Geographical Distribution of *Cacao swollen shoot virus* Molecular Variability in Ghana

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Abstract

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Cacao (*Theobroma cacao* L.) was introduced into West Africa from South America during the nineteenth century. However, cacao swollen shoot disease (CSSD) was first observed in Ghana in 1936 and, later, discovered in Nigeria, Côte d'Ivoire, Togo, and Sierra Leone. The objectives of this work were to assess the genetic diversity and spatial distribution of the *Cacao swollen shoot virus* (CSSV) in Ghana and investigate the origin and spread of the virus by identifying alternative host plants. Results obtained from polymerase chain reaction amplifications and phylogenetic relationship analyses of infected cacao and alternative host plants collected from the cacao-growing regions in Ghana revealed the existence of nine CSSV groups, A, B, C, E, G, J, K, L and M, with six groups detected for the first time in Ghana. The CSSV groups in Ghana are very divergent and correspond to at least five different putative species, according to the International Committee on Taxonomy of Viruses recommendations (A, B-C complex, G, E, and M), with the M species only being detected in the alternate host *Ceiba pentandra*. The spatial distribution of the different molecular groups in Togo, Côte d'Ivoire, and Ghana makes it difficult to predict a single origin for CSSV among the West African cacao-growing countries.

Major cacao-growing countries in West Africa, Côte d'Ivoire, Ghana, Sierra Leone, Nigeria, and Togo have been plagued by viral diseases affecting the cacao plant for several decades. Chief among these viral diseases is cacao swollen shoot disease (CSSD) caused by the *Cacao swollen shoot virus* (CSSV). This virus has been identified in West Africa only, whereas cacao originated from the Amazon basin (Dzahini-Obiatey et al. 2006). CSSV is a member of the virus family *Caulimoviridae*, genus *Badnavirus* and is naturally transmitted to cacao (*Theobroma cacao*) in a semipersistent manner by at least 14 species of mealybugs with *Formicococcus njalensis*, *Ferrisia virgata*, and *Planacoccus citri* being the most studied (Dongo and Orisajo 2007). CSSD was first observed in Ghana in 1936 at Effiduase in the New Juabeng District, when a plant pathologist made a detailed description of a disease reported to him by a cacao farmer (Steven 1936), although the disease was probably present in the nearby Nankese township of Ghana from 1922 onwards (Paine 1945). The disease was later discovered in the other West African cacao-producing countries, such as Nigeria (1944), Côte d'Ivoire (1946), Togo (1949), and Sierra Leone (1963) (Attafuah et al. 1963; Mangenot et al. 1946; Thresh 1959).

Several control strategies have been put in place in Ghana to check the spread of CSSV, including a massive nationwide eradication campaign that began in 1946 and aimed at cutting out diseased cacao plants and any other cacao plant in contact with the diseased plants (Owusu 1983). Some of these strategies included the introduction of block plantings in the 1950s, in which large areas of infected cacao trees were cleared and replanted in contiguous blocks (Owusu 1983). The "plant-as-you-cut" scheme was introduced in the 1970s (Thresh et al. 1988); under this intervention, the government cut down CSSV-infected trees, then, replanted and maintained the farms for a time, before returning them to their owners. Another intervention was the Suhum rehabilitation project funded by the World Bank, in which diseased farms were cut down and replanted in blocks with fast-growing and early-yielding cultivars (Owusu 1983). Despite these interventions, CSSD is still prevalent in Ghana and is causing economic losses to cacao production in Ghana and the other West African cacao-growing countries.

Over the years, Ghana's cocoa production has faced major adverse challenges, which have contributed to the country losing her position as the leading producer of cocoa beans in the world (Anim-Kwapong and Frimpong 2005). CSSD together with other viral and fungal diseases has been a major factor in the decline of cacao production in Ghana and the other countries. It severely reduces the yields of an infected cacao tree and kills the tree within 5 years (Posnette 1947). Between these two extreme phases (reduced yield and tree death), the leaves gradually turn yellow and fall, and then, the branches dry out from their tips (die back). CSSV infection exhibits myriad symptoms in the cacao plant; these include red vein banding in young leaves, different shades of chlorosis in mature leaves, swelling on stems, chupons, and roots. A large number of isolates have been distinguished by the symptoms they cause in cacao in the different countries concerned, and this number would probably be even larger if finer differences in symptom expression were considered.

