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Molecular survey of the Texas Phoenix decline phytoplasma population in Florida, USA

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A nested polymerase chain reaction (PCR) assay was used to amplify 16S-23S intergenic spacer (IGS) region from DNA samples individually extracted from 25 *Sabal palmetto* (cabbage palms) showing symptoms of Texas Phoenix decline (TPD) in West Central Florida. The IGS region was also amplified from DNA from other palm species showing symptoms of TPD and lethal yellowing (LY). A subset of the aforementioned phytoplasma DNA samples (*Sabal* and other palm species) together with additional samples from various hosts collected from different geographical localities were further studied to compare the collected phytoplasma strains using sequence analysis of the glycoprotease (*gcp*) genes. Restriction fragment length polymorphisms (RFLP) analysis of the PCR-amplified 16S-23S IGS region and the *gcp* gene using a three restriction enzymes showed that the population of the phytoplasmas infecting *S. palmetto* in West Central Florida is probably homogenous. The *S. palmetto* phytoplasma also appeared similar to all the 16SrIV-D phytoplasmas infecting other palm species and different from all phytoplasmas belonging to the 16SrIV-A subgroup. We recommend more work using genes or genomic regions other than the 16S-23S IGS region and the *gcp* gene to be done.

Key words: 16S-23S intergenic spacer region, glycoprotease gene, phytoplasma, Texas Phoenix decline, lethal yellowing.

INTRODUCTION

Until 2005, the only phytoplasma disease of palms known to occur in Florida was Lethal Yellowing (LY) caused by phytoplasma subgroup 16SrIV-A. While *Cocos nucifera* L. (coconut palm) is the primary palm susceptible to this subgroup, the disease has been observed in 35 other palm species (Harrison and Jones, 2004). During the 40 plus years that LY has been active in Florida, no palm species native to Florida has been affected by this disease. Even with the detection of a decline phytoplasma disease in Florida in 2005, native palm species remained unaffected (Harrison et al., 2008). Thus, it was a surprise in 2008 to determine that the lethal decline of *Sabal palmetto* (Walter) Lodd. Ex Schult. & Schult. f. (cabbage palm), the most common native palm throughout Florida, being observed in west central Florida was caused by a phytoplasma (Harrison et al., 2009). Initial DNA-based characterization of the phytoplasma affecting *S. palmetto* determined that this strain is identical to the subgroup previously documented as affecting *Phoenix* spp. in Texas and later on *Phoenix* spp. and *Syagrus romanzoffiana* in west central Florida, namely subgroup 16SrIV-D (Harrison et al., 2008, 2009). In all its host palms, the disease caused by phytoplasma subgroup 16SrIV-D is called Texas Phoenix decline (TPD). Because *S. palmetto* is the dominant native palm, and often the dominant tree, in natural areas

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throughout Florida, association of the TPD phytoplasma with *S. palmetto* has caused great concern as its biology, its genetic characteristics and the extent of devastation to be expected are not known.

The purpose of this work was to survey the composition of the TPD phytoplasma population in west central Florida and compare with strains outside of Florida and with subgroup 16SrIV-A strains. The 16S-23S IGS region should offer more variation because of less evolutionary constrains on this region than on the 16S rRNA gene. Phytoplasma identification is based on a classification scheme that uses the highly conserved 16S rRNA gene which is useful as the basis of the classification scheme for identifying the major groups of phytoplasmas (Gundersen et al., 1994), but less conserved regions of the genome are necessary for comparing strains within a subgroup. An additional comparison was made among these phytoplasma strains (16SrIV-A and 16SrIV-D) collected from different palm hosts in Florida, Texas, Jamaica, Honduras and Mexico using the *gcp* gene. The protein encoded by the *gcp* gene, o-galactosidase endopeptidase, is possibly a host adaptation and virulence factor and is a member of the M22 peptidase family (Rawlings and Barrett, 1995).

