Aseptic techniques in plant tissue culture

1. 1. Aseptic Techniques in Plant Tissue Culture Kanika and N.C. Gupta National Research Centre on Plant Biotechnology, New Delhi-110012
2. [2.](http://image.slidesharecdn.com/aseptictechniquesinplanttissueculture-140528065359-phpapp02/95/aseptic-techniques-in-plant-tissue-culture-2-638.jpg?cb=1401279312)⎫ Need for maintenance of aseptic conditions ⎫ Sources of contaminations ⎫ Prevention of contamination ⎫ Conclusion ⎫ Suggested reading
3. [3.](http://image.slidesharecdn.com/aseptictechniquesinplanttissueculture-140528065359-phpapp02/95/aseptic-techniques-in-plant-tissue-culture-3-638.jpg?cb=1401279312)ϖ Plant tissue culture medium very rich in nutrition ϖ Microbes can grow very fast on it as compared to plants ϖ This is harmful for the plants ϖ Therefore aseptic conditions are to be maintained in the culture vessel
4. [4.](http://image.slidesharecdn.com/aseptictechniquesinplanttissueculture-140528065359-phpapp02/95/aseptic-techniques-in-plant-tissue-culture-4-638.jpg?cb=1401279312)Possible sources of contamination :- ϖ culture vessel ϖ nutrient medium ϖ Instruments used during various operation ϖ Explant ϖ operator ϖ environment of the tissue culture laboratory
5. [5.](http://image.slidesharecdn.com/aseptictechniquesinplanttissueculture-140528065359-phpapp02/95/aseptic-techniques-in-plant-tissue-culture-5-638.jpg?cb=1401279312)a)Dry heat ♣ Used for glassware, metal instruments etc. dry-heating in an oven at 160-1800C for 3 hours approx. (1 hr heat up period to reach to temp and cooling period) ♣ Wrapping in aluminum foil ♣ Cannot be used for plastic ware, however, certain plastic wares can also be heat sterilized (Instructions of the manufacturer must be read before doing this) Glassware, plasticware and Medium sterilization:-
6. [6.](http://image.slidesharecdn.com/aseptictechniquesinplanttissueculture-140528065359-phpapp02/95/aseptic-techniques-in-plant-tissue-culture-6-638.jpg?cb=1401279312)b) Wet heat • Autoclaving (steam under pressure) or a home pressure cooker steam pressure of 1.05kg/cm2 (temperature 121 0 C ) for 15- 45 minutes • Time required for autoclaving varies with the volume of liquid to be sterilized • Do not close the escape valve until a steady steam comes out of autoclave • Actual time of autoclaving should be started when proper temperature is reached • Over autoclaving should be avoided • Once autoclaving is over, pressure must not lost rapidly, it should be allowed to return to atmospheric pressure slowly as rapid loss of pressure will lead to vigorous boiling of liquids inside the culture vessels • It should be opened when the pressure is zero as this might cause accidents ¬ Now a days pre-sterilized ready-to-use plastic ware is available, which can be used directly to pour medium etc.
7. [7.](http://image.slidesharecdn.com/aseptictechniquesinplanttissueculture-140528065359-phpapp02/95/aseptic-techniques-in-plant-tissue-culture-7-638.jpg?cb=1401279312)Figure. Autoclave unit used for wet sterilization of nutrient media Pressure Gauge Safety Valve Power Switch Water Level Indicator Lid Safety Clamp Heating Indicator Power indicator
8. [8.](http://image.slidesharecdn.com/aseptictechniquesinplanttissueculture-140528065359-phpapp02/95/aseptic-techniques-in-plant-tissue-culture-8-638.jpg?cb=1401279312)♣ Certain components of medium like Zeatin, GA3, pantothenic acid, antibiotics etc. are thermolabile and cannot be autoclaved ♣ These can be sterilized by membrane filtration and added to autoclaved medium once it has cooled down to ~ 400C c) Filter sterilization: ♣ filter membranes of pore size 0.45 µm or less are used ♣ Filter assemblies of different sizes are available ♣ Once the component is filter sterilized, it is collected in a sterile container which can be used immediately or dispensed in smaller amounts to be used later ♣ These filter sterilized components can be stored at 40C or -200C depending on the frequency of their usage
9. [9.](http://image.slidesharecdn.com/aseptictechniquesinplanttissueculture-140528065359-phpapp02/95/aseptic-techniques-in-plant-tissue-culture-9-638.jpg?cb=1401279312)Figure. Filter sterilization techniques Membrane Filter Syringe Filter sterilized Membrane Filter Air Pump Membrane Filter Filter sterilized solution
10. [10.](http://image.slidesharecdn.com/aseptictechniquesinplanttissueculture-140528065359-phpapp02/95/aseptic-techniques-in-plant-tissue-culture-10-638.jpg?cb=1401279312)Instruments :- • Sterilized by dipping in 95% ethanol followed by flaming and cooling • Glass bead sterilizer and infra-red sterilizer are available commercially operated by electricity these instruments are safer and not a fire hazard • Glass bead sterilizer has glass bead in a heated cavity where a temperature of nearly 2500C is maintained instruments are pushed into the cavity for 5-7s • Infrared sterilizer also has a cavity where a temperature of nearly 7000C can be achieved by infra- red wave heating. Exposure of 2-5 s is effective for sterilization of instruments
