

Musa spp.

(2nd edition)

edited by **M. Diekmann and C.A.J. Putter**



Previously published Technical Guidelines for the Safe Movement of Germplasm

Cocoa	1989
Edible Aroids	1989
<i>Musa</i> (1st edition)	1989
Sweet Potato	1989
Yam	1989
Legumes	1990
Cassava	1991
Citrus	1991
Grapevine	1991
Vanilla	1991
Coconut	1993
Sugarcane	1993
Small fruits (<i>Fragaria</i> , <i>Ribes</i> , <i>Rubus</i> , <i>Vaccinium</i>)	1994
Small Grain Temperate Cereals	1995

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INTRODUCTION

Collecting, 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 pests¹ along with the host plant. In particular, pathogens that are often symptomless, such as viruses, pose a special risk. In order to manage this risk, effective testing (indexing) procedures are required to ensure that distributed material is free of pests that are of quarantine concern.

The ever-increasing volume of germplasm exchanged internationally for research purposes, coupled with recent advances in biotechnology, has created a pressing need for crop-specific overviews of the existing knowledge in all disciplines relating to the phytosanitary safety of germplasm transfer. This has prompted FAO and IPGRI to launch a collaborative programme for the safe and expeditious movement of germplasm, reflecting the complementarity of their mandates with regard to the safe movement of germplasm. FAO, as the depository of the International Plant Protection Convention of 1951, has a long-standing mandate to assist its member governments to strengthen their plant quarantine services, while IPGRI's mandate—*inter alia*—is to further the collecting, conservation and use of the genetic diversity of useful plants for the benefit of people throughout the world.

The purpose of the joint FAO/IPGRI programme is to generate a series of crop-specific technical guidelines that provide relevant information on disease indexing and other procedures that will help to ensure phytosanitary safety when germplasm is moved internationally. The scope of the recommendations in these guidelines is confined to small, specialized consignments used in technical crop improvement programmes, e.g. for research and basic plant breeding programmes. When collecting germplasm, local plant quarantine procedures, for example pest risk assessment, should be considered.

These technical guidelines are produced by meetings of panels of experts on the crop concerned, who have been selected in consultation with the relevant specialized institutions and research centres. The experts contribute to the elaboration of the guidelines in their private capacities and do not represent the organizations for whom they work. The guidelines are intended to be the best possible advice for institutions involved in germplasm exchange for research, conservation and basic plant breeding. FAO, IPGRI and the contributing experts cannot be held responsible for any failures resulting from the application of the present guidelines. By their nature, they reflect the consensus of the

¹ The word 'pest' is used in this document as it is defined in the International Plant Protection Convention. It encompasses all harmful biotic agents ranging from viroids to weeds.

crop specialists who attended the meeting, based on the best scientific knowledge available at the time of the meeting. The experts who have contributed to this document are listed after this introduction.

The guidelines are written in a short, concise style, in order to keep the volume of the document to a minimum and to facilitate updating. Suggestions for further reading are given at the end, along with the reference cited in the text (mostly for geographical distribution, media and other specific information). The guidelines are divided into two parts. The first part makes general recommendations how best to move *Musa* germplasm. The second part covers the important pests and diseases of quarantine concern. The information given on a particular pest or disease is not exhaustive but concentrates on those aspects that are most relevant to quarantine.

The present guidelines were developed at an FAO-sponsored meeting held in Rome, Italy from 19 to 21 June 1995. The meeting was hosted by the International Plant Genetic Resources Institute and its programme, the International Network for the Improvement of Banana and Plantain (INIBAP). These guidelines supersede those published in 1989 (Frison and Putter 1989).

Guideline update

In order to be useful, the guidelines need to be updated when necessary. We ask our readers to kindly bring to our attention any developments that possibly require a review of the guidelines, such as new records, new detection methods or new control methods. For your convenience, a form is provided on the last page of this publication.

