Association of ‘Candidatus Phytoplasma cynodontis’ with Bermuda grass white leaf disease and its new hosts in Qassim province, Saudi Arabia

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ABSTRACT

Typical symptoms of phytoplasma such as whitening of the leaves, shortening of the stolons on Bermuda grass, variegated leaves, yellows, stunting, little leaves and yellows on Giant reed, Cooba and sand olive shrub were observed in Qassim province, Saudi Arabia, during the autumn season of 2015. When tested for phytoplasma by universal primers P1/P7 followed by R16mF2/R16mR2, products of approximately 1400 bp (as expected) were amplified from 16 plants with symptoms but not from symptomless plants. Based on sequencing, phylogenetic analysis and virtual restriction fragment length polymorphism patterns of the 16S rDNA F2nR2 fragments of seven Qassim phytoplasma isolates, bermuda grass isolates 170, 175 and 177, giant reed isolate 180, sand olive isolates 181 and 182 and cooba isolate 185, the associated phytoplasma was identified as a member of ‘Candidatus Phytoplasma cynodontis’ which belong to the 16SrXIV-A subgroup. The 16S rDNA gene sequences of seven Qassim phytoplasma isolates exhibited over 99.2% identity with members of ‘Ca. Phytoplasma cynodontis’ group of phytoplasmas. This is the first report of characterization of ‘Ca. phytoplasma cynodonties’ (16SrXIV) associated with Cynodon dactylon in Saudi Arabia and its new hosts, Dodonaea angustifolia, Arundo donax and Acacia salicina.

Introduction

Bermuda grass (Cynodon dactylon), which is native to Africa, is normally grown as a turf grass or as forage for livestock, can be an invasive weed. It is considered to be the most widely used turf grasses in tropics and sub tropics on athletic fields and golf course fairways. Bermuda grasses establish rapidly and spread by vegetative propagules by both stolons and rhizomes (Brosnan & Deputy 2008). Bermuda grass white leaf (BGWL), first described in Taiwan, is a destructive phytoplasmal disease of Bermuda grass caused by the phytoplasma group (16SrXIV) and characterized by whitening of the leaves and shortening of the stolons (Chen et al. 1972; Obura et al. 2010). BGWL disease is reported in many countries including Singapore (Koh et al. 2008), Turkey (Çağlar et al. 2013), Sudan (Dafalla & Cousin 1988), Thailand (Sunnapao 2014), India (Rao et al. 2007; Snehi et al. 2008; Kumar et al. 2015), Madagascar (Nejat et al. 2009; Naderali et al. 2013), Myanmar (Win et al. 2013), Kenya, Tanzania and Uganda (Asudi et al. 2015), and Serbia and Albania (Mitrović et al. 2015). Phytoplasmas, wall-less prokaryotes, classified in the Class Mollicutes, Order Acholeplasmatales and genus Candidatus are uncultivable (Xiaodong et al. 2006). They infect a wide variety of plants such as fruit crops, timber, vegetables, grasses and other ornamental crops by inhabiting the phloem tissue resulting in economically significant epidemics throughout the world (Bekele et al. 2011). Highly conserved 16S rRNA based characterization is applied to identify and classify phytoplasmas. Since the concentration of phytoplasma in phloem tissue of some plants is very low, nested polymerase chain reaction (PCR) is used for amplifying DNA fragments from the first round amplification using internal primers (Gundersen & Lee 1996; Heinrich et al. 2001). Though, phytoplasma infection in Bermuda grass has been previously described (Marcone & Ragozzino 1997; Çağlar et al. 2013; Naderali et al. 2013; Win et al. 2013; Khanna et al. 2015; Mitrović et al. 2015) the information on Bermuda grass infection from Saudi Arabia was not available. However, typical phytoplasmal symptoms were observed in Qassim region of the country. Therefore, a study was carried out to characterize the phytoplasma infecting Bermuda grass in Qassim region using 16S ribosomal RNA.

Materials and methods

Samples collection

During the autumn season of 2015, nine samples of Bermuda grass (C. dactylon) showing whitening of the leaves and shortening of the stolons were collected from three different places from Qassim region (University campus, Al Safra and Mulayada); three samples of sand olive shrub (Dodonaea angustifolia) with stunting, little leaves and yellows; two samples of small evergreen tree, cooba (Acacia salicina) with yellows and two samples of Giant reed (Arundo donax) displaying variegated leaves were collected from Faculty of Agriculture and Veterinary Medicine farm. All samples were stored at 4°C until processed for DNA extraction. Asymptomatic samples of the same species (C. dactylon,
D. angustifolia, A. salicia and A. donax) were also collected for use as negative controls.

