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Phytoplasma Detection in Coconut Palm and Other Tropical Crops

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Abstract: Phytoplasmas are small bacteria with very small genomes which also have extremely low levels of the nucleotides guanine plus cytosine (G+C). They are associated with hundreds of plant diseases globally. The uneven distribution and low concentration of phytoplasmas in the phloem of infected plant, especially in woody hosts, and variations in titre according to season and plant organ are also important obstacles for efficient diagnosis. Polymerase Chain Reaction (PCR), nested PCR and real-time PCR have been employed for phytoplasma detection. PCR is the most versatile tool for detecting phytoplasmas in their plant and insect hosts. Nested PCR with a combination of different universal primers can improve the diagnosis of unknown phytoplasmas present with low titre in the symptomatic host. Universal ribosomal primers nested with group-specific primers are extremely useful when the phytoplasma to be diagnosed belongs to a well-defined taxonomic group. Real-time PCR has been shown to be an effective method of quantifying the titre of phytoplasmas within the plant. This paper also discuss phytoplasma diseases on coconut palm.

Key words: Lethal yellowing, coconut yellow decline, Bermudagrass white leaf

INTRODUCTION

Phytoplasmas, formerly termed mycoplasma-like organisms (MLOs), were first discovered in the early 1900s (Kunkel, 1926). They are small bacteria with very small genomes which also have extremely low levels of the nucleotides guanine plus cytosine (G+C), sometimes as little as 23-29% which is thought to be the threshold for a viable genome and coding DNA that are associated with hundreds of plant diseases globally, affecting hundreds of plant species including many important food, vegetable and fruit crops; ornamental plants; timber and shade trees. Geographically, the occurrence of phytoplasmas is worldwide (Lee *et al*., 2000; Bertaccini, 2007; Dickinson, 2003; Hogenhout *et al*., 2008).

Phytoplasmas require a vector to be transmitted from plant to plant. They are phloem-limited bacterial pathogens that can cause devastating losses in crops and natural ecosystems worldwide and most prevalent in tropical and sub-tropical regions of the world (Lee *et al*., 2000; Bertaccini, 2007). Phytoplasmas are minute cell-wall-less prokaryotes with a diameter less than 1 micrometer ranging from 200 to 800 μm, or roughly the size of a plant cell's chloroplasts, a rounded pleiomorphic or filamentous shape surrounded by a triple layered unit membrane and have cytoplasma, ribosome and strands of nuclear material, DNA is free in the cytoplasm (Bertamini *et al*., 2003). They arose from a gram-positive clostridiumlike bacterial ancestor of the lactobacillus lineage which appear to have suffered extreme genome reductions compared with their Gram-positive relatives.

The sizes of phytoplasma genomes vary considerably, ranging from 530 to 1350 kbp. The Bermudagrass white leaf phytoplasma has a genome size of just 530 kb, the smallest chromosome of any known living organism to date. The aster yellows phytoplasmas genomes, which are the largest (≤1185 kbp) among the phytoplasmas, are still much smaller than those of culturable relatives (*Acholeplasma* sp.) (Lee *et al*., 2000).

Phytoplasmas cause a wide variety of symptoms that ranges from mild yellowing to death of infected plants that suggest profound disturbances in the normal balance of plant hormones or growth regulators (Lee *et al*., 2000). A common symptom caused by phytoplasma infection include general yellowing or reddening of leaves, stunting, sterility of the flowers, bushy or witche's broom, proliferation of auxiliary shoots, reduced yields, increase in size of the internodes and necrosis of the phloem tissues. Some symptoms fairly unique to phytoplasma diseases such as phyllody, the production of leaf like structures in place of flowers and virescence (the development of green flowers and the loss of normal flower pigments) (Lee *et al*., 2000; Welliver, 1999).

