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SEARCH FOR VARIABILITY AMONG MASTREVIRUSES SHOWING SYMPTOMS OF *MAIZE STREAK VIRUS* FROM DIFFERENT REGIONS OF NIGERIA BY POLYMERASE CHAIN REACTIONS

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Abstract: Field surveys were undertaken in 1997–1999 across five ecological zones in Nigeria to collect grass species showing symptoms of *Maize streak virus* (MSV) (Genus *Mastrevirus*: Family *Geminiviridae*). Apart from maize (*Zea mays* L.), 15 grass species were found with symptoms similar to MSV. These hosts showed two types of symptoms vi**z:** mild or severe. Polymerase chain reaction (PCR) and immunocapture IC–PCR were used to diagnose the mastreviruse. Variation among the isolates is illustrated in relationship dendograms. The PCR dendogram showed 35–85% variation among the isolates while the IC–PCR dendogram showed 55–85%. The PCR dendogram clusters the isolates essentially along symptom severity. Isolates that were most distantly related to a standard MSV strain showed mild symptoms in their grass hosts. Simple PCR protocols could help in studying diversity among grass *Mastreviruses* where facilities for genome sequencing are not available.

Key words: Mastreviruses, MSV, Isolates, Polymerase chain reaction (PCR), Immunocapture (IC)–PCR, Graminae, relationship dendogram, diversity

INTRODUCTION

Maize, *Zea mays* L., is a member of the grass family, *Gramineae*, to which all the major cereals belong. Maize is a basic staple food and principal crop in many 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).

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Maize streak virus (MSV) causes the most important virus disease of maize in sub-Saharan Africa. Damage due to MSV can be insignificant in some years, but epidemics of the disease can devastate crops with yield losses up to 100 percent (Thottappilly *et al*. 1993; Bosque–Perez 2000). It was first recorded in South Africa in 1901 by Fuller as "mealie variegation" but has been reported to be widely distributed all over Africa south of the Sahara (Fajemisin and Shoyinka 1976; Rossel and Thottappilly 1985).

MSV is the type member of the genus *Mastrevirus* (family: *Geminiviridae*). Natural hosts of MSV are grasses (Damsteegt 1983; Mesfin *et al.* 1992), but as there seem to be various strains of the virus with considerable specificity of adaptation, it is necessary to distinguish those that can infect maize and cause economic damage. Serological tests are widely used for the detection and to some degree the characterization of geminiviruses (Dekker *et al.* 1998; Pinner and Markham 1990). Polymerase chain reaction serves as corroborative tests particularly when further characterization is intended (Briddon and Markham 1995). The protocols use nucleotide sequence information of several viruses to design oligonucleotide primers. Such primers are used to detect and differentiate viruses at the family, genus or strain levels (Robertson *et al.* 1991; Naidu and Hughes 2003). Mixtures of virus-specific primer pairs have also been used in PCR studies of viruses (Naidu and Hughes 2003). MSV strains isolated from different host species share between 78 and 90% identity with maize adapted strain (Rybicki *et al.* 1998; Willment *et al*. 2001) indicating that host adaptation is probably the main force driving MSV diversification.

Methodologies bases on polymerase chain reaction was used to study diversity among isolates of different grass mastreviruses showing symptoms of MSV in order to have a clearer understanding of the naturally-occurring strains in Nigeria, and their relatedness to a standard maize isolate used at International Institute of Tropical Agriculture (IITA) for resistance screening and transmission studies.

MATERIALS AND METHODS

Field surveys were undertaken across different ecological zones in Nigeria between 1997–1999 to collect streak virus 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. Transmission and serological tests were used to characterize these isolates in an earlier paper (Oluwafemi *et al.* 2007). An isolate of MSV (Soto *et al.* 1982) with severe symptoms in maize, used at IITA by breeders to screen maize germplasm was used as a standard for comparison. MSV–suscsetible, open-pollinated maize variety Pool 16 was used as the host of the isolate

Polymerase chain reaction (PCR) and immunocapture–PCR (which amplifies the DNA of virus previously trapped in the PCR tube by antibodies) techniques were as follows:

DNA Extraction: Total DNA from plants with streak symptoms were extracted by a combination of Dellaporter and CTAB methods (Dellaporter *et al*. 1983; Doyle and Doyle 1987; 1990).

