Banana streak viruses (BSV) constitute a major impediment to the distribution and use of *Musa* germplasm around the world, due to:

- The presence of endogenous, activatable viruses in the B genome
- Activation of infections from the B genome, especially after environmental stresses and tissue culture.
- Widespread occurrence in *Musa* germplasm
- Existence as a “cryptic” species with considerable genomic diversity
- Complexity of detection assays and necessity of distinguishing endogenous from exogenous sequences.

This workshop was organized by John Thomas (University of Queensland, Australia), chair of the MusaNet Conservation Thematic Group. It was the continuation of a discussion started by Australian virologist Andrew Geering (University of Queensland, Australia), and followed up by CIRAD virologist Pierre-Yves Teycheney, at the 2007 ISHS-ProMusa symposium, which was summarized in a ProMusa blog. Developments in our understanding of the molecular structure of integrated BSV prompted a further examination of this subject.

**Workshop aim:**

The objectives of the workshop were to:

1. identify and discuss barriers caused by BSV on the distribution and use of genetic resources by the community, and to propose possible solutions to these issues.
2. make recommendations to collection holders, and especially ITC, on strategies to facilitate distribution of BSV-infected germplasm.

**Workshop format:**

The workshop was chaired by John Thomas (University of Queensland, Australia). Short presentations were made as situation statements on the following subjects:

- current needs for germplasm that is restricted by BSV integration/activation issues (Edson Amorim / EMBRAPA, Brazil)
- risks associated with distribution of this germplasm (Andrew Geering)
- responsibilities of importers and exporters of the germplasm (Bart Panis, Bioversity International)
- possible alternatives to using this germplasm (Pierre-Yves Teycheney)

These short presentations were followed by a roundtable discussion on these issues and possible ways to overcome restrictions on the use of *Musa* germplasm. Over 30 participants from a variety of disciplines (including virologists, breeders, germplasm curators, horticulturists and program managers) attended the workshop.
Presentations

John Thomas outlined the background to the workshop.

Andrew Geering gave an overview of the properties of BSV, its taxonomy and mealybug transmission. He noted the paucity of data on the impact of BSV on production, with most available information being anecdotal. BSV is a major contaminant of international germplasm, with the majority of accessions with the B genome being infected and the activatable viruses already widely distributed internationally. He concluded by noting that under international conventions, it is ultimately the importing country that bears the responsibility for the material it imports.

Pierre-Yves Teycheney described the directions CIRAD was taking within their breeding program to address the BSV issue. The two avenues are being explored at CIRAD are:

- Risk assessment of spreading BSV through large scale distribution of existing interspecific hybrids, and
- Creation of improved M. balbisiana genitors devoid of infectious eBSV

The latter is possible since the elucidation of the structure of BSV integrants and the ability to differentiate infectious and non-infectious eBSV. This work by CIRAD is the major advance since the ProMusa workshop in 2007.

Edson Amorim noted that the AAB cultivars predominate in Brazilian production. The EMBRAPA germplasm collection is being evaluated for its integrated BSV status, with the cooperation of CIRAD. Symptom expression in the field is not common, but sometimes occurs after environmental stress.

Bart Panis outlined the structure and function of the in vitro germplasm collection held at the Bioversity International Transit Centre (ITC). He presented information on virus indexing and virus therapy, and noted the policy of the ITC only to provide material that has indexed virus-free.

Discussion

**General importance of BSV in the field**

The discussions were wide ranging. It is evident that there is limited documented and published data on the impact of BSV on production (e.g. Daniells et al 2001; Lassoudière 1974, 1979; Murekezi 2005). In addition there are several examples of unpublished observations and anecdotal data. Jerome Kubiriba (NARO) and Anthony James (QUT) mentioned a limited outbreak of BSV in germplasm imported from ITC and IITA. This comprised about 20 symptomatic plants out of a few hundred, and consisted of eBSV species only, sometimes as mixed infections. BSMYV was found in germplasm but not in the field. Thierry Lescot (CIRAD) gave several examples of BSV outbreaks in Costa Rica, Colombia, Peru and Ecuador. In most cases these were B genome-containing cultivars, but one example, from Ecuador, was Cavendish infected with BSOLV. He and Brian Irish (USDA, Puerto Rico) both mentioned the variability in levels of activation of endogenous BSV under different levels of stress (e.g. FHIA 21). Uma Subburaya (NRCB, India) also noted a progressive yield decline and environmental effects on symptoms in cv. Mysore, infected with BSMYV.

A wide range of symptomatology was also described, with fruit symptoms sometimes occurring, including irregular bunch emergence, thin, splitting peel, mosaic patterns and necrotic spotting. However, the appearance of these symptoms in both leaves and fruit is erratic. It is most likely that only the worst cases of BSV infection are noticed and publicised, and less severe cases go unrecognised or unreported.

Miguel Dita (EMBRAPA, Brazil) raised the question about the effect of individual BSVs on a range of individual cultivars, and it became apparent that very little is known in this area. A major practical
consideration is working with cultivars containing the B genome, where inoculation with a specific BSV could be confounded by uncontrolled activation of eBSVs.


Current status of BSV-infected accessions in collections

At the ITC, accessions with active, episomal infections, either from field infection or activation of an endogenous allele, are held in quarantine. Cultivars lacking the B genome (no acitvatable endogenous BSV) are subjected to virus elimination therapy, which if successful allows release of the germplasm.

