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TRANSMISSION, ELISA AND SDS-PAGE RESULTS OF SOME *MAIZE STREAK VIRUS* ISOLATES FROM DIFFERENT PARTS OF NIGERIA

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Abstract: Field surveys were undertaken in 1997–1999 across five ecological zones in Nigeria to collect isolates of *Maize streak virus* (MSV), genus *Mastrevirus*. Apart from maize (*Zea mays* L.), 15 other grass species were found with MSV symptoms in Nigeria. These hosts showed two types of symptoms viz: mild (with or without mottle) or severe (typical symptoms in maize). When *Cicadulina storeyi* China was used to attempt transmission of these isolates of MSV to seedlings a susceptible maize hybrid CML 254 X CML 247, six isolates were not transmissible to maize. Seven isolates that were transmissible to maize produced mild symptoms. The viral agents causing typical or severe streak symptoms in *Axonopus compressus* (Sw.) P. Beauv., *Brachiaria distichophylla* (Trn.) Stapf, *Dactyloctenium aegyptium* (Linn.) P. Beauv. and *Setaria barbata* (Lam.) Kunth produced symptoms that were typical of MSV in farmers fields, when transmitted to maize. Out of 33 plant species that seedlings were challenged with MSV, only eight proved susceptible. Four of them showed mild symptoms while the other four showed severe symptoms of MSV. Only three isolates collected during the surveys did not react with a MSV polyclonal antiserum produced in mice in Double Antibody Sandwich-Enzyme-Linked Immunosorbent Assay (DAS-ELISA). These isolates were found in *Andropogon gayanus* Kunth (from Kaduna), *Thelepogon elegans* Roth ex Toem & Schult (from Kadawa) and *Rottboellia cochinchinensis* (Lour.) Clayton (from Jos) exhibited mild streak/mottle symptoms. Specific monoclonal antibodies, raised against MSV, reacted with 12 out of 25 samples tested. The DAS-ELISA data also showed significant variation in concentration of the virus in the different plant hosts. The relationship dendogram through SDS-PAGE among eight purified virus isolates show 55–90% variation. At 0.55 coefficient of similarity, the dendogram divided the samples into two groups while at 0.9 coefficient of similarity, the 8 isolates were identified as distinct genetic entities.

Key words: isolates of MSV, transmission, enzyme-linked immunosorbent assay, (ELISA), Sodium Dodecyl Sulphate-Polyacrylamide Gel Elctrophoresis (SDS-PAGE), *Cicadulina storeyi* China as a vector of MSV, *Graminae*

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INTRODUCTION

Maize, *Zea mays* L., is a member of the grass family, *Graminae*, to which all the major cereals belong. Maize is a basic staple food and principal crop not only in West Africa but also in many other parts of sub-Saharan Africa where it is also used for animal feed and as a raw material for the manufacture of starch, ethanol and other products (Okoruwa 1995). High yields of maize are limited by a number of constraints including climate, soil fertility and lack of improved technology, but mostly due to pests and diseases. (Efron et al. 1989). In tropical Africa, maize is mostly attacked by stem borers of the genera *Sesamia, Eldana, Busseola* and *Chilo* (Bosque-Perez and Mareck 1990) and *Cicadulina* species, vectors of *Maize streak virus* (MSV) genus *Mastrevirus* and *Maize mottle chlorotic stunt* genus *Mastrevirus*. Other diseases such as leaf blight, rusts, stalk and ear rots and systemic foliar diseases are also important (Fajemisin 1986).

Maize streak is the most important virus disease of maize in sub-Saharan Africa. The causal agent is *Maize streak virus* (MSV). It was first recorded in South Africa in 1901 by Fuller as "mealie variegation" (Fuller 1901). It has since been reported to be widely distributed all over Africa South of the Sahara (Fajemisin and Shoyinka 1976; Rossel and Thottappilly 1985). MSV is transmitted by several species of leafhoppers in the genus *Cicadulina* (*Homoptera: Cicadellidae*) (Storey 1925, 1928; Fennah 1959; Rose 1962; Fajemisin et al. 1976). No other natural method of spread is known (Storey 1928; Storey and McLean 1930; Fajemisin et al. 1976).

Despite many years of investigations on the aetiology, transmission and epidemiology of MSV, much work remains to be done about the pathological status of the different isolates of the virus in grass weeds and their effects on the epidemiology of the disease in maize. We studied transmission and serological variation among the the different strains of MSV in alternative grass hosts in order to have a clearer understanding of the variability of the Nigerian isolates and their epidemiological importance.

MATERIALS AND METHODS

In order to meet the objectives of this study, experiments were conducted between June 1997 and June 2000, at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Field surveys were undertaken (between 1997 and 1999) across different ecological zones in Nigeria to collect leafhopper vectors and MSV isolates as they occur in weeds within and around maize fields.

Samples of different *Gramineae* plant species showing streak symptoms were collected from farmers' maize fields and/or from grassland areas surrounding maize fields across the major ecological zones of Nigeria. The plants were uprooted with a handtrowel and transplanted immediately in the field into 20 cm-diameter plastic pots. Transplanted plant samples were also arranged into metal trays and transported to an insect-proofed screenhouse at IITA, Ibadan. Plant samples with streak virus symptoms were identified following the descriptions of Akobundu and Agyakwa (1987) and technical assistance of Mr. John Ogazie (IITA Weed Science Laboratory).