PCR Cocktail: The reaction mixture per reaction contained the following: Thermo buffer 2.5 µl (1X concentration) (Promega Biotech); $MgCl_2$ 2.0 µl (2.5 mM/µl); 5%

v/v Tween 20 2.5 µl; Deoxynucleotide triphosphate (dNTP) (2.5 mM/µl each of dATP, dCTP, dGTP, & dTTP) 1.0 µl; Virus forward primer 1.0 µl (2.5 pM/µl); Virus reverse primer 1.0 µl (2.5 pM/µl); *Thermus acquaticus* (Taq) polymerase 0.4 µl (2 units); Sample DNA 5.0 µl (1 ng/µl); Sterile distilled H_2O 9.6 µl. Two drops (about 30 µl) of sterile mineral oil was layered on the reaction mixture to prevent evaporation during the PCR process.

PCR Condition: PCR cycles were: 1 cycle of initial denaturation at 94°C for 1 min; 33 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 2 min; 1 cycle of 94°C for 1 min, 50°C for 1 min, 72°C of final elongation for 7 min; Storage 4°C. Reactions were cycled in a DNA Thermal Cycler 4800 or 9600 (Perkin–Elmer Cetus, USA). The PCR reaction products were kept at 4°C until when electrophoresis was done.

Oligonucleotide primers used for MSV detection through PCR: The primers used for MSV detection are listed in table 1. The first four were as published by Rybicki and Hughes (1990) while the fifth sequence was by Ed Rybicki (personal communication). These five primers were purchased from Integrated DNA Technologies Inc., USA. Primer-pairs 1and 5 were designed to amplify MSV while 2, 3, and 4 were originally designed by Rybicki and Hughes (1990) to amplify the following closely related viruses: *Chloris striate mosaic virus, Digitaria streak virus* and *Wheat dwarf virus* respectively at the C2 ORF region.

Table 1. Oligonucleotide primers used in polymerase chain reactions to detect MSV

Serial No.	Primer code	Nucleotide sequence 5′ to 3′
1	Forward 1F	TTC ATC CAA TCT TCA TC
1	Reverse 1R	GGA AAA TCT ACT TGG GC
2	Forward 2F	TTG AGC CAA TCT TCG TC
2	Reverse 2R	GGA AAG ACT TCC TGG GC
3	Forward 3F	TGC AGC CAG TCT TCA TC
3	Reverse 3R	GGA AAG ACT TCT TGG GC
4	Forward 4F	TTC ATC CAA TCT TCA TC
4	Reverse 4R	GGA AAG TCT ACT TGG GC
5	Forward 5F	TTG GVC CGM VGA TGT ASA G
5	Reverse 5R	CCA AAK DTC AGC TCC TCC G

Immunocapture (IC)–PCR: IC–PCR detection of streak viruses in plant leaf samples was carried out following the protocol of Jansen *et al*. (1990) and Hoffmann *et al*. (1997). Leaf samples were ground (1 g leaf: 10 ml buffer) in PBS–Tween containing 2% polyvinylpyrrolidine (PVP) using sterile mortars and pestles. The macerated samples were filtered through glasswool and the resulting extracts used for IC–PCR amplification assays.

Eppendorf tubes (0.5 ml) were coated with MSV antibodies by loading 50 µl of MSV polyclonal antiserum (from John Innes Institute, UK, through Dr. Peter Markham) diluted 1:1000 in a coating buffer (15 mM Na_2Co_3, 34.9 mM $NaHCO_3$, pH 9.6). The tubes were incubated for 2–3 hr at 37°C and then washed with PBS–Tween (three times with three min soaking). Streak virus saps (50 µl) were then added to

the coated tubes and the tubes were incubated overnight at 4°C. The tubes were washed three times with PBS–Tween after which 25 µl of PCR cocktail was added to each tube.

IC–PCR cocktail and conditions were similar to the standard PCR except that the trapped antibody-antigen complex in the tubes provided the genetic material of the virus for amplification. In addition, the volume of sterile distilled water was increased from 9.6 µl to 14.6 µl per tube.

The DNA bands were separated in 1.4% w/v agarose, stained in ethidium bromide, observed under UV light and their positions relative to the DNA marker noted. Photographs of the bands in the gel were taken by using gel documentation computer software system.

