

“Al-Wijam”, a new phytoplasma disease of date palm in Saudi Arabia

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Abstract

Date palm (*Phoenix dactylifera* L.) is one of the most important cash crops in Saudi Arabia, occupying 150,744 ha where 23 million trees produce 970,488 t of dates annually. The Eastern region is one of the most important in the country, growing date palm for centuries with more than 4 million trees located at Al-Hassa oasis. A disease called Al-Wijam has been found affecting date palm in Saudi Arabia. Main symptoms are leaf stunting, yellow streaking and a marked reduction in fruit and stalk size, which leads to failure in fruit production at harvest. Leaf samples with and without symptoms of Al-Wijam, and specimens of leafhoppers were collected in Al-Hassa oasis during 2003-2005. Total DNA was extracted from plant samples and batches of three insects and indexed by a nested PCR reaction with phytoplasma generic primers P1/P7-R16F2n/R2. PCR products were characterized by RFLP and direct sequencing. The 16S rDNA sequences were compared with those of other phytoplasmas in GenBank. Phytoplasma rDNA was amplified from 28/40 samples with symptoms of Al-Wijam and 16 insect batches. No PCR products were obtained from asymptomatic palms. RFLP patterns for all amplicons were identical for each enzyme when digested with *RsaI*, *HinfI*, *TaqI*, *HpaII*, *KpnI*, *DraI*, *HhaI* and *Sau3AI*. The 16S rDNA sequences of the phytoplasma identified in Al-Wijam affected date palm (DQ913090) and *Cicadulina bipunctata* (Melichar) (DQ913091) shared 100% similarity, and had 98% similarity to that of Aster yellows phytoplasma (AF322644) a member of the 16SrI, ‘*Candidatus* Phytoplasma asteris’ group. The identification of the phytoplasma associated and a putative vector are important contributions to our knowledge of the disease in the Al-Hassa oasis, Saudi Arabia.

Key words: ‘*Candidatus* Phytoplasma asteris’, *Cicadellidae*, date palm, *Phoenix dactylifera*, phytoplasma, Saudi Arabia

Introduction

‘Al-Wijam’ diseases of *Phoenix dactylifera* L. (date palm) was first reported as widespread in Al-Hassam (Saudi Arabia). Symptoms are stunting and yellow streaking of the leaves, with fruits and fruit stalks reduced in size (around 30%); intensity of symptoms varies with variety. A phytoplasma was suspected to cause the disease following histopathology and antibiotic therapy studies. El-Zayat *et al.*, (2000), reported their finding of a phytoplasma similar to that causing lethal yellowing of coconut palm in Florida.

Phytoplasmas have been associated with diseases in date palm such as white tip die-back and slow decline in North Africa (Cronjé *et al.*, 2000a; 2000b), yellowing in Kuwait (Al-Awadhi *et al.*, 2002), and lethal decline in Texas (Harrison *et al.*, 2002). Date palm lethal decline (LD) is associated with a phytoplasma belonging to the 16SrIV Group, subgroup D. Other phytoplasmas of the 16SrIV group, a diverse group of phytoplasmas, cause lethal diseases of coconut and other palms in Central America, the Caribbean, east and west Africa (Harrison and Jones, 2003). *Myndus crudus* (van Duzee) is considered as the vector of the phytoplasma which causes coconut lethal yellowing, a member of the 16SrIV, subgroup A in Jamaica and Florida, however, phytoplasmas in general are vectored by Auchenorrhyncha insects: leafhoppers, planthoppers and psyllids.

We report here on the identification and molecular characterization of a phytoplasma associated with “Al-Wijam” in Al-Hassa and the identification of a putative vector of the disease.