Most studies on the virus have centered on the description of symptoms in infected cacao plants and, also, on factors favoring the spread of the disease by vectors. Molecular studies on the virus started in 1990, once it was discovered that CSSV possessed nonenveloped bacilliform particles and a double-stranded circular DNA genome of about 7.5 kb (Lot et al. 1991). Further molecular analyses of the CSSV genome revealed five putative open reading frames (ORF) located on the plus strand whose biochemical functions were thoroughly investigated. ORF1 encodes a 16.7-kDa protein, whose function is yet to be determined (Hagen et al. 1993). The ORF2 product is a 14.4-kDa nucleic acid–binding protein (Jacquot et al. 1996). ORF3, which is the largest, codes for a polyprotein of 211 kDa, which contains from 5′ to 3′ consensus sequences characteristic of a cell-to-cell movement protein, an RNA binding domain of the coat protein, an aspartyl proteinase, a reverse transcription (RT), and a ribonuclease H (RNase H). ORFX (13 kDa) and ORFY (14 kDa)

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overlap ORF3 and encode proteins of unknown functions. Molecular work on CSSV isolates from various areas of West Africa has identified and categorized the different isolates in several distinct groups (A, B, C, D, E, F) based on the sequence alignment of the first part of ORF3 (Kouakou et al. 2012; Oro et al. 2012). Oro et al. (2012) identified the A, B, and C groups in Togo, and similar work by Kouakou et al. (2012) in Côte d'Ivoire identified the B, D, E, and F groups in that country. Only groups A, B, and E have been detected in Ghana so far (Kouakou et al. 2012; Oro et al. 2012). Additionally, when using the International Committee on Taxonomy of Viruses recommendations, which consider nucleotide diversity in the RT/RNaseH region, four different species (A, B-C complex, D, and E) sharing a less than 80% nucleotide identity threshold could be described.

Ghana is the country where the disease was first described and where it is currently the most widespread. It was important to study the variability and the distribution of CSSV isolates and species involved in CSSD in Ghana as a means to identify the various groups present in the country, as has been done in Togo and Côte d'Ivoire. Considering the vast economic losses in cacao revenues due to CSSD, it was additionally appropriate to study the molecular variability and phylogenetic relationship among the different CSSV groups in Ghana, as this would make it possible to screen cacao varieties for resistance to CSSV with isolates representative of the diversity present in each area and also help to improve and validate diagnostic tools for CSSV detection. To achieve these goals and also try to determine whether Ghana could be the origin of CSSV, we carried out molecular characterization of CSSV isolates in the six main cacao-growing regions of Ghana where CSSD has been reported, namely Ashanti, Brong-Ahafo, Central, Eastern, Volta, and Western. CSSV was amplified from DNA extracted from leaves of infected cacao and wild hosts, using polymerase chain reaction (PCR), and the resulting amplified fragments were sequenced. Phylogenetic analyses of sequenced fragments (5′ end of ORF3 of the CSSV genome) were then carried out to compare the new sequences to those sequences already identified in Ghana and the neighboring West African cacao-growing countries (Togo and Côte d'Ivoire). We were able to detect CSSV groups A, B, C, E, G, J, K, L, and M in the cacao-growing regions of Ghana and at the Cocoa Research Institute of Ghana (CRIG) research station in Tafo, with groups G, J, K, L and M being detected for the first time.

Materials and Methods

Sample collection. In all, 846 samples were collected from 2013 to 2014, of which 791 were symptomatic leaves from infected cacao trees. They were harvested and sun-dried between January and March 2013 and 2014, respectively, in the cacao-growing regions of Ghana (Fig. 1). Young leaves showing red vein banding symptoms were preferred; however, in their absence, older leaves showing various leaf symptoms were collected. Asymptomatic leaves from trees showing shoot and stem swellings were also collected. In all about three to five leaves were collected from each tree. Around 20 asymptomatic leaf samples were also collected from wild hosts (alternative host plants) located on or near plots or farms where infected cacao leaves were sampled. Ten samples collected in 2010 by H. Dzahini-Obiatey at the CRIG research station in Tafo, Ghana, were also included in this study.

