Cocoa swollen shoot virus in Ghana: A review of diagnostic procedures

H. DZAHINI-OBIATEY, L. A. OLLENNU & P. C. ACULEY
Cocoa Research Institute of Ghana, P. O. Box 8, Akim Tafo, Ghana

ABSTRACT

A quick and more reliable diagnostic method has for a long time been identified as one input that will greatly enhance the control of the cocoa swollen shoot disease in Ghana. Many diagnostic procedures have been developed for detecting the virus that causes the disease; yet, the detection of latent infections is still unpredictable. This paper reviews work done in diagnosing swollen shoot disease to develop a quicker, more reliable, and cost-effective method that could be applicable in programmes for controlling cocoa swollen shoot disease. Enzyme-linked immunosorbent assay (ELISA), virobacterial agglutination tests (VBA), and other biochemical analyses have been discussed as diagnostic tools that could be developed to effectively replace the use of visible symptom expressions as the only method for field detection of the disease.

Subject review article. Received 18 Nov 03; revised 29 May 07.

RÉSUMÉ

DZAHINI-OBIATEY, H., OLLENNU, L. A. & ACULEY, P. C.: *Le virus de la virose cacaoyer au Ghana: Une révision de procédures de diagnostic.* Il y a lontemps qu'une méthode diagnostique rapide et plus fiable a été identifiée comme un intrant qui va considérablement améliorer la lutte contre la maladie de virose cacaoyer au Ghana. Beaucoup de procédures de diagnostic ont été développées pour le dépistage du virus qui cause la maladie; pourtant le dépistage d'infections latentes est encore imprévisible. Cet article passe en revue la recherche faite envers la diagnose de la maladie de virose cacaoyer en vue de développer une méthode plus rapide, plus fiable et d'un bon rapport coût-efficacité qui pourrait être applicable aux programmes de la lutte contre la maladie de virose cacaoyer. Essai immunosorbant enzyme-liée (EISEL), essais d'agglutination virobactérien (AVB) et d'autres analyses biochimiques ont été discutés comme des outils diagnostiques qui pourraient être développé pour remplacer efficacement l'usage des expressions de symptôme visible comme la seule méthode pour le dépistage de la maladie sur le terrain.

Introduction

To formulate adequate control measures against a plant virus disease, correct identification of the virus causing the disease in the field is essential. Disease symptoms on the plants in the field are almost always inadequate to give an accurate identification. Visual inspection has been used for many years in diagnosing cocoa swollen shoot disease, despite its serious limitations (Thresh & Owusu, 1986). This is because quicker and more reliable methods are unavailable.

Several other methods have been used in identifying plant viruses, some of which have led to the wrong classification of some of these viruses (Kurstak, 1981). Hamilton *et al.* (1981) reviewed these methods and advanced recommendations that could be used as guidelines for identifying and characterizing plant viruses. The subject has also been exhaustively discussed elsewhere (Walkey, 1985). Methods such as the reproduction of disease using isolated virus, host range studies, symptom expression, cross-protection, mode of transmission (graft, vector, mechanical or sap), types of particles observed in negative stains by

electron microscopy, cytopathology by light and electron microscopy, *in vitro* properties in crude sap (thermal inactivation temperature, dilution end point, longevity in crude sap), and serology (precipitin tests, immunoelectron microscopy, immunosorbent assay) have been evaluated. Other methods that have been tried included sedimentation properties (rate-zonal, equilibrium), analysis of nucleic acids (type of nucleic acids, strandedness, number and size of polynucleotide, base composition and sequence), and coat protein (number of polypeptide, molecular weight of polypeptide, amino acid composition and sequence).

In diagnosing a plant virus, one or more of the identifiable methods mentioned above would have to be used to find out whether the virus is already known. Cocoa swollen shoot virus (CSSV) disease is diagnosed by adapting and using most of these methods as well as other newly developed ones.

This paper reviews work done on the diagnostic procedures against the background of searching for a fast and reliable diagnostic procedure to apply in the cocoa swollen shoot disease control programmes.