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Phytoplasma detection: Diagnosis of phytoplasmas is routinely done by PCR and can be divided into three phases: total DNA extraction from symptomatic tissue; PCR amplification of phytoplasma-specific DNA; characterization of the amplified DNA by sequencing, RFLP analysis or nested PCR with group-specific primers (Marzachi, 2004). The success of these protocols relies on obtaining nucleic acid preparations of good quality, if possible enriched in phytoplasma DNA. The titre of phytoplasma cells in the phloem of infected plants varies according to the season, plant organ and the plant species and it is often very low in woody hosts. For these reasons, although phytoplasmas seriously threaten the cultivation of some very important crop species, their diagnosis is not simple (Firrao *et al*., 2007). Thus several methods have been developed to extract total phytoplasma DNA for the detection of phytoplasmas, aiming to concentrate it while reducing plant phenolics and polysaccharides (Ahrens and Seemuller, 1992; Daire *et al*., 1997; Green *et al*., 1999; Marzachi *et al*., 2000; Prince *et al*., 1993; Zhang *et al*., 1998). The main goal of each protocol is to concentrate phytoplasma DNA whilst decreasing plant polysaccharide and polyphenolic molecules that can inhibit the Taq DNA polymerase used in the PCR assay. Amplification will not occur if DNA preparations contain PCR-inhibiting substances. Most of the protocols involve a phytoplasma enrichment step followed by total DNA extraction, but some researchers also suggest addition of a proteinase digestion step. The Cetyl Trimethyl Ammonium Bromide (CTAB) method of Doyle and Doyle (1990), phytoplasma enrichment method of Kirkpatrick *et al.* (1987) and small scale DNA extraction method of Zhang *et al.* (1998) have been used to extract total nucleic acids from infected coconut tissues (Harrison *et al*., 2002a; Llauger *et al*., 2002; Sharmila *et al*., 2004; Warokka *et al*., 2006).

DNA extraction methods of a modification of an MLO-enrichment procedure described by Kirkpatrick *et al.* (1987) and Ahrens and Seemuller (1992), the Cetyl Trimethyl Ammonium Bromide (CTAB) method of Doyle and Doyle (1990) and the small scale procedure (Zhang *et al*., 1998) with some modification provided a good template for DNA amplification of phytoplasma (Nejat *et al*., 2009a; Warokka *et al*., 2006). On the other hand, the use of the small scale method is much faster and more convenient than the MLO-enrichment procedure for DNA extraction from plant tissue and insect vectors. This method also significantly decreased the amount of the tubes, reagents and chemicals and is relatively inexpensive compared to the MLO-enrichment procedure and commercially available nucleic acid extraction kits (Zhang *et al*., 1998).

The amount of phytoplasma DNA recovered from total DNA was significantly affected by the plant part used for DNA extraction (Kollar *et al*., 1990). Furthermore, the known low concentrations and uneven distribution of the phytoplasmas in the phloem vessels of infected plants increases the dependence of detection on the appropriately chosen material (Davis and Lee, 1993; Jones *et al*., 1999; Seemuller *et al*., 1998). Detection of LY phytoplasma by PCR has indicated highest sensitivity when the DNA sample has been extracted from immature palm leaf bases (heart tissues) rich in functional phloem (Harrison *et al*., 1994). Detection of LY by PCR employing ribosomal or non-ribosomal primer pairs has also been obtained with the DNA sample that has been extracted from leaves or inflorescence, trunk tissues and roots of coconut palm (Harrison *et al*., 1999). Such studies have also revealed that the LY phytoplasmas are more readily detected in immature than mature tissues (Escamilla *et al*., 1995; Harrison *et al*., 1995). Mpunami (1997) reported that phytoplasma DNA was detected in all meristematic tissues sampled, including the area below the growing point, the spear leaf, the petioles of very young leaves, inflorescences and root tips. This is in agreement with Warokka (1999) who showed that the highest concentrations were found in the area below the growing point, the petioles of young unopened leaves and the root tips. Therefore, the inability to locate the pathogen in mature leaves reflects perhaps an irregular distribution of phytoplasma rather than its absence from these organs (Harrison *et al*., 1994). The Coconut Yellow Decline (CYD) phytoplasma detection was consistent and reproducible for the affected coconut palm ecotypes when template DNAs for PCR were derived from either spear leaves, inflorescences or trunk tissues (Nejat *et al*., 2009b).

Since, phytoplasmas occur in low titre in the phloem tissues of their host-plants and their concentration may be subject to seasonal fluctuations. In woody plants, it is known that the persistence of phytoplasma in the aerial parts depends on the seasonal state of the phloem (Braun and Sinclair, 1976). The phytoplasmas overwinter in the root system and recolonize the stem in spring. In pears and apples, phytoplasmas disappeared from the above-ground parts of the trees due to cold temperatures during the winter months and that they survived in the roots of the scion to recolonize the stem and branches in the following spring (Seemuller *et al*., 1984). The reason behind this behaviour is thought to be the degeneration and complete inactivation of sieve tubes in the above ground parts of trees in winter (Evert, 1960; Jarausch *et al*., 1999; Schaper and Seemüller, 1982). Seemuller *et al.* (1998) reported that the highest level of phytoplasma population in proliferation-diseased apple

trees was reached in September or October in the young shoots and trunks, using fluorescence microscopy using DAPI.

