Schneider et al. 1995) in the first reaction. Two microliters of the product of the first reaction, diluted 1:20 in sterile water, were used as a template for nested PCR with primers R16mF2/R16mR2 as previously described (Omar 2017). Positive reactions were conformed through electrophoresis using a 1.5% agarose

Table 1 Information about the symptomatic samples collected in this study

Cultivar	Species	Sampled plant no.	Phytoplasma positive	Isolate name	16Sr group	'Ca. Phytoplasma' species	Genbank accession No.
Sukkari	*Phoenix dactylifera* L.	28	3	Suk-20 Suk-49 Suk-165	16SrII	'*Candidatus* Phytoplasma australasia'	MH157916
Khalas		8	2	Kh-1 Kh-2	16SrII	'*Candidatus* Phytoplasma australasia'	MH157918
Nabtat		17	4	Ns-122 Ns-123 Ns-125 Ns-134	16SrII	'*Candidatus* Phytoplasma australasia'	MH155427
Male Date palms		21	5	DPM-17 DPM-27 DPM-29 DPM-31 DPM-217	16SrII	'*Candidatus* Phytoplasma australasia'	MH157915
Mexican fan palm	*Washingtonia robusta*	7	4	Wr-128 Wr-130 Wr-145 Wr-146	16SrII	'*Candidatus* Phytoplasma australasia'	MH157917

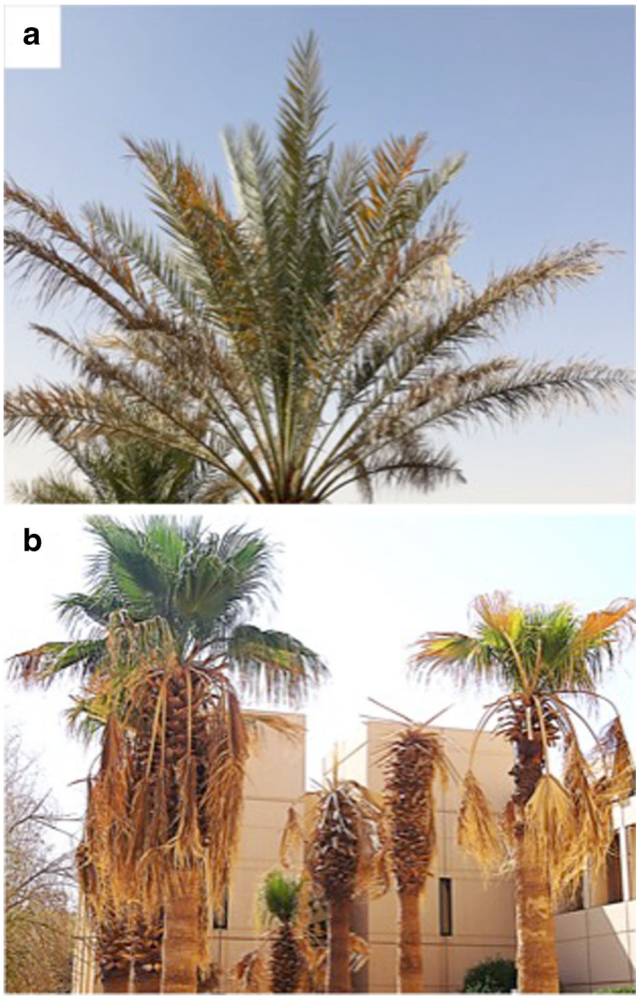

Fig. 2 Symptoms observed in palm trees in Saudi Arabia. **a** Date palm trees. **b** Mexican fan palm trees

gel stained with ethidium bromide and visualized using a transilluminator.

The amplicon generated from 14 date palms and four Mexican fan palms was purified using MEGA quick-spin Plus Fragment DNA Purification Kit (Intron Biotechnology, Korea), following the manufacturer's recommendations. PCR products generated from all the samples were directly sequenced with primers R16mF2/R16mR2 (Macrogen, Korea). To obtain the complete F2nR2 sequence, the amplicon generated from Suk-49 (Table 1), was cloned into the vector pGEM-T Easy (Promega, Madison, WI USA) according to the manufacturer's recommendations, then plasmids were transformed into chemically competent *E. coli* TOP10 (Invitrogen), and three clones per sample were sequenced using plasmid-targeted primers T7/SP6.

