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Analysis of the distribution and structure of integrated *Banana streak virus* DNA in a range of *Musa* cultivars

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SUMMARY

Banana streak virus strain OL (BSV-OL) commonly infects new *Musa* hybrids, and this infection is thought to arise *de novo* from integrated virus sequences present in the nuclear genome of the plant. Integrated DNA (Musa6+8 sequence) containing the whole genome of the virus has previously been cloned from cv. Obino l'Ewai (*Musa* AAB group), a parent of many of the hybrids. Using a Southern blot hybridization assay, we have examined the distribution and structure of integrated BSV-OL sequences in a range of *Musa* cultivars. For cv. Obino l'Ewai, almost every restriction fragment hybridizing to BSV-OL was predicted from the Musa6+8 sequence, suggesting that this is the predominant type of BSV-OL integrant in the genome. Furthermore, since only two junction fragments of *Musa*/BSV sequence were detected, and the Musa6+8 sequence is believed to be integrated as multiple copies in a tandem array, then the internal *Musa* spacer sequences must be highly conserved. Similarly sized restriction fragments were detected in four BB group cultivars, but not in six AA or AAA group cultivars, suggesting that the BSV-OL sequences are linked to the B-genome of *Musa*. We also provide evidence that cv. Williams (*Musa* AAA group) contains a distinct badnavirus integrant that is closely related to the 'dead' virus integrant previously characterized from Calcutta 4 (*Musa acuminata* ssp. *burmannicoides*). Our results suggest that the virus integrant from cv. Williams is linked to the A-genome, and the complexity of the hybridization patterns suggest multiple sites of integration and/or variation in sequence and structure of the integrants.

INTRODUCTION

Banana streak virus (BSV) is widely distributed throughout banana and plantain (*Musa* spp.) production areas of the world (Dahal *et al.*, 1998; Diekmann and Putter, 1996). In recent times, BSV has risen significantly in prominence, particularly because of the problems it has created within the *Musa* breeding programmes. New hybrids have been developed with resistance to other important pathogens such as *Mycosphaerella fijiensis*, but because the hybrids have frequently been infected with BSV, their exploitation has been curtailed. Recent work suggests that this infection has arisen from expression of virus sequences that are integrated into the *Musa* genome (Harper *et al.*, 1999; Ndowora *et al.*, 1999).

The genetic composition of cultivated *Musa* is complex. The majority of cultivars are either derived from *Musa acuminata* or are hybrids of this species and *Musa balbisiana* (Jones, 1999); their genetic composition is normally represented with the letter codes A and B, representing the contributions of the two wild species, respectively. Cultivars can be diploid (AA, BB) or tetraploid (AAAB, AABB, ABBB) but the vast majority are triploid, and AAA, AAB and ABB genotypes all occur (Jones, 1999). Nearly all cultivars presently grown have arisen through a process of traditional selection. Breeding programmes have only commenced relatively recently and are located in Africa, Central and South America and India (Ortiz *et al.,* 1995).

Cv. Obino l'Ewai (*Musa* AAB group) and the wild banana accession Calcutta 4 (*M. acuminata* ssp. *burmannicoides*) have been used for breeding at the International Institute for Tropical Agriculture in Nigeria. Despite the apparent freedom of both parents from BSV infection, the progeny of crosses have frequently been infected with the Onne strain of BSV (Dahal *et al.*, 1999; Harper and Hull, 1998), now renamed BSV-OL (R. Hull, personal communication). BSV-OL sequences have been found integrated into the nuclear genome of cv. Obino l'Ewai. One integrant (Musa6+8) that has been characterized contains the full complement of the virus genome, although the sequence is interspersed with a 'scrambled' region containing inverted and non-contiguous viral sequences and perhaps *Musa* sequences (Ndowora *et al.*, 1999). It is thought that new hybrids become infected through expression of this integrant. The current model for episomal expression of the BSV-OL integrant involves two homologous

