International Scholars Journals

African Journal of Virology Research ISSN 3421-7347 Vol. 13 (3), pp. 001-008, March, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Status of cocoa swollen shoot virus disease in Nigeria

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Accepted 9 October, 2018

Cocoa Swollen Shoot Virus Disease (CSSVD) is one of the major diseases affecting cocoa production in West Africa, especially Ghana. The lack of any published article on the continued presence or absence of this viral disease has necessitated this investigation. Cocoa leaf samples from plants showing symptoms of leaf chlorosis, interveinal chlorosis with mottling and swollen shoot were collected from Cocoa Research Institute of Nigeria, Ibadan, and two farmers' plots at Offa-Igbo, both in Oyo State, Nigeria. Detection of Cocoa Swollen Shoot Virus (CSSV) in the leaf samples was done by Enzyme Linked Immunosorbent Assay (ELISA) using antiserum raised against mild and severe strains of the virus. CSSV was readily detected in 60% of the samples by Antigen Coated Plate ELISA (ACP-ELISA) but not with the Protein A Sandwich ELISA (PAS-ELISA). Of the positive samples, 16.67% reacted to both the mild and severe strains of the virus. CSSV was detected in most of the positive samples from farmers' plot 1 (68.96%) than from farmers' plot 2 and CRIN respectively (54.16 and 55.55%). The result has re-established the natural occurrence of the virus in Nigeria and thus calls for a re-evaluation of the economic importance of the disease in Nigeria.

Key words: Cocoa, cocoa swollen shoot virus, ELISA.

INTRODUCTION

About 80% of the world's cocoa production is in West Africa where it contributes significantly to the economies of countries in this sub-region, as well as many other countries in Central America and South East Asia. In West Africa, Nigeria is the third largest producer of Cocoa accounting for approximately 12% of the world production (ICCO, 2004). The production of cocoa in Nigeria has witnessed a downward trend since the early 1970s and a lot of factors including ageing trees, shortcomings in applying certain agronomic techniques by the farmers, drop in cocoa bean quality and particularly the effects of several pests and diseases have been implicated.

One of the most important cocoa diseases in West Africa is the Cocoa Swollen Shoot Virus disease (CS-SVD), caused by Cocoa Swollen Shoot Virus (CSSV). The disease has been a major factor in the decline of cocoa production in Ghana and Nigeria over the the past several years (Thorold, 1975; Woods, 1987). It was initially thought to be an agronomic problem until Posnette (1943) diagnosed it as a viral disease. In Nigeria, swollen shoot outbreaks were discovered in 1944 in two areas, the larger being to the east of Ibadan. The responsible pathogen, CSSV, spread from tree to tree by mealy bugs and over 8 species of mealy bugs are known to be capable of transmitting the virus (Padi, 1997). *Planococcoides njalensis* (Laing) and *Planoco-ccus citri* are known to be the most important mealy bug vectors by virtue of their being the most abundant implicated with transmission of CSSV.

There are many strains of the virus, which differ in the symptoms they produce. The virulent strains cause various types of leaf chlorosis, root necrosis, swellings on branches and twigs, with greater development of phloem and xylem, followed by die-back.

Trials carried out during the 1950s in Nigeria showed that the effect of the viral disease alone was relatively

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slight and it was only where trees were attacked by capsids (mirids) that viral infection accelerated the decline and possible death of the trees (Thresh, 1960). However, Adegbola (1981) reported that infection with the swollen shoot virus causes a disease which, apart from reducing the productive life of the cocoa tree, slowly but surely kills the entire tree. He was able to show that despite regular farm routine maintenance and mirid control practices affected by spraying with Gamma-benzene hexachloride, appreciable loss in pod production of CSSV infected cocoa trees was obvious with time in years. Generally, however, the spread is gradual, a slow but steady process by which an outbreak become pro-gressively larger, causing even increasing numbers of trees to become infected.