At CIRAD, natural hybrids (local cultivars) that have indexed virus-free are screened with molecular markers for endogenous BSV, and then micro-propagated and reintroduced to the country of origin of the planting material. The importer is informed about the possible BSV expression due to infectious eBSV when it exists in the B genome of the cultivar. For newly created hybrids having B genomes, CIRAD breeders are now working again with interspecific hybrids and seeded BB diploids having either non-infectious eBSV or no eBSV, and thus posing no risk of BSV activation.

At IITA, the Inter-African Phytosanitary guidelines for Musa germplasm exchange in Africa are followed:

“(i) On the exchange of Musa spp Internationally, and recognising that the BSVDNA are integrated to the Musa chromosomes, and BSV being worldwide in distribution, newly bred Musa hybrids can be exchanged without restrictions among interested countries.

(ii) The exporting agency must however, ensure that all procedures for virus testing of Musa germplasm are complied with and only virus tested free materials are exchanged.”

Consequences of maintaining the status quo

- Unavailability of some existing hybrids and landraces for field assessment (e.g. Taxonomic Reference Collection, provitamin A assessment), use as breeding parents and distribution to endusers (e.g. cultivars with black Sigatoka and panama wilt resistance). As an example, of 300 plantain accessions held in the ITC, only 20 (<10%) are virus-free.

- Cost of maintaining in vitro germplasm. The question arises that if the material is to be stored indefinitely with no hope of distribution or virus therapy, should it be discarded? But if rare genotypes held in vitro are not maintained, this will ultimately lead to an erosion of genetic resources and reduction of diversity in the field.

Consequences of distributing eBSV-containing accessions

- Possible outbreaks of BSV in new plantings. The risk of activation is dependent on both cultivar and environmental and physiological stresses. Some hybrids such as FHIA 21 seem particularly prone to BSV activation. Some hybrids e.g. Goldfinger (FHIA 001, AAAB) and natural selections e.g. Ladyfinger (AAB) in Australia virtually never express BSV.

- New BSV species could potentially be imported with germplasm. However the eBSVs, especially BSOLV, are the most common BSVs internationally already.

- In most circumstances, natural spread of BSV via insect vectors is extremely slow, making control through eradication of infected plants a practical consideration. If an accession
demonstrates lower levels of activation, then infected plants could be removed and replaced, with negligible risk to other nearby plantings

Means of limiting or avoiding the negative effects of BSV

- Fred Bakry (CIRAD) described a strategy of sourcing germplasm and shipping back to the same country with natural hybrids and landraces. Experience in West Africa indicated only low levels (e.g. 4%) of tissue culture derived plants expressing BSV symptoms. This method presents no additional threat to the importing country, but utilizes only the germplasm already in that country.

- Virus elimination through meristem tip culture and chemotherapy, cryotherapy or electrotherapy as undertaken for the ITC collection. This is only feasible in non-B genotypes, but would limit the distribution of the less common BSVs that are not integrated,

- Conventional (non-tissue culture) multiplication of planting material, such as macropropagation, to avoid activation during tissue culture

- Use of B genome genitors lacking activatable eBSV. This is a major advance for future breeding programs. However, a problem is the small percentage of B genome-containing hybrids lacking activatable eBSV and the small number of activatable eBSV-free hybrids that can be obtained through artificial crosses. This means that a limited range of B genome diversity will be available for breeding.

Proposed ways forward

- Wait until new CIRAD hybrids are released. This is a very safe approach, but will result in long delays before new hybrids are available and a more limited B genome diversity in the hybrids.

- Release “quarantined “germplasm with activatable BSV. This will result in the availability of a much wider range of genotypes and diversity. Is it possible to release this material with a statement of its eBSV molecular status and possibly its propensity for activation under a standard set of environmental conditions (e.g. after micropropagation, compared to a reference cultivar)?

- Release limited quantities of germplasm for conventional in situ propagation and eventual wider release of individual plants not expressing BSV. Macropropagation methods may be appropriate here.

- If they are not to be distributed in the short to medium term, BSV-infected accessions could be held in cryopreservation only, thus significantly reducing the cost of storage.

Observations and Recommendations

- Further controlled and well-documented field studies are required on the economic impact of BSV.

- Responsibility for the importation of Musa germplasm rests with the importing country.

- The exporter is obliged to fully describe the material being exported.

The International Standards for Phytosanitary Measures, ISPM 7, Phytosanitary Certification System (2011) states:
“The NPPO of the exporting country has the sole authority to undertake phytosanitary certification…”

“Phytosanitary certification should be based on official information from the importing country. The NPPO of the exporting country should, to the extent possible, have available current official information concerning the phytosanitary import requirements of relevant importing countries.”

In the case of BSV, could this Phytosanitary Certification include a “health statement” which could mention the status of eBSV and likelihood of activation?

- The workshop did highlight a need for the distribution of some BSV-infected and quarantined accessions from ITC.

- It would be useful for a small panel of specialists to be brought together to advise Bioversity International on ways to best characterize accessions and report their BSV status and propensity for activation, and thus allow a mechanism for responsible distribution of all germplasm.

- If a modest number of cultivars were targeted for conditional release, it may be possible to map their integrants, and also grow out a standard number of tissue cultured plantlets under standard conditions to assess the relative rate of activation compared e.g. to a reference hybrid.