Total DNA extraction. About 20 mg of dried cacao leaf portion from each sample was ground in microcentrifuge tubes in the presence of ceramic beads with an MP disrupter (Labtech). DNA was extracted, using the DNeasy plant mini kit from Qiagen (Hilden, Germany) according to the manufacturer's protocol. In each case, DNA was eluted in two elutions of 75 µl each, making a total of 150 µl, and the concentration of each DNA was estimated using a Thermo Scientific spectrophotometer (Labtech), which uses NanoDrop 2000 technology.

PCR analysis. Primer pairs ORF3A-F (5′GTYRTACCRRAYA YYATGATGAC3′) and ORF3A-R (5′GTYTYCCRTTRSYRGA YTCYTCCCATAC3′), together with Badna1 CSSV (5′CTTTT ATGAATGGTTAGTGATGCCCTTTGG3′) and Badna4 CSSV

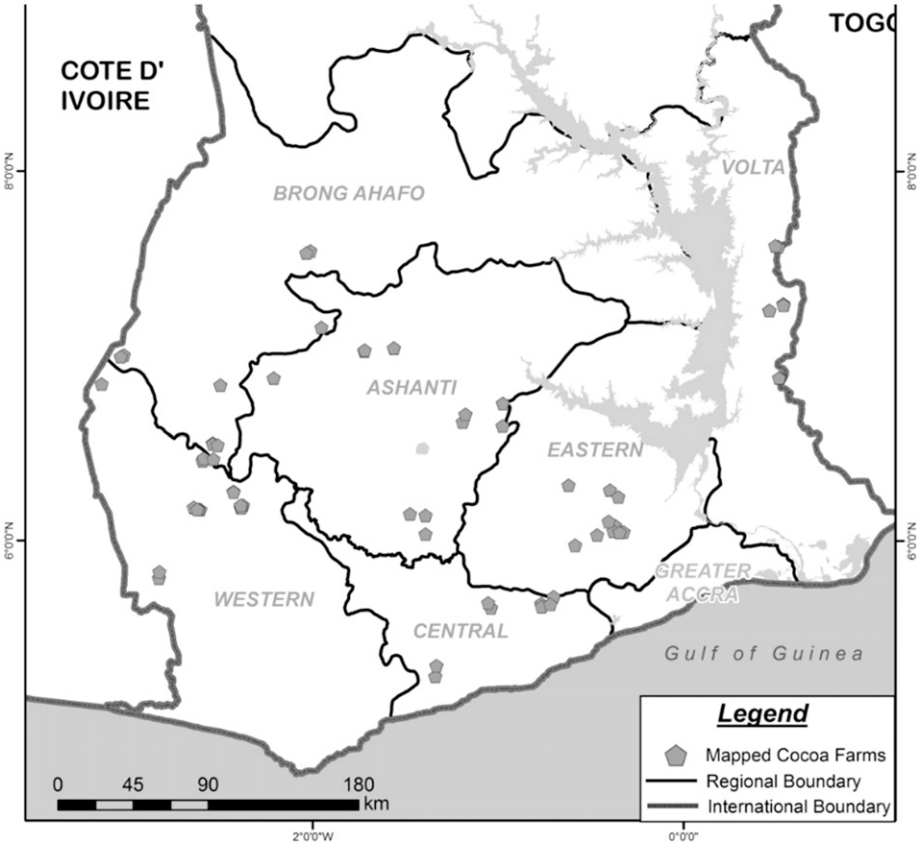

Fig. 1. Map of southern Ghana showing regions and locations affected by cacao swollen shoot disease where leaf samples were collected for analysis.

(5′TCCACTTACATACGGCCCCCCATCC3′), were used. The primer pair (ORF3A-F/R) corresponds to a conserved region at the beginning of the ORF3 domain of the CSSV genome. It amplifies a 534- to 537-bp fragment corresponding to positions 1,848 to 2,381 of the complete DNA sequence of the CSSV isolate Agou1 (L14546). This primer pair was obtained by aligning the set of CSSV sequences available in 2009, including the CSSV detected in Côte d'Ivoire during the outbreaks of CSSD in the center of that country and which diverged from the characterized CSSV groups A, B, and C (Kouakou et al. 2012). The primer pair (Badna1/4 CSSV), corresponding to a conserved region in the RT/RNaseH region (third part) of ORF3 of the CSSV genome, was used to amplify CSSV DNA from isolates representative of the different groups. PCR amplifications were performed in 25 μl of reaction mixture containing 10 μl of plant DNA ($^1/_{10}$ dilution of original DNA obtained from leaves), each dNTP at a final concentration of 0.2 mM, each primer at a final concentration of 1 μM and 1 U of GoTaq DNA polymerase (Promega) in the reaction buffer supplied by the manufacturer. The two PCR reactions were carried out in a Biometra T personal thermal cycler, using an initial denaturation step at 94°C for 2 min, followed by 35 cycles at 94°C for 30 s, 49°C for 30 s, and 72°C for 1 min, and a final elongation step at 72°C for 10 min. When the result was negative, another PCR amplification was performed in a mixture containing another dilution of the plant DNA (1 or 5 μl).