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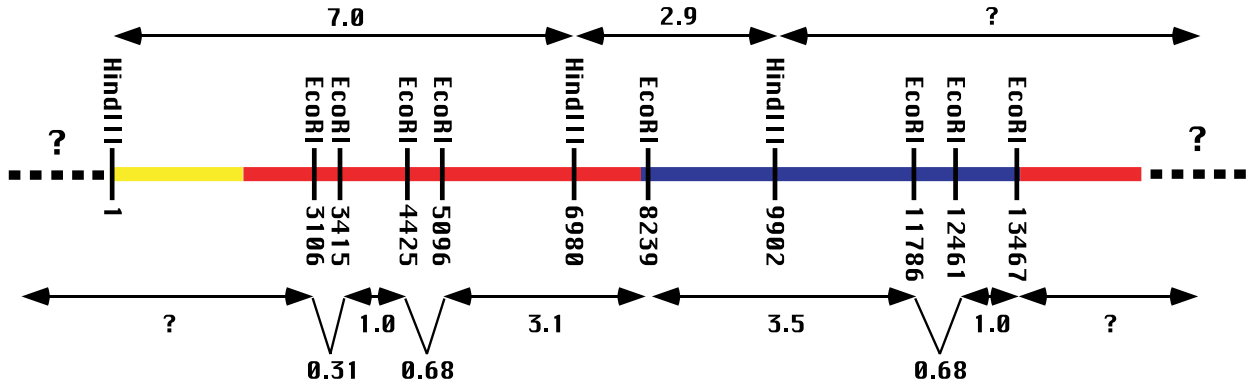

Fig. 1 Restriction map of the Musa6+8 integrant. The yellow region is the MusaOL sequence, the red region is contiguous BSV-OL sequence, the blue region is the 'scrambled' region containing several segments of noncontiguous and inverted BSV-OL sequence, and the dotted lines represent uncharacterized *Musa* sequence (5′ end) and/or BSV-OL sequence (3′ end).

recombinations leading to excision of the 'scrambled' region and circularization of the DNA to produce a transcriptionally active form of the virus (Ndowora *et al.*, 1999). Propagation by tissue culture is thought to be a trigger for episomal expression of the integrant (Ndowora *et al.*, 1999).

A second type of integrant (Musa1) has been isolated from Calcutta 4, and this integrant has a greater sequence similarity to *Cacao swollen shoot virus* than to BSV-OL (LaFleur *et al.*, 1996; Ndowora *et al.*, 1999). This integrant contains sequence comprising only part of a badnavirus genome and the sequence is rearranged and partially inverted. This integrant is therefore considered incapable of giving rise to infection.

Although significant progress has been made in understanding the nature of integrated viral sequences in the *Musa* genome, several significant questions remain. The structure of the BSV-OL (Musa6+8) integrant previously identified in cv. Obino l'Ewai has not been completely determined, as a clone containing the 3′ junction of virus and *Musa* sequence has not been isolated. Fluorescent *in situ* hybridization (FISH) results of Harper *et al.* (1999) suggest that there are two loci of integration of BSV-OL into the *Musa* genome. Furthermore, DNA-fibre stretch hybridization (FSH) results suggest that at each locus of integration, there are multiple repeats of BSV-OL sequence, and each repeat of the virus sequence is flanked on one side by a repetitive *Musa* sequence (MusaOL). The structure of these additional BSV-OL integrants and the role they play in the infection process are unknown. PCR experiments using degenerate badnavirus primers suggest that Cavendish bananas (*Musa* AAA group, Cavendish subgroup), which are the most commercially important group of bananas in the world, also contain integrated badnavirus sequence (Lockhart & Olszewski, unpublished data), but the nature of this sequence is unknown.

In this paper we describe experiments done to test for the presence of BSV integrants in various cultivars of *Musa*. We also provide additional information on the structure of BSV integrants in these cultivars.

