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The Distribution of Badnavirus-Infected *Musa* Germplasm from the International Transit Centre, Katholieke Universiteit Leuven, Belgium

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Abstract

Bioversity International is reviewing its moratorium on the distribution of virus-infected *Musa* germplasm from the International Transit Centre (ITC), and the ProMusa Crop Protection Working Group has been invited to comment on policy changes. This paper was written to form a basis of discussion among the working group members during the ISHS/ProMusa symposium. It argues that the distribution of *Musa* germplasm should be guided by the International Plant Protection Convention, which states that it is the responsibility of the importing country, not the exporter, to impose the phytosanitary measures. There may be special circumstances where the release of badnavirus-infected germplasm from the ITC could be justified.

INTRODUCTION

One of the core activities of the Commodities for Livelihoods programme of Bioversity International is to conserve *Musa* diversity. The global banana collection is maintained at the International Transit Centre (ITC) hosted by the *Katholieke Universiteit Leuven* in Belgium. The ITC holds over 1000 accessions of *Musa*, the majority of which are traditional cultivars. These accessions are kept under tissue culture and also cryopreserved in liquid nitrogen (-196°C) for long-term storage. A maximum of five plants of each accession is available free to anyone in the world upon completion of a Standard Material Transfer Agreement. Since its establishment, the ITC has distributed more than 60,000 germplasm samples of 450 accessions to 88 countries (Anon., 2006). Use of the germplasm is varied, from fundamental scientific studies, such as the analysis of genetic diversity or gene discovery, to attempts to find replacement cultivars with improved horticultural or disease resistance traits.

Nearly all *Musa* germplasm held in the ITC has been virus indexed at one of the recognised international testing centres using standard protocols (Diekmann and Putter, 1996). Indexing for *Banana bunchy top virus*, *Cucumber mosaic virus* and *Banana bract mosaic virus* was until recently undertaken by ELISA, but now PCR techniques are used. To allow detection of as broad a range of badnaviruses as possible, viruses in this group are indexed by immunosorbent electron microscopy using a partially purified virus preparation (virus miniprep) and a mixture of polyclonal antisera to trap virus particles onto the electron microscope grid. This indexing protocol does not allow the identification of badnaviruses to species level, but additional testing of a sample of the accessions by immunocapture PCR suggest that the main species encountered are *Banana streak OL virus* (BSOLV) and *Banana streak GF virus* (BSGFV) (J.N. Parry and A.D.W. Geering, unpublished results). Both BSOLV and BSGFV are endogenous badnaviruses

(the DNA of these viruses is integrated into the B genome of *Musa*), and thus it is likely that the infection is *de novo*, having developed when the plants were initiated into tissue culture (Gayral et al., 2008; Ndowora et al., 1999). Likewise, the indexing protocol for the *Flexiviridae* is also generic and although *Banana mild mosaic virus* (BanMMV) is likely to be the most common virus in the collection, the possibility that some plants are infected with banana virus X cannot be excluded.

The current policy of Bioversity International is that only those *Musa* accessions in the ITC that are virus free are available for distribution. Viruses have been detected in about one third of the collection (Table 1), thus significantly reducing the genetic diversity available to the international community. The viruses that are most commonly found are *Banana mild mosaic virus* (BanMMV, unassigned member of family *Flexiviridae*) and unidentified species from the genus *Badnavirus* (family *Caulimoviridae*). The viruses are not randomly distributed amongst the different *Musa* genotypes. Two hundred and forty-two out of a total 372 accessions containing the B genome are infected with a badnavirus and/or BanMMV. Of these infected accessions, 162 are plantains (which represents 82% of the total number of plantains in the collection).

Therapies exist to clean germplasm of BanMMV but not endogenous badnaviruses such as BSOLV and BSGFV. The question arises as to what do with badnavirus-infected germplasm in the ITC, as –for this germplasm to have any value– it must be able to be utilised in some way.

PRINCIPLES GUIDING THE INTERNATIONAL MOVEMENT OF PLANTS AND PLANT PRODUCTS TO PREVENT THE SPREAD OF PESTS

The International Plant Protection Convention (IPPC) is the principal treaty by which countries abide to help prevent the spread of pests (including pathogens) in plants and plant products (Anon., 2007). As of 2 August 2007, there were 163 contracting governments to the IPPC. The IPPC is governed by the Commission on Phytosanitary Measures (CPM), which prepares International Standards for Phytosanitary Measures (ISPMs) in order to achieve international harmonisation of quarantine policies and to facilitate trade by preventing countries from using unjustifiable measures as trade barriers.

Under the IPPC, an importing country has the right to impose phytosanitary measures for quarantine or regulated non-quarantine pests, but not for non-regulated pests (i.e. pests that are indigenous or introduced and widespread). A quarantine pest is defined as "a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled". A regulated non-quarantine pest is defined as "a non-quarantine pest whose presence in plants for planting affects the intended use of those plants with an economically unacceptable impact and which is therefore regulated within the territory of the importing contracting party". The IPPC further stipulates that the phytosanitary measures must also not be any more stringent than those presently in place if the pest is already in the country. It is the duty of the importing country to publish and transmit their phytosanitary requirements, which may require consignments to enter through specified points of entry if the imports need to be inspected, treated or accompanied by a phytosanitary certificate. The importing country can also "make special provision, subject to adequate safeguards, for the importation, for the purpose of scientific research, education, or other specific use, of plants and plant products and other regulated articles, and of plant pests".