Sequencing and sequence analysis. Two isolates per farm detected by the PCR diagnosis (primer pair ORF3AF/R) were chosen for sequencing. Direct sequencing of the amplified fragments was done by Beckman Coulter Genomics (U.K.). Seaview version 4.0 software was used to analyze the DNA sequences, and nucleotide sequences were aligned using the Clustal W multiple alignment algorithm (Thompson et al. 1994). Phylogenetic relationships between CSSV sequences were estimated with PhyML (maximum likelihood method) (Guindon and Gascuel 2003) with 500 bootstrap repeats, and phylogenetic trees were visualized with the Darwin 5 program (Perrier et al. 2003).

Results

CSSV detection in the six cacao-growing regions of Ghana and at the CRIG research station. Using the primer pair ORF3A-F/R on the 846 samples from 76 plots or farms, CSSV was amplified in 515 of the samples, which translated into a 60.9% rate of detection. Of 76 plots or farms, 65 recorded at least one or more CSSV amplification; this translated into an 85.5% detection rate at plot level. A total of 332 leaf samples had no CSSV amplification; this amounted to 39.2% of the total number of leaf samples collected (Table 1). The Eastern region had the highest percentage of detection (85.3%), followed by Brong-Ahafo (64.6%), Central (58.9%), Western (46.2%), Ashanti (45.9%), and Volta (41.8%) (Table 1). In all, five CSSV amplifications were recorded out of the 20 leaf samples collected from alternative host plants located in or near sampled farms; this resulted in a 25% detection rate in the wild host (Table 2).

Of the 10 cacao leaf samples collected from the CRIG research station, only four tested positive with the primer pair ORF3A-F/R.

Phylogenetic analyses of CSSV isolates. In this study, 103 partial ORF3 sequences were obtained; CSSV isolates that shared over 80% nucleotide sequence identity were grouped together. These gave rise to sequences that aligned with groups A, B, C, and E, which were already known (Fig. 2); other sequences formed clades that were different from the already-known groups and were, thus, designated as new groups and, subsequently, named G, J, K, L, and M respectively (Fig. 2). Most of the isolates or sequences obtained were confined to the B and E groups (Fig. 2). Sequences belonging to the B group were very homogeneous, whereas sequences or isolates in the E group were very heterogeneous.

Sequences in the B group shared over 95% identity with each other, similar to isolates or sequences in the A and C groups. Groups B and C shared more than 80% sequence identity and are considered

Table 1. Total number of cacao leaf samples collected from each region, percentage of detection, *Cacao swollen shoot virus* (CSSV) groups identified, and number of leaves labeled as suspects

Region	No. of positive samples/ total no. of samples	Detection (%)	Farms with positive samples (%)[a]	No. of suspected samples[b]	CSSV groups identified after sequencing
Ashanti	40/87	45.9	63.3 (7/11)	20	A and B
Brong-Ahafo	93/144	64.6	76.9 (10/13)	15	E and B
Central	82/139	58.9	100 (9/9)	15	B and L
Eastern	180/211	85.3	100 (14/14)	–	B
Volta	23/55	41.8	63.6 (7/11)	5	A, B, and C
Western	97/210	46.2	100 (18/18)	–	B, E, J, K, and A
Total	515/846	60.9	85.5 (65/76)	55	A, B, C, E, J, K, and L

[a] In parentheses: number of farms containing positive samples/total number of farms.
[b] Leaves collected from plants showing shoot and stem swellings (asymptomatic leaves) CSSV symptoms and from plants with indistinct leaf symptoms (colorations).