The eighteen sequences generated in this study were assembled using the Staden package (Bonfield and Whitwham 2010), and compared with reference sequences from GenBank using BLAST (http://www.ncbi.nlm.nih.gov). An alignment was constructed using the CLUSTAL W option of the MEGALIGN program for pairwise sequence similarity calculation among the amplified sequences from the palm trees.

The 16S F2nR2 sequences were analyzed using the *i*Phyclassifier (Zhao et al. 2009) to classify the group/ subgroup of the strains detected. The in silico RFLP pattern obtained from 16S rRNA-encoding DNA was compared with the RFLP pattern from previously identified strains and a similarity coefficient (F) was calculated for each pair as described (Wei et al. 2007).

Phylogenetic analysis was conducted for F2nR2 sequences using the Maximum-likelihood method with MEGA v6.0 (Tamura et al. 2013), and bootstrapping 1000 times to estimate stability. *Acholeplasma ladlawii* strain PG-8A (U14905) was used as outgroup to root the tree.

Results from BLAST showed that the ~1.2 kb F2nR2 sequences obtained from palm trees showed 98% - 99% nucleotide identity among them and with the reference strain for the '*Candidatus* Phytoplasma australasia' and subgroup 16SrII-D (GenBank accession no. Y10097). One sequence per date palm cultivar and/or species was deposited to GenBank under the accession numbers MH157916 for the phytoplasma strain affecting Sukkari date palms, MH157918 for the strain affecting Khalas date palms, MH155427 for the strain affecting Nabtat Ali date palms, MH155427 for the phytoplasma affecting male date palms, and MH157917 for the phytoplasma affecting the Mexican fan palm trees (Table 1).

The F2nR2 sequence generated through cloning was identified as 16SrII-D by RFLP digestion pattern (Fig. 3a), and the coefficient of similarity for this sequences was 1.00 with the reference strain of the '*Ca. P. australasia*' species (16SrII-D, GenBank accession no. Y10097) (Fig. 3a).

These results were confirmed through phylogenetic analysis. All the sequences generated in this study branched with phytoplasma strains belonging to subgroup 16SrII-D, including the reference strain for the species '*Ca. P. australasia*' (GenBank accession no. Y10097), and it is closely related to strains from the Middle Eastern countries. However, sequences amplified from palm trees grouped together and branched together by plant host (Fig. 3b).

In previous studies the use of palm trunk tissue has been suggested as a better material to extract the total DNA and to develop further molecular analysis (Oropeza et al. 2002), but it is not a written rule. In fact, in studies such as Vázquez-Euán et al. (2011), the authors sampled leaf and stem tissue indiscriminately in order to determine if the plants were affected or not by phytoplasmas. The decision of sampling leaf tissue may explain the low number of positive plants detected in the study in comparison with the total number of plants sampled for each variety and/or species (Table 1). For this reason, in further epidemiological analysis we will consider the analysis of leaf and trunk tissue of symptomatic plants for the detection of phytoplasma DNA. In this study the symptoms observed in the field were associated with phytoplasma