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GENERAL RECOMMENDATIONS

- Germplasm should be obtained from the safest source possible. There is, for example, a pathogen-tested *Musa* germplasm collection accessible at INIBAP's Transit Centre at the Laboratory of Tropical Crop Improvement, Katholieke Universiteit Leuven, Kardinaal Mercierlaan 92, 3001 Heverlee, Belgium. The International Institute of Tropical Agriculture's indexed *Musa* germplasm can be obtained from their Plantain and Banana Improvement Program, IITA, c/o L.W. Lambourn & Co., Carolyn House, 26 Dingwall Road, Croydon CR9 3EE, UK.
- All germplasm should be moved in the form of tissue culture. If this is not possible, full quarantine measures 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 specified in these guidelines. However, in some instances tests may be omitted if there is strong, 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 attached.

TECHNICAL PROTOCOLS

Vegetative material

1. Select a sucker from a plant without symptoms of systemic infection.
2. The sucker should be trimmed to remove soil, roots and any other extraneous material, leaving part of the central corm containing the meristem and about 10 cm above it. The overall dimension of the block of tissue will be about 20 cm high and 10-15 cm in diameter. The block should be air-dried for 2-3 days and wrapped in newspaper. The material should be labeled and dispatched in a cardboard box. No plastic should be used for wrapping.
3. The material should be sent to an appropriate tissue culture laboratory in the country of origin, or if this is not possible, a tissue culture laboratory which preferably should not be in a banana-growing area.
4. Meristems should be excised, surface-disinfected and cultured.
5. The meristem culture should be cloned to seven plantlets, of which five should be

sent to an indexing facility and two should remain in culture for future multiplication.

6. At the indexing facility, four plants should be established in a vector-free, insect-proof greenhouse under conditions conducive to vigorous plant growth (the fifth serves as a back-up).
7. After 3 months of growth, tissue samples should be taken from the three youngest expanded leaves and indexed for viruses as described below.
8. Three months later, tissue samples should again be taken from the three youngest expanded leaves and indexed for viruses as described below. In addition, electron microscopic observations should be undertaken to look for the presence of other viruses. The minipreps described in the BSV section can be used for this.
9. If all tests are negative, the four indexed plants may be released and the cultures derived from the two remaining subclones may be further propagated and distributed *in vitro*. For the movement of *in vitro* material, neither charcoal, fungicides nor antibiotics should be added to the medium. *In vitro* cultures should be shipped in transparent tubes and visually inspected for bacteria, fungi and arthropods. Contaminated material should be destroyed.

Seed

Seed should be free of pulp, air-dried, inspected for the absence of insect pests and fumigated when necessary. They should be sent to an appropriate tissue culture laboratory in the country of origin, or if not possible, preferably in a non-banana growing area.

1. Seed should be surface-disinfected with 0.5% sodium hypochlorite for 10 minutes at room temperature to eliminate externally seed-borne pathogens.
2. The seed coat should be removed before culturing *in vitro*.
3. Seedlings should be indexed in the same way as material derived from meristem culture.

DEFINITIONS OF TERMS AS USED IN THIS PUBLICATION

Cosmopolitan

This expression is used to describe the distribution of pathogens which are reported to occur in all continents, and in many countries of these continents.

Germplasm

A set of different genotypes conserved or used in breeding programmes.

Transit centre

Operated by INIBAP at the Katholieke Universiteit Leuven, Belgium in order to facilitate a safe exchange of *Musa* germplasm. With over 1000 accessions the Transit Centre contains the largest *in vitro* collection in the world.

Virus Indexing Centres (VIC)

Operated by INIBAP at Virology Research Units having expertise with *Musa* viruses, namely at CIRAD-FLHOR, Montpellier, France (Officer-in-Charge: Dr Marie-Line Iskra-Caruana); QDPI, Brisbane, Australia (Officer-in-Charge: Dr John Thomas); TBRI, Pingtung, Taiwan (Officer-in-Charge: Dr Sin-Wan Lee). Other laboratories, such as the one at IITA, also index *Musa* germplasm for viruses.

DESCRIPTIONS OF DISEASES

Abaca mosaic

Cause

A potyvirus, possibly a strain of sugarcane mosaic potyvirus, causes the disease. The flexuous rod particles measure about 680 nm.

Significance

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

Symptoms

Leaves show yellowish or light green streaks (Fig. 1). Petioles and midribs are mottled with dark green and yellowish streaks, even when no symptoms appear on the leaves (Fig. 2).

