



Technical guidelines for the safe movement of *Musa* germplasm

Edited by JE Thomas



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The Global Musa Genetic Resources Network (MusaNet) is a global collaborative framework for *Musa* genetic resources and a partnership of all key stakeholders, aiming at ensuring the long-term conservation on a cooperative basis, and facilitating the increased utilization of *Musa* genetic resources globally.

Our vision is a world in which *Musa* genetic resources diversity is valued, secured and supporting all life.

To create this world our mission is: “To build upon existing strengths in the global, regional and national collections by bringing people to optimize the effort to conserve, add value and promote the use and safe distribution of a wide range of *Musa* genetic diversity as a foundation for further breeding or direct use by farmers.”

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1. Introduction¹

These guidelines describe technical procedures that minimize the risk of pest introductions due to the movement of germplasm for research, crop improvement, plant breeding, exploration or conservation. It is important to emphasize that these guidelines are not meant for trade and commercial consignments concerning export and import of germplasm.

Collection, conservation and utilization of plant genetic resources and their global distribution are essential components of international crop improvement programmes.

Inevitably, the movement of germplasm involves a risk of accidentally introducing plant pests along with the host plant. In particular, pathogens that are often symptomless, such as viruses, pose a special risk. To minimize such risks, preventive measures and effective testing procedures are required to ensure that distributed material is free of pests of potential phytosanitary importance. Eradication of pathogens from a region or country is extremely difficult, and even low levels of infection or infestation may result in the establishment of pathogens in new areas.

The international, and inter-regional, movement of plant germplasm for research, conservation and basic plant breeding purposes (including plant biotechnology) requires complete and up-to-date information concerning the phytosanitary status of the plant germplasm. In addition, the relevant and current national regulatory information governing the export and importation of plant germplasm in the respective countries is essential.

The recommendations made in these guidelines are intended for small, specialized consignments used in research programmes, e.g. for collection, conservation and utilization for breeding of plant genetic resources and crop improvement by conventional and modern technologies. When collecting and transporting germplasm, International Standards for Phytosanitary Measures (ISPMs) established by the Food and Agriculture Organization of the UN (FAO)'s International Plant Protection Convention (IPPC; <https://www.ippc.int/core-activities/standards-setting/ispms>) should be considered.

This revision of the technical guidelines for banana has been produced by the Conservation Thematic Group of MusaNet, an international network for *Musa* genetic resources coordinated by Bioversity International (www.musanet.org). These guidelines supersede those published in 1989 (Frison and Putter 1989) and 1996 (Diekmann and Putter 1996). The experts who contributed to the

¹ Adapted from End et al. (2010).

production of these technical guidelines are listed in Section 2 of this publication. They have contributed to the preparation of the technical guidelines in their personal capacity and do not represent or commit the organizations for which they work. The guidelines are intended to provide the best possible phytosanitary information to institutions involved in small-scale plant germplasm exchange for research purposes. Bioversity International and the contributing experts cannot be held responsible for any problems resulting from the use of the information contained in these guidelines.

1.1. Guidelines structure

The guidelines cover virus diseases only, as they are the major pathogen contaminants found in *in vitro Musa* germplasm. Section 3 lists the International Transfer Centre and indexing laboratories, while Section 4 makes general and technical recommendations on safe procedures to move banana germplasm. The largest section, Section 5, covers pests of phytosanitary concern for the international or regional movement of *Musa*. The information given on a particular pest is not exhaustive but rather concentrates on those aspects that are most relevant to the safe movement of germplasm. No specific information on treatment is given in the pest descriptions. A pest risk analysis (PRA) will produce information on which management options are appropriate for the case in question. General precautions are given in the technical recommendations.

1.2. Guidelines update

These guidelines reflect the consensus and knowledge of the specialists who have contributed to this revision, but it is anticipated that the information will need to be regularly updated as new information becomes available. We ask our readers to kindly bring to our attention any developments that may require a review of the guidelines, such as new records, detection methods or control methods.

Correspondence regarding this publication should be addressed to Bioversity International, Parc Scientifique Agropolis II, 34397 Montpellier Cedex 5, France.

1.3. Definitions

- Germplasm** Germplasm is living tissue from which new plants can be grown. It can be a seed or pollen or vegetative material (e.g. a sucker, leaf, piece of stem, corm, meristem or regenerable cells).
- Pest** Any species, strain or biotype of plant, animal, or pathogenic agent, injurious to plants or plant products (FAO 1996).

2. Contributors and acknowledgements

Contributor	Address	Email
John Thomas (Editor)	The University of Queensland, Ecosciences Precinct, Level 2C West, GPO Box 267, Brisbane, Queensland 4001, Australia	j.thomas2@uq.edu.au
Murray Sharman	Queensland Dept of Agriculture and Fisheries, Ecosciences Precinct, Level 2C West, GPO Box 267, Brisbane, Queensland 4001, Australia	Murray.Sharman@daf.qld.gov.au
Ludivine Lassois	University of Liege, Gembloux Agro-Biotech, Passage des Déportés, 2, B-5030 Gembloux, Belgium	Ludivine.lassois@ulg.ac.be
Sebastien Massart		massart.s@fsagx.ac.be
Caroline De Clerck		Caroline.declerk@ulg.ac.be
Marie-Line Caruana	CIRAD, UMR-BGPI TA A-54/K, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France	marie-line.caruana@cirad.fr
Matthieu Chabannes		matthieu.chabannes@cirad.fr
Pierre-Yves Teycheney	CIRAD, Station de Neufchâteau, Sainte-Marie, 97130 Capesterre-Belle-Eau, Guadeloupe	pierre-yves.teycheney@cirad.fr
P. Lava Kumar	IITA-Nigeria Ibadan, PMB 5320, Ibadan, Oyo State, Nigeria	L.kumar@cgiar.org

Contributor	Address	Email
Ines Van den houwe	Bioversity International Transit Centre, c/o KU Leuven, Division of Crop Biotechnics - Laboratory of Tropical Crop Improvement, Willem de Croylaan 42 box 2455 , BE3001 Heverlee, Belgium	i.vandenhouwe@cgiar.org
Nicolas Roux (MusaNet Coordinator)	Bioversity International, 1990 Boulevard de la Lironde, Parc Scientifique Agropolis II, 34357 Montpellier, France	n.roux@cgiar.org

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3. Transit centre and virus indexing centres

3.1. International Transit Centre

The International Transit Centre (ITC) is operated by Bioversity International under the auspices of FAO, and is hosted by the Katholieke Universiteit Leuven, Belgium. Its role is to facilitate safe exchange of *Musa* germplasm. With over 1,500 accessions, the ITC contains the largest banana *in vitro* collection in the world.

3.2. Virus indexing centres

Virology research units having expertise with *Musa* viruses, and collaborating with Bioversity International for the virus indexing of banana, currently include the Centre de coopération internationale en recherche agronomique pour le développement (CIRAD), Montpellier, France (Officer-in-Charge: Dr Marie-Line Iskra-Caruana); University of Queensland, Brisbane, Australia (Officer-in-Charge: Dr John Thomas); University of Liege (ULg), Gembloux Agro-Biotech, Gembloux, Belgium (Officer-in-Charge: Dr. Sébastien Massart) (currently Bioversity's Virus Indexing and Sanitation Centre (VISC)); and the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (Officer-in-Charge: Dr Lava Kumar).

4. General recommendations and technical protocols

4.1. General recommendations

The following general recommendations apply for all international *Musa* germplasm exchanges.

- Germplasm should be obtained from the safest source possible. There is for example a pathogen-tested *in vitro Musa* germplasm collection available at the ITC, from which safe germplasm can be ordered online (<http://www.crop-diversity.org/banana>). All germplasm moving from one continent to another should transit through the ITC or, if possible, be obtained from the ITC. Other sources of safe germplasm may be available where indexing laboratories have the capacity and expertise to test for the complete range of viruses.
- All germplasm should ideally be moved in the form of tissue culture. If this is not possible, full quarantine measures, including containment in secure post-entry quarantine facilities, must be taken until the vegetative material or seed is cultured *in vitro*.
- Germplasm should be tested for all viruses known to affect *Musa* according to the protocols described in these guidelines. However, in some instances tests may be omitted if there is reliable evidence that particular viruses are not present in the country of origin of the germplasm.
- Indexing procedures and results should be documented, e.g. in a germplasm health statement. A sample copy is included at the end of this publication (Appendix 2).
- The movement of germplasm should be carefully planned in consultation with quarantine authorities and the relevant indexing laboratory, and should comply with the regulatory requirements of the importing country.
- The ISPM measures as published by the Secretariat of the International Plant Protection Convention (IPPC) should be followed during movement of germplasm. In accordance with IPPC regulations, any material being transferred internationally must be accompanied by a phytosanitary certificate.

4.2. Technical protocols

Vegetative material

The following protocol should be followed to prepare vegetative material for virus indexing and exchange (distribution) of germplasm.

