Elucidation of Cocoa Swollen Shoot Disease pathogenesis and insect vector viral retention

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Ongoing work

Investigating the nature of the putatively integrated Badnavirus sequence internationally present in the genomes of cocoa.

I have been continuing work on the novel integrated sequence found in samples from the International Cocoa Quarantine Centre (Reading). My work has involved the detection and sequencing of integrated sequences and I am a co-author on a paper on this topic produced in collaboration with Emmanuelle Muller (CIRAD, France), Jim Dunwell, Ihsan Ulah and Andrew Daymond (University of Reading) and Joel Allainguillaume and Andy Wetten (UWE) which has been published in the journal Scientific Reports. In this paper we outline the first evidence of a series of distinct, conserved Badnavirus sequences, distantly related to swollen shoot virus, integrated in the genome of asymptomatic cocoa plants. I am in the process of conducting mealybug-based transmission tests between plants possessing the integrated sequence (IS) and IS-free recipient cocoa in order to test the hypothesis that the sequences are only vertically transmissible (rather than via the known CSSV mode of transmission).

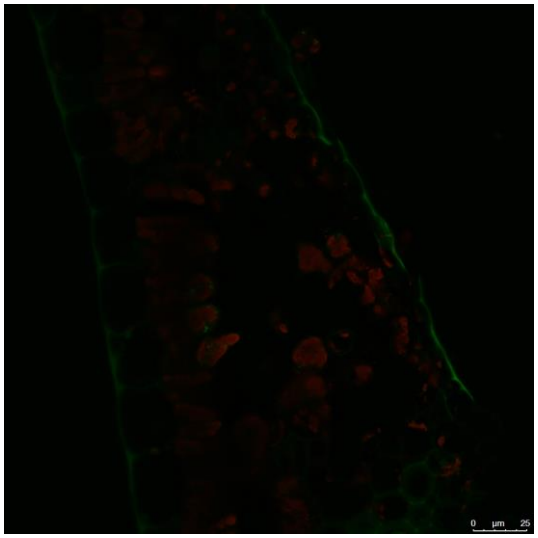
In collaboration with ICQC, Reading, a series of further crosses between plants containing the distinct IS types were planned in 2020. The beans from the resultant pods have been grown and seedlings have been sampled. These samples are undergoing DNA extraction and PCR for analysis to further determine the heritability of the sequences.

Confocal microscopy

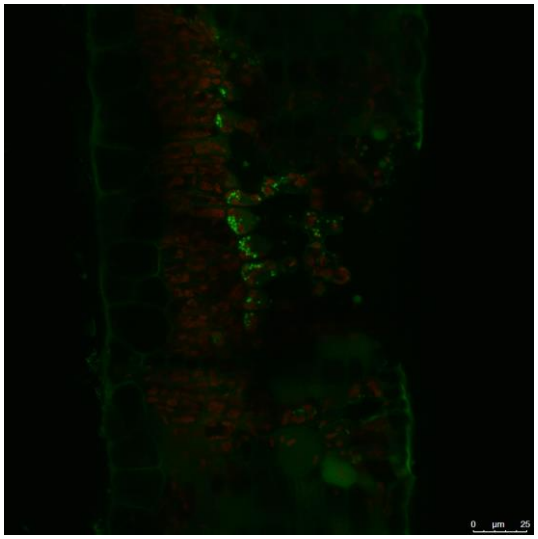
I have been testing different methods of specimen preparation and mounting and using an indirect immunofluorescence method to identify CSSV presence in infected *T. cacao* and *P. citri* tissues. My primary antibodies have been kindly donated by Jackie Barnett and Sue Tyler; these bind to specific CSSV coat proteins. I have been testing two different primary antibodies – one which Jackie and Sue have found has a higher affinity for binding to CSSV. My secondary antibody has an attached fluorescent FITC tag; this antibody will bind to the primary antibody and act as a marker.

My negative samples come from CSSV-free *T. cacao*, and my positive samples are taken from CSSV positive (strain New Juaben) symptomatic *T. cacao*. Both my positive and negative samples are treated with the same indirect immunofluorescent method prior to imaging.

So far, my images have been promising, with a clear increase in fluorescence in my positive samples when compared to my negative samples, suggesting CSSV virion presence particularly within the spongy mesophyll cells (both primary antibody types have resulted in fluorescence predominantly from these cells) Current work is focussed on the minimisation of non-specific binding in order to confirm that the fluorescence increase is due to CSSV presence.



CSSV Negative leaf sample: Primary AB 1 µg/mL, Secondary AB 0.1 µg/mL.X63 mag. In red are chloroplasts, green shows autofluorescence from plant tissues.



CSSV Positive leaf sample: Primary AB 1 µg/mL, Secondary AB 0.1 µg/mL. X63 mag. In red are chloroplasts, increased green fluorescence indicates possible CSSV presence.

I am also looking at using Fluorescence *in situ* hybridization (FISH) to locate CSSV DNA within *T. cacao* cells. I am looking to optimize this once I have completed my immunofluorescent microscopy work.

Stem sample extractions

Stem samples can be used as an alternative to DNA extraction from leaf samples for the detection of CSSV. 6 cm stem samples were cut lengthways and peeled back at the cambium, exposing phloem tissue. Phloem tissue is limited to the outer peel, while xylem and cambium tissue remains in the core. The peeled sample is submerged in water and left to incubate. Using qPCR, CSSV can be reliably detected when samples are diluted (necessary in order to reduce the concentration of PCR inhibitors). This method has also been used to provide insight into the location of the virus within the stem; peel and core were separated and incubated individually. A higher concentration of CSSV was detected in the peel than in the core, as expected given CSSV has been described as a phloem

limited virus, and the phloem tissue is present in the peel only. As well as qPCR, Endpoint PCR using Thermo Scientific Phire Hot Start II DNA Polymerase has been shown to successfully amplify bark-extracted CSSV DNA. This could allow for this method to be used in remote areas where qPCR equipment is not available, but thermocyclers are.

All samples have been collected and analysed. I have now moved onto data handling and manuscript writing.

Other activities

I have been working as Membership Secretary for the British Society for Plant Pathology (BSPP) and have been a member of the BSPP Education Committee. I am planning on attending and presenting a poster at the BSPP Presidential Conference (Our Plants, Our Future) held in Birmingham in December.

References

Muller, E., Ullah, I., Dunwell, J.M., Daymond, A.J., Richardson, M., Allainguillaume, J. and Wetten, A. (2021) Identification and distribution of novel badnaviral sequences integrated in the genome of cacao (*Theobroma cacao*). *Scientific Reports*. 11, 8270. (https://www.nature.com/articles/s41598-021-87690-1)