Virus acquisition feeding was tested by confining adult *Cicadulina storeyi* China (as a vector of MSV) on infected maize leaves (about 1.5 cm length) placed in small

glass vials (8 cm height) containing water. Cotton wool was placed over the water to prevent insects from drowning and to keep the leaves in place. The infected leaves together with the insects were covered with PVC tubes. Virus inoculation access feeding was carried out by confining viruliferous leafhoppers on test plants (planted in 12 cm pots) using PVC cages.

All biological studies involving virus acquisition and inoculation feeding were conducted inside a "transmission room". The room is a containment facility with 12 hr photoperiod and a temperature of 22±2°C. The relative humidity was not controlled but was about 70% during the day, and at night, fluctuated between 80–100%. A hygrothermograph (Model 8368-00 by Cole-Parmer) was used to monitor both temperature and relative humidity. All biological studies in the transmission room (air – conditioned, containment facility) or in the screenhouse or glasshouse were carried out by confining leafhoppers on host seedlings or pieces of infected leaves using PVC tube cages measuring 7 cm in diameter and 25 cm high.

Handling of insects inside the transmission room was done with a transmission cage. The transmission cage is a wooden 96 cm x 96 cm x 96 cm hood painted black inside except the back that has a light panel. Dark green or black cloth was used to cover the front, and the investigator, while working. Insects released into the cage were attracted to the light panel and were easily picked by an aspirator.

Source of seeds for the experiments

Two maize (*Zea mays* L.) genotypes that were susceptible to MSV were used. Seeds of an open-pollinated, maize cultivar *Pool 16* were supplied by the maize-breeding unit of IITA. A streak susceptible hybrid *CML254 X CML247* was produced for this work at IITA. Ten seeds each of the inbred lines were supplied from Centro International de Majoramieto de Maiz y Trigo (CIMMYT), Mexico through Dr. Diallo. The seeds were multiplied and selffed twice before being crossed. Pearl millet (*Pennisetum americanum* L.) (cv. ex-bornu) and sorghum (*Sorghum bicolor* L.) (cv. KSV-8) seeds were obtained from Institute of Agricultural Research (IAR), Ahmadu Bello University, (ABU) Zaria.

Source of maize streak virus isolate

The severe MSV isolate used was initially collected from widely scattered areas of Nigeria (Soto et al. 1982). It has been used for several years at IITA, Ibadan, to screen for MSV resistance and transmission studies. The MSV-susceptible, open – pollinated maize variety *Pool 16* was the host plant for this isolate.

Transmission of MSV isolates from grasses to maize seedlings using *C. storeyi* as a vector

Groups of 100 non-viruliferous *C. storeyi* were confined for 48 h on leaves or plant parts (with streak symptoms) of grasses collected from different parts of Nigeria to acquire MSV while feeding. The vectors were thereafter transferred singly to 10–14 day old seedlings of MSV-susceptible, maize hybrid, *CML 254 X CML 247* for inoculation feeding period of 48 h. The vectors were then removed and the test plants sprayed with 2.5% Pyrethrum I and II (SanoPlant^{R} produced by Maag Bio, Dielsdorf, Germany). The inoculated seedlings were kept in a screenhouse and observed at two days interval for symptom development. Symptom severity and time to appearance

of symptoms (latent period) were determined. A scale of 0–5 was used to evaluate the response of the maize test plants to the different MSV isolates (0 = no streaking, 1 = very few streaking, 2 = light streaking, 3 = mild/moderate streaking, 4 = severe streaking on at least 60% of leaf area, 5 = severe streaking on 75% of leaf area or more and stunting) (Soto et al. 1982). Screenhouse evaluation and observation of symptoms was completed after four weeks. Fifteen maize seedlings per treatment were used and the experiment was repeated twice.

Transmission of MSV from maize to grass seedlings

Seedlings of 33 grass species that are regularly present in maize fields in Nigeria were challenged with IITA isolate of MSV (a severe strain of MSV that is being used to screen IITA maize germplasm for MSV resistance). Groups of 100 non-viruliferous *C. storeyi* were confined on MSV-infected maize seedlings for 48 h to acquire the virus while feeding. The insects were then tranferred to the test plants at 2–3 leaf stage. Three insects were caged on each seedling and were allowed three days of inoculation acceess feeding. The vectors were then removed by spraying with 2.5% Pyrethrum I and II. The inoculated seedlings were kept in a screenhouse for symptom development. Symptom severity and time of appearance of symptoms (incubation period) were determined. A scale of 0–5 was also used to evaluate the response of the different test plants. Screenhouse evaluation and observation of symptoms was completed after two months. Fifteen seedlings per treatment were used (i.e. grass species) and the experiment was repeated twice.

Characterization of streak virus isolates with enzyme – inked immunosorbent assay (ELISA)