- Select a sucker from a plant without symptoms of systemic pathogen infection.
- Trim the sucker to remove soil, roots and any other extraneous material, leaving part of the central corm containing the meristem and about 10 cm of the pseudostem tissue above it. The overall dimensions of the block of tissue will be about 20 cm high and 10–15 cm in diameter.
- Air-dry the block for 24 h and wrap in newspaper. Label the material and dispatch in a cardboard box using the fastest transportation method. Plastic should not be used for wrapping.
- Send the material to the nearest appropriate tissue culture laboratory in the country of origin, or if this is not possible, to a tissue culture laboratory preferably in a non-banana-growing area.
- The meristem tip should be excised, surface disinfected (Hamill et al. 1993) and cultured aseptically (Strosse et al. 2004; Van den Houwe and Swennen 2000; Vuylsteke 1989; Wong 1986).
- A single meristem-tip-derived subculture should be further cloned to seven plantlets, of which five should be sent to an indexing facility and two should remain in culture for future multiplication.
- At the indexing facility, four plants should be established in a potting mix in a vector-free, insect-proof greenhouse under conditions conducive to vigorous plant growth. The fifth plant serves as a back-up. The plants to be indexed should be physically isolated from other bananas so as to minimize the possibility of cross-contamination.
- After three and six months of growth, tissue samples should be taken from the three youngest expanded leaves and indexed for viruses as described in Appendix 1.
- In addition, electron microscopic observations should be undertaken at both three and six months to look for the presence of any undescribed viruses.

- If all tests are negative, the four indexed plants may be released and the cultures derived from the two remaining plants *in vitro* may be further propagated and distributed *in vitro*.
- If found to be infected, the material should be subjected to a virus eradication treatment if available. The plant material must not be released until its virus-free status has been confirmed using the testing procedures described in Appendix 1.
- For the movement of *in vitro* material, neither charcoal nor antibiotics should be added to the culture medium, as these may mask contamination in the culture.
- *In vitro* cultures should be shipped in transparent tubes, preferably plastic, and visually inspected for bacteria, fungi and arthropods. Contaminated germplasm should be destroyed. Special care should be taken to protect the material from extremes of temperatures (not below 15°C and not above 35°C) during shipment.

Seeds

Some viruses may be transmitted through seeds. The following protocol should be followed before seeds are moved internationally.

- Collect seeds from plants that are free from pests and disease symptoms.
- Make sure seeds are free of pulp, and there is no visual evidence of insects, mites or nematodes. Air-dry and, if necessary, fumigate. Banana seeds are sensitive to desiccation and therefore should be moved immediately to an appropriate tissue culture laboratory in the country of origin, or if not possible, to a tissue culture laboratory preferably in a non-banana-growing area.
- Seed should be surface-disinfected. The embryos should be isolated and germinated *in vitro* to establish sterile cultures (Bakry 2008; Pancholi et al. 1995; Uma et al. 2011).
- Materials derived from embryo culture should be indexed and moved in the same way as material derived from meristem tip culture, as described above.

5. Virus diseases of *Musa*

5.1. Banana bunchy top

Cause

Banana bunchy top virus (BBTV) has been consistently associated with the disease. This persistently aphid-transmitted virus is restricted to the phloem tissue, and has 18–20 nm isometric virions (Figure 1) with a coat protein subunit of 20.1 kDa and a multicomponent single-stranded DNA genome. It is a member of the genus *Babuvirus* (family *Nanoviridae*). Two geographical groups are described: the South Pacific group (isolates from Australia, Africa, the Pacific islands and the Indian sub-continent) and the Asian group (South-east Asia and China).

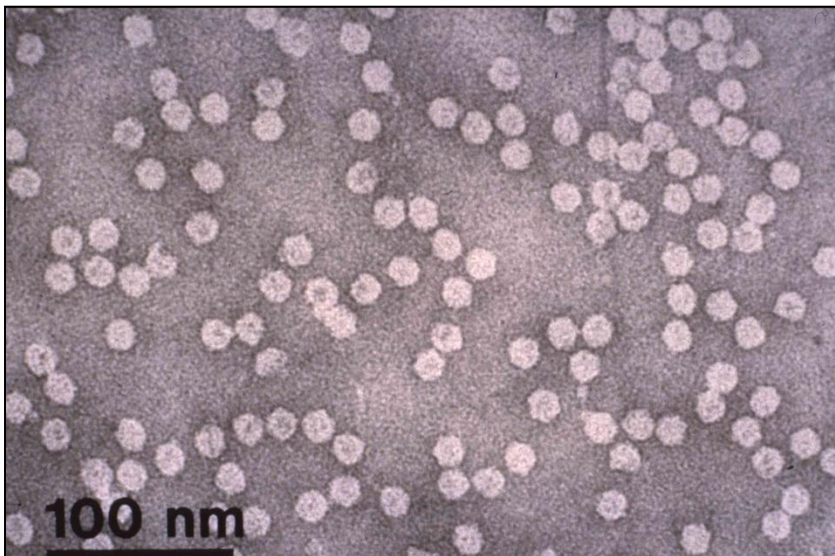


Figure 1. Purified preparation of BBTV particles (JE Thomas).

Significance

The most devastating effects of the virus in recent years have been reported in Pakistan (Soomro et al. 1992), Hawaii (Ferreira et al. 1989) and sub-Saharan Africa (Adegbola et al. 2013; Kumar et al. 2011; Lokossou et al. 2012).

Several countries (e.g. Australia, Taiwan and the Philippines) have implemented comprehensive roguing/sanitation programmes.

Symptoms

Typical severe symptoms include dark green streaks of variable length in the leaf veins, midribs and petioles. These streaks may, however, be rare or absent in some banana cultivars, abaca (*Musa textilis*) and *Ensete* spp. Leaves become progressively shorter and develop marginal chlorosis (Figure 2). As the disease progresses, leaves become more upright or 'bunched' at the apex of the plant (Figures 3 and 4).



Figure 2. A healthy banana leaf (left) and a BBTV-infected banana leaf (right) (C De Clerck).



Figure 3. Bunched appearance of the apex caused by BBTV (C De Clerck).

Plants can be infected at every development stage, but symptoms do not appear until at least two new leaves are produced (Thomas 2008).

Depending on when the plant becomes infected, it may produce no fruit or the bunch may not emerge from the pseudostem. When infection takes place very late in the season, there may be no leaf symptoms but dark green streaks may be seen on the tips of the bracts.

Plants coming from infected planting material show strong symptoms on all leaves, with leaves remaining bunched at the top of the pseudostem and showing chlorotic edges (Figure 4). Aphid-inoculated plants show symptoms only on leaves formed after infection. All the suckers from an infected plant will be infected.

Mild symptoms of vein clearing as well as symptomless infections have been reported from Taiwan (Su et al. 2003) and Vietnam. Attenuation of initial severe symptoms has been reported in the cv. Veimama from Fiji (Magee 1953).



Figure 4. Severe BBTV symptoms on plants grown from infected planting material; Burundi, near Bujumbura (C De Clerck).

Hosts

All *Musa* species and cultivars are potential hosts for BBTV. To date, no resistant cultivar has been identified but varietal differences in susceptibility have been reported (Espino et al. 1993; Hooks et al. 2009; Stover 1972; ADW Geering and JE Thomas, unpublished). Cultivars in the Cavendish subgroup are severely affected. *Ensete ventricosum* has been experimentally infected.

Canna indica (Pinili et al. 2013; Su et al. 1993), *Hedychium coronarium* (Su et al. 1993), *Alpinia zerumbat* and one selection of *Colocasia esculenta* (Pinili et al. 2013) have been reported as experimental hosts of BBTV using Asian subgroup isolates of the virus. Attempts to confirm these results have been unsuccessful with South Pacific subgroup isolates (Geering and Thomas 1997; Hu et al. 1996; Manickam et al. 2002). There are no reports of natural infections of hosts outside the *Musaceae*.

Geographical distribution

The map in Figure 5 highlights the countries affected by BBTV. The affected countries are also listed below.

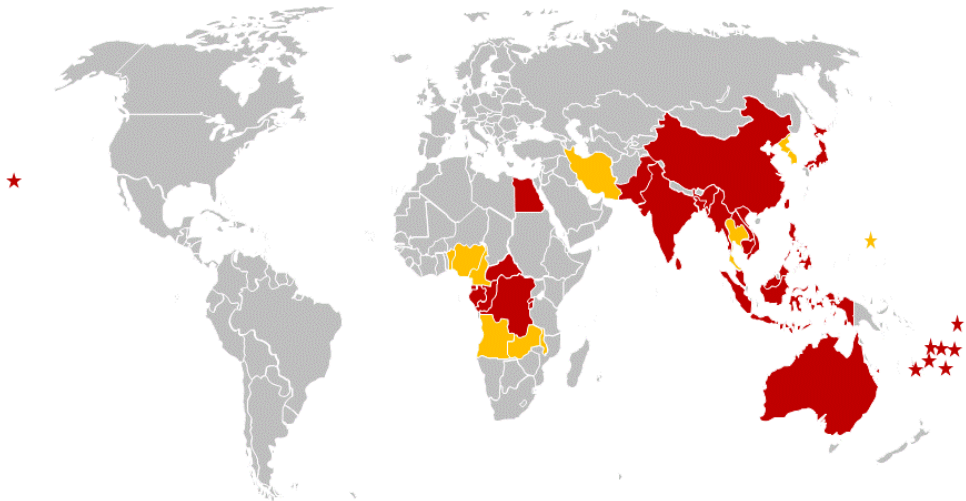


Figure 5. Distribution of BBTV. Countries in red were identified in the 1996 Technical Guidelines (Diekmann and Putter 1996) while those in orange (and the countries in bold in the list below) have been identified since 1996.

Countries affected by BBTV (by region)

Note: new records which were not in the 1996 Technical Guidelines (Diekmann and Putter 1996) are printed in bold; * unconfirmed (this status conferred by the quoted author).

