Breeding Banana and Plantain for Resistance to Diseases and Pests

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Serological and Genomic Heterogeneity of Banana Streak Badnavirus: Implications for Virus Detection in *Musa* Germplasm

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Abstract

A number of serologically and genomically distinct isolates of banana streak badnavirus (BSV) have been identified. Three distinct serotypes have so far been characterized: BSV-MA (Morocco isolate), BSV-RW (Rwanda isolate) and BSV-Mys/T (Trinidad Mysore isolate). There is also evidence for the existence of other serologically distinct isolates. Because of poor cross-reactivity among BSV isolates in serological and nucleic acid hybridization assays, indexing of *Musa* germplasm by these methods is unreliable. A strategy based on PCR amplification of conserved badnavirus genomic sequences appears to provide the most reliable method for the detection of all possible members of a polymorphic badnavirus species, including BSV. Oligonucleotides that prime polymerase chain reaction (PCR) amplification of the majority of known badnaviruses have been designed and tested. The PCR-based amplification method has been shown to be capable of detecting all isolates of BSV so far tested.

Viral leaf streak of banana (Figs 1 and 2 with colour photos grouped between pages 240 and 241) was first reported from Côte d'Ivoire (Yot-Dauthy, Bové 1966; Lassoudière 1974) as "mosaïque à tirets", to distinguish this disease from that caused by cucumber mosaic virus (CMV) infection, which produces a more diffuse mosaic pattern ("mosaïque en plages", Yot-Dauthy, Bové 1966). The causal agent of viral leaf streak of banana was identified in Morocco in 1986 (Lockhart, 1986). The disease was called banana streak, and the causal agent was named banana streak virus (BSV). Banana streak has been identified to date in the following areas: Australia, Brazil, Canary Islands, China, Côte d'Ivoire, Jordan, Madagascar, Madeira, Morocco, Rwanda, Tanzania, and Trinidad. It is probable that the disease occurs in all banana-producing areas, although the amount of field infection may vary greatly.

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Mysore, a widely-distributed cultivar, has recently been found to be universally infected with BSV. An analysis of the economic effect of BSV infection in banana was reported from Côte d'Ivoire (Lassoudière 1979). BSV infection can have a pronounced effect on bunch size and fruit shape in banana, but this effect is difficult to predict or quantify. Pronounced streak symptoms, which are directly correlated with virus titre, occur sporadically throughout the year. It is possible that, when inflorescence initiation coincides with an episode of increased BSV replication and symptom expression, the resulting bunches are most severely affected.

BSV is a member of the genus Badnavirus (Lockhart, Olszewski 1992). Badnaviruses have nonenveloped bacilliform particles measuring 120-150 x 30 nm and containing a circular double-stranded DNA genome (Lockhart 1990) 7.4-7.8 kb in size. Other definitive members of the badnavirus group include cacao swollen shoot (CSSV), rice tungro bacilliform (RTBV), *Commelina* yellow mottle (CoYMV), *Canna* yellow mottle (CaYMV), *Dioscorea* bacilliform (DBV), sugarcane bacilliform (ScBV), *Piper* yellow mottle (PYMV), *Kalanchoë* top-spotting (KTSV) and schefflera ringspot (SRV) viruses.

The vast majority of badnaviruses occur in clonally-propagated tropical crops and are spread in nature by vegetative propagation. All but two members of the badnavirus group have been shown to be transmitted in a semipersistent manner by mealybug (Pseudococcidae) vectors (Brunt 1970; James et al. 1973; Lockhart et al. 1992). BSV is readily transmitted by the citrus mealybug, *Planococcus citri* Russo. Symptoms appear in emerging leaves 6-8 weeks after inoculation by viruliferous mealybugs (Fig.3: see colour photos grouped between pages 240 and 241).

Like the majority of badnaviruses, BSV has a restricted host range. The only other known host of BSV-MA is sugarcane (*Saccharum* sp.) (Lockhart, Autrey 1988). However, preliminary host-range studies with other BSV isolates indicate differences in the ability to infect species within the Musaceae as well as sugarcane. All isolates of ScBV tested so far are transmitted by both *P. citri* and *Saccharicoccus sacchari* from sugarcane to banana (AAA Dwarf Cavendish). Isolates of ScBV associated with pronounced leaf-streaking symptoms in *Saccharum officinarum* (Lockhart et al. 1992) produced similar symptoms in Dwarf Cavendish (Fig.4: see colour photos grouped between pages 240 and 241), whereas ScBV isolates from asymptomatic *S. officinarum* clones produced markedly less pronounced symptoms in banana. BSV-MA and the first described ScBV isolate from the *S. officinarum* clone Mex. 57-473 were found to be closely related serologically, and were initially considered to be the same virus, or else closely-related variants of the same virus (Lockhart, Autrey 1988). More recent studies have shown, however, that both BSV and ScBV, as well as several other badnaviruses, are serologically heterogeneous (Lockhart, unpublished), and that the degree of serological relatedness may vary widely among the isolates of a given virus.

