

Movement and Exchange of Germplasm

Abed Kagundu

**Head, Biosafety and Phytosanitary
Service - KEPHIS**



General Provisions

- ALL intending to move germplasm MUST understand the country requirements including obtaining a Plant Import Permit (PIP) prior to shipment of such plants if required
- The permit specifies the requirements for plant health indicating prohibitions, restricted quarantine importations and additional declaration with regard to pre-shipment treatments.
- The original permit should reach the plant health authorities in the country of origin for adherence to import permit requirements of the country of import.
- Plant consignment should be accompanied by a copy of a permit and a Phytosanitary certificate, international model or its equivalent from the country of origin.



General Provisions

- Issuance of permits may vary; some countries do not issue PIPs.
- All plant materials must be declared to NPPO at the entry point.
- Prohibited or non-compliant plant materials are destroyed or shipped back to source or treated at the importer's cost..
- Compliant plant materials: Confirmed clean, healthy plant materials Permitted, are released to owners after the inspection.



General requirement contd..

- Importers of propagating/planting material must observe plant breeders rights. Protected varieties should only be imported/used with the consent of the breeders.
- Diseased or insect infested plant materials irrespective of value may be destroyed at the point of entry or shipped back to the country of origin at the owners cost.
- All plant or plant products for export must meet the current phytosanitary requirements of the importing country
- All seed for export (and only by registered seed merchants) must meet the minimum standards and be accompanied by the relevant certificates



Exchange of plant and Research Material

Importation of Plant Germplasm

- Making a declaration of intention to import to the NPPO indicating the type/identity of the article/plant material, quantity, purpose and source/destination.
- New sources; where the plant material is being obtained for the first time from a new source, there would be need for **pest risk analysis** (PRA) information to generate import conditions.
- The NPPO remits import requirements to the source country through the applicant (importer) outlining the conditions to be met by the plant material in question.
- The source country NPPO evaluates and confirms compliance of the plant materials to the conditions of the importing country.
- The source country NPPO prepares a phytosanitary certificate if the import conditions are met.



Exportation of Plant Germplasm

- Some of the conditions can only be confirmed during active growth; in such a case the NPPO should be involved as early as possible
- NPPO verifies compliance to the import conditions of importing country by inspection and or laboratory analysis.
- The NPPO would then prepare a phytosanitary certificates only for compliant plant materials.
- In some cases there may be need to prepare and sign a Material Transfer Agreement (MTA) between the exporting institution, community where target plant germplasm occurs, scientist, exporter and the importing party.



Categories of import material

- Material for Research *Cf* Commercial Consignments
- **Genetically Engineered Material**
- **DNA, Plasmids, Transformed Bacteria etc.**
- **Varieties not released in the country (New Varieties)**
- Tissue culture/Indexed plants
- Seed
- Rooted plants
- Tubers
- Vines



Entry of material

Ports of Entry

- In most of east and central Africa consignments are not restricted to specific ports but will be subjected to phytosanitary inspection upon arrival

Transit

- No additional measures need to be undertaken for consignments, which transit a third country en-route to Kenya

Re-Export

- In some circumstance a re-export is required and in this case a re-export phytosanitary certificate may be issued



Moving diseased material

- For material containing regulated pests, the NPPO may allow that recognized techniques (e.g. meristem tip culture, thermotherapy) be used in combination with conventional micropropagation to eliminate the pest from the candidate plant.
- Laboratory testing must be used to confirm the success of this approach before exchange commences.
- Such materials are taken to provisional quarantine facilities for further observation depending on the risk levels.
- These materials are only released either after being found clean or after cleaning/indexing.



Facilities for producing or maintaining germplasm

- The NPPO of the exporting country is responsible for ensuring that the phytosanitary aspects of these facilities and of the related propagation system meet the importing country's phytosanitary import requirements.
- The NPPO of the exporting country is also responsible for phytosanitary certification.



Facilities for cleaning germplasm

- A testing programme on the candidate plant should be applied in an official testing laboratory.
- This laboratory should meet general requirements to ensure that all potato micropropagative material moved to maintenance and propagation facilities is free from the pests regulated by the importing country.
- Conventional micropropagation does not consistently exclude some pests, for example, viruses, viroids, phytoplasmas and bacteria.