Table 2. Sampling region, type, and number of alternative host plants analyzed

Location	Type of wild hosts	No. of wild hosts	No. of CSSV positive samples	CSSV isolate groups identified
Western region	*Ceiba pentandra*	5	2	C
	Sterculia tragacantha	4	–	–
	Cola gigantea	1	–	–
Eastern region	*Sterculia tragacantha*	1	–	–
	Ceiba pentandra	1	1	M
Brong Ahafo region	*Ceiba pentandra*	3	1	nd[a]
CRIG (CSSV museum)[b]	*Cola chlamydantha*	1	–	–
	Ceiba pentandra	1	–	–
	Adansonia digitata	1	–	–
	Sterculia tragacantha	1	1	nd

[a] nd indicates the sequence is not determined.
[b] CRIG = Cocoa Research Institute of Ghana; CSSV = *Cacao swollen shoot virus*.

as a single group, B-C. Isolates in the E group shared varying degrees of sequence similarities within the group; however, all isolates in this group shared a minimum of 80% identity in their nucleotide sequences. Sequences from groups A, B-C, E, G, J, K, L, and M taken in pairs shared less than 80% sequence identity.

When isolates from groups A, B-C, E, G, and M were amplified using the primer pair Badna1/4 CSSV in the third part of ORF3, corresponding to the RNase H/RTase region, the sequences also shared less than 80% sequence identity. However, after sequencing of the same region for isolates representative of groups E, J, K, and L, these

Fig. 2. Maximum likelihood phylogenetic tree of cacao swollen shoot disease (*Cacao swollen shoot virus* [CSSV]) sequences based on alignment of the 5′ end of open reading frame 3. Numbers on the branches represent percent bootstrap values (500 replicates) over 70. The names of the CSSV groups A, B, C, E, J, K, L, and M are indicated. Other CSSV sequences representative of groups D, G, and B groups were used in comparison along with the *Citrus mosaic virus* sequence (CiYMV) (AF347695) used as the outgroup. The names of sequences include the abbreviation of the country (CI for Côte d'Ivoire, G for Ghana, To for Togo), the abbreviation of the locality name or a sampling number, and the year of sampling (1993 to 2014 coded as 93 to 14). The scale bar shows the number of substitutions per base.

groups were found to be very close to each other (less than 10% nucleotide divergence).

Geographical distribution and structuring of CSSV isolates in Ghana. CSSV sequences obtained from this work belonged to nine molecular groups (A, B, C, E, G, J, K, L, and M) (Fig. 3). The total number of CSSV molecular groups detected in Ghana proved to be well above the number of CSSV groups detected in Togo (three) and Côte d'Ivoire (four). Group B was detected in all the regions sampled and seemed to be the most widespread in Ghana (Fig. 3), whereas isolates belonging to group A were only detected on farms or plots located in the Western, Volta, and Ashanti regions of Ghana, the majority of which were amplified in samples from the Volta region. Isolates belonging to group C were detected in the Volta region only; it is the first time this group of isolates has been detected in Ghana, but it had been detected in Togo.

Isolates belonging to group E were mostly from the Western and Brong Ahafo regions of Ghana, with the majority from the Western region (Fig. 3). These isolates exhibited varying subtle CSSD symptoms, mostly including swelling on shoots or stems, various forms of leaf mosaic, and only sometimes, red vein banding in young flush leaves. Group G was only detected at the CRIG research station.

In most cases, sequences from the same farm or plot always belonged to the same group, but there were some instances where isolates belonging to two different groups were identified on the same plot or farm (Table 3). For two farms in the Volta region, one was infected by the A and C isolates, while the other farm was infected by both the A and B isolates. For the three farms in the Western region, one had isolates belonging to groups E and J. The second farm had CSSV isolates belonging to the A and B groups. The third farm in the Western region was infected by isolates belonging to the B and E groups. A single farm in the Ashanti region also had cacao trees infected with isolates belonging to groups A and B. A single farm in the Eastern region had isolates belonging to the B and M groups (Table 3).

