CRITERIA FOR ASSESSMENT OF TISSUE CULTURE UNITS

A tissue culture lab is governed by infrastructure facilities, quality control mechanism and competence of the technical supervision. Each of these areas have a prominent role in ensuring the production of good quality planting material. The group constituted by the Department of Agriculture and Cooperation, Ministry of Agriculture, Government of India, has attempted to assign weightage to different activities in the form of a score card having a total weight of 100. The details are given in Table below:

Score Card for assessing TC units

S. No.	PARTICULARS	MARKS
1.	**Infrastructure**	
A.	*Lab facilities*	
	• Washing room	5
	• Media preparation room	10
	• Inoculation room	10
	• Growth room	10
B.	*Hardening facilities*	
	• Transfer area	10
	• Greenhouse/shade area	10
	• Nursery	5
2.	**Quality control**	
	• Selection of clones and maintenance of germplasm	5
	• Explant	5
	• Virus indexing	5
	• Number of multiplication cycles and Clonal uniformity	10
	• Overall quality of the plants	5
3.	**Technical supervision and monitoring**	
	• Monitoring of the production process and the staff involved therein	10
	• Technical competence of the production supervisory staff	
	• Operators	
	Total	**100**

The description of the parameters for evaluating the TC unit is given in **Annexure**. The TC units getting an overall score of less than 65 should not be considered eligible for distribution of micropropagated planting material till such time when the facilities are improved as per norms. The lab facilities including hardening facilities carry the maximum marks of 60 followed by quality control with 30 marks. These two areas are most crucial for enabling the production and supply of disease free planting material. Technical supervision and monitoring are also important which involve strict supervision of all the activities that are performed in the TC unit (media, lab, inoculation lab, growth room and hardening area). The technical competence of the supervisory staff will also have a bearing on the output.

Annexure

PARAMETERS FOR EVALUATING A TISSUE CULTURE LAB

S.No.	Particulars	Requirement	Marks
1. ***A.***	**Infrastructure** ***Lab facilities*** • **Washing room** - Facilities for washing, drying and storing of glassware - Quality of washing - Overall cleanliness	a) Depending on the volumes, washing may be done manually or through a machine but the quality of washing must be good. b) Contaminated cultures should not be stored. They should be washed as soon as possible. c) All the contaminated cultures must be autoclaved before washing with a detergent. If the contamination levels are very high then the glassware (infected cultures only) after autoclaving should be left overnight in the chromic acid before washing with detergent the following day. d) The glassware must be washed under running tap water to ensure that no traces of media or detergent is left behind e) After washing with ordinary water, the culture vessels should be rinsed with deionized water before drying. f) Drying may be done by leaving the jars in an inverted position, overnight. Petriplates and other glassware may be dried in an oven. g) There should be a proper mechanism for disposal of used agar h) Overall cleanliness must be maintained.	5
	• **Media Preparation** - Availability of equipment for media preparation and autoclaving - Quality of chemicals - Quality of culture vessels - Maintance of records - Operational efficiency of media preparation (amount of media prepared everyday, proper labeling of media, etc.) - Cleanliness	a) The media preparation lab must have all the basic equipment such as weighing balance(electronic). PH meter, conductivity meter, microwave oven, de-ionizer/distillation unit/RO water facility, autoclave, etc. b) The chemicals should be of AR grade from a reputed company such as MERCK of QULAIGENS. c) The details of the media must be recorded and the trays/racks containing media should be properly labeled. d) All the parameters pertaining to autoclaving such as the time when the autoclave was switched on, when the desired pressure was obtained,	10

		autoclaving time, etc. must be recorded. e) As much as possible, high operational efficiency should be maintained to save on manpower. f) After autoclaving, the medium should ideally be stored for 2-3 days so that if something goes wrong with autoclaving, microbial contamination is detected before the medium is put to use. g) The medium must be stored in clean area where very high level of sterility (at least Class 1,000) is maintained	
	• **Inoculation room** - Equipment - Sterility levels - Technical competence of the operators - Operational efficiency (number of cultures handled by each operator, labeling of cultures, contamination losses, etc.	The inoculation room should have at least sterility level of Class 1,000. The room must be fumigated periodically with sterilant The airflow of the laminar airflow cabinet should be checked periodically. Besides flaming, the tools (forceps, scalpels, etc.) should also be autoclaved periodically Instead of rectified spirit, use of glass bead sterilizers should be favored as the former is a potential fire hazard Regular monitoring of air borne microbes in the lab is must Operators working in the lab must remove their foot wears outside the room and wear clean (preferably autoclaved) lab coats Entry to the lab must be trained/technically sound During sub-culturing at a time only one clone/genotype should be handled to avoid any mixing Due emphasis should be given to the efficiency of the operators (the number of jars handled, multiplication rates, contamination losses, etc.) Proper record of species, clone, passage number, media, operator name, etc. should be maintained	10
	• **Growth room** - Availability of equipment such as BOD, shakers, etc - Adequate facility to maintain stringent conditions for temperature and RH - Sterility levels	a) The growth room should be equipped with racks, AC, heat convector, temperature and humidity controller, photoperiod stimulator, shakers b) High sterility levels (Class 10,000) should be maintained with periodic check on airborne contaminants c) The room must be fitted with UV lights. It should also be fumigated periodically especially during the monsoon to keep the contamination under control	10

