Identification and Molecular Characterization of a Phytoplasma Associated with Al-Wijam Disease of Date Palm in Saudi Arabia

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Abstract

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Date palm (*Phoenix dactylifera* L.) has been affected by a disease called Al-Wijam 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. A lethal yellow phytoplasma was identified in Al-Wijam infected date palms growing in the Al-Hassa oasis, Eastern region. More than 30 leaf samples with and without Al-Wijam symptoms and 60 leafhopper samples were collected in a survey in Al-Hassa oasis during 2003-2005. Total DNA was extracted from plants and batches of three insects and indexed by a nested PCR reaction with phytoplasma generic primers P1/P7-R16F2n/R16R2. PCR products were characterized by RFLP and direct sequencing. The 16S rDNA sequences were compared with those of other reference phytoplasmas in the GenBank. Phytoplasma rDNA was amplified from 28 Al-Wijam samples and 16 insect batches. No PCR products were obtained from healthy palms. RFLP patterns for all PCR amplifications were identical with *Rsa*I, *Hinf*I, *Taq*I, *Hpa*II, *Kpn*I, *Dra*I, *Hha*I and *Sau3A*I enzymes. The 16S rDNA sequences of the phytoplasma identified in date palm (DQ913090) and *Cicadulina bipunctata* (Melichar) (DQ913091) were 100% identical in between and 98% with that of Aster yellows phytoplasma (AF322644) from 16SrI, *Candidatus* Phytoplasma asteris group. This is the first report of a *Ca.* P. asteris phytoplasma associated with a disease in date palm in Saudi Arabia, and the identification of a potential vector of Al-Wijam disease.

Keywords: Aster yellow, *Cicadellidae*, Date palm, *Phoenix dactylifera*, Phytoplasma, Saudi Arabia.

Introduction

Date palm, (*Phoenix dactylifera* L.), is one of the most important cash crops in Saudi Arabia. The area planted with date palm is 150,744 ha where 23 million trees produce 970,488 t of dates annually (5). The Eastern region of Saudi Arabia 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.

"Al-Wijam" disease was first reported as widespread in Al-Hassa (18). Symptoms are characterized by a stunting and yellow streaking of the leaves, with fruits and fruit stalks reduced in size (around 30%) and varies based on varieties.

Association of viral, fungal and nematode pathogens with the disease was not consistent (1, 2, 18). A phytoplasma pathogen was suspected to cause Al-Wijam-affected palms following histopathology and antibiotic therapy studies (2). This was further supported by El-Zayat *et al.* (19), who reported a phytoplasma similar to that causing lethal yellow of coconut palm in Florida.

Phytoplasmas are prokaryote organisms of the class *Mollicutes*, which affect more than 700 plant species from tropical to temperate countries (25). They cannot be cultivated *in vitro* and are mainly transmitted by leafhopper or planthoppers of the order *Hemiptera* (30). Phytoplasmas have been associated with diseases in date palm such as white tip die-back (WTD) and Slow decline in Sudan in North Africa (11, 12), yellowing in Kuwait (3), and lethal decline in Texas (23).

Lethal yellowing (LY) and lethal decline (LD) are lethal coconut phytoplasma diseases that are spread in many countries, including these of West and East Africa, including Mozambique and Kenya (33), Tanzania, Ghana (41, 42); the Caribbean and Central America, including Belize (20), Jamaica (44), Honduras (35), Mexico (7, 10), Guatemala (31) and Cuba (29).

Myndus crudus (Van Duzee) (24) is known as the vector of LY (24) in Jamaica and Florida, however, there are reports of other *Hemiptera* species found associated with either LY or LD diseases (15, 33)

The phytoplasma associated with Al-Wijam in Saudi Arabia (19) was found to be similar to that causing lethal yellows in Florida, however, no attempts were made earlier to identify the potential *Hemiptera* vectors out of the disease agent.

In this paper, we report the association of a phytoplasma of 16SrI group, *Ca*. P. asteris, with Al-Wijam in Al-Hassa, Saudi Arabia, and the identification of a cicadellid candidate as the disease vector.

Materials and Methods

Sample collection

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. DNA of the reference phytoplasma strain of European aster yellows (EAY, 16SrI) from the

phytoplasma collection at Rothamsted Research, UK, was used for direct comparison of restriction fragment length polymorphism (RFLP) patterns.

PCR and RFLP analysis

DNA was extracted from leaf tissue and batches of three insects by the method of Doyle & Doyle, (17). Aliquots of final DNA preparations were used as template for a nested PCR (nPCR) assay with phytoplasma 16S rDNA primers P1 (14) and P7 (37) using a programmable thermocycler (TECHNE, TC-412) following previous described PCR conditions (6). Nested PCR products were digested with restriction endonucleases *Rsa*I, *Hinf*I, *Taq*I, *Hpa*II, *Kpn*I, *Dra*I, *Hha*I and *Sau3A*I (SIGMA, UK) according to manufacturer's instructions. Digestion products were electrophoresed in 1.5% agarose gels, and visualized after staining with ethidium bromide by UV transillumination. RFLP patterns were compared with previously published patterns (27, 28).