MATERIALS AND METHODS

Plant materials and DNA extraction

The first set of samples, consisting of interior stem tissue shavings, was collected from the lower stems of *S. palmetto* with foliar symptoms indicative of decline. These symptomatic palms were located in the adjacent counties of Hillsborough and Manatee in west central Florida, USA; where diseased *S. palmetto* were most numerous. Twenty-four (24) samples were obtained (: EGS1-11, Sab1-7, and SP1, 2, 4, 6, 7 and 9) (Table 1). A second set of stem samples was obtained from TPD symptomatic *Phoenix* palms, including *P. canariensis* Chab. (Canary Island date palm) (PC1, PC2, SEG, PCT3 and SA1), *P. dactylifera* L. (edible date palm) (RPA) and *P. sylvestris* (L.) Roxb. (silver date palm) (S5-PS); and one symptomatic *S. romanzoffiana* (Cham.) Glassman (queen palm) (S1-QP) (Table 1). This second set of samples also formed part of a previous study by Harrison et al. (2008). A third set of samples were apical meristem (bud) tissues from a TPD symptomatic cabbage palm in west central Florida (Sabal1), a LY symptomatic coconut palm from Broward county in southeastern Florida (LYFL), a LY symptomatic coconut palm in Jamaica (LYJAM) and two LY symptomatic coconut palms in Mexico (LYMEX3 and LYMEX5) (Table 1). The fourth set of samples (CID3, CLDO, COYOL, JLL and PCT4), which were either stem or apical bud samples, was obtained from existing phytoplasma collections (Table 1). The areas from which the samples were collected are shown in Figure 1A and B.

For all the stem samples, tissue was removed from palms by drilling into the stem using a portable electric drill fitted with a wood boring bit as previously described by Harrison et al. (2002). The stem shavings were collected into clean sealable plastics bags. For the bud samples, tissues were collected by felling the palm and excising immature leaf bases of the stem apex. Total nucleic acids were extracted from 3 g of stem tissue or from 100 g of bud tissues. DNA from bud tissues was extracted following the phytoplasma enrichment method of Harrison et al. (1994). From stem tissue, DNA was extracted using CTAB extraction buffer according to the procedure of Doyle and Doyle (1990). For both extraction techniques, nucleic acid was precipitated with 95% ethanol and pellets were recovered by centrifugation at 12000 x g for 15 min. The pellets were resuspended in 200 µL TE buffer [10 mM Tris, 1 mM ethylenediaminetetraacetic acid (EDTA, pH 8)]. Presence of DNA in the pellets was confirmed by agarose gel electrophoresis.

Polymerase chain reaction

DNA preparations from the symptomatic plants were evaluated by PCR assay, together with a negative control, which consisted of DNA from a healthy plant, and a water control (no DNA template). The PCR reaction was conducted using primer pair 16S1064F 5'-TTGGAGGAAGGTGGGGATTAC-3'/23SRev 5'-TTCGCCTTTCCCTCACGGTACT-3' (which is also a phytoplasma diagnostic primer pair) for the first reaction and primer pair TPD-16-23SF 5'-AGCTTAAACGCGAGTTTTTGGCAA-3'/TPD-16-23SR5'-GTTTCGCTCGTCGCTACTACCAGA-3' for the nested round. These primers were designed specifically for this study to amplify the 16S-23S intergenic spacer region. For the *gcp* gene nested PCR reaction, GCPF3 5'GATAGGCCCAGGTTCTTA3' and GCPR2 5' TCCGGAGGAAAACGAGTTA 3' were followed by GCPF1 5' GGTACACGTCTAGCTGTTGTTA 3' and GCPR1 5'CCGGAGGAAAACGAGTTATT3'. Depending on the sharpness of the primary PCR band, nested dilutions ranged from 1/10 to 1/30. All primers used in this study were designed using sequences of the LY phytoplasma obtained by 454 sequencing. Positions of these primers on the LY genome cannot yet be ascertained as the sequencing of this genome is not complete. Each PCR reaction contained 33.8 µL H_2O; 5 µL buffer (1.675 µL H_2O; 1.25 µL 1 M KCl; 1 µL 1 M Tris; 0.5 µL 5% Tween 20; 0.5 µL 1% gelatin; 0.075 µL 1 M $MgCl_2$); 0.1 µg of each of the two primers; 0.04 mM of each of the dNTPs and 1 unit of Taq DNA polymerase (New England BioLabs, Waverley, MA, USA). The DNA template was between 50 and 100 ng. The total volume per PCR reaction was 50 µL and the reaction was run for 35 cycles. Each cycle consisted of 94°C for 1 min, 55°C for 2 min, and 72°C for 3 min.

The 35 thermal cycles were preceded by 1 min initial denaturation at 94°C and succeeded by 7 min final elongation at 72°C. At the end of the PCR run, 10 µL of the PCR mixture was mixed with 7 µL of gel loading dye, electrophoresed through 1% agarose gel using TAE buffer and visualized by UV transillumination following staining with ethidium bromide.