As stated above, BSV, like the majority of badnaviruses, occurs in a vegetatively-propagated host plant. Because all of the progeny plants derived from a BSV-infected source plant are automatically virus-infected, it is important to develop sensitive and reliable virus indexing methods to ensure that plants used for propagation are virus-free. A reliable virus indexing procedure is also essential to ensure safe movement of *Musa* germplasm between countries. Methods available for virus indexing include the following.

- Visual inspection, based on observation of characteristic, diagnostic symptoms.
- Detection of virus particles in sap extracts by electron microscopy (EM).
- Biological indexing using indicator plants.
- Detection of virus-associated double-stranded RNA (dsRNA) by electrophoresis.
- Serological methods, including immunoprecipitation, enzyme immunoassay (EIA) and immunoelectron microscopy (IEM).

Sensitive and reliable detection of BSV in banana has not been achieved using these methods for the following reasons.

1. Diagnosis of BSV infection in banana by visual inspection is unreliable because foliar symptoms appear sporadically and may be completely absent for several months before reappearing. Furthermore, symptoms of BSV infection can be confused with those caused by CMV infection.

2. Biological indexing using indicator plants is not applicable to BSV detection because, like most badnaviruses, BSV has a restricted natural and experimental host range, and has not been transmitted to any herbaceous indicator plants. Mechanical transmission of BSV from infected to healthy banana has not been successful and, although the virus is readily transmitted by mealybugs, symptoms appear only after 6-8 weeks.

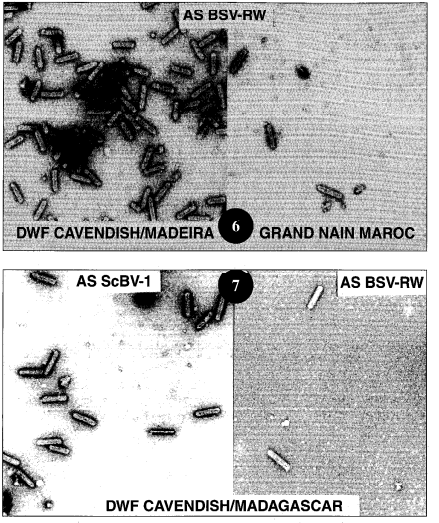
3. Electron microscopic examination of crude leaf extracts does not provide a reliable method for BSV detection because, even in banana leaf tissue showing pronounced symptoms, the concentration of virions is low, and virus can be detected with difficulty, or not at all, in tissue showing mild symptoms. EM detection of BSV is therefore most unreliable in the most critical range—i.e., in cases of mild or suggestive symptoms.

Detection by electrophoresis of virus-specific dsRNAs in plants has been shown to be an extremely sensitive method for detecting viral infection (Dodds

et al. 1984). This technique is applicable to the majority of plant viruses, whose genomes consist of single-stranded RNA (ssRNA), the dsRNAs representing intermediates in the replication of these viruses. BSV and other badnaviruses, which have dsDNA genomes, do not have dsRNA replicative intermediates and therefore cannot be detected using this technique.

Serological detection currently provides a rapid, sensitive, and convenient indexation method for most plant viruses, and both EIA and IEM have been found to be effective and reliable for BSV in initial studies (Lockhart 1986). However, more recent studies with a wider range of isolates of BSV, ScBV, and several other badnaviruses have revealed a significant degree of serological heterogeneity among isolates of a given badnavirus (Lockhart, unpublished), an observation that had been noted previously in the case of CSSV (Brunt 1970). With respect to BSV, this serological heterogeneity among isolates can be illustrated in the EIA and IEM data presented in Figures 4-6 (for Figs 4 and 5, see colour photos grouped between pages 240 and 241). These data point out the serological heterogeneity existing among known BSV isolates, and the consequent ineffectiveness of antibodies against any given isolate to detect all other isolates. One approach to circumventing this difficulty would be to employ a mixture of antisera for BSV detection. However, such an approach can be employed with a reasonable expectation of success only when a much wider range of BSV isolates has been characterized serologically, as it is possible that the isolates characterized so far may represent only a fraction or a geographical subset of all possible BSV serotypes.

Nucleic acid hybridization has been found to be an extremely sensitive method for the detection of viruses and other plant pathogens. The target nucleic acid is immobilized on the nitrocellulose membrane, and is detected by a radioactively- or nonradioactively-labelled DNA probe of sufficient complementary-sequence homology to bind to the target molecule under given wash conditions. This technique, sometimes called dot-blot hybridization, was initially considered to be particularly attractive for the detection of BSV and other badnaviruses because, as distinct from the case of ssRNA plant viruses, it is unnecessary first to prepare a complementary DNA (cDNA) probe. Preliminary studies, however, revealed a wide degree of genomic heterogeneity among BSV and ScBV isolates. There was no cross-hybridization between BSV isolates, and only BSV-MA hybridized to any of the ScBV isolates. This lack of cross-hybridization using genomic viral DNA was subsequently confirmed in labelling experiments employing the PCR-generated products of several BSV isolates, as illustrated in Figures 8-11. These results implied that a specific probe would be needed for each BSV isolate, and that there would be a high probability that this probe would be incapable of detecting other isolates of the virus.



