



Economic and Social Council

Distr.: General
22 December 2022

Original: English

Economic Commission for Europe

Steering Committee on Trade Capacity and Standards

Working Party on Agricultural Quality Standards

Specialized Section on Standardization of Seed Potatoes

Fiftieth session

Geneva, 16–17 March 2023

Item 8 of the provisional agenda

Draft guide on minituber production

Draft guide on minituber production

Submitted by the rapporteur's group

Summary

The following contains the draft guide on minituber production, developed by a rapporteur's group consisting of Australia (rapporteur), France, Germany, the Netherlands, New Zealand, South Africa, the United Kingdom of Great Britain and Northern Ireland, and Euroseeds.

The Specialized Section is invited to review and consider the text of the draft guide on minituber production for approval.

The document is submitted according to ECE/CTCS/2022/8 section VI, ECE/CTCS/2022/2 Decision 2022-15, ECE/CTCS/WP.7/2022/2 paragraph 71, and A/77/6 (Sect. 20).



1. Introduction

This guide has been developed by the Specialized Section on Standardization of Seed Potatoes of the United Nations Economic Commission for Europe (UNECE) Working Party on Agricultural Quality Standards in order to be a reference tool for the production and certification of seed potato minitubers.

When the production of potato microplants (plants including microtubers produced by micropropagation/tissue culture techniques) and minitubers (G0 seed potatoes) is used for subsequent multiplication within a seed potatoes certification 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 and varietal purity
- is pest free
- is traceable to the origin of production.

The UNECE standard S-1 concerning the marketing and commercial quality control of seed potatoes (UNECE Standard) defines a set of conditions and minimum quality requirements to be satisfied for the production and the marketing of Pre-basic tissue culture (PBTC) seed potatoes.

For phytosanitary risk management and phytosanitary certification, International Standards for Phytosanitary Measures (ISPM)¹ are recommended to the National Plant Protection Organizations.

Potato microplants and minitubers (Field Generation 0 (FG0)) should be produced in accordance with the producer's procedures, which may be approved by the Certifying Authority (CA). Hence this guide is a resource for the producers and for the CA. In addition to annexes I, II, III and IV to the UNECE Standard, it provides recommendations for the production of microplants and minitubers within a seed potato certification scheme.

2. Production of micropropagative material (in vitro material)

The production of micropropagative material starts with the mother plant. This is an identified plant or tuber that has varietal identity. Varietal identity is assessed using morphological and/or molecular characters.

From this mother plant, material is taken to establish an initial stock. Initial stock or nuclear stock refers to the pathogen-tested microplants that form the basis of a tissue culture seed potato propagation cycle. Microplants of the initial stock are propagated by taking nodal cuttings under aseptic conditions to produce large quantities of microplants.

The resulting microplants are retained for further multiplication cycles or grown to maturity to provide harvestable tubers usually of the class PBTC as outlined in the UNECE Standard.

Micropropagation is carried out in a tissue culture laboratory that is approved by the CA.

[Note: To check consistency in the use of terms between micropropagative material and other terms such as initial stock, and consider adding a table of terms or a diagram.]

¹ The following ISPM of IPPC of the Food and Agriculture Organization of the United Nations (FAO) are recommended as guidelines (available for download on www.ippc.int):

- ISPM 10. 1999. Requirements for the establishment of pest free places of production and pest free production sites. Rome, IPPC, FAO. (Adopted 1999, published 2016)
- ISPM 33. 2010. Pest free potato (*Solanum* spp.) micropropagative material and minitubers for international trade. Rome, IPPC, FAO. (Adopted 2010, published 2019)
- ISPM 34. 2010. Design and operation of post-entry quarantine stations for plants. Rome, IPPC, FAO. (Adopted 2010, published 2016).

2.1 Organizational requirements for the tissue culture laboratory

The tissue culture laboratory must demonstrate good laboratory practices to maintain high plant health and traceability throughout the entire production. The laboratory must comply with the following requirements:

- (a) Appropriate aseptic laboratory procedures must be applied and documented to avoid contamination of the cultured plant material, e.g. use of sterile tools and sterile growing media.
- (b) Appropriate cleaning of all laboratory surfaces is required, including media preparation and growth room to avoid pathogen contamination and to ensure an aseptic and pest free environment.
- (c) Regular visual monitoring of the growing tissue culture plants must be conducted to ensure no contamination of tissue culture stocks has occurred.
- (d) Management practices must ensure that the varietal identity is maintained at all times.
- (e) Records and quality management systems must be in place to ensure traceability and integrity of all material produced.
- (f) Laboratory staff must be suitably trained.
- (g) Dedicated clothing for laboratory staff must be provided (e.g. lab coat, overshoes).
- (h) Access to the facility must be controlled and limited to authorized personnel.