The titre of phytoplasma in coconuts also depends on environmental and climatic conditions in Malaysia. The lower temperature and higher humidity in the wet season might allow circulation through phloem into the aerial parts of the tree to continue for longer. It may also reflect an increase in the concentration and the even distribution of phytoplasma in phloem tissue. As a consequence, CYD could continue to be distributed through the tree, thus enhancing the possibility of detection in December, without necessarily indicating an increase in CYD concentration.

Detection and identification of phytoplasmas is necessary for accurate disease diagnosis. Molecular diagnostic techniques for the detection of phytoplasma introduced during the last two decades have proven to be more accurate and reliable than biological criteria long used for phytoplasma identification (Lee *et al*., 2000). Polymerase Chain Reaction (PCR) is the most versatile tool for detecting phytoplasmas in their plant and insect hosts (Smart *et al*., 1996). Phytoplasma diagnostics has been routinely based on phytoplasma-specific universal (generic) (Table 1) or phytoplasma group specific Polymerase Chain Reaction (PCR) primers designed on the basis of the highly conserved 16S ribosomal RNA (rRNA) gene sequences (Ahrens and Seeuller, 1992; Davis and Lee, 1993; Deng and Hiruki, 1991a, b; Harrison *et al*., 1996; Jomantiene *et al*., 1998; Schaff *et al*., 1992; Smart *et al*., 1996). Phytoplasma group-specific primers have also been designed on ribosomal protein, SecA, SecY and elongation factor genes sequences (Table 2) (Gundersen *et al*., 1996; Hodgetts *et al*., 2008; Lee *et al*., 2006; Marcone *et al*., 2000; Schneider *et al*., 1997) and alternative diagnostic methods based on other gene sequences have been established, such as Heteroduplex Mobility Assays (HMAs) (Wang and Hiruki, 2005), Single Stand Conformation Polymorphisms (SSCP) (Music *et al*., 2008), Terminal Restriction Fragment Length Polymorphism (T-RFLP) (Hodgetts *et al*., 2007) and real-time PCR (Baric and Dalla-Via, 2004; Hren *et al*., 2007; Wei *et al*., 2004).

For sensitive detection of phytoplasmas in plants with very low levels of inoculum, specific primers based on Mollicutes 16S rRNA genes have been used to selectively amplify phytoplasma DNA from mixtures with host DNA (Ahrens and Seemüller, 1992; Davis *et al*., 1992; Deng and Hiruki, 1990, 1991a, b; Lee *et al*., 1993; Namba *et al*., 1993). Proof of phytoplasma association can be obtained by several DNA-based techniques including PCR, nested PCR and recently, real-time PCR for routine disease diagnosis and epidemiological studies. Generally, the 16S rDNA is amplified by universal primer pair P1/P7 followed by nested PCR with R16F2n/R16R2 primer pair (Deng and Hiruki, 1991b; Gundersen and Lee, 1996; Lee *et al*., 1993; Schneider *et al*., 1995). The choice of primer sets for phytoplasma diagnosis by nested PCR mostly depends on the phytoplasma we are looking for. Nested PCR with a combination of different universal primers can improve the diagnosis of unknown phytoplasmas present with low titre in the symptomatic host. Universal ribosomal primers nested with group-specific primers are extremely useful when the phytoplasma to be diagnosed belongs to a well-defined taxonomic group (Marzachi, 2004).

Unfortunately, some primers can induce dimers or unspecific bands. They also have sequence homology in the 16S-spacer region to chloroplasts and plastids increasing the risk of false positives (Heinrich *et al*., 2001). The universal phytoplasma primer pair, R16F2n/R16R2 amplified the 16S rRNA gene from coconut chloroplast derived from spear leaf and inflorescence samples of some asymptomatic coconut palms. These results indicate that R16F2n/R16R2 can amplify plant DNA. Secondly, *Bacillus* sp., were amplified with these primers from the inflorescences of some asymptomatic coconut palms. *Bacillus megaterium* has previously been isolated from