Facilities for cleaning germplasm

- Because both infested and pest free potato propagative material (tubers, plants *in vitro* etc.) may be handled in the same facility, strict procedures should be implemented to prevent contamination or infestation of pest free material. Such procedures should include:
 - prohibition of entry of unauthorised personnel and control of the entry of authorized staff
 - provision for the use of dedicated protective clothing (including dedicated footwear or disinfection of footwear) and hand washing on entry (with particular care being taken if staff members work in areas of higher phytosanitary risk, e.g. the testing facility)
 - chronological records of actions in handling material so that production can, if necessary, be checked easily for contamination and infestation if pests are detected
 - stringent aseptic techniques, including disinfection of work areas and sterilization of instruments (e.g. by autoclaving) between handling materials of a different phytosanitary status



Facilities for cleaning germplasm

- The facility should be monitored for the regulated pests and pest vectors during the production cycle and, if necessary, pest control measures or other corrective actions should be undertaken and documented. The facility should be well maintained and cleaned after each production cycle.



Staff Competence

Staff should be trained and competent in:

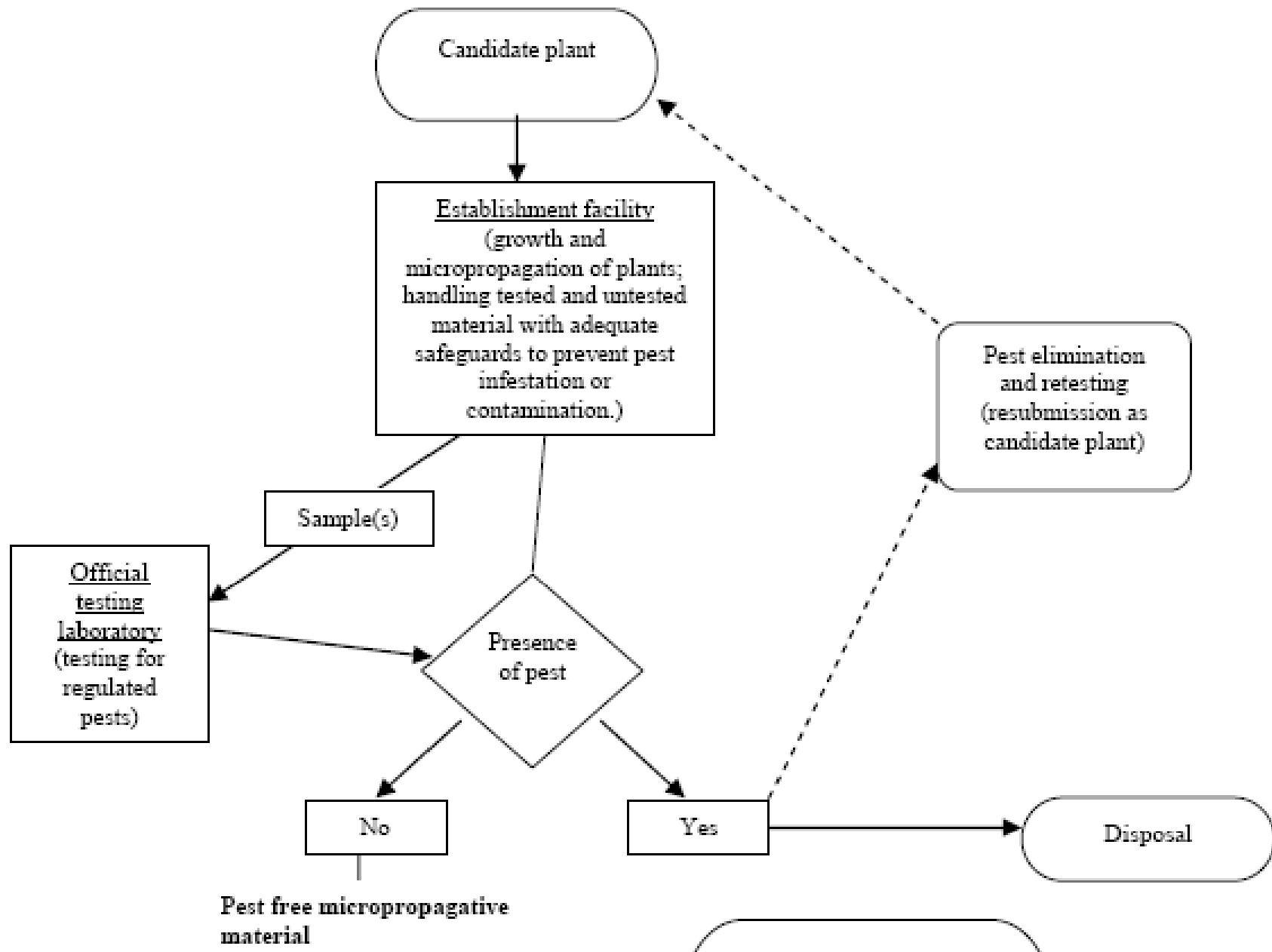
- techniques for the establishment of pest free micropropagative material, the maintenance of pest free material and diagnostic testing as relevant
- following administrative, management and record-keeping procedures.
- Procedures for maintaining staff competence should be in place and training should be updated, in particular, when phytosanitary import requirements change.