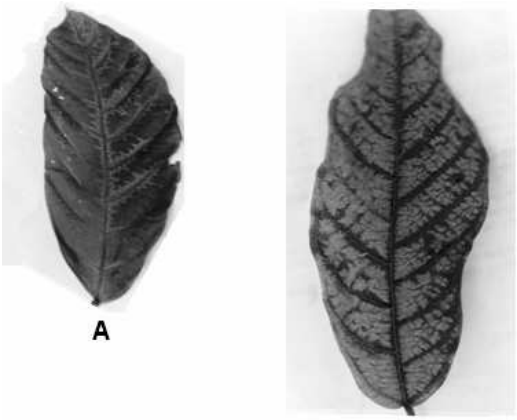

Fig. 1. CSSV severe 1A showing characteristic fern pattern (A), and pepper and salt mosaic (B) symptoms on affected leaves.

Diagnostic procedures

Symptom expression

The CSSV disease was first noticed in Ghana as stem-swellings and tip-die back disease. The disease was subsequently identified to be caused by a virus (Posnette, 1940). Further studies showed other symptoms such as leave patterns (Posnette, 1941), root swellings (Attafuah, 1957), and reduced pod size and bean weight (Posnette, 1947). The patterns formed on the leaves are so unique and intense that they could be easily distinguished from other plant viruses (Fig. 1 and 2) and, thus, form limited basis for CSSV diagnosis and classification (Dale, 1962; Thresh & Tinsley, 1959). Symptom expressions, particularly in the leaves, which is slow to manifest or may not at all be noticed, is still being used in diagnosing CSSV, particularly in screening for resistant materials during breeding programmes,despite its numerous limitations (Thresh & Owusu, 1986; Thresh *et al*., 1988). It has been suggested that these limitations may be partly responsible for the slow pace of progress in resistant breeding programmes for the disease in Ghana (Dzahini-Obiatey, 1993).

Fig. 2. CSSV Kpeve showing the mottle type of leaf symptoms.

Host range studies and cross-protection

Host range studies of an unknown virus and

the symptoms it produces are often important clues to identifying it. Several workers have investigated the host range of CSSV isolates found in Ghana (Posnette, Robertson & Todd, 1950; Dale & Attafuah, 1957; Tinsley & Wharton, 1958). It was determined from these studies that the infectivity of CSSV isolates was limited to members of the following plant families: Sterculiaceae, Bombacaceae, Tiliaceae, and Malvaceae. In a related host range studies, Legg & Bonney (1967) used seven species of mealybugs on six isolates of CSSV to classify *Cola* isolate (naturally isolated from *Cola chlamydantha***)** as a 1A-type isolate and *Adansonia* isolate (originally isolated from *Adansonia digitata*) as a mottle type. Apart from the above-mentioned plant families, several other unsuccessful attempts have been made to determine the susceptibility of other herbaceous plants to CSSV isolates; and to get local lesion and alternative hosts (Legg & Lovi, 1968; Owusu & Lovi, 1986; Donkor & Dzahini-Obiatey, 1993).

In cross-protection, plants already infected with one virus may be totally or partially immune from the effects to the same or related strains. Such interference has been used as a basic criterion for determining relationships (Price, 1940; Bawden, 1950; Bennett, 1953) and, hence, in diagnosing pathogens. Cross-protection has been demonstrated and used in cocoa viruses (Crowdy & Posnette, 1947; Posnette, 1950; Posnette & Todd, 1955) to make limited classification of some CSSV isolates (Thresh & Tinsley, 1959; Dale, 1962). The phenomenon, particularly the protection of the mild strains against the severe ones, is now being investigated for exploitation as a possible means of controlling CSSV (Owusu, Ollennu & Dzahini-Obiatey, 1996; Ollennu, Owusu & Dzahini-Obiatey, 1999). The use of cross-protection in diagnosing and classifying viruses in general is, however, constrained because of the incomplete or no detectable cross-protection between virus strains that have been shown to be antigenically related (Kassanis, 1963), as well as the examples of occurrence of cross-protection between obviously unrelated viruses (Kassanis, 1963; Hamilton, 1980). The use of cross-protection in CSSV diagnosis, therefore, needs to be treated cautiously.