Analysis of PCR & IC–PCR data: The presence or absence of DNA bands by each primer-pair for each MSV isolate was transformed into a binary character matrix ("1" for the presence and "0" for the absence of DNA). Pairwise distance matrices were then computed by NTSYS–pc 2.0 software package (Rohlf, 1993). Dendograms were created by UPGMA cluster analysis (Sneath and Sokal 1973).

RESULTS

Table 2 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 (Oluwafemi *et al.* 2007) and the abbreviations used in describing results.

Table 3 shows that the virus isolates reacted differently to the primer-pairs tested. In PCR assays, primer-pairs 1 and 5 could amplify most of the isolates, although the intensities of the amplified bands were different. Isolates from *Brachiaria lata* (mild), *B. deflexa, Paspalum conjugatum, P. notatum, P. scrobiculatum* and *Setaria barbata* produced faint bands while the other isolates produced bold bands. Virus isolates in *Panicum maximum* (Ife) and the three *Rottboellia cochinchinensis* samples (from Ife, Zaria and Jos) and *Thelepogon elegans* (mild/mottle streak symptom) were not amplified by primer-pair 1. Primer-pair 5 did not amplify the virus isolates in *Brachiaria lata* (Mokwa), *Digitaria horizontalis* (Mokwa), *P. maximum* (Ife) *R. cochinchinensis* (Jos & Zaria) and *T. elegans* (mild/mottle streak symptom). Primer-pair 3 could amplify the viruses in seventeen of the isolates, primer-pair 4 amplified 22 isolates while primer-pair 2 amplified only five of the isolates.

In IC–PCR assays, most of the isolates reacted positively to the 5 primer-pairs. Primer-pairs 1 and 5 amplified all the isolates. Primer-pair 2 amplified 18 isolates. Only one isolate each was not amplified by primer-pair 3 and primer-pair 4.

The relationship dendograms among the different streak isolates from PCR and IC–PCR experiments are presented in figures 1 and 2 respectively. At 0.35 coefficient of similarity, the PCR dendogram divided the samples into two clusters while at 0.85 the samples were divided into ten. The PCR dendogram thus show 35–85% variation among the isolates. The IC–PCR divided the isolates into two clusters at 0.55 coefficient of similarity but at 0.85 coefficient of similarity, the isolates were divided into five clusters only. The IC–PCR dendogram thus show 55–85% variation among the isolates.

Table 2. List of grass species found with virus-like streak symptoms in various locations in Nigeria, with the locations where samples were collected, type of symptoms observed and abbreviation used in describing results

Isolates	Location	Symptoms	Abbreviation
Andropogon gayanus Kunth	Kaduna	mild	Andropo
A. gayanus	Mokwa	mild	
Axonopus compressus (Sw.) P. Beauv.	IITA, Ibadan	severe	Axo.se
A. compressus	IITA, Ibadan	mild	Axo.mild
Brachiaria deflexa (Sch.) C.E. Hubbarb ex Robyns	Moor Plantation, Ibadan	mild	B.de.MP
B. deflexa	Catholic Redemptorist Camp, Ibadan	mild	B.de.CR
B. distichophylla (Trn.) Stapf	IITA, Ibadan	severe	B.di.se
B. lata (Schumach) C.E. Hubbarb	Mokwa	severe	B.la.MK
B. lata	IITA, Ibadan	severe	B.la.se
B. lata	IITA, Ibadan	mild/ mottle	B.la.mm
Dactyloctenium aegyptium (Linn.) P. Beauv.	IITA, Ibadan	severe	Dactylo
Digitaria horizontalis Willd	Ikenne	mild	Dig.Ik
D. horizontalis	Mokwa	severe	Dig.MK
D. horizontalis	Zaria	severe	Dig.Zar
D. horizontalis	Jos	severe	Dig.Jos
D. horizontalis	IITA, Ibadan	severe	Dig.IITA
Eleusine indica Gaertn	Mokwa	mild	Eleu.MK
Panicum maximum Jacq	IITA, Ibadan	mild	Pan.IITA
P. maximum	Ife	mild	Pan.Ife
Paspalum conjugatum Berg.	IITA, Ibadan	mild	Pasp.c.
P. notatum Flüggé	IITA, Ibadan	mild	Pasp.n.
P. scrobiculatum L.	IITA, Ibadan	mild	Pasp.s.
Rottboellia cochinchinensis (Lour.) Clayton	Ife	mild	Rot.Ife
R. cochinchinensis	Zaria	mild	Rot.Zar
R. cochinchinensis	Jos	mild	Rot.Jos
Rhynchelitrum repens (Wild.) C.E. Hubbarb	Jos	mild	Rhy.Jos
Setaria barbata (Lam.) Kunth	IITA, Ibadan	severe	Setaria
Thelepogon elegans Roth ex Toem & Schult	Jos	mild/ mottle	
T. elegans	Kadawa	mild/ mottle	Thel.mm
T. elegans	Kadawa	severe	Thel.se
Zea mays L.	IITA, Ibadan	severe	MSV.IITA
Z. mays	Kaduna	severe	MSV.Kd