Hosts

- natural: *Musa textilis* (abaca, Manila hemp), *Marantha arundinacea*, *Canna indica*.
- experimental: several experimental hosts, including banana.

Geographical distribution

Philippines.

Transmission

The virus is transmitted by vegetative propagation and tissue culture, as well as by aphids (mainly *Rhopalosiphum maidis* and *Aphis gossypii*) in a nonpersistent manner. Mechanical transmission is extremely difficult.

Detection

The virus can be detected by ELISA using antibodies for sugarcane mosaic virus (Eloja and Tinsley 1963).

Treatment

No information reported.

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Fig. 1. Mosaic symptoms caused by abaca mosaic virus. (Dr L. Magnaye, BPI, Davao City)

Fig. 2. Dark green mottling on banana petioles caused by abaca mosaic virus. (Dr L. Magnaye, BPI, Davao City)



Banana bract mosaic

Cause

The disease is caused by banana bract mosaic potyvirus (BBMV). The virus consists of flexuous filamentous particles about 700-750 nm in length.

Significance

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

Symptoms

Symptoms progressively develop as distinct, dark coloured, broad streaks on the bracts of the inflorescence (Fig. 3). A shortening of bunch internodes is also characteristic. After removal of dead leaf sheaths, the presence of large, dark coloured stripes of varying length, sometimes with a mosaic pattern, is diagnostic of the disease (Fig. 4). Greenish to brownish broad, irregularly scattered spindle streaks develop along the petioles, possibly with raised veins (Fig. 5). Leaf symptoms of chlorotic spindle-shaped lesions may or may not occur.

Hosts

Musa species and cultivars.

Geographical distribution

Philippines (Magnaye and Espino 1990), India (where the disease is commonly found on French plantain in Kerala State and is called 'Kokkan') and Sri Lanka (Thomas *et al.* 1996).



Fig. 3. Conspicuous mosaic in a bract caused by BBMV.
(Dr D.R. Jones, INIBAP, Montpellier)

Transmission

No mechanical transmission has been reported in *Musa*. In addition to transmission through vegetative propagation and tissue culture, transmission by the aphids *Rhopalosiphum maidis*, *Aphis gossypii* and *Pentalonia nigronervosa* has been reported.

Detection

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

Treatment

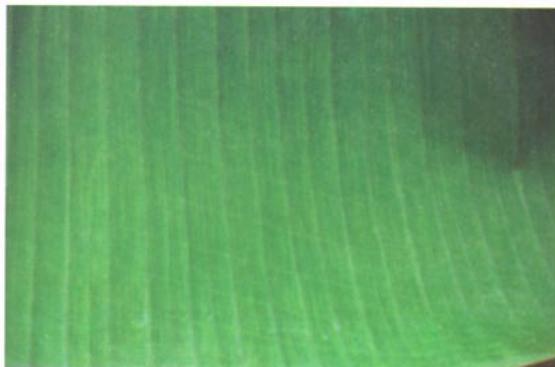
No information reported.

For bibliography see p. 23



Fig. 4. Large, dark coloured stripes underneath a dead leaf sheath, caused by BBMV (Dr L. Magnaye, BPI, Davao City)

Fig. 5. Subtle chlorotic streak and raised vein symptoms of BBMV on a leaf of Cardaba (ABB/BBB)
(Dr D.R. Jones, INIBAP, Montpellier)



Banana bunchy top

Cause

Banana bunchy top virus (BBTV) has been consistently associated with the disease. The virus has 20 nm isometric virions with a coat protein subunit of 20.1 kDa and a multicomponent single-stranded DNA genome.

Significance

The virus causes substantial disease outbreaks if the aphid vector *Pentalonia nigronervosa* is present. A serious epidemic has been reported recently from Pakistan (Soomro *et al.* 1992). Several countries (e.g. Australia, Taiwan, 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 (Figs. 6, 7). These streaks, however, may be rare or absent in abaca (*Musa textilis*) and *Ensete* spp. Leaves become progressively shorter and develop marginal chlorosis (Fig. 8). As the disease progresses, leaves become more upright or 'bunched' at the apex of the plant (Fig. 9). 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, no leaf symptoms may appear, but dark green streaks may be seen on the tips of the bracts. Mild symptoms of vein clearing as well as symptomless infections have been reported from Taiwan. Attenuation of initial severe symptoms has been reported in the cv. Veimama from Fiji.

