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# GLOBAL SCENARIO ON PHYTOPLASMAL DISEASES IN PALMS

Vinayaka Hegde[1,]*, Merin Babu[2] and A Josephrajkumar[2]

[1]ICAR-Central Plantation Crops Research Institute, Kasaragod 671124, Kerala, India
[2]ICAR-Central Plantation Crops Research Institute, Regional Station, Kayamkulam, Kerala, India
*Email: hegdev64@gmail.com (corresponding author)

**ABSTRACT**

**Worldwide phytoplasma diseases pose great threat to the sustainability of the palm family members viz., coconut, arecanut, date palm, oil palm and ornamental palms causing setbacks in economic and livelihood security. So far, at least eight *Candidatus* Phytoplasma species belonging to different 16Sr groups have been reported to be associated with coconut. This includes *Ca.* P. asteris (lethal wilt disease in India; 16Sr IB), *Ca.* P. oryzae (Coconut root (wilt) disease in India, Weligama leaf wilt disease in Sri Lanka; 16Sr XIB), *Ca.* P. cynodontis (Coconut yellow decline in Malaysia; 16Sr XIV), *Ca.* P. malaysianum (Malayan yellow dwarf phytoplasma in Malaysia; 16Sr XXXIIB), *Ca.* P. palmicola (Cape St. Paul wilt in Ghana, Awka wilt in Nigeria; 16Sr XXIIB), *Ca.* P. cocostanzaniae (lethal yellowing in East Africa; 16Sr IVC), *Ca.* P. noviguineense (Coconut bogia syndrome in Papua new Guinea) and *Ca.* P. palmae (Coconut lethal yellowing in Americas and Caribbean region; 16Sr IVA,B,E). Though there are several reports on insects transmitting coconut phytoplasmal diseases, except for LY in Florida and RWD in India, the vectors remain elusive. Susceptibility of date palms to phytoplasmal diseases viz., lethal yellowing, Al-wijam, White Tip Dieback (WTD) and Slow Decline (SD) or El Arkish' are emerging concerns in Arabian countries and North Africa. Oil palm is a potential host of diverse groups (16SrI, 16SrXI, 16Sr XIV and 16SrXXXII) of phytoplasma in many parts of the world. Yellow leaf disease caused by 16SrI, 16SrXI and16SrXIV group phytoplasmas is one of the major diseases limiting the productivity of arecanut in south India. Many ornamental palm species harbour a wide array of phytoplasma groups that could serve as an intermediary mode of transmission to cultivated palms. Large scale movement of ornamental palms through international trade warrants the need for strengthening the international quarantine networks and bio-security measures to contain the spread of these phytopathogenic mollicutes. Despite rapid progress accomplished in taxonomy using molecular approaches many palm phytoplasma isolates are yet to be characterized for the assignment of specific taxonomic status. Due to its confinement to phloem, non-uniform distribution and sub-minimal titres in palms, molecular detection is many atimes intriguing. Palm-phytoplasmal interaction and transmission mechanism by auchenorrhynchan fauna are poorly understood. As a victim of climate change, phytoplasma diseases in synergy with survival superiority of insect vectors are extremely challenging to comprehend and evolve effective suppression mechanism. Management approaches involving surveillance, eradication and containment of the disease in newly emerging areas along with resistance breeding to ensure sustainable income to the farmers are discussed in this chapter.**

**Key words:** Arecaceae, mollicutes, vectors, management, diagnosis

Palms (Family: Arecaceae) are monocotyledonous flowering plants widely distributed across the world. This family consists of 181 genera and approximately 2600 species (Baker and Dransfield, 2016). Palms occupy a unique position in tropical and sub-tropical ecosystems with their high species diversity and adaptation to grow in a wide range of habitats. Palms also form an integral part of the culture, cuisine, customs and traditions of several rural communities. They serve as the primary source of income for farmers in many resource-poor countries and are acknowledged as livelihood service providers. Biotic and abiotic stresses limit the growth and productivity of palms and are reported worldwide. Diseases caused by phytoplasmas are a real threat to palm cultivation calling for global solidarity to tackle such menace. The presence of phytoplasmal diseases in palms has a long history of more than 100 years upsetting the economy of several countries (Gurr et al., 2016). They were found pathogenic to more than 50 different palm species growing in different continents

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(Yankey et al., 2018). The recent increase in reports of palm phytoplasmal diseases from different geographical locations and expansion of the host range of existing phytoplasmas to new palm species are viewed very seriously by the researchers. Being fatal on several instances, phytoplasma diseases in palms and the insect vectors associated have drawn international attention to evolve holistic solutions in the multilateral system. This review outlines the global scenario of phytoplasmal diseases in palms.

