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Draft guide on minituber production

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Submitted by the secretariat

The following draft guide on minituber production, coordinated by Australia, is for review by the Specialized Section. The document is presented for review and discussion by the Specialized Section.

The document is submitted according to ECE/CTCS/2021/7 section V, ECE/CTCS/2021/2 Decision 2021-07-07, and A/76/6 (Sect. 20).

1. Introduction

This guide has been developed by the Seed potato specialized section of the UNECE working party on Agricultural Quality Standards in order to be a reference tool given recommendations for the production and certification of seed potato minitubers.

When the production of potato microplants (plants including micro tubers produced by micropropagation/tissue culture techniques) and minitubers (G0 seed potatoes) is used for subsequent multiplication within a seed Scheme, it is very important that this step allows for the production of high quality material.

The focus for the production of potato microplants and minitubers is to ensure that the material produced;

- has maintained varietal identity, varietal **identity and varietal** purity and **trueness to type [comment from DE: From our point of view "trueness to type" is not clearly defined. The requirements are "varietal identity and varietal purity". NC need to review UNECE standard Annex 1. The parent material must be true-to-type for the variety. NC — Pending resolution of Annex suggest re- worded to "as maintained varietal identity, and purity".]**
- is pest and disease free.

[1] Comments and additional text are indicated in bold and underlined.

- is traceable to the origin of production.

The UNECE standard S-1 for seed potatoes defines a set of conditions and minimum quality requirements to be satisfied for the production and the marketing of Pre-basic TC seed potatoes.

For the phytosanitary risk management and phytosanitary certification, International Standards for Phytosanitary Measures (ISPM)[2] are recommend for the National Plant Protection Organization (NPPO).

The production of potato microplants and minitubers (G0 seed potatoes) should be conducted within specific producer's procedures, **which are supported or approved by the Certification Authority (CA). [Comment from Australia – UNECE standard Annex 1 The facilities and procedures used for this production must be subject to official approval by the CA — pending resolution of the Annex.]** Hence this guide is a resource for the producers and for the CA. In addition to annexes I, II, III and IV of the UNECE Standard, it provides recommendations for the production of microplants and minitubers within a seed potato certification scheme.

2. Production of the initial micropropagation stock material

Micropropagative multiplication involves the process of propagating microplants of initial stock by taking nodal cuttings under aseptic conditions to produce large numbers of microplants. The resulting microplants are retained for further multiplication cycles or grown to maturity to provide harvestable tubers usually of the class **Pre-basic TC (tissue culture) (PBTC) as outlined in the UNECE standard.**

2.1 Requirements for the tissue culture laboratory

The tissue culture laboratory used to produce microplants must maintain the high health status of the initial stock, avoid pathogen contamination, and ensure the integrity of the material that is produced. It shall comply with the following requirements:

1. Appropriate sterile laboratory procedures are applied and documented to avoid contamination of the cultured plant material, e.g., use of sterile tools, laminar flow hoods and sterile growing media for aseptic multiplication of plant material, dedicated clothing for operators (e.g., lab coat, overshoes). The laboratory should demonstrate good laboratory practices to maintain high plant health and traceability.
2. Management practices should be such to ensure that integrity of variety is kept at all times.
3. Regular visual monitoring of the growing tissue culture plants is conducted to ensure no contamination of tissue culture stocks has occurred.

[2] The following ISPM's are recommended as guidelines: **[New references provided by S. Africa]:**

- **ISPM No. 10 - Requirements for the establishment of pest free places of production and pest free production sites (Adopted 1999, published 2016)**
  ISPM No. 33 - Pest free Potato (Solanum spp.) micro-propagative material and mini tubers for international trade (Adopted 2010, published 2019)
  ISPM No. 34 - Design and operation of Post-Entry Quarantine Stations for plants (Adopted 2010, published 2016)
- ISPM No. 10 - REQUIREMENTS FOR THE ESTABLISHMENT OF PEST FREE PLACES OF PRODUCTION AND PEST FREE PRODUCTION SITES (1999) [**check all to make sure this is latest version]**
- ISPM No. 33 - PEST FREE POTATO (SOLANUM SPP.) MICROPROPAGATIVE MATERIAL AND MINITUBERS FOR INTERNATIONAL TRADE (2010)
- ISPM 34 DESIGN AND OPERATION OF POST-ENTRY QUARANTINE STATIONS FOR PLANTS (2010)

4. Appropriate cleaning of all laboratory surfaces including media preparation and growth room. Appropriate management of the tissue culture laboratory to ensure no mites, spiders, or other insects can reside.
5. Records and quality management systems are in place to ensure traceability of all lines.
6. Laboratory staff are suitably trained.