Hosts

Musa species and cultivars; *Ensete ventricosum* has been experimentally infected. There is some evidence for the existence of alternative hosts: *Canna indica* and *Hedychium coronarium* (Su *et al.* 1993).

Geographical distribution

Note: new records which were not in the 1989 guidelines are printed in **bold**; * = unconfirmed (this status conferred by the quoted author).



Fig. 6. Dark green streaks on the petiole caused by BBTV.

(Dr M.L. Iskra-Caruana, CIRAD, Montpellier)

Fig. 7. Dark green streaks on a banana leaf caused by BBTV.
(Dr J. Thomas, QDPI, Indooroopilly)



Africa:

Burundi (Sebasigari and Stover 1988)

Central African Republic (Fouré and Lassoudiere, unpublished)

Congo (Wardlaw 1961)

Egypt (Magee 1953)

Gabon (Manser 1982)

Rwanda (Sebasigari and Stover 1988)

Zaire (Manser 1982)

Oceania:

Australia (Magee 1927)

Fiji (Magee 1927)

Guam (Beaver 1982)

Kiribati (Shanguganathan 1980)

Wallis Island (Simmonds 1933)

New Caledonia" (Buddenhagen 1968)

Tonga (Magee 1927)

Tuvalu (Ellice Island: Campbell 1926)

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

Western Samoa (Magee 1927)

Asia:

Bangladesh* (Fouré and Manser 1982)

China (Anonymous 1979)

Hong Kong* (Buddenhagen 1968)

Indonesia (Sulyo and Muharam 1985)

India, Sri Lanka (Magee 1953)

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

Fig. 8. Marginal chlorosis due to BBTV on a plant derived from tissue culture.
(Dr M.L. Iskra-Caruana, CIRAD, Montpellier)



Fig. 9. Bunched appearance of the apex, caused by BBTV.
(Dr J. Thomas, QDPI, Indooroopilly)



Kampuchea* (Stover 1972)

Laos* (Stover 1972)

Malaysia (Su *et al.* 1993)

Myanmar* (Buddenhagen 1968)

Pakistan (Soomro *et al.* 1992)

Philippines (Castillo and Martinez 1961)

Taiwan (Sun 1961)

Vietnam (Vakili 1969)

The virus is reported to occur in symptomless plants or in plants with mild symptoms from India, Malaysia, South Africa, Taiwan and Thailand (Su *et al.* 1993). The phenomenon of symptomless infections of BBTV in bananas is currently being investigated in other laboratories.

Transmission

The virus is transmitted vegetatively, through tissue culture and by the aphid vector *Pentalonia nigronervosa*. No mechanical transmission has been reported.

Detection

The virus can be reliably detected by ELISA (enzyme-linked immunosorbent assay). Monoclonal and polyclonal antibodies are commercially available (Wu and Su 1990b; Dietzgen and Thomas 1991). Samples from midribs of leaf tips should be indexed 3 months after plantlets have been established from tissue culture. DNA probes are available for BBTV DNA components 1 to 6 (Burns *et al.* 1995).

Treatment

Meristem-tip culture (Thomas *et al.* 1995b), possibly combined with heat therapy (Ramos and Zamora 1990; Wu and Su 1991), has been successful in achieving a proportion of virus-free plantlets. Testing after treatment is essential.

For bibliography see p. 23

Banana mosaic

Cause

The disease is caused by cucumber mosaic cucumovirus (CMV), a virus with a tripartite single-stranded RNA, packaged in icosahedral particles about 28 nm in diameter. The two major serogroups of the virus, coinciding with two hybridization groups, occur in *Musa*.

Significance

Occasionally, severe outbreaks occur. Plantlets derived from tissue culture are more prone to infection. Numerous strains exist, varying from those not causing symptoms to those inducing mild or severe symptoms in banana. The heart-rot strain found in Morocco is particularly destructive (Wardlaw 1972).

