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Full Length Research Paper

Relatedness of *Maize streak virus* in maize (*Zea mays* L.) to some grass isolates collected from different regions in Nigeria

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***Maize streak virus* (MSV; family *Geminiviridae,* genus *Mastrevirus*) is the most important virus of maize (*Zea mays* L.) in sub-Saharan Africa. The relatedness of this virus to others showing streak symptoms from grasses on or near maize fields from five ecological areas of Nigeria was studied using genetic scanning analyzer. The relationship dendogram showed 50-95% variations as the 30 isolates were grouped into two main clusters at 0.50 coefficient of variation, five subgroups at 0.06 and 25 at 0.95 coefficient of variation, respectively. The dendogram suggests five family trees at 60% similarity. Split decomposition data showed three clusters implying three evolutionary trees among the streak isolates in Nigeria, as indicated by the three major groupings. The first cluster had four subgroups. MSV (IITA) is within the first tree, which also had 14 other grass isolates. The second tree comprised only three isolates, which were all transmissible to maize and produced typical or severe symptoms in their grass hosts. The third tree had 12 isolates, which were diverse from each other. Despite basic differences in the theoretical background of UPGMA cluster analysis and Split Decomposition, these two approaches of phylogeny reconstruction yielded similar results.**

Key words: Mastreviruses, maize streak virus (MSV), isolates, polymerase chain reaction (PCR), gene scan, relationship, dendogram, split decomposition.

INTRODUCTION

Maize streak virus (MSV; family *Geminiviridae,* genus *Mastrevirus*) is the most economically important virus of maize (*Zea mays* L.) in sub-Saharan Africa and causes serious yield reductions and threat to food security throughout Africa (Thottappilly et al., 1993). The symptoms caused by MSV are characterized by broken to almost continuous, narrow, chlorotic streaks centered on secondary and tertiary leaf veins (Fajemisin and Shoyinka, 1976). Streaks are distributed uniformly over the leaf surface. The parallel, chlorotic streaks may partially or almost completely fuse, leaving irregular green lines or islands centered between veinlets. In susceptible maize varieties, prominent, intermittent or continuous, white chlorotic spots and stripes may start developing within 4-5 days after infection (Soto et al., 1982). Streaking mostly develops as from the growing point at their base of the leaves. Chlorotic stripes develop into a more homogenous, yellowish white chlorosis over and along the veins on most of the leaf lamina. In highly susceptible genotypes, chlorotic streaks tend to coalesce and develop into almost uniform chlorosis (Efron et al., 1989). The latter may result in progressive necrosis and, eventually, die-off if infection occurred at early growing stages (Soto et al., 1982; Efron et al., 1989). MSV symptoms have been observed on many crops and weeds, all belonging to the Graminae (Soto et al., 1982;

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Efron et al., 1989; Bosque-Perez et al., 1998; Bosque-Perez and Buddenhagen, 1999). However, MSV is of economic importance only to maize.

Apart from MSV, there are six other African streak virus species within the *Mastrevirus* genus of the *Geminiviridae*: *Eragrostis streak virus* (ESV) (Shepherd et al., 2008); *Panicum streak virus* (PanSV) (Briddon et al., 1992); *Sugarcane streak virus* (SSV) (Hughes et al., 1992); *Sugarcane streak Egypt virus* (SSEV) (Brigarre et al., 1999); *Sugarcane streak Reunion virus* (SSREV) (Peterschmitt et al., 1991) and *Urochloa streak virus* (USV) (Oluwafemi et al., 2008). African streak virus species are transmitted in a persistent manner by several species of leafhoppers in the genus *Cicadulina* (Oluwafemi et al., 2007a) and geographically are restricted to Africa and its neighbouring islands. There are several strains of MSV. Out of the five distinct strains of MSV that have been described, only one (MSV-A) causes severe disease in maize. The other strains (MSV-B, -C, -D, and –E) are described as 'grass-adapted' (Varsani et al., 2008). In Nigeria, samples of grasses showing streak viruses have been collected, and transmission, serological and polymerase chain reaction (PCR) diagnostic studies have been reported (Mesfin et al., 1992; Oluwafemi et al., 2007b, 2008). Understanding the epidemiology of MSV, as it occurs in maize, requires knowledge of the natural host ranges and the relatedness of different strains and species of African streak viruses. This report attempts to show how streak viruses or strains of MSV, found in some grasses in Nigeria relate to a specific MSV isolate used in International Institute of Tropical Agriculture (IITA, Ibadan, Nigeria). This IITA MSV is a MSV-A strain used for resistance screening of maize germplasm.