## Phytoplasmal diseases of palms- concern across continents

The discovery of Mycoplasma-Like Organisms (MLOs) by Doi et al. (1967) unveiled the etiology of many palm diseases. Phytoplasmal diseases have been reported from several economically important palm species in America, Africa, Asia and Oceania. The devastating losses of coconut plantations due to phytoplasmal diseases have been recorded since the late 19th century. Some of them are lethal and widespread, whereas others are only debilitating affecting palm health, or are limited to certain geographic regions. The most well-known phytoplasmal disease of coconut is the Lethal Yellowing (LY) in the Americas. After its first scientific report from Jamaica in 1891 (Fawcett, 1891), the disease was recorded from Cuba (De La Torre, 1906), Haiti (Llauger et al., 2002), Florida (Martinez and Roberts, 1967), Mexico (Romney and Harries, 1978; Harrison et al., 2002a), Dominican Republic (Carter and Suah, 1964), Bahamas (Leach, 1946) and Honduras (Ashburner et al., 1996). The disease caused the death of five million Jamaica Tall palms in Jamaica, 6.5 lakh palms in the Yucatan peninsula and one lakh coconut palms in Florida (Mora 2002; Romney, 1983). The coconut production in Haiti declined from 60 million to 30 million nuts a year due to LY. The destructive disease killed about 90% of the palms in the Honduran Atlantic coast and about 80% of palms in the western region of Haiti (Donis, 2002; Doyle, 2001; Myrie, 2002).

Phytoplasmal diseases have annihilated millions of palms namely coconut (*Cocos nucifera* L.), arecanut (*Areca catechu* L.), date palm (*Phoenix dactylifera* L.) oil palm (*Elaeis guineensis* Jacq.) and other ornamental palms across several countries in Africa. Kaincope disease (Maladie de Kaincope) in Togo (Nienhaus and Steiner, 1976), Awkawilt disease (AWD) or bronze leaf wilt in Nigeria (Bull, 1955), Kribi disease in Cameroon (Dollet et al., 1977), Cape St. Paul Wilt Disease (CSPWD) in Ghana (Dabek et al., 1976), Cote d'Ivoire lethal yellowing (CILY) in Cote d'Ivoire (Konan Konan et al., 2013) and lethal decline (LD) reported from Tanzania (Schuilling and Mpunami, 1990), Mozambique (Mpunami et al., 1999) and Kenya are the major phytoplasmal diseases of coconut palms occurring in Africa. The Bogia Coconut Syndrome (BCS) reported from Papua New Guinea (Kelly et al., 2011) is an emerging threat to coconut cultivation in Oceania. CSPWD has wiped out about one million coconut trees in Ghana (Nipah et al., 2007). In Nigeria, 'Awka wilt' killed over 98% of the West African Tall (WAT) and LD destroyed about 56% of palms in southern Tanzania (Mpunami et al.,1999; Odewale et al., 2010) highlighting the seriousness of the disease in the international coconut sector.

In Asia, phytoplasmal diseases of coconut palms have been reported from India, Sri Lanka, Indonesia and Malaysia. This includes Root (Wilt) Disease (RWD), Lethal Wilt Disease (LWD) and Tatipaka from India, Weligama Coconut Leaf Wilt Disease (WCLWD) from Sri Lanka, Kalimantan Wilt (KW) of Indonesia and Coconut Yellow Decline (CYD) from Malaysia (Butler, 1908; Rao et al., 1956; Sitepuet al., 1988; Wijesekara et al., 2008; Nejat et al., 2009a; Babu et al., 2021). Among these RWD, WCLWD and Tatipaka are non-lethal whereas LWD, KW and CYD are fatal to palms. WCLWD causes about 40 to 60% reduction in yield (Weerakkody, 2010) and the crop loss due to RWD was estimated as 968 million nuts (Anonymous, 1985).

Another economically important palm species that is vulnerable to phytoplasma infection is the date palm (*Phoenix dactylifera* L.). The crop is considered as an important component of farming systems in dry and semi-arid regions and is suitable for both small and large-scale farming (Khushk et al., 2009). It is an important subsistence crop in most of the world's desert areas which earns a good amount of foreign exchequer as well. The susceptibility of *Phoenix* spp. to lethal yellowing type disease has been reported as early as in the 1970s (Thomas, 1974). McCoy et al. (1980) first reported the LY of date palms from Texas. The disease is also known as lethal bronzing disease (LBD) or Texas Phoenix Palm Decline (TPPD) was later reported from edible date palm in Florida (Harrison et al., 2008) and central Mexico (Padilla et al., 2011). White Tip Dieback (WTD) affecting young date palms of 5-8 years old and Slow Decline (SD) or 'El Arkish' of mature date palms occurs in the Northern Sudan region in North Africa (Cronje et al., 2000a &b). The disease is prevalent along the Nile between Dongola and Merowe-Karema causing an annual yield loss of about 6%. Phytoplasma diseases limit the productivity of date palms in Middle East countries also. The Al-Wijam disease is a major concern to date palm cultivation in Saudi Arabia (Alhudaib et al., 2007a & b). Furthermore, phytoplasma diseases of date palms have been reported from Kuwait (Al-Awadhi et al., 2002), Egypt (Al-Khazindar, 2014), Iran (Zamharir and Eslahi, 2019) and recently from Oman (Hemmati et al., 2020).

