. POTATO – TISSUE CULTURE - RAISED MINITUBER - (PTCMT) STANDARDS FOR CERTIFICATION

I. Application and Amplification of General Seed Standards for PTCMT

a. The General Seed Standards are basic and, together with the following specific standards constitute the standards for approval of PTCMT. As the name implies, these standards are applicable to tissue culture raised mini tubers multiplied under laboratory and greenhouse conditions as laid here.

b. The General Standards are amplified as follows to apply specifically to the PTCMT:

1. Eligibility requirements for PTCMT production:

The PTCMT to be eligible for production shall be from a source meeting the following standards for laboratory and greenhouse facilities.

i. Laboratory and greenhouse facilities used for production of plantlets/microtubers or minitubers shall be maintained free of potato pests or vectors of potato 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 to be used in a laboratory or greenhouse operation should be free from impurities.

ii. Hygienic conditions shall be strictly observed during micro propagation, 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 seed multiplication.

iii. All micro propagation and greenhouse facilities must be approved, as per the standard/guidelines. These facilities must have a changing area between the double doors.

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 for producing PTCMT must be of Registered/Notified variety[=] and confirmed identity. It must be duly documented with respect to origin.

vi. The plants of a potato varieties being initiated for tissue culture should be tested in an *accredited* laboratory[2] *for freedom from the* following:

PVA, PVS, PVM, PVY, PVX, PLRV, PALCV, PSTVd and endophytic or epiphytic bacteria and fungi. Tests must be carried on a minimum of ten plantlets of each variety selected at random. For virus testing ELISA or an equivalent method should be used, for viroid RT-PCR should be used, and for fungi and bacteria light microscopy and culturing on media should be used.

2. *Sources 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 morns).

ii. The initiating facility must maintain following information on each variety for review and audit by the competent authority 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 ten plantlets, selected at

random, for each variety by an accredited laboratory. No plant should contain PVA, PVS, PVM, PVY, PVX, PLRV, PALCV, PSTVd and other endophytic or epiphytic bacteria and fungi.

iv. Valid pathogen testing results are required prior to the initiation of micro tubes production cycle or planting of test tube plantlets in the greenhouse.

v. PTCMT shall be produced and multiplied from approved source *in vitro* plants or microtubers, as per the requirements.

vi. PTCMT may be used as breeder seed for further production certified classes of seed as prescribed in the Indian Minimum Seed Certification Standards.

vii. Concerned laboratory should issue a certificate to the effect that the PTCMT has been produced with the standards as prescribed under their supervision.

II. Greenhouse/Controlled Environment Requirements

1. All micropropagation and greenhouse facilities must meet the standards given above under eligibility requirements.
2. The soil used for PTCMT production should not be infested with pathogen and pests of potato, particularly the following:
   - wart *(Synchytrium endobioticum* (Schilb.) Perc.) and or cyst forming nematodes;
   - brown rot *(Pseudomonas solanacearum (E.F.* Sm.) E.F. Sm.) or non-cyst forming nematodes within the previous three years;
   - common scab *(Streptomyces scabies* (Thaxt.) Waks. & Henrici).

III. Inspection of Greenhouse/Controlled Environment facility used for production of PTCMT

1. The grower must notify the Competent Authority of his production plans well in advance of the planting.
2. The crop must be grown from approved basic source *in vitro* plants or micro tubers, which were produced, in an aseptic environment.
3. A minimum of three inspections shall be made as follows:

    i. The first inspection shall be made 35 days and 45 days after planting for plains and hills respectively to verify growing conditions, extent of disease infection and off types and also to confirm isolation requirement of one meter between different varieties as to avoid mechanical admixture;

    ii. The second inspections shall De made at 60-65 days after planting to verify off types, disease infection if any and pathogen testing, on a representative sample, comprising of *1% of the plants with a minimum of 5 and a maximum of 25 plants* sampled *for each* variety:

    iii. The third inspection shall be made immediately after haulms cutting/ destruction in order to verify that haulms have been cut/destroyed by the prescribed date and proper manner.

    iv. Effective sanitation practices including insect and disease monitoring and prevention must be adhered to.

    v. Basic Stock can be planted in commercially available medium, which has not been recycled. If nursery beds are used, the substrate should be properly sterilized before planting.

    vi. The greenhouse must be free from all potato and solanaceous plant debris before planting.

    vii. No field-produced seed potatoes (including pathogen tested clonal selections), non-seed potatoes, nor any other solanaceous species of

plants can be grown in the protected environment while used to produce Basic Stock.

viii. Varieties must be separated by appropriate partitioning of greenhouse to prevent varietal mixture.

ix. If testing performed by an accredited laboratory reveals the presence of banned virus (es), fungus or bacteria all the crops in the protected environment will be ineligible for multiplication and the entire material will be destroyed.

x. In the eventuality of detection of insect (particularly aphids, thrips and white flies) vectors (for which yellow sticky traps should be put at least at three places in a greenhouse) by competent Authority, the grower must provide post harvest test results to this authority. A representative sample, representing each variety grown in the protected environment must be post harvest tested and if the results are negative for PVA, PVS, PVM, PVY, PVX, PLRV and PALCV, the crop will be assigned basic stock status or otherwise rejected.

