

# Standard Operating Procedures For Recognized Tissue Culture Production Facility

---

(Name & Address of Recognized Tissue Culture Production Facility)

COPY  
NO: \_\_\_\_



सत्यमेव जयते

National Certification System for Tissue Culture Raised Plants (NCS-TCP)  
Department of Biotechnology, Government of India  
New Delhi

October 2008

<b>SOPs for Tissue Culture Production Facility</b>		
<b>Section-0</b>	<b>Table of Contents</b>	<b>Page 1 of 1</b>
<b>October 2008</b>		

### Table of Contents

<b>Section</b>	<b>Title</b>	<b>Page Number</b>
Section-0	Contents	2-2
Section-00	Control of Document	3-5
Section 1	Introduction (Scope/Purpose/Definitions/References)	6-8
Section 2	Organization structure and role & responsibilities	9-10
Section-3	Selection of Mother Plant/Establishment of mother stock (nursery)	11-12
Section-4	Preparation of ex-plants	13-13
Section-5	Cleaning/Washing/Drying of glassware	14-14
Section-6	Preparation of media	15-18
Section-7	Inoculation	19-19
Section-8	Incubation of cultures	20-20
Section-9	Virus indexing and Genetic fidelity testing of tissue culture raised plants	21-24
Section-10	Packing/labeling/dispatch of ex-agar plants	25-25
Section-11	Entry/Exit to tissue culture laboratory	26-26
Section-12	Maintenance of cleanliness & sterile conditions/servicing of HEPA filters	27-27
Section-13	Monitoring of Microbial Contamination, Fumigation/disinfection of facility	28-28
Section-14	Management of primary hardening facility (green house)	29-30
Section-15	Management of secondary hardening facility (shadenet house)	31-31
Section-16	Dispatch and monitoring of performance of tissue culture raised plants	32-33
Section-17	Calibration of Measuring and Monitoring Equipments	34-35
Section-18	Document Management and Record Control	36-38
Section-19	Training of workers/staff	39-40
Section-20	Communication/Auditing/Review	41-45

SOPs for Tissue Culture Production Facility		
Section-00	Control of Document	Page 1 of 2
October 2008		

## **1. Document Issue and Revision:**

This document issue and revision is controlled by the Document Approval Authority (Department of Biotechnology) As and when a section of this document is revised, the revised section is issued in its entirety together with a revision number, identifying the new issue status and the issue date of each section. The revised sections are automatically issued to each of this document copy holders listed in Section 2 of 'Control of Document':

Section	Title	Issue Number	Date of Issue / approval  October 10, 2008	Revision Number	Date of Rev/ Approval
Section-0	Contents	1			
Section-00	Control of Document	1			
Section 1	Introduction (Scope/Purpose/Definitions/References)	1			
Section 2	Organization structure and role & responsibilities	1			
Section-3	Selection of Mother Plant/Establishment of mother stock (nursery)	1			
Section-4	Preparation of ex-plants	1			
Section-5	Cleaning/Washing/Drying of glassware	1			
Section-6	Preparation of media	1			
Section-7	Inoculation	1			
Section-8	Incubation of cultures	1			
Section-9	Virus indexing and Genetic fidelity testing of tissue culture raised plants	1			
Section-10	Packing/labeling/dispatch of ex-agar plants	1			
Section-11	Entry/Exit to tissue culture laboratory	1			
Section-12	Maintenance of cleanliness and sterile conditions/servicing of HEPA filters	1			
Section-13	Monitoring of Microbial Contamination, Fumigation/disinfection of facility	1			
Section-14	Management of primary hardening facility (green house)	1			
Section-15	Management of secondary hardening facility (shadenet house)	1			
Section-16	Dispatch and monitoring of performance of tissue culture raised plants	1			
Section-17	Calibration of Measuring and Monitoring Equipments	1			
Section-18	Document Management & Record Control	1			
Section-19	Training of workers/staff	1			
Section-20	Communication/Auditing/Review	1			3

SOPs for Tissue Culture Production Facility		
Section-00	Control of Document	Page 2 of 2
October 2008		

Upon the receipt of a revised section, this document copy holder should remove the obsolete section and replace it with revised section together with revision number. This issue status table identifies the latest issue of all section of this manual which has been provided to all document copy holders listed in section 2 of 'Control of document'.

This document copy holders are responsible for ensuring all sections of this manual are at the correct issue status prior to use. The document approval authority as evidenced by signature/date/stamp of approving authority and document issuing authority as evidence by signature/date/stamp of issuing authority will be placed on title page and approval of revision section indicated against the revision number in the issue table above.

## 2. Document distribution

This document distribution and subsequent revisions distribution are controlled and issued by the Document Issuing Authority (Accreditation Unit of the DBT established at Biotechnology Consortium India Ltd). This document is issued to all those personnel responsible for various activities and processes described in this document are undertaken.

This document is issued on a controlled copy basis. The only copies of this document that are permitted are those held by the copy holders identified in the table below. This ensures that when changes to this manual are made, all copy holders receive those changes. This manual is currently issued to the following copy holders:

Document Copy No	Copy Holder	Contact Address
Original	Accreditation Unit (Biotech Consortium India Ltd)	Anuvrat Bhawan, 5 <sup>th</sup> Floor, 210, Deendayal Upadhyaya Marg, New Delhi-02
1	Certification Agency	Department of Biotechnology. Block-2, 7 <sup>th</sup> Floor, CGO Complex, New Delhi-03
2	Referral Centre under NCS-TCP	Advanced Centre for Plant Virology, Division of Plant Pathology, IARI, New Delhi-12
3	Referral Centre under NCS-TCP	Centre for DNA Fingerprinting and Diagnostics, ECIL Road, Nacharam, Hyderabad-500076
4	K. F. Bioplants Pvt. Ltd.	S. No. 178, Kirtane Baug, Mundhawa Road, Magarpatta, Hardaspur, Pune. Maharashtra-411036
5	Reliance Life Sciences Pvt. Ltd.	R-282, TTC Area of MIDC, Thane Belapur Road, Rabale, Navi Mumbai Maharashtra-400701
6	Shri Ramco Biotech	3rd Floor, Sabari Complex, 24 Residency Road, Bangalore Karnataka-560025
7	H.U Gogle Agro Biotech Co.	Karmala Road, Jamkhed, Ahmednagar Maharashtra-413201
8	Pudumjee Plant Laboratories Ltd.	Thergoan, Chinchwad, Pune Maharashtra-411033
9	Growmore Biotech Ltd.	41-B, SIPCOT-II, Hosur Tamil Nadu-635109
10	Develela Biotech	Anand Vihar, Opposite Energy Park, VIP Road, Raipur Chhatisgarh



SOPs for Tissue Culture Production Facility		
Section-1	Introduction	Page 1 of 3
October 2008		

### **1.1. Scope:**

This document provides guidance and describes the standard operating procedures (SOPs) for tissue culture production facility involving various steps of production of tissue culture plants.

### **1.2. Purpose**

The purpose of this document is to facilitate adoption of standard operating procedures by all the recognized tissue culture production facilities under National Certification System for Tissue Culture Raised Plants established by the Department of Biotechnology, Ministry of Science & Technology for undertaking certification of tissue culture production facility in accordance with the guidelines established by the Department of Biotechnology (certification agency) in exercise of the powers conferred under section 8 of the Seeds Act, 1966.