**DNA extraction**

Total nucleic acids were extracted from fresh leaves of all symptomatic and asymptomatic samples. Hundred milligram of each sample were powdered in liquid nitrogen and transferred to a 1.5-ml Eppendorf tube for subsequent processing of DNA extraction using i-genomic plant DNA extraction Mini Kit (iNtRON Biotechnology Inc., Cat. No. 17371, Korea). The DNA was eluted in 100 µl of elution buffer and were kept at −20°C to use as DNA template in PCR assays.

**Amplification of phytoplasma 16S rDNA gene fragments**

The universal phytoplasma primer pair P1/P7 in first round (Deng & Hiruki 1991; Schneider et al. 1995) were used to amplification of 1.8 kbp DNA fragment comprising the nearly full length 16S rDNA. The pair primers, R16mF2 (5’-CATGCAAGTGGACGGA-3’) and R16mR2 (5’-CTTACCCCACAATCTCGA-3’) were used in second round nested PCR assays which amplify an internal DNA fragment of 1400 bp from the 16S rDNA gene (Baric & Dalla Via 2004). PCR assay was performed in a thermal cycler (SwiftTM MaxPro Thermal Cycler, ESCO healthcare). The PCR reaction mixture (20 µl in the first PCR round) contained 1 µl (50 ng) nucleic acid as template, 1 µl of each primer (10 pmol), (4 µl) of 5× FIREPol® Master Mix (Solis BioDyne, Estonia) and 13 µl of Nuclease free water (Promega, USA), and the volume of 40 µl was used in the second round of PCR with primers R16mF2/R16mR2 with double the amount of the 5× FIREPol® Master Mix (Solis BioDyne, Estonia), 1 µl of each primer

![Image](a), ![Image](b)

**Figure 1.** Bermuda grass white leaf (BGWL) exhibiting (a) whitening and little leaves, bushy growth and shorten internodes, and (b) whitening leaves.

**Figure 2.** Giant reed (A. donax) displaying witches’ broom and variegated leaves (a) and variegated upper leaves (b).

**Figure 3.** Cooba tree (A. salicia) with yellowing and narrow leaves (a) and healthy cooba tree (b).
(10 pmol), 1 µl of the primary PCR product and 29 µl of Nuclease free water (Promega). The PCR program with P1/P7 primers was followed with initial desaturation at 94°C for 3 min, 34 cycles with denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 2 min and followed by a final extension step at 72°C for 7 min. The same PCR program was used in the second round with primers R16mF2/R16mR2 except the annealing temperature of 46°C. The amplified PCR products were analyzed by electrophoresis through a 1.5% agarose gel, stained with ethidium bromide, and DNA bands were visualized using a UV transilluminator (G:BOX F3 system, Syngene).

**Sequencing PCR products and phylogenetic analysis**

Seven internal amplified fragments of 16Sr DNA by the primer pairs R16mF2/R16mR2 were sequenced in both orientations (Macrogen Inc., Korea). The sequences obtained from Qassim 'Candidatus Phytoplasma cynodontis' isolates, 170, 175, 177, 180, 181, 182 and 185 were deposited in the GenBank databases under accession numbers, LT220876, LT220879, LT220880, LT220881, LT220882, LT220883 and LT220884, respectively. The obtained sequences were gathered and edited using GAP4 program (Bonfield et al. 1995). Multiple sequence alignments and nucleotide sequence similarity were carried out using ClustalW (Thompson et al. 1994). The 16S rDNA gene sequences of seven Qassim phytoplasmas isolated from Bermuda grass (isolate 170, 175 and 177), giant reed (isolate 180), sand olive (isolates 181 and 182) and cooba (isolate 185) plants in this study were compiled in FASTA format and compared with each other and with 11 other reference phytoplasmas which belong to different 16S rDNA subgroups and with 12 'Ca. phytoplasma cynodontis' strains reported from different countries using ClustalW (Thompson et al. 1994). Phylogenetic tree was constructed using the neighbor-joining phylogenetic method implemented in MEGA4 program (Tamura et al. 2007) with 1000 bootstrap replications. *Acholeplasma laidlawii* was used as outgroup.

