

A strain-independent screen for Cacao Swollen Shoot Virus (CSSV)

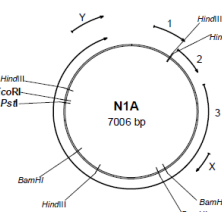
Developing a rapid molecular test to diagnose CSSV and other West African viruses

Cacao Swollen Shoot Virus (CSSV), the mealy bug transmitted causal pathogen of Cocoa Swollen Shoot Virus Disease (CSSVD), is the most debilitating virus affecting cocoa and continues to severely restrict cocoa production in many regions of West Africa. Depending on the viral strain and the variety of cocoa, the symptoms vary from fleeting red-vein banding in young leaves for the mildest strains, to severe stem swellings, defoliation and eventual tree death for the most virulent isolates.

Despite decades of efforts aimed at controlling the spread of the disease through a cordon sanitaire approach, the condition continues to cause major crop losses. The international exchange of cocoa germplasm that could facilitate the breeding of virus tolerant/resistant varieties is presently constrained by lengthy graft-based quarantine procedures. The availability of a molecular test capable of detecting any of the varied strains of CSSV will strengthen and potentially accelerate the quarantine procedure. It will also provide a valuable insight into the overall molecular variability of the virus and its occurrence in non-cocoa host plants.

In order to establish a rapid, sensitive test for *Cacao Swollen Shoot Virus* (CSSV), DNA sequencing was conducted on the large collection of strains of the pathogen held at the Virus museum of the Cocoa Research Institute of Ghana (CRIG). From initial analysis of sequences across a range of CSSV gene coding regions, four primer pairs have been developed which, when used in combination, allow the detection of all strains of the virus so far tested. Putative CSSV products generated by the primers were analysed by a combination of high throughput (454) and direct DNA sequencing which revealed the presence of 30 distinct strains of CSSV in the collection. Other non-CSSV viruses could also be detected by use of the primer set (consistently those trees that generated only one out of four positive reactions – either the Reverse Transcriptase or the RNase H product). The survey has provided the first evidence of the presence of CSSV sequences in the indigenous tree *Ceiba pentandra* that predate sequences in Ghanaian cocoa. The suite of primers can now be used directly to enhance cocoa quarantine activities, while in a tagged form their effectiveness for the 454-based sequencing of large numbers of samples will be utilised in a new survey of CSSV throughout West Africa funded by CR(UK) Ltd.

- ORF1 – Open Reading Frame 1
- MP – Movement Protein
- RTase – Reverse Transcriptase
- RNase H – Catalyzes RNA cleavage



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