### **1.3. Definitions & Terms:**

<b>Accredited Test Laboratory</b>	A test laboratory accredited by the Department of Biotechnology for virus/quality (genetic fidelity) testing of tissue culture raised plants and certification.
<b>Acclimatization</b>	It is a physiological adaptation of plants to climate or environment such as, light, humidity, temperature, etc.
<b>Recognized tissue culture production facility</b>	A tissue culture production facility recognized by the Department of Biotechnology for quality production of tissue culture plants.
<b>Clone</b>	A progeny of plant derived through vegetative propagation having identical genetic make-up with that of parent plant.
<b>Controlled document:</b>	Documents formally identified. These documents are registered, maintained and their change, as well as, their implementation is regulated.
<b>Controlled Record:</b>	A record that requires to be kept and maintained under safeguard for future reference
<b>Culture medium</b>	It is a liquid or gelatinous substance containing nutrients for the growth of explants.
<b>Corrective action</b>	Action to eliminate the cause of a detected non-conformity.

<b>Data</b>	Quantified information in documents.
<b>Document:</b>	Procedures, work instructions, references, specifications or regulatory material for the administration of the system.
<b>Explant</b>	An explant is any portion of the plant that will be used to initiate the culture.
<b>Hardened plant</b>	In-vitro derived plants which have developed good root and stem system to grow in the field conditions and ready for field plantation.
<b>Inoculation</b>	Transferring of sterilised explants on to the nutrient media to in a culture tube/bottle.
<b>Incubation</b>	Maintenance of inoculated explants in bottle/tube in an environment of controlled conditions of temperature, light, humidity and nutrients.
<b>Internal Audit</b>	Independent activity to verify, through an exam and evaluation of objective evidence, if the processes and elements applicable to the quality system have been developed, documented and implemented.
<b>Internal document</b>	Document generated outside the limits of the administrative system for example: a regulatory document that is referred to a procedure or work instruction.
<b>Micropropagation</b>	It is the practice of rapidly multiplying a large number of progeny plants from a desired plant using modern plant tissue culture methods.
<b>Mother plant</b>	A plant which acts as a source of material for multiplication by micropropagation.
<b>NCS-TCP</b>	National Certification System for Tissue Culture Raised Plants (NCS-TCP) established by the Department of Biotechnology, Ministry of Science & Technology.
<b>Non-Conformity</b>	Any situation that differs from standard procedures, guidelines or regulations
<b>Objective evidence</b>	Data supporting the existence or verify something.
<b>Plantlet</b>	A baby plant produced in vitro on an auxenic culture medium from a meristematic plant tissue.

SOPs for Tissue Culture Production Facility		
Section-1	Introduction	Page 3 of 3
October 2008		

<b>Plant Tissue Culture</b>	Plant tissue culture is a technique of culturing plant cell, tissue or organ in artificial, controlled and aseptic conditions. It mainly covers micropropagation, organogenesis and somatic embryogenesis.
<b>Pest</b>	Any species, strain or biotype of plant, or pathogenic agent, injurious to plants or plant products.
<b>Procedure</b>	Document that describes, "Who does the job", "when", "where", and "why".
<b>Protocol or work instruction</b>	A written instruction to carry out a specific task or activity or job.
<b>Record:</b>	Document (electronic or print), product or sample statement, which will confirm that a procedure (or part of the procedure) has been carried out.
<b>Somaclonal variation</b>	It is the term used to describe the variation seen in plants that have been produced by plant tissue culture. Chromosomal rearrangements are a major source of this variation.
<b>Stock culture</b>	Tissue culture derived from mother plant
<b>Standard Operating Procedures (SOPs)</b>	Standard operating procedures (SOPs) are sets of written instructions that document the routine or repetitive activity followed by an organization. The development and use of SOPs are an integral part of a successful quality system as it provides individuals with the information to perform a job properly.
<b>Virus Indexing:</b>	Testing of the plants for known viruses and ensuing their elimination before micropropagation.

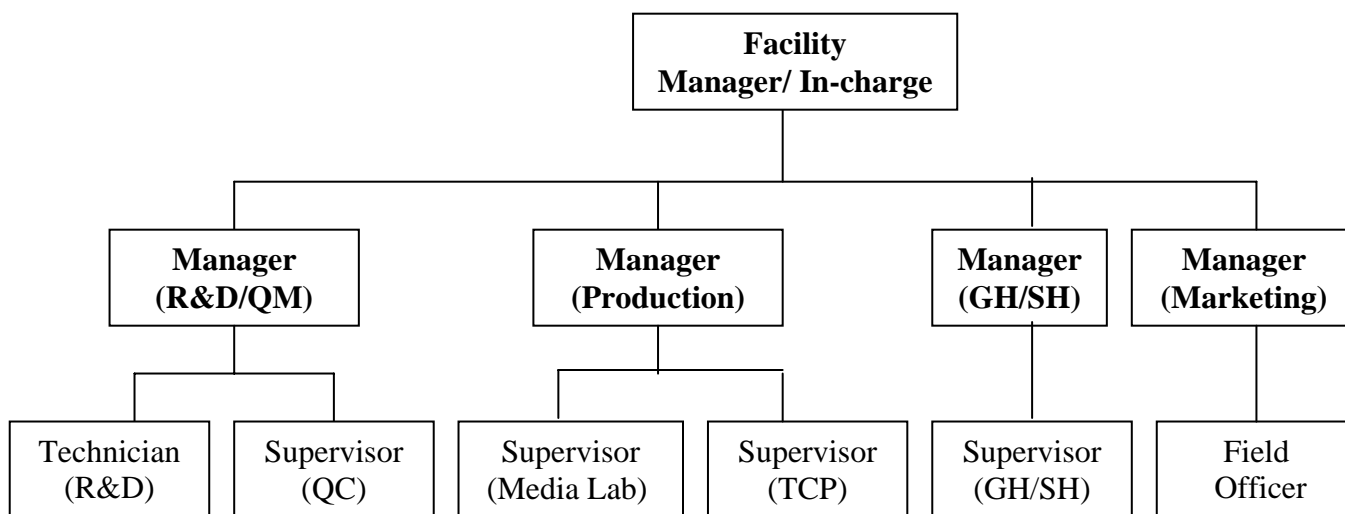
#### **1.4. References:**

*Guidelines for recognition of tissue culture production facilities (2006), Department of Biotechnology, New Delhi.*

### **1.1. Organization structure:**

The Organisational structure and job titles may vary with different tissue culture production facility depending on level of production. However the following organisation structure of tissue culture production facility is considered to be a minimum for recognition of tissue culture production facility.