MATERIALS AND METHODS

Field survey

Field surveys were undertaken across northern, southern, eastern and western parts of Nigeria between 1997-1999 and 42 suspected MSV leaf samples were collected from grass weeds within and around maize fields. Out of 42 leaf samples collected during the surveys, 30 MSV isolates were obtained (Table 1).

Genetic analysis

DNA extraction and polymerase chain reaction (PCR) were carried out on 30 MSV isolates as described previously by Oluwafemi et al. (2008). PCR amplification of 5 µl aliquot DNA of the MSV isolates was carried out with a primer-pair (Forward: TTG GVC CVM VGA TGT ASA G; Reverse: CCA AAD NKC ASC TCC TCC G, where D=A/G/T; N=A/C/G/T; K=G/T; S=C/G; V=A/C/G; M=A/C) (supplied by Dr. S. Winter, Plant Virus Division, DSMZ, Germany). The reaction mixture per PCR tube was the same as the standard PCR except the addition of 0.5 µl tamra (F) dNTPs label mix (PE, 1997). 2 µl of each PCR product plus 24 µl deionized formamide were placed into 0.5 ml Eppendorf tube. The samples were placed in a thermal cycler (PCR machine) for 5 min at 95°C. The tubes were then immediately placed in an ice-water bath and then placed into the sample tray. The sample tray was then placed on the auto-sampler and then subjected to gene scanning using an ABI PRISM® 310 genetic analyzer (PE, 1997).

Statistical analysis

The genetic data of 30 MSV isolates were obtained by ABI PRISM® 310 genetic analyzer and then translated into matrix data (1 for presence of peak, 0 for absence of peak at a position). Pairwise distance matrices were then computed by NTSYS-pc 2.0 software package (Rohlf, 1993) using the Jaccard coefficient of similarity (Jaccard, 1908). Dendograms were created by UPGMA cluster analysis (Sneath and Sokal, 1973). Binary character matrices were also analysed by split decomposition (Bandelt and Dress, 1992a, 1992b), a distance method that was applied in the study of the evolution of the foot-and-mouth virus disease based on capsid protein gene sequences (Dopazo et al., 1993).

RESULTS

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 have been documented (Table 1). At 0.50 coefficient of variation, the dendogram grouped the 30 isolates into two main clusters (GrpA and GrpB) while at 0.6 coefficient the dendogram grouped the 30 isolates into five subgroups (GrpA1, GrpA2, GrpA3, GrpB1 and GrpB2) (Figure 1). The GrpA1 group consisted of MSV (IITA) and eight other isolates. The GrpA2 group had three isolates (*Axonopus, Setaria* and *Digitaria*). The GrpA3 group had 11 isolates, the GrpB1 had only three isolates while the GrpB2 group had four isolates. At 0.95 coefficient of variation, the dendogram grouped the 30 isolates into 25 separate entities. Twenty-three isolates were identified as distinct from each other. MSV isolates in *Axonopus compressus* (IITA) and *Digitaria horizontalis* (Ikenne), both with mild streak symptoms, were grouped together as one entity. Five other isolates with mild symptoms from 3 locations were also grouped together as one entity. These were isolates in *Brachiaria deflexa* (Moor Plantation, Ibadan), *Panicum maximum* (Ife), *Rottboellia cochinchinensis* (Ife), *Paspalum conjugatum* (IITA) and *P. notatum* (IITA). The dendogram show 50-95% variations among the isolates.

According to split decomposition analysis, all the 30 isolates were grouped into two main clusters (GrpA and GrpB) and six subgroups (GrpA1, GrpA2, GrpA3, GrpA4, GrpB1 and GrpB2) (Figure 2). GrpA1 group consisted of MSV (IITA) and four other isolates. GrpA2 group had three isolates (*Axonopus, Setaria* and *Digitaria*). GrpA3 group had only 3 isolates while GrpA4 had four isolates. GrpB1 and GrpB2 had 4 and 11 isolates, respectively.