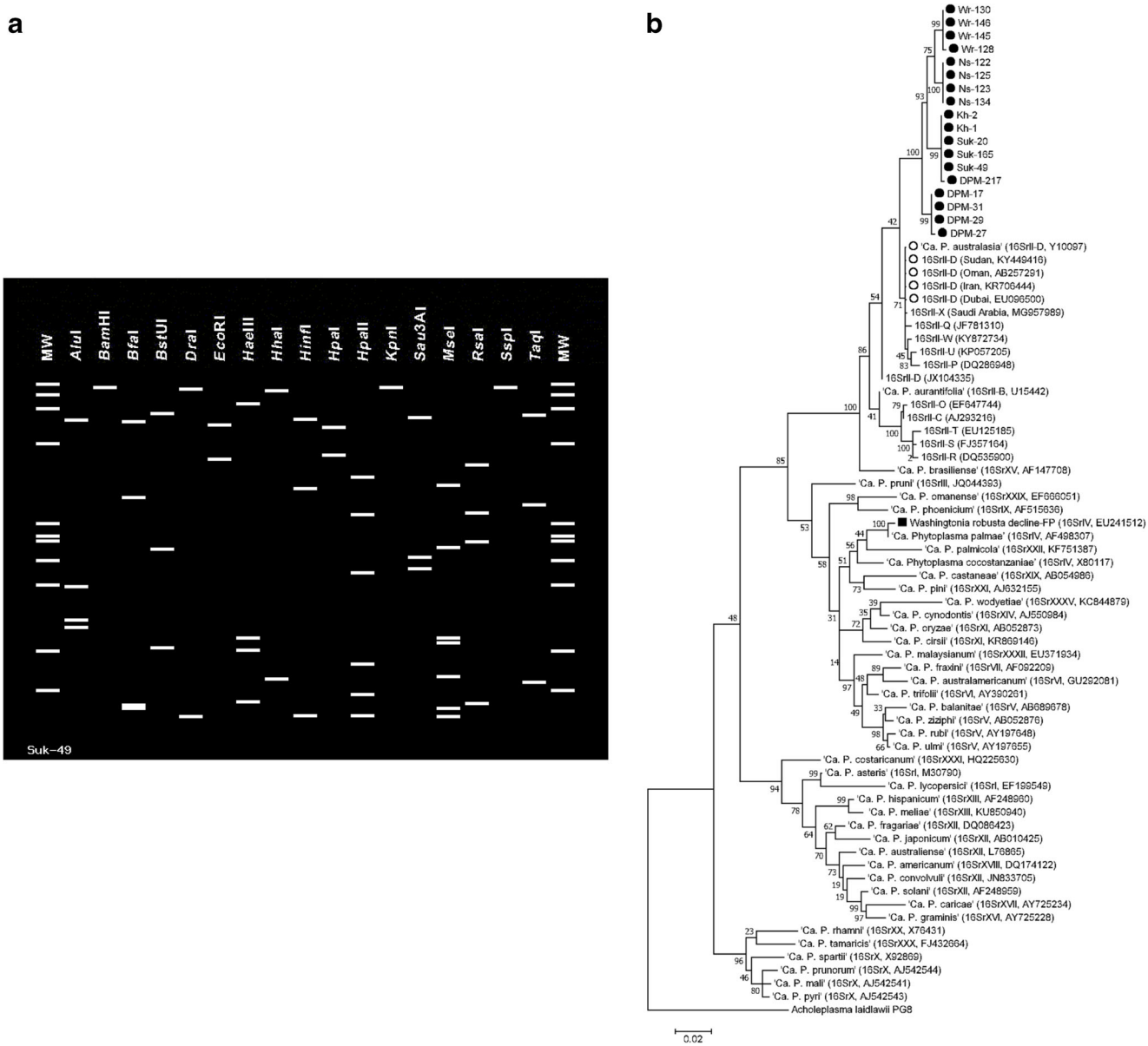

Fig. 3 Identification of the 16SrII phytoplasma strains affecting palm trees in Saudi Arabia. **a** Distinctive RFLP patterns obtained with *i*PhyClassifier from in silico digestion of 16S rRNA gene F2nR2 fragment from Suk-49 (MH157916). In the computer-simulated digestions, we used the set of 17 enzymes previously defined in the scheme of phytoplasma classification. Lanes labelled MW represent *HaeIII*-digested phage ϕX174 DNA. **b** Phylogenetic tree for 16SrII related phytoplasma strains reconstructed and the strains affecting palm trees in Saudi Arabia and reference strains for '*Candidatus* Phytoplasma' spp. described to date through the maximum likelihood method of the 16S rRNA gene sequences. Accession numbers are indicated in parentheses, and the strain acronym is given if applicable. *Acholeplasma laidlawii* PG8 was used as outgroup in the original tree. The phylogenetic tree was bootstrapped 1000 times to achieve reliability. Bar, 5 substitution in 1000 positions. Black circle is marking strains from Saudi Arabia, and not filled circles are making other 16SrII strains detected in Middle East. Black square is marking the strain previously detected in Florida affecting Mexican fan palms