#### **Organizational Chart of Tissue Culture Production Facility**



### **1.2. Responsibilities**

#### **1.2.1. Facility Manager/ In-charge:**

The Facility Manager/ In-charge of tissue culture production facility will be responsible for overall management of tissue culture production facility and the responsibilities include:

- recruitment of technical/administrative personnel
- approval of purchase of equipments and quality chemicals
- adoption of standard operating procedures (SOPs) for tissue culture production facilities
- planning resources for development of tissue culture production facility
- review of implementation of quality system and procedures
- contracting tissue culture production

<b>SOPs for Tissue Culture Production Facility</b>		
<b>Section-2</b>	<b>Organization structure and Responsibilities</b>	<b>Page 2 of 2</b>
<b>October 2008</b>		

### **1.2.2. Manager (R&D/QM):**

The Manager (R&D/QM) is responsible for:

- research and development/standardization/validation of tissue culture protocols for the initiation of new plant species
- selection of mother plants and maintenance of pest-free mother stock (nursery)/ stock cultures
- maintenance of appropriate records related to selection of mother plants/stock cultures
- quality management and internal auditing of activities related to tissue culture production
- implementing SOPs
- organizing virus-indexing and quality (genetic fidelity) testing of tissue culture plants
- calibration of measuring/monitoring equipments

### **1.2.3. Production Manager**

The Production Manager is responsible for:

- planning, execution and supervision of tissue culture production activities
- maintenance of tissue culture multiplication records
- overall maintenance of cleanliness and sterile conditions of tissue culture laboratory facilities

### **1.2.4. Green House Manager**

The Green house manager is responsible for:

- management of tissue culture hardening facilities (greenhouse) as well as nursery (secondary hardening) raised under shade net facility
- maintenance of appropriate records related to green house/shade net facilities
- evaluation of field performance of tissue culture plants in the field

### **1.2.5. Marketing Manager:**

The Marketing manager is responsible for:

- planning, execution and supervision of marketing/shipment of tissue culture plants
- providing package of practices for growing tissue culture plants in farmer's field
- demonstration of tissue culture technology to the farmers for improvement of agriculture production
- receiving customer's feed back.

SOPs for Tissue Culture Production Facility		
Section-3	Selection of Mother Plants/Establishment of mother stock (nursery)	Page 1-2 of 2
October 2008		

### **3.1. Selection of Mother Plants:**

- 3.1.1. The selection criteria employed should be limited to a few important phenotypic traits estimated to have a relatively high heritability (the ability of the parents to transmit their characteristics to the progeny) such as stem straightness, branching and flowering habits
- 3.1.2. The information collected about the mother plant will be recorded in format prescribed in Annexure-3A.
- 3.1.3. The selected mother plants will be healthy and free from pest and diseases.
- 3.1.4. The selected mother plants will be appropriately labelled giving Ref No/Date, Name of plant species (common/scientific name)/variety, plant parts, location, name of the collector
- 3.1.5. The selected mother plants will be appropriately packed in card board cartons and transported to tissue culture production facilities with in the same date of collection.
- 3.1.6. If the selected mother plants are of foreign origin, the same will be imported subject to existing phytosanitary regulations covered under PQ Order, 2003 and amendments issued there under.

### **3.2. Establishment of mother nursery:**

- 3.2.1. The selected mother plants (elite clones with proven yield potential and improved agronomic characteristics/horticulture traits) will be thoroughly screened before planting in a mother nursery in isolated area and /or under protected condition (such as glasshouse) at the tissue culture production facility, where appropriate.
- 3.2.2. Each plant will be appropriately labeled giving accession number, plant species/variety, date of planting and the particulars will be recorded in a mother stock register maintained by the facility.
- 3.2.3. Each plant will be tested especially virus-free before planting in mother nursery and maintained in virus-free condition until used in tissue culture production.

### Annexure-3A

#### Information Sheet on Selection of Mother Plant

1. Ref. No/Date	
2. Plant species (common/scientific name)	
3. Variety	
4. Name of the owner/ contact person (Telephone/fax/mobile/mailling address)	
5. Geographic location (latitude/longitude / altitude) from which mother plant collected	
6. Climate/soil type/topography of the area:	
7. Location address (Village/Mandal/Taluk/ District/State)	
8. Survey No/Field/Plot No	
9. Total planted area	
10. Source of material for raising the planting	
11. Month/year of planting & Age of the crop	
12. Type of cultivation	Open field/protected area (green house/glass house growing)
12. Description of plant parts collected	
13. Description of phenotypic characteristics of the plant:	
a. Plant Height	a.
b. Girth of plant	b.
c. Growth habit	c.
d. Flowering type	d..
e. Fruiting type	e.
f. Yield	f.
g.. phenotypic markers	g.
h. Any other Characteristics ( _____ ) (Specify)	h.
14. Month/year of collection	
15. Any information on pest/disease status observed	
16. Signature/Name/Designation of collecting person	

<b>SOPs for Tissue Culture Production Facility</b>		
<b>Section-4</b>	<b>Preparation of Ex-plants</b>	<b>Page 1 of 1</b>
<b>October 2008</b>		

- 4.1. The ex-plants for initiation of tissue culture will be taken only from mother stock (nursery) tested and maintained in virus-free condition.
- 4.2. The explants taken for tissue culture will be healthy and disease-free and the cutting tools used for excising plants will be appropriately disinfected with 70% alcohol and also the hands will be cleaned and washed thoroughly with suitable detergent.
- 4.3. The working surface will be appropriately disinfected with 70% alcohol or sterile filter paper pads used for excising plants
- 4.4. The explants will be sterilized by keeping in appropriate sterilent solution such as 0.1% - 0.2% mercuric chloride solution for 5-20 minutes and wash it 3-4 times in sterile distilled water before inoculating to culture media depending on the types of tissue
- 4.5. In the case of meristem tissue culture plants, the ex-plants will be cut 1.0 to 1.5 mm close to the shoot-tip with the help of sterile scalpel and planted on culture media with the help of sterile forceps (tweezers).
- 4.6. The culture bottles will be appropriately labeled giving information about accession number, plant species/variety, date of transfer & name of technician.
- 4.7. The culture bottles will be incubated in growth rooms under controlled temperature/humidity/conditions for proliferation of shoot buds.
- 4.8. The proliferated shoot buds in clumps are individually transferred to another nutrient medium for shoot elongation and root development.
- 4.9. The stock cultures thus obtained will be maintained in a refrigerator and the particulars of stock cultures maintained will be recorded in a stock culture register.
- 4.10. The stock cultures will be utilized for further multiplication for obtaining desired number of plantlets.

<b>SOPs for Tissue Culture Production Facility</b>		
<b>Section-5</b>	<b>Cleaning/washing/drying of glassware</b>	<b>Page 1 of 1</b>
<b>October 2008</b>		

- 5.1. All the glassware used for tissue culture work will be thoroughly cleaned/washed by trained workers under the supervision of media laboratory supervisor.
- 5.2. The cleaning/washing will be done either manually or through automatic washing machine.
- 5.3. The water used for cleaning and washing will be of potable quality and mixed with a detergent such as teepal.
- 5.4. All the contaminated culture bottles will be autoclaved before washing with detergent and the agar collected into a suitable container and disposed by composting in a pit covered with soil.
- 5.5. After cleaning/washing the glassware will be rinsed with clean water to remove the detergent.
- 5.6. After rinsing the glassware such as bottles or jars will be kept in inverted position on drying stand.
- 5.7. The glassware such as Petri dishes or pipettes will be sterilised in hot air oven by wrapping in a clean kraft paper or keeping in suitable air-tight container at 160 °C for a minimum period of 1 hour.
- 5.8. The scalpels and forceps used will be wrapping in a clean kraft paper and sterilised in a hot air oven at 160 °C for a minimum period of 1 hour.