Double antibody sandwich (DAS) enzyme-linked immunosorbent assay (ELISA) procedure described by Peterschmitt et al. (1991) and Clark and Adams (1977) was used. Wells of microtitre plates were coated (100 µl/well) with MSV polyclonal antibodies diluted 1:1000 in coating buffer (15 mM Na_2CO_3, 34.9 mM $NaHCO_3$ pH 9.6). The plates were incubated for 2–3 h at 37°C followed by three quick washes (washed three times for three min each) in PBS-Tween (137 mM NaCl, 8.2 mM Na_2HPO_4, 2.7 mM KCl, 1.5 mM KH_2PO_4 pH 7.4 containing 0.05% w/v Tween – 20). Plant samples were ground in sterile mortar containing extraction buffer (PBS-T plus 2%w/v PVP) in 1:10 dilution and then filtered through glasswool or cotton muslin. Extracted sap was added into the wells of ELISA plates (100 µl/well). The plates were incubated at 4°C overnight (16–18 h). At the end of incubation, the plates were washed three times for three minutes each in PBS-Tween. The plates were then blotted dry. Unbound reaction sites were blocked using skimmed milk (Marvel) dissolved (5% w/v) in PBS-Tween by adding 200 µl to each well. The plates were incubated at 37°C for 1 h after which they were drained but not washed. MSV polyclonal (diluted 1:1 000 in PBS-Tween) or monoclonal antibodies (from John Innes Institute, UK) diluted in 1: 500, were added to the wells (100 µl/well). The plates were incubated at 37°C for 2–3 h and washed three times. Goat anti-mouse alkaline phosphatase conjugate 100 µl, diluted 1:40 000 in conjugate buffer (PBS-Tween – PVP and 0.2% egg albumin) was then added to the wells (100 µl/well). The plate was incubated at 37°C for 2–3 h after which they were washed as described previously. A substrate, 4-p-nitrophenyl phosphate disodium salt (from Sigma) was dissolved (0.01 g/10 ml or one tablet per 10 ml) in substrate buffer

(10% diethanolamine, pH 9.8 with HCl). This was added into the wells (100 µl/well). Absorbance readings at 405 nm were recorded using ELISA plate reader Dynatech MR 500 after incubation at room temperature for 2 h and overnight at 4°C.

Tripple Antibody Sandwich (TAS) ELISA: The method is as essentially described above with DAS-ELISA with the substitution of polyclonal antiserum for monoclonal antibody also from John Innes Institute, UK.

Characterization of streak virus isolates with Sodium Dodecyl Sulphate- Polyacrylamide Gel Elctrophoresis (SDS-PAGE)

Some MSV isolates in maize (i.e. transmitted from grass hosts to maize) were purified using the method of Larsen and Duffus (1983) as modified by Reyaud and Peterschmitt (1992). These isolates were chosen because of the perennial nature of their host grasses. SDS-PAGE of purified virus isolates was carried out using a vertical gel apparatus from Bio-Rad (Model 220). A discontinuous system employing 12% separating gel overlaid with 4% stacking gel was adapted from Laemmli (1970). The molecular mass of the samples was determined by comparison to medium range molecular mass markers.

Comparison of electrophoretic patterns of the purified isolates was undertaken using Jaccard's similarity index (Sneath and Sokal 1973) as a measure of homology (D'Urzo et al. 1990). The gel was photographed with Grab-It software. The bands were scored with Advanced Gel Documentation software version 3.0. The data obtained were translated into matrix (1.0) and dendogram was constructed using NTSYS 2.0 version PC PROGRAM.

RESULTS

Field sampling of MSV isolates

Table 1 shows a list of grass species that were found with virus-like streak symptoms during the survey trips, with the locations where samples were collected, type of symptoms observed and abbreviation used in describing results. Out of the 16 plant species, *Digitaria horizontalis, Panicum maximum* Jacq, *Setaria barbata* (Lam.) Kunth, *Brachiaria lata* (Schumach) C.E. Hubbarb, *B. deflexa* (Schumach) C.E. Hubbarb ex Robyns and *B. distichophylla* (Trn.) Stapf were more commonly encountered weeds with virus-like streak symptoms. Two types of streak symptoms viz: mild (with or without mottle) or severe (typical streak symptom in maize) were found in *Axonopus compressus* (Sw.) P. Beauv., *B. lata, Digitaria horizontalis* Willd, *Rottboellia cochichinensis* (Lour.) Clayton and *Thelepogon elegans* Roth ex Toem & Schult.

Transmission of MSV isolates from grass to maize

Table 2 shows the results of experiments to transmit MSV from different grass species to a MSV-susceptible maize hybrid *CML254 X CML 247* with the use of adult *C. storeyi* as vectors. Streak virus-like isolates (with symptoms typical of MSV in maize) in *Andropogon gayanus, Panicum maximum, Paspalum conjugatum* and *P. notatum* could not be transmitted to the maize test plants. Streak virus-like isolates with mottle/streak symptoms in *Rottbellia cochinchinensis* and *Thelepogon elegans* were also not transmissible to the maize test plant. The agent causing typical streak or severe streak symptoms in *Axonopus compressus, Brachiaria distichophylla, Dactyloctenium*

Table 1. List of grass species found with virus-like streak symptoms in various locations in Nigeria

