Plant Viruses ©2007 Global Science Books

Banana Streak Virus: a Highly Diverse Plant Pararetrovirus

Tony Remans[1,3] • Shazia Iram[1] • Louise Shuey[1] • Yasmina Jaufeerally-Fakim[2] • Peer M. Schenk[1*]

[1] School of Integrative Biology, The University of Queensland, St. Lucia, Queensland 4072, Australia
[2] Biotechnology Unit, Faculty of Agriculture, University of Mauritius, Reduit, Mauritius
[3] Current address: Environmental Sciences, Hasselt University, Agoralaan Building D, 3590 Diepenbeek, Belgium

Corresponding author: * **p.schenk@uq.edu.au**

ABSTRACT

Plants are attacked by two major groups of DNA viruses: the single-stranded DNA viruses *Geminiviridae*; and the double-stranded DNA viruses which belong to *Caulimoviridae* (including the genera *Caulimovirus* and *Badnavirus*). Badnaviruses are frequently found in banana, rice, cocao, sugarcane and other plant species. Banana streak virus (BSV) is a badnavirus which typically is found integrated in the B genome of *Musa*. The integrated form is able to remain dormant for several years before viral replication starts and symptoms appear. The virus is known to be transmitted by at least three mealy bug species but it mainly spreads through vegetative propagation. *Banana streak Mysore virus* (BSMyV), *Banana streak GF virus* (BSGFV), and *Banana streak OL virus* (BSOLV) are the three major BSV species identified so far. BSV eradication from infected banana plants is very rare due to genome integration and clonal propagation practices. Interestingly, cryopreservation and use of selective inhibitors of *Hepatitis B virus* replication are currently the only two strategies which have been successfully used for this purpose.

Keywords: badnavirus, BSV, genome integration, sugarcane bacilliform virus

CONTENTS

DISEASE SYMPTOMS AND FIRST ISOLATES

Banana streak virus (BSV) is a plant pararetrovirus that infects banana and causes chlorotic and necrotic streak symptoms on leaves and pseudostem (Lockhart 1986). Affected plants may be stunted and the fruit may be distorted, have a thinner peel and occur in smaller bunches (Lassoudière 1974). Banana streak disease symptoms were first reported from the Ivory Coast in 1968 and the causal virus was first isolated and characterised from banana in Southern Morocco (Lockhart 1986). BSV symptoms have since been observed worldwide in almost every producing area of banana and plantain (Dallot *et al.* 2001). The virus is transmitted through vegetatively propagated plant material and semi-persistently by mealy bugs, but not mechanically or through soil (Jones and Lockhart 1993). There are at least three mealy bug species found to be associated with the transmission of this virus and among these *Planococcus citri* is the most prevalent. Symptomatic leaves appear sporadically during the year and may be followed by new leaves expressing few or no symptoms (Daniells *et al.* 2001; Kubiriba *et al.* 2001; Fargette *et al*. 2006).

BANANA STREAK VIRUS IS A PLANT PARARETROVIRUS OF THE GENUS *BADNAVIRUS*

Two major groups of plant pararetroviruses in the family *Caulimoviridae* are the genus *Caulimovirus* (icosahedral shaped viruses including for example the *Cauliflower mosaic virus*, CaMV) and the genus *Badnavirus* (containing bacilliform shaped DNA viruses). Other distinct plant pararetroviruses include *Cassava vein mosaic virus* (CsVMV) and *Petunia vein clearing virus* (PVCV), which differ from both groups and from each other in genome organisation (Hohn and Fütterer 1997; Richert-Pöggeler and Shepherd 1997). BSV is a plant pararetrovirus belonging to the group of the badnaviruses (Lockhart 1986).

GENOME AND REPRODUCTION CHARACTERISTICS OF PLANT PARARETROVIRUSES

Plant pararetroviruses are non-enveloped viruses, containning a circular, non-covalently closed double-stranded DNA genome of about 7 to 8 kb in length. All described badnaviruses have three long open reading frames (ORFs) that are positioned exclusively on one strand. These ORFs

Received: 19 December, 2006. Accepted: 22 January, 2007.

