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Key Components for Establishment and Operation of Commercial Plant Tissue Culture Unit in Accordance to National Standards

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ABSTRACT: *Standards prescribed under the National Certification System for Tissue Culture Raised Plants (NCS-TCP) provide guidance to entrepreneurs for meeting the quality requirements in establishment and operation of commercial unit in terms of infrastructure, package of practices and quality of end products. Layout of the facility is the basic requirement for ensuring unidirectional man and material movement and avoiding possibility of cris-cross movement between sterile and non-sterile zone. Facility should have clearly demarcated area for entry, washing, media preparation, media storage, inoculation, growth room and plantlets washing/grading area. Proper consideration should be given to ensure connectivity to various segments of facility and availability of basic equipment particularly for maintenance of minimum sterility class 100,000 in media storage, inoculation, growth rooms and corridor connecting the sterile area.*

Package of practices for production of specific plant species is critical once the infrastructure is ready in compliance with the requirements. Standard production practice includes defining criteria of elite mother plant, maintenance of record for mother plants, virus indexing of initiated in vitro *cultures for all the known viruses listed under the NCS-TCP prior to mass multiplication, restricting the multiplication of shoots within the prescribed cycles, maintenance of cleanliness and monitoring of airborne microbes, grading of plantlets while transferring it from laboratory to hardening facility, monitoring of growth of plantlets, management of insect/pest and maintenance of batch corresponding to original material in order to have complete traceability. Availability of trained and dedicated facility in-charge, training and active supervision are other important aspect. Facility needs to document and maintain records for all critical operations. Batch certification of tissue culture raised plants to be done as per the sampling strategy defined in the Standard Operating Procedures (SOPs). Farmers should be provided plants with hand-out for cultivation practices and labels issued by Accredited Test Laboratory (ATL). Data on performance of tissue culture plants and farmer's feedback should also be maintained.*

Key words: Standards, NCS-TCP, Sterility Class, SOPs, Virus Indexing, ATL

INTRODUCTION

Micro propagation is the true to type propagation of selected elite plants using *in vitro* culture techniques[1]. Micro propagation technology has been applied successfully for the large scale production of hundreds of plant species including ornamental, fruit crops and trees. The operational steps in micro propagation are labour-intensive and repetitive that escalates production cost. The comparatively high production cost of micro propagation over conventional methods of propagation are compensated for by the several benefits: (a) rapid propagation because of short propagation cycle (b) large volume propagation of high value crop (c) easy storage and transport of large number of plants (d) plant production independent of season (e) quick supply of plants with the change in demand[2].

In USA, inception of commercial micro propagation was reported in 1970s with the propagation of Orchids whereas Indian micropropagation industry started almost a decade later. In 1996, the cumulative installed annual capacity was noted at 190 million plants[3]. Indianplant tissue culture sector has witnessed a significant growth in the last 10 years and a number of companies with

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production capacity ranging from a few lakhs to 30 million plants per annum are operational with the aggregate production capacity of more than 300 million plants per annum. There is agreat potential which can be realized by propagation of healthy and high yielding planting material. In the absence of effective quality parameters in micro-propagation may result into spread of virus and/or somaclonal variants which may hamper its growth resulting intopoor acceptance of this promising technology. Therefore, a quality management system is critical for the growth of the tissue culture industry. Accordingly, National Certification System for Tissue Culture Raised Plants (NCS-TCP) has been established by the Department of Biotechnology (DBT), Government of India which is first of its kind in the world. It is a constantly evolving and well organized system aimed at facilitating production of quality tissue culture plants and providing well defined system for certification of quality tissue culture plants[4].