DISCUSSION

Split decomposition decomposes any dissimilarity matrix

Table 1. 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.

S/N	Grass species	Location	Symptoms	Isolate
1	*Andropogon gayanus* Kunth	Kaduna	Mild	Andropo
2		Mokwa	Mild	
3	*Axonopus compressus* (Sw.) P. Beauv.	IITA, Ibadan	Severe	Axo.se
4		IITA, Ibadan	Mild	Axo.mild
5		Ilesha		
6	*Brachiaria deflexa* (Sch.) C.E. Hubbarb ex Robyns	Ikenne	Mild	
7		Moor Plantation, Ibadan	Mild	B.de.MP
8		Catholic Redemptorist Camp, Ibadan	Mild	B.de.CR
9	*B. distichophylla* (Trn.) Stapf	Ikenne, Mokwa	Mild/ severe	
10		IITA, Ibadan	Severe	B.di.se
11	*B. lata* (Schumach) C.E. Hubbarb	Ikenne	Mild/ severe	
12		Mokwa	Severe	B.la.MK
13		IITA, Ibadan	Severe	B.la.se
14		IITA, Ibadan	Mild/ mottle	B.la.mm
15	*Dactyloctenium aegyptium* (Linn.) P. Beauv.	IITA, Ibadan	Severe	Dactylo
16	*Digitaria horizontalis* Willd	Kaduna, Zaria	Mild/ severe	
17		Ikenne	Mild	Dig.Ik
18		Mokwa	Severe	Dig.MK
19		Zaria	Severe	Dig.Zar
20		Jos	Severe	Dig.Jos
21		IITA, Ibadan	Severe	Dig.IITA
22	*Eleusine indica* Gaertn	IITA, Ibadan		
23		Mokwa	Mild	Eleu.MK
24	*Panicum maximum* Jacq	Ikenne, Odeda, Ikire, Ilesha, Mokwa, Kaduna, Zaria, Kadawa & Jos	Mild	
25		IITA, Ibadan	Mild	Pan.IITA
26		Ife	Mild	Pan.Ife
27	*Paspalum conjugatum* Berg.	IITA, Ibadan	Mild	Pasp.c.
28	*Paspalum notatum* Flüggé	IITA, Ibadan	Mild	Pasp.n.
29	*Paspalum scrobiculatum* L.	IITA, Ibadan	Mild	Pasp.s.
30	*Rottboellia cochinchinensis* (Lour.) Clayton	IITA, Ibadan	Mild	
31		Ife	Mild	Rot.Ife
32		Zaria	Mild	Rot.Zar
33		Jos	Mild	Rot.Jos
34	*Rhynchelitrum repens* (Wild.) C.E. Hubbarb	Jos	Mild	Rhy.Jos
35	*Setaria barbata* (Lam.) Kunth	Kaduna, zaria	Mild/severe	
36		IITA, Ibadan	severe	Setaria
37	*Thelepogon elegans* Roth ex Toem & Schult	Jos	mild/ mottle	
38		Kadawa	mild/ mottle	Thel.mm
39		Kadawa	severe	Thel.se
40	Zea mays *L.*	Ikenne, Odeda, Ikire, Ife, Ilesha, Mokwa, Zaria, Kadawa & Jos		
41		IITA, Ibadan	severe	MSV.IITA
42		Kaduna	severe	MSV.Kd