through molecular methods for four date palm cultivars widely grown in the Qassin region, Saudi Arabia, and detected for first time worldwide the presence of 16SrII phytoplasma in symptomatic Mexican fan palm trees. While Mexican fan tree has been previously reported as a phytoplasma host, the phytoplasma type was 16SrIV (Harrison et al. 2008).

A high number of ornamental and economically important crop plants have been affected by 16SrII phytoplasma strains in Saudi Arabia (Omar 2016, 2017; Omar et al. 2017). Reason why the presence of 16SrII phytoplasma strains associated with palm trees is not surprising, and it is pointing to a fast spreading of the pathogen. It is very interesting that although the presence of phytoplasmas affecting date palms in Middle East has been reported (Fig. 1), in Sudan the strain affecting date palms belongs to the 16SrXIV group, in Egypt to 16SrI, and in Iran to 16SrVII (Cronjé et al. 2000; AlKhazindar 2014; Zamharir et al. 2016).

Although date palms have been reportedly affected by phytoplasmas in Saudi Arabia in several scientific meetings (Alhudaib et al. 2007, 2014), this is the first report of '*Ca. P. australasia*'- related strains affecting four different date palm tree varieties in the Qassim region in Saudi Arabia, and the first report of Mexican fan palm trees affected by 16SrII phytoplasma worldwide.

Much work remains to be done to characterize the phytoplasma strain affecting date palm and Mexican fan palm trees in Saudi Arabia, and most importantly to identify the vector or vectors responsible for the spread of 16SrII phytoplasma group in Central Saudi Arabia (Al-Qassim region), but this study is without a doubt a good first step.

Acknowledgments The authors thank Qassim University, represented by the Deanship of Scientific Research on the material support for this research under the number (1453-cavm-2016-1-12-S) during the academic year 1437AH/2016 AD.

References

Aleid SM, Al-Khayri JM, Al-Bahrany AM (2015) Date palm status and perspective in Saudi Arabia. In: Al-Khayri JM, Jain SM, Johnson DV (eds) Date palm genetic resources and utilization. Asia and Europe, vol 2. Springer, Dordrecht, pp 49–95

Alhudaib K, Arocha Y, Wilson M, Jones P (2007) "Al-Wijam", a new phytoplasma disease of date palm in Saudi Arabia. B Insectol 60: 285–286

Alhudaib K, Rezk A, Alsalah M (2014) Phytoplasma disease in date palm in Saudi Arabia. Proceedings of the Fifth International Date Palm conference pp. 313–320

AlKhazindar M (2014) Detection and molecular identification of Aster yellows Phytoplasma in date palm in Egypt. J Phytopathol 162:621–625

Bertaccini A (2007) Phytoplasmas: diversity, taxonomy, and epidemiology. Front Life Sci 12:673–689

Bonfield JK, Whitwham A (2010) Gap5 - editing the billion fragment sequence assembly. Bioinformatics 26:1699–1703

Cronjé P, Dabek AJ, Jones P, Tymon AM (2000) First report of a phytoplasma associated with a disease of date palms in North Africa. New Dis Rep 1:4

Deng SJ, Hiruki C (1991) Genetic relatedness between two nonculturable mycoplasma-like organisms revealed by nucleic acid hybridization and polymerase chain reaction. Phytopathology 81: 1475–1479

Harrison NA, Helmick EE, Elliott ML (2008) Lethal yellowing-type diseases of palms associated with phytoplasmas newly identified in Florida, USA. Ann Appl Biol 153:85–94. https://doi.org/10.1111/j.1744-7348.2008.00240.x