IV. Field Standards

A. Field Standards of PTCMT at greenhouse

a. <u>General requirements</u>

1. Isolation: Minimum 1 meter between the different varieties grown in greenhouse so as to avoid mechanical admixture.

2. All micropropagation and greenhouse facilities must be notified (approved) by DAC, as per the standards given above under eligibility requirements.

b. *Specific requirements*

Factor	Maximum permissible limits
* Offtypes	0.05%
**Plants showing symptoms of	
- Mild mosaic	0.05%
- Servere mosaic, leaf roll, yellows and apical leaf curl	0.05%
** Plants infected by brown rot (syn. Bacterial wilt) (Ralstonia solamacearum)	nil

*Maximum permitted before dehaulming

**Maximum permitted at final inspection, though the diseases mentioned above are not expected to be present in tissue culture raised plants but it essential to maintain a good crop hygiene.

c. Seed Standards for PTCMT

Factor	Standards for PTCMT
Weight of mini tuber (minimum)	1.0gm
Germination/sprouting (minimum)	90%
Varietal Purity (minimum)	99%
Pure seed	98%
Virus	0.01%

B. Field standards for Foundation Crops and Certified Crop raised out of Potato-Tissue Culture raised Mini Tuber (PTCMT1 (shall be same as prescribed for conventional method)[3] at Annexure-III. .

[1] *in vitro multiplication for custom production 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] *The following* laboratories *of* the *'National Facility for Virus Diagnosis and* Quality *Control of* Tissue *Culture Raised Plants' are currently* accredited *for virus testing: (1) Advanced* Centre *for Plant Virology, Indian Agricultural Research Institute, New Delhi (2) Indian* institute *of Horticultural Research, Hessaraghatta Lake PO, Bangalore, (3) Institute of Himalayan Bioresources Technology,' Post Box. No.6, Palarnpur (HP), in addition Central Potato Research* Institute is also *accredited.* Of these *four laboratories, Advanced Centre for* Plant *Virology, Indian Agricultural* Research Institute *will* act *as Referred lab.*

[3] Details not required here but given for reference

LABORATORIES FOR CERTIFICATION OF PTCMT — MINIMUM REQUIREMENT

Under the National Facility for Virus Diagnosis and Quality Control of Tissue Culture Raised Plants, the following three centers are working for the virus testing.

1. Indian Agricultural Research Institute, New Delhi (Referral Laboratory)
2. Indian Institute of Horticultural Research, Bangalore
3. Institute of Himalayan Bioresource Technology, Palampur

All the three centers of the National Facility for Virus Diagnosis and Quality Control of Tissue Culture Raised Plants have excellent infra-structure facilities and well trained and experienced scientists to test for plant viruses using state-of the-art diagnostic techniques like ELISA, PCR, dot blot hybridization, Western blotting, immuno-electromicroscopy, etc. Considering the availability of expertise and necessary infra structure, these laboratories are accredited to test and certify plants for freedom from viruses. Apart from these three laboratories considering the availabii:.y of expertise and necessary infrastructure for this work, the Central Potato Research Institute, Shimla is also accredited for virus testing of PTCMT.

1. Advanced Centre for Plant Virology, Indian Agricultural Research Institute, New Delhi- 110012 (Referral Laboratory)

The Centre was established in 1988 for characterization of plant viruses and development of management practices. Since then, a large number of viruses have been characterized; their diagnostic reagents prepared and technologies developed for the management of important viral diseases of plants. This centre is well equipped with all the necessary facilities required for virus work like ultra centrifuges, electron-microscope, electrophoretic systems, PCR machines, nucleic acid sequencer, insect-proof glass houses. environment controlled glasshouse meeting biosafety requirement and plant growth chambers. The Centre has scientists trained in various laboratories in the USA and Europe on different aspects of virology including molecular virology. It is a leading National Centre for work on plant viruses.

