Phytoplasmas identical to coconut lethal yellowing phytoplasmas from Zambesia (Mozambique) found in a pentatomide bug in Cabo Delgado province

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Abstract

Phytoplasmas are associated with several syndromes of lethal yellowing type of coconut. Only in Florida the insect vector of the local coconut lethal yellows was identified to be *Myndus crudus* (Cixiidae). In Mozambique, phytoplasmas are associated with a coconut lethal yellowing type syndrome. In the Cabo Delagdo province, some pentatomids of the species *Platacantha lutea* were found carrying the same phytoplasmas as those identified in the diseased coconut on which they were found. As some pentatomids are vectors of other phloem-restricted pathogens associated with a coconut lethal yellowing type syndrome, hypothesis of a possible pentatomid as a vector is proposed.

Key words: Pentatomidae, *Platacantha lutea*, vector, Derbidae, coconut lethal yellowing.

Introduction

Several syndromes of lethal yellowing type (LYTS) occur worldwide on coconut and other palm species. For most of them, phytoplasmas are specifically associated with the syndrome. However, the phytoplasmas belong to different 16SrDNA groups and inside each group, it is possible to identify several subgroups. For instance, the phytoplasmas associated with the most studied LYTS - coconut lethal yellowing (LY) occurring in the Caribbean- belong to the group 16SrIV. But in this group, the number of subgroups has been continuously increasing these last years, now reaching six subgroups (Harrison *et al.*, 2008).

Other phytoplasmas are associated with LYTS in West Africa, as for instance those associated with Cape St Paul Wilt in Ghana (CSPW), "maladie de Kaïncopé" in Togo or Awka disease in Nigeria (Dollet *et al.*, 2008). Until the end of the 90s, they were listed in the same groups as the LY phytoplasmas, the 16SrIV (Harrison et *al.*, 1994; Lee *et al.*, 1998). Then, they were included in a new group, 16SrXXII (Wei *et al.*, 2007). In Mozambique - East Africa- phytoplasmas very close to those associated with CSPW occur in coconuts, causing a LYTS (Mpunami *et al.*, 1999).

Among all these LYTS only one insect vector has been identified: *Myndus crudus* (Homoptera: Cixiidae), vector of the coconut LY in Florida (Howard *et al.*, 1983). Search for the insect vector(s) of LYTS in other countries stays fruitless. In Ghana another cixiid – *Myndus adiopodoumensis*, now known as *Myndodus adiopodoumensis* (Ceotto and Bourgoin, 2008)- was suspected to be the vector, but it was impossible to prove it (Philippe *et al.*, 2009). In only one insect - Derbidae – the CSPW phytoplasma was detected by PCR but no transmission of the syndrome could be obtained (Philippe *et al.*, 2007). In Tanzania, some insects were found PCR positive for phytoplasmas but experimental tran-

smission never occurred (Mpunami *et al.*, 2000). Then, because of the diversity of the phytoplasmas involved in the coconut LYTS and because of the diversity of the fauna existing in the different regions where these LYTS occur, we can imagine there could be a diversity of insect vectors involved in the transmission of the different coconut LYTS. We describe in this article, for the first time, the possibility of a pentatomid bug as a phytoplasma vector of the coconut LYTS in Cabo Delagado province.

Materials and methods

A survey was made in Zambesia and Cabo Delgado provinces in September 2007. Samples were taken from palms with LYTS. Every time when it was possible diseased trees were cut and leaves and flowers examined. Samples from flowers or trunk and insects were kept dried until arrival in the laboratory. DNA extractions were performed with CTAB or with DNeasy Blood and tissue Kit QIAGEN, and direct PCR with primers P1/P7 or nested PCR (P1/P7 followed with G813/AKSR) were run according to already described protocols (Tymon *et al.*, 1997). PCR products were cloned and sequenced. Sequences were analysed with BLAST. Insect species were determined by J. M. Maldes.

Results

In Zambesia province all diseased coconut samples had a 16SrDNA sequence very close to the sequences published by Mpunami *et al.*, (1999) corresponding to the local LYTS (16SrXXII). No insect – apart from several derbids – were found on diseased coconuts.

In Cabo Delgado province, in Mecufi, (south of Pemba), in a hamlet south of the village four palms at

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Figure 1. *P. lutea* found on a diseased coconut in Mecufi, Cabo Delgado province. (In colour at www.bulletinofinsectology.org)

different stages of LYTS were observed. One palm at the beginning of the yellowing was cut and dissected. The removal of the leaves one by one revealed more than 40 predominantly green pentatomid bugs (figure 1) identified as *Platacantha lutea* Westwood, 1837 (Pentatominae – Eysarcorini). 7 *P. lutea* out of 12 individuals tested and a sample of the dissected palm tree were found positive by direct PCR. Using nested PCR 10 of 12 *P. lutea* were positive. The 16SrDNA sequences obtained from the bugs and from the palm were all identical or very close (99%) to the 16SrXXII sequences.