Cloning

Cloning instead of direct sequencing was done to ensure that the full length of the PCR-amplified regions was sequenced. PCR products were purified using Wizard PCR-preps purification kit (Promega Corp, Madison, WI) and were quantified by visualizing on agarose gel with a serial dilution of uncut lambda DNA. The PCR fragments were ligated (mixed with and incubated at 4°C overnight) with pGEM-T vector (Promega Corp, Madison, WI). The ligated PCR product was transformed into Top 10 chemically competent *Escherichia coli* cells (Invitrogen Life Technologies, Carlsbad, CA, USA). The transformed bacterial cultures were grown at 37°C on Luria-Bertani (LB) media amended with isopropyl ß-D-1-thiogalactopyranoside and X-gal for blue/white colony screening. After 24 h incubation white colonies, which were regarded as carrying the cloned PCR fragment, were selected, inoculated into LB broth and incubated at 37°C with gentle shaking for 24 h. Cells

Table 1. Phytoplasma samples included in the study, listed with palm species, location strain identity and GenBank accession number of the sequenced gene fragment.

Phytoplasma sample identity	Palm species	Phytoplasma subgroup	Location[a]	GenBank accession number 16S-23S IGS[a] and *gcp*[b] gene	Year the sample was collected
*[1]EGS1	*Sabal palmetto*	16SrIV-D	Hillsborough	HQ414247[a]	2009
*[1]EGS2	*S. palmetto*	16SrIV-D	Hillsborough	HQ414253[a]	2009
*[1]EGS3	*S. palmetto*	16SrIV-D	Hillsborough	HQ438068[a]	2009
*[1]EGS4	*S. palmetto*	16SrIV-D	Hillsborough	HQ438069[a]	2009
*[1]EGS5	*S. palmetto*	16SrIV-D	Hillsborough	HQ438070[a]	2009
*[1]EGS6	*S. palmetto*	16SrIV-D	Hillsborough	HQ438071[a]	2009
*[1]EGS7	*S. palmetto*	16SrIV-D	Hillsborough	HQ438072[a]	2009
*[1]EGS8	*S. palmetto*	16SrIV-D	Hillsborough	HQ438073[a]	2009
*[1]EGS9	*S. palmetto*	16SrIV-D	Hillsborough	HQ414258[a]	2009
*[1]EGS10	*S. palmetto*	16SrIV-D	Hillsborough	HQ414254[a]	2009
*[1]EGS11	*S. palmetto*	16SrIV-D	Hillsborough	HQ414259[a]	2009
*[1]Sab1	*S. palmetto*	16SrIV-D	Manatee	HQ438224[a]	2008
*[1]Sab2	*S. palmetto*	16SrIV-D	Manatee		2008
*[1]Sab3	*S. palmetto*	16SrIV-D	Manatee		2008
*[1]Sab4	*S. palmetto*	16SrIV-D	Manatee		2008
*[1]Sab5	*S. palmetto*	16SrIV-D	Manatee		2008
*[1]Sab6	*S. palmetto*	16SrIV-D	Manatee		2008
*[1]Sab7	*S. palmetto*	16SrIV-D	Manatee	HQ414246[a]	2008
*[1]SP1	*S. palmetto*	16SrIV-D	Hillsborough		2008
*[1]SP2	*S. palmetto*	16SrIV-D	Hillsborough	HQ414248[a]	2008
*[1]SP4	*S. palmetto*	16SrIV-D	Hillsborough		2008
*[1]SP6	*S. palmetto*	16SrIV-D	Hillsborough	HQ438074[a]	2008
*[1]SP7	*S. palmetto*	16SrIV-D	Hillsborough	HQ414250[a]	2008
*[1]SP9	*S. palmetto*	16SrIV-D	Hillsborough		2008
*[3]Sabal1	*S. palmetto*	16SrIV-D	Hillsborough	HQ414252[a]; HQ613883[b]	2010
*[2]RPA	*Phoenix dactylifera*	16SrIV-D	Hillsborough	HQ438059[a]; HQ613878[b]	2007
*[2]PC1	*P. canariensis*	16SrIV-D	Hillsborough	HQ438060[a]	2007
*[2]PC2	*P. canariensis*	16SrIV-D	Hillsborough		2007
*[2]SEG	*P. canariensis*	16SrIV-D	Hillsborough	HQ613879[b]	2007
*[2]S5-PS	*P. sylvestris*	16SrIV-D	Manatee	HQ438065[a]	2007
*[2]S1-QP	*Syagrus romanzoffiana*	16SrIV-D	Manatee	HQ438064[a]	2007
*[4]CID3	*P. canariensis*	16SrIV-D	Sarasota	HQ613886[b]	2007
*[2]PCT3	*P. canariensis*	16SrIV-D	Texas	HQ438067[a]; HQ613877[b]	2001
*[4]PCT4	*P. canariensis*	16SrIV-D	Texas	HQ613884[b]	2001
*[4]JLL	*Phoenix* sp.	16SrIV-D	Texas	HQ613888[b]	2006
*[4]CLDO	*Cocos nucifera*	16SrIV-D	Honduras	HQ613887[b]	Before 2006
*[4]COYOL	*Acrocomia aculeate*	16SrIV-D	Honduras	HQ613876[b]	Before 2006
*[1]SA1	*P. canariensis*	16SrIV-A	Manatee	HQ438063[a]; HQ613880[b]	2007
*[3]LYFL	*C. nucifera*	16SrIV-A	Broward	HQ613875[a]; HQ613890[b]	Before 2002
*[3]LYJAM	*C. nucifera*	16SrIV-A	Jamaica	HQ414262[a]; Identical to LYFL[b]	Before 2002
*[3]LYMEX3	*C. nucifera*	16SrIV-A	Mexico	HQ613881[b]	Before 2002
*[3]LYMEX5	*C. nucifera*	16SrIV-A	Mexico	HQ441261[a]; HQ613882[b]	1995