Africa

Angola (Kumar et al. 2008)

Benin (Lokossou et al. 2012)

Burundi (Sebasigari and Stover 1988)

Cameroon (Oben et al. 2009)

Central African Republic (Foure and Lassoudiere, unpublished)

Congo Republic (Wardlaw 1961)

Democratic Republic of Congo (formerly Zaire) (Manser 1982)

Egypt (Magee 1953)

Equatorial Guinea (Manser 1982)

Gabon (Manser 1982)

Malawi (Kenyon et al. 1997)

Nigeria (Adegbola et al. 2013)

Rwanda (Sebasigari and Stover 1988)

Zambia (Gondwe et al. 2007)

Asia

Bangladesh* (Fouré and Manser 1982)

China (Thomas and Dietzgen 1991)

Hong Kong* (Buddenhagen 1968)

Indonesia (Sulyo and Muharam 1985)

India (Magee 1953)

Iran (Bananej et al. 2007)

Japan (Ogasawara-gunto, formerly Bonin Island: Gadd 1926; Okinawa: Kawano and Su 1993)

Kampuchea* (Stover 1972)

Korea* (Kiritani 1992)

Laos (Chittarhat et al., unpublished, 2015)

Malaysia (Su et al. 1993)

Myanmar (Furuya and Natsuaki 2006)

Pakistan (Soomro et al. 1992)

Philippines (Castillo and Martinez 1961)

Sri Lanka (Magee 1953)

Taiwan (Sun 1961)

Thailand (Wongsuwan and Chawpongpan 2012)

Vietnam (Vakili 1969)

Oceania

Australia (Magee 1927)

Fiji (Magee 1927)

Kiribati (formerly Gilbert Islands; Shanmuganathan 1980)

Marianas Islands (Guam: Beaver 1982; Saipan, Tinian and Rota: Miller et al. 2011)

New Caledonia (Kagy et al. 2001)

Tonga (Magee 1927)

Tuvalu (formerly Ellice Islands: Campbell 1926)

USA (American Samoa: Magee 1927; Hawaii: Dietzgen and Thomas 1991)

Wallis Island (Simmonds 1933)

Western Samoa (Magee 1927)

Transmission

The virus is transmitted vegetatively, through tissue culture and conventional planting material, and by the aphid vector *Pentalonia nigronervosa* (banana aphid; Figure 6). The virus does not replicate in the aphid and is not transmitted from an infective adult to the newly hatched nymphs. The virus does not replicate in leaves developed prior to the infection. Aphids are thus only able to acquire the virus from symptomatic leaves formed after infection. Recently, *P. caladii* was also shown to be a vector (Watanabe et al. 2013). No mechanical transmission has been reported.



Figure 6. Banana bunchy top aphid vector *Pentalonia nigronervosa* (C De Clerck).

Detection

The virus can be detected by ELISA (enzyme-linked immunosorbent assay) on samples from field plants, tissue culture plants and infective aphids (Thomas and Dietzgen 1991; Thomas et al. 1995). Monoclonal and polyclonal antibodies are commercially available (Dietzgen and Thomas 1991; Wu and Su 1990). Polymerase chain reaction (PCR) assays are a sensitive alternative for detection (Hu et al. 1996; Mansoor et al. 2005; Sharman et al. 2000b) and DNA probes and primers are available for BBTV DNA components 1 to 6 (Burns et al. 1995; Stainton et al. 2012). Recently real time PCR (Chen and Hu 2013), loop-mediated isothermal amplification (LAMP; Peng et al. 2012b), electrochemiluminescence PCR (Tang et al. 2007) and impedance spectroscopy (Majumder et al. 2013) based methods have also been described for the detection of BBTV. However, these latter methods have not yet been tested against a genetically diverse range of BBTV isolates to test their wider applicability. Multiplex PCR assays have also been reported (Sharman et al. 2000b; Liu et al. 2012).

The optimal tissue for indexing is the midrib of the youngest leaves, though the virus can also be detected in the leaf lamina, pseudostem, dormant meristems, the bunch stalk, bracts and fruit peel from infected plants. The virus can sometimes be detected in the leaf immediately preceding the youngest symptomatic leaf.

Therapy

Meristem or shoot tip culture (<1 to 2 mm), possibly combined with heat therapy (Helliot et al. 2001; Lassois et al. 2013; Ramos and Zamora 1990; Thomas et al. 1995), has been successful in achieving a proportion of virus-free plantlets. Testing after treatment (ideally after an 8–12 month period in the greenhouse) is essential in case the virus is reduced to undetectable levels, but not eliminated, during tissue culture.

5.2. Abaca bunchy top

Cause

Abaca bunchy top virus (ABTV) is a member of the genus *Babuvirus* (family *Nanoviridae*) and has a multicomponent single-stranded DNA genome, a predicted coat protein size of ca. 19.5 M_r, and is presumed to have ca. 20 nm isometric virions similar to BBTV.

Significance

There are no reports on the economic effects of abaca bunchy top disease, though the virus is distantly related to and causes similar symptoms to BBTV, and is expected to have a similar impact on production.

Symptoms

The symptoms caused by ABTV are indistinguishable from those caused by BBTV. Banana cv. Cavendish displays vein clearing and dark green streaking on the leaf lamina, reduced leaf size, bunching of young leaves and plant stunting (Su et al. 2003). Leaf samples from the only abaca plants confirmed to be infected with ABTV displayed vein clearing flecks (Figure 7) and narrow, brittle leaves with chlorotic, upturned margins consistent with descriptions of abaca bunchy top disease in the early literature (Ocfemia 1930).



Figure 7. Vein clearing flecks in a banana leaf infected with ABTV (JE Thomas).

Hosts

Abaca (*Musa textilis*) and banana cv. Cavendish are the only reported hosts.

Geographical distribution

The virus has been reported only from the Philippines and Malaysia (Sarawak). However, some diagnostic ELISA and PCR tests for BBTv will cross-react with ABTV, therefore it is possible that some detections of ABTV may have been mistakenly identified as BBTv.

Transmission

The virus is transmitted vegetatively, through tissue culture and conventional planting material, and by the aphid vector *Pentalonia nigronervosa*. By analogy to the related BBTv, ABTV is likely to be transmitted in a persistent circulative manner by its aphid vector.

Detection

The virus can be detected by ELISA in field plants. Some commercially available BBTv monoclonal and polyclonal antibodies (from Agdia, Inc., USA) are known to cross-react with ABTV, albeit with a reduced sensitivity.

Specific PCR assays are a sensitive and reliable alternative for detection (Sharman et al. 2008). Some PCR primers for BBTV are known to cross-react with ABTV (Su et al. 2003).

ABTV is likely to be phloem limited, and the optimal tissue for indexing is the midrib of the youngest leaves.

Therapy

There are no reports on attempts to eliminate ABTV from infected germplasm, but it is likely that meristem tip culture will be effective, as it is for BBTV (Ramos and Zamora 1990; Thomas et al. 1995; Wu and Su 1991).

5.3. Banana bract mosaic

Cause

The disease is caused by the potyvirus *Banana bract mosaic virus* (BBrMV). The virus has flexuous filamentous particles about 700–750 nm in length (Figure 8), with a coat protein of ca 39.3 kDa, and a single stranded RNA genome.

Significance

Up to 40% yield loss is reported in the Philippines, where comprehensive roguing/sanitation programmes are implemented (Magnaye 1994). Fruits fail to fill on infected plants in India (DR Jones, unpublished). On export bananas, deformations and streaks on the fruit are a cause for rejection.

Symptoms

Chlorotic spindle-shaped lesions parallel to the veins may occur on the leaf lamina, especially on younger leaves (Figure 9). Dark coloured broad streaks and mosaic patterns on the bracts of the inflorescence are characteristic of the disease (Figure 10). Chlorotic streaks may occur on the peduncles, and a shortening of bunch internodes is also characteristic. After removal of outer leaf sheaths, the presence of irregular coloured stripes or mosaic patterns is diagnostic of the disease (Figure 11). These markings can be chlorotic, yellow or reddish, depending on the pseudostem colour. Greenish to brownish broad, irregularly scattered spindle streaks develop along the petioles (Figure 12).

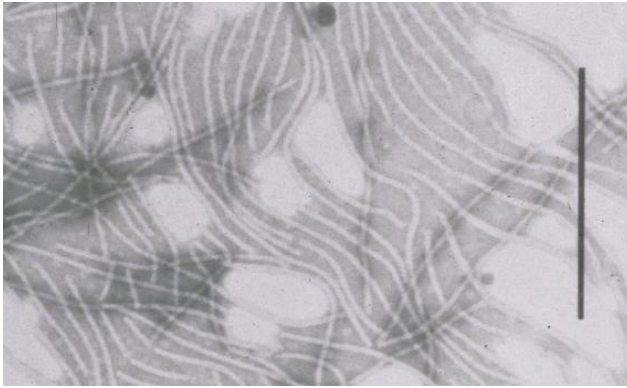


Figure 8. Purified virions of BBrMV; bar represents 400 nm (JE Thomas).



Figure 9 (left). Chlorotic spindle-shaped lesions parallel to the veins on the leaf lamina of a BBrMV-infected banana (JE Thomas).

Figure 10 (right). Dark coloured broad streaks and mosaic patterns on the bracts of the inflorescence of a BBrMV-infected banana (JE Thomas).



Figure 11 (left). Irregular stripes and mosaic patterns on the pseudostem of a BBrMV-infected banana, after removal of the outer leaf sheaths (JE Thomas).

