IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Plant Tissue Culture: A Comparative Study on Sterilization Methods

M. Sai Yaswanthi

Department of Botany

Abstract:

Plant Tissue Culture is a technique of plant biotechnology used to grow plants in vitro under controlled aseptic conditions on suitable medium. Whole plant is produced from single cell, groups of cells, tissues and organs, which is the characteristic of plant cells called “Totipotency”. As world population increasing, huge demand for food as well as other plant products is increased. To meet this requirement, plant tissue culture became one of the solutions. Such technique has defined procedure to grow plants. But during the process, contamination in cultures is a common problem which affects the growth, yield and productivity of cultured plants. To overcome this problem, sterilization methods are implemented. Using better method for sterilization during culture process, gives best results i.e., plants with desired characters in large number without contamination.

Introduction:

Tissue Culture is a method of growing, multiplying and regenerating novel plants from genetically engineered cells, tissues and organs of plants on defined solid or liquid media under controlled aseptic environment. The excised plant portion used to start tissue culture i.e., bits of leaf, stem, root or any part of plant called as “Explant". There are following types of cultures: Callus culture, Cell suspension culture and Organ cultures. By using these cultures, we are obtaining disease free plants, androgenic and gynogenic haploid plants, genetically improved plants, somatically hybridized plants etc.

Review of literature:

Process of plant tissue culture contains following stages:

●Preparation of nutrient culture medium

●Creating aseptic environment conditions for culture using different sterilization methods

●Inoculating explant into medium

●Development of plantlets in the laboratory

●Making plantlets to grow in natural environmental conditions

Before initiating the process, the vessels, instruments, medium, plant material must be freed from contamination. It occurs due to impurities, chemicals or pathogens that makes cultured tissues unfit for further development of healthy plantlets.

Different sterilization methods are developed to avoid contamination. They are:

1.Sterilization by heat:

A) Moist heat: Autoclave is the instrument uses steam (moist heat) to sterilize glass apparatus, media(solid/liquid), discarded cultures, gloves, rubber tunes etc. Heating the materials with steam generated due to boiling of water which is set at 121°c temperature and 15p.s.i/1.03 bar pressure for 15 to 40 minutes. Pressure cooker can also be used.

B) Dry heat: Hot air oven is used to sterilize glass ware, plastic ware, powder (starch, zinc oxide etc..), metal equipment like scalpels, scissors and blades. Heating materials at 160 to 180°c temperature for 3 hours is done.

2. Sterilization by filtration: Thermolabile substances such as growth hormones, urea and vitamins as liquid solution are sterilized by membrane filtration. Commonly used filters are membrane filters made up of cellulose acetate, cellulose nitrate etc. The pore size exists between 0.2 to 0.45 microns that decides the size of microorganisms to be removed.

3. Sterilization by air: Air comprise of millions of particles along with microbes that lands on medium and materials and cause contamination during the time of inoculations and transfers from one culture medium to other. To maintain clean and sterile air, laminar air flow with HEPA (High Efficiency Particulate Air) filters and germicidal UV light along with cool white fluorescent tunes for uniform illumination is used. The blower blows air through filters sort microorganisms larger than 0.3 microns size with 99% efficiency.

4) Surface sterilization: Explants are surface sterilized with appropriate chemical agents to kill contaminating microbes. Different chemicals used for sterilization based on type of explant and plant species selected and its tolerance towards the chemical are:

a) Calcium Hypochlorite: It is available in powder form, used by diluting to 9 to 10% and filtered. Explant is exposed for 3 to 30 minutes.

b) Sodium Hypochlorite: Also called Bleach used after diluting to 10 to 20%such that the final concentration becomes 0.5 to 1.0% bleach with 30 to 40 minutes exposure time.

c) Mercuric chloride: It is used at 0.1 to 1% concentration with 2 to 10 minutes exposure time.

d) 70% Ethanol: Ethanol or Isopropyl alcohol is at 70% concentration with 30 to 60 seconds exposure time is used.

All these are most commonly used for their satisfactory results. Along with them, Bromine water (1-2%), Silver nitrate (1%), Hydrogen peroxide (10-12%) are also used.

Recently for cleaning glass apparatus, chromic acid or chromic-sulphuric acid is using instead of detergents. After soaking for 24 hours in chromic acid, they are washed under tap water with high pressure set up along with brushing.

Objectives:

>> Study the usage of different sterilization methods and instruments

>> Observe the cultures with less or free of contamination using different sterilization methods.

>> Compare the methods to obtain best for culturing.

Research Methodology:

A sample media is prepared from artificial powders available at market by dissolving in distilled water. Add agar, sugar and other supplements required and adjust the pH. Choose a plant for culture and select an explant from it. Before initiating the process, sterilize the laboratory area. Then implement different sterilization methods for apparatus and culture materials one after the other. Prepare sample cultures by inoculating explants in culture media. Observe their growth such that which way of sterilization is given the best results.

Conclusion:

Contamination is one of the largest problems in plant tissue culture, as it leads to loss of yield and productivity. Infections may continue through generations easily if required precautions are not taken. Hence suitable and efficient sterilization methods should be used to reduce or avoid contamination without tissue damage. Sterilized medium gives resistance to plantlets against diseases, reduce the growth cycle time of some plants and increase the multiplication rate of some other plants.

Dry heat sterilization is better than Autoclave because Autoclave needs more time and metal equipment may rust and become blunt. Media preparatory machines are available now to prepare sterilized media using different heating technique take quite less time.

Bleach and calcium hypochlorite are better than mercuric chloride and ethanol as they more toxic to plant tissues when compared.

References:

● M.K. Razdan and S.S. Bhojwani (1996) “Plant Tissue Culture: Theory and practice” pg.no. 19 to 39

● U. Satyanarayana (2017) “Biotechnology” pg.no. 497 to 517

● Hirenkumar Sherathiya (2012) “Practical manual for Plant Tissue Culture: Basic Techniques of Plant Tissue Culture and Molecular Biology”

● Nancy Bhatia (9th September 2021) https:/labassociates.com “4 methods of sterilization used in plant tissue culture”

● https:/www.plantcelltechnology.com/blog/the-five-ideal-explant-sterilizing-methods-in-tissue-culture