[a]Location is county within Florida, USA unless otherwise noted. *Number showing samples belong to the same set.

were lysed, using lysis buffer, to recover the ligated plasmid vectors. The plasmids were purified, resuspended in TE buffer and submitted for sequencing. The plasmid purification kit was supplied by Promega Corp, Madison, WI. Sequencing of cloned fragments

Figure 1A. All phytoplasma strains used in this study were collected from the southern USA and Central America. States in the United States and countries in Central America from which the phytoplasma strains were collected are shown with red dots.

Figure 1B. A map of Florida showing counties from which most of the phytoplasma strains were obtained. The counties from which the strains were collected are shown in red dots.

was done using the M13 forward and M13 reverse primers by the University of Florida's Core DNA Sequencing Service Laboratory, Gainesville.

Sequence analysis

Sequences of the cloned fragments were assembled with SeqMan software (Lasergene™ 7.1; DNASTAR, Madison, WI, USA). Database sequence similarity searching was performed using BLAST in NCBI (website: http://www.ncbi.nlm.nih.gov/BLAST). Sequences were compared pairwise using ClustalW (Larkin et al., 2007). A phylogenetic tree was constructed from the alignment by the neighbor-joining method using MEGA 4.1 software (Tamura et al., 2007). Only representative sequences were used to infer the phylogenetic trees. Sequences obtained from this study were deposited in GenBank and the accession numbers are listed in Table 1.

Restriction fragment length polymorphisms

Analysis of restriction fragment length polymorphisms was used to supplement sequencing data which may be subject to sequencing errors. Polymerase chain reaction products of the PCR amplified 16S-23S intergenic spacer region were digested separately using restriction enzymes, *Ase*I, *Hha*I and *Rsa*I at 37°C for a minimum of 16 h. For the *gcp* gene, *Rsa*I was selected for the RFLP analysis. All the restriction enzymes used in this study were purchased from New England BioLabs, Waverley, MA, USA. These enzymes best differentiated between the phytoplasma strains as shown in a virtual test of sequence data using pDRAW32 (AcaClone, http://www.acaclone.com). Products of the restriction digests were separated by electrophoresis through 8% denaturing polyacrylamide gel in TBE buffer (90 mM Trisborate, 2 mM EDTA). Profiles were visualized using a UV transillumination following staining with ethidium bromide.