Invited Mini-Review

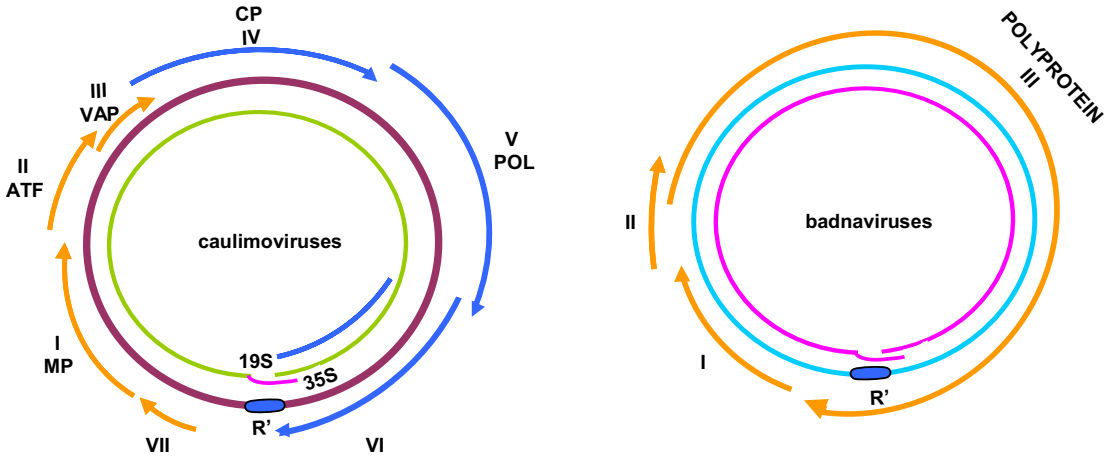

Fig. 1 Schematic representation of genomic maps and transcripts of the two major groups of plant pararetroviruses, the caulimoviruses (left) and the badnaviruses (right). The viral genome consisting of double-stranded DNA is represented by the full circle in the middle, with the box marked R' indicating the region of the genome that is transcribed twice in the terminally redundant transcript. The thick arrows outside represent the major viral open reading frames (ORFs). MP (movement protein), ATF (aphid transmission factor), VAP (virion associated protein), CP (capsid protein), POL (protease, reverse transciptase, RNAse H).

are separated by a large intergenic region, which contains a transcription start site and a poly-adenylation signal that are positioned such that they allow transcription of the full-length genome into a terminally redundant pregenomic RNA (pgRNA) (**Fig. 1**). This pgRNA serves as template for replication *via* reverse transcription and also as polycistronic mRNA for translation into viral proteins. The synthesis of the minus strand during replication is primed by $tRNA^{Met}$ (Rothnie *et al.* 1994). In contrast to caulimoviruses, they may also have an additional promoter outside the intergenic region for transcription of shorter transcripts (see **Fig. 1**: 19S transcript). Badnaviruses have only one transcriptional initiation site, which is present in the intergenic region between the end of ORF III and the beginning of ORF I (Pooggin *et al*. 1999). The functions of ORFs I (encoding 20.8 kDa protein) and II (encoding 14.5 kDa protein) are not well understood, although evidence exists that the ORF II protein participates in assembly of the virus particle (Herzog *et al*. 2000) and could function as an aphid transmission factor (Stavalone *et al*. 2001). ORF III encodes a polyprotein of 208 kDa with movement protein, coat protein, aspartic protease, reverse transcriptase and ribonuclease H (RNase H) functions (Hohn and Fütterer 1997). This polyprotein is thought to be post-translationally cleaved into functional units by aspartic protease activity. The 14.5 kDa protein contains a coiled-coil domain, which would allow the protein to self interact to form a tetramer, and a short, basic domain in the C-terminus, providing non-specific ssRNA and dsDNA binding properties (Geering *et al.* 2005).

The 5′ end of the pgRNA (transcription start site) has been mapped for some pararetroviruses and is located 31 to 34 nucleotides downstream of a TATA-box (Pooggin *et al.* 1999 and references therein; Schenk *et al.* 2001; Remans *et al.* 2005). In all these viruses, the pgRNA promoter is located close to the left border of the large intergenic region and overlaps with the end of the coding region at its 5′ end. Consequently, most of the intergenic region of the *Caulimoviridae* is transcribed into a large pgRNA leader of 350-800 nt long that contains several short ORFs (Hohn *et al.* 2001). There is no conventional AUG start codon in the beginning of ORF I. The analyses of primary and secondary structures of pgRNA leader sequence preceding ORF I help to identify the start of this ORF. The pgRNA leader contains several short ORFs which terminate upstream of the stable hairpins. The strong secondary structure of the pgRNA leader is suggestive of a ribosome shunt mechanism of translation, as proposed for other members of *Caulimoviridae* (Pooggin *et al.* 1999). According to the shunt model of translation initiation, sORF I is reached by the scanning ribosome and is translated. Following termination of sORF I translation, the ribosome shunts over the secondary structure, bypassing the remaining sORFs and a putative encapsidation signal on the top of the hairpin, and resumes linear scanning at the shunt landing site. Translation initiation of ORFs II and III in the BSV genome probably occurs by leaky scanning of the ribosome. A large region of the BSMysV genome between the pgRNA leader and ORF III was found to have no AUG codons, except for the ORF II AUG start codon. This lack of AUG codon ensures that the shunting ribosomes reached ORF III by leaky scanning (Geering *et al.* 2005). For an overview of translation reinitiation and leaky scanning mechanisms in plant viruses see Ryabova *et al.* (2006).

SEQUENCE SIMILARITY BETWEEN BSV ISOLATES AND OTHER BADNAVIRUSES

So far, several BSV isolates have been identified. Lockhart *et al.* (1993) described four distinct isolates of BSV, which showed significant sequence divergence. The 7.4 kb genome of one isolate of BSV from Nigeria (BSV-Onne) has been completely sequenced (Harper and Hull 1998). Studies on BSV in Uganda also identified very wide diversity of this pathogen (Harper *et al*. 2004, 2005). Four Australian isolates from the banana cultivars 'Williams' (Cavendish), 'Mysore', 'Goldfinger' and 'Red Dacca' were designated BSV-Cav, BSV-Mys, BSV-GF and BSV-RD, respectively (Geering *et al.* 2000). For these BSV isolates, 1.3 kb (for BSV-Cav, BSV-GF and BSV-RD) and 2.1 kb (for BSV-Mys) of the genome comprising the 5′ border of the intergenic region and the 3′ end of ORF III (including the RNase H region and, for BSV-Mys only, both the RNase H and the reverse transcriptase region) were sequenced. The sequence obtained from BSV-RD was shown to be virtually identical to that of the Nigerian BSV-Onne, whereas the other isolates from cultivars 'Cavendish', 'Mysore' and 'Goldfinger' were divergent in nucleotide sequence with 68.39 to 77.52% sequence similarity in the conserved RNase H domain (Geering *et al.* 2000; Geering, pers. comm.).