It is important to plan well in advance for establishment of tissue culture production facility (TCPF) in accordance with the Standards prescribed under the National Certification System for Tissue Culture Plants (NCS-TCP) so that any structural modification(s) at later stage leading escalation of project cost could be avoided. Recognition of TCPF under the NCS-TCP is necessary requirement in order to get access of market of government supply through tenders[5]. It is also one of requirement for availing incentives available under Mission for Integrated Development of Horticulture[6]. In addition to these benefits, NCS-TCP recognition provides high level of visibility and credibility to the company resulting into their market access which is required for sustainability and long term existence in the market through meeting the customer expectations of quality plants. This article describes establishment of tissue culture facility and its operation in line with NCS-TCP

A. ESTABLISHMENT OF COMMERCIAL PLANT TISSUE CULTURE FACILITY:

1. Location

Suitability of location is determined in terms of availability of water, power, human resources, connectivity, proximity of amenities etc. Approval on land use and layout has to be obtained from the concerned authorities. Location of the facility is out of the scope of NCS-TCP. However, it is desired that facility should not be located in polluted, unhygienic area as well as it should also not pose any health hazard/ risk of fire accidents for the population living in surrounding area due to discharge of wastage or storage of any inflammable material.

2. Layout

Clearly demarcated area for different components of laboratory operation namely washing room, media preparation, media storage, inoculation, growth room, plant transfer/grading room, primary and secondary hardening area are needed for effective functioning of the operation as well to meet the point to point area specifiedcriteria. Entry to sterile area should be restricted for operators working in inoculation and growth room. Non-sterile entry/exit should be kept separate. Interconnectivity and minimum movement can be ensured by placing pass-boxes between certain area such as autoclaving to media storage, Inoculation to growth rooms and growth rooms to dispatch area. Interconnectivity of sterile area is to be provided with sterile corridor. Layout may vary depending on the demography, production capacity and plant species (like potato where the laboratory would need some additional facility). A basic layout of the tissue culture laboratory is at Figure 1for the purpose of illustrating the requirement.

3. Building and Infrastructure

Building should be made with RCC structure, smooth wall/surface and wall should have plastic paint/ water proof emulsion. Fire-fighting system covering emergency exit, fire/smoke alarm, fire extinguisher, fire alarm and path showing fluorescent strip is essential to ensure safety of staff. Power-backup is the basic need to support the consistent operation. Due attention is required while designing and constructing the entry to sterile area of laboratory (which is critical to act as barrier for entry of dust/ spores). Entry area should be equipped with hand and leg wash facility, air shower, dress-change cubicle and dress storage cabinet. Bottle washing area needs to be connected to the media preparation roomthrough a pass-box or closed corridorfor transfer of washed vessel smoothly. There should be availability of running tap water, separate basin for different stages of washing as well as for jars received from hardening area/after decontamination. A separate autoclave (other than used for sterilizing media) is required for de-contaminating the infected culture and media and the same might be kept at