Discussion

Detection and amplification rates. The detection rates (Table 1) were very similar to the results obtained by Kouakou et al. (2012) in a study on the molecular variability of CSSV in Côte d'Ivoire using the same primer pair (ORF3A-F/R). They recorded 63.5% as the overall rate of detection and 80% as the detection rate for farms or plots that had one or more CSSV amplification. A total of 39.2% of our samples went undetected or had no CSSV amplification; this may be due to the fact that some of our samples were old or matured leaves, as it was found in studies by Muller et al. (2001) that CSSV concentration decreases or becomes low and unavailable as leaves age or mature. It has been proven experimentally that CSSV concentration varies within the same leaf showing symptoms of CSSD infection (Dzahini-Obiatey and Fox 2010). Misidentification of CSSD symptoms could also be a possible cause of failure to detect CSSV in leaves that were not infected but might have been collected during sampling. These factors might have accounted for our samples that went undetected. Also, given the variability that was observed in populations of CSSV in countries like Togo (Oro et al. 2012) and Côte d'Ivoire (Kouakou et al. 2012), it was likely that similar or greater variations were to be expected in Ghana, hence, the primers used in this work may not have been polyvalent enough to amplify CSSV DNA from all the samples.

Geographical differentiation and biodiversity of CSSV in Ghana. The C group was predominant in the Volta region of Ghana, a region that shares a border with Togo (Fig. 1). This group was detected on three plots or farms in the same location (Akpafu Adokor in Hohoe district). The detection of this group was quite interesting, since it has only been detected until now in Togo in the Kloto region (Oro et al. 2012) close to the Volta region, illustrating the fact that this viral group has spread only locally. The A group was detected in three regions (Volta, Ashanti, and Western); this group has also been detected in Togo. In the Volta region, it was detected in two separate locations (Akpafu Adokor and Dzindziso). Dzindziso is a small village situated near the Ghana and Togo border and is noted for having long stretches of cacao farms extending into Togo (the Litimé region). This group has also been detected in the Litimé region of Togo; however, it was also detected in samples from the CRIG museum in 2000 (SS365B/Ghana 30 isolate) (Oro et al. 2012). This isolate was collected in the Eastern region. The B group was detected in all six regions studied in this work and has also been detected in neighboring countries where CSSV is prevalent, notably Togo (Oro et al. 2012) and Côte d'Ivoire (Kouakou et al. 2012). The ubiquitous nature of the B group may mean it is readily transmitted by mealybugs, probably because of higher concentrations in the cacao phloem than the other groups (Posnette 1980). This group tends to induce more easily the typical red vein banding symptom seen in young or flush leaves of infected cacao plants, since most of the samples collected in the Eastern region, whether young or mature, had the conspicuous red vein banding or vein clearing symptom.

Another group, designated E, was detected in a very large proportion of the samples in this work; an isolate (Punekrom/Ghana 26) belonging to this group was once detected in the CRIG museum by Kouakou et al. (2012). This group was predominantly detected in the Western and Brong-Ahafo regions. The large-scale detection of the E group in the Western region was expected in a way, but in another way, it was surprising and interesting, as this region is now regarded as the region with the highest level of CSSV infection in Ghana (Domfeh et al. 2010; Dzahini-Obiatey et al. 2010). Current levels of the disease in parts of this region (the northern part) have led to the designation of an area of about 29,000 hectares in Essam district as an area of mass infection (AMI) (CSSDCU 2007). Other parts of the same region, such as Sefwi Bekwai, Enchi, Juabeso, and Sefwi Buako have also been reported to have high levels of CSSV infection (Domfeh et al. 2010). The high incidence of the disease in the Western region is partly due to opposition from farmers against the cutting down of infected cacao trees and also the lack of diagnostic leaf symptoms in infected cacao trees contrasting with infected cacao trees in the Eastern region and other parts of the country. Samples belonging to the E group exhibited unrecognizable symptoms until advanced stages of the disease, when stem swellings and die-back were observed. The other regions in which CSSD is reported have cacao trees showing distinctive symptoms such as red vein banding and vein clearing, which is absent in some parts of the Western region (Domfeh et al. 2011).

Five other groups (G, J, K, L, and M) were also detected for the first time in Ghana in this work. The J and K groups were detected in the Western region, whereas the L and M groups were detected in the Central and Eastern regions, respectively. The G group was restricted to the CRIG research station, Tafo in the Eastern region, and the CRIG museum (unpublished results). Using the 80% nucleotide identity threshold in the RT/RNase H domain for discriminating badnavirus species, isolates from groups A, B-C, E, G, and M could be considered as five different putative species, G and M being new.