Isolates	Location	Symptoms
Andropogon gayanus Kunth	Kaduna	mild
A. gayanus	Mokwa	mild
Axonopus compressus (Sw.) P. Beauv.	IITA, Ibadan	severe
A. compressus	IITA, Ibadan	mild
A. compressus	Ilesha	mild
Brachiaria deflexa (Sch.) C.E. Hubbarb ex Robyns	Ikenne	mild
B. deflexa	Moor Plantation, Ibadan	mild
B. deflexa	Catholic Redemptorist Camp, Ibadan	mild
B. distichophylla (Trn.) Stapf	Ikenne, Mokwa	mild/severe
B. distichophylla	IITA, Ibadan	severe
B. lata (Schumach) C.E. Hubbarb	Ikenne	mild/severe
B. lata	Mokwa	severe
B. lata	IITA, Ibadan	severe
B. lata	IITA, Ibadan	mild/mottle
Dactyloctenium aegyptium (Linn.) P. Beauv.	IITA, Ibadan	severe
Digitaria horizontalis Willd	Kaduna, Zaria	mild/severe
D. horizontalis	Ikenne	mild
D. horizontalis	Mokwa	severe
D. horizontalis	Zaria	severe
D. horizontalis	Jos	severe
D. horizontalis	IITA, Ibadan	severe
Eleusine indica Gaertn	IITA, Ibadan	
E. indica	Mokwa	mild
Panicum maximum Jacq	Ikenne, Odeda, Ikire, Ilesha, Mokwa, Kaduna, Zaria, Kadawa & Jos	mild
P. maximum	IITA, Ibadan	mild
P. maximum	Ife	mild
Paspalum conjugatum Berg.	IITA, Ibadan	mild
Paspalum notatum Flüggé	IITA, Ibadan	mild
Paspalum scrobiculatum L.	IITA, Ibadan	mild
Rottboellia cochinchinensis (Lour.) Clayton	IITA, Ibadan	mild
R. cochinchinensis	Ife	mild
R. cochinchinensis	Zaria	mild
R. cochinchinensis	Jos	mild
Rhynchelitrum repens (Wild.) C.E. Hubbarb	Jos	mild
Setaria barbata (Lam.) Kunth	Kaduna, zaria	mild/severe
S. barbata	IITA, Ibadan	severe
Thelepogon elegans Roth ex Toem & Schult	Jos	mild/mottle
T. elegans	Kadawa	mild/mottle
T. elegans	Kadawa	severe
Zea mays L.	Ikenne, Odeda, Ikire, Ife, Ilesha, Mokwa, Zaria, Kadawa & Jos	severe
Z. mays	IITA, Ibadan	severe
Z. mays	Kaduna	severe

Table 2. Transmission of streak virus isolates from the original host to a susceptible maize hybrid using single adults of *C. storeyi* as vectors (with 72 h acquisition and inoculation access feeding periods)

Plant Species	Symptom severity in maize[a]	Symptom severity when transmitted from maize to maize
Andropogon gayanus Kunth	0	0
Axonopus compressus A[b] (Sw.) P. Beauv.	severe (4)	severe (4)
A. compressus B	mild (3)	mild (3)
Brachiaria deflexa (Schumach) C.E.Hubbard ex Robyns	mild (3)	mild (3)
B. distichophylla	severe (5)	severe (5)
B. lata A (Schumach) C.E.Hubbard	severe (4)	severe (4)
B. lata B	mild (3)	mild (3)
Dactyloctenium aegyptium (Linn.) P.Beauv.	severe (5)	severe (5)
Digitaria horizontalis A Willd	mild (3)	mild (3)
D. horizontalis B	mild (3)	mild (3)
Eleusine indica Gaertn	mild (3)	mild (3)
Panicum maximum Jacq	0	0
Paspalum conjugatum Berg.	0	0
P. notatum Flüggé	0	0
P. scrobiculatum	mild (3)	mild (3)
Rottboellia cochinchinensis B (Lour.) Clayton	mild (2)	mild (2)
R. cochinchinensis C	0	0)
Setaria barbata (Lam.) Kunth	severe (4)	severe (4)
Thelepogon elegans B	mild (3)	mild (3)
T. elegans C	0	0
Zea mays Linn.	severe (5)	severe (5)

[a] based on a scale of 0–5, where 0 = no streaking, 1 = light streaking, and 5 = severe streaking on 75% or more of the leaf area and stunting.
[b] A = severe isolate; B = mild isolate: C = mottle/ streak

aegyptium and *Setaria barbata* was transmissible to maize by *C. storeyi* giving symptoms that were typical of MSV in farmers' fields. *Axonopus compressus* (mild isolate), *B. deflexa, Brachiaria lata* (mild isolate), *Digitaria horizontalis* (mild isolate) *Paspalum scrobiculatum, Rottboellia cochinchinensis* and *Thelepogon elegans* produced mild symptoms when transmitted to maize by *C. storeyi*. When the MSV isolates from grasses that were transmissible to maize were further transmitted from maize to maize, using *C. storeyi* as vectors, the original symptoms (mild/severe) in the grass hosts were obtained in maize.

Transmission of MSV from maize to healthy grass seedlings

The results of experiments to transmit a severe isolate of MSV from maize, using *C. storeyi* as a vector, to some grasses (found in farmers' fields as weeds) are presented in Table 3. Out of 33 plant species tested, only eight proved susceptible to MSV. Twenty five plant species remained symptomless after 60 days post-Inoculation Access Feeding (IAP). Four of these grass species produced typical severe symptoms of MSV while the other four produced mild symptoms in maize.

Table 3. Transmission of a severe MSV maize isolate to 34 plants species and from them back to maize using *C. storeyi* as a vector (given 72 h acquisition and inoculation access feeding periods, three insects per plant, 40 seedlings per plant species)