**In silico restriction enzyme digestions and virtual gel plotting**

R16F2n/R16R2 region of the 16S rDNA gene sequences were digested in silico with 17 restriction enzymes (*AluI, BamHI, BflI, BstUI, DraI, EcoRI, HaeIII, HhaI, Hinfl, Hpal, HpaII, KpnI, Sau3AI (MboI), MseI, Rsal, SspI, TaqI*) that have been used for phytoplasma classification (Lee et al. 1998), and virtual gel plotting was performed using iPhyclassifier (Zaho et al. 2009).

**Results and discussion**

**Disease symptoms**

The natural symptoms of the diseases caused by 'Ca. Phytoplasma cynodontis' were variable according to the host on

![Figure 4](image-url) Sand olive shrub (*D. angustifolia*) showing stunting, small leaves and yellowing (a), and normal sand olive shrub (b).

![Figure 5](image-url) Agarose gel electrophoresis of nested PCR products from the 16SrRNA gene using primers R16mF2 and R16mR2. Lane 1, 2, 3 and 4 are *C. dactylon*, *A. donax*, *D. angustifolia* and *A. salicia* symptomless samples; lane 5–13 are *C. dactylon* samples with whitening leaves, lane 14 and 15 are *A. donax* samples with variegated leaves; lane 16–18 are *D. angustifolia* samples with stunting, little leaves and yellows; lane 19 and 20 are *A. salicia* samples with yellows, M: 100 bp DNA ladder (Solis BioDyne).
Bermuda grass (*C. dactylon*) showing whitening and little leaves, bushy growth and shorten internodes (Figure 1(a)), whitening of leaves (Figure 1(b)). Similar symptoms have been described on Bermuda grass (Rao et al. 2007; Salehi et al. 2009; Khanna et al. 2015). Whereas different phytoplasmal symptoms were observed on the other hosts, *A. donax*, *A. salicia* and *D. angustifolia* plants, showing witches’ broom and variegated leaves (Figure 2(a) and 2(b)), yellowing and narrow leaves (Figure 3(a) and 3(b)), and stunting, small leaves and yellowing (Figure 4(a) and 4(b)) in these hosts. *Ca. Phytoplasma cynodontis* is associated with many other grass and plant species displaying white and small leaves, yellows, bushy growth, severe chlorosis and inflorescence necrosis symptoms in certain countries such as blue grass (*Poa annua*) in Italy (Lee et al. 1997), Brachiaria grass (*Brachiaria distachya*), carpet grass (*Axonopus compressus*), ivy ground (*Coccinia grandis*) and crowfoot grass (*Dactyloctenium aegyptium*) in Thailand (Wongkaew et al. 1997; Sdoodee et al. 1999; Jung et al. 2003; Sunpapao 2014), golden beard grass (*Chrysopogon aciculatus*) in Korea (Win & Jung 2012), Delhi grass (* Dichanthium annulatum*), *Oplismenus burmannii* (Retz.) P. Beauv. and *Digitaria sanguinalis* (L.) Scop in India (Rao et al. 2007; Salehi et al. 2009; Khanna et al. 2015).

**Figure 6.** Virtual restriction fragment length polymorphism pattern derived from in silico digestions of R16F2n/R16R2 fragments of *Candidatus Phytoplasma cynodontis* Qassim isolates.

**Figure 7.** Phylogenetic tree constructed by the Neighbor-Joining method from 16S rDNA gene sequences of *C. dactylon* (isolates 170, 175 and 177), *A. donax* (isolate 180), *D. angustifolia* (isolates 181 and 182) and *A. salicia* (isolate 185) and other 33 phytoplasma members and strains belong to different 16S rDNA subgroups. *Acholeplasma palmae* was used as outgroup. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. GenBank accession numbers for sequences are written right beside the phytoplasmas.
Table 1. 16S rDNA nucleotide sequences similarities among ‘Ca. Phytoplasma cynodontis’ isolates.