Based on amino acid homology in the conserved region of the RNase H domain of the ORF III polyprotein, the four Australian BSV isolates are more closely related to each other than they are to any other badnavirus (**Table 1**; Geering *et al*. 2000). The next closest relative to the Australian BSV isolates is CoYMV (*Commelina yellow mottle virus*). BSV-Cav is most closely related to BSV-Onne, having 95.1% amino acid sequence identity in the conserved RNase H region. The other BSV isolates were less similar to each other, with amino acid sequence identities ranging

Table 1 Amino acid sequence identity of pairwise combinations of badnaviruses for the RNaseH domain of the open reading frame (ORF) III polyprotein. *Banana streak virus* isolates are BSV-Onne, BSV-Cav, BSV-Mys and BSV-GF; other viruses are *Commelina yellow mottle virus* (ComYMV), *cacao swollen shoot virus* (CSSV), *dioscorea bacilliform virus* sequence variants 'a' (DBVa) and 'b' (DBVb) and *sugarcane bacilliform virus* (ScBV). [adapted from Geering *et al.* 2000].

	BSV- Onne	BSV- Cav	BSV- Mys	BSV- GF	CoYMV	CSSV	DBV[a]	DBV[b]	ScBV
BSV-Onne	100	95.1	78.2	73.8	69.7	68.0	70.5	68.9	59.0
BSV-Cav		100	74.8	76.2	71.3	68.9	71.3	69.7	57.4
BSV-Mys			100	66.4	66.4	60.5	65.6	64.7	51.3
BSV-GF				100	68.0	65.6	61.5	60.7	56.6
CoYMV					100	68.9	72.1	71.3	57.4
CSSV						100	69.7	68.0	61.5
DBV[a]							100	98.4	59.0
DBV[b]								100	58.2
ScBV									100

from 66.4 to 78.2% (**Table 1**). This suggests that these BSV viruses may have sufficiently diverged to be recognised as distinct virus species, as their similarity is not much greater than the similarity between distinct species of the genus *Badnavirus* such as CoYMV and DBV (*Dioscorea bacilliform virus*) (Geering *et al*. 2000).

According to the decision by the *Caulimoviridae* study group of the International Committee on Taxonomy of Viruses (ICTV), the BSV sequences having less than 80% similarity in nucleotide sequence in the polymerase (RT + RNAse H) region will be considered as different badnavirus species. Based on this criteria *Banana Streak Mysore Virus* (*BSMyV*), *Banana Streak GF Virus* (*BSGFV*) and *Banana Streak OL Virus* (*BSOLV*) (named after their host genotypes 'Mysore', 'Goldfinger' and 'Obino l'Ewai', respectively) are the three BSV species so far identified in the *Badnavirus* genus (Fargette *et al*. 2006).

BSV SEQUENCES HAVE BEEN FOUND TO BE INTEGRATED IN THE BANANA GENOME

Interestingly, several plant pararetroviruses have been found to be integrated into their host genomes (for a review see Staginnus and Richert-Poggeler 2006). Although plant pararetroviruses do not employ integration during the infection cycle, a complete sequence identical to that of BSV-Onne was found in the chromosomal DNA of some *Musa* genotypes (Harper *et al.* 1999; Ndowora *et al.* 1999). A sequence homology of 99% was shared between the BSV sequences integrated in Obino L'Ewai and the genomic DNA of episomal virus which was isolated from the *in vitro*-propagated progeny of the same plant. This is contrary to the high degree of genomic heterogeneity which is a characteristic of BSV isolates occurring in nature (Lockhart and Olszewski 1993). This integrated form of BSV is associated with the "B-genome" of banana and plantains (Geering *et al.* 1999) and a related, but incomplete integrant that is closely related to CSSV is associated with the "A-genome" (Ndowora *et al.* 1999). Banana cultivars (*Musa* spp.) originate from two wild progenitor species: *Musa acuminata* and *Musa balbisiana*. A haploid genome of these diploid wild species is represented by the letters A and B, respectively. Most domesticated cultivars have triploid genotypes such as AAA, AAB or ABB, although diploid and tetraploid cultivars exist as well.

The multiple copies of viral sequence that are integrated into the genome of banana are arranged in tandem, but are interspersed with a long "scrambled" region that consists of highly rearranged BSV sequences (Harper *et al.* 2002). The viral sequence that is integrated into the genome of cv. 'Obino l'Ewai' (*Musa* AAB Group) represents the complete genome of BSV-Onne, but it has 6091-bp of that scrambled non-contiguous viral sequence (Harper *et al.* 1999; Ndowora *et al.* 1999). BSV integrates into the plant genome at two loci. It has been observed through *in situ* hybridisation to stretched DNA fibres that one locus is approximately 150

