Rapid and cost effective 'on-site' detection of Cacao swollen-shoot virus (CSSV).

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Abstract

The UK chocolate industry is worth >£4 billion per year and demand is ever increasing. Most of the cocoa beans imported and consumed in the UK come from West Africa, where Cacao Swollen Shoot Virus disease (CSSD) has been identified as the main disease threat to productivity. Currently infected trees are identified by observation of characteristic leaf changes eg.red-vein banding and chlorosis and or the presence of swollen stems and roots. However it can take months to years for these symptoms to appear, during which time the infection is spread by the mealybug insect vector Pseudococcidae to surrounding trees. Immunoassays developed to detect Cacao Swollen Shoot Virus (CSSV) in infected leaves have not proved to be sensitive enough due to the high background interaction with plant material. Current laboratory tests available to confirm CSSV infection involve detection of CSSV viral DNA using polymerase chain reaction (PCR) technology (Dzahini-Obiatey, 2010 and Oro *et al.*, 2012) this can currently only be performed in a laboratory environment by specialist staff.

The work presented is the strategy and progress of a multidisciplinary project that aims to develop a unique biosensor system for detection of presymptomatic CSSD, that is applicable as a field-based system. This will be achieved by developing an inexpensive novel assay system and integrated hand-held device for the detection of CSSV strain New Juaben, viral coat protein. The sensor is based on the novel arrangement of layers of porous materials through which the sample and regents will flow. The basis of the assay is a novel immobilized binding reagent and a highly efficient reporter system. This is a platform technology with a range of possible adaptations and this is the first of many possible applications across the agri-food, environmental and biomedical fields. The assay developed will be validated in collaboration with the local branch of WCF Ghana-CocoaAction a private-public sector initiative.

Introduction

The UK chocolate industry is worth >£4 billion per year and demand is ever increasing. Most of the cocoa beans imported and consumed in the UK come from West Africa where Cacao Swollen Shoot Virus disease (CSSV) has been identified as the main disease threat to productivity, with a current estimated annual revenue loss of 289 million pounds in Ghana alone. This multidisciplinary project aims to develop a unique system for detection of pre-symptomatic CSSV that is applicable as a field-based system. This will be achieved by developing an inexpensive novel assay system and integrated hand-held device for the detection of a CSSV protein. This will be validated in collaboration with the local branch of 'CocoaAction, Ghana'.

The chocolate industry has identified CSSV infection as the major constraint to productivity in West Africa with up to 15% loss of total crop output per annum. There are no commercially available tests for detection of CSSV infection of cacao trees. Current control measures include visual inspection by local government agencies of trees and removal of those infected and their 'contacts'. In Nigeria this was abandoned in favour of the use of 'Condon Sanitaire' (i.e. isolation of infected areas). Surveys show around 17% of the Ghana cacao growing region is infected. Ghana has had a nationwide cutting out and rehabilitation programme (34m trees cut down since 2006). Despite many years of these procedures CSSV infection has spread and is increasing. Current losses are hard to estimate, Mars has seen areas of mass infection in a locality (100% losses), through to farms with a single tree showing symptoms (1% losses).

The objective of this investigation is to develop a specific, sensitive, rapid, and inexpensive and field based test for pre- symptomatic CSSV to enable producing countries to better manage the disease. It is intended that the test will be incorporated into current surveillance procedures by local governments and this will be supported by industry such that Cacao farmers in West Africa will not incur any cost in the use of the test. The control and containment of CSSV infection will significantly improve the yield of cacao and therefore protect and possibly improve the income of cacao farmers in West Africa.

Background

Attempts have been made previously to develop immunoassays for detection of CSSV in plant material. Techniques used previously include ELISA assays (Sagemann *et al.*, 1983) and virobacterial agglutination tests (Hughes and Ollennu, 1995). These assays have had limited success due to high background leading to insensitivity of the tests and their viral strain dependence. Molecular techniques have been used to detect and characterise strains of CSSV, in particular Polymerase Chain Reaction (PCR), (Muller *et al.*, 2001). A combination of immune-capture combined with PCR has been found to be more sensitive than ELISA with other badnaviruses (James *et al.*, 2011). This method still has serious limitations due to strain variability because the antiserum cannot recognise new strains. All these assays have been used as research tools and require laboratory facilities and trained personnel, to date no successful 'on-site' assay has been developed.

Strategy

The project is multidisciplinary including aspects of plant virology, biosensing technology, molecular biology, and engineering. The key steps in the development of the detection methodology for this crop virus include the selection of candidate novel molecules produced *in-vitro* that have been designed to bind to and capture CSSV in an assay format allowing detection of captured virus. The novel molecules produced are based on the knowledge of the sequence of the CSSV coat protein sequence. The novel capture molecule has a well characterised label incorporated which will be detected in a novel way. A key engineering aspect of the project involves developing a diagnostic device that will integrate and measure the captured virus in a prototype for the validation of the technique with plant samples from naturally infected cacao trees in Ghana.

This project aims to prove that the use of novel binding reagents produced *in-vitro* can reduce high background associated with previous immunoassays, this coupled with a rapid and sensitive biosensor will enable the detection of CSSVD in pre-symptomatic trees.

Recent sequencing of CSSV present in a wide variety of samples from West Africa, European Cocoa Association (ECA) CSSV diversity project and Chingandu *et al.*, 2017 a, 2017b have shown diversity in the badnavirus species causing CSSD. Once proof of concept has been achieved in the current study, additional binding reagents will be produced based on the coat protein sequences available, such that the binding reagents will detect multiple CSSV species and other badnavirus species associated with CSSD.
'Cocoa Action, Ghana' will enable the validation of the developed technology with a wide variety of CSSV field isolates. It will also facilitate its future use as a product through increased pace and scale of uptake of the technology not only in Ghana, but also other cocoa producing countries in West Africa. The deployment of this technology in West Africa will be important to enable increased cocoa production to supply the rapidly growing Asian markets.

Phases of development

In addition to the development and optimisation of the biosensor through selection of candidate binding molecules, materials, components and integration of the assay with the sensor. The easy preparation of the plant sample for use in the field with the biosensor is being optimised, this is essential to enable the biosensor to be used at the point of use.
In addition to the evaluation of sensitivity, specificity and diagnostic accuracy of the biosensor with field samples, evaluation of routes to the implementation of its use is also an essential as part of this project. Cocoa Action, Ghana through their collaborations will provide advice on the possible routes for the CSSD diagnostic to become a part of normal screening procedures.

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