Table 3. Immunocapture (IC–PCR) and polymerase chain reactions (PCR‡) of some MSV isolates to five primer-pairs designed to amplify grass-infecting geminiviruses at the C2 ORF region

MSV isolates	IC–PCR					PCR				
	Primer–pairs#					Primer–pairs				
	1	2	3	4	5	1	2	3	4	5
MSV (IITA) in maize	+	–	+	++	+	++	–	++	+	++
Andropogon gayanus	+	–	+	++	+	++	–	–	–	++
Axonopus compressus (mild)	++	–	++	++	++	++	–	++	++	++
A. compressus (severe)	++	–	++	++	++	++	–	–	++	++
Brachiaria deflexa (CRC)	++	–	++	++	++	+	–	–	–	+
B. deflexa (Moor Plantation)	++	++	++	++	++	++	++	++	++	++
B. distichophylla (severe)	++	–	++	++	++	++	–	++	–	++
B. lata (Mokwa)	++	+	++	++	++	++	–	++	++	–
B. lata (mild)	+	–	+	++	++	+	–	–	–	+
B. lata (severe)	++	–	+	++	++	++	–	++	+	++
Dactyloctenium aegyptium	++	+	++	++	++	++	–	–	+	++
Digitaria horizontalis (IITA)	++	+	++	++	++	+	–	–	–	++
D. horizontalis (Ikenne)	++	+	++	++	++	++	–	++	++	++
D. horizontalis (Jos)	++	+	++	++	++	++	–	++	++	++
D. horizontalis (Mokwa)	+	+	++	++	++	++	–	++	++	–
D. horizontalis (Zaria)	++	+	++	++	++	++	–	+	+	++
Eleusine indica (Mokwa)	+	++	++	++	++	++	–	++	++	++
MSV (Kaduna) in maize	+	+	+	++	++	+	–	–	+	++
Panicum maximum (Ife)	++	–	–	++	++	–	+	–	++	–
P. maximum (IITA)	+	–	+	++	+	++	–	–	–	++
Paspalum conjugatum	+	+	+	++	++	+	+	++	+	++
P. notatum	++	–	+	++	++	+	–	++	++	+
P. scrobiculatum	++	+	+	–	++	+	+	+	+	+
Rottboellia cochinchinensis (Ife)	++	+	++	+	++	–	+	+	+	+
R.. cochinchinensis (Jos)	++	+	++	++	++	–	–	–	–	–
R. cochinchinensis (Zaria)	++	++	++	++	++	–	–	–	–	–
Rhynchelitrum repens (Jos)	++	++	+	++	++	++	–	+	+	++
Setaria barbata	++	–	++	++	++	+	–	–	++	+
Thelepogon elegans	++	++	++	++	++	++	–	++	++	++
Thelepogon elegans (mottle)	++	++	++	++	++	–	–	–	+	–

‡ – negative reactions that produced no bands
\+ – positive reactions that produced faint bands
++ – positive reactions that produced bold bands
\# – Key for primer-pair: 1 = MSV, 2 = WDV, 3 = CSM, 4 = DSV, 5 = MSV