16S rDNA sequencing and phylogenetic analysis

Phytoplasma rDNA amplified by PCR using the primer pair P1/P7 was purified on spin columns (QIAquick gel extraction kit; QIAGEN). The PCR products were sequenced in both directions using primer pair P1/P7 by the Sequencing Service, School of Life Sciences, University of Dundee, UK (http:// www.dnaseq.co.uk), with Applied Biosystems Big-Dye version 3.1 chemistry on an Applied Biosystems model 3730 automated capillary DNA sequencer. The 16S rDNA sequences of phytoplasmas identified in our study were compared with others in Genbank (Table 1) by BLAST (4). Sequences were aligned and a phylogenetic tree constructed by the program MEGA version 3.1 (26) using 1000 bootstrap datasets to support the branch values. *Acholeplasma palmae* and *A. laidlawii* were used as the outgroups to root the tree.

Results

Sample collection

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 the Al-Hassa oasis.

PCR and RFLP analysis

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*. Samples were representative with the 1250 bp expected second round PCR amplification (Figure 2). No PCR products were obtained from apparently healthy palms. Except for *Taq*I, the RFLP patterns for all PCR amplicons from date palm and *C. bipunctata* were identical with *Rsa*I, *Hinf*I, *Hpa*II, *Hha*I, *Kpn*I, *Sau3A*I, and *Dra*I enzymes (Figures 3A and 3B) to that of the reference control EAY, suggesting these phytoplasmas belong to 16SrI group, *Ca.* P. asteris. In the case of the phytoplasma identified in *A. decedens*, it showed differences when compared to the reference control with *Hha*I and *Hinf*I enzymes.

16S rDNA sequencing and phylogenetic analysis

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.

Sequence similarity between the phytoplasma 16S rDNAs from date palm and *C. bipunctata* to those of previously characterized phytoplasma strains *Ca. P. asteris*, AAY, AshWB, BD, OY was 98%; 95% to *Ca. P. australiense*; 94% to MPV and *Ca. P. japonicum*; 92% to *Ca. P. pyri*, 90% to FCoLY and LDT and less than 89% with other known phytoplasma group, representatives. Sequence similarity of 16S rDNA amplified from *A. decedens* phytoplasma to those of StolSer1, StolSer2, VK and Stolbur was 99%; 98% to *Ca. P. graminis*; 97% to *Ca. P. caricae* and *Ca. P. americanum*; 96% to *Ca. P. fragariae*, 95% to *Ca. P. australiense*, 94% to *Ca. P. japonicum* and MPV, 92% to *Ca. P. pyri*; 90% to FCoLY; 89% to LDT and less than 88% with the rest of phytoplasma groups.

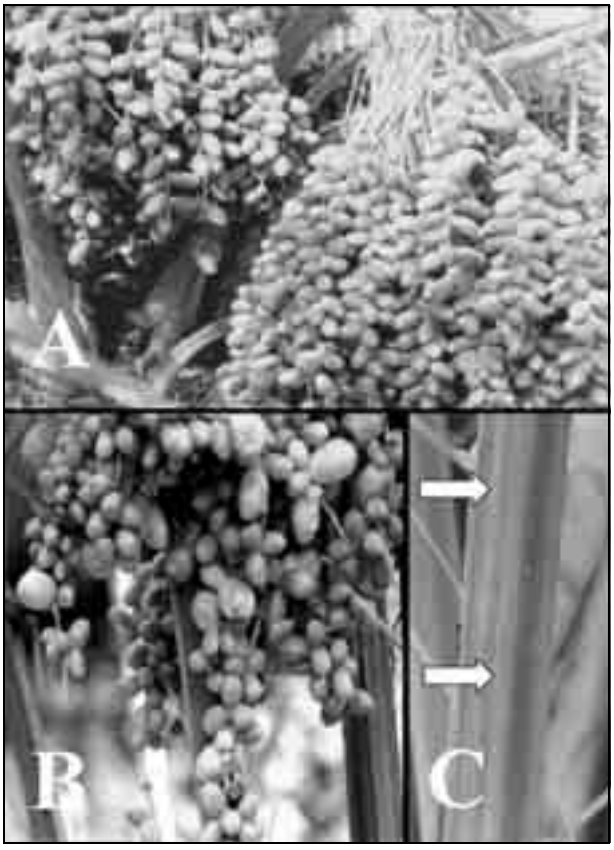

Figure 1. Date in kimir stage, (A) healthy date palm fruit, (B) date from a Al-Wijam affected palm showing stunting symptom, (C) yellow streak on the rachis of leaf from a Al-Wijam affected palm (indicated by arrows).