The susceptibility of oil palm to phytoplasma poses a threat to the global vegetable oil industry. Oil palm is grown extensively in South-East Asian, African and South American countries. Among the various phytoplasmal diseases, lethal wilt is a threat to oil palm cultivation. It was recorded as early as 1994 from Colombia (Alvarez et al., 2014). By 2010, the disease killed a total of 97619 palms in about 690 ha area. In India, Spear Rot Disease of oil palm (SRD) was reported in Kerala to the extent of 1.04% with a range of 0.12% to 7.18% in different plantations (Kochubabu, 1993). The presence of phytoplasma has been reported from oil palm in Ecuador (Bolanos et al., 2019), Mozambique (Bila et al., 2015) and Malaysia (Nejat et al., 2013).

Yellow Leaf Disease (YLD) is the most serious phytoplasma malady affecting arecanut cultivation in Asia. The disease has been reported from India (Nambiar, 1949), China and Sri Lanka (Kanatiwela- de Silva et al., 2015). The YLD considerably reduces the production and quality of the betel nut.

**Symptoms of phytoplasmal infection in palms- yellowing to wilting**

Symptoms due to phytoplasmal infection in palms vary according to the palm species, cultivar and pathogen group. Most of the coconut phytoplasmal diseases are lethal except Root (Wilt) Disease (RWD) and Weligama wilt (WCLWD). For most of the lethal phytoplasmal diseases of coconut, abnormal shedding of nuts followed by inflorescence necrosis, yellowing and drying of leaves and death of bud form the characteristic symptoms (Fig. 1). In some cases, the yellowing may not be pronounced as in the case of Awka Wilt of coconut and lethal bronze disease in date palm. The symptoms of Texas Phoenix Palm Decline (TPPD) (16SrIV-D) and LY (16SrIV-A) on date palm are similar with one exception; root decay has been observed with TPPD early in the disease process (Harrison and Elliott, 2016). Inflorescence necrosis was found to be absent in bogia coconut syndrome reported from Papua New Guinea (Kelly et al., 2011).The characteristic symptoms of RWD and WLCWD include flaccidity, yellowing and marginal necrosis of leaflets (Fig. 2). The date palm phytoplasma diseases in Arab countries are identified by the presence of yellow streaks on leaf petiole and marked reduction in fruit size. The primary symptoms

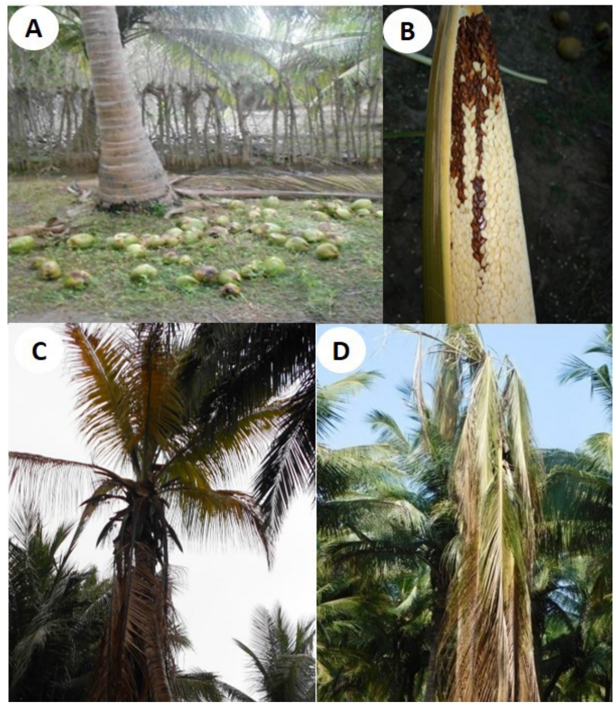


Fig. 1. Impact of root (wilt disease). A, Abnormal shedding of nuts; B, Inflorescence necrosis; C and D, Yellowing and drying of leaves in coconut palm

Fig. 2. Root (wilt) disease affected coconut palm

of the lethal wilt disease of oil palm include vascular discolouration and yellowing of leaves. These leaves dry off, wilt and eventually the palm collapse.

