

CITRUS- TISSUE CULTURE – (CTC) - STANDARDS

I. Applications and Amplification of General seed Standards for CTC

- i. The General Seed Certification Standards are basic and, together with the following specific standards constitute the standards for approval of CTC. As the name implies, these standards are applicable to tissue culture multiplied under laboratory and greenhouse conditions as laid here.
- ii. The General Standards are amplified as follows to apply specifically to the CTC.

1. *Eligibility requirements for CTC production:*

- i. All micropropagation and greenhouse facilities must be approved as per standards/ guidelines set by the competent authority. These must have a changing area between double doors.
- ii. Laboratory and greenhouse facilities used for production of plantlets shall be maintained free of pests or vectors of citrus pathogens. Failure to keep such pests under control may cause rejection of all lots maintained in the facility. All potting or growth media shall be sterile. Water sources used in the laboratory or greenhouse operation shall be treated or otherwise rendered free of all possible pathogens by the applicant.
- iii. Hygienic conditions shall be strictly observed during micropropagation, potting, planting, irrigating, movement and use of equipment and other laboratory and greenhouse practices to guard against the spread of diseases or pests in the facilities used for citrus plant multiplication.
- iv. The greenhouse (protected environment) must be “insect proof” and be equipped with a double-door entrance, provision for footwear disinfection prior to entering the protected environment and insect proof ventilation screening on intakes and exhaust openings. The persons entering the protected environment should use Wellington boots (plastic boots) and change lab-coat in the changing area to reduce the chances of inadvertent introduction of vector insects clinging to clothes
- v. The material being initiated must be of a notified variety and confirmed identity. It must be duly documented with respect to origin.
- vi. All samples of citrus varieties being initiated should be tested in an accredited laboratory and be free of viruses such as Indian Citrus Ringspot Virus (ICRSV), Tristeza Virus (CTV) and Citrus Yellow Mosaic Virus (CYMV) and other endophytic or epiphytic bacteria and fungi.

- vii. The basic material for sub-multiplication need to be obtained afresh from the nodal organization as soon as the maximum permitted number of passages (as confirmed by DNA fingerprinting) of shoot multiplication with old cultures has been completed.
- viii. On application for inspection, the mother cultures as developed above are eligible for certification. The micropropagation facility to be inspected must have been approved by the competent Authority. All stocks must have a valid variety identification and disease testing report at any time during multiplication process.

In vitro multiplication of an imported variety or a non-notified variety can be taken up by the industry exclusively for export purposes. Such varieties, however, should be introduced following the approved guidelines of Government of India.

2. Source of Seed:

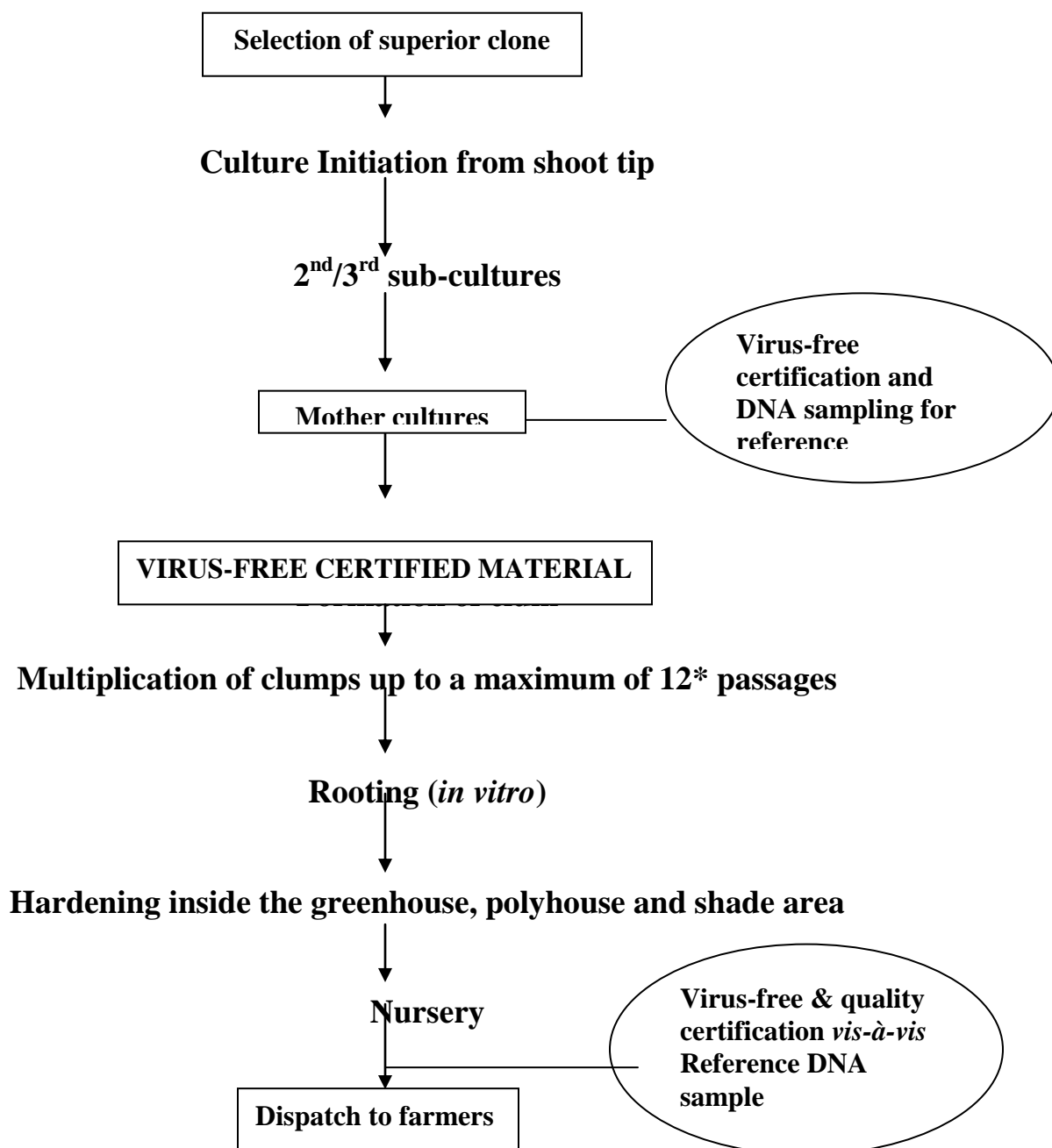
- i. The facility should use recognized aseptic initiation and propagation procedures (i.e. follow procedures and use equipment, which will maintain sterile conditions as per standard tissue culture norms).
- ii. The initiating facility must maintain following information on each variety for review and audit by the competent authority at least once in a year: variety identification, date of initiation, origin and testing results from accredited laboratory.
- iii. Tests must be carried out on a minimum of 0.1% (at least ten) plantlets for each variety by an accredited laboratory. Such tests will be valid so long as cultures of that particular batch are under production (subject to a maximum of 12 passages). No plant should contain viruses such as Indian Citrus Ringspot Virus (ICRSV), Tristeza Virus (CTV) and Citrus Yellow Mosaic Virus (CYMV), and other endophytic or epiphytic bacteria and fungi.
- iv. Valid pathogen testing results are required at the 2nd/3rd subculture stage prior to the bulking up of the cultures.
- v. The guidelines for production of tissue culture plant is at Appendix-I