Table 1. Acronyms GenBank accession numbers of phytoplasma 16S rDNA sequences used to construct the phylogenetic tree.

Acronym	Phytoplasma strain designation	RFLP Group	Accession number
Ca. P. trifolii	*'Candidatus* Phytoplasma trifolii'	16SrVI	AY390261
Ca. P. fraxini	*'Candidatus* Phytoplasma fraxini'	16SrVII	AF092209
Ca. P. ulmi	*'Candidatus* Phvtoplasma ulmi'	16SrV	AFI22910
FD	'Flayescence donée'	16SrV	AF176319
Ca. P. ziziphi	*'Candidatus* Phytoplasma ziziphi'	16SrV	AY072722
LWB	'Loofah witches' broom'	16SrVIII	L33764
Ca. P. cynodontis	*'Candidatus* Phytoplasma cynodotis'	16SrXIV	AJ550984
SCWL	'Sugarcane white leaf'	16SrXI	X76432
Ca. P. oryzae	*'Candidatus* Phytoplasma oryzae'	16SrXI	D12581
FCoLY	'Coconut yellows'	16SrIV	U18747
LDT	'Coconut lethal decline'	16SrIV	X80117
Ca. P. pini	*'Candidatus* Phytoplasma pini'	16SrIV	AJ310849
Ca. P. castaneae	*'Candidatus* Phytoplasma castaneae'	16SrIV	AB054986
Ca. P. phoenicium	*'Candidatus* Phytoplasma phoenicium'	16SrIX	AF515837
PPWB	'Pigeon pea witche's broom'	16SrIX	U18763
VWB	'Vaccinia witche's broom'	16SrIII	X76430
WX	'Western X-disease'	16SrIII	L04682
PYC	'Papaya yellow crinkle'	16SrII	YI0097
PWB	'Peanut witche's broom'	16SrII	L33765
PM	'Papaya mosaic'	16SrIl	YI0096
Ca. P. aurantifolia	*'Candidatus* Phytoplasma aurantifolia'	16SrII	U15442
Ca. P. brasiliense	*'Candidatus* Phytoplasma brasiliense'	16SrXV	AF147708
Ca. P. mali	*'Candidatus* Phytoplasma mali'	16SrX	AJ543541
Ca. P. pyri	*'Candidatus* Phytoplasma pyri'	16SrX	AJ542543
Ca. P. prunorum	*'Candidatus* Phytoplasma prunorum'	l6SrX	AJ542544
Ca. P. spartii	*'Candidatus* Phytoplasma spartii'	16SrX	X92869
Ca. P. allocasuarinae allocasuarinae	*'Candidatus* Phytoplasma allocasuarinae'	16SrX	AY13523
Ca. P. rhamni	*'Candidatus* Phvtoolasma rhamni'	16SrX	X76431
Ca. P. graminis	*'Candidatus* Phytoplasma gaminis'	16SrXVI	AY725228
Ca. P. caricae	*'Candidatus* Phytoplasma caricae'	16SrXVII	AY725234
StolSer1	'Stolbur, Serbia l'	16SrXII	X76427
Canuum	'Stolbur transmitted from C. anuum'	16SrXII	X76427
StolSer2	'Stolbur, Serbia l'	16SrXII	DQ222972
STOL	'Stolbur, Italy'	16SrXII	AF248959
Ca. P. australiense	*'Candidatus* Phvtoplasma australiense'	16SrXII	L76865
SGP	'Strawberry green petal'	16SrXII	AJ243044
SLY	'Strawberry lethal yellows'	16SrXII	AJ243045
PDB	'Paoaya dieback'	16SrXII	Y10095
PYL1	*'Phormium* yellow leaf'	16SrXII	U43569
VK	*'V vinifera* phvtoplasma'	16SrXII	X76428
Ca. P. americanum	*'Candidatus* Phytoplasma americanum'	16SrXII	DOl74122
Ca. P. fragariae	*'Candida/us* Phytoplasma fragariae'	16SrXII	DQ86423
Asymmetrasca	*'A. decedens* phvtoplasma'	16SrXII	DQ913092
MPV	'Periwinkle yirescence'	16SrXIII	AF248960
AAY	'American aster yellows'	16SrI	X68373
Ca. P. asteris	*'Candidatus* Phytoplasma asteris'	16SrI	M30790
AshWB	'Ash witches' broom'	16SrI	AY568302
BD	'Barley deformation'	16SrI	AY734453
OY	'Onion yellows'	16SrI	D12569
Ca. P. japonicum	*'Candidatus* Phytoplasma japonicum'	16SrI	AB0I0425
Date Palm	'Date palm phytoplasma'	16SrI	DQ913090
Cicadulina	' *'Candidatus biounctata* phvtoplasma'	16SrI	DQ913091
A. oalmae	*Acholeplasma palmae*	-	L33734
A. laidlawii	*Acholeplasma laidlawii*	-	M23932