**Palms-a haven for phytoplasmas**

Palms serve as hosts of diverse groups of phytoplasmas. Worldwide palms have been affected by nine 16Sr groups of phytoplasmas viz., 16SrI, II, IV, VII, XI, XII, XIV, XXII and XXXII (Table 1). Among cultivated palms, coconut harbours the highest number of phytoplasma groups followed by date palm and oil palm. Coconut palm is reported as the host of phytoplasmas belonging to diverse groups viz., *Candidatus* P. asteris (16SrI), '*Ca.* P. palmae' (16SrIV-A,B,E), 16Sr IV D, '*Ca.* P. cocostanzaniae' (16SrIV-C), 16SrVII, '*Ca.* P. palmicola' (16SrXXII-A,B), '*Ca.* P. oryzae' (16SrXI), '*Ca.* P. cynodontis' (16SrXIV), '*Ca*. P. malaysianum' (16SrXXXII-B) and '*Ca.* P. noviguineense' (Babu et al., 2021; Arocha-Rosete et al, 2014; Bertaccini et al., 2014; Harrison et al., 2014; Nejat et al., 2013; Pereraet al., 2012; Wijesekhara et al., 2013; Miyazaki et al., 2018). Several species of ornamental palms are reported as hosts of different groups of phytoplasma. Most of the ornamental palms used for landscaping in America were found to be susceptible to phytoplasma belonging to the 16Sr IV subgroup (Harrison et al., 1999; Bahder et al., 2019a).

**Epidemiology of palm phytoplasmal diseases-a grey area**

Epidemiological studies play a key role in formulating strategies to prevent the spread of plant diseases. Phytoplasmal diseases are primarily transmitted by auchenorrhychous insects belonging to the order Hemiptera. As soon as a phytoplasmal disease is reported, research on vectors was also initiated. Being a persistent and circulative pathogen, phytoplasma once acquired by the insect needs to cross the gut as well as the salivary gland barrier to become infective and for transmission. Those insects lacking this ability may not become vectors for transmitting phytoplasma diseases. Most of the insect vectors of phytoplasma are non-destructive feeders except the lace bug, *Stephanitis typica* transmitting RWD and brown marmorated stink bug, *Haylomorpha halys*, transmitting witches broom phytoplasma to *Paulownias* sp. (Weintraub and Beanland, 2006).The most appropriate method for determining the vectoral role of an insect species is the cage transmission test. For palm-phytoplasmal pathosystems, this approach has several logistical constraints as it is very difficult to cage and maintain perennial woody palms for a long time. Hence, only very few insects have been identified as phytoplasmal vectors through conventional transmission trials attempted so far. The first successful report was the transmission of LY in coconut by the planthopper, *Haplaxius crudus* in Florida (Howard et al., 1983). Thirty years later, the role of *H. crudus* as a vector of LY in *Pritchardia pacifica* was confirmed by cage transmission experiments in Yucatan, Mexico (Dzido et al., 2020). The role of a lace bug (*S. typica*) and planthopper (*Proutista moesta*) in transmitting RWD phytoplasma has been conclusively established through cage transmission experiments and detection through electron-microscopy (Mathen et al*.,* 1990; Rajan, 2011). Despite the extensive transmission studies conducted in Ghana from 1990s, the vector of CSPWD in Ghana remains unknown (Philippe et al., 2009; Danyo, 2011). Several hemipteran insects have been reported as putative vectors of palm phytoplasmal diseases based on PCR results (Dollet et al., 2011;Kumara et al., 2015; Kwadjo et al., 2018; Alhudaib et al., 2007a, Pilotti et al., 2014), but their vector status has not yet been established by cage transmission trials. Tanne et al. (2001) developed a feeding medium approach as an alternate method to bypass the use of caged palms in the preliminary vector screening trials. The single-insect feeding medium tests coupled with Loop-Mediated Isothermal Amplification (LAMP) developed by Lu et al. (2016) to identify putative vectors of BCS also help in the rapid screening of potential vectors.

Until 1990s, it was thought that there is no possibility of seed transmission as phytoplasmas are phloem-limited pathogens. During the past two decades, evidence for seed transmission of phytoplasma has been reported from different countries. Harrison and Oropeza (1997) detected the presence of lethal yellowing phytoplasma in seeds from LY-affected coconut palms. The presence of LY phytoplasmal DNA was confirmed by PCR in 18.06% of embryos from fruits of diseased Atlantic Tall coconut palms (Cordova et al., 2003). CSPWD phytoplasma was detected in nine out of the 52 embryos from diseased West African Tall (WAT) palms by PCR (Nipah et al., 2007). The presence of phytoplasma DNA in embryos of nuts collected from the root (wilt) diseased coconut palms was reported by Manimekalai et al. (2014a). The detection of LY phytoplasma in coconut plantlets obtained through *in vitro* culturing of the embryo from the seed nuts of diseased palms by Oropeza et al. (2017) indicates the possibility of phytoplasma transmission through seeds. As the embryo lacks a sieve element connection, the

