Original article

A survey in Southern Nigeria reveals the presence of *Cucumber mosaic virus* subgroup I in *Musa* crops

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A survey in Southern Nigeria reveals the presence of *Cucumber mosaic virus* subgroup I in *Musa* crops.

Abstract — Introduction. *Cucumber mosaic virus* (CMV), genus *Cucumovirus*, is distributed worldwide; it is a common pathogen of *Musa* spp. and it is present in all banana-producing areas. **Materials and methods**. Banana and plantain (*Musa*) leaf tissues were collected from six major *Musa*-growing states of Southern Nigeria in August 2000. A broad-spectrum CMV polyclonal antibody was used in Protein A Sandwich (PAS) ELISA to detect CMV isolates, while a CMV subgroup I-specific polyclonal antibody was used in DAS-ELISA to separate subgroup I isolates. **Results**. Of 108 *Musa* leaf samples collected, 76 samples (70.4%) reacted positively with the CMV polyclonal antibodies used, in which 32 samples (42.1%) belonged to CMV subgroup I. Edo and Ondo states had low CMV subgroup I incidence of 13.6% and 17.2%, even though both states had high CMV incidence of 63.6% and 62.1% in infected samples, respectively. Imo state had the highest CMV incidence of 72.4% and subgroup I incidence of 55.2%. Of the 32 leaf samples infected with CMV subgroup I, 18 samples expressed one type of viral symptom such as interveinal chlorosis, chlorotic streaks, leaf puckering and crisp deformed leaf. Thirteen other samples expressed two or three viral symptoms, showing either vein thickening or general leaf chlorosis and any of the former symptoms. One asymptomatic leaf tissue was also infected with the virus. **Discussion**. Our results confirm that subgroup I and other subgroups are responsible for CMV infection in *Musa* species in Nigeria. The detection of CMV over a large geographical area underscores the importance of virus control measures. CMV can be controlled by use of virus-free suckers.

Nigeria / *Musa* / viruses / bromoviruses / ELISA / identification

Une prospection dans le sud du Nigéria indique la présence en bananeraie du sous-groupe I du virus de la mosaïque du concombre.

Résumé — Introduction. Le virus de la mosaïque du concombre (CMV), genre *Cucumovirus*, est distribué dans le monde entier ; c'est un pathogène commun du genre *Musa* et il est présent dans toutes les zones de production du bananier. **Matériel et méthodes**. Des tissus de feuille de bananiers et plantains (*Musa*) ont été collectés en août 2000 dans six États du Sud nigérian importants pour la culture du bananier. Un anticorps polyclonal du CMV à large spectre a été utilisé dans un test ELISA Sandwich (PAS-ELISA) pour détecter les isolats de CMV, alors qu'un anticorps polyclonal spécifique du sous-groupe I du CMV était utilisé dans un test double sandwich ELISA (DAS-ELISA) pour séparer des isolats du sous-groupe I. **Résultats**. Sur 108 échantillons de feuilles de bananier collectés, 76 échantillons (70,4 %) ont réagi positivement avec les anticorps polyclonaux du CMV utilisés, parmi lesquels 32 échantillons (42,1 %) ont été rattachés au sous-groupe I du CMV. Les États d'Edo et d'Ondo ont été peu concernés par le sous-groupe I du CMV (13,6 % et 17,2 %, respectivement) bien que le CMV ait eu une forte incidence (63,6 % et 62,1 %, respectivement) dans les échantillons infectés. L'État d'Imo a été le plus atteint par le CMV (72,4 %) et l'incidence du sous-groupe I a été de 55,2 %. Sur les 32 échantillons de feuille infectés par le sous-groupe I du CMV, 18 échantillons ont exprimé un seul type de symptôme viral tel qu'une chlorose entre nervures, des stries chlorotiques, un plissement ou une déformation de la feuille. Treize autres prélèvements ont montré soit deux, soit trois symptômes viraux se traduisant, en plus de l'un des précédents symptômes, par un épaississement de la nervure ou une chlorose générale de la feuille. Un tissu de feuille sans symptôme s'est révélé également atteint par le virus. **Discussion**. Nos résultats confirment que le sous-groupe I et d'autres sous-groupes du CMV sont responsables de l'infection des bananiers du Nigéria. La détection du CMV effectuée sur une large zone géographique souligne l'intérêt de mesures de dépistage du virus. Celui-ci peut être contrôlé par l'utilisation de rejets sains.

