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General account of Plant Tissue Culture:

In biological research, tissue culture refers to a method in which fragments of a tissue (plant or animal tissue) are introduced into a new, artificial environment, where they continue to function or grow.

Tissue culture is the *in vitro* aseptic culture of cells, tissues, organs or whole plant under controlled nutritional and environmental conditions often to produce the clones of plants. The resultant clones are true-to-type of the selected genotype. The controlled conditions provide the culture an environment conductive for their growth and multiplication. These conditions include proper supply of nutrients, pH medium, adequate temperature and proper gaseous and liquid environment.

Plant tissue culture technology is being widely used for large scale plant multiplication. Apart from their use as a tool of research, plant tissue culture technique have in recent years, become of major industrial importance in the area of plant propagation, disease elimination, plant improvement and production of secondary metabolites.

Basic Requirements and Techniques of Plant Tissue Culture:

The main requirements of plant tissue culture are:

(1) Laboratory Organisation

(2) Culture Media

(3) Aseptic Conditions

(1). Laboratory Organization:

In a standard tissue culture lab, there must be a few basic facilities like:

- A Media Room for preparation, sterilization and storage of culture media.
- Facilities for washing of lab-wares, explants, etc.
- Space for storage of lab-wares.
- Culture rooms or incubators where conditions of temperature, humidity and light etc. can be maintained.
- Observation and Data Collection area.

(2). Culture Media:

The formulation or the medium on which the explant is cultured is called culture medium. It is composed of various nutrients required for proper culturing. Different types of plants and organs need different compositions of culture media. A number of media have been devised for specific tissues and organs.

Some important of them are:

- MS (Murashige and Skoog) Medium
- LS (Linsmaier and Skoog) Medium

- B5 (Gamborg's) Medium
- White's Medium, etc.

Important constituents of a culture medium are:

(i)Organic supplements:

(a) Vitamins like thiamine (B_1), Pyridoxin (B_6), Nicotinic Acid (B_3), etc.

(b) Antibiotics like Streptomycin, Kanamycin;

(c) Amino Acids like Arginine, Asparagine.

(ii) Inorganic Nutrients:

Micronutrients as Iron (Fe), Manganese (Mn), Zinc (Zn), Molybdenum (Mo), Copper (Cu), Boron (B).

Macronutrients include six major elements as Nitrogen (N), Sulphur (S), Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg).

(iii) Carbon and Energy Source:

Most preferred carbon source is Sucrose. Others include lactose, maltose, galactose, raffinose, cellobiose, etc.

(iv) Growth Hormones:

a. Auxins-mainly for inducing cell division.

b. Cytokinins-mainly for modifying apical dominance and shoot differentiation.

c. Abscisic Acid (ABA)-Used occasionally.

d. Gibberellins-Used occasionally.

(v).Gelling Agents:

These are added to media to make them semisolid or solid. Agar, Gelatin, Alginate etc. are common solidifying or gelling agents.

(vi)Other Organic Extracts:

Sometimes culture media are supplemented with some organic extracts also like coconut milk, orange juice, tomato juice, potato extract, etc.

3. Aseptic Conditions:

Maintenance of aseptic conditions is the most critical and difficult aspect of in-vitro culturing experiments. Aseptic condition mean the conditions free from any type of microorganisms (so as to prevent the loss of experiment by contamination). For this, sterilization (i.e., complete removal or killing of microbes) is done. The most common contaminants in culture are fungi and bacteria.

Measures to be taken for maintaining asepsis during tissue culture are:

- Sterilization of the culture vessels using detergents, autoclaves, etc.
- Sterilization of instruments like forceps, needles etc. by flame sterilization.
- Sterilization of culture medium using filter sterilization or autoclaving methods.
- Surface sterilization of explants using surface disinfectants like Silver Nitrate (1%), H_2O_2 (10-12%), Bromine water (1-2%), Sodium Hypochlorite solution (0.3-0.6%), etc.

The whole procedure of plant tissue culture is to be carried out essentially under aseptic conditions. So, the overall design of the laboratory must focus on the maintenance of aseptic conditions. Secondly, the worker is also required to have

proper knowledge of operating various equipment's like pH meter, balance, laminar air flow, microscope, etc.