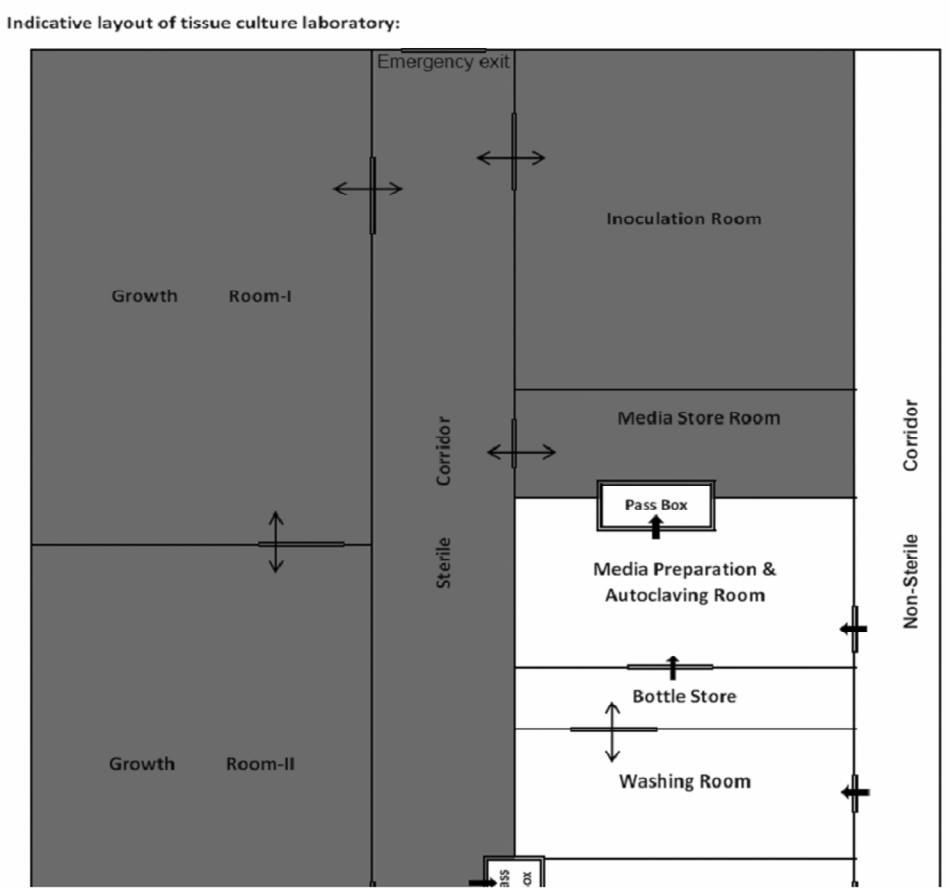

Figure 1. Illustration of laboratory layout showing connectivity with different area. Shades area should be covered under clean air class 100,000

bottle washing area. Washing area should be closed and to be considered as integral part of facility with maintenance of proper hygiene and cleanliness. Media preparation area should be equipped with all the basic equipment electronic balance, pH meter, water purification system, microwave oven, autoclave etc. Media storage room should have adequate space for storing the media for at least three days. In the room, UV light should be installed in order to minimize the microbial load. Inoculation room needs to have connectivity with media storage room and laminar air flow cabinet should be fitted with manometer, UV tube and glass bead sterilizer. Inoculated cultures need to be transferred to growth room through sterile corridor. Culture should be transferred to grading area through pass-box so that reverse movement of material can be avoided. Grading area should be equipped with washing facility, working table, grading scale and pictorial map for organized grading of plantlets.

Maintaining the sterility class 100,000 is critical requirement for meeting the requirement of recognition. It helps in minimizing the contamination level. Cleanrooms are classified according to the number and size of particles permitted per volume of air.ISO 14644-1 (Equivalent to US FED 209 E) Cleanroom Standards define the sterility class of the area which is maintained through module or Air Handling Units fitted with HEPA filter. A cleanroom is an environment typically used to maintainlow level of environmental pollutants such as dust, airborne microbes. It is categorized as Class 1, class 10, class 100, class 1000, class 10000, class 100000. Above Class 100000 is called room air[7]. For sterile area of plant tissue culture facility (which includes media storage room, inoculation room, growth room and clean corridor) class 100000 (Equivalent to ISO 8) is required to be maintained to meet the requirement of NCS-TCP. Recording the particle count data is essential as evidence for maintaining the desired class.

Greenhouse/poly-house and net-house should have double door entry (L-shaped) to prevent the inset/pest. Raised beds/mulching should be

provided to avoid the contact of root to the round soil. Yellow sticky traps need to be placed in hardening areas at the rate of per 10 m^2 for monitoring of insect/pest.

B. OPERATIONAL REQUIREMENT:

1. Standard Operating Procedures (SOPs):

Standard Operating Procedures (SOPs) are set of written instructions which include step by step activities. Adoption of SOPs is an integral part of any quality management system which makes the system/operation process-dependant rather than making it person specific. It helps in traceability through connectivity in records and facilitates documentation of key process. Template SOPs developed under NCS-TCP placed at NCS-TCP website is the must read document for operationalization of TCPF in compliance with the specified requirements[8].