Minimum Quality Standards for growing of plants inside greenhouses/polyhouses

The following requirements must be met for production of plantlets :

- i. Effective sanitation practices including insect and disease monitoring and prevention must be adhered to.
- ii. No field-produced citrus plants can be grown in the protected environment (greenhouse/polyhouse) along with tissue cultured plants.
- iii. Varieties must be separated by physical barriers (such as proper tagging), which will prevent varietal mixture.
- iv. Before dispatch to the farmers, the tissue-cultured plants growing in the nursery should be tested for the absence of the viruses such as Indian citrus ringspot virus (ICRSV), Tristeza virus (CTV) and Citrus Yellow Mosaic Virus (CYMV) and clonal uniformity. For establishing clonal fidelity, the sample size should be 0.1% of the batch size with a minimum of 10 plants.
- v. If testing performed by an accredited laboratory reveals the presence of banned viruses, fungus or bacteria the tissue-cultured plants should not be dispatched from the premises of the production lab and the entire material should be destroyed.
- vi. The concerned laboratory/agency producing the tissue culture raised material should issue a certificate to the effect that CTC have been produced as per guidelines
- vii. The agency producing CTC will follow the labelling procedures as given at Appendix-II

Procedures and standard parameters for production of citrus by tissue culture



**In tissue culture it is well known that lesser the number of subcultures, lower will be the chances of somaclonal variation. However, it must also be realized that if the number of passages are far too small then the entire production process becomes economically unviable. Therefore, efforts should be made to optimise the shoot multiplication process and extend the number of passages only till the clonal uniformity of the progenies is maintained. This could be achieved through a) strict monitoring of shoot multiplication process ensuring that adventitious shoots are not multiplied and b) confirming the clonal fidelity of tissue cultured plants using molecular markers in different passages. Citrus shoots have been sub-cultured upto 12 passages without any loss of clonal fidelity. There is a possibility that the clonal fidelity of the tissue-cultured plants is maintained even beyond 12 passages*

Labeling- Citrus-Tissue Culture (CTC)

1. CTC shall be supplied in containers. A paper-lined label of 12cm x 6 cm containing following information shall be affixed on the container

Certified Tissue Culture Raised Quality Plants/Propagules

NCS-TCP Certified Tissue Culture Raised Quality Plants/Propagules

Certificate of Quality No.:

Label No. :

Name of Production Facility:

Botanical Name:

(Common Name):

Certification No. and validity:

Variety:

of Certificate of Recognition:

Batch No. & Batch Size:

Contact person and Designation:

Stage of Tissue Culture Plants:

Address with phone number:

In agar Ex-agar Hardened

Date of Issue:

Bar Coding :

Name/Sign/Stamp of ATL with date:

‘The container should also have printed on it the kind, variety and name of Institution’

2. The label shall be rubber stamped with signature, name and designation of the concerned Agency. Colour of the label shall be diagonally yellow No. 356 (IS 5-1978) and opaline green (IS No. 275)
3. CTC producing Agency shall maintain the account of labels printed and issued