Active research on CSSVD in West Africa has been concentrated in Ghana and the main thrust of research has been directed at various aspects of disease control, the causal agents and their vectors. To date, the recommended control measures include cutting out of infected trees and breeding for resistance. During the 1950's and 1960's in Nigeria, the government introduced the cutting down policy to control CSSVD but this was greatly opposed by the farmers and was abandoned in favour of trying to contain the disease with a Condon Sanitaire (Wood and Lass, 1989).

In the past 15 - 17 years, no published research work has been carried out on CSSV in Nigeria. Going by the economic importance of the disease in neighboring countries, like Ghana and Togo, where more than 200 million trees have been cut down since 1948, there is an urgent need for a re-evaluation of the importance of the disease in Nigeria. Most cocoa trees in Nigeria are over 40 years old and as such, leaf symptoms of the disease are masked except for stem swellings. The continuous loss of cocoa trees by farmers in Nigeria is commonly attributed to old age and black pod diseases.

It is against this background that this study was conducted to re-establish the presence of the virus and subsequently ascertain its distribution through a survey of the major cocoa growing areas of Nigeria with the view of characterising the isolates and evaluate their economic importance.

MATERIALS AND METHODS

Collection of samples

Leaves of naturally-infected cocoa plants showing symptoms of swollen shoot, interveinal chlorosis with mottling and leaf chlorosis were collected from Cocoa Research Institute of Nigeria (CRIN), Ibadan and two farmers' plots at Offa-Igbo, Oyo State. A total of 80 leaf samples were collected, out of which, 27 were from CRIN, 29 and 24 from farmers' plot 1 and 2 at Offa-Igbo, Oyo State, Nigeria respectively. Equally collected from these sampled centres were asymptomatic leaf samples. The leaf samples were placed in transparent poly bags and brought back to the virology laboratory, International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria for serological detection of CSSV by Enzyme linked Immunosorbent Assay (ELISA).

Serological detection

The collected leaf samples were each tested at Virology Laboratory, IITA by ELISA of extracted sap using antisera raised against two strains of CSSV, mild strain (NA) and severe strain (1A). The polyclonal antisera and healthy control saps were kindly supplied by Cocoa Research Institute of Ghana, Ghana. Two ELISA techniques, Protein A Sandwich ELISA (PAS- ELISA) and Antigen Coated Plate ELISA (ACP-ELISA) were used to detect the presence or absence of the two strains of the virus. The PAS-ELISA protocol was as described by Hughes (1986) using 8x12 well polystyrene microtitre plates. ACP-ELISA was carried out according to Huguenot (1992). In both protocols, 100 µl well^{-1} were used except for the substrate where 200 µl well^{-1} was used. The absorbance was read at 405 nm (A_{405}) using Dynatech MR 5000 microtitre plate reader. Means for test samples and healthy control in duplicate wells were calculated.

Processing of ELISA data

Based on the mean absorbance values obtained, each sample was scored positive or negative for CSSV. A sample was considered infected (positive) when its absorbance was at least twice that of the healthy control (Sutula et al., 1986). The incidence of CSSV was calculated as the ratio of samples containing the virus either singly or in a mixture to the total number of samples processed (Barnett, 1986): % incidence of CSSV = No of samples +ve for the virus singly or in mixture x 100 / Total No of samples tested

RESULTS AND DISCUSSION

Collection of samples

Seventy two (72) out of the 80 leaf samples collected showed symptoms of leaf chlorosis, interveinal chlorosis with mottling and swollen shoot (Figure 1) while 8 of the samples were symptomless.