Nigéria / *Musa* / virus / bromovirus / test ELISA / identification

1. Introduction

Bananas and plantains are the fourth most important food crop worldwide based on gross value of production [1]. Thirty-five percent of the world's *Musa* is produced in the humid forest and mid-altitude zones of sub-Saharan Africa, providing 25% of the carbohydrate for approximately 70 M people [2]. More than three quarters of all plantain produced in Africa is grown in the west and central regions of Cameroon, Côte d'Ivoire, Ghana, Nigeria and Zaire. In Nigeria, the humid rain forest in the south of the country represents the primary area of plantain production [3, 4].

Four viruses are known to commonly infect *Musa* spp. [5]. *Banana streak virus* BSV [6] and *Cucumber mosaic virus* (CMV), genus *Cucumovirus* [7], have been reported in Nigeria.

Cucumber mosaic virus is the type species of the genus *Cucumovirus* in the family *Bromoviridae*. It has been reported to be one of the most common plant viruses of substantial agricultural importance, infecting more than 1 000 plant species [8]. CMV is a single-stranded positive-sense tripartite genome RNA virus. It is distributed worldwide, and it has an extremely broad host range and numerous strains [9] that differ in host range and pathogenicity [10]. CMV is present in all banana-producing areas and it is severe under certain circumstances [11–14]. It causes chlorosis, mosaic and heart rot in bananas [15]; symptoms include yellow streaking or mosaic patterns on leaves, often with a curling of leaf margins. In cool weather, rottening of heart leaves and of the center of the pseudostem (heart rot or sheath rot) also occur [16]. Infected banana planting stock and infected alternative host plants in banana plantations constitute the primary sources of infection [16]. CMV is readily aphid-transmitted to and from banana [13]. Infections with viruses cause disruption both in breeding programs and in certified plantlet production. Strains of CMV have been reported recently for the first time on bananas in a number of developing countries, probably due to the availability of efficient detection and identification methods [17–25]. CMV isolates have been divided into two subgroups, subgroup I and subgroup II, based on serological data, peptide mapping of the coat protein, nucleic acid hybridization, RT-PCR combined with RFLP and nucleotide sequence similarities [9, 16, 26–29].

In Nigeria, CMV has been detected in yam and cowpea [30–32]. In *Musa* germplasm, it has been detected at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, but information on the strains or serogroups present are not available and there is relatively little information about their geographical distribution in the major *Musa*-growing areas of southern Nigeria. Our study was undertaken to identify CMV subgroup I isolates from *Musa* leaf samples collected from the major *Musa*-growing areas of southern Nigeria.

2. Materials and methods

2.1. Collection of *Musa* leaf samples

Musa leaf tissues were collected from six major *Musa*-growing states of southern Nigeria in August 2000. The locations sampled were selected based on earlier reports of virus symptoms. Sampling was done by walking through plantain and banana orchards, mostly in backyard gardens of various houses and farms in villages. False Horn plantain landraces (Agbagba) or similar landraces were visually inspected for virus-like symptoms. Within states, the locations were between 5 km and more than 50 km apart, in each location, and, without bias for plantain or banana, 5–10 leaf samples were collected from symptomatic and from asymptomatic plants to exclude the possibility of infection by a mild strain or latent infection. The incidence of CMV in leaf samples was calculated as the percentage of leaf samples positive for CMV relative to the number of leaf samples collected. The leaf samples were preserved for up to 7 d while in transit by placing them individually in labeled polyethylene sample bags before the bags were placed over ice packs in coolers. All the samples were later taken to the laboratory for CMV detection using Protein

A Sandwich (PAS) Enzyme Linked Immuno Sorbent Assay (ELISA) with a broad-spectrum CMV polyclonal antibody [Horticulture Research International (HRI), UK]. CMV subgroup I-specific polyclonal antibodies (Agdia, USA) were used in Double Antibody Sandwich (DAS) ELISA to identify subgroup I isolates from the infected field samples.