Figure 12 (right). Irregular streaks and mosaic patterns on the leaf midrib of a BBrMV-infected banana (JE Thomas).

BBrMV symptoms may be masked by other virus infections, and the virus has been recovered from plants with a mild *Cucumber mosaic virus* (CMV)-like mosaic symptom, often co-infected with CMV, and from banana streak virus (BSV)-infected plants with typical BSV symptoms (Rodoni et al. 1997; 1999). Symptomless infections have also been reported (Feng 2006).

Hosts

Hosts include *Musa* species and cultivars (Diekmann and Putter 1996), *Alpinia purpurata* (Wang et al. 2010) and *Elettaria cardamomum* (Siljo et al. 2012). BBrMV infects abaca (*Musa textilis*) causing symptoms similar to those caused by the abaca strain of *Sugarcane mosaic virus* (Sharman et al. 2000a; see Section 5.4 on abaca mosaic disease).

Geographical distribution

BBrMV has been reported in the Philippines (Magnaye and Espino 1990), India (where the disease is called ‘Kokkan’) and Sri Lanka (Thomas et al. 1997), Thailand, Western Samoa, Vietnam (Rodoni et al. 1999), Taiwan (Feng 2006)

and Hawaii (Wang et al. 2010). Reports from Colombia and Ecuador (Quito-Avila et al. 2013) are yet to be confirmed.

Transmission

No mechanical transmission has been reported in *Musa*. In addition to transmission through vegetative propagation and tissue culture, transmission by the aphid species *Rhopalosiphum maidis*, *Aphis gossypii* and *Pentalonia nigronervosa* has been reported (Thomas et al. 2000a).

Detection

Leaf symptoms can be erratic and bract symptoms are evident only during flowering. Dead leaf sheaths must be removed to reveal mosaic and streaking on the pseudostem.

The virus can be detected in extracts of leaf lamina, midribs and flower bracts by ELISA using BBrMV-specific polyclonal and/or monoclonal antibodies.

The virus can also be detected by RT-PCR (Iskra-Caruana et al. 2008), immunocapture RT-PCR (Sharman et al. 2000b) or LAMP assays (Siljo and Bhat 2014). Multiplex (IC)-PCR assays are available (Sharman et al. 2000b; Liu et al. 2012).

Therapy

Both meristem culture and heat treatment of shoot cultures have resulted in elimination of BBrMV from infected banana cv. Señorita (Ramos and Zamora 1999).

5.4. Abaca mosaic

Cause

Abaca mosaic is caused by a distinct strain of the potyvirus *Sugarcane mosaic virus* (SCMV-Aba; Eloja and Tinsley 1963; Gambley et al. 2004). The flexuous filamentous particles measure about 680 nm (Figure 13), have a ca. 35 kDa coat protein, and contain a single-stranded RNA genome.

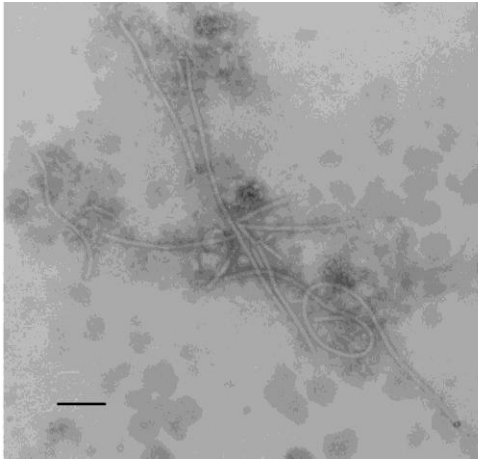


Figure 13. Virions of SCMV-Aba; bar represents 100 nm (JE Thomas).

Significance

The disease has been a significant constraint to abaca production in the Philippines. The disease affects fibre yield as well as fibre quality.

Symptoms

Leaves can show yellowish or light green spindle-shaped streaks (Figures 14 and 17) or a mosaic pattern (Figures 15 and 16). Petioles and midribs are mottled with dark green and yellowish streaks, even when no symptoms appear on the leaves (Figure 16) (Thomas and Magnaye 2000).



Figure 14. Spindle-shaped chlorotic lesions caused by SCMV-Aba on banana cv. Lakatan (JE Thomas).



Figure 15. Mosaic symptoms caused by SCMV-Aba on abaca (JE Thomas).

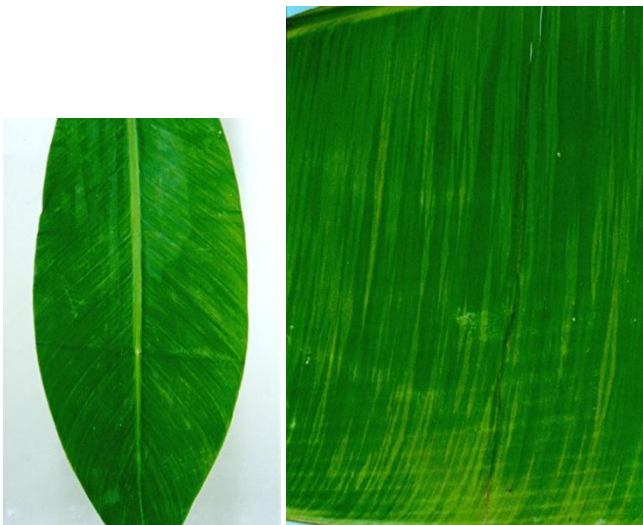


Figure 16 (left). Mosaic pattern caused by SCMV-Aba in banana cv. Buhutan (JE Thomas).

Figure 17 (right). Symptoms caused by a mixed infection of SCMV-Aba and BBrMV in abaca (JE Thomas).

Hosts

Natural hosts include *Musa textilis* (abaca, Manila hemp), *Marantha arundinacea* and *Canna indica*.

There are several experimental hosts, including banana and some species in the Poaceae (Eloja and Tinsley 1963).

Geographical distribution

Abaca mosaic disease has been reported only in the Philippines (Eloja and Tinsley 1963).

Transmission

The virus is transmitted by vegetative propagation and tissue culture, as well as by aphids (mainly *Rhopalosiphum maidis* and *Aphis gossypii*) in a non-persistent manner (Gavarrá and Eloja 1969; Ocfemia and Celino 1938). It is mechanically transmitted with difficulty (Dante A Benigno and Del Rosario 1965; Eloja et al. 1962).

Detection

The virus can be detected by ELISA using antibodies prepared against the abaca strain of SCMV (Gambley et al. 2004), or by RT-PCR (ADW Geering et al., unpublished) – see Appendix 1.

Therapy

No information reported.

5.5. Banana mosaic

Cause

The disease is caused by *Cucumber mosaic virus* (CMV) belonging to the genus *Cucumovirus*, family *Bromoviridae*. CMV has icosahedral particles 28–30 nm in diameter (Figure 18), separately encapsidating the tripartite single-stranded RNA genome components. Subgroup I and II isolates of CMV have both been recorded from banana.

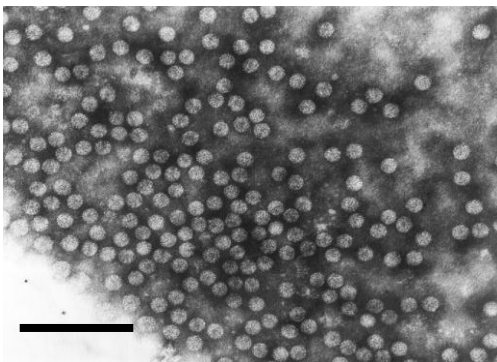


Figure 18. Virions of CMV; bar represents 200 nm (Rothamsted Research, UK).

Significance

Most strains of the virus are so-called ‘common’ strains, which do not produce severe symptoms or cause significant crop damage (Lockhart and Jones 2000a), though sometimes bunches may be distorted or bear smaller or fewer fruit. However, some severe strains are more damaging, with infection resulting in internal pseudostem necrosis (Figure 19) and even plant death (Niblett et al. 1994). The heart-rot strain found in Morocco is particularly destructive (Bouhida and Lockhart 1990). Plantlets derived from tissues culture are more prone to infection.

Hosts

CMV is known to infect over 800 different plant species, including cultivated crops (e.g. tomato, pepper and cucurbits) and weeds (e.g. *Commelina*), which serve as reservoirs for infection of banana.

Geographical distribution

CMV has a cosmopolitan distribution, i.e. it is found on all continents and in many countries, but some strains causing severe symptoms, e.g. heart-rot strain, are limited in distribution.

Symptoms

Symptoms are very variable, and include foliar mosaic (Figure 20), chlorotic streaking or flecking (Figure 21), and occasional leaf deformation, especially in young suckers developing from infected mother plants (Lockhart 2002). Uneven ripening has been also associated with the virus. Infected plants may show no symptoms, particularly suckers. In some varieties, high temperature may suppress symptoms. Symptoms have often been confused with those of BSV.



Figure 19. Internal necrosis (heart rot) symptoms in a CMV-infected banana plant (BEL Lockhart).