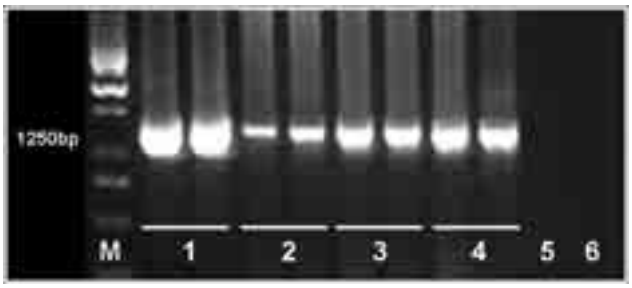

Figure 2. Nested-PCR amplifications with R16F2n/R16R2 primers from Al-Wijam affected date palm and *Cicadellids* associated. Lane 1: EAY reference positive control. Lane 2: *A. decedens*. Lane 3: *C. bipunctata*. Lane 4: leaflet symptomatic. Lane 5: healthy leaflet. Lane 6: water as negative control. Lane M: 1Kb marker (Promega).

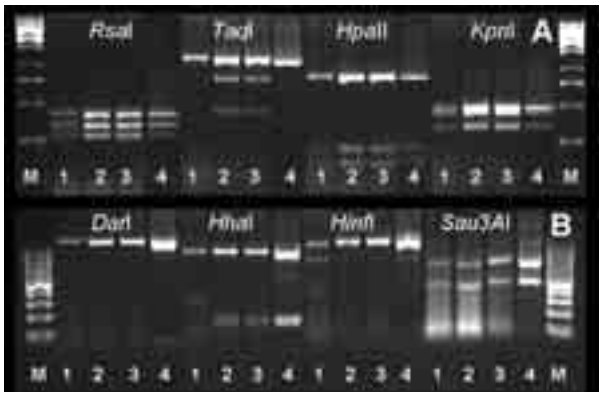

Figure 3. A, B. RFLP analysis of 16S rDNAs amplified by nPCRs with A. *Rsa*I, *Taq*I, *Hpa*II, *Kpn*I, B. *Dra*I, *Hha*I, *Hinf*I and *Sau3A*I enzymes. Lane 1: *A. decedens*. Lane 2: *C. bipunctata*. Lane 3: leaflet symptomatic. Lane 4: EAY reference positive control. Lane M: 100 bp ladder (MBI Fermentas, Lithuania).

Phylogenetic analysis of 52 phytoplasmas, *A. palmae* and *A. laidlawii* produced the consensus tree illustrated in Figure 4. Phytoplasmas identified in date palm and *C. bipunctata* were embraced in the 16SrI group, *Ca*. P. asteris cluster, which was in agreement with grouping according to RFLP results. However, the phytoplasma identified in *A. decedens* was placed in the phylogenetic branch of phytoplasmas belonging to the 16SrXII group, *Ca*. *P. solani*.

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 (19).

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 in agreement with sequencing and phylogenetic results (Figure 4). However, sequence and phylogeny based on the analysis of the 16S rDNA of the phytoplasma identified in *A. decedens*, even sharing similar RFLP profiles with those of the EAY reference control, clearly indicated that this phytoplasma belongs to 16Sr XII group, *Ca*. *P. solani*.