2.2. Sap extraction and detection of CMV using PAS-ELISA

Musa leaf tissue (0.1 g) was homogenized in 1 mL of ice-cold Phosphate Buffered Saline buffer (PBS: 0.14 M NaCl, 0.001 M KH_2PO_4, 0.008 M Na_2HPO_4, 0.003 M KCl in 1 L distilled water, pH 7.4) containing 0.05% (v/v) Tween 20 (PBS-T) and also 2% (w/v) PolyVinylPyrrolidone (Sigma PVP-40 USA). Protein A Sandwich ELISA was done according to the procedure of Odu *et al.* [33]. The absorbance of the contents of the wells at 405 nm was read after 1 h at ambient room temperature (23–28 °C) using a Dynex MRX ELISA plate reader. Mean absorbance values at 405 nm that were twice or greater than the mean of the healthy samples were considered to be infected (positive) with CMV. The healthy, control samples were collected from certified tissue culture *Musa* plants produced by the IITA's tissue culture unit.

2.3. Detection of CMV subgroup I isolates using DAS-ELISA

DAS-ELISA was performed as described by Clark and Adams [34], using CMV subgroup I-specific polyclonal antibodies (Agdia, USA). The plates were washed with Phosphate Buffered Saline containing 0.5% (v/v) Tween 20 (PBS-T) from a wash bottle. Afterwards 200 µL of 3% (w/v) dried skimmed milk in PBS-T was added to each well of the ELISA plate and incubated at 37 °C for 30 min. Without washing, 100 µL of sap (*Musa* spp.) was added to each well. Following overnight incubation at 4 °C and washing, 100 µL CMV IgG (= immunoglobulin G) conjugate A and B (Agdia, USA) in conjugate buffer was added and incubated at 37 °C for 2 h. The plates were washed and then 200 µL of 1 $mg \cdot mL^{-1}$ of p-nitrophenyl phosphate in 10 $mL \cdot mL^{-1}$ substrate buffer (diethanolamine pH 9.6) was added to each well. The absorbance of the contents of the wells was read at 405 nm.

2.4. Identification of CMV subgroups IA and IB by mechanical inoculation using *Nicotiana glutinosa* and *Vigna unguiculata*

Ten *Musa* leaf samples that reacted strongly ($A_{405\ nm} \geq 3.0$) with CMV subgroup I polyclonal antibody in DAS-ELISA and another ten *Musa* leaf samples that reacted strongly with CMV polyclonal antibody in PAS-ELISA but did not react with CMV polyclonal antibody in DAS-ELISA were selected for further differentiation into subgroups IA and IB by mechanical inoculation into *Nicotiana glutinosa* and *Vigna unguiculata*.

Leaf tissues from each isolate in both groups of ten *Musa* leaf samples were ground in inoculation buffer (phosphate buffer pH 7.5 containing 1% ethylenediaminetetra-acetic acid and 0.1% sodium sulfite) and were each mechanically rubbed on carburundum-dusted leaves of six healthy 6-week-old *N. glutinosa* and six 1-week-old *V. unguiculata* TVu 76 in an insect-free and insect-proof screenhouse to determine symptom variations among the two groups. The plants were left in the screenhouse for 4 weeks for symptom development.

3. Results

3.1. Field symptoms

Virus-like symptoms observed in all 108 *Musa* leaf samples collected from twelve locations in six states were similar and included interveinal chlorosis (29.6%), chlorotic streaks (17.6%), leaf puckering (13.0%), crisp deformed leaves (10.2%), general chlorosis (5.6%), mixed symptoms (21.3%) and asymptomatic leaf samples (2.8%). The combination of virus symptoms on *Musa* leaf tissues infected with CMV subgroup I isolates and the reactions in PAS- and DAS-ELISA (*table I*) showed that *Cucumber mosaic virus* was associated with all virus symptom types observed in the field.

Table I.
Virus symptoms associated with *Musa* field samples infected with *Cucumber mosaic virus* (CMV) subgroup I isolates in Southern Nigeria and their reactions in PAS- and DAS- ELISA.