Symptoms

Mild or severe chlorosis, chlorotic streaking or flecking, mosaic patterns and leaf distortion (Figs. 10, 11). The heart-rot strain causes severe yellowing and necrosis, which begins on the cigar leaf and spreads into the pseudostem (Fig. 12). Eventually the pseudostem rots. Uneven ripening has been associated with the virus. Suckers produced from infected plants may show no symptoms. In some varieties, high temperature may suppress symptoms. Symptoms have often been confused with those of BSV.

Hosts

Extremely wide host range, including numerous dicotyledon and monocotyledon species.

Geographical distribution

Cosmopolitan; some strains causing severe symptoms, e.g. heart-rot strain, are limited in distribution.

Transmission

The virus is transmitted in a nonpersistent manner by aphids, including *Aphis gossypii*, *Rhopalosiphum maidis*, *R. prunifoliae* and *Myzus persicae*. In experimental studies, when associated with BBTV, CMV was transmitted by *Pentalonia nigronervosa*. Seed transmission has been reported (Gold 1972).



Fig. 10. Mild symptoms of CMV infection. (Dr H.J. Su, National Taiwan University, Taipei)

Detection

The virus can be reliably detected by ELISA using polyclonal and monoclonal antibodies for CMV, or by mechanical inoculation to a range of diagnostic test plants, e.g. *Chenopodium amaranticolor*, *C. quinoa* and *Vigna unguiculata* (Francki *et al.* 1979).

Treatment

Virus-free plantlets have been obtained by culture of meristem from heat-treated suckers (Gupta 1986) or from lateral buds developed on heat-treated rhizomes (Berg and Bustamante 1974).

For bibliography see p. 25



Fig. 11. Severe symptoms of CMV infection, including leaf distortion. (Dr H.J. Su, National Taiwan University, Taipei)

Fig. 12. Heart-rot symptoms caused by CMV (Dr H.J. Su, National Taiwan University, Taipei)

Banana streak

Cause

The disease is caused by banana streak badnavirus (BSV). The virus consists of nonenveloped bacilliform particles measuring 120-150 x 30 nm. In some isolates longer particles of up to 1500 nm length occur. Particles contain a circular double-stranded DNA genome approximately 7.4 kb in size.

Significance

Few quantitative studies are known; there is the potential for serious yield losses with some isolates (Lassoudiere 1974). Plant death has been reported in Africa. Disease incidence varies between countries and this may be related to strain differences and/or vector activity. Tissue culture plantlets seem to be very susceptible.

Symptoms

Symptoms vary with isolates and cultivars. Most isolates produce broken (Fig. 13) or continuous (Fig. 14) chlorotic streaks or spindle-shaped patterns which are first chlorotic, then become increasingly dark in colour, and finally result in black streaking in older leaves (Fig. 15). Some isolates of BSV occurring in Africa produce severe necrosis which begins with the cigar leaf and results in internal pseudostem necrosis (Fig. 16) and plant death. Other isolates produce very fine, indistinct broken brown interveinal streaks or pin-points. Bunches may be reduced in size. Symptomless infection occurs frequently. Symptoms appear sporadically, and may be absent on leaves produced during many months before reappearing. Symptom appearance and severity are associated with temperature changes, but the precise correlation has not been experimentally determined. Symptoms are often confused with those of CMV.

Hosts

- natural: *Musa* species and cultivars.
- experimental: *Ensete* spp.

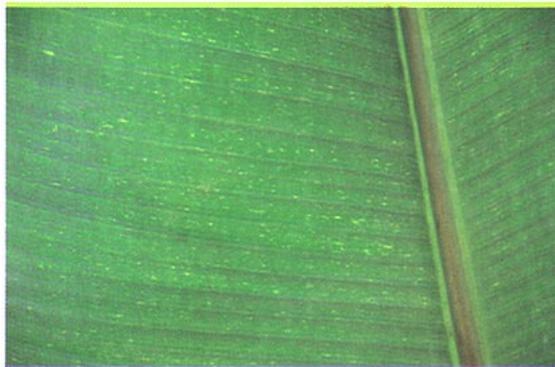


Fig. 13. Discrete leaf symptoms associated with BSV on the plantain hybrid TMP x 548-9 consisting of whitish to yellow flecks.