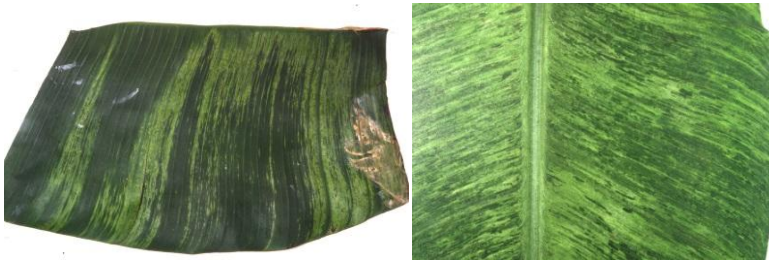


Figure 20. (left) Foliar mosaic streaking in CMV-infected leaves (JE Thomas).

Figure 21. (right) Chlorotic streaking in CMV-infected leaves (JE Thomas).

Transmission

CMV is transmitted from plant to plant in a non-persistent manner by many aphid species including *Aphis gossypi*, *Myzus persicae*, *Rhopalosiphum maidis* and *R. prunifoliae*. These aphids colonize a wide range of plant species and visit but do not colonize banana. As a result, CMV infection of banana occurs almost exclusively by aphid transmission from other plant species rather than from banana to banana (Lockhart 2002). The virus can also be spread over long distances during traditional vegetative propagation using suckers or during *in vitro* multiplication. Seed transmission has also been reported (Gold 1972).

Detection

A range of techniques can be used to detect CMV, including observation of symptoms, electron microscopy, the use of indicator plants, nucleic acid hybridization, serology and PCR. The latter two are recommended for reliable and sensitive routine detection of CMV. A number of ELISA kits are commercially available and several PCR assays have been described (e.g. Hu et al. 1995; Sharman et al. 2000b; Wylie et al. 1993), including multiplex assays (Sharman et al. 2000b; Liu et al 2012).

Therapy

Different methods have been developed and tested for CMV eradication, such as thermotherapy, chemotherapy, meristem culture and cryotherapy. A combination of these techniques can improve the eradication rate (Gupta 1986; Helliot et al. 2002; 2004). The efficiency of the eradication technique depends on (i) the virus strain characteristics, (ii) the type of tissue treated and (iii) the banana genotype. However, meristem culture from heat-treated *in*

vitro plantlet is recommended for best results. Protocols are available in Lassois et al. (2013).

5.6. Banana streak

Cause

Banana streak disease is caused by a group of related viruses (banana streak viruses, BSV) which are plant pararetroviruses belonging to the genus *Badnavirus* of the family *Caulimoviridae* (King et al. 2012). The viruses have non-enveloped bacilliform particles measuring $120\text{--}150 \times 30$ nm (Figure 22). Particles contain a non-covalently closed circular double-stranded DNA genome of approximately 7.4 kb made of three different open reading frames (King et al. 2012). BSV display important serological and molecular variability with sometimes more than 20% nucleotide differences in the most conserved RT-RNase H region of the genome. They are considered different badnavirus species, all causing the same disease. BSV exist in two states: as an episomal form, producing infection in the plant cells, or as viral DNA integrated within the B genome of banana. Both forms can be infectious in banana plants.

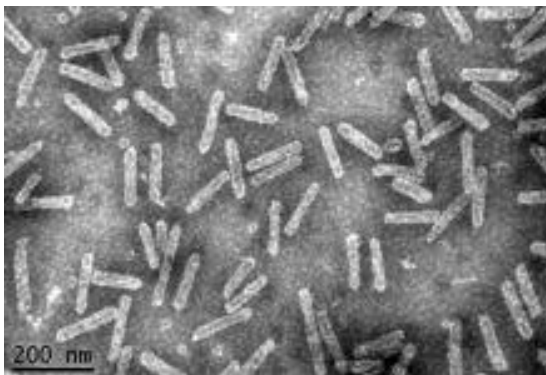


Figure 22. Electron micrograph of *Banana streak Mysore virus* particles (J Vo).

Significance

Few quantitative studies are known. Disease incidence varies between countries and this may be related to species differences, banana genotypes and/or vector activity. There is the potential for serious yield losses with some isolates (Lassoudière 1974). Plant death has been reported in Africa. Studies in

Australia with Banana streak Cavendish virus have demonstrated yield losses of 6–11% (Daniells et al. 2001).

Symptoms

Symptoms vary with isolates and cultivars. Most isolates produce broken or continuous chlorotic streaks or spindle-shaped patterns which are first chlorotic, then become increasingly dark in colour, and finally result in necrotic streaking in older leaves (Figure 23B and C). A range of other symptoms have been observed including failure of bunch emergence, splitting of the pseudostem (Figure 23A), and a 'traveller's palm' leaf arrangement (Daniells et al. 2001; Lockhart and Jones 2000b). Some isolates of BSV occurring in Africa produce severe necrosis, which begins with the cigar leaf and results in internal pseudostem necrosis and plant death. Other isolates produce very fine, indistinct broken brown interveinal streaks or pinpoints. Bunches may be reduced in size. Symptomless infection occurs frequently. Symptoms can appear sporadically, and may be absent for many months before reappearing. Symptom appearance and severity are associated with temperature changes and physiological age, but the precise correlation has not been experimentally determined. Symptoms are often confused with those caused by CMV.

Hosts

Musa species and cultivars are the natural hosts.

Experimental hosts include *Ensete* spp. and sugarcane, *Saccharum officinarum*.

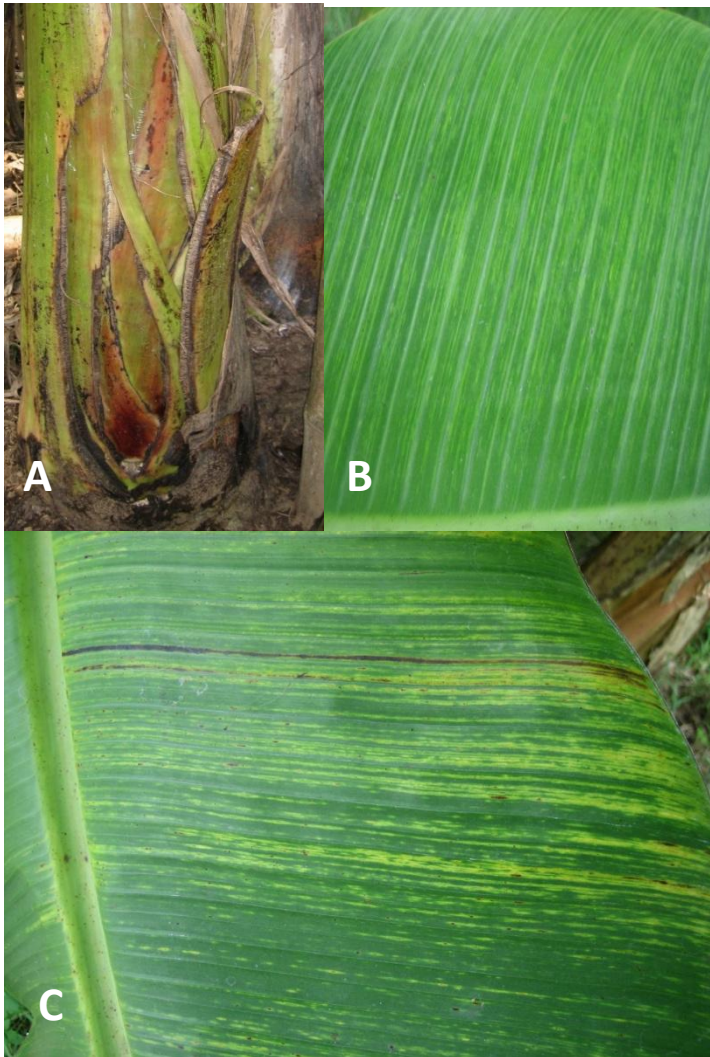


Figure 23. (A) Splitting of the pseudostem associated with BSV infection on plantain. (B) Discrete leaf symptoms associated with BSV on young leaves of a plantain (AAB). (C) Chlorotic and necrotic symptoms of BSV on an older leaf of the same plantain. (Photographs taken at the Centre Africain de Recherches sur Bananiers et Plantains (CARBAP) in Cameroon by M Chabannes.)

Geographical distribution

Figure 24 shows the distribution of BSV. The affected countries are also listed below (*unconfirmed – this status conferred by the quoted author).

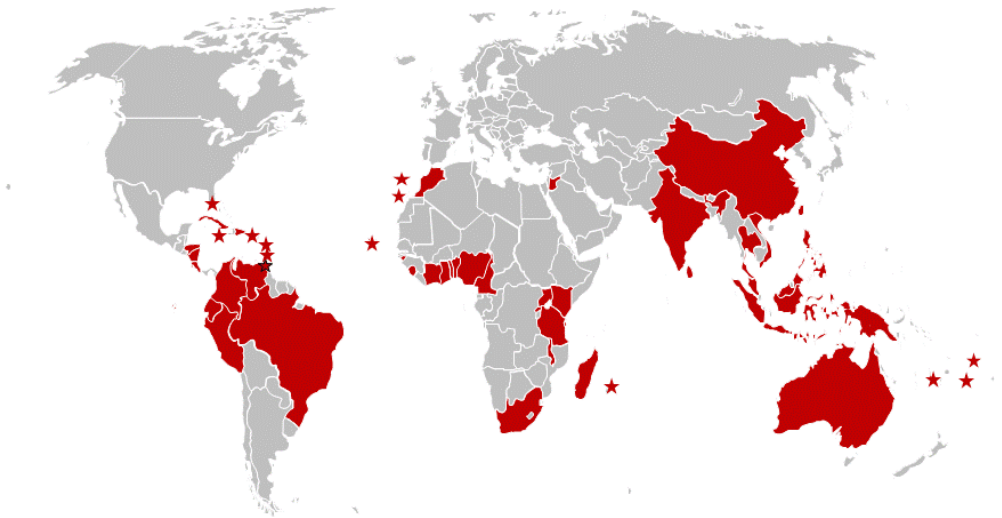


Figure 24. Distribution of BSV (affected countries shown in red).