One symptom type	Reaction in PAS- / DAS-ELISA	Two symptoms type	Reaction in PAS- / DAS-ELISA	Three symptoms type	Reaction in PAS- / DAS-ELISA
Chlorotic streak	+ / +	Leaf puckering + general chlorosis	– (PAS-ELISA) / + (DAS-ELISA)	Vein thickening + leaf puckering + crisp deformed leaf	+ / +
Interveinal chlorosis	– (PAS-ELISA) / + (DAS-ELISA)	Vein thickening + general chlorosis	+ / +	Vein thickening + general chlorosis + crisp deformed leaf	+ / +
Interveinal chlorosis	– (PAS-ELISA) / + (DAS-ELISA)	Leaf puckering + vein thickening	+ / +	Crisp deformed leaf + vein thickening + general chlorosis	+ / +
Interveinal chlorosis	+ / +	Chlorotic streak + general chlorosis	+ / +	Crisp deformed leaf + general chlorosis + vein thickening	+ / +
Interveinal chlorosis	+ / +	Interveinal chlorosis + vein thickening	+ / +	General chlorosis + vein thickening + crisp deformed leaf	+ / +
Leaf puckering	– (PAS-ELISA) / + (DAS-ELISA)	Leaf puckering + crisp deformed leaf	+ / +	General chlorosis + vein thickening + crisp deformed leaf	+ / +
Interveinal chlorosis	+ / +	Crisp deformed leaf + general chlorosis	+ / +		
Crisp deformed leaf	+ / +				
Chlorotic streak	+ / +				
Chlorotic streak	+ / +				
Interveinal chlorosis	+ / +				
Interveinal chlorosis	– (PAS-ELISA) / + (DAS-ELISA)				
Interveinal chlorosis	+ / +				
Interveinal chlorosis	+ / +				
Leaf puckering	Not tested / +				
Interveinal chlorosis	Not tested / +				
Chlorotic streak	+ / +				
Leaf puckering	– (PAS-ELISA) / + (DAS-ELISA)				

Seventy-one *Musa* leaf samples expressed one type of virus symptom described earlier, of which 42 (59.2%) of these leaf tissues reacted positively with the CMV polyclonal antibody. Eighteen leaf samples out of 42 were also infected with CMV I isolates. The symptom distribution for the 18 *Musa* field samples were interveinal chlorosis (10) chlorotic streaks (4), leaf puckering (3) and crisp deformed leaf (1).

Twenty-six leaf samples had two types of virus symptoms; 17 of these were infected with CMV and 7 belonged to subgroup I (*table II*).

Eight other leaf tissues showed a combination of three virus symptoms; all were infected with CMV and six were subgroup I isolates (*table II*). The triple symptoms always showed chlorotic crisp deformed leaves in combination with general chlorosis, vein thickening or leaf puckering. This category of leaf tissues had a higher absorbance values in DAS-ELISA than the single and double symptom types. Two asymptomatic leaf samples were infected with CMV and one isolate belonged to subgroup I. *Cucumber mosaic virus* subgroup I was detected in all states surveyed.

3.2. Detection of CMV and CMV subgroup I isolates

A total of 76 (70.4%) out of 108 *Musa* leaf samples reacted positively to CMV polyclonal antibodies in PAS- and DAS-ELISA: of these, 68 leaf samples reacted to the CMV

polyclonal antibody in PAS-ELISA (*table III*). Thirty-two leaf samples reacted in DAS-ELISA, of which 24 of these also reacted in PAS-ELISA, while the remaining eight samples reacted in DAS-ELISA alone. The absorbance values for the leaf tissues in PAS-ELISA ranged from 0.450 to 4.0, while the absorbance values for the healthy controls ranged from 0.219 to 0.264. The absorbance values for the 32 leaf samples out of 108 (29.6%) infected with subgroup I CMV isolates ranged from 0.666 to 1.218, while the absorbance values for the healthy controls ranged from 0.319 to 0.380, respectively. The incidences of CMV subgroup I isolates in the infected leaf tissues were between 13.6% in Edo state and 55.2% in Imo state. Edo and Ondo states recorded low incidences of 13.6% and 17.2% CMV subgroup I isolates, respectively, even though both states recorded high CMV incidences of 63.6% and 62.1% in the infected leaf samples. Imo state had the highest incidence of CMV (72.4%) and CMV subgroup I isolates (55.2%).