|                | Saudi Arabia (Qassim region) | Italy & Albania | Myanmar | India | China | Serbia |
|----------------|-----------------------------|-----------------|---------|-------|-------|---------|
| Isolates       | 170 175 177 180 181 182 185 |                 |         |       |       |         |
| Saudi Arabia (Qassim region) | 170 100 |                |         |       |       |         |
| 175            | 99.8                         |                 |         |       |       |         |
| 177            | 99.3 99.5                    |                 |         |       |       |         |
| 180            | 99.2 99.4 99.1               |                 |         |       |       |         |
| 181            | 99.4 99.7 99.3 99.1          |                 |         |       |       |         |
| 182            | 99.3 99.5 99.1 98.9 99.8     |                 |         |       |       |         |
| 185            | 99.3 99.6 99.3 99.0 99.3 99.1|                 |         |       |       |         |
| Italy & Albania| AJ550984 99.4 99.7 99.2 99.1| KF383978       | KF383979| KF383980| KF383981| KJ000024| KP019339|
| 99.3 99.6 99.3 99.0 99.3 99.1               |                | KF383979       | KF383980| KF383981| KJ000024| KP019339|
| Myanmar        | AB741630 99.4 99.3 99.2 99.1| AB642601       | AB741630| AB642601|            |         |
| 99.3 99.6 99.1 99.0 99.3 99.2 99.2 99.8 98.8 98.9 99.6 |            | AB642601       | AB741630| AB642601|            |         |
| India          | EU032485 98.8 99.0 98.5 98.5 98.7 98.7 98.6 99.2 99.3 |            | EU032485| GQ403690|            |         |
| 98.3 98.6 98.1 98.1 98.3 98.3 98.2 98.8 98.9 99.6 |            | EU032485| GQ403690| EU032485|            |         |
| China          | EU377477 99.3 99.6 99.1 99.0 99.3 99.3 99.2 99.8 99.9 99.4 99.0 |            | EU377477| GQ403690| EU032485| GQ403690|
| Serbia         | KF383981 99.2 99.4 98.9 98.8 99.1 99.1 99.0 99.6 99.8 99.1 98.7 99.7 100 |            | EU377477| GQ403690| EU032485| GQ403690|
|                | KF383981 |                |         |       |       |         |
|                | KJ000024|                |         |       |       |         |
|                | KP019339|                |         |       |       |         |
et al. 2009, 2010), coconut palm (Cocos nucifera) and Foxtail Palm (Wodyetia bifurcata) in Malaysia (Nejat et al. 2009; Naderali et al. 2013).

**PCR amplification**

PCR was carried out using P1/P7 primers in the first round followed by nested PCR with R16mF2 and R16mR2 primers in the second PCR round. All tested symptomatic samples of C. dactylon, A. donax, A. salicia and D. angustifolia gave DNA fragments of approximately 1400 bp. No amplification was obtained from symptomless plants (Figure 5).

**Virtual restriction enzyme digestions**

Virtual restriction fragment length polymorphism (RFLP) patterns of the 16S rDNA F2nR2 fragments of seven phytoplasma Qassim isolates, three C. dactylon isolates (170, 175 and 177), two isolates of D. angustifolia (181 and 182), one isolate of both A. donax and A. salicia (180 and 185) using iPhyClassifier (Zhao et al. 2009) revealed that pattern similar to that of 'Ca. Phytoplasma cynodontis' which belongs to phytoplasma 16SrVIX group and subgroup A (GenBank accession: AJ550984) with a similarity coefficient of 0.97 (Figure 6). BGWL phytoplasma was previously identified as 16SrXIV-A subgroup based on virtual RFLP in India (Khanna et al. 2015). Additionally, the virtual RFLP analysis was used successfully to (i) reveal the genetic diversity among other phytoplasmas such as cactus witches’ broom phytoplasma strains infecting Opuntia species in China (Cai et al. 2008), (ii) grouped potato purple top phytoplasma strains to four different phytoplasma groups (16SrI, 16SrII, 16SrIII and 16SrXIII) in Mexico (Santos-Cervantes et al. 2010), (iii) identified the Indian arecanut palm yellow leaf disease phytoplasma as a member of 16SrXI-B subgroup (Ramawamy et al. 2013) and (iv) classified Indian cucumber phytoplady (CuP) and squash phyllody (SpP) phytoplasmas to different phytoplasma subgroups 16SrII-M and 16SrII-D, respectively (Salehi et al. 2015).

**Nucleotide sequence identities and phylogenetic analysis**

Sequence analysis conducted on seven Qassim phytoplasma isolates, which compared to other 33 phytoplasma strains from different countries revealed that all Qassim phytoplasma isolates have identities ranging from 99% to 99.8% with each other and they are closely related to 'Ca. Phytoplasma cynodontis' (16S XV-A subgroup) isolates reported from Italy, Albania and Myanmar with high similarities from 99.2% to 99.7% (Table 1). Also, they shared identities of more than 98.9% with three Serbian and Chinese isolates (KJ000024, KF383981, KP019339 and EU377477). However, the lowest identities of 98.1% were seen with two Indian isolates (EU032485 and GQ403690) (Table 1).