Cytopathology by light microscope, sedimentation and in vitro *properties*

Induced distinctive inclusions, redistribution, accumulation of certain substances, un-differentiated tissues and wall modification, among others, are the identifiable features by light microscopy studies that have been found associated with virus infections. In light microscopy studies of CSSV, accumulation of anthocyanin pigments (Knight & Tinsley, 1958), undifferentiated mesophyll and other tissues, and the non-formation of chloroplast in the affected parts of the leaf (Mangenot, Alibert & Basset, 1946; Knight & Tinsley, 1958) were observed in infected plants. These features are highly diagnostic in some viruses, but their use in CSSV diagnosis has been limited probably because of their cumbersome nature.

Sedimentation properties of CSSV have been used extensively in attempts to purify the virus (a process which is diagnostic in itself). Almost all workers purifying CSSV used equilibrium rate-zonal, isopycnic and differential centrifugation methods successfully (Brunt & Kenten, 1963; Kenten & Legg, 1965). Indeed, the successes of most CSSV purification processes have been partly due to the accurate determination and use of sedimentation properties.

In vitro properties such as ability of crude sap of CSSV to store under various conditions have also been tested (Kenten & Legg, 1965; Owusu, Ollennu & Lagudah, 1985), but their diagnostic values were insignificant.

Mode of transmission

The method by which a virus is transmitted is important in virology, and the prospects of any further studies on a virus may be highly dependent on whether it is readily sap or mechanically transmissible. Other means of transmitting plant

viruses, particularly by natural means such as vectors, have been found to be highly diagnostic (Box, 1945; Posnette & Strickland, 1948; Costa, 1976; Harrison, 1977; Pirone & Harris, 1977; Maramorosch & Harris, 1979). Cocoa swollen shoot virus had been transmitted using vectors (Box, 1945), graft (Posnette, 1940), and sap or mechanical transmissions (Brunt & Kenten, 1960). This review focuses on the mode of transmission under two main headings: (i) graft and vector transmission, and (ii) purification for mechanical or sap transmission.

Graft and vector transmission

In the absence of visible pathogens, grafting is one of the criteria for proving a disease to be caused by a virus. The CSSV was first transmitted experimentally by using grafts made of patches from hardened stems (Posnette, 1940). Subsequent transmissions were successfully done by budding, in-arching and cleft grafts, or by grafting on to embryos (Archibald, 1954). Patch-grafting is still in use in identifying and diagnosing new strains and isolates of CSSV.

The search for a natural means of transmitting CSSV culminated in identifying the insect vector, the mealybug (Pseudococcidae), as being responsible (Box, 1945). To date, Pseudococcidae is still the only family of mealybug known to be vectors of CSSV, and over 20 species from this family have been found to be able to transmit the virus (Thresh & Tinsley, 1959; Dale, 1962).

Graft and mealybug transmission of CSSV still have high prominence in identifying and diagnosing CSSV. For example, when an infected material that is suspected to be an isolate of CSSV is collected from the field, it is patch-grafted on to Amelonado cocoa rootstock. When symptoms expressed resemble CSSV, they are then re-transmitted using mealybugs. If the symptoms persist, the virus can then be diagnosed as an isolate/strain of CSSV. Both types of transmission are now being used in screening breeding materials for resistance.

Purification for mechanical or sap transmission

Whether a virus is mechanically transmissible may be an important diagnostic feature, although some viruses are only mechanically transmitted using special additives and inoculation techniques. Some viruses may require highly purified sap preparations while others may not. The ability to successfully purify a virus from its source can also be a diagnostic feature in itself. However, purified sap preparations are often used in other tests such as mechanical transmission, electron microscopy, serology, sedimentation, physicochemical analysis (such as protein and nucleic acid analyses), and in producing antisera.

In CSSV studies, purified virus has been used for all the above-mentioned tests or assays. However, this section focuses on purifying for mechanical transmission only. Purification for other uses has been discussed in the subsequent sections of this review. Purification of CSSV for mechanical inoculation was first demonstrated in cocoa using CSSV cultures in *Adansonia digitata* and *Bombax brevicuspe* (Brunt & Kenten,1960). This was subsequently followed by successful extraction from cocoa tissues (Brunt & Kenten, 1962). The inability to extract infective CSSV with ease from cocoa tissues was attributed to tannins, mucilage, and other inhibitors in cocoa that are thought to inactivate the viral particles. Work in purifying the virus, therefore, became focused on trying to get rid of these substances.