Samples from the E, J, K, and L groups showed less than 10% nucleotide divergence in their RT/RNaseH region, when amplified with the primer pair Badna1/4 CSSV, whereas the sequences identified for these isolates in the first part of ORF3 showed more than 20% divergence, which results in a distortion between the phylogenetic trees constructed from the two genomic parts. This distortion means that recombination took place between the first and third part of ORF3 among isolates from these groups and one or more other unknown isolates. Mixed infections by closely related viruses (as observed in

Table 3. Farms with *Cacao swollen shoot virus* (CSSV) isolates belonging to two different CSSV groups

Farm number	Region	Location	Type of isolates
1	Volta	Akpafu Adorkor (Hohoe)	A and C
11	Volta	Dzindzinso (Jasikan)	A and B
8	Ashanti	Betriako (Tepa)	A and B
5	Western	Aprompe (Sefwi and Buako)	E and J
16	Western	Kaase Tema (Bia and Essam)	A and B
17	Western	Asuoti (Enchi)	B and E
13	Eastern	Akyem Tontro (Tafo)	B and M

some instances) (Table 3) open up the possibility for recombination and reassortment, which can lead to the emergence or origination of new viral species (Elena et al. 2014). Another possibility is that mixed infection by distinct viral isolates is being detected in individual plants (Sagemann et al. 1985). There is now a need to obtain full sequences for these isolates to confirm recombination and try to identify their parental sequences. The possibility of recombinant sequences further increases the variability of viral sequences responsible for causing a disease, hence the difficulties in fighting the disease.

Possible origin of CSSD in Ghana. Given the fact that three of the CSSV groups (A, B, and C) identified in Ghana are the only groups identified in Togo (Oro et al. 2012), it should be easy to say that groups A, B, and C probably originated in or emerged from Ghana, since CSSD was first detected in Ghana in 1936. However, until this study, the CSSV C group had never been detected in Ghana (nor was it detected in the collection at the CRIG museum); this makes it difficult to conclude that CSSV (all groups) emerged from Ghana, since group C was detected earlier in Togo. Likewise for the spatial distribution of CSSV groups in Togo; the C group is shown to be restricted to the Kloto area, which is located close to the border between Ghana and Togo (Oro et al. 2012). Hohoe district is not very far from the border with Togo and there is, therefore, the possibility of farmers exchanging planting materials in the form of seedlings or infected pods between these regions. Mealybugs feeding on cacao trees close to the borders of these countries can also transmit the virus from one location to the other. Since the A and B groups have already been detected and documented in these two countries, it could be that they emerged from one of the two countries and infected the other or they emerged differently at different times in these two countries. On the other hand, when CSSV groups identified in Ghana are compared with those found in Côte d'Ivoire, only groups B and E are common to both countries. In addition to these two groups, Côte d'Ivoire has other groups (D and F) that have not been detected in Ghana up to now and Ghana also has groups A, C, G, J, K, L, and M, which have not been identified or detected in Côte d'Ivoire. This distribution may reflect differences in cacao crop-planting dates in the regions of these countries These observations, again, make it difficult to predict a single origin for CSSV in West Africa among the cacao-growing neighboring countries and we can, therefore, make the assumption that the different putative CSSV species emerged from different sources at different times.

The roles played by alternative host plants (wild host) in all of this cannot be over-emphasized, since CSSV is not present in the Amazon basin, where the cacao tree originated. The exact role played by these wild hosts in the epidemiology of CSSV is, today, difficult to investigate, because the prevalence of CSSD is greater in cacao trees than in these alternative host plants. Cacao-to-cacao transmission of CSSV is easier and very likely when compared with cacao-to-alternate host plant transmission or vice versa. An additional difficulty is that studies on the role of alternative hosts would require the use of vectors, because CSSV is not easily transmitted mechanically. It is considered a phloem virus and its mealybug vectors are phloem feeders (Entwistle and Longworth 1963; Esau 1961). According to Posnette (1980), the less pathogenic strains seem to be in lower concentrations in cacao than the more virulent ones and, in most wild host plants, even the strains that are virulent in cacao attain only low concentrations in the chronic phase and, probably, the virus is often localized in only certain parts of the plant. The occurrence of infected specimens of *Cola chlamydantha* at locations where CSSD is unknown strongly suggests that CSSV infection of *C. chlamydantha* antedates that of cacao (Todd 1951). This also supports the assertion that CSSV was probably transmitted to cacao by the infected wild host plants and that new wild host plants (located near infected cacao trees) became infected by the infected cacao trees, with these newly infected alternative host plants then acting as or remaining as sources of inoculum for fresh outbreaks on new cacao farms after the destruction of previously infected cacao farms.