Africa

Benin* (Pasberg-Gauhl et al. 1996)

Cameroon* (Gauhl et al. 1997; Lockhart 1995)

Cape Verde * (Lockhart 1995)

Côte d'Ivoire* (Lassoudière 1974, M-L Caruana, unpublished)

Ghana* (Pasberg-Gauhl et al. 1996)

Guinea Bissau* (Lockhart 1995)

Kenya* (Lockhart 1995)

Madagascar* (Jones and Lockhart 1993)

Malawi* (Vuylsteke et al. 1996)

Mauritius* (Jaufeerally-Fakim et al. 2006)

Morocco* (Lockhart 1986)

Nigeria* (Gauhl et al. 1999; Pasberg-Gauhl et al. 1996)

Rwanda* (Sebasigari and Stover 1988)

Sierra Leone (Lockhart 1995)

South Africa* (Jones and Lockhart 1993)

Tanzania* (Sebasigari and Stover 1988)

Togo (Lockhart 1995)

Uganda* (Dabek and Waller 1990; Geering et al. 2005; Harper et al. 2005)

Zanzibar* (Vuylsteke et al. 1998)

Asia

China, Peoples' Republic* (Zhuang et al. 2011)

India* (Cherian et al. 2004)

Indonesia (New Guinea)* (Davis et al. 2000; Lockhart 1995)

Jordan (Lockhart 1995)

Malaysia (Ang and Ong 2000)

Philippines* (Lockhart 1995)

Sri Lanka* (Lockhart 1995)

Taiwan* (Su et al. 1997)

Thailand* (Aung et al. 2012; Lockhart 1995)

Vietnam* (Lheureux et al. 2007; Lockhart 1995)

Europe

Spain (Canary Islands) (M-L Caruana, unpublished)

Portugal (Madeira) (Jones and Lockhart 1993)

Oceania

Australia* (Thomas et al. 1994)

New Caledonia* (Lockhart 1995)

Papua New Guinea (Davis et al. 2000)

Tonga* (Thomas et al. 1994)

Western Samoa* (Thomas et al. 1994)

South and Central America

Brazil* (Carnellosi et al. 2014)

Colombia* (Reichel et al. 2003)

Costa Rica* (Pasberg-Gauhl et al. 2000)

Cuba* (Javer et al. 2009; Jones and Lockhart 1993)

Dominican Republic* (P-Y Teycheney et al., unpublished)

Ecuador* (Jones and Lockhart 1993)

Grenada (Jones and Lockhart 1993)

Guadeloupe* (Jones and Lockhart 1993)

Honduras* (Jones and Lockhart 1993)

Jamaica (Jones and Lockhart 1993)

Martinique* (Jones and Lockhart 1993)

Nicaragua (Lockhart 1995)

Peru* (Pasberg-Gauhl et al. 2007)

Trinidad* (Jones and Lockhart 1993)

USA (Florida, Puerto Rico, Virgin Islands)* (Lockhart 1995)

Venezuela* (Garrido et al. 2005)

Transmission

Two means of transmission have been observed: horizontal and vertical transmission.

Horizontal transmission

BSV cannot be transmitted to *Musa* by mechanical inoculation. Field spread is mainly due to the citrus mealybug *Planococcus citri*. Sugarcane bacilliform virus (ScBV), which is closely related serologically to BSV, can be transmitted from infected sugarcane to banana by *P. citri* and the pink sugarcane mealybug (*Saccharicoccus sacchari*) in laboratory conditions, and produces typical streak symptoms (Lockhart and Autrey 1988). Such transmission has never been reported in field conditions. The mealybugs *Dysmicoccus brevipes* and *Planococcus ficus* are also vectors (Kubiriba et al. 2001; Meyer et al. 2008).

Vertical transmission

The virus is transmitted by vegetative propagation to 100% of progeny plants and the use of infected suckers contributes to the spread of the disease.

Since the late 1990s, numerous spontaneous outbreaks of banana streak disease have been observed in many banana-producing areas in banana breeding lines and micropropagated interspecific *Musa* hybrids originating from virus-free parents. The origin of these outbreaks was correlated with the presence of endogenous sequences of banana streak viruses (eBSV) integrated into the *Musa balbisiana* genome of the hybrids and cultivars. Infectious eBSV contain the complete functional viral genome. Three natural widespread BSV species have so far been identified as infectious integrants arising from the *M. balbisiana* genome: *Banana streak OL virus* (BSOLV), *Banana streak IM virus* (BSIMV) and *Banana streak GF virus* (BSGFV) (Chabannes et al. 2013; Gayral et al. 2008; Iskra-Caruana et al. 2010). In banana, abiotic stresses such as micropropagation by *in vitro* culture processes (Côte et al. 2010; Dallot et al. 2001), temperature differences or water stresses and genetic crosses (Lheureux et al. 2003) are known to contribute to triggering the activation of eBSV to form infectious, episomal BSV.

The presence of infective eBSV species in most B genomes poses a unique problem for the distribution of germplasm. No methods for inactivating these eBSVs are currently available. Therefore, a strategy has been proposed and accepted by the research community for the distribution of this germplasm from the ITC, which minimizes the risks associated with the distribution of BSV, especially non-eBSVs, to the recipient country. Details of this strategy can be found at www.musanet.org.

There is evidence that BSV is seed-transmitted in *Musa* (Daniells et al. 1995), though this work was conducted before the phenomenon of integration was discovered. This work involved the use of embryo-rescued hybrids of cv. Mysore (AAB) × I.C.1 (AAAA), and the infection of progeny through activation of integrants, rather than through seed transmission, cannot be discounted.

Detection

The main difficulty in diagnosing BSV is to distinguish active infections (episomal) from integrated viral sequences. Although infectious eBSV are only present in the *M. balbisiana* genome, all *Musa* spp. also carry partial or 'dead' integrated BSV sequences in their genomes. Thus PCR tests on any banana DNA can result in BSV-positive reactions. Diagnostic PCRs must be designed to accommodate this problem.

Serological detection of BSV is complicated by the wide serological diversity among virus isolates, some of which are serologically unrelated (Lockhart and Olszewski 1993). A polyvalent polyclonal antiserum raised against BSV species and Sugarcane bacilliform virus species (Ndowora 1998) is capable of detecting all known isolates by immunosorbent electron microscopy (ISEM) in partially purified extracts, even in asymptomatic leaf tissue (BEL Lockhart, unpublished).

Plants derived from *in vitro* material should be tested at least 3 months after plantlets have been acclimatised and planted out.

Detection of BSV by ELISA

ELISA test kits for BSV are commercially available from Agdia and DSMZ companies. Samples of laminar and midrib tissue from the three youngest expanded leaves, or symptomatic tissue if present, should be tested by ELISA.

Detection of BSV by ISEM

A partial purified extract is used to observe virus particles under the electron microscope (Geering et al. 2000).

Detection of BSV by IC-PCR

Immunocapture PCR (IC-PCR) to detect specific BSV species has been described (Geering et al. 2000; ADW Geering unpublished – see Appendix 1; Le Provost et al. 2006). Primers for the detection of various BSV species are shown in Appendix 1. Immunocapture conditions were optimized to minimize non-specific binding of plant genomic DNA to the immunocapture tubes. Alternatively, to avoid contamination by plant genomic DNA, a DNase I treatment is performed (Appendix 1).

Detection of BSV by RCA

Rolling circle amplification (RCA) is a technique that selectively amplifies circular DNA (such as encapsidated BSV genomic DNA) resulting in a specific amplification of DNA from episomal forms of BSV only. James et al. (2011) have developed and used RCA with the addition of BSV-specific primers for the detection of episomal BSV, and demonstrated the ability of this method to differentiate between episomal and integrated viral genomic sequences. This method avoids the need for antiserum, but does require knowledge of the virus sequence in the selection of restriction enzymes required for visualization of the virus-specific products. Identification of a particular BSV also requires subsequent PCR or sequencing.

Detection of BSV by LAMP

Loop-mediated isothermal amplification (LAMP) assay is a DNA amplification technique that does not require thermal cycling (Notomi et al. 2000). LAMP products can be detected using conventional agarose gel electrophoresis or using visual observation to estimate turbidity or colour changes (Mori et al. 2001). Peng et al. (2012a) have used the LAMP assay to detect BSV particles in infected banana plants. However this technique cannot differentiate between integrated and episomal forms so false positives could be very frequent. Also, primer design is complicated and is likely to be a constraint with the genetic diversity found in germplasm indexing.

Detection of eBSV

Based on the sequences and the structure of the different eBSV described in the seeded diploid (BB) *M. balbisiana* Pisang Klutuk Wulung (PKW), several PCR markers have been developed in order to genotype those integrations in different banana genotypes (Chabannes et al. 2013; Gayral et al. 2008; 2010), including (i) integration markers targeting eBSV insertion sites, (ii) structure markers targeting internally reorganized eBSV structures and (iii) allelic markers which allow the distinction between alleles of the same eBSV species. The targeted eBSV, the type of marker developed, the name and the sequence of the primer as well as the size of the PCR product are described in Chabannes et al. (2013).