The phylogenetic tree confirmed the result obtained from virtual RFLP (Figure 7). Therefore, all Qassim phytoplasma isolates and other 'Ca. Phytoplasma cynodontis' isolates were placed in one clade and were separated from 'Ca. Phytoplasma phytoplasmas' members with bootstrap of 100%. The 'Ca. Phytoplasma cynodontis' clade was regionally divided into four subclades (A, B, C and D). Hence, where the seven Qassim phytoplasma isolates were clustered together in subclade A with bootstrap 95%, the Italian and Albanian isolates formed a subclade B. Further, isolates from Myanmar, China and India were found in subclade C, whereas Serbian isolates were grouped in subclade D. The results presented in this study are in concurrence with previous work which showed that the BGWL phytoplasmas from different geographical regions are identical (Marcone & Ragozzino 1997; Wongkaew et al. 1997; Lee et al. 1998; Tran-Nguyen et al. 2000). As the 16S rDNA sequence identity is greater than 97.5% among Qassim isolates and other members of 'Ca. Phytoplasma cynodontis' (16SrXIV) which is enough for defining the status of novel 'Candidatius' phytoplasma species, the Qassim phytoplasma isolates associated with C. dactylon, D. angustifolia, A. donax and A. salicia plants should be considered as members of 'Ca. Phytoplasma cynodontis’ group. However, another prospective analysis based on RFLP and other molecular markers are needed for better differentiation among Qassim phytoplasma isolates to confirm their taxonomic position. To the best of our knowledge, this is the first report of the characterization of 'Ca. Phytoplasma cynodonties’ (16SrXVII) associated with C. dactylon, two D. angustifolia, A. donax and A. salicia plants in Saudi Arabia.

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**Disclosure statement**

No potential conflict of interest was reported by the author.

**References**

Arocha Y, Horta D, Pinot B, Palenzuela I, Picornell S, Almeida R, Gones P. 2005. First report of a phytoplasma associated with Bermuda grass white leaf disease in Cuba. Plant Pathol. 54:233.

Asudi GO, Van den Berg J, Midega CAO, Schneider B, Seemüller E, Pickett JA, Khan ZR. 2015. Detection, identification, and significance of phytoplasmas in wild grasses in East Africa. Plant Dis. PDIS–11–14–1173. doi:10.1094/pdis-11-14-1173-re

Baric S, Dalla Via J. 2004. A new approach to apple proliferation detection: a highly sensitive real-time PCR assay. J Microbiol Meth. 57:135–145.

Bekele B, Hodgetts J, Tomlinson J, Boonham N, Nikolic P, Swarbrick P, Dickinson M. 2011. Use of a real-time LAMP isothermal assay for detecting 16SrII and XII phytoplasmas in fruit and weeds of the Ethiopian Rift Valley. Plant Pathol. 60:345–355.

Bonfield JK, Smith KF, Staden R. 1995. A new DNA sequence assembly program. Nucleic Acids Res. 23:4992–4999.

Brosnan JT, Deputy J. 2008. Bermuda grass. University of Hawai‘i at Mānoa, and Human Resource, Cooperative Extension Service Publication TM-5. Available November 17, 2015, from: http:// turfgrass.ctahr.hawaii.edu/downloads/Bermudagrass_NEW2.pdf

Çağlar BK, Satar S, Elbeanio T. 2013. Detection and molecular characterization of Bermuda grass (Cynodon dactylon) white leaf phytoplasma from Turkey. Int J Agric Biol. 15:90–94.

Cai H, Wei W, Davis RE, Chen H, Zhao Y. 2008. Genetic diversity among phytoplasmas infecting Opuntia spp.: virtual RFLP analysis identifies new subgroups in the peanut witches’ broom phytoplasma group. Int J Syst Evol Microbiol. 58:1448–1457.

Chen TC, Lee CS, Chen MJ. 1972. Mycoplasmalike organisms in Cynodon dactylon and Brachiaria distachya affected by white leaf disease. Rep Taiwan Sugar Exp Stn. 56:49–55.
Dafalla GA, Cousin MT. 1988. Fluorescence and electron microscopy of *Cynodon dactylon* affected with a white leaf disease in Sudan. J Phytopathol. 122:25–34.