Serological detection

Using ACP-ELISA, CSSV was detected in 48 out of the 72 symptomatic leaf samples assayed giving a percentage incidence of 60. There was no detection of CSSV in symptomless leaf samples. The severe strain of CSSV (1A) was detected in mixed infection in 8 leaf samples from farmers' plot 1 at Offa- Igbo while the rest 40 leaf samples from CRIN and Offa-Igbo reacted positively to the mild strain (NA). Of the CRIN samples, 55.55% (15/27) reacted to the mild strain while 68.96% (20/29) and 54.16% (13/24) from farmers' plots 1 and 2, respectively, reacted to the mild strain (Table 1). CSSV was not detected by PAS-ELISA in any of the leaf samples (Tables 2 - 4). However, CSSV was not detected by ELISA in 8 of the symptomless samples or in 33.33% (24/72) of symptomatic leaf samples collected. This could mean that the viruses contained in them are of

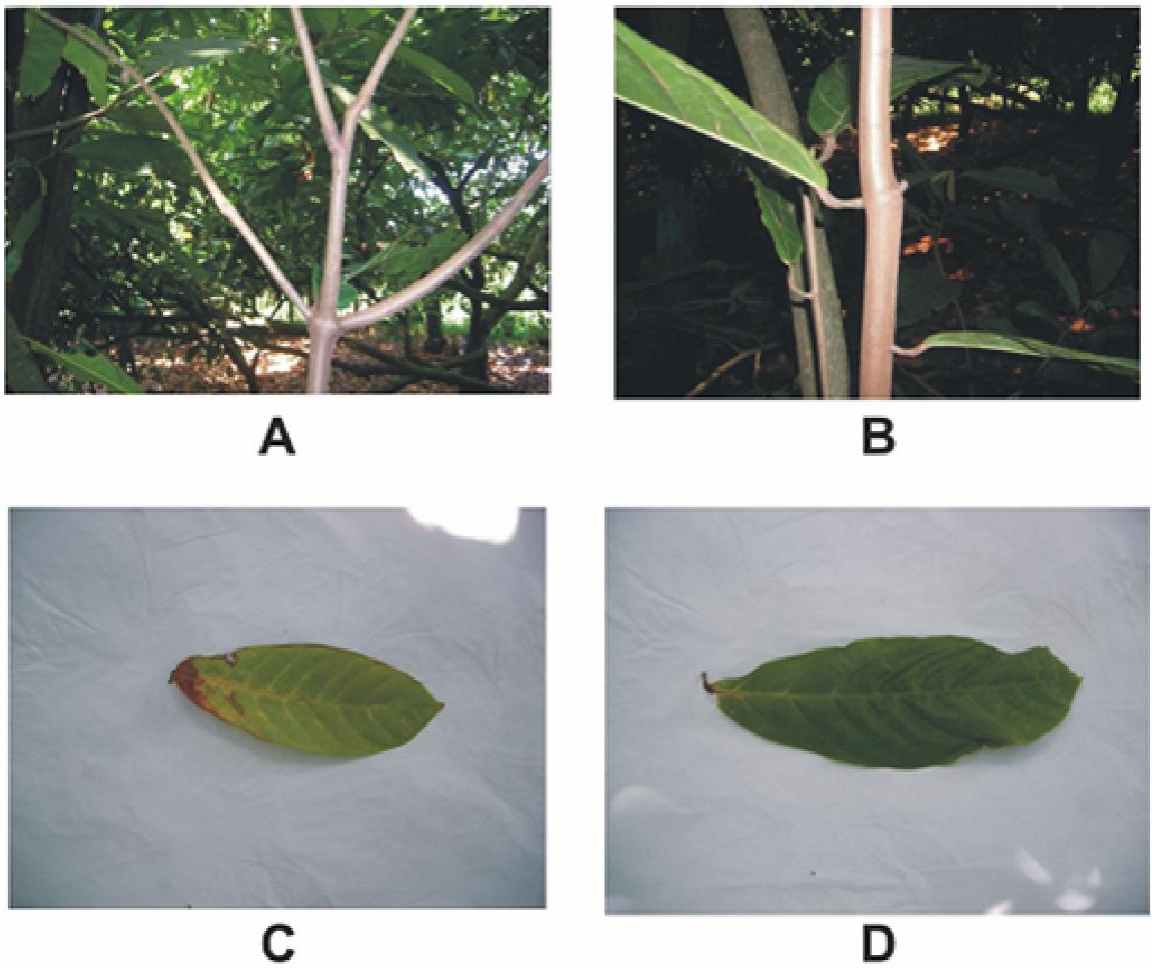

Figure 1. Cocoa plant parts showing different symptoms. A and B: Swollen shoot. C: Interveinal chlorosis with mottling. D: Leaf chlorosis. (Photo: L. N. Dongo).