Table 1: PCR general primers commonly used for the detection of phytoplasma

Primer set	Nucleotide position	Oligonucleotide sequence	Location	PCR product length	Reaction	References
P1	6-30	5'-AAG AGT TTG ATC CTG GCT CAG GAT T-3'	16S/23S	1800 bp	PCR	Deng and Hiruki (1991b)
P7	68-51	5'-CGT CCT TCA TCG GCT CTT-3'				Schneider *et al*. (1995)
R16F2	152-168	5'-ACG ACT GCT AAG ACT GG-3'	16S/IS	1245 bp	Nested PCR	Lee *et al*. (1993)
R16R2	1397-1373	5'-TGA CGG GCG GTG TGT ACA AAC CCC G-3'				
R16F2n	149-168	5'-GAA ACG ACT GCT AAG ACT GG-3'	16S/IS	1240 bp	Nested PCR	Gundersen and Lee (1996)
R6R2	1397-1373	5'-TGA CGG GCG GTG TGT ACA AAC CCC G-3'				
fU5	369-386	5'-CGG CAA TGG AGG AAA CT-3'	16S	880 bp	Nested PCR	Lorenz *et al*. (1995)
rU3	1251-1231	5'-TTC AGC TAC TCT TTG TAA CA-3'				
SecAfor1	296-315	5'-GAR ATG AAA ACT GGR GAA GG-3'	secA gene	840 bp	PCR	Hodgetts *et al*. (2008)
SecArev3	1115-1138	5'-GTT TTR GCA GTT CCT GTC ATN CC-3'				
SecAfor2	650-668	5'-GAY GAR GSW AGA ACK CCT-3'	secA gene	480 bp	semi-nested PCR	Hodgetts *et al*. (2008)
SecArev3	1115-1138	5'-GTT TTR GCA GTT CCT GTC ATN CC-3'				

Table 2: Several group specific primers used for phytoplasma detection

Primer set	Oligonucleotide sequence	Specificity	Location	Expected size of PCR product	References
fTufAy	5'-GCTAAAAGTAGAGCTTATGA-3'	16SrI	tuf gene	940 bp	Schneider *et al.* (1997)
rTufAy	5'-CGTTGTCACCTGGCATTACC-3'				
AysecYF1	5'-CAGCCATTTTAGCAGTTGGTGG-3'	16SrI	secY gene	1400 bp	Lee *et al.* (2006)
AysecYR1	5'-CAGAAGCTTGAGTGCCTTTACC-3'				
rp(I)F1A	5'-TTTTCCCCTACACGTACTTA-3'	16SrI	Ribosomal protein	1200 bp	Lee *et al.* (2004a)
rp(I)R1A	5'-GTTCTTTTTGGCATTAACAT-3'				
rp(II)F1	5'-GCTCTTACTCGTAAAYATGTAGT-3'	16SrII	Ribosomal protein	1200 bp	Martini *et al.* (2007)
rp(II)R1	5'-TTACTTGATTTTCTGGTTTTGA-3'				
rp(III)F1	5'-TTAGAGAAGGCATTAAAC-3'	16SrIII	Ribosomal protein	1200 bp	Martini *et al.* (2007)
rp(III)R1	5'-CTCTTTCCCCATCTAGGACG-3'				
LY16Sf	5'-CAT GCA AGT CGA ACG GAA ATC-3'	16SrIV	16S	1400 bp	Harrison *et al.* (2002c)
LY16Sr	5'-GCT TAC GCA GTT AGG CTG TC-3'				
LYC24F	5'-CAT ATT TTA TTT CCT TTG CAA TCT G-3'	16SrIV	nonribosomal	1000 bp	Harrison *et al.* (1994)
LYC24R	5'-TCG TTT TGA TAA TCT TTC ATT TGA C-3'				
rp(V)F1A	5'-AGGCGATAAAAAAGTTTCAAAA-3'	16SrV	Ribosomal protein	1200 bp	Lee *et al.* (2004b)
rp(V)R1A	5'-GGCATTAACATAATATATTATG-3'				
rp(VI)F2	5'-GGTTGTTGATTTAATTCGTGGTC-3'	16SrVI	Ribosomal protein	1000 bp	Martini *et al.* (2007)
rp(VI)R2	5'-CCAGATATTCGTCTAGTATCAGAA-3'				
rp(VIII)F2	5'-AGTTGTCGATTTAATTCGTGGCA-3'	16SrVII, VIII	Ribosomal protein	1000 bp	Martini *et al.* (2007)
rp(VIII)R2	5'-CAGCAGATATTTGTCTAGTATCTGCG-3'				
rp(IX)F2	5'-GCACAAGCTATTTTAATGTTTACACCC-3'	16SrIX	Ribosomal protein	800 bp	Martini *et al.* (2007)
rp(IX)R2	5'-CAAAGGGACTAAACCTAAAG-3'				
rpStolF	5'-CGTACAAAATAATCGGGAGA-3'	16SrXII-A	Ribosomal protein	1372 bp	Martini *et al.* (2007)
rpStolR	5'-CGAAACAAAAGGTTTACGAG-3'				