Deng S, Hiiruki C. 1991. Genetic relatedness between two non-culturable mycoplasmalike organism revealed by nucleic acid hybridization and polymerase chain reaction. Phytopathol. 81:1475–1479.

Gundersen DE, Lee I-M. 1996. Ultrastructural detection of phytoplasma by nested-PCR assays using two universal primer sets. Phytopathol. Medit. 35:144–151.

Heinrich M, Botti S, Caprara L, Arthur W, Strommer S, Hanzer V, Katinger H, Bertaccini A, Laimer da Câmara Machado M. 2001. Improved detection methods for fruit tree phytoplasmas. Plant Mol Biol Rep. 19:169–179.

Jung HY, Sawyeranong T, Wongkaew P, Kakizawa S, Nishigawa H, Wei W, Oshima K, Miyata SI, Ugaki M, Hibi T, Namba S. 2003. *Candidatus Phytoplasma oryzae*, a novel phytoplasma taxon associated with rice yellow dwarf disease. Int J Syst Evol Microbiol. 53:1925–1929.

Khanna S, Singh J, Singh R, Kumar P, Rani T, Baranwal VK, Sirohi A, Koh LH, Yap ML, Yik CP. 2008. First report of phytoplasma infection of *Phytoplasma cynodontis* from western Uttar Pradesh, India. Crop Prot. 74:138–144.

Koh LH, Yap ML, Yik CP. 2008. First report of phytoplasma infection of *Phytoplasma asteris* in Singapore. Plant Dis. 92:317.

Kumar S, Jodon V, Tiwari AK, Rao GP. 2015. *Exitis indices* (Distinct): a putative vector for *Candidatus Phytoplasma cynodontis* in India. Phytopathogenic Mollicutes. 5:SS1–SS2.

Lee I-M, Gundersen DE, Davis RE, Bartoszyk I-M. 1997. Revised classification scheme of phytoplasmas based on RFLP analysis of 16S rRNA and ribosomal protein gene sequences. Int J Syst Bacteriol. 48:1153–1169.

Lee I-M, Pastore M, Vhibio M, Danielli A, Attathorn S, Davis RE, Bertaccini A. 1997. Detection and characterization of aphytoplasma associated with annual blue grass (*Poa annua*) white leaf disease in southern Italy. Eur J Plant Pathol. 103:251–254.

Marcone C, Ragazzino A. 1997. Detection of Bermuda grass white leaf disease in Italy and characterization of the associated phytoplasma by RFLP analysis. Plant Dis. 81:862–866.

Mitrović J, Smiljeković M, Seemüller E, Reinhardt R, Huttel B, Bertaccini A, Kube M, Duduk B. 2015. Differentiation of *Candidatus Phytoplasma cynodontis* based on 16S rRNA and groEL genes and identification of a new subgroup, 16SrXIV-C. Plant Dis. 99:1376–1382.

Naderal N, Nejat N, Vadmalai G, Tan YH. 2013. First report of two distinct phytoplasma species, *Candidatus Phytoplasma cynodontis* and *Candidatus Phytoplasma asteris*, simultaneously associated with yellow decline of *Wodyetia bifurcata* (foxtail palm) in Malaysia. Plant Dis. 97:1504.

Nejat N, Sijari K, Abdollahi SN, Vadmalai G, Dickinson M. 2009. First report of a 16S rDNA *Candidatus Phytoplasma cynodontis* group phytoplasma associated with coconut yellow decline in Malaysia. Plant Pathol. 58:389.

Obura E, Masiga D, Midega CAO, Wachira F, Pickett JA, Deng AL, Khan ZR. 2010. First report of a phytoplasma associated with Bermuda grass white leaf disease in Kenya. New Dis Rep. 21:23.

Padovan A, De La Rue S, Eitchner R, Davis R, Schneider B, Bernuetz A, Gibb KS. 1999. Detection and differentiation of phytoplasmas in Australia: an update. Aust J Agric Res. 50:333–342.

Ramaseswamy M, Nair S, Soumya VP, Thomas GV. 2013. Phylogenetic analysis identifies *Candidatus Phytoplasma oryzae* related strain associated with yellow leaf disease of areca palm (*Areca catechu L.*) in India. Int J Syst Evol Microbiol. 63:1376–1382.