While performing the tissue culture experiments there must present the first aid kits and fire extinguishers in the laboratory to avoid any mishap or accident. In addition, proper attention should be given while handling the toxic chemicals and all the chemicals should be kept in correct labeled containers and bottles.

General Technique of Plant Tissue Culture:

General technique of plant cell, tissue and organ culture is almost the same with a little variation for different plant materials. There are certain basic steps for the regeneration of a complete plant from an explant cultured on the nutrient medium.

These basic steps for in-vitro culturing of plants are:

(a) Selection and Sterilisation of Explant:

Suitable explant is selected and is then excised from the donor plant. Explant is then sterilized using disinfectants.

(b) Preparation and Sterilisation of Culture Medium:

A suitable culture medium is prepared with special attention towards the objectives of culture and type of explant to be cultured. Prepared culture medium is transferred into sterilized vessels and then sterilized in autoclave.

(c) Inoculation:

Sterilized explant is inoculated (transferred) on the culture medium under aseptic conditions.

(d) Incubation:

Cultures are then incubated in the culture room where appropriate conditions of light, temperature and humidity are provided for successful culturing.

(e) Sub culturing:

Cultured cells are transferred to a fresh nutrient medium to obtain the plantlets.

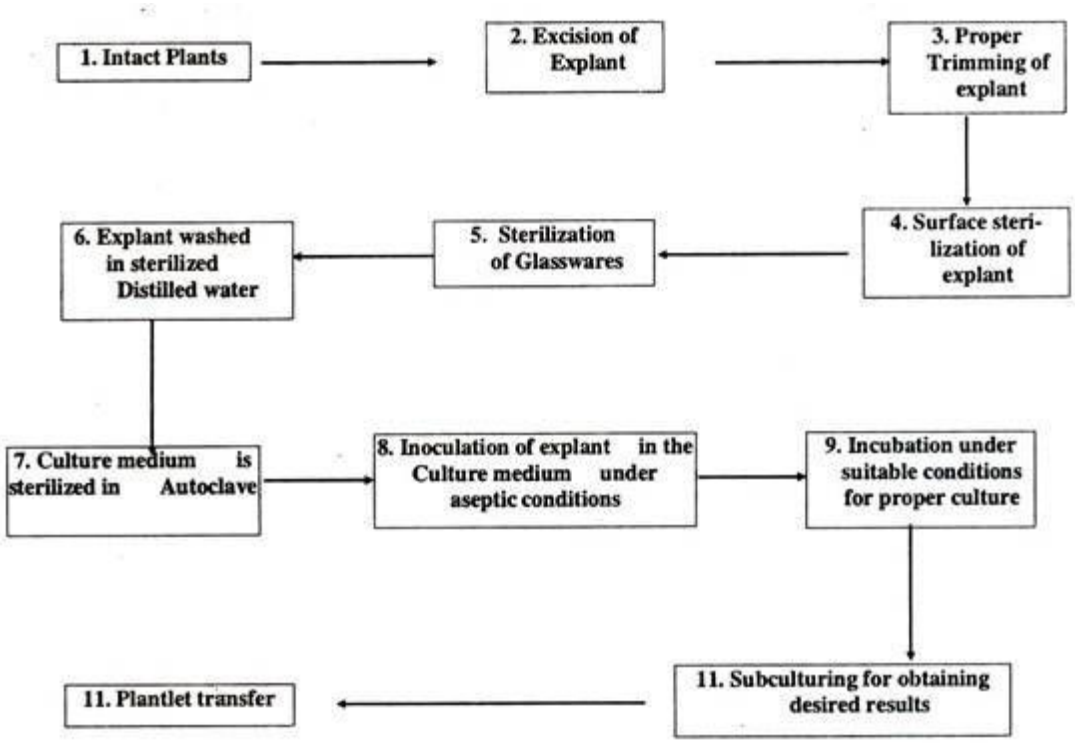

Fig. 1. Steps in general technique of Plant tissue culture.

(f) Transfer of Plantlets:

After the hardening process (i.e., acclimatization of plantlet to the environment), the plantlets are transferred to green house or in pots.