Table 3: Oligonucleotide primers and TaqMan probes sequences used for phytoplasma detection by real-time PCR (Galetto and Marzachi, 2010)

Specificity	Location	Primer sequence	Probe	References
Universal	16S rDNA	5'-CGTACGCAAGTATGAAACTTAAAGGA-3'	5'-TCTTCGAATTAAACAACATGATCCA-3'	Christensen *et al.* (2004)
		5'-TGACGGGACTCCGCACAAGCG-3'		
Universal	16S rDNA	5'-AGGTTGAACGGCCACATTG-3'	5'-TTGCTCGGTCAGAGTTTCCTC-3'	Galetto *et al.* (2005)
		5'-ACACGGCCCAAACTCCTACGGGA-3'		
Universal	16S rDNA	5'-AAATATAGTGGAGGTTATCAGGGATACAG-3'	5'-AACCTAACATCTCACGACACGAACT-3'	Hren *et al.* (2007)
		5'-ACGACAACCATGCACCA-3´		

the trunk samples of date palms (*Phoenix canariensis* Chabaud) affected by the lethal decline phytoplasma using universal phytoplasma primer pair (P1/P7) in Texas (Harrison *et al.*, 2002c) more specific universal phytoplasma primers are currently being developed (Hodgetts *et al.*, 2007, 2008; Martini *et al.*, 2007) and it may be that these will be more suitable for diagnostics from samples such as coconuts which appear to harbor other Gram-positive bacteria in significant populations particularly on the spear leaves and inflorescences. Primers fU5/rU3 were used in nested PCR more reliable to detect from the spear leaves and inflorescences of palms (Nejat *et al.*, 2009c).

The use of nested PCR has been reported for diagnostic purposes particularly in trees and insect vectors when phytoplasmas occur in low titre in the phloem vessels of their host-plants and their concentration may be subjected to seasonal fluctuation (Gundersen and Lee, 1996; Jacobs *et al.*, 2003; Leyva-Lopez *et al.*, 2002; Marzachi, 2004).

The PCR program using parameters as described by Lee *et al.* (1993), using 1 min (2 min for an initial denaturation) at 94°C, 2 min at 55°C and 3 min at 72°C for 35 cycles and a final extension at 72°C for 10 min is time-comsuming require about 9 h for nest PCR. The following PCR program is shorter: 35 cycles of 1 min at 94°C, 1 min at 57°C and 90 sec at 72°C with a final elongation of 10 min at 72°C.

Real-time PCR method has many advantages over the conventional PCR in terms of accuracy, dynamic range, short analysis time, high automation capability, high-throughput capacity and absence of post-PCR manipulations that prevents carryover contamination (Higuchi *et al.*, 1993; Schaad and Fredrick, 2002). The application of this method to plant pathogens is increasing and in the case of phytoplasmas real-time PCR has been up to now applied for both phytoplasmas detection and quantification. Real-time PCR has been shown to be effective methods of quantifying the titre of phytoplasmas within the plant.

Recently three protocols for the universal diagnosis of phytoplasmas using direct real-time PCR amplification of the 16S rDNA gene have been developed (Christensen *et al.*, 2004; Galetto *et al.*, 2005; Hren *et al.*, 2007). Real-time PCR have been developed for diagnosis Flavescence Dorée (FD) and Bois Noir (BN) phytoplasmas infecting grapevines, Columbia Basin potato purple top and CYD novel phytoplasma infecting

Malayan yellow and red dwarf ecotypes (Aldaghi *et al*., 2007; Baric and Dalla-Via, 2004; Baric *et al*., 2006; Crosslin *et al*., 2006; Jarausch *et al*., 2004; Nejat *et al*., 2010).