Table 1. Host range and distribution of palm phytoplasmas

| 16Sr Group | Host | Disease | Distribution | Reference |
|---|---|---|---|---|
| 16SrI | *Cocos nucifera* | Lethal yellowing (LY) | Cuba | Camilo et al. (2019) |
| | | Cote d'Ivoire lethal yellowing (CILY) | Côte d'Ivoire | Kwadjo et al. (2018) |
| | *Phoenix dactylifera* | Al-Wijam Date palm yellows | Saudi Arabia | Al hudaib et al.(2008) |
| | | Egypt | | Al Khazindar (2014) |
| | *Roystonea regia* | Royal palm yellow decline | Malaysia | Naderali et al. (2015) |
| 16Sr IB | *C. nucifera* | Lethal wilt | India | Babu et al. (2021) |
| | *Elaeis guineensis* | Lethal wilt | Colombia | Alvarez et al. (2014) |
| | *Areca catechu* | Yellow leaf disease (YLD) | India | Chaitra et al. (2014) |
| | *Cyrtostachys renda* | Yellow decline | Malaysia | Naderali et al.(2014) |
| 16Sr IID | *P. dactylifera*<br>*Washingtonia robusta* | LY | Saudi Arabia | Omar et al. (2018) |
| | *P. dactylifera* | Streak Yellows | Oman | Hemmati et al. (2020) |
| 16Sr IVA | *C. nucifera* | LY | Florida, Cuba. Guatemala, Jamaica, Honduras, St.Kitts and Nevis, Mexico | Harrison et al. (1994); Howard et al. (1983); Myrie et al. (2006); Brown et al. (2007); Harrison et al. (2008); Myrie et al. (2012); Hernandez et al. (2020) |
| | *P. dactylifera*<br>*Livistona chinensis*<br>*Pritchardia pacifica*<br>*Adonidia merrillii* | LY | Florida, Antigua | Harrison et al. (1999); Myrie et al. (2014); Bahder and Helmic (2018) |
| | *Aiphanes lindeniana*<br>*Allagoptera arenaria*<br>*Arenga engleri*<br>*Borassus flabellifer*<br>*Caryota mitis*<br>*Caryota rumphiana*<br>*Chelyocarpus chuco*<br>*Corypha taliera*<br>*Crysophila warsecewiczii*<br>*Cyphophoenix nucele*<br>*Dictyosperma album*<br>*Dypsis cabadae*<br>*Dypsis decaryi*<br>*Gaussia attenuata*<br>*Howea belmoreana*<br>*Howea forsteriana*<br>*Hyophorbe verschaffeltii*<br>*Latania lontaroides*<br>*Livistona rotundifolia*<br>*Nannorrhops ritchiana*<br>*Phoenix canariensis*<br>*Phoenix reclinata*<br>*Phoenix rupicola*<br>*Phoenix sylvestris*<br>*Pritchardia affinis*<br>*Pritchardia remota*<br>*Pritchardia thurstonii*<br>*Ravenea hildebrandtii*<br>*Syagrus schizophylla*<br>*Trachycarpus fortunei*<br>*Veitchia arecina* | LY | Florida | Harrison et al. (1999) Bahder and Helmic (2018) |