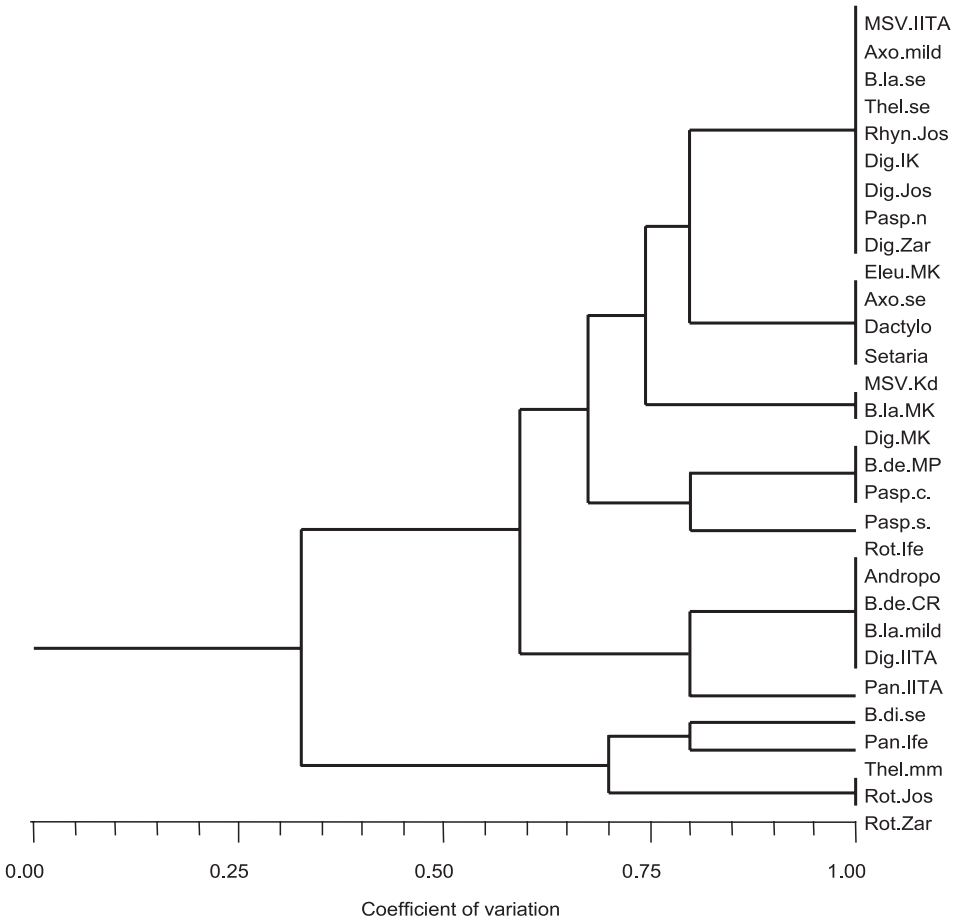

Fig. 1. Dendogram from PCR experiments (from five pairs of primers) showing the variation among 30 isolates of streak virus in comparison to the severe IITA MSV being used to screen maize germplasm for streak resistance