2.2 Requirements for the technical infrastructure used in a tissue culture laboratory

In a tissue culture laboratory producing potato microplants the following equipment/infrastructure is required:

- (a) A laminar flow or biosafety cabinet for aseptic multiplication, alternatively a designated clean room may be used.
- (b) An autoclave or an alternative for media and tools sterilization.
- (c) An artificial lights system for in vitro growth of tissue culture plantlets.
- (d) Controlled temperature growth room to maintain optimum temperature for in vitro growth.

[Note: to include pictures of the items above.]

2.3 Conditions to be satisfied for micropropagation material

The microplants which constitute micropropagation material shall comply with the following:

- (a) The in vitro propagating material shall have originated from an in vitro facility which respects the conditions detailed in point 2.1 and point 2.2.
- (b) The initial stock used to produce the PBTC seed potatoes shall be known to be free from the following pests:
 - *Clavibacter michiganensis* spp. *sepedonicus* (ring rot)
 - *Ralstonia solanacearum* (brown rot)
 - *Pectobacterium* spp. and *Dickeya* spp. (syn. *Erwinia* spp.)
 - *Candidatus Liberibacter solanacearum*
 - *Candidatus Phytoplasma solani*

- Potato spindle tuber viroid
- Potato viruses X, Y, S, M and A
- Potato leafroll virus

Evidence of pest freedom, such as laboratory testing or other official information e.g. pest free areas may be required by the CA. Freedom from other pests may be required at the discretion of the CA.

Records must be maintained of testing protocol, testing results and sources of the initial stock.

Material that has positive detections for any of the above pathogens must not be used for minituber production and should be removed from tissue culture laboratories.

Material that is found to have a known pest may be treated to have the pest removed. In such cases laboratory testing must be used to confirm the success of this treatment before multiplication commences.

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 potato certification scheme. The initial stock material shall be referenced, and have its origin documented for entering into the scheme. The Certifying Authority should have access to documentation on traceability of this material and access to the following information regarding the introduction of initial stock material if necessary.

- (a) Name of variety maintainer (e.g. breeder, gene bank).
- (b) Origin of the material.
- (c) Type of material (tissue culture plantlets or microtuber).
- (d) Variety – denomination and clone number.
- (e) Quantity of material (number of microplants).
- (f) Date when the material was supplied.
- (g) A diagnostic report approved by the CA of the pest free status of the material.
- (h) Variety description as a reference for certification requirements.
- (i) Information on any treatments applied e.g. heat treatment to remove viruses.

2.5 Monitoring of initial stocks

The Certifying Authority 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 are in compliance with the requirements.

The Certifying Authority may conduct initial and periodical evaluations.

3. Production of minitubers (FG0) as Pre-basic tissue culture class seed

The UNECE Standard provides a set of conditions specified in Annex I – minimum conditions to be satisfied in the production of PBTC seed potatoes.

Potato minituber production involves the multiplication of micropropagative material and subsequently growing plants in a controlled facility to produce minitubers.

This production is done using peat, hydroponic, and aeroponic production systems. Regardless of the systems used for production of minitubers, the standards for certification should be uniformly applied.

[Note: To insert example pictures of minituber production peat, aeroponic etc.]

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

Only micropropagation material should be planted to produce the potato minitubers. The micropropagation 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

A risk assessment of plant health concerns should be considered regarding the establishment of the minituber facility at a certain location.

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

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

The operator of the minituber facility must take all reasonable husbandry practices for the prevention or spread of pests and diseases. The growing potato crop must have been kept free from potato viruses and bacterial diseases, and the varietal identity of the potato crop must be maintained.

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.

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

Only one generation of minitubers should be produced.

3.3.1 Technical equipment and infrastructure

The minituber facility infrastructure should ideally include the following:

- (a) An anteroom with double door access in the entrance area. The entrance area may be equipped with a footbath for disinfecting footwear and wash bay for washing and disinfecting hands, or alternative measures should be taken to avoid contamination from shoes or hands.
- (b) All access doors, openings and ventilation openings should be sealed with insect-proof mesh with reference to local pests and vectors. The mesh size should be appropriate to exclude the targeted pests. For example, the mesh size to exclude aphids should be in the order of 193.5 micron or 75 mesh.

| <i>Insect</i> | <i>Body Length</i> | <i>Body width</i> | <i>Reference</i> |
|---------------------------------------|--------------------|-------------------|------------------|
| Green Peach Aphid | 1.2–1.9 mm | 0.69–0.82 mm | [to add link] |
| Onion thrips (<i>Thrips tabaci</i>) | 0.7–1.3 mm | [TBC] | [to add link] |

(c) All openings should be sealed between the external and the internal environment of the structure.