Materials and methods

Leaf samples from 40 date palms with and without “Al-Wijam” symptoms and 60 specimens of *Cicadellidae*: 42 of *Cicadulina bipunctata* (Melichar) and 18 of *Asymmetrasca decedens* (Paoli) were collected from a survey in Al-Hassa oasis during 2003-2005. Samples were indexed by a nested PCR with phytoplasma 16S rDNA primers P1/P7-R16F2n/R2. PCR products were digested with restriction endonucleases *RsaI*, *HinfI*, *TaqI*, *HpaII*, *KpnI*, *DraI*, *HhaI* and *Sau3AI* (SIGMA, UK). P1/P7 PCR products were purified and sequenced by the Sequencing Service, School of Life Sciences, University of Dundee, UK (<http://www.dnaseq.co.uk>). The 16S rDNA sequences of phytoplasmas identified were compared with others in Genbank. Sequences were aligned and a phylogenetic tree constructed (MEGA version 3.1) using 1000 bootstrap datasets and *Acholeplasma laidlawii* as the outgroup to root the tree.

Results

Newly opened leaves and spathes became stunted and shorter than healthy ones. Stunting and yellowing increase through the years and yellow streaks appear on the petioles. Fruits and fruit stalk were reduced in size by 36-40% in different varieties. In the advanced stages, there was significant stunting and yellowing depending on the variety, until the palm died. Figure 1 shows characteristic symptoms of “Al-Wijam” disease observed in



Figure 1. Symptoms of stunted dates and leaf rachis yellow streaks from an “Al-Wijam” affected palm.

the Al-Hassa oasis. Phytoplasma rDNA was amplified from 28 date palm leaf samples showing typical Al-Wijam symptoms, and 16 leafhopper batches: 12 of *C. bipunctata* and 4 of *A. decedens*. No PCR products were obtained from apparently healthy palms. Except for *TaqI*, the RFLP patterns for all PCR amplicons from date palm and *C. bipunctata* were identical with *RsaI*, *HinfI*, *HpaII*, *HhaI*, *KpnI*, *Sau3AI*, and *DraI* enzymes to that of the reference control EAY, suggesting these phytoplasmas belong to 16SrI group, ‘*Ca. P. asteris*’. Phytoplasma 16S rDNA sequences from date palm and *C. bipunctata* were 100% identical and shared 96% identity with 16S rDNA amplified from *A. decedens* phytoplasma. Phylogenetic analysis showed that phytoplasmas identified in date palm and *C. bipunctata* belonged to the 16SrI group, ‘*Ca. P. asteris*’ cluster, which supported the RFLP results. However, the phytoplasma identified in *A. decedens* was placed in the phylogenetic branch of phytoplasmas belonging to the 16SrXII-A (Stolbur) group.

Discussion

The amplification of phytoplasma DNA from 28/40 date palm plants showing typical “Al-Wijam” symptoms demonstrates the association of this pathogen with the disease in Al-Hassa oasis as previously described (El-Zayat *et al.*, 2000). The phytoplasma identified in date palm and *C. bipunctata* showed RFLP profiles that clearly placed them in the 16SrI group, ‘*Ca. P. asteris*’, which is supported by the sequencing and phylogenetic data. The phytoplasma identified in *A. decedens* belongs to 16SrXII-A group and no evidence of this phytoplasma was found in any date palm samples. This is the first report of a 16SrI group phytoplasma being associated with a disease in date or any other palm species. The well documented coconut lethal yellowing diseases are associated with phytoplasmas of the 16SrIV group and are found in southern USA, central America, the Caribbean and east and west Africa. Phytoplasmas from

g. 16SrXI, ‘*Ca. P. oryzae*’ and 16SrXII groups have been reported for Kalimantan wilt in Indonesia.

‘*Ca. P. asteris*’ is the only phytoplasma group distributed worldwide and the most diverse in its plant and insect hosts. It has been found in sandalwood in India, and safflower and carrot in Israel. Results of our experiments extend this to date palms and *C. bipunctata*. The 16SrXII-A group phytoplasma was only detected in *A. decedens* and not in “Al-Wijam” affected date palms, suggesting that *A. decedens* may be an occasional feeder or visitor to the field, and could be associated with a disease of other plants rather than that of date palm. The 16S rDNA of the phytoplasma identified in *C. bipunctata* captured from “Al-Wijam” affected date palms in Al-Hassa oasis, has shown a 100% identity with that of the date palm, and accordingly it has been identified as a putative vector of “Al-Wijam” disease. Results are an important contribution to the knowledge on the biodiversity of phytoplasmas associated with “Al-Wijam” disease in the region and help us devise sustainable control strategies.

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