

TOPIC : Composition of culture media and its preparation
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Plant Tissue Culture, Ethnobotany, Biodiversity
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INTRODUCTION

- **Culture media** can be defined as the substance required for growing plant materials/ microorganisms *in vitro* (in a test tube, culture disc) conditions.
- Culture media are largely responsible for *in vitro* growth and morphogenesis of plant tissues. The basic nutrient requirements of cultured plant cells are very similar to the plants growing in natural condition.
- In general, a media contains macronutrients, micronutrients, vitamins, amino acids or other nitrogen supplements, sugar (s), solidifying agents or support systems and growth regulators. The component of a media without growth regulators is called a **basal media**. In addition, media can also include antibiotics or natural complexes or organic supplements as per the demand of the experiment.

Properties of Culture Media

- Plant tissue culture media must contain different nutrients for different tissues from different explants to grow efficiently.
- It should also contain sufficient moisture, suitable level of oxygen, and pH adjusted as per requirement of the growing material.
- Media must be sterile before use.



Composition of Plant Tissue Culture Media

- Complex mixture of inorganic salts
- Organic supplements : vitamins and/or amino acids.
- Carbon source
- Plant growth regulators
- Solidifying agents
- Water

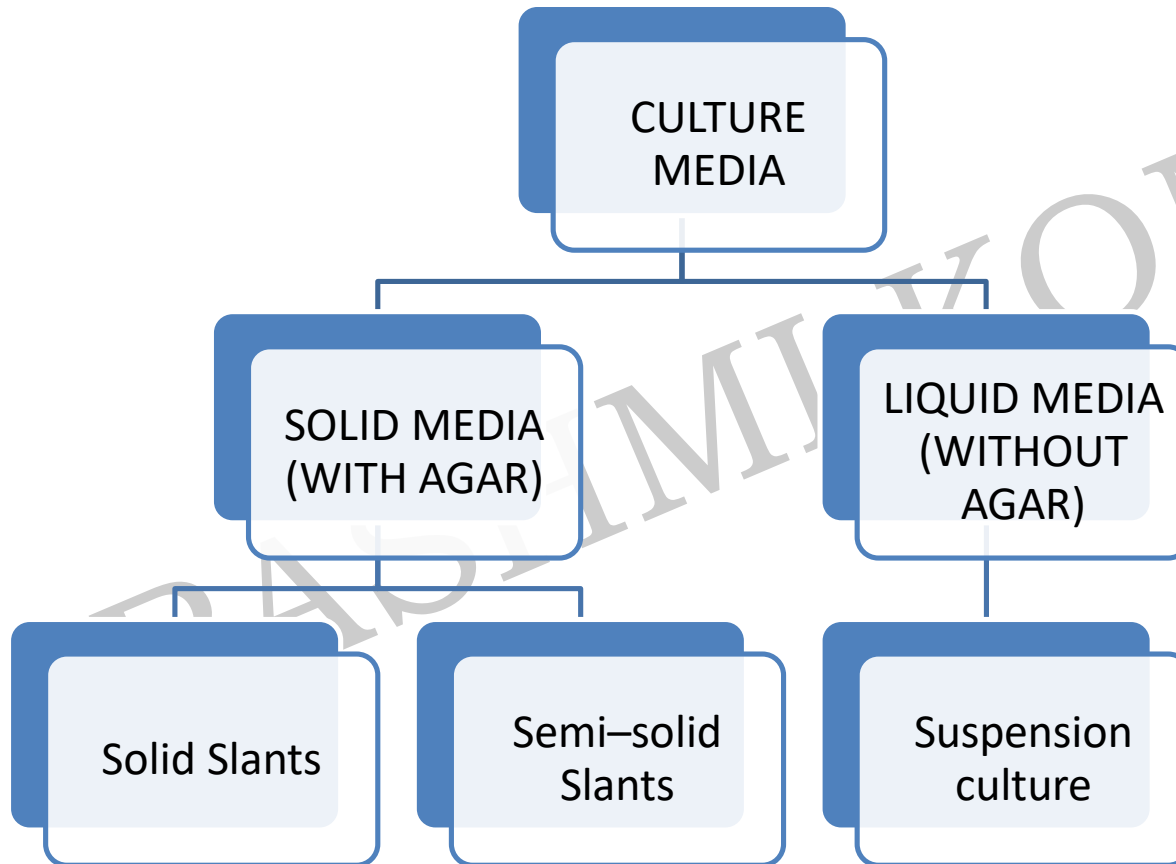
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Defined and semi-defined media

- In a defined media the exact composition of the components of the media are known. It is also referred to as **synthetic media**.
- In a semi-defined media chemically undefined components and their composition like protein hydrolysates, fruit juice, vegetable extract, plant extract, coconut milk are added. It is regarded as a **natural media**.