*(contd.)*

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|---|---|---|---|---|
| | *R. regia*<br>*Acrocomia mexicana* | LY | Mexico | Narvaez et al. (2016) |
| 16SrIVB | *C. nucifera* | Yucatan coconut decline | Yucatan (Mexico), Cuba, Honduras | Tymon et al. (1998); Roca et al. (2006) |
| | *Acrocomia aculeata* | Coyol palm decline | Honduras | Roca et al. (2006) |
| | *A. merrillii* | LY | Mexico | Hernandez et al. (2020) |
| 16SrIVC | *C. nucifera* | Lethal decline | Tanzania, Kenya | Mpunami et al. (1999) |
| 16SrIV D | *C. nucifera*<br>*Carpentaria acuminata*<br>*L. chinensis*<br>*Butia capitata*<br>*Bismarckia nobilis*<br>*P. dactylifera*<br>*P. sylvestris*<br>*Phoenix roebelenii*<br>*Syagrus romanzoffiana*<br>*Sabal palmetto* | Lethal Bronzing disease (LBD)/ Texas Phoenix Palm Decline (TPPD) | Florida | Harrison et al. (2008); Jeyaprakash et al. (2011); Dey et al. (2018); Bahder et al. (2019b) |
| | *P. canariensis* | | Florida, Texas, Lousiana, Mexico | Harrison et al.(2002b); Bahder et al. (2019b); Ferguson et al. (2020); Palma et al. (2020) |
| | *Trachycarpus fortunei* | | Lousiana | Singh and Ferguson, (2017) |
| | *A. merrillii* | | Mexico, Florida | Lara et al. (2017); Bahder et al. (2019b) |
| | *Sabal mexicana*<br>*Pseudophoenix sargentii*<br>*P. pacifica*<br>*W. robusta*<br>*Brahea brandegeei* | | Mexico | Vazquez-Euan et al.(2011); Narvaez et al.(2017); Poghosyan et al.(2019) |
| 16Sr IVE | *C. nucifera* | LY | Dominican republic | Martinez et al.(2008) |
| 16SrIVF | *W. robusta*<br>*P. dactylifera* | LY | Florida | Harrison et al.(2008); Harrison and Elliot (2016) |
| 16SrVII | *C. nucifera* | LY | Cuba | Camilo et al. (2019) |
| 16SrXIB | *C. nucifera* | Root (wilt) disease | India | Manimekalai et al.(2014b) |
| | *A. catechu* | Yellow leaf disease (YLD) | India | Manimekalai et al. (2013) |
| 16Sr XI | *C. nucifera* | Weligama coconut leaf wilt disease (WCLWD) | Sri Lanka Kanatiwela- de Silva et al.(2015) | Perera et al. (2012) |
| | *A. catechu* | YLD | | |
| | *C. nucifera* | Kalimantan wilt | Indonesia | Warroka, (2006); Bertaccini et al. (2014) |
| 16SrXII | *C. nucifera* | LY | Cuba | Camilo et al. (2019) |
| 16Sr XIII | *C. nucifera* | Kalimantan wilt | Indonesia | Sitepu et al. (1988); Warroka (2006); Bertacciniet al. (2014) |
| 16Sr XIV | *C. nucifera* | Coconut yellow decline | Malaysia Naderali et al. (2013) | Nejat et al. (2009a & b) |
| | *Wodyetia bifurcata* | Yellow decline | | |
| | *P. dactylifera* | White tip die back<br>Slow decline | North Africa | Cronje et al. (2000 a & b) |
| 16SrXXIIA | *C. nucifera* | Awka wilt disease<br>Lethal decline | Nigeria<br>Mozambique | Harrison et al. (2014) |
| | *Borassus aethiopum*<br>*E. guineensis* | LY | Mozambique | Bila et al. (2015) |
| 16SrXXIIB | *C. nucifera* | CILY<br>Cape St. Paul Wilt Disease (CSPWD) Ghana | Cote d'Ivoire | Harrison et al. (2014) |

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*(Table 1 contd.)*

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|---|---|---|---|---|
| 16Sr XXXII- B | *C. nucifera* | Coconut yellow decline | Malaysia | Nejat et al. (2013) |
| **Palm phytoplasmas not assigned to 16Sr groups** | | | | |
| | *C. nucifera* | Bogia Coconut Syndrome (BCS) | Papua New Guinea | Miyazaki et al. (2018) |
| | | Kaincope disease | Togo | Nienhaus and Steiner (1976) |
| | | Kribi disease | Cameroon | Dollet et al. (1977) |
| | | Tatipaka Disease | India | Rao et al. (1956) |
| | | Blast | Coted'Iviore | Quillec et al. (1978) |

mechanism by which the phytoplasma reaches the embryo remains unknown. The possibility of seed transmission poses a threat to international germplasm exchange. For vegetative propagated palms particularly ornamental palms, the planting material itself may serve as primary inoculums introducing the disease to newer geographical locations.

## Diagnosis-a long way to go to the field

Preliminary identifications of phytoplasmal diseases are based on symptoms only. The association of phytoplasma with the disease is usually established by microscopy or molecular detection techniques. The low titre of phytoplasma and their uneven distribution in palms is always a matter of concern for effective diagnosis.

### I. Microscopy

#### i. Light microscopy

Though phytoplasmas could not be directly visualized under a light microscope, accumulation of DNA in extra-nuclear sites indicative of phytoplasmal infection could be demonstrated by staining the tissues with Dienes' reagent (Deeley et al., 1979) or the fluorochrome DAPI (4'- 6'diamidino - 2 phenyl indole) (Russel et al.,1975). Light microscopy has been used to establish the association of phytoplasma with yellow streak disease of date palm (Ammar et al., 2005) RWD (Solomon et al., 1987) and Tatipaka disease of coconut (Rajamannar et al., 994).

#### ii. Transmission electron microscopy (TEM)

TEM occupies a prime position in the history of plant-pathogen diagnosis as it enabled Doi et al., (1967) to identify and describe a new class of phloem limited phytopathogens-the phytoplasma associated with yellows disease. TEM remained the most reliable diagnostic tool for establishing phytoplasmal etiology till the late 1990s. This technique played a pivotal role in elucidating the association of phytoplasma with several diseases of palms particularly LY and RWD of coconut and YLD in arecanut (Beakbane et al*.,* 1972; Plavsic et al., 1972; Solomon et al*.,* 1983; Nayar and Seliskar 1978). Even though TEM is not preferred as a routine diagnostic technique as it is expensive and time-consuming, still it is considered as a valid tool to study host-phytoplasma interaction.

