

Review

Lethal yellowing disease of the coconut palms (*Cocos nucifera* L.): An overview of the crises

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Phytoplasmas are minute cell, wall-less prokaryotes with a diameter less than 1 micrometer ranging from 200 to 800 μm . They have cytoplasm, ribosome and strands of nucleus materials. They arose from gram-positive Clostridium-like bacterial ancestor of the lactobacillus lineage, which appears to have suffered extreme genome reductions when compared with their gram-positive relatives. The inability to culture phytoplasmas in any axenic media and the low concentration in phloem of infected plants are obstacles for efficient diagnosis. Polymerase chain reaction (PCR) amplification is the most important tool for detecting phytoplasmas in plant and insect hosts. Real-time PCR method has so many advantages over the conventional PCR. Recent reports across coconut growing regions tell increase in the incidence of lethal yellowing disease (LYD). Palms with greater than 25% leaf discoloration due to LYD should be removed. Fields replanted with the tolerant varieties must be monitored using molecular technique. The overview crises of more than 100 years after the incidence of phytoplasmas and more than 50 years of research efforts to control LYD of coconut palms world wide are presented.

Keywords: Polymerase chain reaction and lethal yellowing disease

INTRODUCTION

Coconut cultivation faces a strong phytopathological constrain 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. About 10 million families rely on coconuts as their main source of food and incomes (IPGRI, 2004). Today, coconut cultivation faces a strong phytopathological constraint generated by the lethal yellowing disease (Cordova et al., 2003). It has destroyed several thousands hectares of coconut fields (Dery et al., 1995). Problem with lethal yellowing go back more than 100 years in the Caribbean. It was discovered in Cayman Islands in 1834 and 50 years later, showed up in the Western end of Jamaica (CIRAD, 2008). Phytoplasma,

formally termed mycoplasma-like organisms (MLOs), were first discovered in the early 1900s (Kunkel, 1926). Geographically, the occurrence of phytoplasmas is world wide (Bertaccini, 2001; Hogenhout et al., 2008). Its incident differs among the affected regions and is referred to as Cape Saint Paul wilt in Ghana (Dabek et al., 1976), Kain-cope disease in Togo (Nienhaus and Steiner, 1975), Kribi disease in Cameroon (Dollet et al., 1977) and Awka wilt disease in Nigeria (Ekpo and Ojomo 1990).

Weligama wilt disease of coconut in Sri Lanka is in the 16SrXI *Ca*. Phytoplasma *oryza* group based on its 16S rRNA sequence (Accession No. EU635503) is a group of phytoplasma commonly found in sugarcane, whilst Kalimantan wilt (KW) disease of coconut in Indonesia belongs to the two phylogenetic groups 16SrXI *Ca*. Phytoplasma *oryza* and 16SrIII Mexican periwinkle virescence (Warokka, 2005). Kerala wilt disease (KWD) of coconuts in India which is a different 16S rRNA phytoplasma group phylogenetically (16SrIV-C) (Edwin and Mohankumara, 2007). In Malaysia, a 16SrXIV *Ca*. Phytoplasma *cynodontis* was found associated with coconut yellow decline in Malayan tall and Malayan red

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Abbreviations: MLOs, Mycoplasma-like organisms; LYD, lethal yellowing disease; MYD, Malayan yellow; AY, Aster yellows; STOL, stolbur; BGWL, Bermudagrass white leaf.

dwarf ecotypes and a novel phytoplasma was identified in Malayan yellow ecotype (Nejat and Vadamalai, 2010).

The Awka wilt or lethal yellowing disease (LYD) of the coconut palm was first reported in Nigeria in 1917 at the Awka district in the former Eastern region (Ekpo and Ojomo, 1990). The disease has practically wiped off great majority of the palms in South-east, South-south and is now extending towards the South-west of Nigeria. Crownless coconut palms are a common sight in these areas (Omamor, 2010). In Nigeria, the coconut grove populations are estimated at 13,615 hectare with over 2 million coconut trees of mainly the WAT cultivar, providing livelihood for over 30,000 rural families for whom coconut is the source of food, wood-fuel, building material, drink e.t.c. (Osagie et al., 2008). The first indication of LYD in a ten hectare coconut plantation was in 1995 when a general disease survey was conducted. By the dry season of the 11th year (2006), 98.8% of the West African tall (WAT) palms were dead, while an average of 72% of the dwarfs were lost (Odewale et al., 2010). Recently Wei et al. (2007) have allocated the Nigerian coconut lethal decline group (LND) 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 origin of the strains (Negat and Vadamalai, 2010).