3.3. Differentiation of CMV isolates into subgroups IA and IB

The CMV isolates could not be assigned to subgroup IA or IB with confidence following the procedure of Daniels and Campell [35]. The CMV isolates mechanically inoculated into *N. glutinosa* and *V. unguiculata* did not show any differences in the symptoms they caused on the two plants (*table IV*). The symptoms caused were vein-clearing on *N. glutinosa* and necrotic lesions on *V. unguiculata*.

Table II.
Virus symptoms on *Musa* leaf samples collected in Southern Nigeria and detection of *Cucumber mosaic virus* (CMV).

Symptom types	No. of *Musa* leaves	Leaves with positive reaction in PAS-ELISA		Leaves with positive reaction in DAS-ELISA	
		Number	%	Number	%
Single	71	42	59.2	18	25.4
Double	26	17	65.4	7	26.9
Triple	8	8	100.0	6	75.0
Asymptomatic	3	1	33.3	1	33.3

4. Discussion

Our work is the first report of CMV subgroup I isolates in *Musa* field samples collected in southern Nigeria. Virus-like symptoms on *Musa* spp. in the field were variable and included interveinal chlorosis, chlorotic streaks, leaf puckering, vein thickening, crisp deformed leaves and general chlorosis. In some cases the leaves showed a combination of these symptoms. Interveinal chlorosis and chlorotic streak were the most

Table III.
Serological detection of *Cucumber mosaic virus* (CMV) in *Musa* leaf tissues collected from six major plantain- and banana-growing states in Southern Nigeria.

States in southern Nigeria	No. of sites sampled	PAS-ELISA		DAS-ELISA	
		Number of CMV isolates positive by PAS-ELISA / number of leaves tested	%	Number of CMV isolates positive by DAS-ELISA / number of leaves tested	%
Anambra	1	4 / 9	44.4	4 / 9	44.4
Delta	2	7 / 11	63.6	2 / 11	18.2
Edo	2	14 / 22	63.6	3 / 22	13.6
Imo	3	21 / 29	72.4	16 / 29	55.2
Ondo	2	18 / 29	62.1	5 / 29	17.2
Rivers	2	4 / 8	50.0	2 / 8	25.0
Total	12	68 / 108	63.0	32 / 108	29.6

Table IV.
Reactions of *Nicotiana glutinosa* and *Vigna unguiculata* leaves to mechanical inoculations with *Cucumber mosaic virus* (CMV) isolates (Nigeria).

Symptom type	Number of CMV isolates positive by PAS-ELISA only for 6 leaves inoculated		Symptom type	CMV isolates positive by DAS-ELISA only for 6 leaves inoculated	
	N. glutinosa	*V. unguiculata*		*N. glutinosa*	*V. unguiculata*
Leaf puckering	6	4	Chlorotic streaks, flecks	0	4
Interveinal chlorosis	4	4	Vein thickening, leaf puckering, crisp deformed leaves	0	4
Leaf puckering	6	0	Vein thickening, puckering, crisp deformed leaves	0	0
Vein thickening, interveinal chlorosis	4	0	Vein thickening	0	0
Asymptomatic	6	0	Chlorotic streaks	2	0
Chlorotic streaks	0	0	Asymptomatic	6	0
Chlorotic flecks	2	0	Chlorosis, thick veins	0	0
Chlorotic flecks	4	0	Interveinal chlorosis	6	0
Chlorotic streaks	0	4	Interveinal chlorosis	0	4
Chlorosis	0	4	Leaf puckering	4	4

predominant virus-like symptoms on *Musa* spp. These symptoms were observed in all the states surveyed.