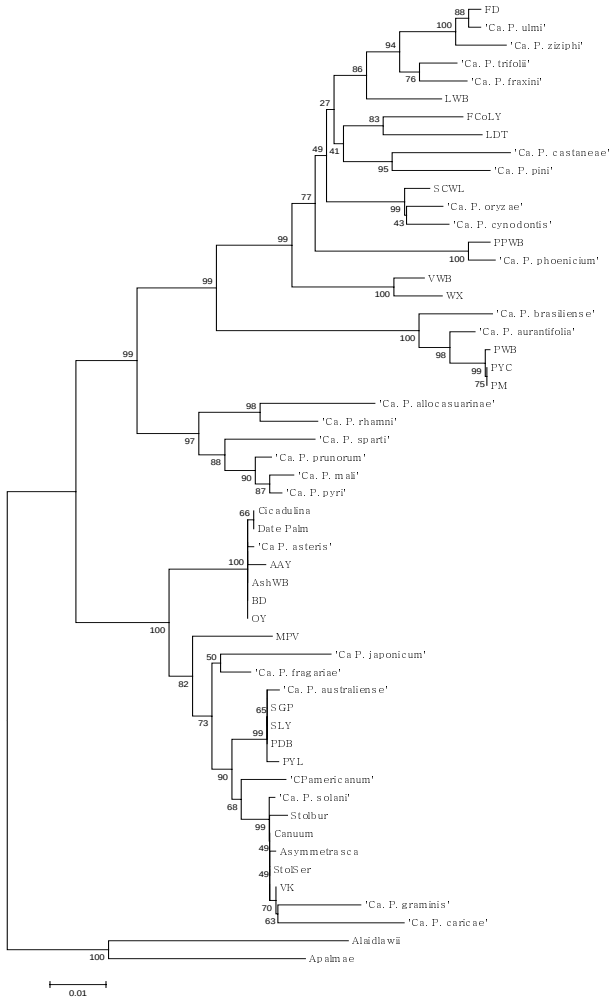

Figure 4. Phylogenetic tree of 16S rDNA sequences constructed by neighbour-joining method showing relationships between phytoplasmas detected in Date palm, *C. bipunctata,* and *A. decedens* with the rest of GenBank reference phytoplasmas analyzed. Numbers above the branches are bootstrap values obtained for 1000 replicates. The branch lengths are proportional to the number of inferred characters state transformation. Abbreviations of phytoplasmas are defined in Table 1.

RFLP analysis has proved to be a simple and rapid tool for the preliminary classification and identification of unknown phytoplasmas in a relatively short time (27, 38). However, it is possible that phytoplasmas belonging to different groups share similar RFLP patterns, whereas, isolates within a given group exhibit distinct RFLP profiles, thus, phytoplasma groups classified on the basis of this method are not always consistent with phylogenetic

grouping (27). Therefore, the sequence of the 16S rDNA gene reflects phylogenetic distance more accurately than restriction patterns which depend on significantly fewer genetic characters (38).

LY and LD type-diseases have been associated with phytoplasmas of 16SrIV group, *Ca. P. palmae* in East Africa (13, 16, 32, 41, 42), North America (22, 23), and Central America and the Caribbean (10, 29, 35, 44). However, other phytoplasma groups, different to 16SrIV, have been associated with phytoplasma diseases in coconut e.g. 16SrXI, *Ca. P. oryzae* and 16SrXII, *Ca. P. solani* with Kalimantan wilt in coconut in Indonesia (43). Recently in India, a possible new phytoplasma has been associated with Kerala wilt disease of coconut palms (39).

The 16SrI, *Ca. P. asteris* is the only phytoplasma group distributed worldwide and the most diverse in plant and insect hosts (28). It has been found in sandalwood in India (36), and safflower and carrot in Israel (34, 36). Results of our experiments extend this to date palms and putative associated species of *C. bipunctata* of Al-Hassa oasis.

Seemüller *et al*. (38) considered phytoplasmas as belonging to the same group if they showed 97% or more similarity between their sequences, while values less than 95% would place phytoplasmas in different groups or subgroups. El-Zayat *et al.* (19), identified a phytoplasma associated with Al-Wijam disease based on an 87% identity of 16S rDNA with that of the Florida lethal yellows phytoplasma; however, the amount of Al-Wijam samples where the phytoplasma was detected was not specified, and additionally it was not found in Al-Wijam affected date palms collected from our surveys.

The phylogenetic tree (Figure 4) reveals that the phytoplasma identified in date palm and *C. bipunctata* are phylogenetically distant from phytoplasmas of 16SrIV group. This phytoplasma showed a 98% identity of its 16S rDNA with that causing aster yellows (AF322644) of 16SrI group, and phylogeny results clearly support that it is a member of this group. This is the first report of the identification of the 16SrI phytoplasma in date palm, and contributes to the knowledge on the biodiversity of phytoplasmas associated with Al-Wijam disease in the region. The fact that the 16SrXII phytoplasma was only detected in *A. decedens* and not in Al-Wijam affected date palms suggests that *A. decedens* may be an occasional feeder or visitor in the field, and is possibly associated with a disease in other plant host different from Al-Wijam in date palm.

The cixiid *M. crudus* was identified as the primary vector of LY in Florida (24), however, there have been few studies showing other *Hemiptera* species as candidates to vector LY or LD type-diseases. PCR and sequence analysis of the 16S rDNA have identified *Diastrombus mkurangai* and *Meenoplus* spp., (*Delphacidae*) as vector candidates of LD in Tanzania (33). *Sophonia* sp. and *Idioscopus clypealis (Cicadellidae)*, and *Nisia nervosa* (Meenoplidae) have been proposed as the potential vectors for Coconut Kalimantan Wilt in Indonesia (43).

Leafhoppers, planthoppers and psyllids are the only known insect vectors of phytoplasma diseases (9, 30). Nowadays, research on phytoplasma has focused on the study of phytoplasma-insect vector interactions for further development of strategies to control phytoplasma diseases (8, 40) as it appears that vector control is the most effective if not the only way to stop the spread of phytoplasma diseases (21).

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 the potential vector of Al-Wijam disease. This is an important contribution for the future control of the disease in Al-Hassa, and points to *C. bipunctata* as the target for future transmission studies to confirm this species as the vector of the phytoplasma causing Al-Wijam disease in Saudi Arabia.