SOPs for Tissue Culture Production Facility		
Section-6	Preparation of culture media & Autoclaving	Page 1 of 4
October 2008		

### **6.1. Preparation of culture media:**

- 6.1.1. All the preparation and autoclaving of media will be done under the direct supervision of media laboratory supervisor.
- 6.1.2. The laboratory chemicals of either analytical or laboratory grade, as the case may be will be used in preparing the media. The agar used for preparation of media will be of TC grade.
- 6.1.3. The water used for preparation of media will be double glass distilled, RO or demineralised.
- 6.1.4. The stock solutions of macronutrients, micronutrients, vitamins & amino acids will be prepared in distilled water (Annexure-6A) and labeled appropriately indicating name, strength of solution and date of preparation and stored in refrigerator at 4 C. However the stock solutions of auxins such as IAA (Indole Acetic Acid), IBA (Indole Butyric Acid), NAA (Naphthol Acetic Acid) and cytokinins (6-benzylaminopurine (BAP) are prepared separately by dissolving the required quantity of hormones in little quantity of 1 N NaOH and finally made up to required strength (1 mM or 10 mM) by adding distilled water.
- 6.1.5. In the first instance the required quantity of sucrose (30 g/l) and agar (8 g/l) weighed and added to three fourth of required quantity of water and heated on a hot plate with magnetic stirrer until the both ingredients are dissolved followed by addition of required quantities of stock solution of macronutrients, micronutrients, vitamins, amino acids and hormones and the rest of distilled water is added to make the final volume of media.
- 6.1.6. The pH of media will be adjusted between 5.6-5.8 using 01 N HCl solution with the help of pH meter.
- 6.1.7. The medium will then be distributed uniformly into culture bottles or jars with the help of automatic media dispenser or manually through a fixed volume dispensing pipette as the case may be and will be capped tightly.
- 6.1.8. The culture bottles will be appropriately labelled indicating name of media, batch number and date of preparation and the particulars of the same will be entered in a media register (Annexure-6B).

<b>SOPs for Tissue Culture Production Facility</b>		
<b>Section-6</b>	<b>Preparation of culture media/autoclaving/ storage</b>	<b>Page 2-4 of 4</b>
<b>October 2008</b>		

## **6.2. Autoclaving:**

6.2.1. The media will be autoclaved at 1kg/cm<sup>2</sup> (i.e., 15 lbs/sq. inch) for 15-20 min at 121 °C in an autoclave. The culture bottles after autoclaving will immediately be removed to media storage room in clean area to avoid contamination.

## **6.3. Filter sterilisation:**

6.3.1. If any heat labile chemicals are used in preparation of media, the stock solutions of heat labile chemicals will be filter sterilised through a syntex filter with pore size of 0.22 µm and the filtersterilized solution will be dispensed under aseptic conditions to autoclaved media after cooling to 36 °C. The syntex filters will be sterilized by autoclaving before use.

## **6.4. Storage of Culture Media.**

6.4.1. After autoclaving the culture media will be stored for 3 days in storage room in clean area (with sterility level of class 100,000) and observed for microbial contamination before issue for inoculation. If any microbial contamination detected, the contaminated bottles will be removed for autoclaving immediately and the particulars of contamination will be recorded for each batch of media prepared in the media register.

Annexure-6A  
**Stock solutions for Nutrient Medium**

Stock solution	Constituents	Quantity (mg/litre)	Volume of stock solution
Stock Solution-I (20X)	MgSO <sub>4</sub> . 7H <sub>2</sub> O KH <sub>2</sub> PO <sub>4</sub> KNO <sub>3</sub> NH <sub>4</sub> NO <sub>3</sub> CaCl <sub>2</sub> . 6H <sub>2</sub> O	7400 3400 38000 33000 8800	1000 ml
Stock Solution-II (200X)	H <sub>3</sub> BO <sub>3</sub> MnSO <sub>4</sub> .4H <sub>2</sub> O ZnSO <sub>4</sub> . 7H <sub>2</sub> O Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O CuSO <sub>4</sub> . 5H <sub>2</sub> O CoCl <sub>2</sub> . 6H <sub>2</sub> O	1240 4460 1720 50 5 5	1000 ml
Stock Solution-III* (200X)	FeSO <sub>4</sub> . 7H <sub>2</sub> O Na <sub>2</sub> EDTA. 2H <sub>2</sub> O	5560 7460	1000 ml
Stock Solution-IV (200X)	Inositol Thiamine HCl Pyridoxine HCl Nicotinic Acid Glycine	20000 100 100 100 400	1000 ml

\*For preparation of stock solution-III, dissolve separately in 450 ml of distilled water by heating and constant stirring. Mix the two solutions, adjust pH to 5.5 and add distilled water to make up the final volume to one litre.



<b>SOPs for Tissue Culture Production Facility</b>		
<b>Section-7</b>	<b>Inoculation of tissue cultures</b>	<b>Page 1 of 1</b>
<b>October 2008</b>		

- 7.1. All the inoculation of tissue cultures will be carried out by trained workers (operators) in inoculation room under a laminar air flow cabinets fitted with HEPA filters and U.V. germicidal lamps.
- 7.2. The operators will wear the clean lab coats, hair cap and the face mask covering nose and mouth, while carrying out inoculation of cultures to prevent microbial contamination.
- 7.3. The inoculation room will be maintained at a minimum sterility level of class 100,000 with positive pressure to prevent microbial contamination.
- 7.4. The tissue culture multiplication will be carried out as per the production plan established by the facility. Only one clone/genotype will be handled for sub-culturing at any one by an operator. The particulars of stock cultures issued for multiplication will be entered in a computer using pre-installed software for production monitoring.
- 7.5. The operator each time will use sterilised scalpel to excise the tissue after placing it on sterilised pads and transfer the excised tissue to culture media with the help of sterile scalpel. The culture bottle will be capped tightly and wrapped with parafilm at the margin of the lid. If test tubes are used, they will be covered with a clean sterile cotton plug and wrapped with a parafilm to facilitate free exchange of air
- 7.6. The operator will use glass-beed steriliser for sterilising scalpel and forceps and the working bench will be disinfected at the end of each transfer.
- 7.7. The technician at the end of completion of transfer of each clone/genotype will label each culture bottle or jar indicating the stock culture number, plant species/variety, multiplication cycle number and the week of transfer and name of the technician.
- 7.8. The technician will complete the job sheet indicating the number of bottles transferred at the end of complete transfer of each clone/genotype.
- 7.9. At the end of complete transfer of each clone/genotype, the production supervisor will enter the particulars of culture transfers in the computer using pre-installed software for production monitoring.

<b>SOPs for Tissue Culture Production Facility</b>		
<b>Section-8</b>	<b>Incubation of tissue cultures</b>	<b>Page 1 of 1</b>
<b>October 2008</b>		

- 8.1. The culture bottles or jars are kept in trays and incubated on storage racks each frame fitted with four daylight fluorescent tubes to provide light intensity of 1200 lux/m<sup>2</sup>. An automatic time control switch is installed to maintain desired photoperiods in growth room, which will vary with different plant species. Also dark room facilities will be required for certain species. The storage racks will be of either fixed type or movable type and the latter one are space saving.
- 8.3. The temperature of growth room will be measured with the help of temperature sensors connected to thermostatic control and the temperature maintained in growth rooms will be continuously monitored with the help of data loggers. The temperature of incubation will vary between temperate and tropical plant species, which will be set with the help of thermostatic control.
- 8.4. The growth rooms will be maintained at a minimum sterility level of class 100,000 and with positive pressure to prevent microbial contamination.
- 8.5. The production supervisor will make observation on growth of cultures and contaminations at weekly intervals and the particulars will be recorded in a growth room register indicating accession number of clone/genotype, plant species/variety, multiplication cycle number, medium type, date of inoculation, tray number and date of observation and observation made. The contaminated cultures, if any, will be immediately removed for autoclaving after making entry in the register.