2. Indian Institute of Horticultural Research, Bangalore

The Indian Institute of Horticultural Research has a very strong unit for work on plant viruses affecting horticultural crops. It has the basic facilities like ultra centrifuges, electromicroscope, electrophorotic systems, PCR machine, etc. It also has a very good insect-proof glass house facility for growing plants under controlled conditions. The scientists working in the unit are very well trained in India and abroad. The Centre is playing an important role in testing for viruses in horticultural crops.

3. Institute of Himalayan Bioresources Technology, Bangalore

Institute of Himalayan Bioresources Technology has also developed excellent facility for work on plant viruses It has developed diagnostic techniques for testing of viruses affecting horticultural plants particularly the ornamentals The Centre has good' facilities for growing plants under-insect-proof conditions. The' Centre is well equipped with ultracentrifuges, electrophorotic systems, PCR machines, ELISA reader, Sequences, etc. The Centre has a good team of scientists trained in India and abroad. Considering the above capabilities, it would be useful to identify all the three centers as testing centers for the purpose on certification for freedom from viruses.

4. Central Potato Research Institute, Shimla.

Central Potato Research Institute, Shimla was established in 1949 to improve potato production in the country through intensive multi disciplinary research and production of healthy seed potato. As a result of technologies developed at this institute potato production has increased by nearly 15 times and potato productivity 2.57 times, making India the fourth largest producer of potato. The Institute has developed excellent facilities and has highly competent scientists for virus testing of PTCMT. The facilities available at the Institute, include, electron microscope, ultracentrifuges, electrophoritic systems, PCR machines, EUSA reader, nucleic acid hybridization facility etc. They also have very well maintained insect-proof glasshouses. The scientists of the Institute have been trained in India and abroad.

General Requirements for accrediting a laboratory for virus testing:

1. The laboratory must be adequately equipped for virus diagnostic work. It must have basic equipment like ultracentrifuge, electrophoritic system, PCR machine, ELISA reader, etc.

2. The laboratory must have facilities for growing plants under insect-proof conditions

3. The laboratory must have at least two scientists with good training in virology, preferably Ph. D in virology, and experience of working on virus diagnosis.

Appendix - IA

Labeling of Potato -Tissue Culture Mini tuber (PTCMT)

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

Crop	Potato	Label No
Variety		
Class of seed	PTCMT	
Lot No.		
Approved laboratory and reference:		
Date of test		
Germination/sprouting (Minimum)		
Producing agency (Name and address)		

'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. PTCMT (Breeder seed) producing Agency shall maintain the account of labels printed and issued.

Annexure-III

Seed Certification Standards for Potato Tissue Culture raised Minitubers (PTCMT)

I. Application and Amplification of General Seed Certification Standards

a. The General Seed Certification Standards are basic and, together with the following specific standards constitute the standards for certification of seed potato.

b. Classification of seed potato on the basis of area of Production:

There shall be two types of seed potatoes, namely the Hills and Plains - grown and shall be designated as Hill Seed (HS) and Plains Seed (PS) respectively. Hill Seed (HS) shall be grown in the high hills generally 2500 meters above the mean sea level or in situations declared technically suitable for seed production. Plains Seed (PS) shall be grown in such areas where aphid infestation is low during the crop growing season and which are technically suitable for seed production.

II. Land Requirements

A crop of seed potato shall not be eligible for certification if grown on land infested with:

a. Wart *(Synchytrium endobioticum (Schilb.)* Perc. And or cyst forming nematodes;
b. Brown rot *(Pseudomonas solanacearum* (E.F_ Sm.). E.F. Sm. Or non-cyst forming nematodes within the previous three years;
c. Common scab *(Streptomyces scabies* (Thaxt.) Waks. & Henrici).

III. Field Inspection

A minimum of four inspections shall be made as follows:

1. the first inspection shall be made about 45 days after planting the PTCMT in the hills and about 35 days after planting the PTCMT in the plains to verify isolation, offtypes and the extent of disease infection with specific reference to mild and severe mosaics, leaf roll, yellows, brown rot and other relevant factors;
2. the second inspection shall be made about 60-65 days after planting the PTCMT for early varieties and about 70-75 days after planting the PTCMT for late varieties or at appropriate growth stage depending on the crop duration of the variety concerned to check isolation, offtypes and extent of disease infection with specific reference to mild and severe mosaics, leaf roll, yellows, brown rot and other relevant factors;
3. the third inspection shall be made immediately after haulms cutting/destruction in order to verify that haulms have been cut/destroyed by the prescribed date and in proper manner;
4. the fourth inspection shall be made about 10 days after haulms cutting/destruction and before harvesting in order to verify that no re-growth of haulms has taken place.