(d) The floor area of the facility should be covered in such a manner that the roots of potato plants kept in containers cannot penetrate the soil on which the facility is erected (e.g. cement floors or the separation from soil through a dense membrane).

(e) Designated areas for washing and disinfecting containers.

(f) Designated areas for cleaning, sorting, packing and storage of minitubers.

(g) A suitable air filtration system if appropriate.

(h) A suitable irrigation water filtration and sanitation system if appropriate. For example, filtration, ultraviolet light or chemical sanitation e.g. chlorine.

[Note: to include pictures of infrastructure.]

3.3.2 Access control to the minituber production facility

Access to the minituber production facility should be restricted to the authorized personnel only.

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 pests. This could be achieved by:

(a) Use of soil-free medium (for example peat and other substrates used in hydroponic and aeroponic production systems, etc.).

(b) Fumigation/disinfection/sterilization of growth medium for plants.

(c) Appropriate transport and storage conditions of growth medium to avoid contamination.

(d) Use of clean water sources, such as borehole/spring water or municipal water and regular pathogen testing of irrigation water.

(e) Use of inorganic or appropriately treated organic nutrients.

3.3.4 Crop management

Crop management in the minituber production facility includes the following aspects:

(a) Plants are to be clearly labelled according to variety.

(b) There are procedures to prevent the occurrence of variety mixes, during the growing and harvesting processes.

(c) Precautions or corrective actions against diseases or pests, including any pest management programme, should be documented by the facility operator.

(d) Insect monitoring in production facility is recommended (for example, insect traps covered with an adhesive strip) and needs to be documented.

3.3.5 Sanitation

The facility operator should ensure:

(a) Appropriate hygienic practices for handling all plant material.

- (b) Regular removal of plant debris during growth.
- (c) Appropriate discarding procedures.
- (d) Absence of growth of algae on floor or wet walls.
- (e) Thorough sanitation of the facility after each production cycle.
- (f) That all containers used for production are sanitized before use.

3.3.6 Post-harvest handling and storage of minitubers

The minitubers must be handled, packed, stored and transported in such a manner that contamination by diseases/pests and varietal mixtures are prevented. The facility operator shall have appropriate systems for post-harvest handling and storage including:

- (a) Appropriate storage conditions.
- (b) Appropriate identification procedures of minitubers.
- (c) Cleaning and sanitation of any equipment and storage facilities.

3.3.7 Minimum record keeping

Documented or recorded evidence shall be available concerning the:

- (a) Map of varieties planted for each greenhouse.
- (b) Traceability of all the minitubers produced.
- (c) Disease test results.

It is advisable to keep long-term records (e.g. 10 years) 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:

- (a) Qualification.
- (b) Continuous training and evaluation.

4. Evaluation and assessment of minitubers for certification

4.1 Evaluating and assessing the crop

Inspections approved by the CA during the growing period should be conducted.

Inspections should include the visual assessment of plants, tubers, containers, equipment and facilities by an authorized person, to ensure compliance with the regulations as stipulated by the CA.

Additional testing or inspections may be required at the discretion of the CA to confirm freedom from pests and diseases.

To check varietal identity and varietal purity and absence of diseases, the CA may require a post-control in field for the minitubers (FG0) which are produced.

The minimum quality conditions for minitubers are listed in the annexes II, III and IV to the UNECE Standard.

4.2 Evaluating and assessing the facility

In evaluating and assessing the minituber facility, the CA may record:

- (a) The observed absence or presence of pests and diseases as prescribed in the UNECE Standard (annexes I, II, III, IV).
- (b) The type of facility (e.g. polytunnel, greenhouse), type of ground (floor), the use of insect physical barriers.
- (c) The physical location of greenhouse.
- (d) The maintenance of the area around greenhouse (e.g. occurrence of weeds, potential hosts).
- (e) Whether access to the facility is controlled with restricted authorized access.
- (f) Other species that are being grown in the production facility.
- (g) Records of visual inspection or testing.

5. Certification of seed potato minitubers

When the minitubers meet the requirements, the minitubers can be certified as PBTC seed class by the CA.

The containers used for the packaging of minitubers should be new and free of contaminants.

The containers should be closed officially or under official control in such a manner that they cannot be opened without damaging the official sealing device or without leaving evidence of tampering on the official label.

The minitubers must be officially labelled before marketing (Annex V to the UNECE Standard).