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الملخص

الهديب، خالد عبد الله، يايما اروشا، م. ولسن وفيل جونس. 2007. الوصف والتشخيص الجزيئي للفيتوبلازما من مجموعة الاستر المصاحبة لنخيل التمر في المملكة العربية السعودية. مجلة وقاية النبات العربية. 25: 116−122.

يصاب نخيل التمر (*Phoenix dactylifera* L.) بواحة الاحساء بالمملكة العربية السعودية بمرض يدعى الوجام. وتتلخص أعراضه باصفرار مخطط على أنصال الأوراق مترافق مع تقزم في الأوراق الجديدة مما يؤدي إلى انخفاض في انتاج التمر في المراحل المتقدمة. قام الزيات واخرون في عام 2002 بوصف المرض على انه فيتوبلازما من نوع الاصفرار المميت (Lethal Yellow). وتعتبر حشرة نطاط الاوراق (leafhopper) هى الحشرة الناقلة للفيتوبلازما ولكن حتى الان لم تدرس هذه الحشرة. تم جمع أكثر من 30 عينة أوراق نخيل مصابة وغير مصابة بالوجام مع 60 حشرة من المناطق المصابة بواحة الاحساء خلال 2003−2005. وقد تم استخلاص الحمض النووى (DNA) من العينات النباتية والحشرات السابقة وتطبيق تقنية تفاعل السلسلة المتبلمرة (nested PCR) عليها وذلك باستخدام بادئات خاصه للتعرف على وجود الفيتوبلازما (P1/P7-R16F2n/R16R2). تم اختبار الناتج من التفاعل السابق بـــ RFLP وفك شيفرة الحمض النووي لـــ 16S rDNA ومقارنتها بالفيتوبلازما الموجوده في بنك الجينات. تم التعرف على وجود الفيتوبلازما في 18 عينة من أوراق النخيل و14 عينة تم اختبرها من الحشرات. ولم يكن هناك تفاعل ايجابي مع بعض الأوراق المصابة بنفس الأعراض. وقد كان هناك تطابقا واضحاً في اختبار RFLP باستخدام الأنزيمات *Rsa*I، *Alu*I، *Hinf*I، *Taq*I، *Hpa*II، *Kpn*I، *Dra*I، *Hha*I و *Sau3A*I، وقد كانت شيفرة الحمض النووى لـــ 16S rDNA للفيتوبلازما متطابقة

100% مابين شيفرة النخيل (DQ913090) وشيفرة الحشرة الناقلة *Cicadulina bipunctata* (DQ913091)، و 98% مع اصفرار الاستر AF322644 من مجموعة 16SrI, *Candidatus* Phytoplasma asteris. ويعتبر هذا التقرير الأول في دراسة مرض الفيتوبلازما التابع لهذه المجموعة كذلك الأول في تشخيص وتعريف الحشرة الناقلة للفيتوبلازما والتى تصيب النخيل التمر بالمملكة العربية السعودية. وهذا سوف يساعد في المستقبل في عمل دراسات حول انتقال الفيتوبلازما لنخيل التمر.

كلمات مفتاحية: فيتوبلازما، نخيل التمر، نطاط الأوراق، *Cicadulina bipunctata*، المملكة العربية السعودية