### II. Serology

Sero-diagnostic techniques have been employed in the detection of phytoplasma since 1980s. Due to the difficulty in developing polyclonal antisera with high titre value, the application of serodiagnostics is limited in the detection of phytoplasmal diseases of palms. Enzyme-linked immunosorbent assay (ELISA) has been developed and standardized for the detection of RWD (Sasikala et al., 2010) and WCLWD of coconut (Kanatiwela-de Silva et al., 2019) and YLD of arecanut (Rajeev et al., 2011).

### III. Nucleic acid-based detection techniques

The progress in the field of molecular biology imparted momentum in the development of PCR-based methods for the detection of phytoplasma. By 1990s, the 16S rRNA-based taxonomy of phytoplasma was evolved (Lee et al., 1998) and PCR-RFLP has helped in the identification and differentiation of several groups of phytoplasmas. The era of nucleic acid-based detection of palm phytoplasmas initiated the development of DNA probes for the detection of LY phytoplasma of Caribbean origin (Harrison et al*.,* 1992). This was followed by the design of an oligonucleotide primer for selective amplification of LY phytoplasma DNA by polymerase chain reaction (Harrison et al*.,* 1994; Rhode et al*.,* 1993).

Real-time PCR assays using 16S rDNA-based TaqMan primer-probe have been standardized for sensitive, quantitative and rapid detection of LY (Cordova et al., 2014), WCLWD (Wijesekara et al., 2020) and CYD (Nejat et al., 2010) of coconut and YLD of arecanut (Nair et al., 2014). Myrie et al. (2011) developed a multiplex direct-PCR system and real-time

PCR using TaqMan probes based on the 16S rDNA and *GroEL*gene for detection of LY phytoplasma affected palms in Jamaica. For the detection of CSPWD, *secA* gene-based PCR assay was developed by Yankey et al. (2014). Ramjegathesh et al. (2019) developed a qPCR assay conjugated with TaqMan® probe to detect RWD phytoplasma in coconut. The nested PCR (Manimekalai et al., 2010), real-time PCR (Manimekalai et al., 2011) and LAMP (Nair et al., 2016) developed for coconut RWD diagnosis, lack consistency in detection when a large number of root (wilt) affected coconut samples were tested and further refinement of these techniques are necessary for reliable and rapid detection of RWD in the early stage of infection (Hegde et al., 2016). LAMP colorimetric assay for the detection of phytoplasma associated with WCLWD developed by Siriwardhana et al. (2012) also needs further refinement before field application. Bahder et al. (2019a) developed digital PCR Technology (dPCR) for the detection of palm-infecting 16SrIV group phytoplasmas in Florida.

**Management-amalgamating surveillance, resistance and sustainability**

The non-availability of technologies for the curative treatment of phytoplasmal diseases accentuates the need for developing multipronged strategies to de-risk farmers and to contain the disease in newly emerging areas.

**i. Surveillance, eradication and containment-the 'slow down' approach**

This may be considered as a prime strategy to be adopted upon the emergence of a phytoplasmal disease in a new area. Measures must be taken to confine the disease in a limited area and create a buffer zone around the affected area to slow down the spread of the disease. Periodic surveillance, diagnosis and eradication of phytoplasma infected palms have to be continued systematically in the diseased zone as well as the buffer zone. This helps to slow down the disease spread. WLCWD is contained to the Southern Province of Sri Lanka by adopting a three km wide and 80 km long buffer zone demarcated around the diseased area. This buffer zone is being periodically inspected for the occurrence of the disease and diseased palms are removed to curtail further spread (Nainanayake et al*.*, 2013). Though the surveillance, eradication and quarantine measures helped to restrict the spread of the disease, isolated incidences in few places quite far from the boundary are being reported (Nainanayake et al*.*, 2016). The systematic implementation of the surveillance and removal programme successfully contained Tatipaka disease in India (Rajamannar et al., 1994). The removal of infected palms slowed down the disease spread in the Dominican Republic and Ghana (Martinez et al., 2008, 2010, Nkansah et al., 2009)