Therapy

Cryopreservation followed by apical meristem culture has a high rate of success in eliminating episomal BSV infections in cv. Williams, a banana genotype free of eBSV (Helliot et al. 2002). Two anti-retroviral and anti-hepadnavirus molecules have also been effective in eradicating the episomal form of BSV from banana plants (Helliot et al. 2003). Methods are described in Lassois et al. (2013).

5.7. Banana mild mosaic

Cause

The disease is caused by *Banana mild mosaic virus* (BanMMV), an unassigned virus in the *Betaflexiviridae*. The virus has flexuous filamentous particles about 580 nm in length (Figure 25), with a coat protein of ca 26.8 kDa, and a single stranded RNA genome (Gambley and Thomas 2001; Thomas et al. 2000b).

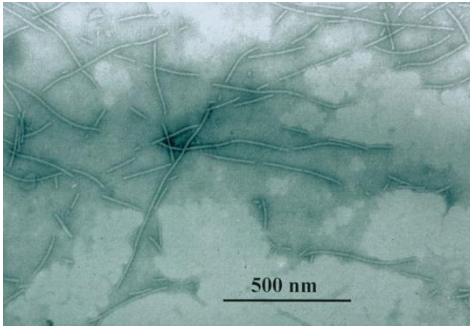


Figure 25. Virions of BanMMV (JE Thomas).

Significance

The economic impact of this virus has not been quantified, but observations suggest that yield may be affected in both banana and plantain in Latin America (Thomas et al. 2000b).

Symptoms

In many cases infected plants are symptomless, and in other cases symptoms are transitory. In cv. Ducasse (syn. Pisang Awak), chlorotic streaks and mosaic (Figure 26) may be present, especially on younger leaves, but these symptoms often disappear as the plant matures. Other cultivars, including Pisang Seribu, show fine silvery streaks on the leaves (Figure 27). Mixed infections with BSV are very common, and in these cases symptoms are dominated by those typical of BSV (JE Thomas and KS Parmenter, unpublished). There was a high correlation between leaf necrosis symptoms and mixed infections of BanMMV and *Cucumber mosaic virus* (CMV) in Guadeloupe, compared to mosaic symptoms with CMV infection alone (Caruana and Galzi 1998). BBrMV-infected samples from the Philippines were frequently found to be mixed infections with BanMMV (M-L Caruana, unpublished).



Figure 26. Mosaic symptoms on a young leaf of banana cv. Ducasse (syn. Pisang Awak) (JE Thomas).

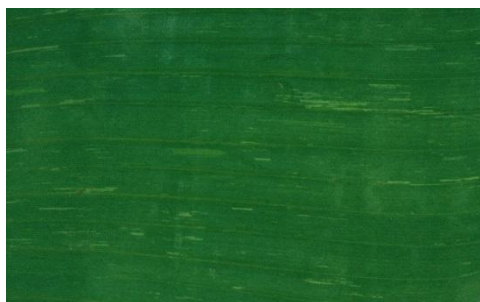


Figure 27. Chlorotic and silvery streaks on the leaf of a BanMMV-infected banana cv. Rose (JE Thomas).

Hosts

Hosts of BanMMV include *Musa* species and cultivars (Thomas et al. 2000b).

Geographical distribution

BanMMV is widespread and probably present in most banana-producing countries.

Transmission

BanMMV is transmitted through vegetative propagation and tissue culture. No mechanical transmission has been reported in *Musa* and no natural vectors have been identified. Attempts to transmit the virus from single infections in cv. Ducasse were unsuccessful with mealybugs (*Planococcus citri*), aphids (*Pentalonia nigronervosa*), via soil collected from around infected plants, or by root-to-root contact (M Sharman and JE Thomas, unpublished). However, circumstantial evidence for plant-to-plant spread by an unknown mechanism has been noted in Guadeloupe. Initially virus-free planting material was shown after field planting to be subsequently infected with CMV and BanMMV, implying co-transmission of the viruses in the field (Caruana and Galzi 1998). Also, analysis of 154 partial RdRp sequences of BanMMV from 68 banana accessions supported the possibility of horizontal transfer of the virus between plants. In general the sequences of different isolates displayed a high level of diversity (mean 20.4%), whereas the variability within individual isolates seemed to be only about 2%. Identical sequences were obtained from two independent pairs of plants, and there was a very steep gradient separating plants sharing identical or near identical sequences. About three-quarters of these plants were located 1–5 metres apart (Teycheney et al. 2005a).

Detection

Polyclonal antisera to BanMMV have been developed (Teycheney et al. 2007; JE Thomas, unpublished), though they are not commercially available. These antisera can be used in immunocapture RT PCR (see below) or in ISEM.

BanMMV displays a high degree of genetic diversity making detection by PCR particularly challenging. A set of 154 partial RdRp sequences showed a mean pairwise nucleotide sequence divergence of 20.4% (Teycheney et al. 2005a) and similarly, a set of 14 complete coat protein sequences showed a mean of 20.6% divergence (JE Thomas, unpublished). A group of partial CP sequences, primarily from the Ivory Coast, was particularly variable both compared with all other isolates (mean 35%) and within the group itself (mean 35%).

Teycheney et al. (2007) have described a nested immunocapture RT PCR assay for the detection of BanMMV, targeting the RdRp region of the flexiviridae genome and based on the primers of Foissac et al. (2005). This assay is not specific for BanMMV, but does not detect *Banana virus X* (Teycheney et al. 2007).

Alternative primers, targeting the coat protein gene (upstream primer) and the poly-A tail (downstream primer), have been developed (Gambley et al. 1999; Teycheney et al. 2005a; M Sharman and JE Thomas, unpublished). The published upstream primer sequence of BanCP2 (Teycheney et al. 2005a) contains a probable typographical error and should read 5'-TATGCNGCNTTYGAYTTCTTRGAYG-3' at positions 7042–7066 on the BanMMV genome. However this primer has from one to three mismatches within the first eight 3' bases in 23 of 54 available partial CP sequences on GenBank. Upstream primer BanMMV CP2 (3'-TGCCAACTGAYGARGAGCTRAATGC-5'; M Sharman, unpublished; see protocol in Appendix 1) similarly has mismatches in the first seven bases against 12 of 54 sequences. However, there is good correlation between ISEM results and IC RT PCR results when the latter primer has been used for extensive testing of banana germplasm (KS Crew and JE Thomas, unpublished). The latter primer has been modified to eliminate the majority of mismatches in the 3' end (BanMMV CP8; 5'-TGCCAACTGAYGARGARYTRAHGC-3'; JE Thomas, unpublished).

Therapy

BanMMV has been eliminated from cvs. Pisang Awak and Pisang Seribu using meristem culture and chemotherapy with a number of chemicals (of which ribavirin was clearly the most effective; Busogoro et al. 2006). Meristem culture alone has inadvertently eliminated BanMMV from selections of cv. Ducasse (syn. Pisang Awak) (SD Hamill, personal communication).

5.8. Banana virus X

Cause

The disease is caused by *Banana virus X* (BVX), an unassigned virus in the *Betaflexiviridae* (Teycheney et al. 2005b).

Significance

The economic significance is not known.

Hosts

The only known hosts are *Musa* spp. (Teycheney et al. 2005b).

Geographical distribution

BVX has been recorded only in Guadeloupe. However, the virus was relatively common in randomly sampled symptomless plants, and was associated with a *Musa* germplasm collection (Teycheney et al. 2005b). Although no surveys have been reported, it is likely to occur more widely in banana-growing countries.

Symptoms

All detections of the virus to date have been in symptomless plants.

Transmission

BVX is not transmitted by sap inoculation to a number of herbaceous test plant species (Teycheney et al. 2005b). No vector has been reported, but the virus is presumably transmitted through vegetative propagation, including tissue culture.

Detection

A nested RT PCR has been reported for the detection of BVX (Teycheney et al. 2007).

Therapy

None reported.

6. References

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Appendix 1. Diagnostic protocols

Diagnostic protocol for abaca mosaic (*Sugarcane mosaic virus, SCMV-Aba*)

RT-PCR (ADW Geering, unpublished)

Primers

SCMV-F15 '-TCT GGA CGG AAA TGT CGG C-3'

SCMV-R15'-CCT GTR TCC TGC AGA CTG G-3'

Product 253 bp

One step RT-PCR reaction mix:

Component	Volume (µL)
RNase-free water	25.0
5 x QIAGEN OneStep RT-PCR buffer	10.0
dNTP mix (10 mM of each dNTP)	2.0
Primer-F1 (2 µM)	5.0
Primer-R1 (2 µM)	5.0
QIAGEN OneStep RT-PCR enzyme mix	2.0
Total	49.0

Into separate 0.2 mL PCR tubes, add 49 µL of reaction mix and 1 µL of RNA.

Place the reaction tubes in a thermal cycler and subject to one cycle at 50°C for 30 min, followed by one cycle at 95°C for 15 min, 35 cycles at 95°C for 20 s, 64°C for 20 s and 72°C for 1 min and one cycle at 72°C for 5 min.

Electrophorese 8 µL of product on 1.5% agarose gel in 0.5 × TBE, stain with ethidium bromide.

Diagnostic protocol for Banana streak virus (BSV)

Detection of BSV by immunocapture-PCR (IC-PCR)

IC-PCR to detect specific BSVs has been described (Geering et al. 2000; ADW Geering unpublished; Le Provost et al. 2006). Immunocapture conditions were optimized to minimize non-specific binding of plant genomic DNA to the immunocapture tubes (Le Provost et al. 2006). Alternatively, and preferably, to avoid contamination by plant genomic DNA, a DNase I treatment is performed after the immunocapture step.