RESULTS

Restriction fragment length polymorphisms

It was previously determined that samples RPA, PC1, PC2, SEG, S1-QP, S5-PS and PCT3 belonged to subgroup 16SrIV-D (Harrison et al., 2008). In the current study, it was determined that all the strains from cabbage palms were subgroup 16SrIV-D, and the strains from *C. nucifera* were subgroup 16SrIV-A (except for CLDO which is a 16SrIV-B strain), as was strain SA1 obtained from *P. canariensis*. From DNA samples from 36 symptomatic plants, PCR fragments ca. 800 bp in length were amplified by nested PCR assay. This PCR fragment incorporated the entire 16S-23S intergenic spacer region. No amplification of products was observed in reactions containing DNA from the healthy palm or the water control. Similarity analysis of the assembled nucleotide sequences derived from these PCR fragments demonstrated the sequences were of phytoplasma origin, giving assurance that the PCR fragments were amplified from phytoplasmas. Based on analysis of RFLP profiles generated by digestion of each amplified PCR fragment with *Ase*I restriction enzyme, 16SrIV-D strains from cabbage palms all had the same profiles, except for SP7 which had an additional band (Figure 2A to C). The 16SrIV-D strains from the *Phoenix* spp., from both Florida and Texas, and *S. romanzoffiana* had the same profiles as the 16SrIV-D strains from *S. palmetto*, which were distinct from profiles for the 16SrIV-A strains, whether from *C. nucifera* or *P. canariensis*. Further RFLP analysis based on the *Hha*I enzyme showed that all the TPD 16SrIV-D strains are similar, no matter the palm source, except for two strains (SP6 and SP7) collected from *S. palmetto*, with each containing an additional band (Figure 2D to F). Restriction enzyme *Rsa*I also differentiated the cabbage palms strains similarly, with SP6 and SP7 again exhibiting additional bands (Figure 2G to I). As earlier, all 16SrIV-D strains from *S. palmetto*, *Phoenix* spp. and *S. romanzoffiana* are similar, but distinct from 16SrIV-A strains. The secondary bands in samples SP6 and SP7 in the RFLP profiles are a common occurrence resulting from digestion of non-specific PCR products.

PCR products corresponding to approximately 1.5 kb nucleotides (with about 1 kb representing *gcp* gene) were amplified using the *gcp* gene primers from fourteen of the samples listed in Table 1. No amplification was observed for the healthy palm or the water controls. The RFLP analysis of the *gcp* gene fragment, using *Rsa*I restriction enzyme, shows that 16SrIV-D strains, from multiple palm hosts, are similar to each other but different from 16SrIV-A strains. The RFLP analysis performed on representtative PCR amplicons is shown in Figure 3.

Molecular comparisons by phylogenetic analysis

Phylogenetic analysis using the sequence of the 16S-23S intergenic spacer region revealed that all the subgroup 16SrIV-D phytoplasmas are genetically distinct from all the 16SrIV-A phytoplasmas (Figure 4). All *gcp* sequences compared on CLUSTALW were trimmed to be of similar size, resulting in only a portion of the *gcp* gene to be analyzed. All group 16SrIV phytoplasmas in this study were distinct from other phytoplasmas with *gcp* gene sequences retrieved from GenBank. Sequence analysis of the *gcp* gene showed that the strains belonging to subgroup 16SrIV-A, namely, the Mexican strains LYMEX3 and LYMEX5, the Florida strain LYFL, the Jamaican strain LYJAM, and SA1 strain from *P. canariensis* are all similar (Figure 5). All the TPD phytoplasma strains (subgroup 16SrIV-D)—Sabal1 from *S. palmetto* in Florida; CID3, PCT3, PCT4, JLL, RPA and SEG obtained from *Phoenix* spp. in Florida and Texas; the coconut lethal decline strain CLDO from *C. nucifera* in Honduras; and COYOL strain from *Acrocomia aculeata* (Jacq.) Lodd. ex Mart. in Honduras—clustered together but were distinctly separated from the subgroup 16SrIV-A