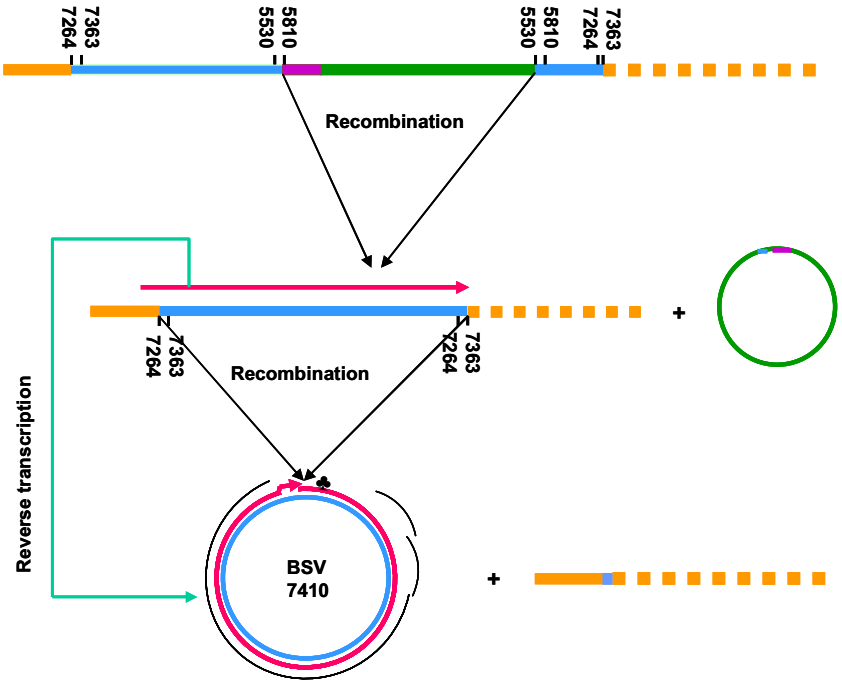

Fig. 2 The models for the generation of an infectious BSV genome by excision of the integrated sequences. Blue and green correspond to BSV sequence in the same and in opposite orientations relative to the transcript, respectively. Orange sequences have no homology to any other sequence. Orange dotted lines are uncharacterised sequences. The purple region contains small segments of BSV and other sequences. The first step in the excision process involves a homologous recombination between a 280 bp direct duplication flanking the central region. This produces a contiguous BSV genome with terminal direct repeats of 98 bp and a circular product containing the central region. For the following step there are two possible ways of yielding an infective BSV genome. The first mechanism involves a second homologous recombination via the 98 bp direct repeats to produce a circular BSV genome that then produces the BSV transcript (pink circular arrow around the blue circular genome), which is then translated to produce viral proteins (black arcs indicate the 3 ORFs) and that also serves as a template for replication of the viral genome *via* reverse transcription. The clover-leaf indicates the annealing site of the tRNAMet that primes first-strand DNA synthesis; homologous recombination to produce an RNA that can be translated and act as a template for reverse transcription in virus replication [modified from Ndowora *et al.* 1999].

kb long while the second locus has a smaller insertion of about 50 kb (Harper *et al* 2002). Two homologous recombination events could theoretically lead to excision of the "scrambled" region and circularisation of the DNA to form a transcriptionally active form of the virus (Ndowora *et al.* 1999; **Fig. 2**). Episomal infections observed in the propagation of virus-free source plants and in the development of new plantain hybrids by *Musa* breeding programs around the world may originate from this integrated BSV. The occurrence of BSV-Onne in these circumstances is thought to be related to the practice of tissue culture, a process that is considered to activate the viral sequences that are integrated into the host genome (Ndowora *et al.* 1999). The hypothesis has been supported by Dallot *et al.* (2001), who found that the proliferation stage of a common micropropagation procedure triggered episomal BSV expression in a tetraploid AAAB *Musa* hybrid. The number of micropropagated plants showing symptoms increased with time in tissue culture, although not all plants that originated from the same microprogagated line showed symptoms. This suggested that episomal infection occurs at random and in a non-simultaneous way in the different cells constituting the proliferation clumps. Rooting of the proliferated plantlets had no significant effect on the occurrence of BSV, nor did the transfer of the plants to the nursery, indicating that the proliferation stage determined episomal BSV expression. Other stress conditions, such as exposure to low temperatures, can also trigger episomal BSV expression (Dahal *et al.* 1998). Under *in vitro* conditions low temperature and wet environment has been observed to enhance the symptom expression but field experiments do not show a clear correlation between temperature and symptom development while wet environment increased the symptom expression in field trials also (Daniells *et al*. 2001). It is known that tissue culture and other stress factors, such as wounding, pathogen attack and some abiotic stress factors such as temperature and heat shock, can activate some retroelements that are not expressed under normal growing conditions (Hirochika 1993), which is consistent with the observation of episomal BSV expression in these circumstances. No data is available for quantification of biotic factors such as tissue culture, wounding or pathogen attack. It has been calculated *in vitro* that low temperature (22ºC) is favourable for BSV activation while higher temperature (28-35ºC) inhibits the virus activation (Daniells *et al*. 2001).

Because the "active" integrated form is associated with the B-genome, infection resulting from expression of the integrated BSV is not a problem with most dessert bananas produced for export as they have an AAA genotype. However, about 90% of the global banana crop is grown for domestic consumption. The majority of these cultivars contain a B-genome and are prone to show episomal infections. These include the bananas and plantains that are produced and consumed locally and that serve as a major carbohydrate component for millions of people worldwide (Anonymous 1997).