2.2 Technical infrastructure used in a tissue culture laboratory

In a tissue culture laboratory used to produce microplants the following equipment/infrastructure may be required;

1. **A laminar flow or biosafety cabinet for aseptic multiplication, alternatively a designated clean room may be used.**
2. **An autoclave or an alternative for media sterilization.**
3. **A light bank of artificial lights for invitro growth of tissue culture plantlets.**
4. **Controlled temperature growth room to maintain optimum temperature for invitro growth.**

[May include pictures of the items above.]

2.3 Conditions to be satisfied for the initial micropropagation material

1. The microplants which constitute the initial micropropagation material shall fulfill specifically the following points.
2. All the in vitro propagating material shall have originated from an in vitro facility which respects the conditions detailed in point 2.1 and may be approved by the CA.
3. The parent material must have varietal identity and purity, this may be defined by morphological and molecular characters.
4. The initial stock must be well labeled to ensure the integrity of the variety.
5. The initial stock must be laboratory tested to be declared and maintained free from at least the following pathogens.
   - Potato Spindle Tuber viroid
   - *Clavibacter michiganensis* subsp. *sepedonicus* (ring rot)
   - *Ralstonia solanacearum* (brown rot)
   - *Pectobacterium* spp. and *Dickeya* spp. (syn. *Erwinia* spp.)
   - Potato viruses, X, Y, S, M and A
   - Potato Leaf roll virus

Other pathogens e.g. Liberibacter and other pests may be tested at the discretion of the CA.

Material that has positive detections for any of the above pathogens must not be allowed entry into the minituber production unit.

Records are to be kept of testing protocol, testing results and sources of original material.

Other plant species may not be produced in the tissue culture laboratory, unless appropriate risk mitigation is in place such as separation between place and time.

2.4 Traceability of initial stocks

The initial stocks will be the foundation for further multiplication within the seed Scheme. The Initial stock material shall be referenced, and its origin well documented prior to entry into the Scheme. The CA should have the guarantee of traceability of this material and access to the following information regarding the introduction of initial stock material if necessary.

1. Name of supplier
2. Origin of the material
3. Type of material (tissue culture plantlets or micro tuber).
4. Variety - denomination
5. Quantity of material (number of microplants).
6. Name of company material supplied to.
7. Date material supplied.
8. A diagnostic report approved by the CA of the status of disease freedom status especially ensuring the material is free from pathogens.
9. At this time, the CA may request a Variety description as a reference for certification requirements.
10. Description of any treatments applied e.g. heat treatment to remove viruses

2.5 Official checks of initial stocks

The CA may set up a system of authorization or approval, which may allow for self-regulation by private laboratories. This is in order to ensure the traceability of the **initial stocks** and the production of the microplant is in compliance with the requirements.

The CA may conduct initial and periodical audits. **In so doing, the CA must ensure that the initial stock used to produce minitubers as Pre-basic TC seed potatoes is free of the pests and diseases listed in 2.2. [Comment from N. Ponserre - UNECE Standard Annex I, point 2 specifies that the initial stock is "officially certified". I propose to delete this condition in the standard which seems to be not appropriate. Comment from N.Crump – pending review of the standard.]**

3. Production of Minitubers (G0) as Pre-basic TC (tissue culture) class seed

[Comment DE: This part is somehow redundant with 2.2 due to the fact that the UNECE standard S-1 specifies many conditions mentioned in detail already under 2.2. However, under 2.2 the standard itself is not mentioned. Maybe this paragraph can be moved to the end of the introduction? The conditions set out in the standard are compulsory for both tissue culture propagation and minituber production. N.Crump - Separation of initial stocks and minituber production.]