The Cape Saint Paul wilt disease (CSPWD), a lethal yellowing type of coconut has been in Ghana since 1932. The disease epidemic which began around Cape Saint Paul in Woe near Keta destroyed thousands of coconut palms and caused collapsed of the coconut industry in the Volta region by the mid 1950s (Leather, 1959). The disease appeared in the Western region at Cape three points in 1964 and in the central region at Ayensudo in 1983. The historic, occurrence, epidemiology and spread of the disease in Ghana have been reported by Dery et al. (1999). Aerial and/or ground surveys were undertaken to assess the current status of the disease spread. The survey showed that, the spread of the disease for the past years has mainly been the expansion of existing foci. From field observations, the estimated rate of spread per year of CSPWD is 0.33 km or about 1 km per 3 years (Nkansah-Poku et al., 2008). In Ghana, about one million coconut palms were killed during the last 30 years (Dollet and Giannolli, 1976).

In Tanzania, coconut palm is an economically important perennial oil crop that supports the livelihood of most farmers in the coastal belt of Tanzania providing food, shelter and rural income. The lethal disease has however, become one of the main factors limiting coconut production in the country. Since the early 1960s, this disease has killed eight million palms or 38% of the total palm population in the mainland (Schuiling et al., 1992). The lethal disease occurs throughout the coastal belt of mainland Tanzania. It is widespread in the southern

regions where it has killed about 56% of the palms during the last 30 years, while only 8.5% are affected in the north (Mpunami et al., 1997). In Tanzania and Mexico, thousands of hectares were destroyed but no precise evaluation is available. In Togo, by 1964, about 60,000 palms or 50 percent of the coconut grooves were also destroyed by the so called Kaincop disease. In Florida, by 1973, at least 20,000 coconut palms (about 6 percent of the total) were affected by the disease (ippmedia.com, 2008).

The Honduras confronts a disease that threatens not only the country's vital tourist industry but also the livelihoods of its coastal peoples. Lethal yellowing disease (LYD) has invaded the coconut palms that fringe the beaches of the Atlantic coast and the Bay islands and is spreading rapidly. There are reports of LYD that date back to the 19th century in Grand Caiman islands. International attention was first drawn to the problem in the 1960s. By 1980, the disease had been responsible for the death of over 7 million palms in Jamaica (Roca de Doyle, 2001).

The Jamaica epidemic was matched by similar outbreaks in Cuba, Haiti, the Dominican Republic, the Bahamas and Florida. In 1977 the disease arrived in Cuzumel-cancun in Mexico and began to move down the Yucatan peninsula to Belize. It reached Honduras in 1995 and in the last six years, it has destroyed 70 per cent of the country's original coconut population (Roca de Doyle, 2001). In Jamaica, the maypan, a hybrid of Malayan yellow (MYD) and Panama tall coconut previously considered highly resistant, is currently being devastated by an epidemic outbreak of lethal yellowing (CIRAD, 2008). It is quite difficult to get a precise evaluation of the losses caused by Ly disease. The coconut industry board records show that, out of the 6 million susceptible Jamaica tall coconut palms in 1961, 90 percent had been killed by 1981 (ippmedia.com, 2008).

PATHOGEN AND HOSTS

Phytoplasma 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 world wide and most prevalent in tropical and sub-tropical regions of the world (Bertaccini, 2007). Phytoplasma 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 chloroplasts, a rounded pleiomorphic or filamentous shape surrounded by a tripled layered unit membrane and have cytoplasm, ribosome and strands of nucleus material, DNA is free in the cytoplasm (Bertamini et al., 2003). They arose from a gram-positive clostridium-like bacterial ancestor of the lactobacillus lineage which appears to have suffered extreme genome reductions compared with their gram positive relatives (Bertamini et al., 2003).

The principal means of transmission of phytoplasmas

between plants is by phloem-feeding insects and whilst the vector for LY, in the southern Florida of USA is the planthopper *Myndus crudus* Van Duzee, the vectors for the African LYDs have not been confirmed. Recently there have been reports in which LY DNA has been detected in coconut embryos (Cordova et al., 2003). The phytoplasma is a systemic pathogen that is found only in the phloem tissue (vascular tissue transporting carbohydrates) of palms. It is not known to survive outside either its plant or insect hosts. The planthopper is a piercing and sucking insect, meaning it feeds on the contents of plant host vascular system, including the phloem. The insect moves the phytoplasma from palm to palm as it moves during its feeding cycles (Harrison and Jones, 2004; Broschat et al., 2002). Electron microscope analysis of tissue from affected palms revealed phloem cells packed with the phytoplasma. This is assumed to cause a physical obstruction to the flow of nutrients, which eventually kills the tree (Roca de Doyle, 2001).