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References

1. **Abdusalam, K., M. Najeeb, M. Rezk and M. Abdel-Megeed.** 1992. Survey of certain fungi associated with Wijamed date palm tree in Al-Hassa Oasis of Saudi Arabia. Annals of Agricultural Science (Ain Shams University, Egypt), 37: 603-611.
2. **Abdusalam, K., M. Abdel-Megeed, M. Rezk and M. Najeeb.** 1993. The influence of oxytetracycline on Wijamed date palm tree. Annals of Agricultural Science (Ain Shams University, Egypt), 38: 301-309.
3. **Al-Awadhi, H., A. Hanif, P. Suleman and M. Montasser**. 2002. Molecular and microscopical detection phytoplasma associated with yellowing disease of date palm *Phoenix dactylifera* L. in Kuwait. Kuwait Journal of Science and Engineering, 2: 29.
4. **Altschul, S., W. Gish, W. Miller, E. Meyers and D. Lipman.** 1990. Basic local alignment search too. Journal of Molecular Biology, 215: 403-10.
5. **Anonymous,** 2006. 19th Statistic Book. Ministry of Agricultural, Saudi Arabia. On-line [http://www.moa.gov.sa:82/indicators/st19_table/statc19.htm] 10/10/2006.
6. **Arocha, Y., M. López, B. Pinol, M. Fernández, B. Picornell, R. Almeida, I. Palenzuela, M. Wilson and P. Jones.** 2005. '*Candidatus* Phytoplasma graminis' and '*Candidatus* Phytoplasma caricae', two novel phytoplasmas associated with diseases of sugarcane, weeds and papaya in Cuba. International Journal of Systematic and Evolutionary Microbiology, 55: 2451-63.
7. **Ashburner, G., I. Córdova and N. Oropeza.** 1996. First report of coconut lethal yellowing disease in Honduras. Plant Disease, 80: 960.
8. **Bai, X., J. Zhang, A. Ewing, S. Miller, A. Radeck, D. Shevchenko, K. Tsukerman, T. Walunas, A. Lapidus, J. Campbell and S. Hogenhout.** 2006. Living with genome instability: the adaptation of phytoplasmas to diverse environments of their insect and plant hosts. Journal of Bacteriology, 188(10): 3682-96.
9. **Carraro, L., N. Loi and P. Ermacora.** 2001. Transmission characteristics of the European stone fruit yellows phytoplasma and its vector *Cacopaylla pruni*. European Journal of Plant Pathology, 107: 695-700.
10. **Córdova, I., C. Oropeza, H. Almeyda and N. Harrison.** 2000. First report of a phytoplasma-associated leaf yellowing syndrome of 'Palma Jipi' (*Carloduvica palmate*) Plant Southern Mexico. Plant Disease, 84: 807.
11. **Cronjé, P., A. Dabek, P. Jones and A. Tymon.** 2000a. First report of a phytoplasma associated with disease of date palms in North Africa. Plant Pathology, 49: 801.
12. **Cronjé, P., A. Dabek, P. Jones and A. Tymon.** 2000b. Slow decline: a new disease of mature date palm in North Africa associated with a phytoplasma. Plant Pathology, 49: 804.
13. **de Almeida, V., S. Fabre and M. Dollet.** 2006. Genetic variability among isolates of coconut lethal yellowing diseases determined by heteroduplex mobility assay. International Organization for Mycoplasmaology, 16th International Congress Cambridge, 70: 55.
14. **Deng, S. and C. Hiruki.** 1991. Amplification of 16S rDNA genes from culturable and non culturable mollicutes. Journal of Microbiological Methods, 14: 58-61.
15. **Dery, S.K., Y.P. N'Cho, A. Sangare and E.D. Arkhurst.** 1997. Cape St. Paul Wilt Disease resistance screening and prospects for rehabilitating the coconut industry in Ghana. Pages 147-152. In: Proceedings of an International Workshop on Lethal Yellowing-Like diseases of coconut, Elmina, Ghana, November 1995. S.J. Eden-Green and F. Ofori (eds.). Natural Resources Institute, Chatham, UK.
16. **Dollet, M., S. Fabre, F. Pilet, R. Quaicoe, J. Mugini and L. Rassaby.** 2006. Variability and diagnosis of coconut lethal yellowing in Africa. International Organization for Mycoplasmaology, 16th International Congress Cambridge, 69: 54.
17. **Doyle, J. and J. Doyle.** 1990. Isolation of plant DNA from fresh tissue. Focus, 12: 13-5.
18. **Elarosi, H., H. El-Said, M. Nagieb and N. Jaheen.** 1982. Al-Wijam decline date palm disease. Pages 388-403. In: First Symposium on Date Palm, King Faisal University, Saudi Arabia, 23-25 March 1982.
19. **El-Zayat, M., K. Abdusalm, A. Shamlool, M. Djerbi and A. Hadidi.** 2000. Phytoplasma detected in date palm trees infected by Al-Wijam in Kingdom of Saudi Arabia. Pages 230-236. In: Proceedings of Date Palm International Symposium, Namibia, 22-25 February 2000.
20. **Escamilla, J., M. Oropeza, N. Harrison, L. Alpizar, I. Córdova and I. Islas-Flores.** 1994. Evaluación de sondas moleculares de AND para el studio de

prganismos tipo micoplasma causantes del amarillamiento letal. Reporte de proyecto. México: CONACYT. 62 pp.