Test plants	% transmission from maize to test plant[a]	Incubation period[b]	Symptom on test plant	Symptom on maize[c]
Andropogon gayanus Kunth	0			
Brachiaria deflexa (Schumach.) C.E. Hubbard ex Royns	0			
B. mutica (Forsk.) Staph	0			
Chloris pilosa Schumach	0			
Chrysopogon acciculatus (Retz) Trin.	0			
Dactyloctenium aegyptium (Linn.) P. Beauv.	0			
Echinochloa colona (Linn.) Link	0			
Eleusine indica (Linn.) Gaertn	0			
Eragrostis tremula Hochst. ex Steud.	0			
Euclaster condylotricha (Hochst. ex Steud.) Stapf	0			
Oryza sativa Linn.	0			
P. notatum Flüggé	0			
P. pedicellatum Trin.	0			
P. polystachion (Linn.) Scult	0			
P. scrobiculatum	0			
P. violaceum (Lam.) L. Rich	0			
P. subangustrum (Schumach.) Stapf & C.E. Hubbarb	0			
Panicum maximun Jacq.	0			
Paspalum conjugatum Berg.	0			
Pennisetum americanum (Linn.) K. Schum	0			
Setaria longiseta P. Beauv.	0			
Setaria pallide-fusca (Schum.) Stapf C.E. Hubbard	0			
Sorghum arundinaceum (Desv.) Stapf	0			
Sorgum bicolor (Linn.) Moench	0			
Sporobolus pyramidalis P. Beauv.	0			
Axonopus compressus (Sw.) P. Beauv.	90	7	severe	severe
Brachiaria lata (Schumach) C.E. Hubbarb	10	10	severe	severe
Cynodon dactylon (Linn.) Pers.	40	24	mild	severe
Digitaria horizontalis Wild	40	14	severe	severe
Eragrostis tenella (Linn.) P. Beauv. ex Roem & Schult	40	10	mild	severe
Lepthochloa filiformis (Linn.) P. Beauv.	90	7	severe	severe
Rhynchelitum repens (Willd.) C.E. Hubbarb	10	30	mild	severe
Setaria barbata (Lam.) Kunth	50	10	mild	severe
Zea mays Linn. (Pool 16)	100	7	severe	severe

[a] n = 20
[b] days from IAP set up to symptom appearance
[c] after transmission from test plant back to maize susceptible hybrid CML254 x CML 247

Serological characterization of MSV isolates

The results of DAS-ELISA on the MSV isolates collected during the surveys are presented in Table 4. All the MSV isolates (except three) gave positive reaction with a polyclonal antiserum produced in mice. These three isolates that tested negative were *Andropogon gayanus* (from Kaduna) exhibiting mild streak symptom, *Thelepogon elegans* (from Kadawa) exhibiting mild streak/mottle symptom and *Rottboellia cochinchichinensi* (from Jos) exhibiting mild streak/mottle symptom. A clone of monoclonal antibodies, raised against MSV, from John Innes Institute, UK reacted with 12 out of the 25 samples that were tested.

Serological variation within the isolates is illustrated in Figures 1 and 2. The ELISA data showed variations that were detectable with MSV polyclonal and monoclonal antisera. While the DAS-ELISA data divided the samples into two groups (30 isolates reacted positively while three samples reacted negatively), the TAS-ELISA monoclonal antibody further separated into two serotypes, those isolates that reacted positively in DAS-ELISA.

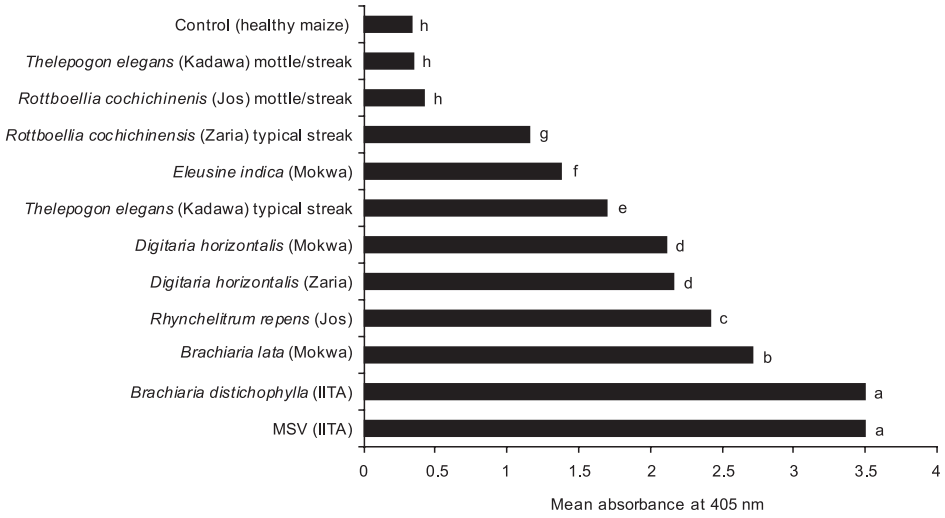


Fig. 1. DAS-ELISA reactions of some streak isolates. Bars follwed by the same letter (a, b, c) indicated that mean absorbance at 405 nm were not significantly different (p = 5%)

The data also showed significant variation in the amount of the virus in the different plant hosts. Figure 1 illustrates the separation of means of the ELISA values of some 11 isolates into 8 statistically different groups. Nine of the isolates that tested positive with the polyclonal antiserum had ELISA titre values that were divided into 7 groups (p = 5%). The MSV isolate in *B. distichophylla* (from IITA) had similar ELISA values to the MSV in maize (IITA) but the two differed from other isolates. The MSV isolate in *B. lata* (Mokwa), *R. repens* (Jos), *D. horizontalis* (Mokwa & Zaria), *T. elegans* (Kadawa), *E. indica* (Mokwa) and *R. cochinchinensis* (Zaria) reacted positively but the ELISA values were significantly different from each other.