RESULTS

Detection of integrated BSV-OL sequence in a range of *Musa* genotypes by Southern blot hybridization assay

Following *Hin*dIII digestion of cv. Obino l'Ewai (OBLE) DNA, three hybridizing fragments are expected, based on the sequence of the BSV-OL integrant (Musa6+8) already characterized from this cultivar (Ndowora *et al.*, 1999; Fig. 1). Two of these fragments, predicted to be 2.9 and 7.0 kb in size, were detected (lane 9, Fig. 2A). The exact size of the third hybridizing *Hin*dIII fragment containing the 3′ junction of virus and *Musa* sequence is unknown, but based on the Musa6+8 sequence, it must be larger than 5.6 kb. A *Hin*dIII fragment of ≈ 16 kb was detected. A fourth *Hin*dIII fragment of 1.2 kb was also detected, and this fragment is not predicted based on available sequence for the Musa6+8 integrant.

Fragments corresponding to those detected in *Hin*dIII-digested OBLE DNA were also detected in the DNA of all BB group cultivars (lanes 1, 3, 5 and 7, Fig. 2A), but not in the DNA of Calcutta 4 (lane 1, Fig. 2B) or the AAA group cultivars (lanes 3, 5, 7, 9 and 11, Fig. 2B). With cvs. Pisang Klutuk Wulung and Pisang Batu and the unidentified BB group banana from Honduras (lanes 1, 3 and 5, Fig. 2A), an additional fragment of ≈ 11 kb was detected that was absent in DNA from cvs. Lal Velchi and OBLE (lanes 7 and 9, respectively, Fig. 2A).

The hybridization pattern predicted following digestion of OBLE DNA with *Eco*RI is more complicated than that predicted following *Hin*dIII digestion. Based on the Musa6+8 sequence, five hybridizing fragments of 0.31, 0.68, 1.0, 3.1 and 3.5 kb are predicted (Fig. 1). Fragments of these approximate sizes were detected (lane 10, Fig. 2A), except for the smallest, which was outside the size range of the blot. In addition to these five fragments, two others containing the 5′ and 3′ junctions of virus and *Musa* sequence should be present and are predicted to be at least 3.1 and 2.0 kb, respectively. The 3.2 and 4.3 kb *Eco*RI fragments

