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Short communication

A streak disease of pearl millet caused by a leafhopper-transmitted geminivirus

Rob W. Briddon, Patricia Lunness, Ian D. Bedford, Leony C. L. Chamberlin, Theo Mesfin and Peter G. Markham

Department of Virus Research, John Innes Institute, John Innes Centre, Colney Lane, Norwich, NR4 7UH, UK (Fax: 603 456844)

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Abstract

The cause of a streak disease of pearl millet (*Pennisetum glaucum*), originating from Nigeria, has been attributed to a geminivirus belonging to the 'African streak virus' cluster. A full-length, infectious clone of the virus was obtained which was transmissible by the vector *Cicadulina mbila* (Naudé). Analysis of the complete nucleotide sequence of the coat protein gene of this virus shows it to be most closely related to sugarcane streak virus. The possible evolutionary implications of this finding are discussed.

Early studies of streak diseases in Africa identified three distinct virus types, which were called the maize, *Panicum* and sugarcane strains of maize streak virus [Bock et al., 1974]. This division was later confirmed by serological analyses [Pinner et al., 1988; Pinner and Markham, 1990; Peterschmitt et al., 1991]. Subsequent sequence analysis of full-length clones led to the conclusion that the three viruses are related but distinct [Hughes et al., 1991; Briddon et al., 1992]. The viruses are now termed maize streak virus (MSV), sugarcane streak virus (SSV) and *Panicum* streak virus (PanSV).

The three streak viruses belong to the genus subgroup I of the Geminiviridae [Briddon and Markham, 1995]. Geminiviruses have a typical twinned particle morphology and a genome consisting of single-stranded, circular DNA. In common with other subgroup I geminiviruses, the streak viruses have a genome consisting of a single circle of DNA (approximately 2700 bases in length) with four open reading frames which are transcribed in a bidirectional manner [Morris-Krsinich et al., 1985]. Transmission of geminiviruses is mediated by insect vectors; in the case of African streak viruses by species of *Cicadulina* leafhoppers, of which the most efficient vector is *Cicadulina mbila* (Naudé) [Rose, 1978; Webb, 1987].

Economically the major pathogen amongst the streak viruses is MSV [Damsteegt, 1983]. The virus causes up to 100% yield losses in maize and other cereal crops [Thottappilly, 1992]. In contrast, PanSV is of no significance to commercially grown crops, being essentially limited to native grass species. When transmitted to susceptible maize varieties only mild symptoms ensue [Briddon et al., 1992]. SSV was a major constraint to the cultivation of sugarcane until the introduction of resistant cultivars. This virus now only occurs in experimental plots where susceptible sugarcane is grown [Bock and Bailey, 1989].

Pearl millet (*Pennisetum glaucum*) plants with faint yellow pin-point lesions, characteristic of very mild infections by streak geminiviruses, were obtained from Nigeria. The virus proved transmissible by *C. mbila* to both *P. glaucum* and *Zea mays* (cv. Golden Bantam). Viral double stranded, super-coiled DNA (scDNA) was extracted from infected *Z. mays* leaf tissue and purified on caesium chloride/ethidium bromide gradients as described previously for PanSV [Briddon et al., 1992]. Single cutting restriction endonuclease sites were determined by digesting the purified viral scDNA with a range of restriction endonucleases followed by its analysis on ethidium bromide stained agarose gels.

Subsequently an aliquot of scDNA was digested with the restriction enzyme *Bam*HI, which was identified as a single cutting site, and ligated into *Bam*HI linearised M13mp18 [Yanisch-Perron et al., 1985]. A single clone (pMil1.0), containing an approximately 2800bp insert, was selected for further analysis (results not shown).

Infectivity of the cloned viral genome was investigated by *Agrobacterium*-mediated inoculation [Grimsley et al., 1987]. An approximately 2000bp *Bam*HI-*Sst*I fragment of pMil1.0 was cloned into the binary vector pBin19 [Bevan, 1984] to produce pMil0.7. The full-length *Bam*HI insert of pMil1.0 was then ligated into the unique BamHI site of pMil0.7 to produced pMil1.7, containing a partial repeat of the viral genome. This pBin19 construct was then transferred by bacterial conjugation into *A. tumefaciens* strain $C58^{nal}$ [Hepburn et al. 1985] essentially as described by Ditta et al. [1989]. *Z. mays* and *P. glaucum* seedlings were inoculated with *Agrobacterium* suspensions as described previously [Boulton et al., 1989]. Infectivity of the cloned virus by *Agrobacterium*-mediated inoculation to *Z. mays* was 33% (average of three experiments involving 35 seedlings per experiment). Symptoms of the virus in *Z. mays* (Figure 1) typically appeared 10 days following inoculation and were indistinguishable from the wild type isolate insect-transmitted to *Z. mays*. We were unable to introduce the virus into *P. glaucum* by agroinoculation, although the virus in agroinoculated *Z. mays* plants could be transmitted by *C. mbila* to *P. glaucum*. Transmission efficiency of the cloned virus from agroinoculated *Z. mays* to both *Z. mays* and *P. glaucum* was typically 3% (average of 3 experiments involving 35 seedlings and one insect per plant given 48 h acquisition access periods and 72 h inoculation access periods). Low transmission efficiencies are a common feature for many grass infecting streak viruses such as PanSV [Briddon et al., 1992]. This may be attributable to low virus concentrations available to feeding insect vectors.

The complete nucleotide sequence of the coat protein gene of the cloned virus was determined by standard dideoxynucleotide chain termination sequencing [Sanger et al., 1977]. This sequence is available in the EMBL and DDJB sequence databases under accession number X86705. The nucleotide and predicted amino acid sequence similarity of the coat protein gene of the virus isolated from millet to the homologous regions of MSV [Nigerian isolate; Mullineaux et al., 1984] PanSV [Briddon et al., 1992] and SSV [Hughes et al., 1993] are shown in Table 1.