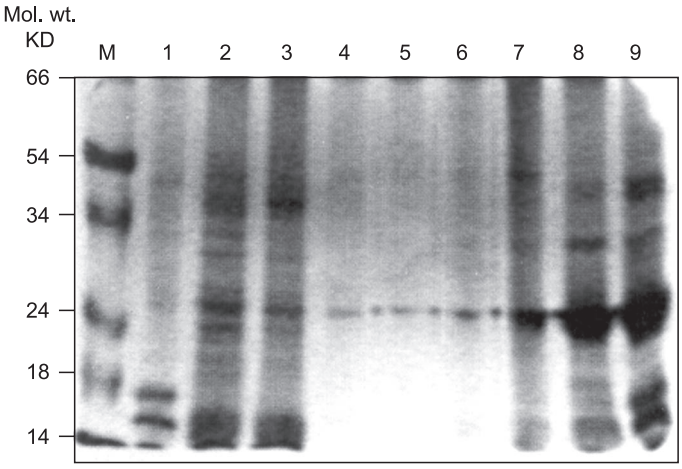
The A_{405} values obtained from monoclonal and polyclonal antisera on the same samples within the same plate were different. Figure 2 illustrates this difference in the two diagnostic protocols. Within the same ELISA plate, the A_{405} values for each of

Table 4. Detection of MSV isolates by ELISA tests

Grass Species	Symptom severity (1–5)	Location of collection	ELISA reactions‡	
			Polyclonal DAS-ELISA	Monoclonal TAS-ELISA
Axonopus compressus	mild (3)	IITA, Ibadan	+	+
A. compressus	severe (5)	IITA, Ibadan	+	+
Andropogon gayanus	very mild (2)	Kaduna	–	–
Brachiaria deflexa	mild (3)	Catholic Camp, Ibadan	+	+
B. deflexa	mild (3)	Moor Plantation, Ibadan	+	–
B. distichophylla	mild (3)	IITA, Ibadan	+	n.t.
B. distichophylla	severe (5)	IITA, Ibadan	+	+
B. lata	mild/mottle (3)	IITA, Ibadan	+	–
B. lata	mild (3)	Mokwa	+	–
B. lata	severe (5)	Mokwa	+	+
Digitaria horizontalis	severe (5)	Mokwa	+	+
D. horizontalis	severe (5)	Zaria	+	+
D. horizontalis	mild/mottle (3)	IITA, Ibadan	+	–
D. horizontalis	mild (3)	Ikenne	+	+
Dactyloctenium aegyptium	mild (3)	IITA, Ibadan	+	n.t.
Eleusine indica	very mild (2)	Mokwa	+	–
MSV in maize	severe (5)	IITA, Ibadan	+	+
MSV in maize	severe (5)	kaduna	+	+
Panicum maximum	mild (3)	IITA, Ibadan	+	–
P. maximum	mild (3)	Ife	+	–
Paspalum conjugatum	mild (3)	IITA, Ibadan	+	+
P. notatum	mild (3)	IITA, Ibadan	+	+
P. scrobiculatum	mild (3)	IITA, Ibadan	+	–
Rottboellia cochinchinensi	mild (3)	Ife	+	–
R. cochinchinensi	mild (3)	Zaria	+	n.t.
R. cochichinensi	very mild (2)	Ife	+	–
R. cochinchinensi	very mild (2)	Jos	+	–
R. cochinchinensi	mild/mottle (3)	Jos	–	–
Rhynchelitrum repens	mild (3)	Jos	+	+
Setaria barbata	mild (3)	IITA, Ibadan	+	n.t.
S. barbata	mild (3)	Kaduna	+	n.t.
Thelepogon elegans	mild (3)	Kadawa	+	–
T. elegans	mild/mottle (3)	Kadawa	–	–

– negative reaction. A_{405} value less than twice the value of healthy (maize) control
+positive reaction. A_{405} value greater than twice the value of healthy (maize) control

n.t. – not tested because of insufficiency of monoclonal antibodies



Mol. wt. KD – molcular weight in kilodaltons
M – Marker
1 – Healthy maize (control)
2 – *Axonopus compressus* (with severe streak symptom)
3 – *A. compressus* (with mild streak symptom)
4 – *Paspalum conjugatum*
5 – *P. scrobiculatu*
6 – *P. notatum*
7 – *Andropogon gayanus*
8 – *Panicum maximum*
9 – MSV in maize

Fig. 2. SDS-PAGE of purified streak virus isolates in maize

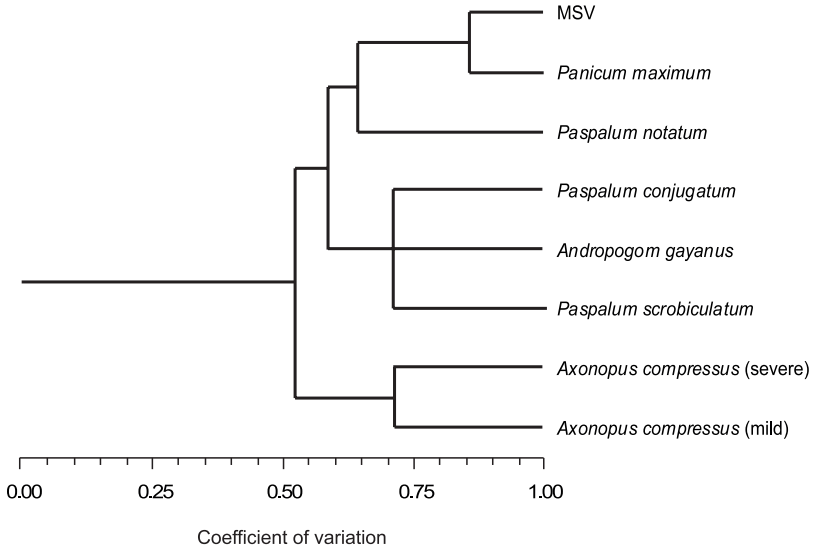


Fig. 3. Relationship dendogram of eight purified streak virus isolates in maize by SDS-PAGE

the isolates were lower for monoclonal than the polyclonal antiserum. The polyclonal antiserum divided the isolates into 7 while the monoclonal antiserum divided the same set of isolates into 4 groups with different virus concentration.

