



First report on the molecular identification of the phytoplasma associated with a lethal yellowing-type disease of coconut palms in Cote d'Ivoire

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Cocos nucifera is considered the most important crop along the coastal belt of West Africa. Particularly in Côte d'Ivoire, coconut palm is cultivated on approximately 50,000 ha (Konan *et al.*, 2008) and produces an average of 45,000 tonnes of copra per year, which represents the main source of income for people living in the coastal region (Allou *et al.*, 2012). Lethal yellowing (LY)-type diseases affecting coconut and other palm species worldwide have mostly been associated with phytoplasma strains of group 16SrIV 'Coconut lethal yellows'. However, LY-type diseases like Cape St Paul wilt in Ghana (CSPW), the "maladie de Kaincopé" in Togo and Awka disease (Lethal Decline, LD) in Nigeria, previously included in 16SrIV group have been recently classified as a new group 16SrXXII (Wei *et al.*, 2007). LY-type disease has quickly developed among coconut palms in the Grand-Lahou in Côte d'Ivoire, currently affecting more than 7000 hectares (ProMED, 2013). Symptoms include leaf yellowing starting in the old leaves quickly moving to the young ones, drying of spikelet progressing to blackening of the whole inflorescence, rotting of heart, immature fruit drop, and crown death of the palm after six months of initial symptoms appearance leaving a scenery of bare trunks, typically known as "telephone poles" (Fig. 1).

Leaves, stems, roots, hearts and inflorescences of LY symptoms-bearing (17 samples) and symptomless (8 samples) coconut palms were collected and subjected to total DNA extraction (N'nan, 2004). Total DNA extracted was used as a template for a nested PCR assay with universal primers that target the phytoplasma 16S rRNA gene, R16mF2/R1 for the first PCR reaction, and R16F2n/R2 for the nested reaction (Gundersen & Lee, 1996). Samples that yielded R16F2n/R2 amplicons (7/17) were purified (EZNA Cycle Pure Kit, Omega Bio-Tek, USA), cloned (pGEM-T Easy Vector, Promega), and sequenced (University of Toronto, Canada). PCR and sequence results were confirmed by phylogenetic analysis (MEGA 4.0, USA). No PCR amplicons were obtained from symptomless coconut plants surveyed. Phytoplasma R16F2n/R16R2 sequences shared 100% sequence identity with each other. The consensus sequence (GenBank Accession No. KC999037) exhibited 99% sequence identity with phytoplasma members of group 16SrIV with the highest score for the CSPW phytoplasma from Ghana (JQ868442). Phylogenetic analysis (Fig. 2) based on the 16S rDNA sequence supported the grouping of the Côte d'Ivoire lethal yellowing phytoplasma (CILY) within the LY cluster, closely related to the Ghanaian CSPW strain.

Earlier reports in Côte d'Ivoire refer to the presence of formerly called mycoplasma-like organisms (MLO) in coconut affected with blast, the main nursery disease of oil palm in Africa (Konan *et al.*, 2008). However,

there have been no previous official records of LY-type disease, or its association with phytoplasma or bacteria-like pathogens. Since the country hosts one of the five multi-site International Coconut Genebanks for Africa and the Indian Ocean, CSPW disease spreading from neighbouring Ghana was always a threat to the coconut trees in Côte d'Ivoire (ProMED, 2013). Regarding the devastating effects caused by CSPW in Ghana, the first report of the detection and identification of a CSPW strain associated with a LY-type disease of coconut palms in Côte d'Ivoire represents a significant impact for the national coconut industry. Therefore, there is an urgent need to develop effective control measures to halt spread of the disease to other areas of the country.

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Figure 1

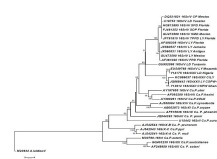


Figure 2

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