		d) Restricted entry	
B.	***Hardening facilities*** • **Transfer area** - Ex agar management - Selection of proper container and potting mix	a) Only one clone to be washed at a time b) Hardening trays should be properly labeled c) Selection of the hardening container and potting mix to be done as per the requirement of the species d) Drying of plants should be avoided by transferring them to the mist room/greenhouse immediately after transfer to the potting mix e) Water used for irrigation must not be hardy (rich in salts) f) Excessive watering of plants to be avoided g) Due consideration should be given to the texture and pH of the soil used for hardening h) All records pertaining to number of plants transferred, date of transfer, etc should be maintained for future reference	10
	• **Greenhouse/ poly house/ shade area** - Necessary facilities for proper hardening of plants through adequate control on temperature and RH	a) Stringent control on temperature and RH b) There shouldn't be any leakage for the inside air to escape c) Facility for ventilation to control excess of RH during monsoon d) Excessive watering of plants to be avoided e) It must be ensured that direct sunlight does fall on the plants but at same time there should be sufficient natural light in the GH f) Adequate provision for artificial light for those species that are high light demander g) Plants should be monitored regularly for their growth and presence of any disease or pest h) Dead plants should be removed immediately to avoid any possible attack of saprophytic fungi i) Fungal infestation in GH particularly during monsoon season is very common. If present, the plants should be sprayed with suitable fungicides j) Wherever possible, use of compost at the GH stage should be avoided because that may invite contamination k) Any kind of treatment given to the plant such as fertilizers, fungicides, pesticides, etc. must be recorded for reference just in	10

		case something goes wrong with the plants l) All moralities taking place in the GI/Polyhouse should be recorded to arrive at the transplantation losses	
	• **Nursery** - Adequate space and facilities for irrigation - Proper management	Nursery should have some shade area where the plants could be kept till they are harden enough to be kept under direct sunlight Only fully decomposed organic manure to be used. Partially decomposed manure will do more harm than any good to the plant There should adequate facilities for irrigation Nursery beds should be properly leveled so as to avoid any water-logging Regular weeding Regular shifting of plants to prevent the roots from entering the ground	5
2.	**Quality control** • **Selection of clones and maintenance of germplasm** - Selection of high yielding clones - Maintaining the germplasm in proper disease-free conditions	a) Following points must be recorded while selecting the mother plant - Geographical location of the mother plant or the area where mother plant is growing - Microclimatic conditions prevailing in that area - Various growth attributes of the mother plant (height, diameter of the stem, yield, etc.) - Origin of the mother plant (seedling raised or vegetatively raised) - Age b) High yielding clones should only be used for micro propagation work c) The mother plants should be maintained in disease-free environment so the chances of getting aseptic cultures remain high	5
	• **Explant** - Apical or axillary bud	a) Choice of the explant is a critical factor in the success of the micropropagation protocol. Since axillary branching method is the most favoured method for in *vitro* clonal propagation, only apical or axillary bud should be used as the explant for micropropagation work. While excising the explant from the mother plant, following points must be properly recorded: - Location of the explant on the mother plant (branches/coppice shoots)	5

		- Season (month) in which the explants have been derived - Any pre-treatment given to the mother plant before excising the explant	
	• **Virus indexing** - Testing the plants for known viruses and ensuring their climination before micropropagation	a) Before starting with the micropropagation work, the material should be tested for the presence of the known viruses (This facility may be developed in house or it may be done at other established centers) b) If the presence of virus is established then these must be removed off through meristem culture or chemo/heat therapy or a combination of techniques c) Only virus-free tissue should be used for further micropropagation work	5
	• **Number of multiplication cycles and Clonal uniformity** - Number of multiplication cycles - Ensuring that multiplication is only through axillary shoots and not adventitious - Ensuring clonal uniformity of plants by molecular methods - Carrying out field trials and confirming the yield before undertaking mass distribution of TC plants	a) In general the multiplication cycles should not exceed 10 passages. However, this number is not fixed and would vary with the species under consideration. b) Operators should be thoroughly trained so that they can draw a distention between the adventitious and axillary shoots. Only axillary shoots should be used for micropropatation work c) The plant tissue should be tested for the presence of systemic bacterial contamination by culturing the tissue after every 3-4 passages on LB medium d) Clonal uniformity may be established morphologically through field trials and with the help of molecular techniques. e) Before wide scale distribution to the farmers or growers, it would be good to reconfirm the superiority of tissue cultured plants. However, this would be valid only for short rotation crops because in perennial crops it will take several years to confirm the superiority of TC plants f) Proper field data must be collected and analysis be done	10
	• **Overall quality of the plants**	a) At the time of dispatch it must be ensured that the plants are fully hardened and are of transplantable size b) A small hand out giving all necessary information about after-care of the tissue cultured plant of that particular species should be provided to all growers for reference	5

3.	**Technical supervision and monitoring** • Monitoring of the production process and the staff involved therein	a) Strict monitoring of the entire production process covering all the activities that are performed in media lab, inoculation lab, growth room and hardening area is a must	10
	• Technical competence of the production supervisory staff	a) The managers, scientists and the supervisory staff involved in production must have very sound technical knowledge of the subject so that they could deal with any eventuality that may arise during course of production. b) There should be at least two supervisors (one in the clean area to monitor lab activities and one in the hardening area for after care and for monitoring field activities) in the production facility	
	• Operators	The operators may or may not have very sound scientific background but they must be tho0roughly trained by the supervisors and the professional staff before they undertake any skilled job such as media preparation or inoculations	

Note : Besides various parameters indicated above, the cost of plantlet production would be very important