Brunt and co-workers at different times used proteins such as egg and blood albumin, hide powder in combination with reducing agents such as cysteine, ascorbate, thioglycollate or sulphide to improve on the infectivity rates of CSSV preparations (Brunt & Kenten, 1962, 1963; Brunt, Kenton & Nixon, 1964). Other workers used polyvinyl pyrrolidone (PVP), coupled with low concentrations of ammonium sulphate, to precipitate CSSV and got even better results (Kenten & Legg, 1965). Treatments with solvents such as ethanol, carbon tetrachloride and ether were tried, and it was observed that further contaminants could be removed with carbon

tetrachloride and ether (Kenten & Legg, 1965). It was later shown that lower concentrations of carbon tetrachloride were even more effective (Owusu *et al.*, 1985). Celite or pectinase or both chemicals were used with â-mercaptoethanol in a sodium dihydrogen phosphate solution or in a phosphate buffer at different *p*Hs to degrade or remove mucilage from CSSV preparations (Adomako, 1974; Adomako, Owusu & Oduro, 1974; Adomako *et al.*, 1983), thereby improving on their infectivity rates (Adomako, 1974; Adomako *et al.*, 1974). Owusu & Lagudah (1986) tried Triton X-100, but observed no significant beneficial effect on the infectivity rates of resultant CSSV preparations. The beneficial effect of proteins on improving infectivity was also confirmed by Dzahini-Obiatey, Ollennu & Owusu (1996a) when they observed the first-ever infective preparations of CSSV N1 (a recently isolated mild isolate of CSSV) only after adding blood serum albumin to the extraction medium. Other reports show that phosphate and its buffers were also important as basic ingredients.

A critical assessment of the improvement brought about by the additives in CSSV preparations over the years showed that the effect had been phenomenal. For example, when mechanical transmission was first demonstrated, the highest infectivity recorded was 46 per cent, while the infective preparations were only occasionally produced (Brunt & Kenten, 1960, 1962). This came to 84.6 per cent with the addition of proteins (Brunt & Kenten, 1963), and the infective preparations produced became more frequent. The PVP and ammonium sulphate precipitation used at high-speed centrifugation of 75000 g for 75 min also yielded preparations with infectivity rates as high as 95 per cent (Kenten & Legg, 1965). Although the infectivities of CSSV preparations (Adomako, 1974) were only up to 80 per cent (which is lower than the 95 % of Kenten & Legg, 1965), the preparation was very clean and free from mucilage. Table 1 presents details on the effects of certain additives to the extraction

TABLE 1

A Comparison of Infectivities Obtained Over the Years Upon Treatment With Important Additives

Reported highest rate of infectivities per experiment (expressed in percentages)	*Important additive and manipulation*	*Reference*
27.7	Virus was cultured in *Adansonia digitata* and concentrated in sucrose	Brunt & Kenten (1960)
46	Thioglycollate, cysteine, sulphide and ascorbate added	Brunt & Kenten (1962)
84.6	Hide powder couple with high speed centrifugation	Brunt & Kenten (1963)
95	1% PVP and ammonium sulphate precipitation at *p*H 8	Kenten & Legg (1965)
80	PEG and celite (undiluted sap)	Adomako (1974)
84.2	1% Triton X-l00 and PEG precipitation (sap diluted 1:15)	Owusu & Lagudah (1986)
85	Carbon tetrachloride (CCl_4) (sap diluted 1:10)	Owusu *et al*. (1985)
80	Blood serum albumin (sap undiluted). Purification stopped at dialysis stage (Dzahini-Obiatey & Ollennu, 1996)	Dzahini-Obiatey (unpublished data)

medium on the infectivity of CSSV preparations over the years.