Identification: The highly conserved 16S rRNA gene sequence has been widely used as the very useful primary molecular tool for preliminary classification of phytoplasmas. A total of 19 distinct groups, termed 16S rRNA groups (16Sr groups), based on actual RFLP analysis of PCR-amplified 16S rDNA sequences or 29 groups based on *in silico* RFLP analysis have been identified (Lee *et al*., 1998, Wei *et al*., 2007).

Phylogeny based on 16S ribosomal DNA (16Sr) sequences divides the phytoplasmas into three distinct clusters (Hogenhout *et al*., 2008). The first cluster (Cluster I) contains the Aster yellows (AY) 16SrI group and the stolbur (STOL) 16SrXII group phytoplasmas. These two groups have diverged but are clearly more closely related to each other than the other phytoplasma groups (Hogenhout *et al*., 2008). The second cluster (Cluster II) contains the Apple Proliferation (AP) 16SrX group phytoplasmas and the third cluster (Cluster III) contains the largest number of phytoplasma groups, including Western X (WX, 16SrIII), Palm Lethal Yellowing (LY, 16SrIV) and Elm Yellows (EY, 16SrV) (Hogenhout *et al*., 2008). To date, phytoplasma diseases belonging to 16SrI, 16SrXIV and novel phytoplasma have been identified in Malaysia (Nejat *et al*., 2009c).

Coconut palm phytoplasma diseases: The coconut palm is a versatile plant with a variety of uses in the world. Every part of it is useful to mankind in form or the other it supplies food, drink, oil and shelter and also raw materials for a number of industries for generations. Coconut cultivation faces a strong phytopathological constraint caused by the lethal yellowing (16SrIV) group phytoplasmas. Lethal yellowing is a highly destructive, fast spreading disease of coconut and at least 35 other palm species. Lethal yellowing in the USA (Harrison *et al*., 1994), Lethal Disease (LD) in Tanzania, East Africa (Schuiling *et al*., 1981), Cape St. Paul Wilt (CSPW) in Ghana, West Africa (Johnson and Harries, 1976; Nipah *et al*., 2007), Kaincope disease in Togo (Nienhaus and Steiner, 1976), Kribi disease in Cameroon (Dollet *et al*., 1977) and Awka or bronze leaf wilt (LDN) in Nigeria (Ekpo and Ojomo, 1990) are all classified as 16SrIV phytoplasmas. Subgroup 16SrIV-A phytoplasmas are associated with Lethal Yellowing (LY) of coconut and other palm species in the Americas (Harrison *et al*., 2002a) whereas, phytoplasmas that induce symptoms similar to LY on coconut in Africa are referred to by other names to reflect strain differences that have previously been identified through 16S rRNA gene analysis. Recently, Wei *et al*. (2007) have allocated the Nigerian coconut lethal decline group (LDN) to a distinct 16Sr group, 16SrXXII-A and this has been confirmed by the work of Hodgetts *et al*. (2008), who showed a high degree of divergence between the different coconut phytoplasmas based on the secA gene which supported their separation into at least three distinct *Ca*. Phytoplasma species that reflect the geographical origins of the strains.

Weligama wilt disease of coconut in Sri Lanka is in the 16SrXI *Ca*. Phytoplasma oryzae group based on its 16S rRNA sequence (ACC. No. EU635503), a group of phytoplasmas commonly found in sugar cane, whilst Kalimantan Wilt (KW) disease of coconuts in Indonesia which belongs to the two phylogenetic groups, 16SrXI *Ca*. Phytoplasma oryzae and 16SrXIII Mexican periwinkle virescence (Warokka, 2005).

Kerala Wilt Disease (KWD) of coconuts in India which as a different 16S rRNA phytoplasma group phylogenetically (16SrIV-C) (Edwin and Mohankumara, 2007; Sharmila *et al*., 2004). In Malaysia, a 16SrXIV *Ca*. Phytoplasma cynodontis phytoplasma was found associated with coconut yellow decline in Malayan Tall and Malayan Red Dwarf ecotypes and a novel phytoplasma was identified in Malayan Yellow dwarf ecotype (Table 4).