SYMPTOMS

As with any disease, diagnosis is based on series of symptoms (Figure 1). Furthermore, no single symptom is diagnostic of LY. Rather, it is the appearance and chronological progression of symptoms that accurately identifies the disease. The difficulty with LY diagnosis is that, symptoms vary according to the palm species and in the case of coconuts, the particular cultivar involved (Harrison and Jones 2004). General symptoms are premature nut fall, bronzing of successively younger leaves and blackening of inflorescence. Infected trees often die 4 to 6 months after appearance of symptoms (Mpunami, 1999). In spite of research efforts, no efficient methods have been identified yet to control this disease (Konan-K et al., 2007).

DIAGNOSIS AND IDENTIFICATION

Detection of phytoplasma for diagnostic purpose has been complicated by several factors including an inability to culture these pathogens in culture media, their small size and presence in low numbers in plant tissues (Thomas and Norris, 1980). The plant symptoms described earlier, are relied upon to make the initial diagnosis. Since the phytoplasma is not culturable, a molecular diagnostic test is used to confirm the presence of the pathogen. Increased sensitivity in phytoplasma detection has been attained through amplification of phytoplasma genomic DNA sequences by the use of PCR assays (Ahrens and Seemuller, 1992). In recent years, molecular markers have been applied to a wide number of genetic and breeding studies (Omamor et al., 2010).

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 and RFLP analysis or nested PCR with group-specific primers (Marzachi, 2004). Detection and identification of phytoplasma is necessary for accurate disease diagnosis. Molecular diagnostic techniques for the last two decades have proven to be more accurate and reliable than biological criteria long used for phytoplasma identification (Lee et al., 2000). For sensitive detection of phytoplasma in plants with very high levels of inoculum, specific primer based on Mollicutes 16S rRNA genes have been used to selectively amplify phytoplasma DNA from mixtures with host DNA (Lee et al., 1993). Unfortunately, some primers can induce dimmers or unspecific bands. They also have sequence homology in the 16S-spectar region to chloroplast and plastids increasing the risk of false positives (Heinrich et al., 2001).

Bacillus megaterium has previously been isolated from trunk of date palms (*Phoenix canariensis*, Chaband) affected by the lethal decline phytoplasma using universal phytoplasma primer pair (P1/P7) in Texas (Harrison et al., 2002), more specific universal phytoplasma primers are currently being developed (Hodgetts et al., 2008; Martini et al., 2007; Nejat and Vadamalai, 2010).

Real-time PCR method has many advantages over the conventional PCR in terms of accuracy, dynamic range, short analysis time, light automation capability, high-throughput capacity and absence of post-PCR manipulations that prevents carryover contamination (Schaad and Fredrich, 2002). The application of this method to plant pathogens is increasing and in the case of phytoplasmas, real-time PCR is now been 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 plants. Recently, three protocols for the universal diagnosis of phytoplasma using direct real-time PCR amplification of the 16SrDNA gene has been developed (Galletto et al., 2005; Hren et al., 2007).

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 silico RFLP analysis have been identified (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 1) contains the Aster yellows (AY) 16SrI group and the stolbur (STOL) 16SrXII group phytoplasmas. These 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 2), contains the apple proliferation (AP) 16SrX group phytoplasmas and the third cluster (Cluster 3), contains the largest number of phytoplasma groups, including