As an emerging technology the plant tissue culture has a great impact on both agriculture and industry, through providing plants needed to meet the ever increasing world demand. It has made significant contribution to the advancement of agricultural sciences in recent times and today they constitute an indispensable tool in modern agriculture.

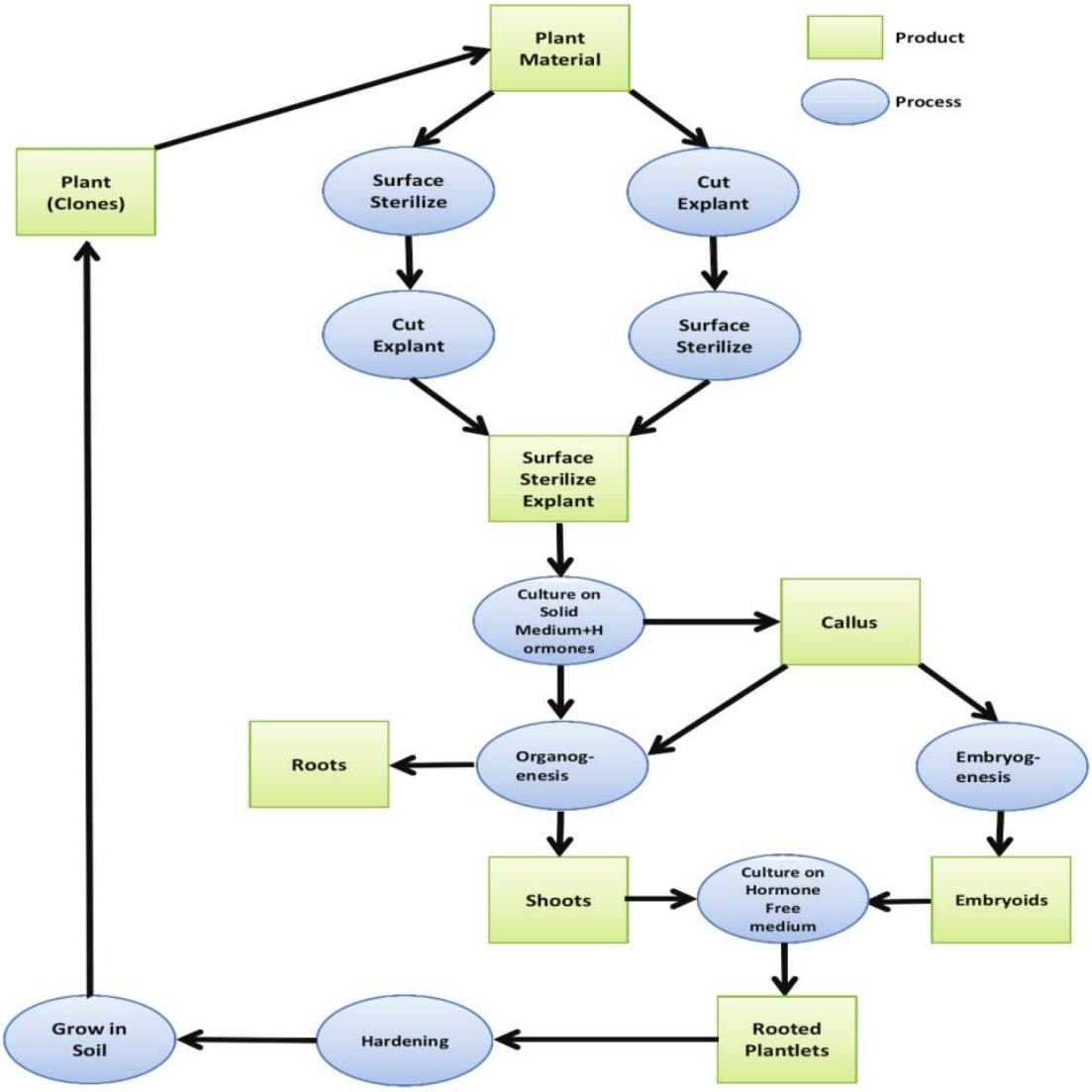

Fig: Summarizing Flow Chart of Plant Tissue Culture Experiment

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