SDS-PAGE: Figures 3 and 4 showed the SDS-PAGE result of eight purified streak isolates and healthy maize leaf as a contol. The band showed that the eight purified isolates had similar molecular weight of about 24 kD (Fig. 3). The relationship dendogram through SDS-PAGE among eight purified virus isolates is presented in Figure 4. At 0.55 coefficient of similarity, the dendogram divided the samples into two groups while at 0.9 coefficient of similarity, the 8 isolates were identified as distinct genetic entities. The dendogram thus show 55–90% variation among the eight isolates.

DISCUSSION

Streak symptoms were observed on annual grasses that grow within and around maize fields. Four of these: *Brachiaria deflexa, B. distichophylla, B. lata,* and *Digitaria horizontalis* have been reported to harbour the maize strain of streak virus (Mesfin et al. 1992). Streak symptoms were also observed on perennial grasses, particularly *Panicum maximum*, which was present in all locations, surveyed. These grasses, on which leafhoppers were collected and streak disease symptoms observed may have epidemiological importance as sources of virus and/or vectors. MSV may bridge maize growing seasons on annual grasses, such as *B. lata* and *Setaria barbata* and/or on perennial grasses such as *Axonopus compressus*. In irrigated areas where maize and grassy weeds (e.g. *Brachiaria* spp., *Digitaria* spp.) grow in close proximity year around, both the vectors and MSV might survive in both the crop and grasses.

The study on transmission of streak virus isolates from original grasses to a susceptible maize hybrid that is adapted to the Tropics shows that not all of the isolates in grasses could be transmitted to maize. Streak isolates from four perennial grasses, *Andropogon gayanus, Panicum maximum*, *Paspalum conjugatum* and *P. notatum* could not be transmitted to maize. Also there was variation in symptoms among those isolates transmissible to maize, some produced typical, severe streak symptoms while others produced mild symptoms in maize. Thus, some isolates appear to be adapted to their grass hosts. These results agree with earlier reports of Bock et al. (1974) and Mesfin et al. (1992), but differ from that of Pinner et al. (1988) who concluded that no adaptation to grass hosts occurred because all 24 grass isolates tested could be transmitted to the sweet corn cv. Golden Bantam.

Previous studies carried out at IITA, have reported differences among streak virus isolates from wild grasses with respect to their transmission to maize (IITA 1984; Rossel and Thottappilly 1985; Mesfin et al. 1992). Other authors (Bock et al. 1974; McClean 1947; Storey and McClean 1930) have also reported difficulties in transmitting isolates from grasses to maize. Rossel and Thottappilly (1985) indicated that they were unable to transmit streak isolates from *B. mutica, R. cochinchinensis* and *P. maximum* to maize. The failure, in this study, to transmit virus isolates with mottle/streak symptoms in *R. cochinchinensis* and *T. elegans* isolates to maize could be attributable to virus-vector-plant interaction that require further study. The virus (es) producing mild streak with mottle symptoms in these grasses may be different from those producing typical streak without mottle symptoms. In this study, MSV isolate in *B. distichophylla* produced severe streak symptom in maize, in confirmation to the report of

Mesfin et al. (1992) but contrary to another earlier report from IITA (IITA 1984). Also, contrary to IITA (1984), MSV isolate in *B. deflexa* produced mild symptoms in maize as reported by Mesfin et al. (1992). Differences in susceptibility of the maize genotype that are used as test plants in various transmissions experiments could be responsible for the variation observed in the symptom development. This could also explain why streak isolates in *P. maximum* were reported as being transmissible to sweet corn cv. Golden Banttam which is an exotic hybrid that is not adapted to the Tropics.

The severe MSV maize isolate at IITA was transmissible to eight out of the 33 grasses and crop species tested. The symptoms were mild on four of the species but severe on the others. When the virus was transmitted back to maize, the symptoms became severe again. This could be attributed to virus-host interaction but which did not affect the virulence of the virus. Further study needs to be carried out to ascertain if the virulence will change were the virus to stay longer on the grass host.

These results indicate that grass species, to which severe MSV were transmissible to, such as *A. compressus* and *B. lata*, and which are known to harbour MSV strain under natural field conditions, are of epidemiological importance. Such grasses could serve as a means of survival to both the virus and the vectors, and their elimination from maize fields should be a major aspect in the integrated control of the MSV disease.

The list of 25 plants into which severe MSV could not be transmitted could indicate a complex of virus-host-vector interactions but also confirms earlier report (Olojede 1987) that the host plant of MSV, in Nigeria, is limited to few plant species. Some of these grass and crop species have been reported as having streak symptoms under natural conditions. Some have also been reported to harbour MSV under experimental controlled conditions (Rose 1978; Mesfin et al. 1992).