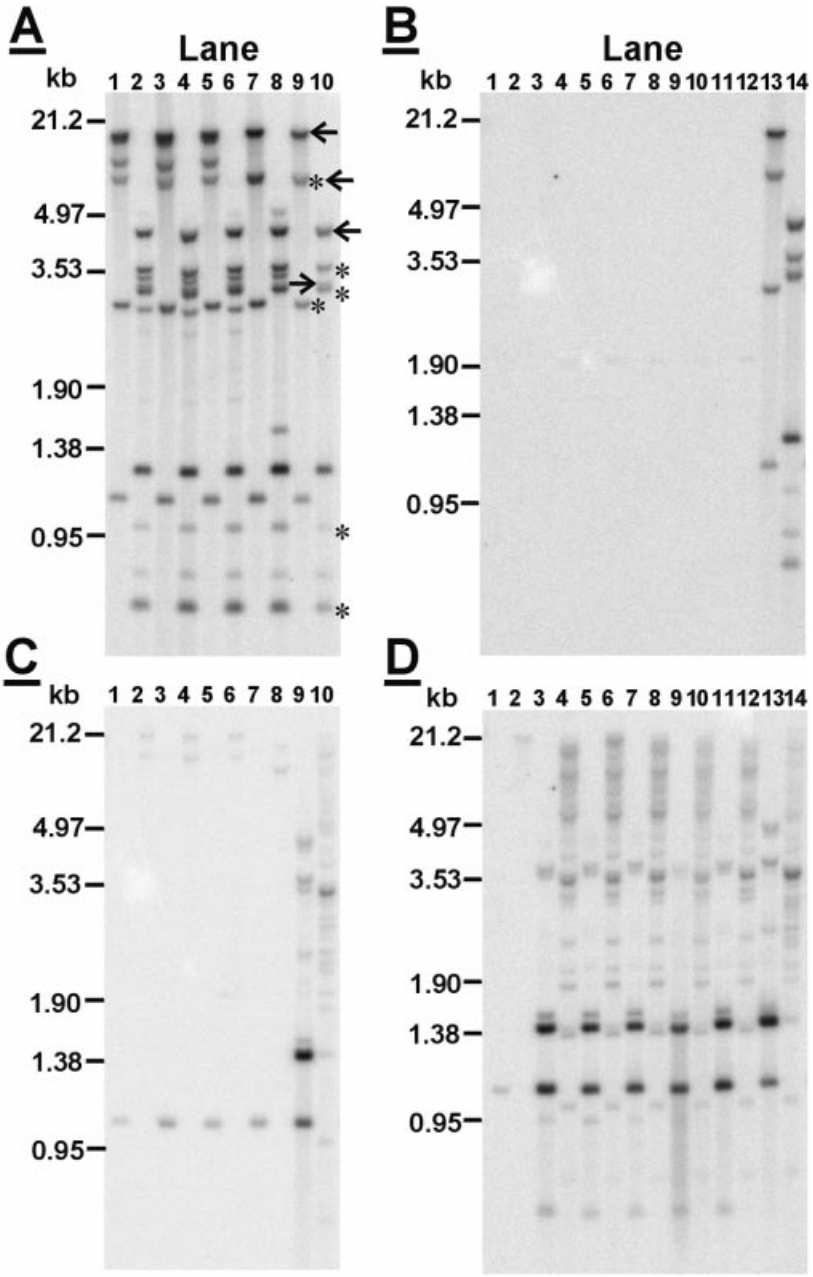

Fig. 2 Detection of BSV-OL (panels A and B) and pWil1 (panels C and D) sequence in genomic DNA of a range of *Musa* cultivars by Southern blot hybridization assay. The DNA was digested with either *Hin*dIII (odd lane numbers) or *Eco*RI (even lane numbers). For panels (A) and (C), DNA was loaded in the following order: lanes 1 and 2, cv. Pisang Klutuk Wulung (BB); lanes 3 and 4, unidentified Honduras BB banana; lanes 5 and 6, cv. Pisang Batu (BB); lanes 7 and 8, cv. Lal Velchi (BB); lanes 9 and 10, cv. Obino l'Ewai (AAB). For panels B and D, DNA was loaded in the following order: lanes 1 and 2. Calcutta 4 (AA); lanes 3 and 4, cv. Williams (AAA); lanes 5 and 6, cv. Giant Cavendish (AAA); lanes 7 and 8, cv. Grand Nain (AAA); lanes 9 and 10, cv. Dwarf Parfitt (AAA), lanes 11 and 12, cv. Gros Michel (AAA); lanes 13 and 14, cv. Obino L'Ewai (AAB). Asterisks mark fragments predicted from the Musa6+8 sequence, and arrows point to *Musa*/BSV-OL junction fragments.

probably represent the ends of the integrated sequence. Two smaller *Eco*RI fragments (0.8 and 1.3 kb) were also detected, and these fragments are not expected based on available sequence for the Musa6+8 integrant.

Fragments corresponding to those detected in *Eco*RI-digested OBLE DNA were also detected in the DNA of all BB group cultivars (lanes 2, 4, 6 and 8, Fig. 2A), but not in DNA of Calcutta 4 or any of the AAA group cultivars (lanes 2, 4, 6, 8, 10 and 12, Fig. 2B). With cvs. Pisang Klutuk Wulung and Pisang Batu and the Honduras BB banana (lanes 2, 4 and 6, Fig. 2A), additional *Eco*RI fragments of 2.9 and 3.4 kb were detected that were absent in the OBLE DNA (lane 10, Fig. 2A). The 3.4 kb fragment was also detected in the cv. Lal Velchi DNA (lane 8, Fig. 2A), but not the 2.9 kb fragment. Furthermore, with the cv. Lal Velchi DNA, additional *Eco*RI fragments of 1.6 and 5.1 kb were detected that were absent in DNA from the other BB group cultivars and OBLE.