(Drs C. Pasberg-Gauhl and F. Gauhl, IITA, Ibadan)

Geographical distribution

Banana streak is found in many *Musa*-producing areas. Symptoms of BSV have been observed in the following countries and localities (* = confirmed by electron microscopy or serology):

Europe:

Spain (Canary Islands) * (Caruana, unpublished)

Portugal (Madeira) * (Jones and Lockhart 1993)

Africa:

Benin * (Lockhart, unpublished)

Cameroon * (Caruana, unpublished)

Cape Verde * (Caruana, unpublished)

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

Ghana * (Lockhart, unpublished)

Guinea * (Caruana, unpublished)

Kenya (Musabyimana, unpublished)

Madagascar * (Jones and Lockhart 1993)

Malawi * (Vuylsteke *et al.* 1996)

Mauritius * (Jones and Lockhart 1993)

Morocco * (Lockhart 1986)

Nigeria * (Jones and Lockhart 1993)

Rwanda * (Sebasigari and Stover 1988)

South Africa * (Jones and Lockhart 1993)

Tanzania * (Sebasigari and Stover 1988)

Uganda * (Dabek and Waller 1990)

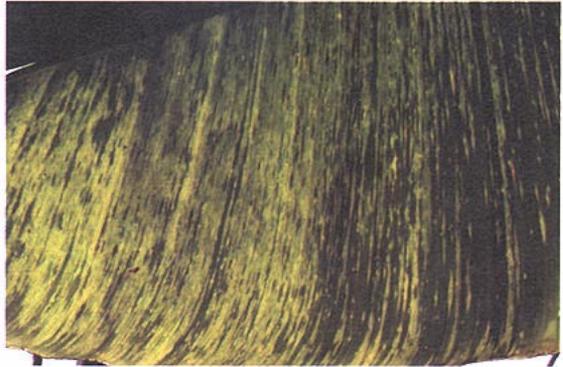


Fig. 14. Continuous chlorotic streaks caused by BSV. (Dr B.E.L. Lockhart, University of Minnesota, St. Paul)

Fig. 15. Chlorotic and necrotic symptoms of BSV on a leaf of Mysore (AAB). (Dr D.R. Jones, INIBAP, Montpellier)

South and Central America:

Brazil (Jones and Lockhart 1993)
Colombia * (Caruana, unpublished)
Costa Rica * (Lockhart, unpublished)
Cuba * (Jones and Lockhart 1993)
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)
Trinidad * (Jones and Lockhart 1993)
USA (Florida, Virgin Islands) * (Lockhart, unpublished)
Venezuela (Jones 1995)

Asia:

China, Peoples' Republic (Jones and Lockhart 1993)
India * (Thomas and Jones, unpublished)
Indonesia (Jones, unpublished)
Malaysia (Jones, unpublished)
Philippines * (Caruana, unpublished)
Sri Lanka * (Thomas and Jones, unpublished)
Thailand (Jones, unpublished)
Vietnam (Jones, unpublished)

Oceania:

Australia * (Thomas *et al.* 1994)
New Caledonia * (Lockhart, unpublished)
Papua New Guinea (Jones, unpublished)
Tonga * (Thomas *et al.* 1994)
Western Samoa * (Thomas *et al.* 1994)