The virus-like symptoms observed on *Musa* spp. in Nigeria were similar to those reported in other countries. In Taiwan, symptoms recorded on bananas include continuous or broken streaks, running from the midrib to the margins of the older leaves [36]. All virus-like symptom types were associated with CMV infection but *Musa* leaf samples were not indexed for the presence of other *Musa* viruses. The detection of CMV in many fields and its widespread distribution over a large geographical area implicates humans in the epidemiology of the virus, probably through exchange of planting material (suckers) which could easily have been obtained free from neighbors or transported over long distances. Abundance of CMV vectors could also have been responsible for the high incidence of the virus and the widespread occurrence of CMV. A *Cucumber mosaic virus* subgroup I polyclonal antibody was used in DAS-ELISA to separate CMV I isolates from other subgroups. This confirms that subgroup I and other subgroups are responsible for CMV infection in *Musa* species in Nigeria. Both CMV subgroups were found infecting bananas in Australia [37]: also, subgroup II CMV isolates have been reported in tropical countries [38]. Some of the one symptom type that had very high virus titre in PAS-ELISA (> 3.0) were observed to be negative in DAS-ELISA (< 0.20) (data not shown); this reaction may be suggestive of CMV subgroup II isolates. Also, the triple symptoms type had high virus titre in both ELISA methods. This may suggest the possibility of infection by isolates with affinity for subgroup II or a single strain with epitopes for both antisera. *Cucumber mosaic virus* subgroup I isolate and the other subgroups induced common symptom types on *Musa* plants; also, the CMV subgroup I isolates induced the same symptom types on test plants as those that did not react with the CMV subgroup I-specific antibody in DAS-ELISA.

Symptomatology is therefore not a reliable method for identifying subgroup I and II isolates of CMV in field samples.

Serological tests were used to separate *Cucumoviruses* and the subgroups. The indirect ELISA method detected more

isolates from both CMV subgroups than the DAS-ELISA; this was expected since the CMV subgroup I antibody was specific only for subgroup I isolates, as opposed to the other antibody that detects both subgroups. Numerous techniques are available for the detection of CMV [9,16, 26–29], and the ELISA tests used in these field samples turned out to be robust, sensitive and cost-effective. They can be used routinely to check planting materials before distribution to growers.

The CMV and the subgroup I isolates were detected from leaf samples collected in all the states surveyed. Since some strains of CMV can cause severe symptoms not only on *Musa* spp. but on other associated crops such as vegetables, the use of virus-free suckers remains the most important control option. For this reason, the establishment of virus-free tissue culture plantlets is recommended.

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Una prospección en el sur de Nigeria indica la presencia en plantación de bananos del subgrupo I del virus del mosaico del pepino.

Resumen — Introducción. El virus del mosaico del pepino (CMV), tipo *Cucumovirus*, está distribuido por todo el mundo; se trata de un patógeno común del tipo *Musa* y está presente en todas las zonas de producción del banano. **Material y métodos**. Se recolectaron tejidos de hoja de bananos y plátanos (*Musa*) en agosto del 2000 en seis estados del sur nigeriano, importantes para el cultivo del banano. Un anticuerpo policlonal del CMV de gran espectro se empleó en un test ELISA Sandwich (PAS-ELISA) para detectar los grupos aislados de CMV, mientras que un anticuerpo policlonal específico del subgrupo I del CMV se empleó en un test doble sandwich ELISA (DAS-ELISA) para separar grupos aislados del subgrupo I. **Resultados**. Entre 108 muestras de hojas de banano recolectadas, 76 muestras (70,4 %) actuaron positivamente con los anticuerpos policlonales de los CMVs empleados, entre las cuales 32 muestras (42,1 %) se volvieron a relacionar al subgrupo I del CMV. Los Estados de Edo y de Ondo se vieron poco afectados por el subgrupo I del CMV (13,6 % y 17,2 %, respectivamente) aunque el CMV tuviese una fuerte incidencia (63,6 % y 62,1 %, respectivamente) en las muestras infectadas. El Estado de Imo fue el más afectado por el CMV (72,4 %) y la incidencia del subgrupo I fue del 55,2 %. Entre las 32 muestras de hoja infectadas por el subgrupo I del CMV, 18 muestras expresaron un único tipo de síntoma viral como, por ejemplo, una clorosis entre bandas, estriaciones cloróticas, un arrugamiento o una deformación de la hoja. Otros trece muestreos mostraron o bien dos, o bien tres síntomas virales que se traducían, no sólo como uno de los síntomas precedentes, sino que también como un engrosamiento nerval o una clorosis general de la hoja. Asimismo un tejido de hoja sin síntoma se mostró afectado por el virus. **Discusión**. Nuestros resultados confirman que el subgrupo I así como otros subgrupos del CMV son responsables de la infección de bananos en Nigeria. La detección del CMV realizada en una amplia zona geográfica subraya el interés por unas medidas de detección del virus. Ésta puede controlarse mediante el empleo de injertos sanos.

Nigeria / *Musa* / virus / bromovirus / ELISA / identificación