IV. Field Standards

A. General Requirements

1. Isolation

The fields of seed potato shall be isolated from the contaminants shown in column 1 of the Table below by the distances specified in columns 2, 3 and 4 of the said Table.

Contaminants	Minimum distance (meters)		
			Certified
1.	Stage I 2.	Stage- II 3.	4.
Fields of other varieties	5	5	5
Fields of the same variety not conforming to varietal purity requirements for certification	5	5	5

B. Specific requirements

		Maximum permissible limits		
Factor	Stage	Foundation		Certified
		Stage-I	Stage-II	
1.	2.	3.	4.	5.
Off types	I & II Inspection	0.050%	0.050%	0.10%
Plants showing symptoms of: - Mild mosaic	1 & II Inspection	1.0%	2.0%	3.0%
-Severe mosaic, leaf roll and yellows	I & 11 Inspection	0.50%	0.750%	1.0%
*Total Virus	-	1.0%	2.0%	3.0%
**Plants infected by brown rot (Syn. Bacterial wilt) *(Pseudomonas solanacearum (E.F.* Sm.) E.F. Sm.)	I & II Inspection	None	None	3 plants per hectare
***Re-growth of plants after destruction of haulms	IV Inspection	0.50%	0.50%	0.50%

*Of the two inspections, the higher virus percentage will be considered for the

purpose of the specified limits of tolerance.

**The presence of brown rot infected plants within the specified limits of tolerance shall be permitted in the areas known to be infected with this disease. In case of plants suspected to be infected with brown rot, the neighboring plants, one on either side should also be rogued along with tubers

***Standards for re-growth after destruction of haulms shall be met at fourth inspection to be conducted about 10 days after haulms cutting.

Note: 1. All off types and diseased plants should be rouged out along with the tubers and destroyed.
2. Gaps in the seed plot should not be more than 10.0%.
3. Haulms must be destroyed as close to the ground as possible before the date specified by the Certification Agency. Failure to destroy haulms in time shall render the crop liable for rejection.

V. Seed Standards

A. Specification in respect of size and weight of seed material for Foundation Stage-1, Foundation Stage-II and Certified class shall be as under:

Size	Mean length and two widths at the middle of tuber	Corresponding weight
a. Hill Seed (HS) Seed size Large size	30mm-60mm above 60 mm	25-150 gm above 150 gm
b. Plains Seed (PS) Seed Size Large size	30 mm - 55 mm above 55 mm	25-125gm above 125 gm

Note:

1. The size of tuber will be decided either on the basis of mean of two widths of a tuber at the middle and that of length or on the basis of corresponding weight of tuber.
2. In a seed lot, tubers not conforming to specific size of seed shall not exceed more than 5.0% (by number).

3. (a) The seed material shall be reasonably clean, healthy, firm and shall conform to the characteristics of the variety. The tubers not conforming to the varietal characteristics shall not exceed 0.050% and 0.10% (by number) for Foundation and Certified seed classes respectively.

(b) Cut, bruised, unshapy, cracked tubers or those damaged by insects, slugs or worms shall not exceed more than 1.0% (by weight).

(c) Greenish pigmentation on tubers will not be a disqualification for certification.

B. Maximum tolerance limit of tubers showing visible symptoms caused by the diseases mentioned below will be as follows!

	Maximum permissible limits		
Diseases	Foundation		Certified
	Stage-I	Stage-II	
1.	2.	3.	4.
Late blight *(Phytophthora* infestations (Mont.) de Bary), dry rot *(Fusarium caeruleum* (Lib.) Sacc.) or Charcoal rot *(Macrophomina phaseoli* (Tassi) G. Goidanich).	1.0% (by number)	1.0% (by number)	1.0% (by number)
Wet rot *(Scierotium rolfsii)* Sacc.)	None	None	None
* Common scab *(Streptomyces* scabies (Thaxt) Waks. & Henrici)	3.0% (by number)	3.0% (by number)	3.0% (by number)
** Black scur *(Rhizoctonia solani* Kuehn.)	5.0% (by number)	5.0% (by number)	5.0% (by number)
*** Total diseases	5.0% (by number)	5.0% (by number)	5.0% (by number)

* Even if a single tuber infected with common scab is detected in a seed lot, the entire seed lot shall be treated with approved fungicide before seed lot is

declared fit for certification. Seed lots having infected tubers more than the prescribed limits will not be certified even after treatment.

**(a) A tuber carrying 10.0% or above scurfed surface will be considered as one infected unit.

(b) Seed lots having black scurf infection more than the prescribed limits could be certified after treatment with approved chemical/fungicide.

(c) For all diseases, the higher disease percentage will be considered for the purpose of the specified limits of tolerance.