Identification of restriction fragments containing the junctions of BSV-OL and *Musa* DNA

In the above-mentioned analyses, restriction fragments were identified as potentially containing the junctions of BSV-OL and

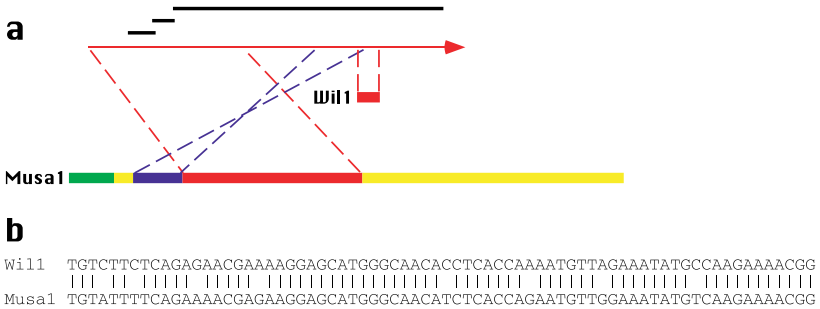

Fig. 3 Comparison of the pWil1 and Musa1 (Ndowora *et al.*, 1999) sequences. (a) The red arrow indicates the generalized structure of a badnavirus transcript and the black lines above indicate the locations of the open reading frames. The lines below the transcript show the structure of the Musa1 integrant (Ndowora *et al.*, 1999) and pWil1. Regions in red and blue correspond to badnavirus sequences in the same and opposite orientations relative to the transcript, respectively. The dotted lines indicate the locations of the different regions of pWil1 and Musa1 on the prototype badnavirus transcript. The yellow regions indicate sequences with no significant identity with any known sequences and are presumed to be *Musa* sequences. The green region corresponds to an incomplete retrotransposon. (b) An alignment of the homologous regions of pWil1 and Musa1.

Musa DNA. To confirm the identity of these fragments, blots with OBLE DNA were probed with pBSV228 and pBSV6701 (data not shown), containing sequences corresponding to the 5′ and 3′ ends of the Musa6+8 integrant, respectively. The pBSV6701 probe only hybridized to the 16 kb *Hin*dIII and 4.3 kb *Eco*RI fragments, suggesting that these fragments contain the 3′ end of the Musa6+8 integrant. The pBSV228 probe hybridized to the 7 kb *Hin*dIII and the 3.2 kb *Eco*RI fragments, suggesting that these fragments contain the 5′ end of the Musa6+8 sequence. The pBSV228 probe also hybridized to the 16 kb *Hin*dIII and 4.3 kb *Eco*RI fragments, suggesting continuation of viral sequence in a contiguous manner beyond the 3′ end of the Musa6+8 sequence, but termination of this sequence at some point prior to the *Hin*dIII site at nucleotide 5810 of the BSV-OL genome (Harper and Hull, 1998).

For cvs. Pisang Batu, Pisang Klutuk Wulung, Lal Velchi and the Honduras BB group banana, the hybridization patterns with the pBSV6701 probe were identical to that of OBLE (data not shown). In addition, like OBLE, the pBSV228 probe hybridized to the 7 and 16 kb *Hin*dIII fragments and the 3.2 and 4.3 kb *Eco*RI fragments from these cultivars. However, the pBSV228 probe also hybridized to the 11 kb *Hin*dIII fragments from Pisang Batu, Pisang Klutuk Wulung, and the Honduras BB group banana, the 1.6 and 5.1 kb *Hin*dIII fragments from cv. Lal Velchi, and the 3.4 kb *Eco*RI fragments from all four cultivars.