There are few examples of plant viral sequences that are integrated into the host genome. Bejerano *et al.* (1996) found geminivirus-related DNA sequences not corresponding to a complete viral sequence in the tobacco (*Nicotiana tabacum*) genome. *Petunia vein-clearing virus* (PVCV) is a pararetrovirus whose genome has been found integrated into the genome of its host, the integration potentially being mediated by a virus-encoded integrase (Richert-Pöggeler and Shepherd 1997). No obvious integrase motif is found in the BSV sequence (Ndowora *et al.* 1999). Jackowitsch *et al.* (1999) found a high number of non-functional integrations of pararetrovirus-like sequences in the tobacco genome, which could deliver resistance to the corresponding tobacco pararetrovirus *via* homology-dependent gene silencing (Mette *et al.* 2002). However, in only one other instance has an episomal infection from an integrated plant virus, caulimovirus-like *tobacco vein clearing virus* (TobVCV) in the genome of *Nicotiana edwardsonii*, been observed (Lockhart *et al.* 2000).

The reports on BSV elimination from infected banana plants are very rare. Cryopreservation has been reported to be one of the successful eradication strategies for BSV from banana. For this the Banana shoots are placed on MS (Murashige and Skoog 1962) semi solid medium and after subculturing small white clumps of highly proliferating meristems are selected for cryotherapy. These white meristematic clumps, (4mm diameter) with at least four apical domes, are immersed in a specific buffer and are given a cryotreatment of 1 hour using liquid nitrogen. The percentage of virus free plants was 59%-76% in control or untreated plants while it increased in the treated plants from 90%-95% (Helliot *et al*. 2002, 2003). It has also been found that acyclic nucleoside phosphate analogues and selective inhibitors of *Hepatitis B virus* replication, adefovir (Adefovir dipivoxil Hepsera™), tenofovir (tenofovir disoproxil fumarate, Viread™) and PMEDAP (2,6-diaminopurine analogue of adefovir), efficiently eliminate BSV from banana (Heliot *et al*. 2003).

BSV PROMOTERS FOR HIGH LEVEL EXPRESSION OF TRANSGENES

Viral promoters have been used extensively for constitutive expression of transgenes in monocot and dicot plants like *Cauliflower mosaic virus* 35S promoter and *Sugarcane bacilliform virus* promoter. Tissue-specific viral promoters have also been isolated from *Commelina yellow mottle virus* (CoYMV) and *Rice tungro bacilliform virus* (RTBV). Two pregenomic RNA promoter sequences isolated from BSV were checked for high-level expression of recombinant genes in stably transformed sugarcane, banana and tobacco plants (Schenk *et al.* 2001). A 2105 bp and a 1322 bp fragment upstream of the first open reading frame of Australian BSV isolates from 'Mysore' and 'Cavendish', respectively, were assessed and were found to give near-constitutive expression of transgenes in vegetative tissues of several monocots including maize, barley, banana, millet, wheat, sorghum and dicot plants like tobacco, canola, sunflower, *Nicotiana benthamiana* and tipu tree (Schenk *et al.* 2001; Remans *et al*. 2005). Importantly, these BSV promoters gave very high levels of Green fluorescent protein (GFP) and β-glucuronidase (GUS) expression in different monocot plants (e.g. up to 1.5% of recombinant protein in sugarcane) and currently present a valuable tool for protein production in a variety of monocot plants, including cereals. A leader sequence of 166 bp and 154 bp was included along with the promoter sequence in BSV-Mys and BSV-Cav respectively. This leader sequences ended at the $tRNA^{Met}$ binding site in both constructs. Highest activity for BSV-Cav was observed in transgenic sugarcane while BSV-Mys showed a high transgene expression level in banana. The promoter activity was higher in vascular tissues which might be due to the repeat sequence CCAGAAG, upstream of TATA box, which is thought to regulate phloem specific expression in RTBV and CFDV promoters (Schenk *et al.* 2001). Potential enhancer and other *cis*-acting regulatory elements were characterised in the BSV-Cav pregenomic RNA promoter (Remans *et al*. 2005).

BANANA STREAK VIRUS MAY HAVE ORIGINATED FROM A SUGARCANE VIRUS

When *Sugarcane bacilliform virus* (ScBV) was experimentally transmitted to banana, the symptoms of infection were typical of banana streak disease (Bouhida *et al.* 1993). Braithwaite *et al*. (1997) obtained a ScBV isolate from sugarcane that has 90% nucleotide sequence identity to the Australian BSV isolate from 'Cavendish' banana (Remans *et al.* 2005). It can therefore be speculated that at least some cases of banana streak disease could have arisen from transmission of badnaviruses from sugarcane to banana. The pink sugarcane mealybug, *Saccharicoccus sacchari*, has been reported to transmit ScBV from sugarcane to banana (Diekmann and Putter 1996).

GENETIC VARIABILITY AND SENSITIVE DETECTION OF BSV

Sequence analysis of 51 samples of BSV from Uganda has demonstrated the high heterogeneity among isolates of this virus (Harper *et al.* 2004, 2005). The reverse transcriptase region was amplified and sequenced as this represents the most conserved part of the badnavirus genome. A similarity of less than 80% was observed among the different sequences. This seems to indicate a large number of viral species. A relatively direct approach to determining the variability of BSV variants can be performed using the different sets of primers from Harper *et al.* (1999) and Geering *et al.* (2000). As an example, a phylogenetic analysis on virus isolates from Mauritius, is presented below, showing that these primers can detect a number of variants.