2. Package of Practices throughout the production process

Standard package of practices for operatingtissue culture facility includes comprehensive list of activities which covers ensuring cleanliness particularly in washing area, proper procedure for discarding the used agar/media, labelling of media as well as culture, trays at each step, monitoring of air borne microbes on monthly basis in addition to particle count at six monthly intervals and regular fumigation, storing the culturemedia for minimum 3-4 days prior to use in order to avoid wastage of precious culture and time due to problem media sterilization, maintenance of laminar air flow cabinet by cleaning of pre-filters, checking the air flow and efficiency of HEPA filter by exposing plates, maintaining uniform temperature in the growth room, grading of plantlets according to specific criteria for the plant species being micro-propagated, monitoring of health of *in-vitro* culture and plants in the hardening area, monitoring of light, temperature and humidity in the greenhouse, insect monitoring in the hardening areas, ensuring that good quality of water (potable) water being used for watering of plants under hardening and avoiding excessive watering and undertaking regular weeding. Entire production process should be strictly monitored and supervised by in-charge and supervisors. Multiplication cycle should be restricted in order to avoid somaclonal variations. Maximum number of cycle can be determined by studying the uniformity at genetic level using molecular markers. Different species have different permissible limits of number of passage (e.g.12 for Apple, 8 for Banana, 15 for Bamboo and 7 for Sugarcane). Facility must ensure that plants are fully hardened at the time of dispatch. In case of ex-agar plants, plantlets should be appropriate size for survival during transport. Hand-out to be given to farmers while supplying plants covering the package of practices for cultivation [9].

3. Testing and Certification of TC Plants

Certification of tissue culture plants is essential after recognition of tissue culture facility. Testing of mother plant tissue (at the time of culture establishment) or stock culture (prior to mass multiplication) needs to be done prior recognition since it is mandatory to get recognition. For the certification of plants (for which facility becomes eligible after recognition under NCS-TCP), testing of samples at two levels needs to be done i.e. testing of initial material (mother plant tissue or stock culture) and batch testing of tissue culture plants derived from the tested stock. Process flow of each level is summarized at 3.1 and 3.2:

3.1 Process Flow for testing of mother plant tissue/ stock culture

Intimation form issent to ATL for Virus Indexing of Plant Tissue/ Stock Culture(s) preferably at least two weeks before the sample(s) to be sent. ATL acknowledges the intimation and inform the company regarding fee to be submitted for testing

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Sample(s) are to besent to ATL along with application covering detailed information of the samples to be tested and requisite fee. Each sample will be assigned unique 20 digits sample registration number by ATLs

↓

The lab-technician after testing, prepares a **test report** and submit to Scientist concerned to verify and sign the test report for issuance to companies

3.2 Process Flow for testing and certification of tissue culture raised plants

Intimation form issent to ATL for testing and certification of tissue culture raised plants preferably at least two weeks before the sample(s) to be sent. ATL acknowledges this intimation and inform the company regarding fee to be submitted for testing. *Application for certification of tissue culture plants is accepted only from the recognized tissue culture facility and only in the case when the batch of tissue culture plants has been produced from indexed stock cultures/mother plant*.

↓

Samples aresent to ATL along with application covering detailed information such as batch number/batch size and requisite fee. Each batch of plants will be assigned unique **40 digits** batch registration number by ATL

↓

The laboratory technicians (Virology/Molecular Biology) after testing prepare a **test report** and submit to concerned scientist of ATL to verify and sign the test report

↓

Head of ATL issues a "**Certificate of Quality"** based on test report (Virus indexing and/or genetic fidelity) to the concerned TCPF

↓

ATL issues required number of **certification labels** to the company only if the samples are free from known viruses and/or true to type along with "Certificate of Quality"