Jarzembowski P, Komorowska B, Ptaszek M, Faltyn A, Proćków J (2018) First report of '*Candidatus* Phytoplasma asteris' associated with witches' brooms on sharp-flowered rush (*Juncus acutiflorus*) in Poland. Plant Dis 102. https://doi.org/10.1094/PDIS-03-18-0383-PDN

Lee IM, Gundersen-Rindal DE, Davis RE, Bartoszyk IM (1998) Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences. Int J Syst Bacteriol 48:1153–1169

Lee IM, Davis RE, Gundersen-Rindal DE (2000) Phytoplasma: phytopathogenic mollicutes. Annu Rev Microbiol 54:221–255

Maixner M, Ahrens U, Seemüller E (1995) Detection of the German grapevine yellows (Vergilbungskrankheit) MLO in grapevine, alternative hosts and a vector by a specific PCR procedure. Eur J Plant Pathol 101:241–250

Ministry of Agriculture (2011) Twenty-fourth statistical yearbook. Total estimated of agricultural crops area and production in Saudi Arabia: estimated area and production of dates crop by region in Saudi Arabia. Department of Economic Studies and Statistics, Ministry of Agriculture, Riyadh, Saudi Arabia, pp 68–70

Omar AF (2016) Association of '*Candidatus* Phytoplasma cynodontis' with Bermuda grass white leaf disease and its new hosts in Qassim province, Saudi Arabia. J Plant Interact 11:101–107

Omar AF (2017) Detection and molecular characterization of phytoplasmas associated with vegetable and alfalfa crops in Qassim region. J Plant Interact 12:58–66

Omar AF, Pérez-López E, Al-Jamhan KM, Dumonceaux TJ (2017) First report of a new jojoba (*Simmondsia chinensis*) witches' broom disease in Saudi Arabia and its association with infection by a '*Candidatus* Phytoplasma australasiae' - related phytoplasma strain. Plant Dis 101:1540

Oropeza C, Cordova I, Narvaez M, Harrison N (2002) Palm trunk sampling for DNA extraction and Phytoplasma detection. University of Florida, Florida

Pérez-López E, Luna-Rodríguez M, Olivier CY, Dumonceaux TJ (2016) The underestimated diversity of phytoplasmas in Latin America. Int J Syst Evol Microbiol 66:492–513

Pérez-López E, Omar AF, Al-Jamhan KM, Dumonceaux TJ (2018) Molecular identification and characterization of the new 16SrIX-J and cpn60UT IX-J phytoplasma subgroup associated with chicory bushy stunt disease in Saudi Arabia. International Journal of Systematic and Evolutionary Microbiology. 192. https://doi.org/10.1099/ijsem.0.002530

Schneider B, Seemüller E, Smart CD, Kirkpatrick BC (1995) Phylogenetic classification of plant pathogenic mycoplasmalike organ- isms or phytoplasmas. In: Razin R, Tully JG (eds) Molecular and diagnostic procedures in Mycoplasmology, vol. 1: pp. 369–380. Academic Press, San Diego

Statistical Year Book. (2016). Ministry of agriculture, Vol. 52. Kingdom of Saudi Arabia. https://www.stats.gov.sa/en/877. Accessed November 2017

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729

Vázquez-Euán R, Harrison N, Narvaez M, Oropeza C (2011) Occurrence of a 16SrIV group phytoplasma not previously associated with palm species in Yucatan, Mexico. Plant Dis 95:256–262

Wei W, Davis RE, Lee IM, Zhao Y (2007) Computer-simulated RFLP analysis of 16S rRNA genes: identification of ten new phytoplasma groups. Int J Syst Bacteriol 57:1855–1867

Zamharir MG, Boshehri SMS, Khajehzadeh Y (2016) *Candidatus* phytoplasma fraxini related (16SrRNA-VII) strain associated with date yellows disease in Iran. Aust Plant Dis Notes 11:31

Zhao Y, Wei W, Lee I-M, Shao J, Suo X et al (2009) Construction of an interactive online phytoplasma classification tool, *i*PhyClassifier, and its application in analysis of the peach X-disease phytoplasma group (16SrIII). Int J Syst Evol Microbiol 59:2582–2593