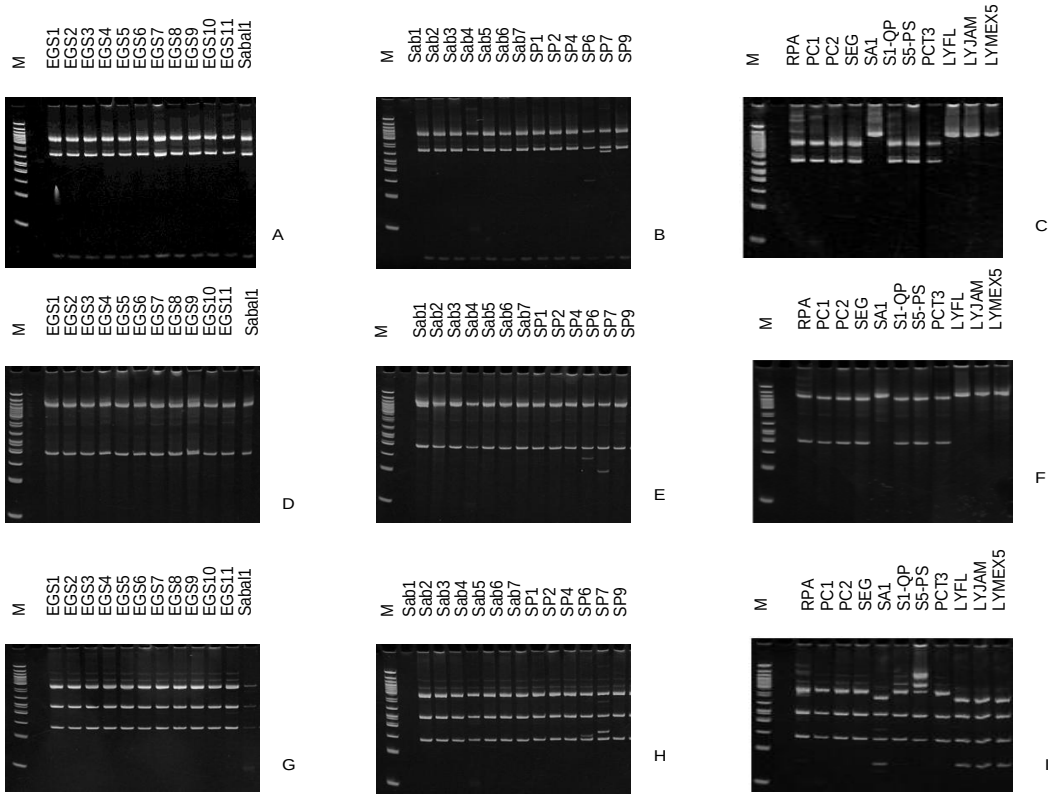

Figure 2. Restriction fragment length profiles of phytoplasma DNA (ca. 800 bp) amplified from symptomatic palms. The PCR amplification was done by primer pair 16S1064F/23SRev followed by primer pair TPD-16-23SF/TPD-16S23SR. A to C) Digestion was with *Ase*I, D to F) Digestion was with *Hha*I and G to I) Digestion was with *Rsa*I. M stands for the pGEM molecular size (bp) markers in descending order: 2465, 1605, 1198, 676, 517, 460, 396, 350, 222, 179, 126, 75, 65, 51 and 36.

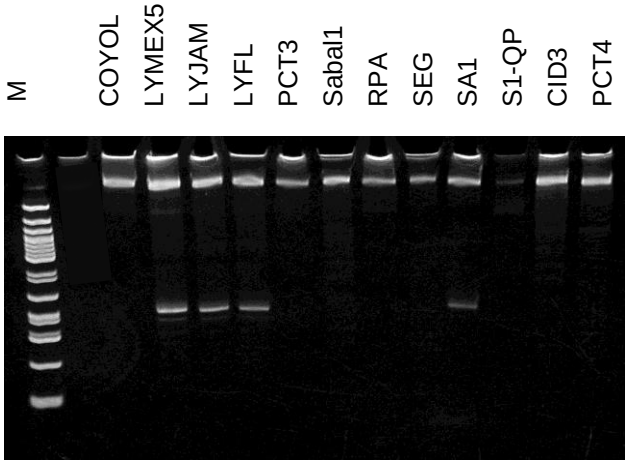

Figure 3. Restriction fragment length polymorphisms of a polymerase chain reaction (PCR) fragment amplified with nested primer pair GCPFI/GCPR1. DNA preparations were from symptomatic tissue of palms from various localities. PCR fragment was digested with enzyme *Rsa*I. M on the first lane is for pGEM molecular size (bp) marker in descending order: 2465, 1605, 1198, 676, 517, 460, 396, 350, 222, 179, 126, 75, 65, 51 and 36.