Figures 6 7. Serological heterogeneity among BSV isolates as revealed by immunoelectron microscopy.
Figure 6 shows differential trapping of BSV isolates from Madeira and Morocco by antibodies against BSV-RW.
Figure 7 shows differential trapping of a Madagascar BSV isolate by antibodies against ScBV-1 and BSV-RW.

Serological and genomic heterogeneity among BSV isolates therefore imposes a serious restriction on the usefulness of serological and nucleic acid hybridization techniques for badnavirus detection in *Musa* germplasm. In order to overcome

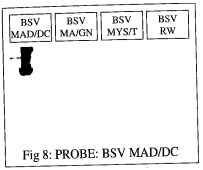


Fig 8: PROBE: BSV MAD/DC

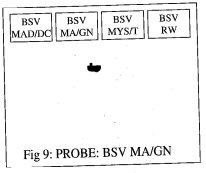


Fig 9: PROBE: BSV MA/GN

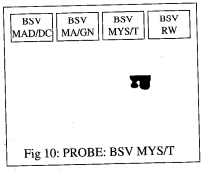


Fig 10: PROBE: BSV MYS/T

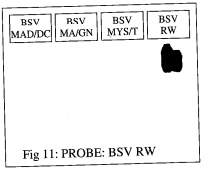


Fig 11: PROBE: BSV RW

Figures 8-11. Genomic heterogeneity among BSV isolates as demonstrated by hybridization assays using PCR-amplified genomic sequences. In each case the detecting probe hybridized only to the homologous target DNA.

these difficulties, an alternative approach was based on PCR amplification (Sidi et al. 1988) of conserved badnavirus genomic sequences. This strategy would permit the detection of any possible variant of BSV. Identification of conserved regions was based on analyses of the complete nucleotide sequences of three badnaviruses, CoYMV, RTBV, and KTSV. Three conserved regions occurring in ORF III (Medberry et al. 1990), representing the $tRNA^{Met}$ binding domain and the ribonuclease H (RNase H) and reverse transcriptase (RT) regions, respectively, were identified. Sequences of three primers for PCR amplification were derived from the consensus sequences of the three conserved regions, as represented in Figure 11. Using the three degenerate primers in two pair combinations vi², BADNAT + BADNA2 and BADNAT + BADNA3, all isolates of BSV tested to date have been detected by PCR amplification. The sequence-specific amplification products, identified by agarose gel electrophoresis, are shown in Figure 12, which illustrates that at least one of the primers' pairs always yields an amplification product. Further studies are in progress to modify

the design of the primers in order to increase specificity of amplification. The PCR-based amplification method therefore appears to represent a workable solution to the problem of coping with the wide range of serological and genomic heterogeneity present among isolates of BSV and other badnaviruses.

	$tRNA^{Met}$ Binding Site							
	ScBV	ACCTCGCTCTGATACCA						
	CoYMV	••AAA••••••••••••						
	RTBV	••A••••••••••••••						
	KTSV	••CAT••••••••••••						
	CaMV	••••••••••••••••••						
	CERV	CTA•G••••••••••••						
	FMV	••A•G••••••••••••						
BADNAT	CACCCCCGGG	ACCAAGCTCTGATACCA						
	SmaI	C T A T C						
		G						
		T						
	Reverse Transcriptase							
	ScBV	ORFIII	Tyr	Ill	Asp	Asp	Ill	Leu
	CoYMV	ORFIII	•	•	•	•	•	•
	RTBV	ORFIII	•	•	•	•	•	•
BADNA2	AAATGCGGCCGC		TAC	ATA	GAC	GAC	ATA	TT
	NotI		T	C	T	T	C	C
				T			T	
	Ribonuclease H							
	ScBV	ORFIII	Ill	Ill	Ill	Glu	Thr	Asp
	CoYMV	ORFIII	•	•	•	•	•	•
	RTBV	ORFIII	•	•	•	•	•	•
BADNA3	AATAGCGGCCGC		ATA	ATA	ATA	GAA	ACA	GAC
	NotI		C	C	C	G	C	T
			T	T	T		G	
							T	

Figure 12. Sequences of primers used to amplify badnaviruses.
The sequences of three primers (BADNAT, BADNA2, BADNA3) that detect badnaviruses are indicated. The primers were derived from consensus sequences generated in comparing the known badnavirus sequences. In the case of BADNAT primer, caulimovirus sequences were also used to design the primer. These primers are all degenerate at some positions as indicated. Restriction endonuclease sites were incorporated into the 5'-ends of the primers to facilitate cloning and analysis of the PCR products generated using these primers.

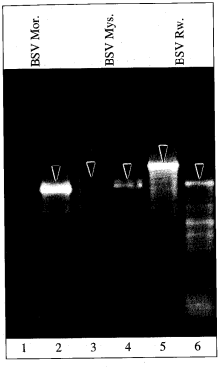


Figure 13. Detection of three BSV isolates by PCR amplification using the same pairs of degenerate primers. Specific amplification products are indicated by arrows. Lanes 1, 3, and 5 show products generated by the primer pair BADNAT + BADNA2; lanes 2, 4, 6 show products generated by the primer pair BADNAT + BADNA3.

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