3.3 Sampling strategy

- **Stock Culture/Mother Plant Tissue:**All mother plant tissue/stock culture must be indexed for all known viruses listed at www.dbtncstcp.nic.in
- **Tissue Culture Raised Plants:** For tissue culture raised plants, following sampling strategy has been adopted:

Batchsize	*Number of tissue culture plants to be sampled*
Up to 1000 Nos	1% plants subject to a minimum of 10 Nos
1001 to 10000 Nos	0.5% of plants subject to a minimum of 10 Nos
10001 to 100000 Nos	0.1% of plants subject to a minimum of 50 Nos

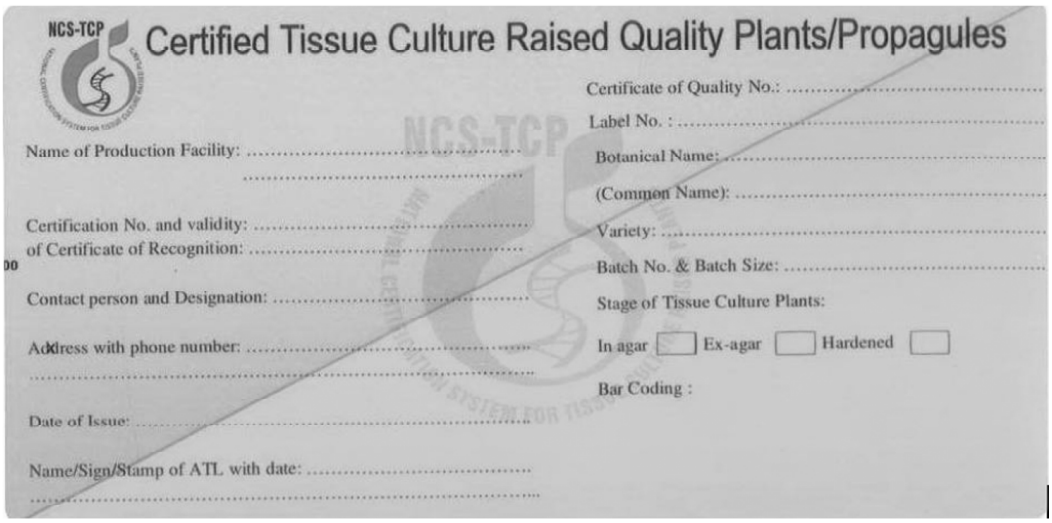
(Source: SOPs under NCS-TCP)

3.4. Labelling and Traceability

Traceability of end products can be achieved through implementation of SOPs, maintenance of effective records for inputs, process, movement of material and labelling of intermediate steps as well as end products i.e., plants raised through tissue culture. Passport data for mother plants/stock culture should be maintained. The origin material needs to be indexed for all the known viruses and allotted a unique 20 digits stock registration number by ATL. This indexed material derives a batch of tissue culture plants and certified batch is given with 40 digits batch registration number which includes additional 20 digits added on the 20 digits stock registration number. SOPs available at the official site of NCS-TCP provide details of these numbers. Certification labels including barcode are issued against each certified batch of tissue culture plants which provides complete history of plants. This labelling and entire concept not only provides effective mechanism of quality assurance by the tissue culture companies but also instils a enhanced level of confidence among farmers [10]. Certification label approved by the DBT has two different colour i.e. diagonally yellow and opaline green is illustrated at Figure 2. Tissue culture raised plants producing recognized company should maintain the account of labels issued to their clients.

4. Documentation and maintenance of records

Documentation and records starting from selection of mother plants to performance of tissue culture raised plants should be maintained. These include (i) Defined criteria (species wise) for the selection of elite plants (ii) Record for mother stock (such as unique code no. and passport data of the mother plants (iii) Decontamination/ autoclave-ing of infected cultures (iv) Calibration of all analytical and measuringequipment (v) Stock solution preparation, media preparation, autoclave cycle (vi) Routine screening of media for any contamination (vii) Efficiency of the operators engaged in media preparation (volume, number of jars, wastage etc.)