Table 1. Incidence of CSSV in cocoa leaf samples from Cocoa Research Institute of Nigeria (CRIN), Ibadan and farmers' plots at Offa-Igbo, Nigeria.

Origin	Symptomless	Symptomatic	Positives/total
CRIN	4	23	15/27 (55.55%)
Farmers' Plot 1 at Offa-Igbo	2	27	20/29 (68.96%)*
Farmers' Plot 2 at Offa-Igbo	2	22	13/24 (54.16%)

*Eight of the positive samples reacted equally to the severe strain (1A) of CSSV.

different strains or the symptoms may have been caused by physical, chemical or other biological agents. It could also be that the concentrations of the viruses in those samples were very low as ELISA does not detect viruses in very low amounts, less than 1 ng/ml (Matthews, 1991). The result has re-established the presence of both the mild and severe strains of CSSV in Nigeria.

With the intensification of cocoa production by the government resulting in new cocoa farm establishment and rehabilitation of old plantations, there is an inherent danger of CSSV spread to new plantings. This calls for an urgent intervention by characterizing and identifying the isolates in all cocoa growing areas of Nigeria, and subsequently assessing the economic importance of the

Table 2. Comparative enzyme-linked immuno sorbent assay (ELISA) values (A_{405}nm) obtained with PAS-ELISA and ACP-ELISA for testing known healthy sap and leaf samples from crin to antisera raised against two strains of CSSV.

Sample no.	Status	Antisera	ACP-ELISA	PAS-ELISA
1	Healthy sap	1A	0.098	0.113
		NA	0.113	0.111
2	IVCM	1A	0.189	0.131
		NA	0.228*	0.118
3	Swollen shoot	1A	0.131	0.123
		NA	0.231*	0.116
4	No symptom	1A	0.167	0.128
		NA	0.124	0.132
5	Swollen shoot	1A	0.178	0.167
		NA	0.227*	0.114
6	IVCM	1A	0.143	0.128
		NA	0.234*	0.137
7	IVCM	1A	0.141	0.119
		NA	0.121	0.112
8	IVCM	1A	0.153	0.180
		NA	0.327*	0.115
9	No symptom	1A	0.142	0.121
		NA	0.152	0.119
10	IVCM	1A	0.171	0.117
		NA	0.225*	0.132
11	IVCM	1A	0.192	0.124
		NA	0.311*	0.119
12	IVCM	1A	0.169	0.127
		NA	0.157	0.134
13	Leaf chlorosis	1A	0.178	0.134
		NA	0.148	0.115
14	Leaf chlorosis	1A	0.154	0.114
		NA	0.242*	0.117
15	Leaf chlorosis	1A	0.149	0.121
		NA	0.148	0.132
16	IVCM	1A	0.148	0.127
		NA	0.165	0.136
17	Swollen shoot	1A	0.156	0.129
		NA	0.229*	0.145
18	Swollen shoot	1A	0.160	0.127
		NA	0.291*	0.161
19	Swollen shoot	1A	0.129	0.124
		NA	0.322*	0.117
20	IVCM	1A	0.153	0.125
		NA	0.143	0.131
21	Swollen shoot	1A	0.171	0.124
		NA	0.248*	0.123
22	No symptom	1A	0.162	0.132
		NA	0.152	0.142
23	No symptom	1A	0.137	0.125

Table 2. Contd.

		NA	0.151	0.140
24	IVCM	1A	0.146	0.127
		NA	0.133	0.132
25	Leaf chlorosis	1A	0.141	0.135
		NA	0.162	0.135
26	Swollen shoot	1A	0.142	0.132
		NA	0.243)*	0.142
27	IVCM	1A	0.170	0.135
		NA	0.266)*	0.131
28	IVCM	1A	0.135	0.143
		NA	0.245*	0.155

*Positive samples (mean absorbance values twice that of healthy control).
IVCM = Interveinal chlorosis with mottling.