PCR amplification of badnavirus sequence from genomic DNA of healthy cv. Williams

A 480 bp DNA fragment was amplified from the genomic DNA of a healthy cv. Williams plant using the degenerate, badnavirus-group primers Mys 3′ and Badna 2. The sequence of one of these clones (pWil1; GenBank accession no. AY028702) was homologous to the badnavirus ORF III, although the coding capacity was interrupted by an internal stop codon. When the sequence of pWil1 was compared with Musa1 (Ndowora *et al.*, 1999), an overlapping region 72 nucleotides in length was found adjacent to the 5′ junction of *Musa* and badnavirus sequence (Fig. 3b). The two sequences had an 88.9% identity in this region. pWil1 contained an additional badnavirus sequence not represented in the Musa1 integrant.

Detection of the Williams-type virus integrant in *Musa* germplasm by Southern blot assay

To test for the presence of the Williams-type virus integrant in the various cultivars, previously prepared blots were stripped and re-probed with pWil1. Complex patterns of hybridization were observed with both *Eco*RI- and *Hin*dIII-digested DNA from the AAA group cultivars (lanes 3–12, Fig. 2D) and OBLE (lanes 13 and 14, Fig. 2D). With *Hin*dIII-digested DNA, fragments of 1.1 and 1.4 kb gave particularly intense signals. By comparison, the patterns of hybridization were much less complex with DNA from Calcutta 4 (lanes 1 and 2, Fig. 2D) and the BB group cultivars (lanes 1–8, Fig. 2C). With *Hin*dIII-digested DNA, only a 1.1 kb fragment was detected. However, the intensity of the signal was much less than that observed with the comparable sized fragment from the AAA group cultivars. With the *Eco*RI-digested DNA, a small number of hybridizing fragments of ≈ 20 kb were detected.

DISCUSSION

Our results provide strong evidence that integrated BSV-OL sequences are associated with the B-genome of cultivated *Musa*. Southern blot hybridization analyses indicated that the Musa6+8 integrant was present in all BB and AAB cultivars tested and that in every case the sizes of the BSV-OL hybridizing restriction fragments corresponded closely to those predicted from the sequence

of the Musa6+8 integrant. We were unable to detect BSV-OL in a range of AAA group cultivars, as well as in Calcutta 4.

FISH and FSH analyses by Harper *et al.* (1999) indicated that BSV-OL sequences are integrated into the nuclear genome of OBLE at two loci, and at each locus there are multiple tandemly arrayed repeats of virus sequence. Their results also suggested that each unit of virus sequence is flanked by a repetitive *Musa* sequence (MusaOL), the sequence identified immediately upstream of the Musa6+8 integrant. The relative simplicity of the hybridization patterns we observed suggest each repetitive unit of BSV-OL sequence in the array matches closely to the structure and sequence of the Musa6+8 integrant. Furthermore, the observation of only two BSV-OL/*Musa* junction fragments in OBLE suggests strong conservation of the internal *Musa* spacer sequences in the array. In contrast, with all BB group cultivars, the hybridization patterns suggest the presence of at least two variants of sequence upstream of the 5′ end of the Musa6+8 integrant.

In contrast to BSV-OL, our results suggest that the badnavirus-like DNA amplified from a healthy cv. Williams plant is linked to the A-genome. Complex hybridization patterns were observed with all AAA and AAB group cultivars, but not with any of the BB group cultivars. The relative complexity of the hybridization patterns suggests multiple sites of integration of the DNA into the genome, and/or variation in sequence and structure of the integrants. Interestingly, very little hybridization was observed between the pWil1 probe and Calcutta 4 DNA. However, the A-genome of Calcutta 4 is significantly different from that of the AAA group cultivars used in our experiments. Calcutta 4 is a selection of *M. acuminata* ssp. *burmannicoides*, whereas dessert bananas (such as those in the Cavendish and Gros Michel subgroups) contain components of four other subspecies of *M. acuminata*, namely sspp. *banksii*, *errans*, *malaccensis* and *zebrina* (F. Carreel, cited in Jones, 1999). Only one weakly hybridizing *Eco*RI fragment of Calcutta 4 DNA was detected using the pWil1 probe. A corresponding fragment was also present in all of the BB group cultivars examined, suggesting that the fragment may contain a repetitive sequence present in a broad range of *Musa*. A related sequence (Musa1; Ndowora *et al.*, 1999) with strong similarity to a 72 bp segment of pWil1 has been isolated from Calcutta 4. However, the DNA of pWil1 and Musa1 are not expected to cross-hybridize because the predicted T_m (Anderson and Young, 1985) for the 72 bp overlap with 11% mismatch is below the T_m of the most stringent washes. The *Hin*dIII fragment from Calcutta 4 hybridizing to the pWil1 probe did not match the size of the fragment predicted from the Musa1 sequence.