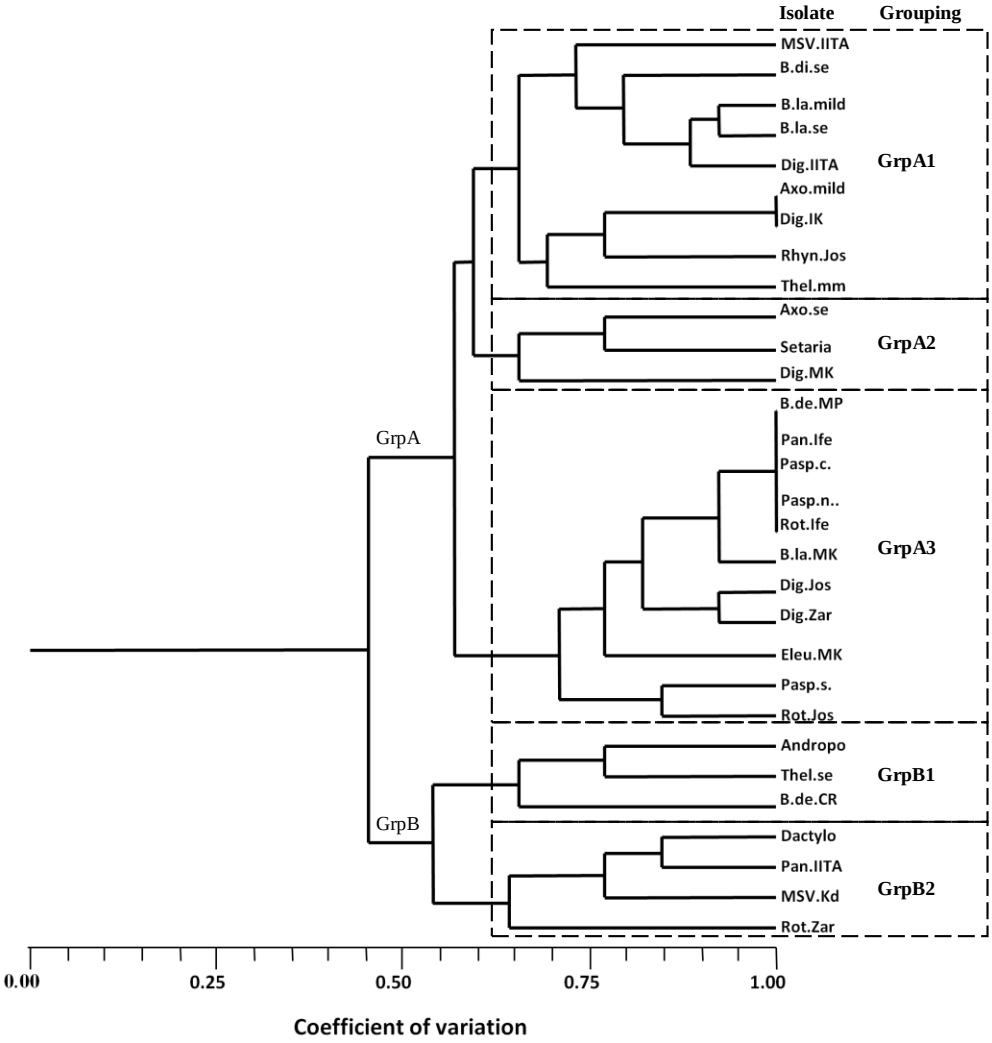

Figure 1. Dendogram showing the variation among 30 isolates of streak virus in comparison to the severe IITA Maize streak virus (MSV) being used to screen maize germplasm for streak resistance.

into a number of binary factors, described as weighted splits, plus a residual term that represents the unresolvable "noise" in the data. Besides, the method permits conflicting alternative groupings and therefore detects some of those distinctive minor features in distance data that are dominated by others and, hence, not necessarily supported by estimated trees. In contrast with UPGMA, which generates some kind of "tree" from any distance data matrix, split decomposition indicates whether true tree-like structures are present or not in the data set (Ramser et al., 1996).

Despite basic differences in the theoretical background of UPGMA cluster analysis and Split Decomposition, these two approaches of phylogeny reconstruction yielded similar results. The dendogram data presented showed that the 30 isolates studied were dissimilar as 23 out of 30 isolates were resolved as distinct entities. Two isolates (*A. compressus* from IITA and *D. horizontalis* from Ikenne) that produced mild symptoms were 100% similar. Another group of isolates that carried mild symptoms were also grouped together as 100% similar. These were *B. deflexa* (Moor Plantation), *P. maximum* (Ife), *P. conjugatum* (IITA), *P. notatum* (IITA) and *R. cochinchinensis* (Ife). The locations were these five isolates were collected were within 70 km apart within the same agroecological zone. This may indicate that these grass species were carrying the same virus strain. This can also be said about the distance between *A. compressus* from IITA and *D. horizontalis* from Ikenne. Although Ikenne is in Humid Forest as compared to IITA in Derived Savannah, the distance between the two locations were about 70 km.

