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# Detection of phytoplasma DNA in embryos from coconut palms in Ghana, and kernels from maize in Peru

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## Abstract

Nested PCR has been used to detect phytoplasma DNA in the inflorecscences and embryos of West African Tall coconut palms infected with Cape St Paul Wilt disease in Ghana and also to detect *'Candidatus* Phytoplasma asteris' DNA in kernels from infected maize plants in Peru. In germination studies, fruits from infected coconut palms had higher germination rates than those from healthy palms indicating that infected fruits retain the ability to germinate. However, no phytoplasmas were detected in seedlings derived through embryo *in-vitro* culture so there is currently no evidence that these pathogens are transmitted to cause disease in progeny palms.

**Key words:** Cape St Paul Wilt, lethal yellowing, phytoplasma, seed transmission, maize kernels, polymerase chain reaction.

## Introduction

Lethal yellowing-like diseases caused by phytoplasmas are a major factor that impacts on coconut productivity worldwide. Lethal yellowing (LY) is the name given to the disease in the Americas, and the similar diseases in Africa are referred to as Lethal yellowing-like (LYD) because the phytoplasmas involved are different strains. In Ghana, the disease is referred to locally as Cape St Paul wilt disease (CSPWD). The symptoms of the disease are characterised by premature fruit drop and blackening of new inflorescences followed by yellowing of the leaves until the crown dies to result in bare trucks or 'telephone poles'.

The principal means of transmission of phytoplasmas between plants is by phloem-feeding insects and whilst the vector for LY in the US 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) along with indications of phytoplasma seed transmission in alfalfa (Khan *et al.,* 2002), tomato, oilseed rape and lime (Botti and Bertaccini, 2006). The possibility of seed transmission has wide ranging implications for coconut breeding and distribution of germplasm as it would mean that progenies from any breeding programmes undertaken in diseased areas could not be planted in disease-free regions, since this would amount to introducing the phytoplasma into these areas. This study was therefore undertaken to investigate whether phytoplasmas are transmitted through embryos.

## Materials and methods

Genomic DNA was extracted from the trunks and inflorescences of healthy and CSPWD-infected west African Tall coconut palms in Ghana and from whole embryos and leaves and roots of embryo cultures. Samples were also extracted from 2 cobs (8 kernels per cob) from two maize plants exhibiting phytoplasma symptoms and one asymptomatic plant in Peru. Samples were screened for phytoplasma DNA using primers P1 and P7, followed by nested PCR with Ghana 813f and Awka SR for the Ghanaian samples, and R16F2 and R16R2 for the Peru samples. PCR products were visualised on agarose gels, cloned using the Promega pGEM®–Teasy Vector System and sequenced using a Beckman CEQ 8000 automated sequencer.

To analyse the germination rates of seeds from CSPWD infected palms, matured seeds were harvested from infected palms along with those that had already dropped from the palms, and were germinated either conventionally on seedbeds or through embryo *in-vitro* culture. The rates of germination were noted and leaves were sampled from germinated seeds three months and six months after nursing for PCR analysis. Embryo culturing was performed as described in Nipah *et al.* (2007) and a total of 2 plantlets from healthy palms, 3 from symptomless and 12 from diseased palms were sampled 3 months and 6 months after culturing and tested for phytoplasma presence.

## Results

Initial PCR on DNA from the inflorescences of CSPWD infected palms in Ghana confirmed the presence of phytoplasma DNA in peduncles, spikelets, male and female flowers. When embryos were tested, PCR products were found in 9 of 52 embryos and these were confirmed as CSPWD by restriction digestion and DNA sequencing.

To test for viability of embryos, 33 seeds from healthy palms and 61 from diseased palms, were nursed in mesh cages at two disease-free locations. Whilst 19 out of 33 healthy seeds (57.6%) germinated, a significantly higher number, 44 out of 61 seeds (72.1%) germinated from diseased palms. Both harvested and dropped seeds from diseased palms showed approximately equal chances of germination. Nested PCR performed on all germinated

seedlings from the nurseries as well as plantlets from embryo cultures after 3 months and 6 months of nursing and culturing respectively, failed to detect the presence of phytoplasma DNA in any of the samples.

In the Peru study, 2 cobs were selected from each of 3 maize plants (2 symptomatic and one asymptomatic for phytoplasma disease). Leaf samples from all three plants tested positive for '*Ca.* P. asteris' phytoplasmas. Eight kernels were pooled from each cob and DNA extracted and tested by PCR. The 4 samples for the cobs derived from the two symptomatic plants were positive for '*Ca.* P. asteris' DNA whilst the 2 samples from the asymptomatic plant were negative.