11. [11.](http://image.slidesharecdn.com/aseptictechniquesinplanttissueculture-140528065359-phpapp02/95/aseptic-techniques-in-plant-tissue-culture-11-638.jpg?cb=1401279312)Figure: Commonly used instruments in plant tissue culture Scissor Scalpel Scalpel holderForceps (Small, Medium & Large)
12. [12.](http://image.slidesharecdn.com/aseptictechniquesinplanttissueculture-140528065359-phpapp02/95/aseptic-techniques-in-plant-tissue-culture-12-638.jpg?cb=1401279312)Plant material ♣ Growing in nature and exposed to a variety of microbes so it is a very rich source of contaminants and needs to be surface sterilized before inoculation into the medium ♣ Variety of surface sterilizing agents are available which vary in their efficacy and toxicity ♣ Too strong treatment can kill the explants whereas too mild treatment may not yield any sterile explant ♣ So sterilizing treatment is selected on the basis of the state of explants ♣ explants are hardy and apparently contaminated a strong treatment can be given if explants are juvenile and soft, a mild treatment should be preferred ♣ In certain cases surface sterilization of the actual explant may not be required. e.g. for culturing the immature ovule the whole ovary is surface sterilized and the ovule is dissected out under aseptic conditions ♣ Generally adding few drops of surfactant in the sterilizing solution enhances its efficiency
13. [13.](http://image.slidesharecdn.com/aseptictechniquesinplanttissueculture-140528065359-phpapp02/95/aseptic-techniques-in-plant-tissue-culture-13-638.jpg?cb=1401279312)ϖenvironment of the tissue culture laboratory
14. [14.](http://image.slidesharecdn.com/aseptictechniquesinplanttissueculture-140528065359-phpapp02/95/aseptic-techniques-in-plant-tissue-culture-14-638.jpg?cb=1401279312)Media room ϖ Maintain cleanliness, removal of contaminated culture, restricted entry Washing room ϖContaminated cultured should be autoclaved and discarded with utmost care, maintain general cleanliness Transfer area ϖA sterile area is required for performing various aseptic manipulations during tissue culture. This ensures that contaminants do not gain entry into the culture vial ϖLaminar air flow cabinets of various shapes and sizes are available commercially. These cabinets allows the tissue culturists to work in the sterile environment for long stretch of time. It provides a covered enclosed area for working
15. [15.](http://image.slidesharecdn.com/aseptictechniquesinplanttissueculture-140528065359-phpapp02/95/aseptic-techniques-in-plant-tissue-culture-15-638.jpg?cb=1401279312)Figure: Laminar air flow cabinet in use Pre-Filter HEPA Filter Acrylic Sheet Working surface Gas Burner
16. [16.](http://image.slidesharecdn.com/aseptictechniquesinplanttissueculture-140528065359-phpapp02/95/aseptic-techniques-in-plant-tissue-culture-16-638.jpg?cb=1401279312)Transfer area ϖ There are UV lights inside the chambers which are switched on for 10-15 minutes before using the laminar air flow cabinet ϖ It has small motors at the base for blowing the air which is first passed through coarse filter. This step ensures removal of large contaminants. Then the air passes through fine filters called HEPA filters. HEPA stands for ‘High Efficiency Particulate Air’ . These filters removes impurities which are larger than 0.3 µm therefore the air coming out of these is clean. ϖ Air coming out of these filter comes with some force which prevent entry of contaminants from the worker or environment into the working area ϖ A gas burner or spirit lamp facility is also available for flaming the instruments Growth room ϖ Maintain cleanliness, removal of contaminated culture, restricted entry
17. [17.](http://image.slidesharecdn.com/aseptictechniquesinplanttissueculture-140528065359-phpapp02/95/aseptic-techniques-in-plant-tissue-culture-17-638.jpg?cb=1401279312)Figure: Regular inspection of culture and removal of contaminated samples reduces the chances of contaminations Tissue Culture trolley & racks Fluorescent Light source Tissue Culture grown plants
18. [18.](http://image.slidesharecdn.com/aseptictechniquesinplanttissueculture-140528065359-phpapp02/95/aseptic-techniques-in-plant-tissue-culture-18-638.jpg?cb=1401279312)Operator: ♣ Clean hand and forearm properly before starting the work ♣ Hands may be cleaned with dilute solution of alcohol or commercial hand sanitizers available in the market ♣ Wearing a surgical mask and head gear and lab coat while working in the laminar will reduce the chances of contamination ♣ Presence or other persons in or near the transfer area should be minimized .