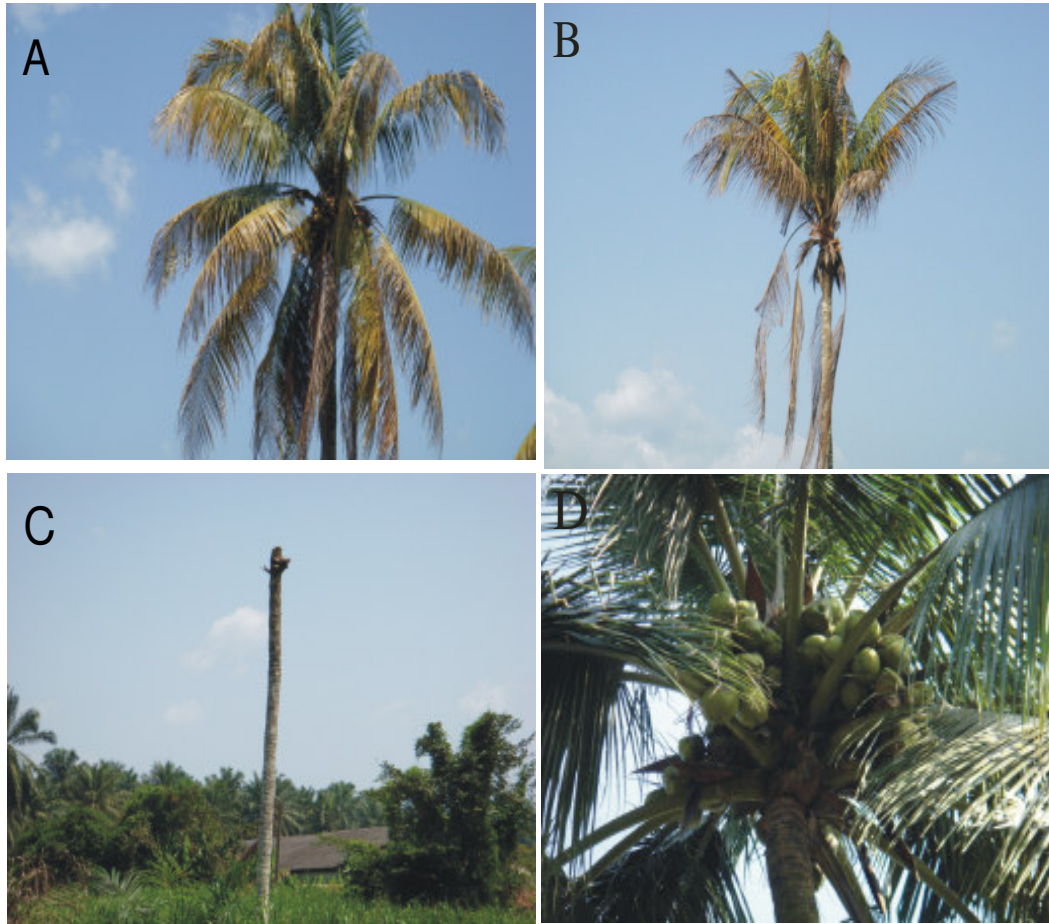


Figure 1. Photographs showing incident of lethal yellowing disease of coconut palm. (a) Yellowing of the fronts, dropping of the nuts and blackening of the inflorescence; (b) advance stage of wilting of the palm; (c) decapitate (crownless) coconut palm; (D) healthy coconut palm.

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., 2009).

Cynodon dactylon L. Pers is commonly called Bermudagrass in many areas of the world. Bermudagrass white leaf (BGWL) is a destructive phytoplasmal disease. It has been detected in Taiwan, 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., 2000).

DISEASE MANAGEMENT

Lethal yellowing diseases have devastated coconut plantations in tropical regions of Africa and sub-tropical

regions of the world, causing severe economic hardship and environmental damage. United Kingdom and African scientists are joining efforts to understand the disease and to develop new varieties of coconut that are able to resist infection. Coconut breeders have identified some palm varieties which show resistance or tolerance to the diseases. They want to study these coconuts and exploit their resistance to produce improved varieties for future plantations (DFID, 2008).

In controlling phytoplasmal diseases, the primary concern is often prevention rather than treatment, which include 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 phytoplasma (Lee et al., 2000). Symptoms of these phytoplasmas may be suppressed by regular tetracycline application but commercial control is primarily being achieved by replanting with resistant varieties (ISID, 2008). To reduce the rate of spread of the disease, eradication of diseased palms and those showing disease symptoms must be felled with a chain

saw machine, fronds pruned off and trunk cut into pieces of meters to facilitate quick drying. All fields replanted with the tolerant varieties must be monitored. All cases of disease development based on visual symptoms must be recorded. First infection cases in each plot must be verified and then confirmed by PCR analysis (Nkasah-Poku et al., 2009). Omamor et al. (2010) using RAPD demonstrated that, presently infected LYD coconut varieties are phylogenetically similar with (non-LYD) apparently healthy coconut palm. New resistant varieties well adapted to local conditions and requirements are urgently needed. Developing resistant varieties is a lengthy process and takes years using conventional method. The process could be speeded up with the help of biotechnology. Molecular markers, for example, could be used to detect possible resistant genes in varieties that have not yet been tested. Marker assisted breeding has been used successfully for diseases in other tree species, but no resistance markers have yet been published for coconut palms. Biotechnology could also hold the key to the mass production of resistant varieties by tissue culture (Roca de Doyle, 2001).

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