Our results indicate that different badnavirus integrants are restricted in their distribution to certain species or even subspecies of *Musa*. *M. acuminata* sspp. *banksii*, *errans*, *malaccensis* and *zebrina* have a natural distribution including Malaysia, Indonesia, New Guinea and the Philippines, whereas *M. acuminata* ssp. *burmannicoides* is from Burma and nearby regions and *M. balbisiana* is from India, northern Indochina and the Philippines (Jones, 1999). Assuming that integration occurred relatively recently, then the disjunct distributions of badnavirus integrants in the different species of *Musa* may simply reflect restricted geographical distributions of the viruses at the times of the integration events. Alternatively, if the integration occurred at a much earlier time in the evolution of *Musa*, then the integrants may have been selectively lost in some species through processes of deletion or sequence decay. Hull *et al.* (2000) have suggested that the presence of a virus integrant may provide a selective advantage to plants by conferring virus resistance through induction of transcriptional or post-transcriptional gene silencing. It is therefore possible that if there was a significant geographical variation in the incidences of badnaviruses and their vectors, there may have been strong selection pressure to maintain the integrants in some but not all species of *Musa*.

A greater insight into the causes of banana streak disease epidemics in banana plantations will be obtained from a better understanding of the distribution of BSV integrants. For example, there were severe epidemics of BSV-OL in export plantations of cv. Grand Nain (*Musa* AAA group) in Costa Rica in early 1999 (Pasberg-Gauhl and Lockhart, 2000; Lockhart, unpublished results). An extrapolation of our results is that infection in these plantations did not arise *de novo* from integrated virus sequences present in the cultivar, but probably arose after an initial introduction of virus by mealybugs, followed by the vegetative multiplication of infected plants. Badnavirus integrants also promise to provide useful markers for taxonomic studies of the family Musaceae.

EXPERIMENTAL PROCEDURES

Sources of *Musa* tissue

Sources of *Musa* leaf tissue were as follows: Calcutta 4 (*M. acuminata* ssp. *burmannicoides*) was obtained from P. Rowe, Fundacion Hondureña de Investigacion Agricola, Honduras; cvs. Giant Cavendish (AAA group), Grand Nain (AAA group), Dwarf Parfitt (AAA group) and Gros Michel (AAA group) were obtained from R.C. Ploetz, University of Florida; cvs. Pisang Batu (BB group), Pisang Klutuk Wulung (BB group) and Lal Velchi (BB group) were obtained from F. Carreel, CIRAD-FLHOR, Guadeloupe; cv. Obino l'Ewai was obtained from G. Thottappilly, International Institute for Tropical Agriculture, Nigeria.

Extraction of genomic DNA

DNA was extracted from *Musa* tissue essentially as described by Gawel and Jarret (1991). Spooled DNA, dissolved in TE pH 8.0, was further purified on a Sepharose 4B (Sigma) spin column essentially as described by Murphy and Kavanagh (1988).