Apart from the ingredients in the extracting media, other equally important factors like shade were also found to be beneficial to the infectivity of CSSV during mechanical inoculations (Kenten & Legg, 1965). In general, introducing and using proteins (Brunt & Kenten, 1963; Brunt *et al.*, 1964; Kenten & Legg, 1965; Dzahini-Obiatey & Ollennu, 1996; Dzahini-Obiatey *et al.*, 1996a), particularly hide powder (Brunt & Kenten, 1963; Brunt *et al.*,1964; Kenten & Legg, 1965), had been the most effective input. It could be useful to find out how proteins act to improve infectivity, and whether such information could be beneficially exploited in CSSV purification in general.

Electron microscopy

One of the fastest ways to diagnose a virus is to examine its purified preparations by electron microscopy, using negative stain procedure. Brunt *et al*. (1964) were the first to observe CSSV particles to be bacilliform-shaped when negatively stained in phosphotungstate. Thereafter, electron microscopy became one of the tools for detecting and assessing the purity of CSSV preparations. Upon a significant improvement on CSSV purification methods, Adomako *et al.* (1983) detected viral particles of varying lengths in negatively stained uranyl acetate. Their micrographs had numerous particles per microscope field, and the result was fairly reproducible and so became the acceptable standard for purifying CSSV. However, a very important step in the method, the celite filtration column (Adomako *et al.*, 1983; Table 1), caused some inexperienced workers to lose virus during purification. Nevertheless, the method or its slightly modified form is used to obtain virus preparations with varying purity and content (Adomako, 1985; Adomako & Owusu, 1986; Sackey & Armah, 1993, 1994; Sackey, 1994a; Dzahini-Obiatey *et al.*, 1996 b, 1997 a, b).

For example, Adomako (1985) observed 10-100 particles per electron microscope field at a magnification of 40,000; whereas most other workers had less. He also assessed the effect of Triton X-100, hemicellulase, and driselase on the yield of the virus and found out that Triton X-100 had a damaging effect on the virus and its yield; while hemicellulase and driselase were insignificant. The Triton X-100 damaged the virus particles and caused no virus yield in some experiments. This contrasted with the observed increase in infectivity on treatment with the same chemical (Owusu *et al.*, 1985). Sackey (1994a) had mostly long, filamentous clostero-like particles in the mild strain he purified. Dzahini-Obiatey *et al.* (1997 a, b) had about 100 particles per field, a figure comparable to that of Adomako *et al.* (1983) only after coating the grids with antiserum from banana streak virus, BSV, a member of the badnavirus group (Fig. 3). The same viral preparation showed only very few virus particles when viewed under electron microscope elsewhere. Dzahini-Obiatey *et al*. (1997a,b) are also now investigating the possibility of reducing the time spent on purifying CSSV using the method described by Adomako *et al.* (1983).

The electron microscopy, although a very fast diagnostic tool in plant virology, is very expensive to run and cannot be used routinely, particularly for screening during breeding programme. For example, only one serviceable electron microscope serves all scientists in Ghana now. The CSSV electron microscopy requires highly purified virus preparations for it to work, and this also takes time to produce.

Serology

Serological tests may be decisive in identifying an unknown virus and important for studying the relationship between related virus isolates or strain. Such tests are based on the avidity that individual antibodies have for their own specific (homologous) antigens. Most plant viruses are effective antigens when injected into a suitable animal (usually a rabbit) and stimulate the production of antibodies that can be used in various serological tests. Highly purified virus