## Discussion

The possibility of seed transmission of phytoplasmas has major implications for quarantine services. Botti and Bertaccini (2006) have reported the seed transmission of some phytoplasmas into seedlings of tomatoes and oilseed rape, and plantlets of lime. Such a mode of transmission had previously been regarded as unlikely because there is no direct connection between the phloem sieve elements of plants and the developing embryo or seed, but there have been reports of phytoplasmas in companion cells (Sears and Klomparens, 1989) and parenchyma cells (Siller *et al.,* 1987). The presence of LY DNA in embryos of coconut palms has also been reported previously (Cordova *et al.,* 2003; Harrison and Oropeza*,* 1997). However, since coconut LY-like diseases usually include premature nut fall, it is possible that seeds from diseased palms lose viability following infection preventing the possibility of seed transmission of phytoplasmas into progeny palms.

This study has indicated that phytoplasma DNA can be detected in embryos derived from infected plants on a regular basis, with CSPWD DNA being detected in Ghanaian coconut palms, and '*Ca.* P. asteris' DNA detected in Peruvian maize kernels. It therefore appears clear that phytoplasma DNA can find its way into embryos, but until microscopic evidence confirms that this is within viable phytoplasmas there remains a possibility that it is the phytoplasma DNA alone that enters the embryos. The questions that also need to be addressed are whether such embryos can germinate, and also whether the phytoplasma DNA found in the embryo is associated with viable organisms that can survive the process of seedling development to result in disease. It was not possible in this study to divide an embryo to test for the presence of phytoplasma in one part and germinate the remaining part. However, should the assumption that infected embryos can not germinate be correct, one would expect seeds from infected palms to generally record lower germination percentages in comparison to those from a healthy source. The opposite was the case in this study. Furthermore the ability to germinate was found to be independent of whether the matured seed had dropped or was freshly harvested from the diseased palm. This indicates that matured embryos containing phytoplasma DNA can still retain the ability to germinate regardless of whether the seed has dropped or not. This concurs with the previous findings of Romney (1983), who showed that seedlings raised from seed nuts collected from LY diseased Jamaican Tall palms remained disease-free when planted in disease-free areas. Since our current study failed to detect any phytoplasmas in the seedlings and plantlets derived from infected palms, we conclude that although phytoplasma DNA can be detected in embryos, there is as yet no evidence that CSPWD can be seed transmitted to cause disease in the resultant palm.

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## References

BOTTI S., BERTACCINI A., 2006.- Phytoplasma infection through seed transmission: further observations, pp. 76. In: *Proceedings of the 16th International Congress of the International Organization for Mycoplasmasmology* (AYLING R. D., CITTI C., NICHOLAS R. A. J., Eds).- Cambridge, UK, 2006. Veterinary Laboratories Agency.

CORDOVA I., JONES P., HARRISON N. A., OROPEZA C., 2003.- *In situ* PCR detection of phytoplasma DNA in embryos from coconut palms with lethal yellowing disease.- *Molecular Plant Pathology,* 4: 99-108.

HARRISON N. A., OROPEZA C., 1997.- Recent studies on detection of lethal yellowing disease phytoplasmas in the Americas, pp. 221-234. In: *Proceedings of an International Workshop on Lethal Yellowing-Like Diseases of Coconut* (EDEN-GREEN S. J., OFORI F., Eds). Elmina, Ghana, 1995. Chatham, UK. Natural Resources Institute.

KHAN A. J., BOTTI S., PALTRINIERI S., AL-SUBHI A. M., BERTACCINI A. F., 2002.- Phytoplasmas in alfalfa seedlings: infected or contaminated seeds?, pp. 148. In: *14th International Organization of Mycoplasmology Conference*, Vienna, Austria.

NIPAH J. O., JONES P., DICKINSON M. J., 2007.- The Cape St Paul Wilt disease is transmitted to the embryos of infected West African tall coconut palms.- *Plant Pathology,* in press.

ROMNEY D., 1983.- Brief review of coconut lethal yellowing.- *Indian Coconut Journal,* 13: 1-8.

SEARS B. B., KLOMPARENS K. L., 1989.- Leaf tip cultures of the evening primrose allow stable aseptic culture of mycoplasma-like organisms.- *Canadian Journal of Plant Pathology,* 11: 343-348.

SILLER W., KUHBANDER B., MARWITZ R., PETZOLD H., SEEMÜLLER E., 1987.- Occurrence of mycoplasma-like organisms in parenchyma cells of *Cuscuta ordorata* (Ruiz et Pav.).- *Journal of Phytopathology,* 119: 147-159.

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