Using primers BSV4673 and BSVr5317 (Harper *et al.* 1999), isolates from some local *Musa* accessions in Mauritius produced a band of similar size (650 bp) to the BSV (OL- Obino l'Ewai) isolate (Jaufeerally-Fakim *et al.* 2006). Using *Sugarcane bacilliform virus*-infected material as template a band of the same size was also produced. Among the samples tested, most of them, including isolates from Williams and Pisang Mas, produced a slightly larger band than the BSV (OL) isolate of about 750 bp. Therefore with this set of primers two variants could be detected. When the same isolates were tested with the primer set R1/F1 Cavendish (Geering *et al.* 2000), none of the Mauritius isolates produced the same-sized band as the BSV (OL). All were around 800 bp, which is slightly larger than the BSV (OL). One isolate, S1, gave an even larger amplicon of about 850 bp. ScBV isolates also produced a band of the same size as the Mauritius BSV isolates. Hence using these two sets of primers at least three variants could be detected among the Mauritius isolates.

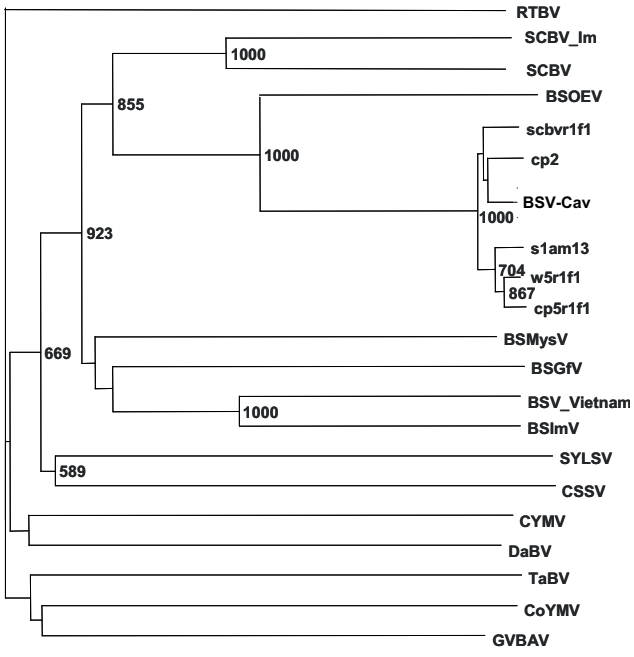

Fig. 3 Dendrogram based on the sequences of part of the ORF III region of different badnaviruses. Additional isolates from Australia (BSV-Cav), Vietnam (BSV_Vietnam), and Mauritius (scbvr1f1; cp2; giorfii; s1am13; w5r1f1; cp5r1f1) are included. BSGFV *Banana streak Goldfinger virus*; BSMysV *Banana streak Mysore virus*; BSImV *Banana streak Imove virus*; BSOEV *Banana streak Obino l'Ewai virus*; CoYMV *Commelina yellow mottle virus*; CSSV *Cacao swollen shoot virus*; CYMV *Citrus yellow mosaic virus*; DaBV *Dioacorea alata bacilliform virus*; GVBAV *Gooseberry vein banding associated virus*; RTBV *Rice tungro bacilliform virus*; SCBV *Sugarcane bacilliform virus*; SCBV_Im *Sugarcane bacilliform virus* Ireng Maleng; SYLSV *Spirea yellow leafspot virus*; TaBV *Taro bacilliform virus* (all sequences are referenced in Jaufeerally-Fakim *et al.* 2006).

An alignment of the sequences of the PCR products of four Mauritius BSV Cavendish isolates (cp2, s1am13, w5r1f1, cp5r1f1) and one ScBV from Mauritius (scbvr1f1) with the Australian isolate (BSV-Cav; Accession no. AF215815) together with other badnaviruses was carried out (**Fig. 3**). The dendrogram obtained indicates that the Mauritius isolates are very close to each other, although one isolate was closer to the ScBV and the Australian BSV isolate. Three Mauritius sequences were grouped together, while the Mauritius ScBV sequence was closer to the Mauritius BSV than the other isolate of ScBV. The sequence alignment showed interesting sites where the Mauritius ScBV was more similar to the Mauritius BSV than to the other strain of ScBV or to BSV-Mys (data not shown). None of the primers designed for BSV-Red Dacca, BSV-Mysore and BSV-Goldfinger could amplify the Mauritius isolates.

Recent developments in molecular diagnostics have led to an increase in the use of real-time PCR (Delanoy *et al.* 2003) and multiplex immunocapture PCR (Provost *et al.* 2006) for rapid and sensitive detection of episomal BSV. A 5′ nuclease assay for episomal BSV has been optimised through the use of primers designed from the conserved sequences of BSV-Obino l'Ewai, BSV-Mysore, BSV-Red Dacca and BSV-Goldfinger. This approach should be useful in the monitoring of episomal BSV in *Musa* accessions.

CONCLUDING REMARKS

Banana streak virus is a major concern world-wide in all banana growing areas. The major limitations in the study of this virus at the molecular level had been the laborious DNA extraction procedure from banana, absence of an alternate host for this virus and the inability if this virus to be mechanically inoculated. Propagation of virus free plants from virus free source plants is usually unsuccessful as the virus integrated in the banana genome becomes activated by the process of tissue culture. With indications of the virus being integrated in most banana and plantain cultivars, BSV is also a restraint to the international movement of *Musa* germplasm (Ortiz 1996). BSV is activated by stress conditions that also trigger activation of retroelements. Phylogenetic analyses have suggested that plant pararetroviruses are more closely related to retroelements than to other viruses (Rothnie *et al.* 1994). This could indicate that BSV has not integrated itself in the banana genome, but that it has always been present as a retroelement in the genome and at a certain time became transferable as a virus particle. Other bad-naviral sequences have been found integrated in the tobacco genome and perhaps more are still to be found in other species. The badnaviruses that have no apparent complete integrated form in their host species may represent a later stage in which the connection with the host genome is lost.