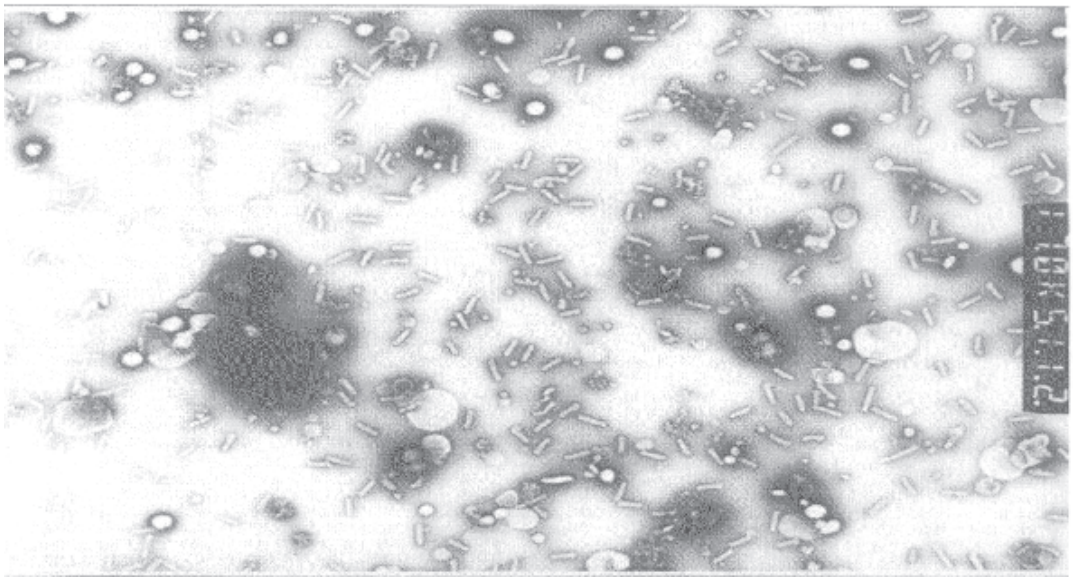

Fig. 3. An electron micrograph of the virus-rich preparation of CSSV Nsaba. The picture was taken at a magnification of 17,000. Note the white rod-shaped viral particles in the micrograph.

preparations are essential for such antibody production. The serum containing the antibodies, when separated from the remaining blood components, is called antiserum. The antiserum is used in reactions such as precipitin, immunodiffusion, agglutination, electron microscopy, enzyme-linked immunosorbent assay (ELISA), and other serological tests. Rapid and accurate diagnosis of viruses is often possible directly on crude preparations, using some tests mentioned above. Some of them are highly cost-effective and render many other diagnostic tests redundant.

Producing highly purified virus preparations has been a limiting factor in antiserum production in CSSV. In Ghana, the purity of CSSV sap preparations is determined by using infectivity rates or electron microscopy. A virus preparation is considered highly purified if it is highly infective (Brunt & Kenten, 1963; Kenten & Legg, 1965, 1967), or has very high number of viral particles per microscope field of view (Adomako *et al.*, 1983; Adomako, 1985; Dzahini-Obiatey *et al*., 1997a, b). The first CSSV antiserum was produced and used in agar-gel-double diffusion tests by Kenten & Legg (1971) to diagnose and study the relationship between some isolates of the virus. Other serological application of antisera in CSSV studies had been in immunosorbent electron microscopy (ISEM) (Adomako *et al*., 1983; Sagemann *et al*., 1983, 1985), in ELISA (Sagemann *et al*., 1983, 1985), in virobacterial agglutination test (VBA) (Hughes & Ollennu, 1993), and in immuno-capture polymerase chain reaction (ICPCR) (Sackey *et al*., 1995a, 1996a).

The underlining drive in all these tests and studies is to develop alternative means of diagnosing CSSV at a relatively faster rate than the visual symptoms method, which could still be relied on to detect the virus in the field. The ELISA, developed for use in detecting CSSV (Sagemann *et al*., 1983), was fast and reliable; but it could not effectively detect latent infections. Recent attempts to refine this method for field application has been constrained by the inability to produce good antisera for the numerous isolates found in Ghana. The VBA (Hughes & Ollennu, 1993), which could detect visible and latent

infections when it was first developed, is now thought to be overreactive because it has produced some false positive results. Attempts were made recently to re-evaluate and refine VBA (Owusu, Dzahini-Obiatey & Takramah, 1997).

The effects of ICPCR and ISEM on latent infections are unknown because they have not been evaluated. Serological studies have invariably contributed to the wealth of information on classifying CSSV, albeit some limitations on some of them. For example, Sagemann *et al*. (1985) were able to compare the relationship between 46 isolates of CSSV, using antisera for a few of them, in ELISA and ISEM. Hughes & Ollennu (1993) characterised eight isolates using similar antisera, whereas Sackey *et al*. (1996a) did so for two isolates.