Immunocapture

1. Coat tubes with 50 µL of a 1/200 dilution of Agdia sugarcane bacilliform virus antibodies in carbonate coating buffer at room temperature for 4 h (note that BSMYV does not react well with the SCBV antibodies and an alternative source of antibodies is required for its detection).
2. Wash tubes 3 × 3 min with PBS-T.
3. Add 50 µL of sample extracts in extraction buffer (e.g. 0.05 M tri-sodium citrate, 0.5 mM EDTA, 0.05% Tween 20, 1% skim milk powder, 0.5% monothiolglycerol (adjusted to pH 8 with citric acid)).
4. Wash tubes 3 × 3 min with PBS-T and rinsed with ddH₂O.

DNase treatment

For DNase (RNase-free) treatment, 30 µL of DNase mix (3 µL 10 × buffer, 3 µL of DNase I and 24 µL of water) are added to the tubes after immunocapture of the virus particles and incubated for 1 h at 37°C. The supernatant is removed and the tubes washed once with water. DNase I is then inactivated by incubation at 95°C for 10 min.

PCR

Primers for the detection of various BSVs are shown in the table on page 57. The PCRs for BSOLV, BSMYV, BSCAV, BSIMV and BSGFV can be multiplexed as described below.

PCR detection of single viruses

To each immunocapture tube, add:

Component	Volume (μL)
5 × PCR buffer	5.0
dNTP mix (10 mM of each dNTP)	0.5
MgCl ₂ (50 mM)	0.75
Forward primer (1 μM)	2.5
Reverse primer (1 μM)	2.5
ddH ₂ O	13.8
<i>Taq</i> polymerase (5 U/ μL)	0.2
Total volume	25 μL

Place the reaction tubes in a thermal cycler and subject to one cycle at 94°C for 1 min, 35 cycles at 94°C for 10 s, 60°C for 15 s and 72°C for 1 min and one cycle at 72°C for 5 min.

Electrophorese 8 μL product on a 1.5% agarose gel in 0.5 × TBE.

Multiplex PCR (ADW Geering, unpublished)

The PCRs for BSOLV, BSMYV, BSCAV, BSIMV and BSGFV can be multiplexed. PCR conditions are as above, except with an annealing temperature of 58°C, and final concentration of 100 nM of each of the primers (except for BSMYV forward and reverse primers at 200 nM each).

Genomic DNA contamination

Genomic DNA contamination can be monitored using *Musa* sequence tagged microsatellite site primers AGMI025 [5'-TTA AAG GTG GGT TAG CAT TAG G-3'] and AGMI026 [5'-TTT GAT GTC ACA ATG GTG TTC C-3'] (Lagoda et al. 1998). Those primers were used in multiplex with BSV specific primers described above (Le Provost et al. 2006).

PCR primers for the detection of six BSV species:

Target virus ^A	Name of primer	Primer sequence	Size of prod. (bp)	Cycle no.	Anneal temp. (°C)
BSOLV	OL-F1	5'-ATCTGAAGGTGTGTTGATCAATGC-3'	522	30	58
	OL-R1	5'-GCTCACTCCGCATCTTATCAGTC-3'			
BSGFV	GF-F1	5'-ACGAACTATCAGACTTGTTCAAGC-3'	476	30	58
	GF-R1	5'-TCGGTGGAATAGTCTGAGTCTTC-3'			
BSMYV	Mys-F1	5'-TAAAAGCACAGCTCAGAACAAACC-3'	589	30	58
	Mys-R1	5'-CTCCGTGATTTCTTCGTGGTC-3'			
BSCAV	Cav-F1	5'-AGGATTGGATGTGAAGTTTGAGC-3'	782	30	60
	Cav-R1	5'-ACCAATAATGCAAGGGACGC-3'			
BSIMV	Im-F1	5'-TGCCAACGAATACTACATCAAC-3'	384	25	58
	Im-R1	5'-CACCCAGACTTTTCTTCTAGC-3'			
BSVNV	Viet-F1	5'-GCTCCATCCCTCTGACACAAGACTGGCA-3'	514	30	62
	Viet-R1	5'-CCAAGTGGAGTAGCGTGTTCACCGTG-3'			

^ABSOLV (*Banana streak OL virus*), BSGFV (*Banana streak GF virus*), BSMYV (*Banana streak MY virus*), BSCAV (*Banana streak CA virus*), BSIMV (*Banana streak IM virus*), BSVNV (*Banana streak VN virus*).

Diagnostic protocol for *Banana mild mosaic virus* (BanMMV)

BanMMV IC-PCR protocol (M Sharman, unpublished)

Primers

Poty1	5' GGA TCC CGG GTT TTT TTT TTT TTT V 3'
BanMMV CP2	5' TGC CAA CTG AYG ARG AGC TRA ATG C 3'
PCR product	ca. 250 bp

RT-PCR can be carried out using an immunocapture step as described below. Alternatively, a total nucleic acid extract can be prepared and used in RT-PCR (Teycheney et al. 2007, or as described below omitting the immunocapture step).

Immunocapture step

1. Coat thin-walled tubes with 2.5 µg/ml (50 µl per tube) of BanMMV IgG diluted in carbonate coating buffer. Incubate for ~2–3 h at room temperature.
2. Wash 3 × 3 min with PBS-T.
3. Add 50 µL of extract made at 1:10 in extraction buffer (0.05 M sodium citrate, 0.5 mM EDTA, 0.05% Tween 20, 1% skim milk powder, 0.5% monothiolglycerol pH 8.0). Incubate overnight at 5°C or 4–5 h at room temperature.
4. Wash 3 × 3 min with PBS-T, briefly rinse with ddH₂O, and spin down.

Synthesis of cDNA

To the IC tubes add:

20 µM Poty1 primer	1.5 µL
ddH ₂ O	23 µL

Heat to 80°C for 10 min, chill on ice and spin down.

From a master mix, add to IC tubes on ice:

Component	Volume (μL)
5 \times 1st strand buffer	8
0.1 M DTT	4
10 mM dNTPs	2
SuperScript III 200 U/ μL (Invitrogen)	0.5
0.6 $\mu\text{g}/\mu\text{L}$ BSA	0.5
RNase guard \sim 40 U/ μL (Amersham)	0.5

Incubate at 50°C for 45 min then 70°C for 15 min, chill and spin down. Store on ice or at -20°C.

Poty1- BanMMCP2 PCR

Use the following PCR mix per tube:

Component	Volume (μL)
10 \times PCR buffer	2.5
MgCl ₂ (50 mM)	1.0
dNTPs (10 mM)	0.5
Poty1 (10 μM)	1.0
BanMMCP2 (20 μM)	1.0
Taq 5 U/ μl (Gibco)	0.3
ddH ₂ O	16.7
cDNA template	2.0
Total volume	25 μL

Place the reaction tubes in a thermal cycler and subject to one cycle at 94°C for 1 min, 35 cycles at 94°C for 20 s, 55°C for 30 s and 72°C for 1 min and one cycle at 72°C for 3 min.

Electrophorese 8 μL of product on 1% agarose gel, stain with ethidium bromide.

Appendix 2. Example germplasm health statement

BIOVERSITY INTERNATIONAL GERmplasm HEALTH STATEMENT

ITC Accession Number:

Accession Name:

Origin of Accession:

The material designated above was obtained from a shoot-tip cultured *in vitro*. Shoot-tip culturing is used to eliminate the risk of the germplasm carrying fungal bacterial and nematode pathogens and insect pests of *Musa*. However, shoot-tip cultures could still carry virus pathogens.

SCREENING FOR VIRUS PATHOGENS

A representative sample of four plants derived from the same shoot-tip as the germplasm designated above has been grown under quarantine conditions for at least 6 months, regularly observed for disease symptoms and tested for virus pathogens as indicated below following methods recommended in the Bioversity International Technical Guidelines for the Safe Movement of *Musa* Germplasm (2015) for the diagnosis of virus diseases.

- PCR-based methods BBTV – Banana bunchy top virus
 CMV – Cucumber mosaic virus
 BBrMV – Banana bract mosaic virus
 BSV – Banana streak viruses
 BanMMV – Banana mild mosaic virus
- Electron microscopy isometric virus particles - includes CMV and unknown viruses
 bacilliform virus particles – includes unknown BSVs
 filamentous virus particles – includes BBrMV, BanMMV and unknown viruses

[P] = test positive, [N] = test negative, [] = test not undertaken.

DISTRIBUTION OF VIRUS PATHOGENS AND OTHER INFORMATION

(Example: BBTV and BBrMV are not known to occur in country of origin)

eBSVs are present in the B genome of *Musa* (banana). Consequently, almost all accessions containing the B genome may develop BSV infection and may express symptoms during any stage of growth.

The information provided in this germplasm statement is based on the results of tests undertaken at Bioversity International's Virus Indexing Centre by competent virologists following protocols current at the time of the test and on present knowledge of virus disease distribution. However, neither Bioversity International nor its Virus Indexing Centre staff assume any legal responsibility in relation to this statement.

Signature

Date

This statement provides additional information on the phytosanitary status of the plant germplasm described herein. It should not be considered as a substitute for the official "Phytosanitary Certificate" issued by the plant quarantine authorities of Belgium.



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