Results from DAS-ELISA results from a MSV polyclonal atiserum indicated that there were two types of viruses that elicit streak symptoms in grasses: those that reacted with the MSV polyclonal antiserum and the following three others that did not: the virus (es) in *A. gayanus*, mild/mottle isolates in *T. elegans* and *R. cochinchinesis.* Since the viruses (es) in these plants were also not transimissible to maize, they probably were separate viruses entirely. Further work is needed to ascertain their aetiology.

The isolates that were serologically related to MSV were further divided into two groups by a monoclonal antiserum (MAb). The non-availability of more MAbs greatly hinders the characterization of the streak isolates by serology. Serological difeerences among MSV isolates have been found using polyclonal and monoclonal antibodies (Dekker et al. 1988; Pinner et al. 1988; Pinner and Markham 1990), but Peterschmitt et al. (1991) characterized MSV isolates from 11 African countries and reported that all belonged to the same serotype.

The symptoms of *A. gayanus* were identical to those described by Fajemisin et al. (1976), Bock (1974), Rossel and Thottappilly (1985) and Pinner et al. (1988). However, the presence of mottle in addition to the streak symptoms in *T. elegans* and *R. cochinchinensis* could support the idea that they are different viruses.

Significant variation in the titre of the virus in the different plant hosts as indicated by ELISA values could play an important role in the epidemiological importance of such isolates. Low virus titre could indicate a measure of resistance as the virus may encounter difficulties in replicating and multiplying in such host plants. Vectors may also encounter more difficulties in acquiring the virus from such host. Asanzi et al. (1994)

reported that maize genotypes with high level of resistance were not good sources of MSV acquisition. This factor could be used on breeding for MSV resistance.

The SDS-PAGE results indicated that the isolates in the seven perennial grasses studied were 50–90% similar. This could help to explain the variation obtained in the transmission and serology experiments. The two types of isolates from *Axonopus compressus* separated from others at 55% were readily transmissible to maize. They also gave positive reactions to both polyclonal and monoclonal antibodies tested. *Panicum maximum* isolate that showed closest relationship to MSV in maize has been described as a distinct virus, called *Panicum streak virus* (Briddon and Markaham 1995; Briddon et al. 1992). Isolates in *Andropogon gayanus*, and *Paspalum* spp. require further studies. They may as well be separate geminiviruses, especially *Andropogon* that was not transmissible to maize and did not react with polyclonal and monoclonal antibodies of MSV in maize.

The results of this study imply that out of the seven perennial grasses, only *Axonopus compressus* may carry isolate of MSV that are of epidemiological relevance in *Maize streak virus* disease and commercial production of maize in Nigeria.

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POLISH SUMMARY

PRZENOSZENIE SIĘ ORAZ WYNIKI TESTÓW ELISA I SDS-PAGE WYBRANYCH IZOLATÓW *MAIZE STREAK VIRUS* POCHODZĄCYCH Z RÓŻNYCH REGIONÓW NIGERII

W latach 1997–1999 podjęto badania w pięciu strefach ekologicznych Nigerii w celu zebrania izolatów *Maize streak virus* z rodzaju *Mastervirus*. Oprócz kukurydzy (*Zea mays* L.) stwierdzono 15 innych gatunków traw z symptomami infekcji MSV. Badane rośliny żywicielskie miały dwa rodzaje objawów, mianowicie łagodne (z plamami lub bez) albo ostre (typowe objawy, jak w przypadku kukurydzy). Kiedy w próbie przeniesienia izolatów MSV na sadzonki podatnej hybrydy kukurydzy CML 254 X CML 247 użyto *Cicadulina storeyi* China, sześć z nich nie przeniosło się. Siedem izolatów, które się przenosiły spowodowało wystąpienie łagodnych objawów. Czynniki wirusowe powodujące typowe lub ostre objawy na *Axonopus compressus* (Sw.) P. Beauv., *Brachiariadistichophylla* (Trn.) Stapf, *Dactyloctenium aegyptinum* (Linn.) P. Beauv. i *Setaria barbata* (Lam.) Kunth spowodowały wystąpienie objawów typowych dla infekcji MSV na polach farmerów kukurydzy, na które zawleczono wirusa. Spośród 33 gatunków roślin, których sadzonki infekowano MSV, tylko osiem było podatnych. Cztery z nich miały łagodne objawy, natomiast cztery pozostałe ostre.

Jedynie trzy uzyskane izolaty nie reagowały podczas testu DAS-ELISA (Double Antibody Sandwich – Enzyme Linked Immunosorbent Assay) z poliklonalnym antyserum wyhodowanym na myszach. Izolaty te zebrano z *Andropogon gayanus* Kunth (z Kaduny), *Thelepogon elegans* Roth ex Toem & Schult (z Kadawy) i *Rottboellia cochinchinensis* (Lour.) Clayton (z Jos); rośliny wykazywały łagodne objawy (pasemka/plamki). Zastosowane specyficzne przeciwciała monoklonalne skierowane przeciwko MSV reagowały z 12 próbkami z 25 badanych. Dane DAS-ELISA wykazały również znaczne zróżnicowanie stężeń wirusa w różnych roślinach żywicielskich.

Drzewo pokrewieństwa wynikające z SDS-PAGE pomiędzy ośmioma oczyszczonymi izolatami wirusa wykazało 55–90% zróżnicowania. Przy współczynniku prawdopodobieństwa 0,55 dendrogram dzieli próbki na dwie grupy, natomiast przy współczynniku 0,9 osiem powyższych izolatów stanowiło odrębne jednostki genetyczne.