Table 3. Comparative enzyme-linked immuno sorbent assay (ELISA) values (A_{405}nm) obtained with PAS-ELISA and ACP-ELISA for testing known healthy sap and leaf samples from Offa-igbo (farmers' plot 1) to antisera raised against two strains of CSSV.

Sample no.	Status	Antisera	ACP-ELISA	PAS-ELISA
1	Healthy sap	1A	0.112	0.110
		NA	0.101	0.091
2	Swollen shoot	1A	0.311**	0.077
		NA	0.287*	0.120
3	Swollen shoot	1A	0.254**	0.143
		NA	0.321*	0.124
4	Swollen shoot	1A	0.132	0.036
		NA	0.244*	0.078
5	Swollen shoot	1A	0.123	0.115
		NA	0.267*	0.117
6	Swollen shoot	1A	0.152	0.134
		NA	0.278*	0.172
7	Swollen shoot	1A	0.351**	0.158
		NA	0.323*	0.128
8	Swollen shoot	1A	0.288**	0.172
		NA	0.243*	0.132
9	Swollen shoot	1A	0.256**	0.019
		NA	0.249*	0.115
10	IVCM	1A	0.121	0.023
		NA	0.256*	0.126
11	IVCM	1A	0.141	0.131
		NA	0.243*	0.142
12	Leaf chlorosis	1A	0.111	0.125
		NA	0.115	0.134
13	IVCM	1A	0.114	0.151
		NA	0.123	0.142

Table 3. Contd.

14	Swollen shoot	1A	0.276**	0.171
		NA	0.268*	0.153
15	Leaf chlorosis	1A	0.132	0.123
		NA	0.125	0.141
16	IVCM	1A	0.118	0.131
		NA	0.113	0.125
17	IVCM	1A	0.141	0.159
		NA	0.252*	0.172
18	IVCM	1A	0.121	0.182
		NA	0.311*	0.157
19	Swollen shoot	1A	0.112	0.159
		NA	0.253*	0.143
20	IVCM	1A	0.123	0.151
		NA	0.112	0.162
21	Swollen shoot	1A	0.321**	0.118
		NA	0.279*	0.119
22	No symptom	1A	0.132	0.137
		NA	0.109	0.128
23	No symptom	1A	0.178	0.137
		NA	0.134	0.156
24	IVCM	1A	0.116	0.087
		NA	0.232*	0.110
25	Swollen shoot	1A	0.241**	0.097
		NA	0.228*	0.113
26	IVCM	1A	0.132	0.171
		NA	0.231*	0.154
27	IVCM	1A	0.142	0.115
		NA	0.311*	0.127
28	IVCM	1A	0.151	0.073
		NA	0.238*	0.052
29	IVCM	1A	0.123	0.115
		NA	0.145	0.117
30	IVCM	1A	0.126	0.153
		NA	0.168	0.168

*Positive samples (mean absorbance values twice that of healthy control).
**Samples that reacted positively to the severe strain 1A.
IVCM = Interveinal chlorosis with mottling.

Table 4. Comparative enzyme-linked immuno sorbent assay (ELISA) values (A_{405}nm) obtained with PAS-ELISA and ACP-ELISA for testing known healthy sap and leaf samples from Offa-Igbo (farmers' plot 2) to antisera raised against two strains of CSSV.

Sample no.	Status	Antisera	ACP-ELISA	PAS-ELISA
1	Healthy sap	1A	0.087	0.103
		NA	0.093	0.106
2	IVCM	1A	0.119	0.098
		NA	0.228*	0.112

Table 4. Contd.