ACKNOWLEDGEMENTS

We wish to thank Dr. Andrew Geering for his valuable comments and acknowledge the Alexander von Humboldt-Foundation, Berlin for providing a Feodor-Lynen Fellowship to Peer Schenk and the University of Queensland for international postgraduate scholarships to Tony Remans and Shazia Iram.

REFERENCES

Anonymous (1997) *FAO Production Yearbook 1996*, FAO, Rome

Bejerano ER, Khashoggi A, Witty M, Lichtenstein C (1996) Integration of multiple repeats of geminiviral DNA into the nuclear genome of tobacco during evolution. *Proceedings of the National Academy of Sciences USA* **93**, 759-764

Bouhida M, Lockhart BEL, Olszewski NE (1993) An analysis of the complete sequence of a sugarcane bacilliform virus genome infectious to banana and rice. *Journal of General Virology* **74**, 15-22

Braithwaite K, Geijskes J, Geering A, McMichael L, Thomas J, Smith G (1997) Genetic variation in sugarcane bacilliform virus and banana streak virus in Australia. In: *Abstracts of the Pathology and Molecular Biology*

Workshop, Umhlanga Rocks, KwaZulu-Natal, South Africa, International Society of Sugar Cane Technologists

Dahal G, Hughes J d'A, Thottappilly G, Lockhart BEL (1998) Effect of temperature on symptom expression and reliability of banana streak badnavirus detection in naturally-infected plantains and bananas (*Musa* spp.). *Plant Disease* **82**, 16-21

Daniells JW, Geering ADW, Bryde NJ, Thomas JE (2001) The effect of *Banana streak virus* on the growth and yield of dessert bananas in tropical Australia. *Annals of Applied Biology* **139**, 51-60

Dallot S, Acuña P, Rivera C, Ramírez P, Côte F, Lockhart BEL, Caruana ML (2001) Evidence that the proliferation stage of micropropagation procedure is determinant in the expression of *Banana streak virus* integrated into the genome of the FHIA 21 hybrid (*Musa* AAAB). *Archives of Virology* **146**, 2179-2190

Delanoy M, Salmon M, Kummert J, Frisson E, Lepoivre P (2003) Development of Real-Time PCR for the rapid detection of episomal banana streak virus. *Plant Disease* **87**, 33-38

Diekmann M, Putter CAJ (1996) FAO/IPGRI Technical Guidelines for the Safe Movement of Germplasm. No. 15. *Musa.* (2nd Edn) Food and Agriculture Organization of the United Nations, Rome/International Plant Genetic Resources Institute, Rome

Fragette D, Kanaté G, Fauquet C, Muller E, Peterschmitt M, Thresh JM (2006) Molecular ecology and emergence of tropical plant viruses. *Annual Review of Phytopathology* **44**, 235-260

Geering ADW, McMichael LA, Dahal G, Olszewski NE, Lockhart BEL, Thomas JE (1999) The origins of banana streak badnavirus. *12th Biennial Conference of the Australian Plant Pathology Society*, Canberra, p 53

Geering ADW, McMichael LA, Dietzgen RG, Thomas JE (2000) Genetic diversity among banana streak virus isolates from Australia. *Phytopathology* **90**, 921-927

Geering ADW, Pooggin MM, Olszewski NE, Lockhart BEL, Thomas JE (2005) Characterization of Banana streak Mysore virus and evidence that its DNA is integrated in the B genome of cultivated *Musa*. *Archives of Virology* **150**, 787-796

Harper G, Hull R (1998) Cloning and sequence analysis of banana streak virus DNA. *Virus Genes* **17**, 271-278

Harper G, Osuji JO, Heslop-Harrison JS, Hull R (1999) Integration of banana streak badnavirus into the *Musa* genome: Molecular and cytogenetic evidence. *Virology* **255**, 207-213

Harper G, Hull R, Lockhart B, Olszewski N (2002) Viral sequences integrated into plant genomes. *Annual Review of Phytopathology* **40**, 119-136

Harper G, Hart D, Moult S, Hull RR (2004) Banana streak virus is very diverse in Uganda. *Virus Research* **100**, 51-56

Harper G, Hart D, Moult S, Hull R, Geering A, Thomas J (2005) The diversity of Banana streak virus isolates in Uganda. *Archives of Virology* **150**, 2407-2420

Helliot B, Panis B, Poumay Y, Swennen R, Lepoivre P, Frison E (2002) Cryopreservation for the elimination of *Cucumber mosaic* or *Banana streak virus* fron banana (*Musa* spp). *Plant Cell Reports* **20**, 1117-1122

Helliot B, Swennen R, Poumay Y, Frison E, Lepoivre P, Panis B (2003) Ultrastructural changes associated with cryopreservation of banana (*Musa* spp.) highly proliferating meristems. *Plant Cell Reports* **21**, 690-698

Helliot B, Panis B, Frison E, De Clercq E, Swennen R, Lepoivre P, Neyts J (2003) The acyclic nucleoside phosphate analogues, adefovir, tenofovir and PMEDAP, efficiently eliminate banana streak virus from banana (*Musa* spp.). *Avtiviral Research* **59**, 121-126