The UNECE standard S-1 provides a set of conditions specified in Annex I. The facilities used for potato minituber production must be free of diseases/pests specified in the respective Standard.

Methods of **potato** mintuber production involve **the multiplication of initial stocks via tissue culture and subsequently** growing plants **in a controlled facility to** produce **tubers**. **This production is done using** pest free **media such as** peat, hydroponic, and aeroponic production systems. Regardless of the systems used for production **of minitubers**, the standards for certification should be uniformly applied.

[Insert example pictures of mintuber production peat, aeroponic etc.]

3.1 Eligible plant material to be used for the production of minitubers

Only initial micropropagation stock material should be planted to produce the potato minitubers . The initial micropropagation stock material shall have originated from an *in vitro* facility which respects the conditions detailed in item 2.

3.2 The location of the minituber production facility

[DE suggested to delete - We propose deleting this point as it is not directly linked to the location of the facility. It is more or less a matter of timing, i.e. management issue.]

The location of the minituber facility should be assessed in relation to plant pest and disease concerns.

Measures should be implemented to ensure the minituber facility has adequate physical and operational safeguards in place to prevent introduction of specified diseases/pests.

Considerations on a location may also include:

- The placement of the facility in a disease/pest-free area, or an area that is free or sufficiently isolated from sources of specified diseases/pests.
- The inclusion of a buffer zone around the facility for specified diseases/pests.
- The placement of the facility in a region with low disease/pest prevalence and low vector pressure.

3.3 The potato minituber production facility/greenhouse

The operator of the minituber facility must take all reasonable husbandry practices for the prevention or spread of pests and diseases. In addition, the growing potato crop must have been kept free from potato viruses, bacterial diseases and from plants that are not true to the variety.

The generation of **potato** minitubers shall be produced from micropropagative material in a facility protected from external contaminations, insect-proof and on growing medium free from pests and diseases.

Other plants or plant species can be a risk of contamination if grown in the immediate minituber production facility at the same time as **potato minituber** production.

[Comment from DE: Maybe this requirement is too strict? Depending on the definition of "production facility". It might be helpful to add a paragraph about the technical equipment that such a facility should/could have.]

An assessment of the risk of growing other plants or plant species may be required **by the CA.**

One generation only of minitubers should be produced.

[N.Crump - Not sure if this is the case for all areas?]

3.3.1 Technical equipment and infrastructure

The minituber facility infrastructure should ideally include the following

1. **An anteroom with double door access in the entrance area. The entrance area shall be equipped with a footbath for disinfecting footwear and wash bay for washing and disinfecting hands.**
2. **All access doors, openings and ventilation openings should be sealed with insect proof mesh with reference to local pests and vectors. The mesh size for the virus netting to isolate the structure, should be in the order of 193.5 micron (75 Mesh)**

   [Comment from Finland: need to convert to microns.]

3. **All openings should be sealed between the external and the internal environment of the structure.**

   [Comment from Australia - N.Crump – is this required.]

4. **The floor area of the greenhouse should be covered in such a manner that the roots of plants kept in containers and, cannot penetrate the soil on which the greenhouse is erected (e.g. Cement floors or the separation from soil through a dense membrane).**

5. **Designated areas for washing and disinfecting containers and cleaning, sorting, packing and storage of minitubers.**

6. **An appropriate air filtration system, if appropriate.**

7. **Water used for irrigation filtration and sanitation systems.**

[May include pictures of infrastructure.]

3.3.2 Access control to the minituber production facility

Access to the minituber production facility should be restricted:

1. Access to the facility should be controlled and limited to authorized access only.
2. Provision should be made for the wearing of protective clothing, disinfection of footwear and hand cleansing.

3.3.3 Growth Medium, nutrients and water used for minituber production

The growing medium, fertilizer, and any irrigation water used shall be free from disease causing organisms. This could be achieved by:

1. **Use of soil-free medium.**
2. **Fumigation / disinfection / sterilization of growth medium for plants.**
3. **Appropriate transport and storage conditions of growth medium to avoid contamination.**
4. **Use of borehole / spring water or municipal water.**
5. **Appropriate treatment of water such filtration, Ultraviolet light or chemical sanitation e.g. chlorine.**
6. **Regular testing of water.**
7. **Use of inorganic or appropriately treated organic nutrients.**

3.3.4 Plant containers

The plant containers used by the minituber production facility should be of such a nature that they can be easily sanitized and are isolated from the ground.