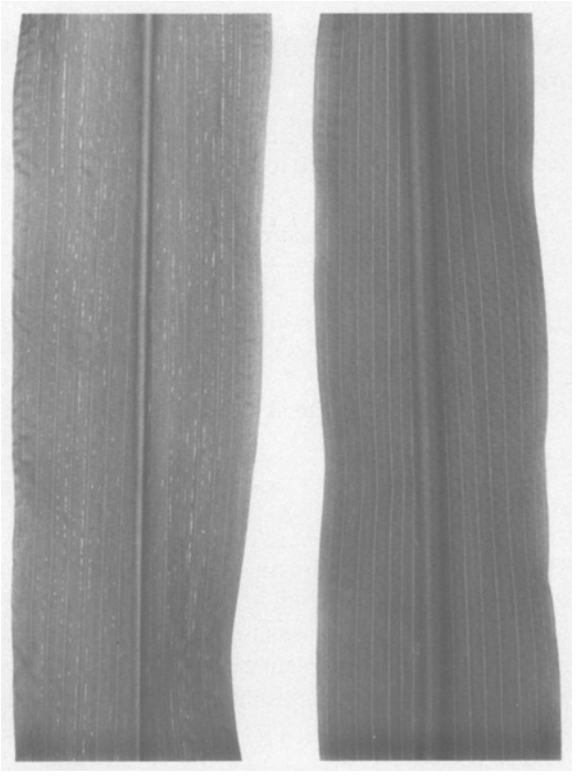

Figure 1. Symptoms of SSV-Mil infection on *Z. mays* following agroinoculation (left) compared to a healthy leaf (right).

The sequence obtained from the millet virus shows the highest levels of similarity to that of SSV. Phylogenetic analysis of the sequence (Figure 2) shows the millet virus to be most closely related to the published sequence of SSV, although the mutation distance between these viruses is considerably larger than between isolates of MSV.

Recently we have shown that isolates of MSV, originating from geographically distant regions, show very low variability (a maximum of 10.9% and 2.0% at the nucleotide and amino acid levels respectively for the coat protein gene [Briddon et al., 1994]). The variation between SSV and the virus originating from millet is considerably higher (20.4% and 10.9% respectively) and is reflected in the higher mutation distance between them than between the MSV isolates (Figure 2). A similar high mutation distance between SSV strains originating from South Africa and Mauritius, based upon limited sequence of the complementary-sense genes, has led Rybicki and Hughes [1990] to suggest

Table 1. Pairwise percentage nucleotide sequence identities between the coat protein gene sequences of streak viruses

	SSV-Mil	SSV	PanSV
SSV	79.6 (89.1)		
PanSV	66.4 (80.8)	67.6 (82.4)	
MSV	65.8 (76.2)	63.7 (79.1)	64.7 (79.0)

Percentage amino acid sequence similarities between the predicted sequences of the coat proteins are shown in brackets.

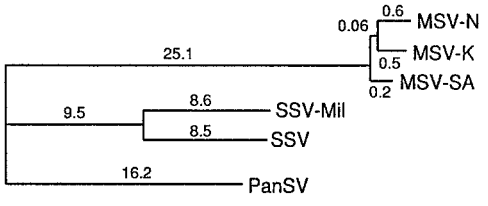

Figure 2. Unrooted phylogenetic tree based on alignments of the predicted amino acid sequences of the coat proteins of PanSV, SSV and maize streak virus isolates originating from Nigeria [MSV-N; Mullineaux et al., 1984], Kenya (MSV-K; Howell, 1984) and South Africa [Lazarowitz, 1988]. The shortest tree (tree with the smallest sum of squares), calculated using FITCH in the PHYLIP (version 3.5) library of programs (J. Felsenstein, Department of Genetics, University of Seattle, Washington, USA), is shown. Vertical distances are arbitrary. Horizontal distances are proportional to mutation distances as indicated by numbers. The sum of squares and average percent standard deviation were 0.00052 and 0.432% respectively. A total of 2433 trees were examined.

that these may be considered distinct viruses rather than strains of a single viral species. At present, a nucleotide sequence identity of 90% has been suggested as the criterion for distinguishing distinct viruses from strains of a single virus species for geminiviruses [Padidam et al., 1995]. Two recent reviews on the phylogeny of geminiviruses have shown that comparisons of the coat protein sequences of geminiviruses provides an accurate estimation of the overall phylogenetic relationships [Rybicki 1994; Padidam et al., 1995]. Thus the millet virus may possibly be considered a distinct virus within the African streak virus cluster. However, due to the close relationship with SSV, we propose to provisionally designate this virus the millet strain of SSV (SSV-Mil) whilst awaiting more detailed characterisation of the virus. The low levels of sequence variability shown for the coat protein gene of MSV are not typical of the other streak viruses. Both PanSV isolates (R.W. Briddon, unpublished results) and SSV isolates [Rybicki and Hughes, 1990] appear to have far greater sequence variation than that shown for MSV, which suggest that the constraints on variation are greater for MSV than for the other streak viruses.

Both maize and sugarcane are crops which have been introduced to Africa. Since neither species is infected by geminiviruses in their native America, it would seem reasonable to assume that both maize streak and sugarcane streak viruses originated in native grasses and adapted to maize and sugarcane upon their introduction. The sequence similarity between SSV and the virus isolated from millet may indicate that grasses harboured viruses that evolved to occupy the niche created by the introduction of sugarcane to Africa and the agricultural selection of millet.

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