SOPs for Tissue Culture Production Facility		
Section-9	Virus/Quality (genetic fidelity) testing of tissue culture raised plants	Page 1 of 4
October 2008		

- 9.1. The recognized tissue culture production facility will submit an application for virus/quality (genetic fidelity) testing and certification of tissue culture raised plants (Annexure-9A) to the Director/HOD of accredited test laboratory sufficiently in advance and get it registered after payment of prescribed fees notified by the Department of Biotechnology from time to time.
- 9.2. The recognized tissue culture production facility will submit samples for testing as per the guidelines provided by the accredited test laboratory or permit drawl of samples through the authorised representative of accredited test laboratory, as the case may be.
- 9.3. The sample collected will be blotted dry to remove excess moisture before packing. The sample will be placed in between paper towels, packed in self sealing/zip-lock polythene bags of appropriate size. The sample will be affixed with a label (Annexure-3C) and kept in a ventilated card board box and /or thermocool box for forwardal to accredited test laboratory.
- 9.4. The packing box will be marked on top of the box with the address of accredited test laboratory with appropriate instructions such as "*Handle with care/Tissue Culture Plants/Rush Delivery*" and either couriered or delivered in person to the concerned accredited test laboratory within 24 hrs period.
- 9.5. A separate record of samples referred for virus testing/quality (genetic fidelity) testing and the report of results of testing received from accredited testing laboratory will be maintained.
- 9.6. Where a facility undertakes in-house testing for virus and or/ quality (genetic fidelity), it should be carried out as per the protocols approved/validated by the referral laboratory recognised by the Department of Biotechnology under national certification system for tissue culture raised plants.
- 9.7. In case of clones/genotypes or stock cultures, the virus testing will involve hundred percent testing of each individual clones/genotypes or the stock cultures just prior to initiation for tissue culture multiplication.
- 9.8. However in case of ex-agar plants/tissue culture hardened plants appropriate samples will be drawn for each batch of production and separately for each plant species/variety for virus/quality (genetic fidelity) testing just prior to dispatch for shipment/marketing.

<b>SOPs for Tissue Culture Production Facility</b>		
<b>Section-9</b>	<b>Virus indexing and quality (genetic fidelity) testing of tissue cultures of plants</b>	<b>Page 2-4 of 4</b>
<b>October 2008</b>		

- 9.9. If the test results for virus testing proved positive the affected clones/genotype or stock culture will be rejected for tissue culture multiplication and the same will be destroyed as per the direction of Accredited Test Laboratory.
- 9.10. If the test results of batch testing proved positive for virus and or/ the results of quality (genetic fidelity) testing exceeded the tolerance limits prescribed by the crop specific standards established by the Department of Biotechnology, the entire batch will be rejected for shipment/marketing and the same will be destroyed as per the direction of Accredited Test Laboratory.

## Annexure-9A

**Application for Virus/Quality (genetic fidelity) Testing & Certification of Tissue Culture Raised Plants**

1. Name/Location Address of the Recognized tissue culture production facility:						
2. Certificate No./date of issue/validity:						
3. Name of authorised person and his contact details (Telephone/ Fax/Mobile/E-Mail):						
4. Details of tissue culture plants required to be sampled:						
Plant species	Variety	Accession/ Batch No	Batch size	No of packages	Tests required for	Category of tissue culture material*
						<input type="checkbox"/> Clonal material <input type="checkbox"/> Invitro-culture <input type="checkbox"/> Ex-agar washed plants <input type="checkbox"/> Primary hardening plants <input type="checkbox"/> Secondary hardening plants <b>*Tick out in appropriate box</b>
5. Purpose of testing/certification*:					<input type="checkbox"/> Import Quarantine requirements <input type="checkbox"/> Mother stock for initiation <input type="checkbox"/> Domestic sale & distribution <input type="checkbox"/> Phytosanitary certification <b>* Tick out in appropriate box</b>	
6. Particulars of payment of testing fees:						
Amount in Rs:						
Demand Draft/Banker's Cheque No./Date of Issue:						
Bank Name/Branch:						
7. Date by which sampling:						
8. Date by which certification is requested:						
9. Any additional information:						
<b><u>Declaration</u></b>						
I/we hereby declare that the information furnished above is complete and correct to the best of my/our knowledge and belief. I/we will meet the TA/DA of technical personnel deputed for sampling in addition to the testing fees as indicated above and provide necessary facilities for sampling.						
Date: _____						
Place: _____						
_____ (Signature/Name/Stamp of Applicant/Date)						
<b>For Office (Testing Facility) Use</b>				<b>Application Reg. No. _____/Date _____</b>		
Check list	Status		Scrutinized by	Action by TCPF	Applicant Response	
Application complete	Yes	No				
Payment of Fees	Yes	No				
Final Action Taken:						
_____ (Signature/Name of Director or HOD of Accredited Test laboratory/Date)						

Annexure-9B

**Sampling Label**

Sample No*:	<table border="1"> <tr> <td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td> </tr> </table> <p>(12 digit code)</p>														
Application Reg. No/Date:															
Plant Species/Variety:															
Lot/Accession No:															
Lot size:															
Tissue Culture Production Facility:															
Date of Sampling:															
Sample size/No of samples drawn:															
Samples drawn by:	<p>_____</p> <p>(Signature/Name/Designation Stamp/Date of Authorised person of/by Accredited Test laboratory)</p>														
In the presence of:	<p>_____</p> <p>(Signature/Name of authorized person from Tissue Culture Production Facility)</p>														
<p>* Sample Number will be assigned by Accredited test laboratory and maintained throughout certification</p>															

SOPs for Tissue Culture Production Facility		
Section-10	Packing/labeling/dispatch of ex-agar plants	Page 1 of 1
October 2008		

- 10.1. The plantlets after attaining good growth will be removed from culture bottles or jars by trained workers and gently washed in a stream of potable water to remove the agar sticking on to the roots.
- 10.2. The plantlets after washing will be blotted dry to remove excess moisture and graded by placing on the glass surface using the standard chart of the plant species/variety as back ground and the plantlets which does not confirm to the required standard will be discarded.
- 10.3. The grading and virus testing of ex-agar plantlets will be carried out as per the certification standards for tissue culture plants established by the Department of Biotechnolgy for domestic purpose and or/as per the requirements specified by the importer in respect of export consignments, where applicable.
- 10.4. The ex-agar plants after grading will be wrapped in tissue paper and placed in thermocool boxes kept in a cardboard carton for overseas shipment. The package will be sealed and labelled giving information about the Name of tissue culture production facility; plant species/variety; no of plantlets; date of packing and the address of consignee. The packages will carry appropriate instruction such as **“HANDLE WITH CARE-TISSUE CULTURE PLANTS”**.
- 10.5. The sealed packages will be stored in cold storage room at appropriate temperature until shipment.

<b>SOPs for Tissue Culture Production Facility</b>		
<b>Section-11</b>	<b>Entry/Exit Control to tissue culture laboratory</b>	<b>Page 1 of 1</b>
<b>October 2008</b>		

### **11.1. Entry Control:**

- 11.1.1. The entry into tissue culture laboratory will be restricted/regulated through a double door entry.
- 11.1.2. All the persons, who are authorised entry, will remove their chapals/shoes including shocks and leave them at shoe stand and wear the sanitary sandals and wash their hands and feet with soap before the entry.
- 11.1.3. All the persons will change cloths at the dress changing area and wear clean sanitised laboratory overcoat, head cap and face mask covering the nose and mouth. They will preferably pass through an air shower for 30 seconds or alternatively through an air curtain. They will dip their hands in basin filled with disinfectant and feet in foot bath filled with disinfectant before entering into the sterile areas of tissue culture production facility.
- 11.1.4. The persons entering will not be permitted to carry out any articles such as writing aids/mobiles/wallets etc in side the facility with out passing through UV sterilizer. No smoking/chewing or eatables will be permitted inside the facility. Suitable lockers will be provided to the staff/workers for storing their belongings at the entry.
- 11.1.5. The facility will display signs such as “**NO ENTRY WITH OUT PERMISSION**” at the entry and also display step by step entry protocols for the guidance and compliance by all persons entering the sterile areas of tissue culture production facility.