Rao GP, Mall S, Marcone C. 2010. *Candidatus Phytoplasma cynodontis* (16SrXIV group) affecting *Opismenus burmannii* (Retz.) P. Beauv. and *Digitaria sanguinalis* (L.) Scop. in India. Australas Plant Dis Notes. 5:93–95.

Rao GP, Mall S, Singh M, Marcone C. 2009. First report of a *Candidatus Phytoplasma cynodontis*-related strain (16SrXIV) associated with white leaf disease of *Dichanthium annulatum* in India. Australas Plant Dis Notes. 4:56–58.

Rao GP, Raj SK, Nehi SK, Mall S, Singh M, Marcone C. 2007. Molecular evidence for the presence of *Candidatus Phytoplasma cynodontis* the Bermuda grass white leaf agent, in India. Bull Insectol. 60:145–146.

Salehi M, Izadpanah K, Siampour M, Taghizadeh M. 2009. Molecular characterization and transmission of Bermuda grass white leaf phytoplasma in Iran. J Plant Pathol. 91:655–661.

Salehi M, Siampour M, Esmailzadeh Hosseini SA, Bertaccini A. 2015. Characterization and vector identification of phytoplasmas associated with cucumber and squash phythodry in Iran. Bull Insectol. 68:311–319.

Santos-Cervantes ME, Chávez-Medina JA, Acosta-Pardini J, Flores-Zamora GL, Méndez-Lozano J, Leyva-López NE. 2010. Genetic diversity and geographical distribution of phytoplasmas associated with potato purple top disease in Mexico. Plant Dis. 94:388–395.

Schneider B, Seemüller E, Smart CD, Kirkpatrick BC. 1995. Phylogenetic classification of plant pathogenic Mycoplasma like organisms or phytoplasmas. In: Razin S, Tully JG, editor. Molecular and diagnostic procedures in mycoplasmosi. San Diego, CA: Academic press; p. 369–380.

Soodree O, Schneider B, Padovan A, Gibb KS. 1999. Detection and genetic relatedness of phytoplasmas associated with plant disease in Thailand. J Biochem Mol Biol Biophys. 3:133–140.

Sneh S, Khan MS, Raj SK, Mall S, Singh M, Rao GP. 2008. Molecular identification of *Candidatus Phytoplasma cynodontis* associated with Bermuda grass white leaf disease in India. Plant Pathol. 57:770.

Sunpapao A. 2014. Association of *Candidatus Phytoplasma cynodontis* with the yellow leaf diseases of ivy gourd in Thailand. Australas Plant Dis Notes. 9:127.

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol. 24:1596–1599.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673–4680.

Tran-Nguyen L, Blanche KR, Egan B, Gibb KS. 2000. Diversity of phytoplasmas in northern Australian sugarcane and other grasses. Plant Pathol. 49:666–679.

Win NKK, Jung H-Y. 2012. *Candidatus Phytoplasma cynodontis* associates with white leaf disease of golden beard grass (*Chrysopogon aciculatus*). Trop Plant Pathol. 37:76–79.

Win NKK, Kim Y-H, Oyga S. 2013. Molecular characterization of white leaf phytoplasma associated with the Graminae in Myanmar. J Fac Agr Kyushu Univ. 58:225–229.

Wongkaew P, Hanboonsong Y, Sirithorn P, Choosai C, Boonkrong S, Xiaodong B, Zhang J, Ewing A, Miller SA, Radek AJ, Shevchenko DV, Win NKK, Jung H-Y. 2012. Differentiation of phytoplasmas associated with sugarcane and gramineous weed white leaf disease and sugarcane grassy shoot disease by RFLP and sequencing. Theor Appl Genet. 95:660–663.

Xiaoandong B, Zhang J, Ewing A, Miller SA, Redek AJ, Shevchenko DV, Tsukerman K, Walunas T, Lapidus A, Campbell JW, Hogenhout SA. 2006. Living with Genome instability: The adaptation of phytoplasmas to diverse environments of their insect and plant hosts. J Bacteriol. 188:3682–3696.

Zhao Y, Sun Q, Wei W, Davis RE, Wu W, Liu Q. 2009. *Candidatus Phytoplasma tamarisci*, a novel taxon discovered in witches’ broom diseased salt cedar (*Tamarix chinensis* Lour.). Int J Syst Evol Microbiol. 59:2496–2504.