The VBA and ELISA stand out as the most likely alternatives to visual symptom expression methods, but they would have to be re-evaluated and refined further to meet the challenges of over-reaction and inability to detect latent infections (Table 2). When this happens, screening of disease-resistant cocoa will then proceed faster. Developing VBA and ELISA will, however, depend on producing very good antisera, which in turn depends on producing very good virus preparation.

Nucleic acid technologies

Determining the type of nucleic acid of a virus by its buoyant density in caesium salt gradients, sensitivity to pancreatic RNase or DNase, or by its base composition is often diagnostic. Other useful information such as the strandedness (single or double) of the nucleic acid by methods like melting techniques (Shepherd, Bruening & Wakeman, 1970), nuclease activity and gel-electrophoresis (Luisoni *et al.*, 1979; Morris & Dodds, 1979) may also be required.

A CSSV isolate was first determined to be a double-stranded DNA virus with an approximate genome size of 7.4 kbp by using nuclease activity with gel-electrophoresis, restriction enzyme digest, and Southern blot analysis (Lot, Djiekpor & Jacquuemond, 1991). The genome was thereafter cloned and sequenced to determine segments that encode for specific activities such as the cell-to-cell movement, coat protein gene (Hagen *et al.*, 1993). The full-length genomes were also subsequently developed into infectious clones (Hagen *et al.*, 1994). Noting that CSSV is a DNA virus, Sackey *et al.* (1995a) also started parallel studies on the numerous Ghanaian isolates of CSSV that apparently vary from the Agou 1 strain [the isolate from which the first clone was made (Lot *et al.*, 1991; Hagen *et al.*, 1993, 1994)]. These workers used nucleic acid techniques such as polymerase chain reaction, PCR (Sackey, 1994b; Sackey *et al.*, 1995b; Dzahini-Obiatey *et al.*, 1996c), DNA hybridisation with radioactive (Dzahini-Obiatey, 1993; Sackey & Hull, 1994) and non-radioactive-labelled probes (Sackey *et al.*, 1995b, 1996b, 1999), and a combination of PCR and DNA hybridisation (Sackey *et al.*, 1995 a, b, c; 1996 a, b) to study the Ghanaian isolates of CSSV. Limited classification of some isolates was, thus, possible.

Some of these techniques can now be used in diagnosing and further characterizing the numerous CSSV isolates in Ghana. The dot blot hybridisation technique in particular can have large-scale application in the field (Table 2) and can, thus, fit into CSSV control programmes. The very low yield of CSSV DNA from cocoa tissues during extraction will, however, have to be addressed before nucleic acid techniques in general can become useful. Attempts are now underway to overcome this by finding other ways of extracting CSSV DNA as well as looking for other tissues for culturing CSSV (Dzahini-Obiatey, Aculey & Owusu, 2001). The possibility of cloning the CSSV DNA before any biochemical manipulations is also being considered.

Discussion and conclusion

The foregoing review shows that rapid and effective diagnosis of CSSV will advance the progress of the studies and control of the virus. In CSSV control, diagnosis enables latent infections to be distinguished from healthy plants

TABLE 2

Biochemical Detection Methods for CSSV and their Field Application Potentials

Method	*No. of samples that can be assayed at a time*	*Field application potential*	*Limitation*	*Remedy*	*Reference*
ELISA	96	. Extremely high . Require good antiserum . No or very little processing of tissues required	. Good results depend on very potent antiserum . Non detection of latent infections	. Refinement of methods to produce good antiserum and to detect latent infections . ELISA was recently refined to detect asymptomatic infection (Dzahini-Obiatey *et al.;* in press)	. Sagemann *et al.* (1985) . Dzahini-Obiatey *et al*., 2002a (in press)
VBA	12	. Very high . Require good antiserum . Very little processing of tissue required	. Good antiserum always required . Oversensitive; thus, prone to false positive	. Needs refinement and re-evaluation	. Hughes & Ollennu (1993)
lCPCR	40	. Medium . Require antiserum . Extensive processing of tissue required	. Cost of input very high and almost prohibitive	. Not much can be done about input cost	. Sackey *et al*. (1995a, 1996a)
PCR	40	. Low . Extensive processing of tissue required	. Cost of input very high . DNA has to be extracted for every assay at every time, making assay error-prone	. Not much can be done about input cost but extreme care can be taken during each DNA extraction to minimise errors	. Sackey & Hull (1994) . Sackey *et al*. (1995b) . Dzahini-Obiatey *et al*. (1996c)