Cynodon dactylon L. Pers is commonly called Bermudagrass in many areas of the world. Bermudagrass white leaf (BGWL) is a destructive, phytoplasmal disease

Table 4: Coconut palm phytoplasma diseases

Disease caused	Accession No.	Phytoplasma group	Country	Reference
Coconut lethal yellowing	AF498307	*Candidatus* Phytoplasma palmae (16SrIV-A)	Jamaica	Harrison *et al*. (2002c)
lethal yellowing	AF500329	*Candidatus* Phytoplasma palmae (16SrIV)	Mexico	Harrison *et al*. (2002b)
Cape St Paul Wilt disease	Y13912	*Candidatus* Phytoplasma palmae (16SrIV)	Ghana	Tymon *et al*. (1998)
Tanzanian lethal disease	X80117	*Candidatus* Phytoplasma palmae (16SrIV)	Tanzania	Tymon *et al*. (1998)
Nigerian Awka disease	Y14175	16SrXXII-A	Nigeria	Tymon *et al*. (1998)
Kaincope disease Togo	-	Unknown	Togo	Nienhaus and Steiner (1976)
Kribi disease	-	Unknown	Cameroon	Dollet *et al*. (1977)
Kalimantan wilt	-	*Ca*. Phytoplasma oryzae (16SrXI) and Mexican periwinkle virescence (16SrXIII)	Indonesia	Warokka (2005)
Kerala wilt	AY158660	*Candidatus* Phytoplasma palmae (16SrIV-C)	India	Edwin and Mohankumara (2007)
Coconut yellow decline	EU328159	*Candidatus* Phytoplasma cynodontis (16SrXIV) and unclassified phytoplasma	Malaysia	Nejat *et al*. (2009a, c)

Table 5: Phytoplasmas related to the *Candidatus* Phytoplasma cynodontis (16SrXIV) group

Disease caused	Accession No.	Natural host	Country	References
Coconut yellow decline	EU636906	*Cocos nucifera* (coconut, Malayan tall)	Malaysia	Nejat *et al.* (2009b)
Coconut yellow decline	EU328159	*Cocos nucifera* (coconut, Malayan red dwarf)	Malaysia	Nejat *et al.* (2009a)
Bermudagrass white leaf	EU294011	*Cynodon dactylon*	Malaysia	Nejat *et al.* (2009c)
White tip die-back	AF100411	*Phoenix dactylifera* (date palm)	Sudan	Cronje *et al.* (2000a)
slow decline	AF268000	*Phoenix dactylifera* (date palm)	Sudan	Cronje *et al.* (2000b)
Bermudagrass white leaf	AF100412	*Cynodon dactylon* (Bermudagrass)	Sudan	Dafalla and Cousin (1988)
Bermudagrass white leaf		*Cynodon dactylon*	Taiwan	Chen *et al.* (1972)
Bermudagrass white leaf	EU032485	*Cynodon dactylon*	India	Snehi *et al.* (2008)
Bermudagrass white leaf	Y14645	*Cynodon dactylon*	Indonesia	Schneider *et al.* (1999)
Bermudagrass white leaf	AJ550984	*Cynodon dactylon*	Italy	Marcone *et al.* (1997)
Bermudagrass white leaf		*Cynodon dactylon*	Pakistan	Zahoor *et al.* (1995)
Bermudagrass white leaf	AF248961	*Cynodon dactylon*	Thailand	Sdoodee *et al.* (1999)
Annual blue grass white leaf		*Poa annua*	Italy	Seemuller *et al.* (1998)
Brachiaria white leaf		*Brachiaria distachya*	Thailand	Seemuller *et al.* (1998)
Dactyloctenium white leaf		*Dactyloctenium aegypcium*	Thailand	Seemuller *et al.* (1998)

of Bermudagrass has been detected in Thaiwan, Pakistan, China, Italy, Iran, Thailand, India and Malaysia. The causal agent of BGWL is *Candidatus* Phytoplasma cynodontis (Marcone *et al*., 2004). In Northern Sudan, a 16SrXIV *Ca*. Phytoplasma cynodontis phytoplasma was found associated with slow decline and white tip die-back in date palm (Cronje *et al*., 2000a) (Table 5).

Control of phytoplasma diseases: In controlling phytoplasmal diseases, the primary concern is often prevention rather than treatment. The most promising strategy for avoiding phytoplasma disease is the use of disease resistance varieties of crops. Phytoplasmas are normally controlled by planting disease-free stocks (Propagate from seed, or from phytoplasma-free plants), control of insect vectors and weed plant hosts act as sources of inoculums, Rogue out and destroy symptomatic plants and avoid planting susceptible crops next to crops harboring the phytoplasma (Lee *et al*., 2000; Welliver, 1999).

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