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Corrigendum

Analyses based on the 16S rRNA and secA genes identify a new phytoplasma subgroup associated with a lethal yellowing-type disease of coconut in Côte d'Ivoire

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Analyses based on the *16S rRNA* and *secA* genes identify a new phytoplasma subgroup associated with a lethal yellowing-type disease of coconut in Côte d'Ivoire

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Abstract

A lethal yellowing-like disease named as Côte d'Ivoire Lethal Yellowing (CILY) has been spreading throughout the Ivoirian coastal coconut plantations of the Grand-Lahou department in Côte d'Ivoire causing losses of about 12,000 tons of copra/year, and has also become a threat for the coconut Genebank. Leaf, stem apex, heart and inflorescence samples of coconut palms exhibiting CILY symptoms that resemble those associated with Cape Saint Paul Wilt Disease (CSPWD) in Ghana, were sampled and nested PCR-tested for phytoplasma presence. The phytoplasma identified was further characterized based on the sequences of the *16S rRNA* and *secA* genes. Phytoplasma universal primers yielded expected amplification products from 61 out of 84 samples from symptomatic trees tested, while samples from symptomless palms yielded no DNA amplification. Both the 16S rDNA and secA sequences of the CILY phytoplasma showed a 99% sequence identity with that of the CSPWD phytoplasma from Ghana, and clustered with previously identified West Africa phytoplasma strains of group 16SrXXII that includes the Ghanaian CSPWD strain. The phytoplasma was assigned to the sub-group 16SrXXII-B based on virtual RFLP of the 16S rDNA sequences. Results support possibility of disease spread from the neighbouring Ghana, posing a threat for the Ivorian coconut industry.

Keywords: 16SrXXII phytoplasma, coconut, RFLP, Cape Saint Paul wilt disease, '*Candidatus* Phytoplasma'

Introduction

Côte d'Ivoire is among the first 20 out of 92 world coconut-producing countries (UNCTAD, 2012), and is the top African, Caribbean and Pacific exporter of coconut oil (from copra), that nowadays accounts for 2.5% of the world vegetable oil production. Coconut palm is cultivated on approximately 50,000 hectares (1 to 5 ha/farms), and produces an average of 45,000 tons of copra/year (Allou *et al.*, 2012) that is the main source of income for people living in the coastal region.

Côte d'Ivoire Lethal Yellowing (CILY) has destroyed 350 ha and is currently threatening over 7,000 ha throughout the coastal coconut plantations of the Grand-Lahou Department. CILY has also become a phytosanitary risk for the Ivorian multisite International Coconut Genebank that provides service for Africa and the Indian Ocean region. CILY 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, known as "telephone pole" (Figure 1), which resembled symptoms caused by the Cape Saint Paul Wilt Disease (CSPWD) phytoplasma in Ghana.

The present study aimed to characterize the CILY phytoplasma based on the *16S rRNA* and *secA* genes through PCR, sequencing, and RFLP analyses. Phylogenetic relationships were determined by comparisons with

Figure 1. Coconut palms from Grand-Lahou department in Côte d'Ivoire showing bare trunks, known as 'telephone poles', corresponding to the final stage of the disease.

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phytoplasmas associated with Lethal Yellowing-like and Lethal Disease-like from the Caribbean and West/East Africa. Results provide tools to determine the possible origin of the disease, and to develop further effective control strategies.

Materials and Methods

Total DNA was extracted from leaf, stem apex, heart and inflorescence samples of 84 coconut palms exhibiting CILY symptoms (N'nan, 2004). Total DNA was used as a template for nested PCR assays with universal primers that target the phytoplasma *16S rRNA* gene (Gundersen and Lee, 1996), R16mF2/mR1 for the direct PCR reaction, and R16F2n/R2 for the nested reaction. Primers SecAFor1/rev3 and SecA For5/rev2 were used in nested PCR reactions to amplify the *secA* gene (Dickinson and Hodgetts, 2013).

Representative R16F2n/R2 and secA amplicons were purified on spin columns (Omega Bio-Tek, USA), cloned (pGEM-T Easy Vector, Promega), and sequenced bi-directionally. Consensus sequences were compared with GenBank reference sequences and aligned using Clustal W (Thompson *et al.*, 1994). Phylogenetic trees were constructed using the neighbor-joining method with MEGA4.0 (Tamura *et al.*, 2007) with default values and 1,000 replicates for bootstrap analysis. *In silico* restriction analysis and virtual gel plotting was conducted using pDRAW32 (http://www.acaclone.com).

Results

Phytoplasma DNA was amplified from 61 out of 84 CILY symptom-bearing samples. No PCR amplicons were obtained from symptomless palms. Unique *Alu*I, *Bfa*I, *Hae*III, *Hpa*I, *Hpa*II, *Mse*I, *Taq*I, and *Tsp509*I RFLP patterns were obtained for the CILY phytoplasma that clearly differentiated it from 16SrIV and 16SrXXII-A strains. The R16F2n/R2 and secA sequences of the CILY phytoplasma exhibited a 99% identity with those of the Ghana CSPWD phytoplasma. Phylogenetic analysis based on the 16S rDNA and *secA* gene sequences and virtual RFLP support the CILY phytoplasma, as a member of group 16SrXXII.

Discussion

The fact that the CILY phytoplasma identified in the Grand-Lahou is very closely related to the Ghanaian strain CSPWD, highlights the complex epidemiology of two very closely related phytoplasmas that affect the same plant host in two different and nearby geographic locations.

Results supports previous suspicions of CILY phytoplasma spreading from the neighbouring Ghana, and colonizing the most susceptible local coconut varieties and hybrids by its possible adaptation to a new bio-ecological niche with a different epidemic capacity.

The identification of the CILY phytoplasma and its designation in a new 16SrXXII subgroup contribute to the knowledge of the biodiversity of coconut LY-associated phytoplasmas in West Africa, as well as, to enhance the study about the epidemic aspects of the disease. CILY phytoplasma possesses a great threat for the survival of the Genebank coconut germplasm, since it is located to just 120 kilometers from the CILY outbreak.

The presence of CILY phytoplasma also becomes a threat for the entire Ivorian coconut industry and prompts to urgently assess the phytosanitary situation of the new 16SrXXII-B subgroup in the coconut production areas of the Grand-Lahou towards identifying potential sources of CILY resistance, and providing new tools to develop effective management strategies to prevent disease spread.

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