Herzog E, Guerra-Peraza O, Hohn T (2000) The rice tungro bacilliform virus gene II product interacts with the coat protein domain of the viral gene III polyprotein. *Journal of Virology* **74**, 2073-2083

Hirochika H (1993) Activation of tobacco retrotransposons during tissue culture. *The EMBO Journal* **12**, 2521-2528

Hohn T, Fütterer J (1997) The proteins and functions of plant pararetroviruses: knowns and unknowns. *Critical Reviews in Plant Science* **16**, 133-161

Hohn T, Corsten S, Dominguez D, Fűtterer J, Kirk D, Hemmings-Mieszczak M, Pooggin M, Schärer-Hernandez N, Ryabova L (2001) Shunting is a translation strategy used by plant pararetroviruses (Caulimoviridae). *Micron* **32**, 51-57

Jakowitsch J, Mette MF, van der Winden J, Matzke MA, Matzke AJM (1999) Integrated pararetroviral sequences define a unique class of dispersed repetitive DNA in plants. *Proceedings of the National Academy of Sciences USA* **96**, 13241-13246

Jaufeerally-Fakim Y, Khorugdharry A, Harper G (2006) Genetic variants of Banana Streak Virus in Mauritius. *Virus Research* **115**, 91-98

Jones DR, Lockhart BEL (1993) *Banana streak disease.* Fact sheet no.1. INIBAP, Montpellier, France

Kubiriba J, Legg JP, Tushemereirwe W, Adipala E (2001) Disease spread patterns of Banana streak virus in farmers' fields in Uganda. *Annals of Applied Biology* **139**, 31-36

Lassoudière A (1974) La mosaïque dite "à tirets" du bananier Poyo en Côte d'Ivoire. *Fruits* **29**, 349-357

Lockhart BEL (1986) Purification and serology of a bacilliform virus associated with banana streak disease. *Phytopathology* **76**, 995-999

Lockhart BEL, Olszewski NE (1993) Serological and genomic heterogeneity of banana streak badnavirus: implications for virus detection in *Musa* germplasm. In: Ganry J (Ed) *Breeding Banana and Plantain for Resistance to Diseases and Pests*, CIRAD/INIBAP, Montpellier, 393 pp

Lockhart BE (1995) Banana streak badnavirus infection in *Musa*: epidemiology, diagnosis and control. *Food and Fertilizer Technology Center Technical Bulletin* **143**, 1-11

Lockhart BE, Menke J, Dahal G, Olszewski NE (2000) Characterization and genomic analysis of tobacco vein clearing virus, a plant pararetrovirus that is transmitted vertically and related to sequences integrated in the host genome. *Journal of General Virology* **81**, 1579-1585

Mette MF, Kanno T, Aufsatz W, Jakowitsch J, van der Winden J, Matzke MA, Matzke AJM (2002) Endogenous viral sequences and their potential contribution to heritable virus resistance in plants. *The EMBO Journal* **21**, 461-469

Ndowora T, Dahal G, LaFleur D, Harper G, Hull R, Olszewski NE, Lockhart B (1999) Evidence that badnavirus infection in *Musa* can originate from integrated pararetroviral sequences. *Virology* **255**, 214-220

Ortiz R (1996) The potential of AMMI analysis for field assessment of *Musa* genotypes to virus infection. *HortScience* **31**, 829-832

Pooggin MM, Futterer J, Skryabin KG, Hohn T (1999) A short open reading frame terminating in front of a stable hairpin is the conserved feature in pregenomic RNA leaders of plant pararetroviruses. *Journal of General Virology* **80**, 2217-2228

Provost Gl, Iskra-Caruana ML, Acina I, Teycheney PY (2006) Improved detection of episomal *Banana streak viruses* by multiplex immunocapture PCR. *Journal of Virological Methods* **137**, 7-13

Remans T, Grof CPL, Ebert PR, Schenk PM (2005) Identification of functional sequences in the pregenomic RNA promoter of the Banana streak virus Cavendish strain (BSV-Cav). *Virus Research* **108**, 177-186

Richert-Pöggeler KR, Shepherd RJ (1997) Petunia-vein clearing virus: a plant pararetrovirus with the core sequences for an integrase function. *Virology* **236**, 137-146

Rothnie HM, Chapdelaine Y, Hohn T (1994) Pararetroviruses and retroviruses: a comparative review of viral structure and gene expression strategies. *Advances in Virus Research* **44**, 1-67

Ryabova LA, Pooggin MM, Hohn T (2006) Translation reinitiation and leaky scanning in plant viruses. *Virus Research* **119**, 52-62

Schenk PM, Remans T, Sagi L, Elliott AR, Dietzgen RG, Swennen R, Ebert PR, Grof CPL, Manners JM (2001) Promoters for pregenomic RNA of banana streak badnavirus are active for transgene expression in monocot and dicot plants. *Plant Molecular Biology* **47**, 399-412

Staginnus C, Richert-Poggeler KR (2006) Endogenous pararetroviruses: two-faced travellers in the plant genome. *Trends in Plant Science* **11**, 485-491

Stavalone L, Herzog E, Leclerc D, Hohn T (2001) Tetramerization is a conserved feature of the virion-associated protein in plant pararetroviruses. *Journal of Virology* **75**, 7739-7743