TABLE 2 (continued)

Biochemical Detection Methods for CSSV and their Field Application Potentials

Method	*No. of samples that can be assayed at a time*	*Field application potential*	*Limitation*	*Remedy*	*Reference*
Dot hybridisation	Unlimited	. High . Very little processing of tissues required . Very minute quantities of sample and reagents required	. Cost of input high	. Not much can be done about input cost	. Sackey & Hull, 1994
Southern hybridisation	Up to 16	. High . Moderate processing of tissues required	. Cost of input high	. Not much can be done about input cost	. Sackey *et al.* (1995b, 1996b)
Denatured proteins on PAGE gels	Up to 16	. Low . Moderate processing of tissues required	. Cost of input high . Proteins will have to be for every assay, making it error-prone	. Not much can be done about input cost . High precaution needed to minimise error	. Dzahini-Obiatey *et al.* (2002b) (in press)
Western hybridisation	Up to 16	. Medium . Moderate processing of tissues required	. Cost of input high	. Not much can be done about cost	. H. Dzahini-Obiatey & P. C. Aculey (unpublished data)

during eradication of affected trees, so that fewer trees are cut during treatment. This approach may be more acceptable to farmers who are always against cutting of cocoa trees. When the diagnostic procedure is rapid, it accelerates screening during breeding for resistance against CSSV, and a lot more materials are assessed within a shorter time than it happens now. The drive to develop diagnostic procedures will also indirectly enhance the wealth of information generated on the virus.

The preceding sections of this review also show how important purifying CSSV is to developing the diagnostic methods, especially in electron microscopy, mechanical transmissions, nucleic acid technologies, and in producing antiserum for serological applications. The review further shows how factors such as reducing agents, proteins, other chemical additives, shade, and differential centrifugation have been found to improve the quality of purified CSSV preparations at one time or the other. The effect of protein had been particularly significant in producing highly infective preparations (Brunt & Kenten, 1963; Kenten & Legg, 1965). It is, therefore, suggested that future work in purification that has to be for sap transmission work should continue to evaluate and use proteins; while sap for electron microscopy

could concentrate on removing tannin and mucilage. Sources other than cocoa tissues could also be considered as alternatives for culturing CSSV for purification. Dzahini-Obiatey *et al*. (2002b) have started a project that is aimed at extracting CSSV and its DNA from viruliferous mealybugs.

In the quest to improve on CSSV purification methods, care should, however, be taken in selecting ingredients for the extraction medium; because the same chemical could impact differently on the virus under different conditions. For example, the damaging effect of 1 per cent Triton X-100 on viral particles (Adomako, 1985) contradicts the near beneficial effect of the same concentration of the same chemical on the infectivity of the virus (Owusu & Lagudah, 1986). It will, however, be interesting to find out whether electron microscopy and infectivity assays assess different components of the same virus. It will also be interesting to find out what causes infectivity. Could it be the coat protein, other associated proteins, or the viral particles themselves?

The discussion also clearly shows the potentials of ELISA and VBA as rapid, reliable, and cost-effective means of diagnosing CSSV. It will be useful to refine and re-evaluate them to enhance their detecting abilities to encompass latent infections, a definite step toward finding application for them in the CSSV disease control programmes. Nucleic acid techniques, particularly the dot blot hybridisation technique that also has high cost-effective diagnostic values, could be developed to complement ELISA and VBA. Developing good diagnostic methods for CSSV definitely holds a future in controlling the disease.

Acknowledgement

The authors are grateful to A. Y. Akrofi and Drs E. Boye Frimpong and F. M. Amoah for their useful comments on the manuscript. This paper is published with the kind permission of the Executive Director of the Cocoa Research Institute of Ghana.

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