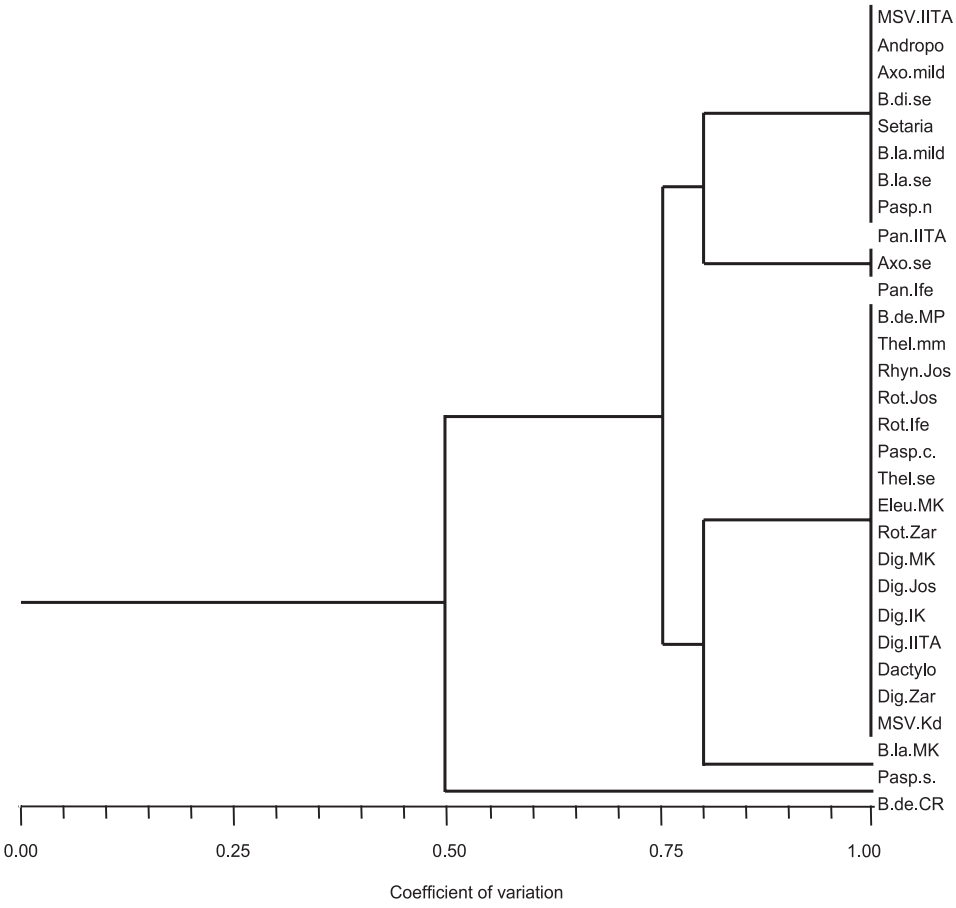

Fig. 2. Dendogram from IC-PCR experiments (from five pairs of primers) showing the variation among 30 isolates of streak virus in comparison to the severe IITA MSV being used to screen maize germplasm for streak resistance

DISCUSSION

The 30 isolates studied were dissimilar although the coefficient of similarity at which the samples began to split into clusters depended on the assay method. PCR started clustering the isolates below 0.35 coefficients while IC–PCR clustered at 0.5. Thus the isolates could be said to share a minimum of 30% similarity. This would be studied further using restriction analysis with many enzymes and genomic sequencing. Previous workers reported that full genomic sequences determined for three MSV isolates from wild annual grass species and wheat share 90% identity with those of the isolates from maize (Schnippenkoetter *et al*. 2001) while virtually all isolates from maize shared greater than 95% nucleotide sequence identity (Briddon *et al*. 1994: Rybicki *et al.* 1998). Isolates from wheat and annual grasses were considerably more

diverse and showed more than 89 and 78% nucleotide sequence identity with maize isolates (Rybicki *et al.* 1998; Willment *et al*. 2001). Our work shows the diverse nature of the grass isolates even when facilities for nucleotide sequencing were not available. As such, simple PCR protocols could be considered useful in studying MSV diversity when different primers are available, since MSV and other african streak viruses are known to have a single genomic component and identical arrangement of genes (Rybicki *et al.* 2000; Martin *et al.* 2001). The big variation reported here may indicate that there were many viruses, apart from MSV, in these collections. The ICTV guideline for mastrevirus species demarcation is less than 75% identity with a previously described species (Fauquet *et al*. 2003). The virus isolates in *Panicum maximum* plants has been described as a distinct virus, *Panicum streak virus* (Briddon *et al*. 1992; Schnippenkoetter *et al*. 2001). Further work on sequencing may eventually name more of these isolates as separate viruses.

The PCR dendogram identified four isolates as most distantly related to MSV (IITA). These were virus isolates that produced mild symptoms in *R. cochinchinensis* from Zaria and Jos, *P. maximum* (Ife) and *T. elegans* (Kadawa). The next cluster that was most distantly related to MSV contained four mild and one severe isolates. Thus, the PCR dendogram clustered the isolates essentially along symptom severity. The first three clusters were made up of isolates that produced severe symptoms in their grass hosts. The exceptions were *A. compressus, R. repens* and *P. notatum;* which had mild symptoms. The other clusters were all made up of isolates that elicited mild symptoms in their grass host plants. This is in agreement with the dendogram of MSV isolates from South Africa (Martin *et al*. 1999). These workers presented a dendogram that had 4 clusters viz: one cluster had mild isolate; two clusters had moderate isolates while the fourth cluster had moderate and severe isolates.

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REFERENCES

Bosque-Perez N.A. 2000. Eight decades of *Maize streak virus* research. Virus Res. 71: 107–121.