MEDIA TYPES ON THE BASIS OF CONSISTENCY



Selection of basal media

Selection of basal media depends on two factors, the genotype of the plant species and the type of explants used for culture i.e. cells, tissue, organ, protoplast. The composition of a media is formulated by considering the specific requirements of a given culture system.

Plant culture media contain the following constituents

1. Inorganic nutrients
2. Organic supplements
3. Carbon sources
4. Growth regulators
5. Solidifying agents
6. pH of medium

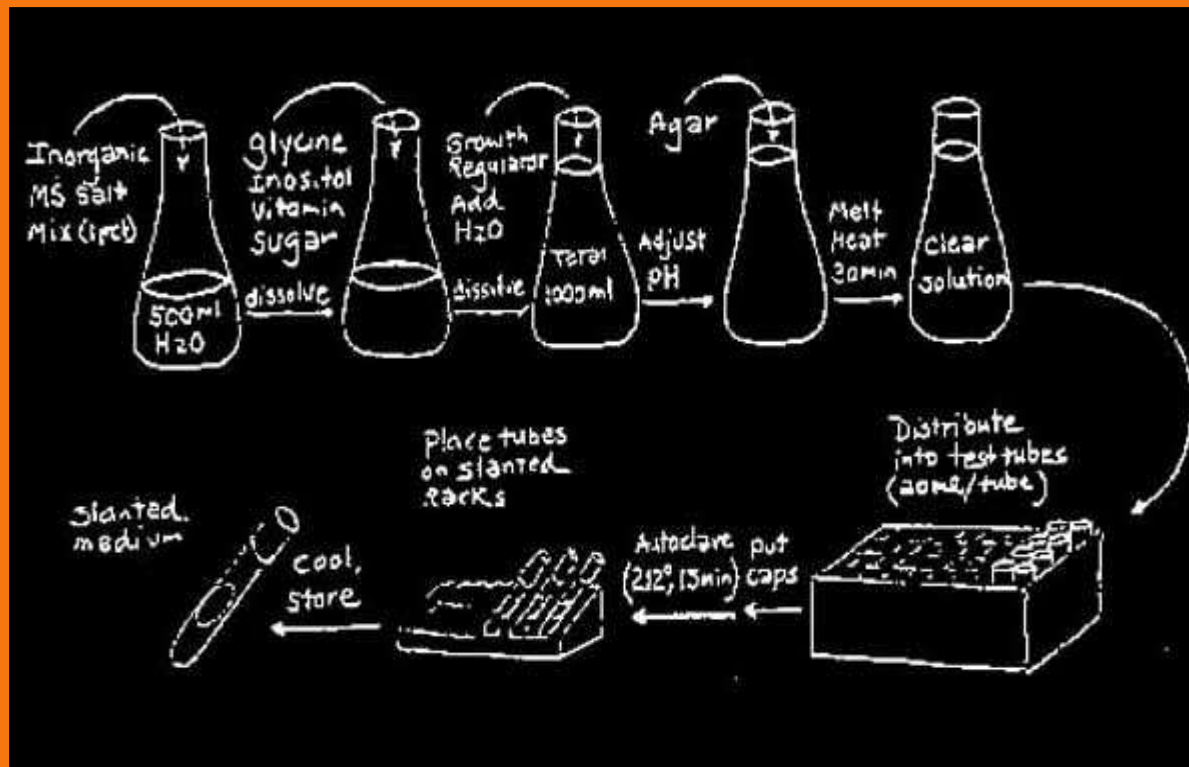
Preparation of culture media

Murashige & Skoogs (1962) media :

I. Preparation of Stock Solution

Separate stock solution for major, minor, iron, vitamins and amino acids are prepared by dissolving the required amount of constituents in distilled water. For preparing stock solution of iron, distilled water is boiled for half an hour. Plant growth regulators are dissolved in very small quantity in suitable solvent. All the stock solutions are stored in a refrigerator at 4°C and taken out 30 minutes before use.