### **11.2. Exit control:**

- 11.2.1. All the persons will leave the laboratory overcoat, head cap and face mask at the dress changing area in disposing bin and leave the sanitary sandals and wear their own cloths. They will collect their belongings, if any before leaving the place.
- 11.2.2. The used overcoats, head cap and face mask will be removed for washing and autoclaving.

SOPs for Tissue Culture Production Facility		
Section-12	Maintenance of cleanliness and sterile conditions/ servicing and changing of HEPA filters	Page 1 of 1
October 2008		

### **12.1. Maintenance of cleanliness and sterile conditions of the facility:**

- 12.1.1. The sterile areas of facility will be cleaned with vaccum cleaner and the floor area will be mopped with disinfectant daily.
- 12.1.1. The waste collected in the disposal bins inside the clean room areas of tissue culture laboratory will be removed daily.
- 12.1.2. The glass pans of the windows/doors will be wiped with clean chamoised cloth or wet sponge periodically to remove the dust, if any collected.
- 12.1.3. The inoculation room, media storage room and growth room will be maintained at a minimum sterility level of class 100,000 and provided with positive pressure.
- 12.1.4. The tissue culture facility will be either provided with centrally air-conditioned plant providing ducted cool-air filtered through HEPA filters (0.22  $\mu\text{m}$ ) @ two air changes per min or with individual air-handling units provided with HEPA filters (0.22  $\mu\text{m}$ ) feeding specific areas of the facility.
- 12.1.5. The inlets of receiving air will be located at the top side or at shelf level and the outlets of exhausting air will be located close to the floor level on opposite side to facilitate uniform air circulation and minimize contamination.

### **12.2. Servicing and Changing of HEPA filters:**

- 12.2.1. The HEPA filters fitted on air-conditioning ducts or air handling units will be serviced at least six monthly intervals by a qualified service engineer and if found damaged/clogged the filters will be replaced by new ones.
- 12.2.2. The HEPA filters fitted in laminar air flow cabinets will be serviced as and when necessary and if found damaged/clogged will be replaced by new ones.
- 12.2.3. The clogging of HEPA filter will be revealed by drop in pressure of manometer fitted on to laminar air flow cabinet and the damage of filters will be revealed by physical examination of the filter.
- 12.2.4. The facility will ensure that the servicing of air-conditioning equipments and changing of HEPA filters are covered under annual maintenance contract and strict schedule is followed for in-house maintenance and a record will be maintained for this purpose.

SOPs for Tissue Culture Production Facility		
Section-13	Monitoring of Microbial Contamination/ Disinfection of facility	Page 1 of 1
October 2008		

### **13.1. Monitoring microbial contamination**

- 13.1.1. The microbial contamination will be monitored in the sterile areas of the facility by exposing agar plates at periodic intervals and the same will be incubated for 72 hours at  $25 \pm 2$  °C and examined for microbial colonies. A record of periodical monitoring of microbial contamination will be maintained indicating date/time of monitoring, area monitored, no of plates exposed, date/time of observation and no of microbial colonies encountered and colony types (fungal or bacterial) and the action taken.
- 13.1.2. Besides the above, counting of dust particles through a particle counter at regular intervals either manually or through an automatic particle counter to ensure required sterility levels are maintained with in the tissue culture laboratory facility and a record of particle counts carried out at periodic intervals will be maintained.

### **13.2. Disinfection of facility**

- 13.2.1. The facility will be disinfected with 4% formalin with an exposure period of at least 24-48 hours usually at monthly intervals or as and when necessary depending on microbial counts/ level of contamination.
- 13.2.2. The disinfected area will be thoroughly aerated and checked before allowing any entry of workers/staff inside the facility.
- 13.2.2. At the end of each day's work, the U.V. germicidal lamps fitted inside the laminar air cabinets will be switched-on overnight to disinfect the laminar air flow cabinet and also the dress changing area.

<b>SOPs for Tissue Culture Production Facility</b>		
<b>Section-14</b>	<b>Management of primary hardening facility (green house)</b>	<b>Page 1-2 of 2</b>
<b>October 2008</b>		

- 14.1. The green house facility will have controlled micro-climatic conditions such as relative humidity, temperature, light intensity and air circulation. It will have controlled misting arrangements.
- 14.2. The green house facility will have double door entry and vector-proofing of all external openings (covering with a screen of 40-60 meshes per linear inch) to the facility in order to prevent entry of insect vectors such as aphids, leaf hoppers, whiteflies and thrips.
- 14.3. The ex-agar washed and graded plantlets will be transplanted in pro-trays (micro-pots) in soil less peat mix growing medium.
- 14.4. Each batch of the plantlets will be appropriately labelled indicating plant species/variety, Accession Number of clone/genotype, batch of production, no of plant lets, date of transplanting and the particulars will be recorded in green house register (Annexure-14A).
- 14.5. The pro-trays will be held under specially designed poly tunnels on benches above the ground. If protrays held on the ground, the soil or sand floors will be covered with impermeable sheet to avoid soil or sand coming in direct contact with protrays. The poly tunnels provided with adjustable openings to provide optimum microclimatic conditions and to facilitate examination of plantlets.
- 14.6. The plantlets will be monitored regularly for their growth and presence of any infestation/infection will be recorded in green house register.
- 14.7. The dead plantlets will be promptly removed to avoid attack by saprophytic fungi and the plantlet mortalities will be recorded in green house register.
- 14.8. Vector monitoring will be carried out at periodic intervals by placing yellow sticky cards close to the micropots at the rate of one sticky card for every 10 m<sup>2</sup> space inside the facility and the same will be recorded indicating date/time, pest species trapped, average number/trap, action taken and name signature of supervisor.
- 14.9. Any kind of treatment given to the plant such as application of fertilizers or micro-nutrient sprays or application of pesticides will be recorded in green house register indicating date/time of application, fertiliser or other chemicals applied, dosage rate and if pesticides applied (including botanicals and microbial pesticides), pest against which applied and name/sign of applicator.



<b>SOPs for Tissue Culture Production Facility</b>		
<b>Section-15</b>	<b>Management of secondary hardening facility (shadenet house)</b>	<b>Page 1 of 1</b>
<b>October 2008</b>		

- 15.1. The shade net facility will have double door entry with all the sides covered with insect-proof screen of 40-60 meshes for linear inch with top covered with polythene sheet against rain protection during monsoon period.
- 15.2. If soil is used it will be treated by vapour heat at 80 °C for 1 hour and only fully decomposed organic manure will be used and mixed with soil before treatment for raising tissue cultured plants.
- 15.3. The plants will be raised individually in poly-bags or other suitable containers on benches or raised beds on the ground. The soil floors are covered by an impermeable plastic film to prevent plants coming in direct contact with soil and weed growth.
- 15.4. Each batch of the plants will be appropriately labeled indicating plant species/variety, accession Number of clone/genotype, batch of production, date of transplanting and the particulars will be recorded in shadenet house register, which is maintained in similar way as that of green house register.
- 15.5. The shadenet house will have drip irrigation and or fertigation arrangements for watering individual plants in poly bags or other containers.
- 15.6. The plants will be monitored regularly for the presence of any pest/diseases and if any plants are suspected of virus symptoms, the affected plants will be immediately segregated and isolated and tested for the presence of virus before the same will be destroyed and the results will be recorded in the shadenet house register.
- 15.7. The dead plant will be promptly removed to avoid attack by saprophytic fungi and the seedling mortalities will be recorded in the shadenet house register.
- 15.8. Vector monitoring will be carried out at periodic intervals by placing yellow sticky cards close to the pots at the rate of one sticky card for every 10 m<sup>2</sup> space inside the facility and the same will be recorded indicating date/time, pest species trapped, average number/trap, action taken and name signature of supervisor.
- 15.9. Any kind of treatment given to the plant such as application of fertilizers or micro-nutrient sprays or application of pesticides will be recorded in shadenet house register indicating date/time of application, fertiliser or other chemicals applied, dosage rate and if any pesticides applied (including botanicals and microbial pesticides), pest against which it is applied and name/sign of applicator.