The fact that CSSV was amplified from alternative host plants (Table 2) in this work makes it more interesting, since all the amplified

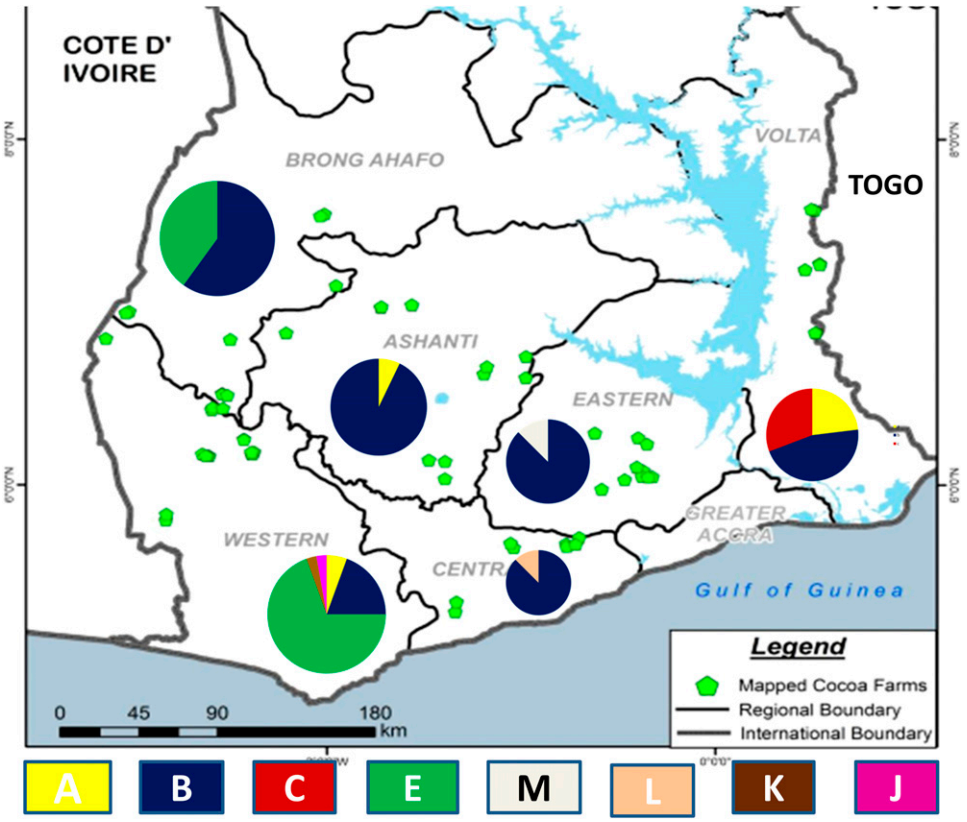

Fig. 3. Regional map of southern Ghana showing the distribution of *Cacao swollen shoot virus* (CSSV) isolates detected. Group G was only detected at the Cocoa Research Institute of Ghana research station, not in the fields. Pie charts in each region show proportions of the CSSV isolate groups present (described by a corresponding letter and color as presented in lettered boxes).

CSSV isolates from the wild host *Ceiba pentandra* always belonged to different groups than those amplified from the infected cacao trees of the same plot. For instance, on one farm in the Eastern region (Akyem Tontro), the CSSV isolate M was amplified from an alternative host plant, whereas CSSV isolates belonging to group B were amplified from the infected cacao trees of the same farm and plot. There were two instances in the Western region (Kaase Yankwa) in which the detected CSSV isolates from alternative host plants belonged to group C, whereas the isolates detected from the infected cacao belonged to group B. Even though it has been demonstrated that CSSV can be transmitted to these wild hosts and vice versa (Tinsley and Wharton 1958), CSSV is now established in cacao trees to the extent that its spread from cacao to cacao is more common and more possible than the other way round. However, CSSV viral species M has not yet emerged on cacao trees and could, potentially, create new outbreaks in this region. Such observations will support the hypothesis of the origin of the disease in alternative hosts.

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