Phytosanitary Certification

- Relevant records and the plants with Accession numbers should be subjected to appropriate phytosanitary procedures to ensure that the micropropagative material meets the importing country's phytosanitary import requirements.
- The use of material certification labels may assist with lot identification, in particular when these labels specify the reference number of the lot, including where appropriate the producer's identification number.
- Below is a schematic representation of the process of exchange





Pest free micropropagative material

Maintenance and propagation facility
(micropropagation of pest free material under aseptic conditions)

Export if it meets importing country's phytosanitary import requirements

Pest free micropropagative material

Minituber facility
(production under conditions to prevent pest infestation or contamination)

Export if it meets importing country's phytosanitary import requirements

Pest free minitubers

Export if they meet importing country's phytosanitary import requirements

Moving Sweet Potato

- Several of the viruses which infect sweet potatoes are incompletely characterized.
- Meristem-tip culture has been demonstrated to be effective for eliminating viruses in sweet potato and is recommended whenever germplasm is moved.
- Additional therapy measures, such as heat-therapy, may be necessary.
- It is recommended, when clones are put in tissue culture, to routinely place the initial explants for 2 or 3 weeks on a medium that promotes the growth of bacteria (e.g. 40 g Trypticase Soy Agar in 1000 ml of water).
- Those meristems that remain free of bacterial growth are transferred to a medium allowing meristem growth (F. Quak, personal communication).
- One to four nodes from the regenerated plant should be subcultured and maintained *in vitro* to prevent recontamination. The remainder (basal part) of the plant should be transplanted and grown in an insect-free greenhouse for virus testing.



Moving sweet potato contd..

- Leaves from plantlets regenerated from meristem-tips can be serologically assayed, as a preliminary screening, when the plantlets are subcultured.
- At this stage, negative results, using assays available, should not be interpreted as freedom from virus.
- The basal part of the plantlet should be grown in an insect-free greenhouse until it has at least 10 nodes on the main stem.
- Two nodes from each sweet potato plant should then be grafted on to *I. setosa*. The sweet potato plant should then be trimmed and allowed to regrow to a similar size and retested in the same manner.
- Both source and indicator plants should be grown in as near optimal conditions as
- possible to stimulate rapid and luxuriant growth. *I. setosa* should be held for a minimum of 4 weeks for observation of symptom expression.
Symptomless *I. setosa*
- If all tests are negative, plantlets originated from the same meristem can be distributed.



Designation	Distribution	Classification	Vector	Additional indicator plants
Sweet potato feathery mottle virus (SPFMV)	Worldwide	potyvirus	aphid	—
Sweet potato virus II (SPV-II)	Taiwan	potyvirus	aphid	<i>N. benthamiana</i>
Sweet potato latent virus (SPLV)	Asia	potyvirus?	unknown	<i>N. benthamiana</i>
Sweet potato mild mottle virus (SPMMV)	East Africa	potyvirus?	<i>Bemisia tabaci</i>	<i>N. tabacum</i> <i>N. glutinosa</i> <i>N. benthamiana</i>
Sweet potato ring-spot virus (SPRSV)	Papua New Guinea	nepovirus?	unknown	—
Sweet potato caulimovirus-like virus (SPCLV)	widespread	?	?	<i>N. megalosiphon</i>
Sweet potato yellow dwarf virus (SPYDV)	Taiwan	potyvirus?	<i>Bemisia tabaci</i>	<i>G. globosa</i> <i>D. stramonium</i> <i>Cassia occidentalis</i>

Virus testing

- Symptomatology
- Indicator plants
- ELISA
- PCR
- NASH

ELISA Reader



Thermotherapy treatment

- Whole plant treatment
- In vitro thermotherapy



Meristem tissue culture

- Meristem media
- Stereo microscope



multiplication

- Multiplication media
- Growth rooms
- E.g Cassava, Irish potato



Acclimatisation

- Greenhouses



Conservation & distribution

- In vitro conservation media
- Preparation for distribution





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Thank you