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Draft guide on minituber production

Revised guide on minituber production *

Submitted by Australia

The following revised version of the draft guide on minituber production was submitted by the delegation from Australia. It contains the comments and proposals introduced at the 2019 session of the Specialized Section and the 2019 Rapporteurs meeting (Montana, United States). The document is presented for review and approval by the Specialized Section. Comments on the text are indicated in brackets.

This document is submitted according to ECE/CTCS/2017/10 section II c, ECE/CTCS/2018/2 section VII a, and A/74/6 (Sect.20), para 20.37; and its supplementary information.

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 allow 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 purity and trueness to type.
- is pest and disease free.

* Submitted on the above date due to include all revisions.



- 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)¹ are recommended 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). 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 material

2.1 Requirements for the tissue culture laboratory

The tissue culture laboratory used to produce microplants shall maintain the high health status of the nuclear stock, avoid pathogen contamination, and ensure the integrity of the material that is produced. [Comment by United States: Check text.] 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.
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 needed to ensure traceability of all lines.
6. Laboratory staff are suitably trained.

2.2 Conditions to be satisfied for the initial micropropagation material

The microplants which constitute the initial micropropagation material (or the *in vitro* nuclear stock) shall fulfill specifically the following points:

¹ The following ISPM's are recommended as guidelines:

- ISPM No. 10 - REQUIREMENTS FOR THE ESTABLISHMENT OF PEST FREE PLACES OF PRODUCTION AND PEST FREE PRODUCTION SITES (1999)
- 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)

1. 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.
2. The parent material should be true-to-type for the variety or breeding line, this may be defined by morphological and molecular characters.
3. The nuclear stock must be well labeled to ensure the integrity of the variety.
4. The nuclear stock must be laboratory tested to be declared and maintained free from at least the following pathogens:
 - Potato Spindle Tuber viroid
 - *Clavibacter michiganensis* spp. *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.

2.3 Traceability

[Comment by France: Traceability is already mentioned in the introduction.]

The nuclear material will be the foundation for further multiplication of plant material within the seed Scheme. This 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 nuclear 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.

2.4 Official checks

It is recommended that the CA set up a system of authorization or approval, which may include delegation of authority of the tissue culture laboratory. This is in order to ensure the

traceability of the material and the production of the microplant is in compliance with the requirements.

The CA may conduct initial and periodical audits.

In any cases, 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 by France: UNECE Standard Annex I, point 2 specifies that the initial stock is “officially certified”. Proposal to delete this condition in the standard which seems to be not appropriate.] [Comment by the United States: to be discussed.]

3. Production of Minitubers (G0)

The UNECE standard S-1 provides a set of conditions specified in annex I. The facilities used for minituber production must be free of diseases/pests specified in the respective Standard. The only material that may enter the minituber production facility are disease/pest-free potato micro propagation material. Produced minitubers used to enter the Scheme for seed certification shall be certified as pre-basic TC.

Methods of minituber production may involve growing plants and producing minitubers in soilless media including peat, hydroponic, and aeroponic production systems. Regardless of the systems used for production, the standards for certification should be uniformly applied.

(Note: Insert example pictures of minituber production.)

3.1 Eligible plant material

1. Only *in vitro* micro-propagative material should be planted to produce the minitubers (G0).
2. All the *in vitro* propagating material shall have originated from an *in vitro* facility which respects the conditions detailed in point 2.

3.2 The location of the minituber facility

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
- Production takes place in period of low disease/pest and vector pressure where possible.

3.3 The 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 crop must have been kept free from potato viruses, bacterial diseases and from deviations of variety and type. [Comment by the United States: pending review of the standard.]

The generation of minitubers (G0) shall be produced from micropropagative material in a facility protected from external contaminations, insect-proof and on growing medium free from pests and diseases.

No other plants or plant species may be produced in the minituber production facility at the same time as G0 production.

One generation only of minitubers should be produced. [Comment by Australia: Not sure if this is the case for all areas.] [Comment by France: It is compulsory in the European Union for example, but I have also understood that it is not the case in other parts of the world. Proposal to keep it but to change “can” to “should” because it is a recommendation which is important. Possibly, explain why it is important, eg “to keep a high quality guarantee”.]

3.4 Visual inspections

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

Authorisation of producers of minitubers may be granted by the CA.

The CA may include systematic 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* and *Clavibacter michiganensis subsp. sepedonicus*. [Comment by United States: Please check taxonomy references to species.]

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.

3.5 Auditing

3.5.1 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 type of greenhouse
2. The physical location of greenhouse
3. The maintenance of the area around greenhouse eg weed and alternative host free
4. Controlled entry with restricted authorized access
5. The use of an anteroom with double door access in the entrance area where protective clothing and overshoes can be donned. The entrance area shall be equipped with a footbath for disinfecting footwear and wash bay for washing and disinfecting hands.
6. 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 by United States: mesh size converted to microns.]
7. All openings should be sealed between the external and the internal environment of the structure
8. 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)
9. Designated areas for washing and disinfecting containers and cleaning, sorting, packing and storage of minitubers

10. An appropriate air filtration system, if appropriate
11. Water used for irrigation filtration and sanitation systems.

3.5.2 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.

4. Production of potato minitubers

4.1 Growth Medium, nutrients and water

The growing medium, fertilizer used, and any water used shall be free from disease causing organisms or have been effectively decontaminated. Options may include:

1. Use of soil-free medium
2. Fumigation / disinfection / sterilization of growth medium for plants
3. Transport and storage conditions of growth medium to avoid contamination
4. Use of borehole / spring water or municipal water
5. Appropriate treatment of water
6. Regular testing of water
7. Use of inorganic or appropriately treated organic nutrients.

4.2 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.

4.3 Crop management

Appropriate management systems have to be in place to ensure:

1. Plants in the minituber production facility 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.

4.4 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 as referenced in 4.2.

4.5 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.

4.6 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.

4.7 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.

4.8 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. [Comment by United States: Change text according to outcome of revised definition of Pre-Basic Tissue Culture (ECE/CTCS/WP.7/GE.6/2020/3.)]