Briddon R.W., Lunness P., Chamberlin L.C., Pinner M.S., Brundish H., Markham P.G. 1992. The nucleotide sequence of an infectious insect-transmissible clone of the geminivirus *Panicum streak virus*. J. Gen. Virol. 73: 1041– 1047.

Briddon R.W., Lunness P., Chamberlin L.C., Markham P.G. 1994. Analysis of the genetic variability of *Maize streak virus*. Virus Genes 9: 93–100.

Briddon R.W., Markham P.G. 1995. Use of PCR in the detection and characterization of geminiviruses. Bull. OEPP/EPPO Bull. 25: 315–320.

Dellaporta S.L., Woods J., Hicks J.B. 1983. A plant DNA mini-preparation: version 11. Plant Biol. Rep. 1: 19–21.

Damsteegt V.D. 1983. Maize streak virus 1. Host range and vunerability of maize germplasm. Plant Dis. 67: 734–737.

Dekker E.L., Pinner M.S., Markham P.G., van Regenmontel M.H.V. 1988. Characterization of *maize streak virus* isolates from different plant species by polyclonal and monoclonal antibodies. J. Gen. Virol. 69: 983–990.

Doyle J.J., Doyyle J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissues. Phytochem. Bull. 119: 11–15.

Doyle J.J., Doyyle J.L. 1987. A rapid total DNA preparation procedure for fresh plant tissues. Focus 12: 13–15.

Efron T., Kim S.K., Fajemisin J.M., Mareck J.H., Tang C.Y., Dąbrowski Z.T., Rossel H.W., Thottapplly G. 1989. Breeding for resistance to maize streak virus: a multidisciplinary team approach. Plant Breeding 103:1–36.

Fauquet C.M., Bisaro D.M., Briddon R.W., Brown J.K., Harrison B.D., Rybicki E.P., Stenger D.C., Stanley J. 2003. Revision of taxonomic criteria for species demarcation in the family Geminiviridae, and an updated list of begomovirus species. Arch. Virol. 148: 405–421.

Fajemisin J.M., Shoyinka S.A. 1976. Maize streak and other maize virus diseases in West Africa. p. 52–61. In: Proceedings International Maize Virus Disease Colloquium and Workshop. 16–19 August, 1976 Ohio Agric. Res. Dev. Centre Wooster, USA (L.E. Williams, D.T. Gordon, L.R. Nault, eds.).

Hoffman K., Sackey S.T., Maiss E.., Adamako D., Vetten H.J. 1997. Immunocapture polymerase chain reaction for the detection and characterization of cacao swollen shoot virus 1A isolates. J. Phytopathol. 145: 205–212.

Janson R.W., Siegel G., Lemon S.M. 1990. Molecular epidemiology of human hepatisis A virus defined by an antigen-captured polymerase chain reaction method. Proc. Natl. Acad. Sci. USA 87: 2867–2871.

Martin D.P., Willment.J.A., Rybicki E.P. 1999) Evaluation of maize streak virus pathogenicity in differentially resistant *Zea mays* genotypes. Phytopathology 89: 695–700.

Martin D.P., Willment J.A., Bilharz R., Velders R., Odhiambo B., Njuguna J., James D., Rybicki E.P. 2001. Sequence diversity and virulence in *Zea mays* of *Maize streak virus* isolates. Virology 288: 247–255.

Mesfin T., Bosque-Perez N.A., Buddenhagen I.W., Thottappilly G. , Olojede S.O. 1992. Studies of *maize streak virus* isolates from grass and cereal hosts in Nigeria. Plant Dis. 76: 789–795.

Naidu R.A., Hughes J.d'A. 2003. Methods for the detection of plant virus diseases. p 233–260. In: "Plant Virology in Sub-Saharan Africa'' (J.d'A. Hughes, B.O. Odu, eds.). Proceedings of a conference organized by IITA, 4–8 June 2001, Ibadan, Ngeria.

Oluwafemi S., Thottappilly G. , Alegbejo M.D. 2007. Transmission, ELISA, ans SDS–PAGE results of some *maize streak virus* isolates from different parts of Nigeria. J. Plant Protection Res. 47(2): 197–212.

Okoruwa, A.E., 1995. Utilization and processing of maize. IITA Research Guide 35, 29 pp.