<b>SOPs for Tissue Culture Production Facility</b>		
<b>Section-16</b>	<b>Dispatch/Monitoring Performance of Tissue Culture Plants</b>	<b>Page 1-2 of 2</b>
<b>October 2008</b>		

- 16.1. The hardened plants will be checked physically for their health (freedom for diseases) and size (plantable) as per specified in certification standards laid down by the Department of Biotechnology.
- 16.2. The hardened plants in poly bags will be either dispatched in ventilated plastic crates/ cardboard cartons in suitable trucks to the concerned growers or farmers for direct planting or else the plants will be removed from poly bags, washed free from soil, wrapped in tissue paper, and packed in ventilated cardboard cartons.
- 16.3. Each package will be affixed with a certification label (Annexure-16A) as prescribed under the certification standard for tissue culture plants established by the Department of Biotechnology. Duly signed/stamped certification labels will be provided by the Accredited Test Laboratory (ATL) to the Tissue Culture Production Facility at the time of issue of certificate of quality of tissue culture raised plants for affixing on the packages. Colour of the label shall be diagonally yellow No. 356 (IS 5-1978) and opaline green (IS No. 275). ATL will issue 10 labels with Certificate of Quality and issuance of other label might be suitably charged.
- 16.4. The Tissue Culture Facility will maintain up to date record of the deployment of the labels in terms of the size of packages, number of plants per package, the name and contact details of the consignee etc.
- 16.5. A small handout or printed leaf let will be placed in each package giving relevant information about after-care of tissue culture plants such as planting, irrigation scheduling, fertigation, agronomy and plant protection etc., for the benefit of farmers/growers.
- 16.6. The Tissue Culture Facility will maintain database of customers and will contact them from time to time to check on various aspects (such as conditions of plants, packaging, initial mortality in the field etc.)
- 16.7. The Tissue Culture Facility will undertake visit to the plantation site of their customer facing problems with the dispatched material and extend technical support troubleshooting.

**Annexure: 16 A**

Label

<b>Certified Tissue Culture Raised Quality Plants</b>		
NCS-TCP logo (Emblem)	_____ _____ (Name and Address of Accredited Test Laboratory)	Accreditation No. of the Accredited Test Laboratory:
Certificate No.	Bar-coding	
Date of issue		
Botanical Name (Common name):		
Variety:		
Lot/Batch No:		
Batch size:		
Number of Plants/ Packages:		
Stages of Tissue Culture Plant	Ex-agar plantlets <input type="checkbox"/> Primary hardened plants <input type="checkbox"/> Secondary hardened plants <input type="checkbox"/>	
Name of Tissue Culture Production Facility:		
Certification No. and validity of Certificate of Recognition		
Name of the contact person		
Address with phone number: :		
Name/Sign/Stamp of Certifying Authority (ATL) with date	_____	

<b>SOPs for Tissue Culture Production Facility</b>		
<b>Section-17</b>	<b>Calibration of Measuring &amp; Monitoring Equipments</b>	<b>Page 1-2 of 2</b>
<b>October 2008</b>		

- 17.1. The Manager (R&D/QM) will be responsible checking and verification of measuring and monitoring equipment and their calibration.
- 17.2. The laboratory analyst (QM) will identify and document measuring/monitoring equipment to provide the necessary accuracy of the measurement. It is required to identify and record each critical equipment using a serial number, lab number/code and or model number and register in a logbook.
- 17.3. The laboratory analyst (QM) will internally calibrate equipments in accordance with written instructions and tolerances provided along with supply of equipment. He will maintain records of calibrated equipment with the information of frequency, conditions, tolerances, method and current status in the format prescribed in Annexure-14A. The calibrated equipment will be labeled with a sticker indicating the status of calibration.
- 17.4. The equipment that requires external calibration will be calibrated by an authorised agency for which annual maintenance/service contract will be entered by the quality manager and ask for a certificate of proof of calibration and file it for future record.
- 17.5. Equipment that does not require calibration will be labeled with a “Not calibrated” sticker. The list of equipments will be verified and updated at least annually
- 17.6. The Manager (R&D/QM) will ensure that the equipments are maintained, stored and handled to preserve their accuracy and protect from damage and deterioration and proper maintenance of record of the calibrated equipment.

Annexure-17A

**Calibration Record Format**

<b>Name of the Equipment/ Make/ Model:</b>					
<b>Date of purchase:</b>					
<b>Supplied by:</b>					
<b>Location of equipment:</b>					
<b>Equipment used for:</b>					
<b>Identification number:</b>					
<b>Tolerance (if applicable) conditions:</b>					
<b>Frequency of calibration:</b>					
<b>Method of calibration:</b>					
<b>Remarks:</b>					
<b>Details of Calibration:</b>					
	<b>date</b>	<b>time</b>	<b>condition</b>	<b>adjust</b>	<b>initial</b>
<b>1</b>					
<b>2</b>					
<b>3</b>					
<b>4</b>					
<b>5</b>					
<b>6</b>					
<b>7</b>					
<b>8</b>					
<b>9</b>					
<b>10</b>					

SOPs for Tissue Culture Production Facility		
Section-18	Document Management & Record Control	Page 1 of 3
October 2008		

### **18. 1. Document management:**

- 18.1.1. The Recognized tissue culture production facility will adopt the standard formats for documentation of information related to tissue culture production as prescribed in the Standard Operating Procedures for Tissue Culture Production Facility established herewith by the Department of Biotechnology or else will establish their own SOPs in line with these established by the Department of Biotechnology. If the SOPs established by the recognized tissue culture production facility deviate from the one established by the Department of Biotechnology, they will be required to be technically justified a copy of the same will be made available to the Accreditation Unit of Department of Biotechnology for record.
- 18.1.2. The tissue culture production facility will not make any changes to the SOPs established by the Department of Biotechnology as this being issued as controlled document
- 18.1.3. Any request for changes in the document (modification/deletion/addition/revision) will be made through a document change application (Annexure-15A). The Accreditation unit of Department of Biotechnology will evaluate the changes and recommend for its revision or reject changes or suggest modifications. The revised document will be approved by the Department of Biotechnology.
- 18.1.5. The Accreditation unit of Department of Biotechnology will issue controlled copies of revised document to all copy holders.
- 18.1.6. The tissue culture production facility will ensure discarding of '**OBSOLETE**' document and replacement with revised document
- 18.1.7. The facility will make available photocopies of controlled document of SOPs to all those technical personnel with production responsibilities for necessary compliance.

