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# Using molecular tools to study the movement of Cacao Swollen Shoot Virus

## *Re-evaluating the possibility of seed transmisionof CSSV*

*Cacao Swollen Shoot Virus Disease* (CSSVD) seriously affects cocoa production in many regions of West Africa. The virus is naturally transmitted by mealy bugs and can also be spread by grafting from an infected tree. Although some related viruses are known to be transmitted through seeds in other plants, reports for CSSV are conflicting. This is in part due to the difficulties of determing infection based on visual symptoms alone. These symptoms, which can include stem swellings and a distinctive red-banding in young leaves, can vary in severity and extent depending on the virus strain. Indeed, the virus can remain latent in the plant, or parts of the plant, without causing any symptoms for long periods of time. The development of diagnostic techniques involving DNA and RNA based polymerase chain reaction (PCR) based methods has enabled the detection of CSSV infection even when there are no visible symptoms. In this project these techniques were used to investigate the movement of CSSV within the plant, with a particular focus on whether or not the virus can be transmitted from one generation to the next through seed or pollen.

A series of self-pollination experiments were set up at the Cocoa Research Institute in Ghana (CRIG) to determine whether seed from CSSV-infected mother trees carried the virus. The study also evaluated whether pollen from infected trees could transfer the virus to seed when it is used to pollinate flowers on healthy trees. No evidence of pollen transmitted virus was found, however it was shown that some of the seeds from CSSV infected mother trees tested positive for the CSSV PCR primer. This indicates that at least a fragment of the CSSV's DNA can be transmitted through the maternal pod and seed tissues. When seedlings were grown on from PCR positive seed, they continued to test PCR positive when tested repeatedly upto 24 months after germination. Nonetheless, when these PCR positive seedlings were tested for active virus using a reverse transcription (RT) PCR screening test, the results were all negative. Furthermore,none of the seedlings have shown any CSSV symptoms and infectivity tests on them have all been negative. These results suggest that although at least part of the viral genome is transmitted through the fruit and seed tissues, the cocoa plant has some mechanism to disable the virus so that it cannot induce symptoms in the seedlings.

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References:

Ameyaw, G.A. (2010) Investigations on seed transmission of cacao swollen shoot virus (CSSV) .PhD Thesis, University of Reading, UK

Ameyaw, G.A., Wetten, A.C. & Allainguillaume, J. (2009). EXPLORING CSSV SEED TRANSMISSION USING MOLECULAR DIAGNOSTIC METHODS. Proceedings of the 17th International Cocoa Research Conference, Bali, Oct 2009, COPAL, Nigeria.

Ameyaw, G. A., Wetten, A., Dzahini-Obiatey, H., Domfeh, O. and Allainguillaume, J. (2013), Investigation on *Cacao swollen shoot virus* (CSSV) pollen transmission through cross-pollination. Plant Pathology, 62: 421–427. doi: 10.1111/j.1365-3059.2012.02640.x

# Investigations on seed transmission of cacao swollen shoot virus (CSSV)

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***A thesis submitted to the University of Reading, UK***

***For the degree of Doctor in Philosophy***

**May 2010**

## Abstract

Cocoa swollen shoot virus disease (CSSVD) seriously affects cocoa production in Ghana, particularly in the areas of mass infection (AMI) in the Eastern Region whilst the disease continues spreading to areas with scatter outbreaks especially in the Western Region. Reports utilising visible diagnostic symptoms on whether the virus is seed-borne and transmissible to seedlings of cocoa (*Theobroma cacao* L.) have been conflicting. Studies show that visible viral symptoms are not always manifested on all affected parts of infected cocoa trees due to a period of CSSV latency. In this work, molecular diagnostic techniques involving DNA and RNA based polymerase chain reaction (PCR) methods were utilised with CSSV primers designed from conserved regions of published CSSV sequences to investigate whether CSSV is sexually transmissible from infected parents to developing seeds and seedlings through a series of pollination experiments. The study also evaluated whether pollen could be a source of CSSV infection into seeds and seedlings through cross pollination experiments by using pollen from CSSV infected cocoa trees onto healthy recipients. Robust precautions were taken to avoid cross contamination during duplicated DNA extractions and only those PCR results accompanied by effective positive and negative controls (from CSSV symptomatic and quarantine assured CSSV free trees respectively) were scored. Following DNA extraction all pods obtained from the self pollination experiments involving CSSV infected cocoa trees were found to contain seeds that were PCR positive for CSSV DNA. The pith (mesocarp) of each of the self pollination generated pods was similarly PCR positive for CSSV DNA, though not all seeds from the pods were positive for the PCR screening. Seed components showed diminishing frequencies of CSSV DNA presence in the order: testa > cotyledon > embryo. Sequential DNA samples from new leaves of some of the seedlings derived from the self-pollinated trees are consistently PCR positive for CSSV DNA over 24 months after germination. Nonetheless, a reverse transcription (RT) PCR screening test has been negative for all those PCR positive seedlings indicating absence of CSSV RNA transcripts in the seedlings. None of the seedlings have exhibited symptoms characteristic of CSSV and infectivity tests on them have been negative. Seeds and seedlings from the cross pollination experiments have also been negative for the PCR analysis implying that pollen could not to be a potential route for CSSV infection. Possible explanations for these events including viral DNA integration into the cocoa genome and possible methylation-directed inactivation of the CSSV DNA are discussed.