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: .....

of Certificate of Recognition: .....

Variety: .....

Batch No. & Batch Size: .....

Contact person and Designation: .....

Stage of Tissue Culture Plants:

Address with phone number: .....

In agar ☐ Ex-agar ☐ Hardened ☐

.....

Bar Coding :

Date of Issue: .....

Name/Sign/Stamp of ATL with date: .....

.....

Figure 2. Certification label provided for certified batch of tissue culture plants.

in media preparation (viii) Efficiency of operatorsin inoculation activity (through monitoring number of jars handled, multiplication rate, contamination losses, rooting percentage and general health of the culture etc.) (ix) Particle count in support of desired sterility class, monitoring of the airborne microbe through microbial plating and fumigating the room periodically with the sterilant (x) In growth room, records for contaminated cultures, temperature and light intensity/duration, calculating multiplication fold at the end of each passage, (xi) Production schedules based on the protocol efficiency (xii) Number of plantlets transferred/ dead plants maintained batch-wise, any treatments given to the plants (fertilizer applications/ insecticide/ fungicidal; sprays (xiii) Incidence of insect pests/ diseases/ vectors (xiv) Farmers' feedback/ data regarding field performance of tissue culture raised plants (xv) Maintain unique code for each batch of production to trace back history of tissue culture plants supplied to farmers[9].

CONCLUSION

NCS-TCP is comprehensive quality management system aimed at strengthening the capacity of tissue culture production facility for production and distribution of quality tissue culture plants through the process of recognition. Testing and certification of tissue culture plants of recognized facility by the Accredited Test Laboratory ensures that plants are free from all known viruses and true to type or uniform. Thus, NCS-TCP not only helps to tissue culture companies but also to consumers/farmers who incur higher cost for planting materials as compared to traditional cultivation.

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REFERENCES

Debergh PC & Read PE (1991), Micropropagation. In: Debergh PC & Zimmerman RH (eds.). The Netherlands; Kluwer Academic Publication, 1 - 13

Evans DE, Coleman JOD & Kearns A (2003), The Basics-Plant Cell Culture. BIOS Scientific Publishers, Taylor & Francis Group, London and New York, pp 20 -21

Govil S and Gupta S C (1997), Commercialization of plant tissue culture in India. Plant Cell, Tiss. Org. Cult. 51(1): 65 - 73 (Online revised version –June 2006)

Shukla SK, Sharma P and Swarup R (2012), Impact of national certification system for tissue culture raised plants (ncs-tcp) on Indian plant tissue culture industry: A unique quality management system for commercial plant tissue culture. 3rd World Congress on Biotechnology, HICC, Hyderabad (13th – 15th September, 2012)

Tender document of Department of Agriculture, Government of Bihar accessed on May 14, (2015),

(http://horticulture.bih.nic.in/Tenders/PDF/Tender_enquiry_for_supply_of_tissue_culture_banana_plant_2015_16.pdf)

Operational Guidelines for Mission for Integrated Development of Horticulture. Page 15 Web-link (http://midh.gov.in/PDF/MIDH_GL(E).pdf)

http://www.cleanairtechnology.com/cleanroom-standards.php(Accessed on May 15, 2015)

http://dbtncstcp.nic.in/downloads/SOP-TCPF.pdf(Accessed on May 15, 2015)

Self-Assessment Report of NCS-TCP Form 2 at link http://www.dbtncstcp.nic.in/downloads/Applicationforms02.pdf (Accessed on May 15, 2015)

Shukla SK (2014) Comprehensive traceability of tissue culture raised plants in commercial micro-propagation. Proceeding of 2nd International Conference on Agricultural & Horticultural Sciences. Hyderabad, India (03-05, February 2014) Pg 172