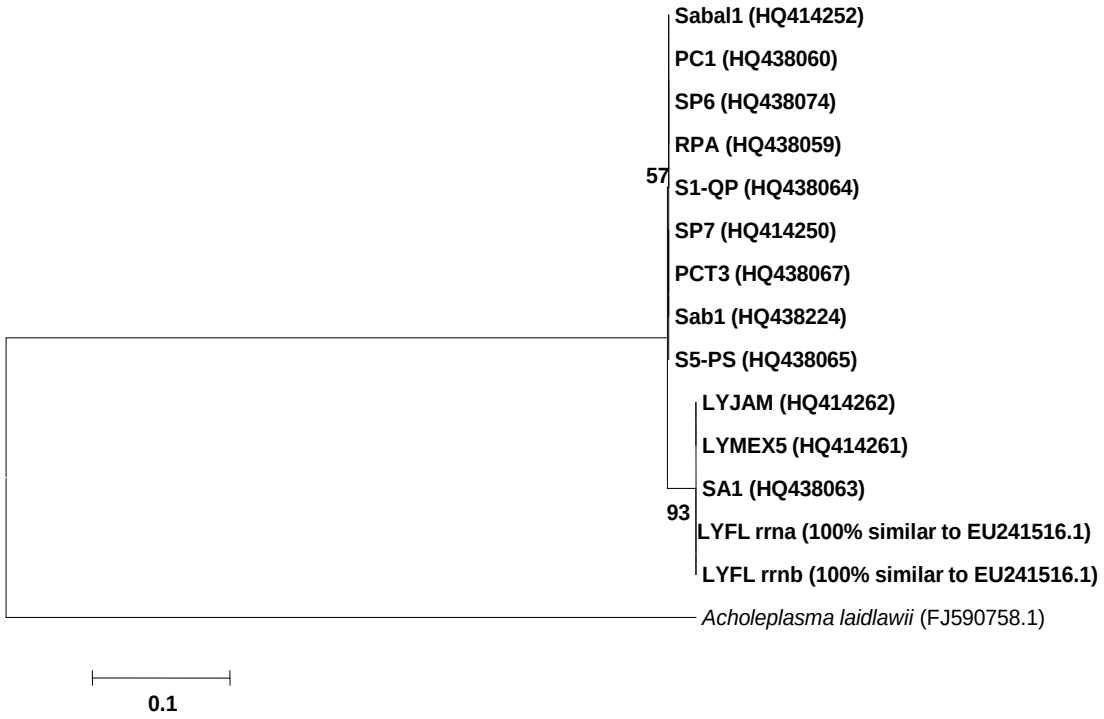

Figure 4. Inferred molecular relationships of phytoplasma strains based on the 16S-23S intergenic spacer sequence. The tree was constructed by the neighbor-joining method and bootstrap values are shown on branches. rrn means ribosomal RNA operon. The GenBank accession numbers are provided in brackets.

strains (Figure 5).

DISCUSSION

Texas Phoenix decline phytoplasma, 16SrIV subgroup D, was first reported in *S. palmetto* in west central Florida in 2008 (Harrison et al., 2008). Although, this phytoplasma subgroup had been previously reported in *P. canariensis* in Corpus Christi, Texas (Harrison et al., 2002), and in west central Florida (Harrison et al., 2008), the attack of *S. palmetto* by a phytoplasma was a surprise as no indigenous palms had been documented as being affected by phytoplasmas in Florida or Texas prior to this time, even though palm phytoplasma diseases had been active in both states since the 1970s (McCoy, 1974). *S. palmetto* is a native species that is important in the natural landscape of the state of Florida and other states in the southern USA.

Characterizing the pathogen population was important in order to understand the phytoplasma population diversity. Interestingly, while this study was in progress, 16SrIV subgroups were detected in *Sabal mexicana* Mart. (A and D), *Pseudophoenix sargentii* H. Wendl. ex Sarg. (D) and *Thrinax radiata* Lodd. ex Schult. & Schult. f. (A and D) in the Yucatan peninsula of Mexico (Vázquez-Euán et al., 2011). All three palm species are native to that area.