21. **Hanboonsong, Y., C. Choosai, S. Panyim and S. Damak.** 2002. Transovarial transmission of sugarcane white leaf phytoplasma in the insect vector Matsumuratettix hiroglyphicus (Matsumura). Insect Molecular Biology, 11: 79-103.
22. **Harrison, N., P. Richardson, J. Kramer and J. Tsai.** 1994. Detection of the mycoplasma-like organism associated with lethal yellowing disease of palms in Florida by polymerase chain reaction. Plant Pathology, 43: 998-1008.
23. **Harrison, N., M. Womack and M. Carpio.** 2002. Detection and characterization of a lethal yellowing (16SrIV) group phytoplasma in Canary Island date palm affected by lethal decline in Texas. Plant Disease, 86: 676-681.
24. **Howard, F.** 1983. World distribution and possible geographic origin of palm lethal yellowing disease and its vector. FAO Plant protection Bulletin, 31:101-113
25. **Jones, P.** 2002. Phytoplasma plant pathogens. Pages 126-139. In: Plant Pathologists Pocketbook. M. Waller, J.M. Lenné and S.J. Waller (eds). CAB International, Part 12. Oxford University Press, USA.
26. **Kumar, S., K. Tamura and M. Nei.** 2004. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Briefings in Bioinformatics, 5:150-163.
27. **Lee, I.M., D. Gundersen-Rindal, R. Davis and M. Bartoszyk.** 1998. Revised classification of phytoplasmas based on RFLP analyses of 16s rRNA and ribosomal proteins gene sequences. International Journal of Systematic and Evolutionary Microbiology, 48: 1153-69.
28. **Lee, I.M., R. Davis and D. Gundersen-Rindal.** 2000. Phytoplasmas: Phytopathogenic Mollicutes. Annual Review of Microbiology, 54: 221-55.
29. **Llauger, R., D. Becker, J. Cueto, E. Peralta, V. Gonzalez, M. Rodriguez and W. Rohde.** 2002. Detection and molecular characterization of phytoplasma associated with lethal yellowing disease of coconut palm in Cuba. Journal of Phytopathology, 150: 390-395.
30. **Maixner, M.** 2005. Risks posed by the spread and dissemination of grapevine pathogens and their vectors. Pages 141-146. In: Plant Protection and Plant Health in Europe: Introduction and Spread of Invasive Species, Symposium Proceedings No. 81, The British Crop Production Council, Alton, Hampshire, UK.
31. **Mejía, F., M. Palmieri, C. Oropeza, M. Doyle, M. Harrison, E. Aguliar, M. Narváez, R. Estrada and G. Ortiz.** 2004. First report of coconut lehal yellowing disease in Guatemala. Plant Pathology, 53: 800.
32. **Mpunami, A., A. Tymon, P. Jones and M. Dickinson.** 1999. Genetic diversity in the coconut lethal yellowing disease phytoplasma of East Africa. Plant Pathology, 48: 109-114.
33. **Mpunami, A., A. Tymon, P. Jones and M. Dickinson.** 2000. Identification of potential vectors of the coconut lethal disease phytoplasma. Plant Pathology, 49: 355-361.
34. **Orenstein, S., A. Franck, L. Kuznetzova, I. Sela and E. Tanne.** 1999. Association of a phytoplasma with a carrot disease in Israel. Journal of Plant Pathology, 81:193-199.
35. **Roca, M. and M. Castillo.** 2006. First report of a 16SrIV group phytoplasma associated with declining Coyol palms in Haonduras. Plant Disease, 90: 526.
36. **Schneider, B., M. Cousin, B. Kirkpatrick and E. Seemüller.** 1993. Classification of plant-pathogenic mycoplasma-like organisms using restriction-site analysis of PCR-amplified 16S rDNA. Journal of Genetic Microbiology, 139: 519-527
37. **Schenider, B., M. Cousin, S. Klinkong and E. Seemüller.** 1995. Taxonomic relatedness and phylogenetic positions of phytoplasmas associated with diseases of faba bean, sunhemp, sesame, soybean and eggplant. Plant Disease, 102: 225-32.
38. **Seemüller, E., C. Marcone, A. Ragozzino and M. Göschl.** 1998. Current status of molecular classification of the phytoplasmas. Journal of Plant Pathology, 80: 3-26.
39. **Sharmila, L., S. Bhasker, M. Thelly, B. Edwin and C. Mohankumar.** 2004. Cloning and sequencing of phytoplasma ribosomal DNA (DNA) associated with Kerala Wilt Disease of coconut palm. Journal Plant Biochemistry and Biotechnology, 13:1-5.
40. **Suzuki, S., K. Oshima, S. Kakizawa, R. Arahida, H. Jung, Y. Yamaji, H. Nishigawa, M. Ugaki and S. Namba.** 2006. Interaction between the membrane protein of a pathogen and insect microfilament complex determines insect-vector specificity. Proceeding of the National Academy of Science, 103: 3945-4330.
41. **Tymon, A., P. Jones and N. Harrison.** 1997. Detection and differentiation of African coconut phytoplasmas: RFLP analysis of PCR-amplified 16S rDNA and DNA hybridisation. Annals of Applied Biology, 132: 91-102.
42. **Tymon, A., P. Jones and N. Harrison.** 1998. Phyloenetic relationships of coconut phytoplasmas and development of specific oligonucleotide PCR primers. Annals of Applied Biology, 132: 437-452.
43. **Warokka, S.** 2005. Studies on the aetiology and epidemiology of Kalimantan wilt disease of coconut in Indonesia: University of Nottingham, PhD thesis. 172 pp.
44. **Wayne, A., M. Wayne, N. Harrison, M. Dollet and O. Been.** 2006. Molecular diagnosis and characterisation of phytoplasmas associated with lethal yellowing disease of coconut palms in Jamaica. International Organization for Mycoplasmaology, 16th International Congress Cambridge 87, 63.

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