PCR, cloning and sequencing

The clone pWil1 contained DNA amplified from the genomic DNA of a healthy *Musa* AAA group cv. Williams plant with Badna 2 and Mys 3′ primers (Ahlawat *et al.*, 1996). The clone pBSV228 contained DNA amplified from purified BSV-OL with BSV-228 (5′ TGCCTACCCAAGAAAAAGTACC 3′) and BSV-1114 (5′ TCCCAGTCACCAGTTGTTCC 3′) primers. The sequence of this clone corresponds to nucleotides 1974–2872 of the Musa6+8 integrant (Fig. 1). The clone pBSV6701 contained DNA amplified from purified BSV-OL with BSV-6701 (5′ TCCAGAACATCCA-GAAATC 3′) and BSV-7246 primers (5′ CAAGGCTCCTTATATAGA-GAG 3′). The sequence of this clone corresponds to nucleotides 14 831–15 376 of the Musa6+8 integrant (Fig. 1). PCR was done using either *Taq* (Gibco-BRL) or *Tfl* (Promega) DNA polymerase and standard PCR procedures (Kramer and Coen, 2000). Amplified DNA fragments were cloned into pCR2.1-TOPO using a TOPO TA Cloning Kit (Invitrogen) according to the manufacturer's instructions. Plasmid DNA was purified using a QIAprep Spin Miniprep kit (Qiagen). Sequencing was done at the Iowa State University DNA Sequencing Facility.

Southern blot hybridization assay

Approximately 10 μg of genomic DNA was digested with either 20 units of *Hin*dIII (Promega) or 24 units of *Eco*RI (Promega) for 2 h at 37 °C. Following concentration by ethanol precipitation, digested DNA was then separated on a 0.7% agarose gel in 1 × TBE. To partially depurinate then denature the DNA, the gel was first soaked in 0.25 N HCl for 30 min, then 0.5 M NaOH + 1.5 M NaCl for 30 min. Finally the gel was neutralized by soaking in 1.0 M Tris-HCl, pH 8.0 + 1.5 M NaCl for 20 min. The DNA was then transferred to a nylon membrane (MSI Magnacharge) by upward capillary transfer (Sambrook *et al.*, 1989) and immobilized by UV irradiation and/or by baking in a vacuum oven at 80 °C for 60–90 min.

Probes were made to clones of the three *Pst*I fragments of BSV-OL DNA (pBSV1, nucleotides 6151–1073; pBSV10, nucleotides 1074–3515; and pBSV23, nucleotides 3516–6150; Ndowora, Lockhart and Olszewski, unpublished data). Probes were also made to the pBSV228, pBSV6701 and pWil1 clones. Cloned DNA was excised from pBSV1, 10 and 23 clones using *Pst*I, from pBSV228 and pBSV6701 using *Eco*RI, and from pWil1 using a combination of *Not*I and *Xho*I. Following gel-purification, the excised DNA was then labelled with [α-^{32}P] dCTP using a Prime-a-Gene Labelling Kit (Promega) following manufacturer's instructions.

Hybridizations were carried out using two methods. In the first method, membranes were pre-hybridized at 42 °C for 2–10 h in a solution of 5 × SSC, containing 50% formamide, 5 × Denhardt's reagent, 0.08 M NaH_2PO_4, 1% SDS and 200 μg/mL herring sperm DNA. Hybridization with heat denatured probe was done overnight at 42 °C in the same solution as pre-hybridization, except that the solution contained 1 × Denhardt's reagent and 0.02 M NaH_2PO_4. In the second method, pre-hybridization and hybridization were both done using QuickHyb Hybridization Solution (Stratagene) following manufacturer's instructions.

Following hybridization, membranes were subjected to two 15 min washes with 5 × SSC, 0.5% SDS at room temperature, followed by two 15 min washes with 1 × SSC, 0.5% SDS at 42 °C, and three 15 min washes at 65 °C with 0.1 × SSC, 1.0% SDS at 65 °C. Bound probe was detected using a PhosphorImager system (Molecular Dynamics). Membranes were stripped of probe by washing for 2 h at 75 °C in 1 mM Tris-HCl, pH 8.0, containing 1 mM EDTA and 0.1 × Denhardt's reagent, then rinsing in 0.1 × SSC (Sambrook *et al.*, 1989).

Sequence analyses

All sequence analyses were done using programs housed at the Australian Genomic Information Service, University of Sydney. Database searches were done using BLAST (Altschul *et al.*, 1990).

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