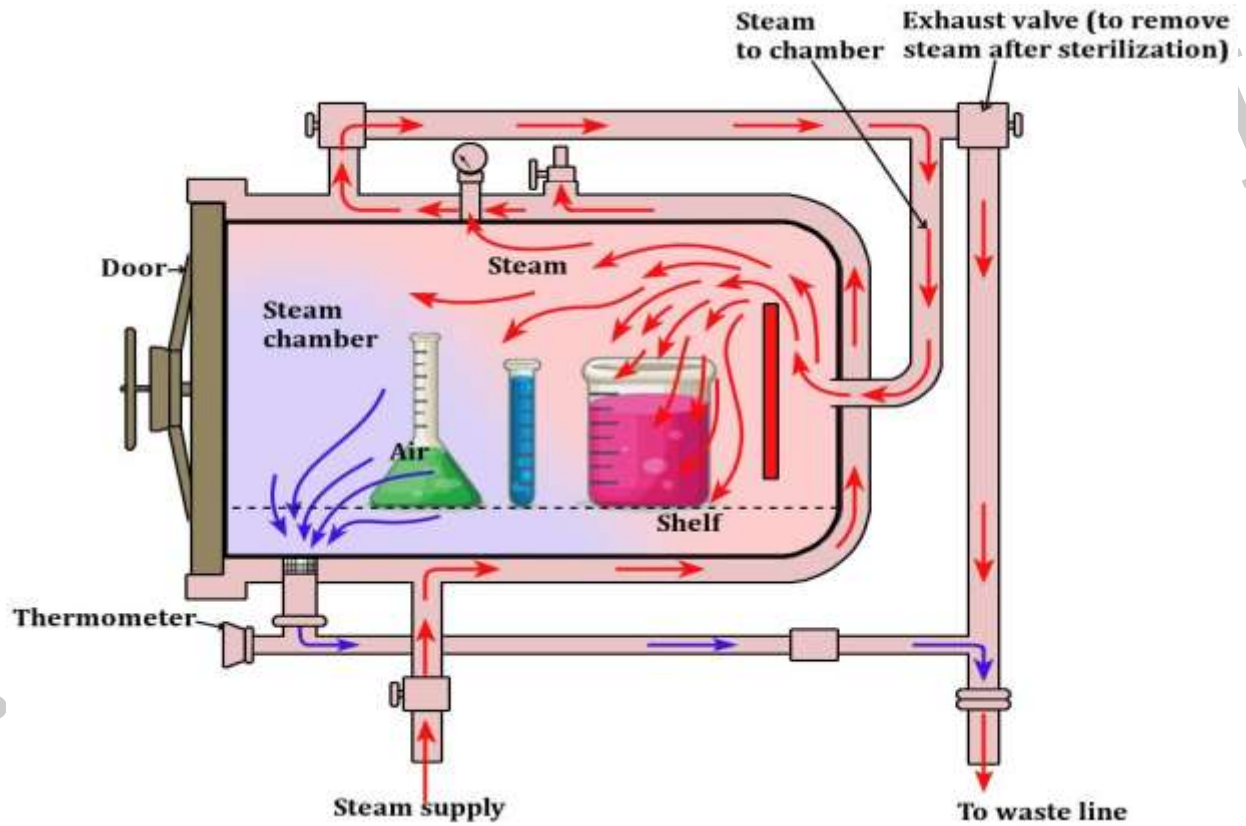
Media preparation steps:



II. Preparation of Medium

Double distilled water is always required for preparation of media. The required quantities of different stocks, plant growth regulators and 30 gm sucrose are taken into a 1000 ml flask as per requirements of the experiment, volume is made up to 950 ml by adding distilled water. The pH of the medium is adjusted to 5.8 by 0.1 N HCl or 0.1 N NaOH. The final volume of the media is made upto one litre and the content is poured in 1litre conical flask containing 7.0 gm agar-agar. The conical flask is plugged with non absorbent cotton plug and placed in an autoclave with steam open for 10-15 minutes or oven for homogenization of agar. About 20 ml of homogenized media is poured into each culture tube. The tubes are plugged with non absorbent cotton plug/plastic caps. These culture tubes containing media are kept in slant racks for solidification.

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Precautions in media preparation

- Always use double distilled water.
- Adjust the medium pH with 0.1 N HCl or 0.1 N NaOH.
- Always adjust pH before adding the agar.
- Never pour excess stock solution back into the original stock solution container.
- Never put excess sucrose or agar back into the original container.
- Always make as much media as required for the experiment.

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LINKS

<https://www.intechopen.com/books/recent-advances-in-plant-in-vitro-culture/plant-tissue-culture-media>

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THANK YOU