PEST RISK ANALYSES

One of the fundamental principles of the IPPC is managed risk, recognising that there is always a risk of spread and introduction of pests when importing plants and plant products and that importing countries should only institute phytosanitary measures consistent with the pest risk involved. The technical tool used to identify appropriate phytosanitary measures is a pest risk analysis (PRA). A PRA may be initiated when "there is an intention to import for selection and/or scientific research a plant species or cultivar not yet introduced that could potentially be a host of pests" (ISPM No. 2 – 2007). It is the responsibility of the importer to prepare the PRA and to communicate any recommendations from this PRA to the exporter.

In preparing a PRA, a number of factors should be considered, including the category of the pest, the economic impact of the pest, the potential for establishment and spread of the pest, and the proposed uses of the plants or plant products. "The conclusion of the pest risk management stage will be whether or not appropriate phytosanitary measures adequate to reduce the pest risk to an acceptable level are available, cost-effective and feasible" (ISPM No. 2 – 2007). If the pest risk is considered unacceptable and there are no measures available to mitigate the risk, then the import can be prohibited. At the other end of the spectrum, if the pest risk is considered negligible, the import may be permitted with few if any phytosanitary measures.

PEST CATEGORISATION

The first stage of a pest risk analysis is to identify pests that may require phytosanitary measures. As previously mentioned, an importing country can only impose phytosanitary measures for regulated pests (quarantine or regulated non-quarantine pest) but not for non-regulated pests (i.e. pests that are indigenous or introduced and widespread). It is beyond the scope of this paper to provide advice on which category BSOLV and BSGFV may fall into in the different banana-producing countries, but some general comments can be made.

BSOLV and BSGFV, being endogenous badnaviruses linked to the B genome of *Musa*, can be expected to be found in any country where *Musa* A×B hybrids are grown, and therefore are unlikely to be categorised as quarantine pests anywhere in the world. In some countries, these viruses may already be abundant and widespread in distribution, and therefore classified as non-regulated pests. Both BSOLV and BSGFV have been recorded in Australia (Geering et al., 2000), but are restricted in distribution and are under active control, and therefore would be categorised as regulated non-quarantine pests.

RECOMMENDATIONS ON THE DISTRIBUTION OF BADNAVIRUS- AND BANMMV-INFECTED *MUSA* GERMPLASM FROM THE ITC

An individual, organisation or country has the right not to give or sell plant germplasm. However, there is no basis under the IPPC for Bioversity International to restrict movement of *Musa* germplasm based on plant health reasons if the importing country is willing to accept the germplasm, with or without accompanying phytosanitary measures. It is the prerogative of the country receiving the germplasm to assess the relative economic risks and benefits of importing the germplasm, and not of Bioversity International. The only obligation on Bioversity International is to ensure the accuracy of the information and additional declarations contained in phytosanitary certificates and to follow any requested phytosanitary measures (ISPM No. 1 - 2006).

There are likely to be circumstances where the benefits of distributing BSOLV- or BSGFV-infected accessions from the ITC to partner organisations or growers may far outweigh the risks. Examples include:

- Export of accessions to regions where the viruses are already well established and widespread;
- Export of accessions for experimental purposes where the viruses could be contained by geographic isolation or by growing them in a glasshouse;
- Export of accessions to countries where economic gains from use of the accessions may far outweigh losses caused by the virus (e.g. if the accessions have resistance to black leaf streak or Fusarium wilt);
- Export of accessions to countries where the risk of spread and establishment of the viruses is low (e.g. where the rate of mealybug spread is very slow).

I recommend that Bioversity International make available badnavirus-infected *Musa* germplasm in the International Transit Centre for distribution to international partners on the provision that the importer is aware of the pathogen status of the plants and that there is evidence that they have taken the necessary steps to determine if phytosanitary measures are needed by consulting with the relevant national plant health authority. Unless further tests are done, it will be impossible to classify the badnavirus infecting the germplasm to species level, and therefore any declaration on a phytosanitary certificate will have to reflect this fact.

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Note from ProMusa:

An online follow-up discussion was held on the ProMusa website, at http://www.promusa.org/index.php?option=com_content&task=view&id=77&Itemid=161.

Tables

Table 1. Virus status of the Bioversity international *Musa* germplasm collection held at the International Transit Centre, Katholieke Universiteit Leuven, Belgium, as of January 2007 with information on type of virus detected (source of data: I. Van den Houwe).

Virus status	**Number of accessions**	**Percentage of collection**
Negative	760	64.3
Positive	411	34.7
Unknown (not indexed)	11	0.9
Total	**1182**	**100**
Type of virus detected	**Number of accessions**	**Percentage of collection**
Badnavirus single infection	152	12.9
Banana mild mosaic virus (BanMMV) single infection	140	11.8
Badnavirus/BanMMV mixed infection	80	6.8
Other single/mixed infections	39	3.3