Sequence analysis of the 16S-23S rRNA IGS region from the strains representing the population of the TPD phytoplasma in west central Florida showed that in this region of Florida, the phytoplasma population is probably homogenous. This apparent homogeneity of the TPD phytoplasma is found across host palm species (*S. palmetto*, *Phoenix* spp. and *S. romanzoffiana*). Sequence homogeneity of the 800 bp 16S-23S IGS region could also mean that only one strain of the phytoplasma, similar to the strain in Texas (PCT3), was introduced into west central Florida, and since its introduction, this strain has multiplied and spread throughout this part of the state. Should this be the case, prediction of the spread of this disease should be easier. However, even if the 16SrIV-D phytoplasma population is homogenous, the question still remains to the supposed sudden extension of the host range to include *S. palmetto*. Analysis of the sequence of the *gcp* gene demonstrated that the TPD phytoplasma strain infecting *S. palmetto* is not different from the TPD strains (16SrIV-D) infecting *Phoenix* spp., *A. aculeata*, and *C. nucifera*, but it is different from the LY (16SrIV-A) strains affecting *C. nucifera* and *P. canariensis*. When analyzing palm phytoplasma strains, the *gcp* gene has not proved more variable relative to the 16S rRNA gene

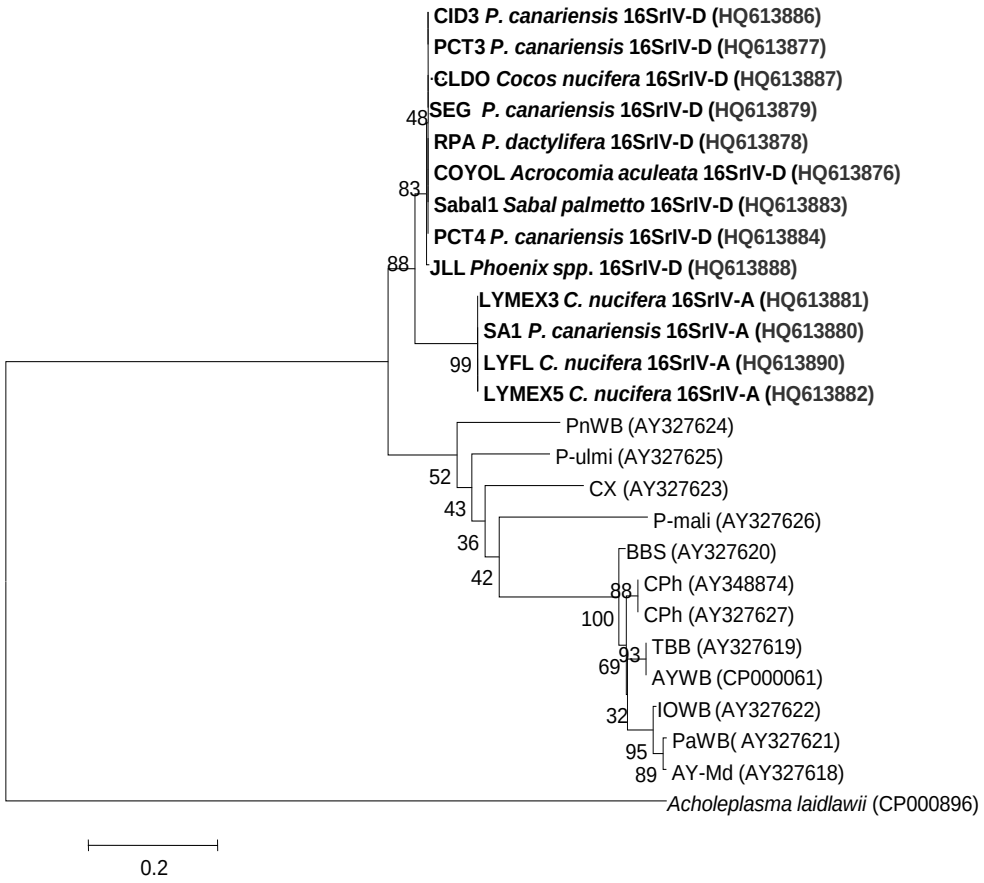

Figure 5. Molecular tree of the glycoprotease (*gcp*) gene sequences of palm lethal disease strains inferred by neighbor-joining method. Approximately, 470 bases of the *gcp* gene were used to infer the tree. Phytoplasma strains (as listed on Table 1) isolated and sequenced in this study are in bold type and the rest of the phytoplasma strain entries (not bold) were retrieved from GenBank. GenBank accession number are provided.

according to which TPD strains are 98.2 to 100% similar (Harrison et al., 2008). It was also shown that the *gcp* gene is not variable among subgroup 16SrIV-A strains.

Further characterization of the 16SrIV-D phytoplasmas using other regions of the genome is necessary to determine if there is a genetic basis for host specificity of these 16SrIV-D phytoplasmas.

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