The procedures for the sanitation of the containers that are used should be audited to ensure the procedures are appropriate to prevent introduction of pest and diseases.

3.3.5 Crop management

Appropriate management systems have to be in place to ensure:

1. **Plants in the minituber production facility to be clearly identified according to variety.**
2. **There are procedures to prevent the occurrence of variety mixes, during the growing and harvesting processes.**
3. **Precautions or corrective actions against disease/pests must be documented by the facility operator.**
4. **Regular and effective fungicide and or insecticide spray programs should be documented by the facility operator.**

5. **Aphid monitoring in greenhouses is recommended. For example, aphid traps covered with an adhesive strip could be distributed through every greenhouse. The date on which the traps were affixed should be noted. All observations during the monitoring action may be noted for each production cycle and retained for an appropriate period of time.**

3.3.6 Sanitation

The facility operator should ensure:

1. **Appropriate hygienic practices for handling all plant material.**
2. **Sanitation during growth includes regular removal of plant debris.**
3. **Appropriate discarding procedures.**
4. **No growth of algae on floor or wet walls.**
5. **The facility should be thoroughly sanitized after each production cycle.**
6. **All containers used for production should be sanitized before use.**

3.3.7 Post-harvest handling and storage

The facility operator shall have appropriate systems for post-harvest handling and storage including:

1. **Appropriate storage conditions. The minitubers must be handled, packed, stored and transported in such a manner that infestation by diseases / pests are prevented.**
2. **Handling procedures should be conducted in a manner to prevent varietal mixtures.**
3. **Sorting, packing and labelling according to requirements for certification.**
4. **New containers be used for packing of minitubers.**
5. **Cleaning and sanitation of any equipment and storage facilities.**

3.3.8 Record keeping for the facility operator

Documented or recorded evidence shall be available concerning the:

1. **Map of varieties planted for each greenhouse.**
2. **Traceability of all the minitubers produced.**
3. **Disease test results.**

It is advisable to keep long-term records as evidence in the event of a dispute e.g. a variety mix.

3.4 Competence training and awareness of personnel

The facility operator should have documented evidence for their staff involved in the production of the minitubers concerning the:

1. **Qualification.**
2. **Continuous training and evaluation.**

4. Auditing and Inspections of minituber production

Official inspections during the growing period should be conducted, with a minimum of two inspections recorded per production cycle.

The official inspections should include the visual inspection of plants, tubers, containers, equipment or facilities by an authorized person, to determine compliance with regulations as determined by the CA.

The CA may include testing of every lot of minitubers to check the absence of viruses (PLRV, PVA, PVM, PVS, PVX, PVY) and of the absence of zero tolerance bacteria e.g. ***Ralstonia solanacearum*** [**Comment US: Check taxonomy references to species]** and *Clavibacter michiganensis subsp sepedonicus*.

To check varietal identity and purity and absence of diseases, the CA may require a post-control in field for the minitubers (G0) which are produced.

4.1 Auditing

4.1.1 Auditing requirements of the facility

Requirements concern appropriate facilities, systems used for traceability, and records of training of staff etc.

In auditing the minituber facility, the CA may **record**:

1. **The observed absence or presence of pests and diseases as prescribed in the UNECE standard.**
2. The type of greenhouse (e.g. Greenhouse, ground (floor) type, **insect physical barriers e.g. mesh or plastic covers**).
3. The physical location of greenhouse.
4. The maintenance of the area around greenhouse (e.g. occurrence of weeds, potential hosts).
5. Access to the facility is controlled with restricted authorized access.
6. Other species that are being grown in the immediate production facility.
7. Records of visual inspection or testing.

4.2 Records relating to production and training. Labelling of material produced.

When the minitubers meet the requirements, the minitubers can be certified as Pre-basic tissue Culture (PBTC) seed class by the CA and can be officially labeled.