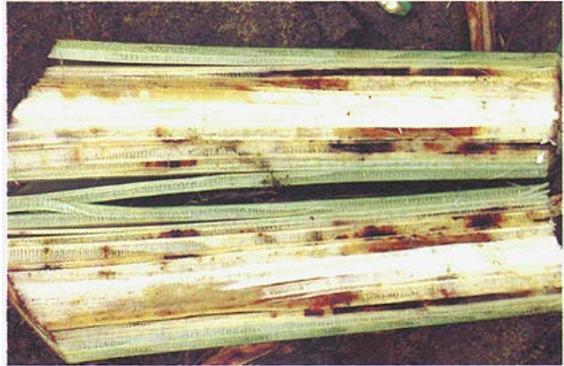
Transmission

BSV has not been transmitted to *Musa* by mechanical inoculation. The virus is transmitted by vegetative propagation to 100% of progeny plants. Field spread is by the citrus mealybug (*Planococcus citri*). Sugarcane bacilliform virus (ScBV), which is closely related serologically to BSV, is transmitted from infected *Saccharum officinarum* to banana by *P. citri* and the pink sugarcane mealybug (*Saccharicoccus sacchari*), and produces typical streak symptoms (Lockhart and Autrey 1988). There is evidence that BSV is seed-transmitted in *Musa* (Daniells *et al.* 1995).

Detection

Serological detection of BSV is complicated by the occurrence of a wide degree of serological diversity among virus isolates, some of which are unrelated serologically to

Fig. 16. Longitudinal section of the pseudostem (plantain hybrid TMP x 597-4) showing necrotic tissue associated with severe BSV infection. (Drs C. Pasberg-Gauhl and F. Gauhl, IITA, Ibadan)



each other (Lockhart and Olszewski 1993). A recently developed antiserum raised against many isolates is capable of detecting all known isolates by ISEM in partially purified extracts, even in asymptomatic leaf tissue (Lockhart, unpublished). Samples of laminar and midrib tissue from the three youngest expanded leaves should be tested 3 months after plantlets have been established from tissue culture.

Miniprep protocol for ISEM: Extract 5-7 g leaf tissue in 18 ml 200mM phosphate buffer pH 6.0, containing 1% Na_2SO_3 . Filter, centrifuge 10 minutes at low speed and discard pellet. Add 1 ml 33% Triton X-100, mix well, and layer over 5 ml 30% sucrose in 100mM phosphate buffer pH 7.2. Centrifuge 1 hour at 35 000 rpm in Beckman Type 50.2 rotor. Discard supernatant and rinse sides of tube with distilled water. Resuspend pellet in 100 μl 10mM phosphate buffer pH 7.2 containing 0.85% NaCl. Centrifuge for 8-10 minutes at 12 000-15 000 rpm in standard microfuge, discard pellet, retain partially purified extract for ISEM examination. Dilute antiserum 1/1000 in 10nM Tris-HCl pH 7.4. Place EM grid on a 10 μl drop of diluted antiserum. Incubate in a Petri dish moist chamber for 15-30 minutes at room temperature. Rinse with 15-20 drops distilled water. Place coated grid on a drop of partially purified extract, incubate overnight at 4°C. Rinse with 10-20 drops of 2% sodium phosphotungstate (PTA), pH 6.8. Examine in electron microscope.

Treatment

No information reported for BSV. Thermotherapy followed by apical meristem culture failed to eliminate or reduce the titre of the related SCBV in sugarcane (Lockhart, unpublished).

For bibliography see p. 25

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INIBAP GERMLASM 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 believed 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 FAO/IPGRI Technical Guidelines for the Safe Movement of *Musa* Germplasm for the diagnosis of virus diseases.

Serology-ELISA BBTV - banana bunchy top virus
 CMV - cucumber mosaic virus
 BBMV - banana bract mosaic virus
 BSV - banana streak virus

Electron isometric virus particle - includes CMV
microscopy bacilliform virus particle - includes BSV
 filamentous virus particle - includes BBMV

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

DISTRIBUTION OF VIRUS PATHOGENS AND OTHER INFORMATION

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

The information provided in this germplasm statement is based on the results of tests undertaken at INIBAP's Virus Indexing Centres by competent virologists following protocols current at the time of the test and on present knowledge of virus disease distribution. However, neither INIBAP 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.

Comments on Technical Guidelines for the Safe Movement of *Musa* Germplasm

Please send to:

Germplasm Health Scientist and
IPGRI

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00145 Rome, Italy

Fax: +39-6-5750309

Chief, Plant Protection Service
FAO

Via delle Terme di Caracalla

00100 Rome, Italy

Fax: +39-6-5225-6347

I would like to bring the following

inaccuracy (ies)

new development (s)

omission (s)

concerns

to the attention of the editors:

Disease

Comments

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Name

Address

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