3	IVCM	1A	0.112	0.121
		NA	0.234*****	0.113
4	IVCM	1A	0.131	0.131
		NA	0.227*****	0.142
5	Swollen shoot	1A	0.124	0.112
		NA	0.311*****	0.132
6	Swollen shoot	1A	0.145	0.126
		NA	0.234*****	0.116
7	IVCM	1A	0.154	0.118
		NA	0.241*****	0.132
8	Leaf chlorosis	1A	0.152	0.118
		NA	0.229*****	0.124
9	IVCM	1A	0.117	0.134
		NA	0.235*****	0.125
10	IVCM	1A	0.134	0.131
		NA	0.251*****	0.141
11	IVCM	1A	0.134	0153
		NA	0.236*****	0.142
12	Leaf chlorosis	1A	0.093	0.131
		NA	0.111	0.113
13	No symptom	1A	0.095	0.117
		NA	0.112	0.189
14	Swollen shoot	1A	0.087	0.145
		NA	0.267*****	0.162
15	No symptom	1A	0.111	0.145
		NA	0.114	0.135
16	IVCM	1A	0.099	0.171
		NA	0.113	0.153
17	IVCM	1A	0.121	0.156
		NA	0.228*****	0.146
18	IVCM	1A	0.091	0.152
		NA	0.226*****	0.147
19	IVCM	1A	0.121	0.152
		NA	0.241*****	0.142
20	IVCM	1A	0.115	0.148
		NA	0.095	0.117
21	IVCM	1A	0.112	0.123
		NA	0.118	0.132
22	Leaf chlorosis	1A	0.127	0.164
		NA	0.167	0.159
23	IVCM	1A	0.097	0.139
		NA	0.114	0.153
25	Swollen Shoot	1A	0.111	0.114
		NA	0.131	0.125

*****Positive samples (mean absorbance values twice that of healthy control).
IVCM = Interveinal chlorosis with mottling.

disease. The result will be of help in the development of cocoa materials resistant to CSSV.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. L. A. Ollennu and Mr. D. Owusu both of Cocoa Research Institute of Ghana, New Tafo, Ghana, for antiserum to CSSV and cocoa leaf healthy sap.

REFERENCES

Adegbola MOK (1981). Long term effect of cocoa swollen shoot virus infection on mature cocoa trees in Nigeria. Proc. 6th int. Cocoa Res. Conf. Caracas, Venezuela 1977: 287-295.

Barnett OW (1986). Survey for plant viruses, Design and considerations In: Mclean GD, Garret RG, Ruesuk WG (eds). Plant virus epidemics. Monitoring, modeling and predicting outbreaks. Academic press, Sydney. pp. 147-166.

Hughes JDA (1986). Viruses of the Araceae and Dioscorea species. Their isolation, characterization and detection. Ph. D Thesis, University of Reading, U.K.

Huguenot C (1992). Practical ELISA diagnosis of cowpea aphid-borne virus. Immunodetection of plant viruses training course, November 16-19, 1992, IITA, Ibadan, Nigeria.

ICCO (2004). Main cocoa producing countries and their cocoa bean production. (http://www.icco.org/questions/production.htm).

Matthews REF (1991). Plant Virology. Third Edition. Academic press, Inc. p. 835.

Padi B (1997). Prospects for the control of cacao mealybugs. Proc. 1st Int. Cocoa pests and diseases. Sem. Accra, Ghana. November 6-10, 1995, pp. 249-263.

Posnette AF (1943). The diagnosis of swollen shoot disease of cacao. Trop. Agric. Trinidad 21: 156-158.

Sutula CL, Gillet JM, Morrissey SM, Ransdel DC (1986). Interpreting ELISA data and establishing the positive-negative threshold. Plant Dis. 70: 722-726.

Thorold CA (1975). Diseases of Cocoa. Oxford, U. K. Clarendon press. p. 423.

Thresh JM (1960). Capsids as a factor influencing the effect of swollen shoot disease on cacao in Nigeria. Emp J. Exp. Agric. 28(3): 193-200.

Woods GAR (1987). Cocoa. Fourth Education Longman Scientific and Technical, p. 619.