### **18.2. Record Control:**

- 18.2.1. The facility will maintain appropriate records as per the formats prescribed herewith for selection of mother plants from field, mother stock (nursery); stock cultures; production of tissue culture plants including hardening.
- 18.2.2. The facility will maintain records related to calibration of equipments and annual maintenance contract of air-conditioned and other equipments.

<b>SOPs for Tissue Culture Production Facility</b>		
<b>Section-18</b>	<b>Document Management &amp; Record Control</b>	<b>Page 2-3 of 3</b>
<b>October 2008</b>		

18.2.3. The facility will maintain a record of tissue culture plants referred for virus/quality testing by accredited test laboratory and the test reports and certification of plants in a single folder. The records related to virus testing/quality testing will be maintained for at least one year.

18.2.5. The records related to clonal selection and maintenance of mother stock (nursery) will be maintained for a period of five years or until such time the stock is maintained to facilitate tracing back of lineage of tissue culture plants to the mother stock.

Annexure-15A

**Document Change Application**

<b>CHANGE REQUESTED BY</b>		<b>APPLICATION DATE:</b>	
<b>DOCUMENT TITLE</b>			
<b>CHANGE REQUESTED</b>			
<b>REASON</b>			
<b>RECOMMENDATION (SELECT ONE)</b>			
<input type="checkbox"/> <b>REJECT (reason)</b>			
<input type="checkbox"/> <b>ACCEPT WITH CHANGES (explain)</b>			
<input type="checkbox"/> <b>ACCEPT</b>			
<b>IF ACCEPTED</b>	<b>SUGGESTED DATE</b>	<b>VALID SINCE</b>	
<b>IF TRAINING</b>	<b>PROPOSED DATE</b>		
<b>NAME/SIGNATURE OF MANAGER (R&amp;D/QM);</b>			<b>DATE</b>
<b>AUTHORIZATION BY THE HEAD OF TISSUE CULTURE PRODUCTION FACILITY:</b>			<b>DATE</b>

<b>SOPs for Tissue Culture Production Facility</b>		
<b>Section-19</b>	<b>Training of workers/staff</b>	<b>Page 1 of 1</b>
<b>October 2008</b>		

- 19.1. Manager (R&D/QM) in consultation with section in-charges (managers) will evaluate the technical personnel to determine training needs.
- 19.2. Manager (R&D/QM) in consultation with section in-charges (managers) will identify internal/external training needs after taking into account the resources available and prepare training programme and request the approval of head of tissue culture production facility for organising the training.
- 19.3. Manager (R&D/QM) and/or section in-charges (managers) will ensure that each technical staff/workers will receive operational training for implementation of SOPs. Besides this, the laboratory technicians will be given hands-on training in tissue culture propagation and further training will be given whenever new tissue culture protocols are developed. The green house workers will be given specific training in management of tissue culture hardening facility (greenhouse) and management of secondary hardening facility (shade net facility). The laboratory analysts attached to the quality laboratory will be given training in virus testing/quality (genetic fidelity) testing and quality management
- 19.4. Manager (R&D/QM) will designate the training experts who will be responsible for preparing and conducting training. If it is external training, he will identify the training institute for conducting the training and recommend to the Managing Director or Head of tissue culture production facility for approval.
- 19.5. Manager (R&D/QM) will distribute training certificates for those who are qualified in the evaluation tests and maintain record of training in respect of each individual staff giving information about the name/designation of staff, type of training (internal/external), area of training, period of training/dates, name/address of training Institute & remarks, if any

<b>SOPs for Tissue Culture Production Facility</b>		
<b>Section-20</b>	<b>Technical Auditing (Internal/External)/Review</b>	<b>Page 1 of 6</b>
<b>October 2008</b>		

### **20.1. Communication:**

20.1.1. The Accreditation Unit of Department of Biotechnology will timely communicate to Recognized Tissue Culture Production Units regarding information on:

- crop specific tissue culture standards
- changes to guidelines for recognition of tissue culture production facilities
- list of Accredited Testing Laboratories
- any other relevant information

20.1.2. The tissue culture laboratories will ensure timely report any changes to contact addresses for updating mailing list

### **20.2. Auditing:**

20.2.1. The Accreditation Unit of Department of Biotechnology (DBT) will establish an auditing panel of technical experts for technical auditing of recognized tissue culture production facilities to ensure that the tissue culture processing activities are performed as per the standard operating procedures established by the concerned tissue culture production facility, which are approved by the Certification Agency.

20.2.2. The Accreditation Unit of DBT will establish a schedule of technical audit and nominate at least two experts from the auditing panel for carrying out the technical audit of activities of recognized tissue culture production facility and intimate the concerned experts at least 10 days in advance, to facilitate making travel arrangements. The concerned recognized tissue culture production facility will be intimated at least one week in advance of the proposed visit.

20.2.3. The nominated experts will have adequate training/experience in auditing quality system and procedures and activities of tissue culture production. The scheduled audits will be carried out once in every year.

SOPs for Tissue Culture Production Facility		
Section-20	Communication/Auditing/Review	Page 2 of 6
October 2008		

- 20.2.4. The scheduled technical audit will involve verification of records and the procedures actually followed both for selection of mother plants, maintenance of pest-free mother stock (nursery), aseptic production of tissue culture plantlets in vitro, virus/quality (genetic fidelity) testing, management of tissue culture hardening facilities both primary (greenhouse) and secondary (shade house) and testing skill competency of the staff involved in tissue culture production and management of tissue culture hardening facilities and compliance with regulatory requirements.
- 20.2.5. At the end of each technical audit, an audit report in prescribed format (Annexure-20A) will be prepared and forwarded to the accreditation unit by the nominated experts. The audit report will indicate non-conformities based on objective evidence, corrective/preventive action to be taken and time schedules by which the measures will be implemented to improve the functioning.
- 20.2.6. The Accreditation Unit of DBT will go through the technical audit report submitted by the nominated experts (auditors) and communicate corrective/preventive actions to be to the concerned tissue culture production facility for implementation at the earliest.
- 20.2.7. The Recognized tissue culture production facility will be re-inspected by an expert to ensure corrective/preventive actions are implemented and a follow-up report of surveillance auditing (Annexure-20B) will be submitted to Accreditation Unit.

### **20.3. Review:**

- 20.3.1. The Apex Committee established by the Department of Biotechnology (Certification Agency) will periodically review the effectiveness of all aspects of the Recognition of tissue culture production facility in consultation with all the representatives of recognized tissue culture production facility and implement changes to the system if required.
- 20.3.2. Such review meetings will be held biannually to discuss the issues and implement action plans for improving efficiency and quality production.

Annexure-20A

**Audit (Scheduled) Report.**

1.	<b>Name &amp; Address of recognized tissue culture production facility</b>		
2.	<b>2.1. Head of Tissue culture production facility (Name &amp; Designation/ contact number/fax/e-mail ):</b>  <b>2.2. Sectional Incharges ( managers)</b>  <b>2.3. Supervisory staff</b>		
3.	<b>Auditing related to the period of</b>		
4.	<b>Date (s) of Auditing:</b>	<b>From:</b>	<b>To:</b>
5.	<b>List of Records Audited/Documents verified:</b>		
6.	<b>Audited by (Name &amp; Designation):</b>		
7.	<b>Details of Auditing reported:</b>		
7.1	<b>General Comments:</b>		





**Document developed on behalf of Department of Biotechnology (DBT) by Accreditation  
Unit (NCS-TCP) at Biotech Consortium India Limited (BCIL),  
New Delhi**