Pinner M.S., Markham P.G. 1990. Serotyping and strain identification of *maize streak virus* isolates. J. Gen. Virol. 71: 1635–1640.

Robertson N.L., French R., Gray S.M. 1991. Use of group specific primers and polymerase chain reaction for the detection and identification of luteoviruses. J. Gen. Virol. 72: 1473–1477.

Rossell, H.W., Thottappilly G. 1985. Virus Diseases of Important Food Crops in Tropical Africa. IITA, Ibadan, Nigeria, 61 pp.

Rohlf F.J. 1993. NTSYS–pc. Numerical taxonomy and multivariate analysis system. Exeter, New York.

Rybicki E.P., Hughes F.L. 1990. Detection and typing of *maize streak virus* and other distantly related geminiviruses of grasses by polymerase chain reaction of a conserved viral sequence. J. Gen. Virol. 71: 2519–2526.

Rybicki E.P., Dennis S., Napier G.Hughes F.L. 1998. Novel grass and wheat strains of *maize streak virus* detected by DNA amplification and sequencing. Mol. Plant Pathol. On-Line http//194.247.68.33/imppol/1988/0929rybycki.

Rybicki E.P., Briddon R.W., Brown J.E. Fauquet C.M., Maxwell D.P., Harrison B.D. Markham P.G., Stanley J. 2000. Virus taxonomy. p. 285–297 In: "Seventh Report of the International Committee on Taxonomy of Viruses" (C.M. Fauquet, D.H.L. Bishop, E.B. Carsten, M.K. Estees, S.M. Lemmon, M.A. Mayo, D.J. McGeoch, C.R. Pringle, R.B. Wickner, eds.). Academic Press, San Diego.

Schnippenkoetter W.H., Martin D., Hughes F., Fuvie M., Willment J., James D. von Wechmar B., Rybicki E.P. 2001. The relative infectivities and genomic characterization of three mastreviruses. Arch. Virol. 146: 1075–1088.

Sneath P.H., Sokal R.R. 1973. Numerical taxonomy: The Principle and Practice of Numerical Classification. W.H. Freeman & Co. San Franscisco, USA, 573 pp.

Soto P.E., Buddenhagen I.W., Asnani V.L. 1982. Development of streak virus-resistant maize populations through improved challenge and selection methods. Ann. Appl. Biol. 100: 539–546.

Thottappilly G., Bosque-Perez N.A., Rossel H.W. 1993. Viruses and virus diseases of maize in tropical Africa. Plant Pathol. 42: 494–509.

Willment J.A., Martin D.P., Rybicki E.P. 2001. Analysis of the diversity of African streak mastreviruses using PCR – generated PFLPs and partial sequence data. J. Virol. Methods 93: 75–87.

POLISH SUMMARY

BADANIA NAD ZRÓŻNICOWANIEM POMIĘDZY MASTERWIRUSAMI POWODUJĄCYMI OBJAWY CHOROBOWE NA ROŚLINACH Z RÓŻNYCH REGIONÓW NIGERII ZA POMOCĄ METOD PCR

W latach 1997–1999 dokonano przeglądu pól w pięciu strefach ekologicznych Nigerii w celu zebrania gatunków traw wykazujących objawy powodowane przez *Maize starek virus* (MSV, Rodzaj *Mastervirus*: Rodzina *Geminiviridae*). Oprócz kukurydzy (*Zea mays* L.) znaleziono 15 innych gatunków traw mających objawy podobne do zakażenia MSV. Żywiciele wykazywali dwa typy objawów, mianowicie średnie i poważne. W celu wykrycia masterwirusów użyto metod PCR (polymerase chain reaction) i IC–PCR (immunocapture polymerase chain reaction). Zróżnicowanie pomiędzy poszczególnymi izolatami ilustrują dendrogramy. Dendrogram PCR wykazuje 35–85% różnicę, natomiast dendrogram IC–PCR 55–85%. Dendrogram PCR zasadniczo grupuje izolaty pod względem nasilenia objawów. Izolaty, które były najmniej spokrewnione z podstawową linią MSV wykazywały średnie objawy na trawach żywicielskich. Nieskomplikowana procedura PCR może być pomocna w badaniach nad zróżnicowaniem masterwirusów w przypadkach, w których sekwencja genomu nie jest dostępna.