Split decomposition tree helps to better understand evolutionary trend among the isolates. There were two

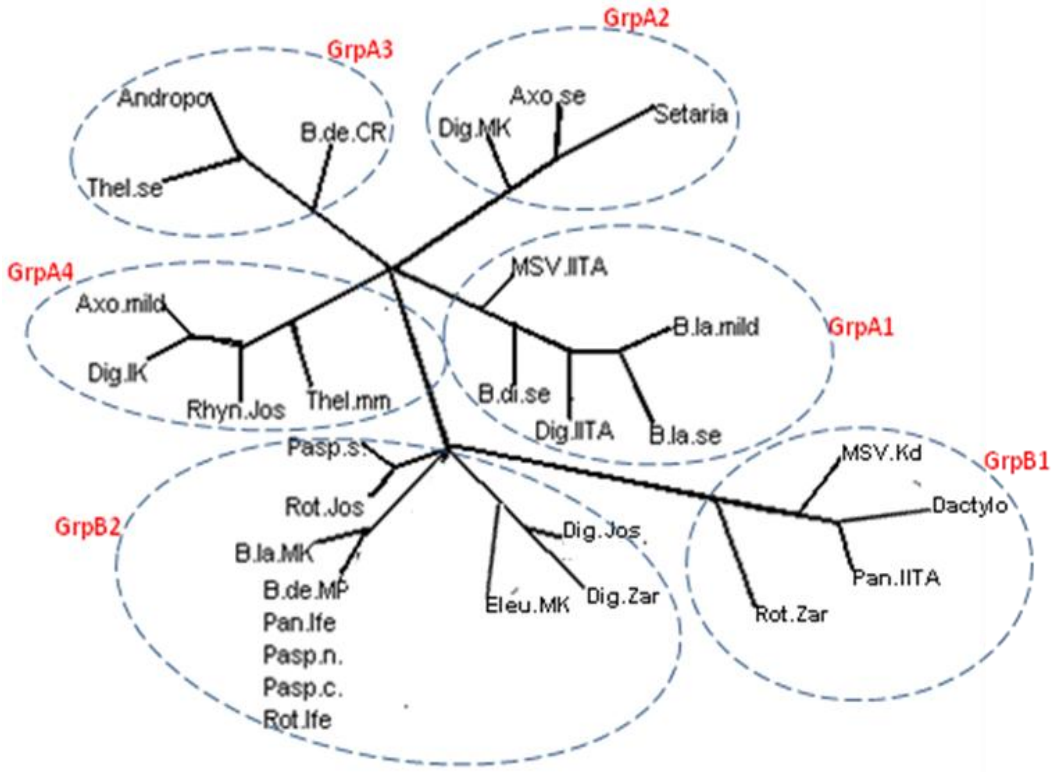

Figure 2. Relatedness of 30 *Maize* streak virus isolates as determined by split decomposition analysis.

evolutionary trees among the streak isolates in Nigeria, as indicated by the two major groupings. Wide geographical and agroecological distance between Ibadan and Kaduna may account for the variation between MSV (IITA) and MSV (Kaduna) as this result indicate that they are different strains of MSV. This third tree (GrpA3) in split decomposition analysis may indicate distinct viruses. The virus causing streak disease in *P. maxmum* has been described as *Panicum streak virus* (PSV), genus *Mastrevirus*, a distinct virus separate from MSV (Briddon et al., 1992). The streak virus from *R. cochinchinensis* from Nigeria is thought to be an RNA virus (Dr. Stephan Winter, personal communication). Further work is needed on the virus causing streak disease in *R. cochinchinensis* in order to ascertain its epidemiological and evolutionary position relative to MSV. Mesfin et al. (1992) reported a virus isolate in *D. aegyptium* which was transmissible to maize, but could not be transmitted back to its original host. Isolates in *Paspalum* spp. were not easily transmissible to maize, and when they were, produced mild symptoms similar to those produced by *P. maximum*.

MSV (IITA) was centrally positioned in the split decomposition tree. This is understandable because the initial virus sources were collected from widely scattered areas of Nigeria at the onset of IITA breeding program for MSV resistance (Soto et al., 1982; Efron et al., 1989). Our work showed a rapid way of comparing these isolates where facilities for full genomic sequencing are not available.

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