Discussion

These results show for the first time that pentatomids (Heteroptera) can carry phytoplasma associated with coconut LYTS. It could be only the consequence of insects feeding in the sap of an infected coconut tree. However when we performed similar tests with a wide range of insects feeding on coconut affected by the CSPW in Ghana we rarely found a positive derbid (Philippe et al., 2007). So far, apart from the only positive result from Myndus crudus (Auchenorrhyncha) in Florida (Howard et al., 1983) the vector(s) of the different LYTS associated with phytoplasmas are not yet clearly identified. May be one must think of an insect outside the Auchenorrhyncha group. As a matter of fact, a pentatomid (Hemiptera) is the vector of a coconut LYTS in Latin America: several species of Lincus sp. (Pentatomidae) are vector of phloem restricted trypanosomatids (Phytomonas sp.) responsible for "Hartrot" of the coconut (Louise et al., 1986; Dollet, 2001).

Acknowledgements

This work was funded by the "Agence Française pour le Développement" (AFD) in coordination with the Ministry of Agriculture and Agricultural Development (MADER), and the National Directorate for Agriculture of Mozambique: "Projecto de Apoio Ao Sector Coqueiro Em Mozambique". The authors thank Serafina Mangana (MADER, Sanidad Vegetal) for her help in the organization of the survey.

References

CEOTTO P., BOURGOIN T., 2008.- Insights into the phylogenic relationships within Cixiidae (Hemiptera: Fulgoromorpha): cladistic analysis of a morphological dataset.- *Systematic Entomology*, 33: 484-500.

DOLLET M., 2001.- Phloem-restricted trypanosomatids form a clearly characterized monophyletic group among trypanosomatids isolated from plants.- *International Journal for Parasitology*, 31: 459-467

DOLLET M., QUAICOE R., PILET F., 2009.- Review of coconut "Lethal Yellowing" type diseases. Diversity, variability and diagnosis.- *Oléagineux Corps gras Lipides*, 16(2): 97-101.

HARRISON N. A., RICHARDSON P. A., KRAMER J. B., TSAI J. H., 1994.- Detection of the phytoplasma associated with lethal yellowing disease of palms in Florida by polymerase chain reaction.- *Plant Pathology*, 43: 998-1008.

HARRISON N. A., HELMICK E. E., ELLIOT M. L., 2008.- Lethal yellowing-type diseases of palms associated with phytoplamas newly identified in Florida, USA.- *Annals of Applied Biology*, 153: 85-94.

HOWARD F. W., NORRIS R. C., THOMAS D. L., 1983.- Evidence of transmission of palm lethal yellowing agent by a planthopper, *Myndus crudus* (Homoptera: Cixiidae).- Tropical Agriculture (Trinidad), 60(3): 168-171.

Lee I-M., GUNDERSEN-RINDAL D. E., DAVIS R. E., BARTOSZYK I., 1998.- Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA ribosomal protein gene sequences.- *International Journal of Systematic bacteriology*, 48: 1153-1169.

LOUISE C., DOLLET M., MARIAU D., 1986.- Research into Hartrot of the coconut, a disease caused by *Phytomonas* (Trypanosomatidae), and into its vector *Lincus sp.* (Pentatomidae) in Guiana.- *Oléagineux*, 4(10): 437-449.

MPUNAMI A. A., TYMON A., JONES P., DICKINSON J., 1999.-Genetic diversity in the coconut lethal yellowing disease phytoplasmas of East Africa.- *Plant Pathology*, 48: 109-114.

MPUNAMI A., TYMON A., JONES P., DICKINSON M. J., 2000.-Identification of potential vectors of the coconut lethal disease phytoplasma.- *Plant Pathology*, 49: 355-361.

PHILIPPE R. NKANSAH-POKU J., FABRE S., QUAICOE R., PILET F., DOLLET M., 2007.- Search for the vector of Cape Saint Paul Wilt (coconut lethal yellowing) in Ghana.- *Bulletin of Insectology*, 60(2): 179-180.

PHILIPPE R., REIGNARD S., DESCAMPS S., NKANSAH-POKU J., PILET F., FABRE S., DOLLET M., 2009.- Study on the transmission of coconut lethal yellowing in Ghana.- *Oléagineux Corps gras Lipides*, 16(2): 102-106.

TYMON A. M., JONES P., HARRISON N. A., 1997- Detection and differenciation of African coconut phytoplasmas: RFLP analysis of PCR-amplified 16S rDNA and DNA hybrydisation.- *Annals of Applied Biology*, 131: 91-102.

WEI W., DAVIS R. E., Lee I-M., ZHAO Y., 2007.- Computer-simulated RFLP analysis of 16S rRNA gene: identification of ten new phytoplasma groups.- *International Journal of Systematic and Evolutionary Microbiology*, 57: 1885-1867.

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