**ii. Breeding for disease resistance- for replanting and replacement**

An enduring solution to manage phytoplasmal diseases lies in breeding varieties resistant to the disease. Resistant varieties form the most viable and cost-effective option in managing phytoplasmal diseases especially for perennials likes palms. Coconut breeders around the world have identified some palm varieties/ accessions with varying degrees of resistance/tolerance to phytoplasmal diseases. Breeding for phytoplasmal disease resistance is being circumscribed by the long generation time, low multiplication rate and ineffective clonal propagation of coconut palms (Cardena et al., 2003). In Sri Lanka, Green dwarf and *Nana* coconut were identified as promising sources for resistance to WCLWD (Perera et al., 2015). In India also green dwarfs and their hybrids were found promising against RWD. Systematic evaluation trials at ICAR-CPCRI has led to the release of two coconut varieties (Kalpasree (Chowghat Green Dwarf), Kalparaksha (Malayan Green Dwarf) and one hybrid (Kalpasankara (Chowghat Green Dwarf x West Coast Tall) for cultivation in the root (wilt) disease prevalent areas (Thomas et al., 2012; Krishna Kumar et al., 2015). In the resistance trials initiated in Jamaica during 1960-1970, tall cultivars appeared susceptible with about 90% mortality whereas in Sri Lanka and India, Malayan Dwarfs and the King Coconut recorded less than 5% mortality only. But, in certain geographical locations in Florida and Jamaica, Malayan Dwarfs showed a high degree of susceptibility over a period of time (Howard et al., 1987). Though Malayan Dwarfs possessed a high degree of resistance to the disease, the cultivar was not preferred by farmers due to its poor productivity under marginal conditions. Breeding programs initiated during the 1970s resulted in the production of Maypan hybrid (Malayan Dwarf x Panama Tall), combining the advantage of higher resistance of the dwarf cultivar with large size and adaptability of the tall. Initially, the hybrid was found to be only 10% susceptible to LY and became the primary foci of coconut replanting programmes, which led to a recovery of the coconut industry in Jamaica (Been, 1995; Ashburner and Been,1997). In the field trials conducted in Florida during 1982-2001, MYD and Maypan hybrids showed high mortality due to LY which indicates that these cultivars can no longer be considered as resistant to the disease (Broschat et al., 2002). Resistance breakdown of Malayan dwarfs and Maypan hybrid by LY triggered research on exploring the possibility of the genetic contamination of parents, change in pathogen/vector that altered the palm-vector-phytoplasma interactions. Though research on this line provided evidence on genetic contamination in Panama Tall and MYD in Jamaica it was insufficient to explain a massive outbreak of the disease (Baudouin et al., 2008; Lebrun et al., 2008). Resistance trials conducted on the northern coast of Yucatan, indicated that coconut populations from the Pacific coasts of Mexico are also promising resistance sources of germplasm to deal with L Y (Zizumbo-Villarreal et al., 2008). The Sri Lankan Green Dwarf (SGD) × Vanuatu Tall (VTT) hybrid which shows CSPWD resistance is being used for replanting in Ghana (Dery et al., 1997; Mariau et al., 1996; Quaicoe et al., 2009; Dare et al., 2010). In Nigeria, green dwarfs were reported to have field resistance to AWD (Odewale et al., 2006).

Majority of the field resistance trials against coconut phytoplasma conducted around the world are projecting dwarfs especially green dwarf cultivars from Asian countries as sources of resistance. In order to broaden the genetic base, more diverse populations from different geographical locations need to be explored.

**iii. Integrated management approaches ensure sustainable income to farmers**

Attempts to control phytoplasmal diseases by foliar and soil application of various chemicals, antibiotics and vector management did not yield any encouraging results (Howard and Oropeza, 1998; McCoy et al., 1976). Integration of on-farm quarantine, surveillance, removal and destruction of symptomatic palms and replanting with disease-resistant high yielding varieties as well as systematic farm management significantly reduced the lethal yellowing disease incidence over years in the coconut plantation of a Jamaican farmer Michael Black (Serju, 2012; Myrie et al., 2011; Gurr et al., 2016). Black's integrated management approach will help the farmers to keep the disease outbreaks in individual farms at a manageable level.

\For non-lethal phytoplasmal diseases like RWD in India and WCLWD in Sri Lanka, coconut farmers are advised to adopt integrated management practices viz., removal of disease advanced palms, replanting with resistant/tolerant cultivars, integrated nutrient management, intercropping and mixed farming along with scientific cultivation practices to ensure a satisfactory and sustainable yield even in a disease prevalent area (Sahasranaman et al.,1983; Amma et al.,1983; Bavappa et al.,1986; Muralidharan et al., 1991; Krishnakumar et al., 2015).

## CONCLUSION

During recent years tremendous progress has been made in identifying and characterizing the phytoplasmal pathogens of palms. Despite great advancements in molecular biology, the development of inexpensive, sensitive, reliable, farmer-friendly on-farm diagnostic kits still remains a distant dream. A ready-to-use diagnostic kit is a prerequisite for the implementation of surveillance programmes in disease emerging areas. The epidemiological factors especially the phytoplasmal vectors remain unresolved in the majority of palm diseases and need greater impetus. More detailed studies on vector-host-pathogen-environment interactions are crucial to elucidate the dynamics of disease progression. Accurate identification of insect vectors, characterization of phytoplasma harboured by them and effective transmission trials to establish vectoral ability are still a long way ahead. The application of advanced biotechnological tools like CRISPR may help in accelerating disease resistance breeding programmes. There is ample scope for research in developing alternate management strategies to improve the health of diseased palms. Intense research is required to design and validate location-specific sustainable palm-based cropping systems to de-risk the farmers.

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