CULTURE MEDIA

TO,
FIFTH SEMESTER STUDENTS

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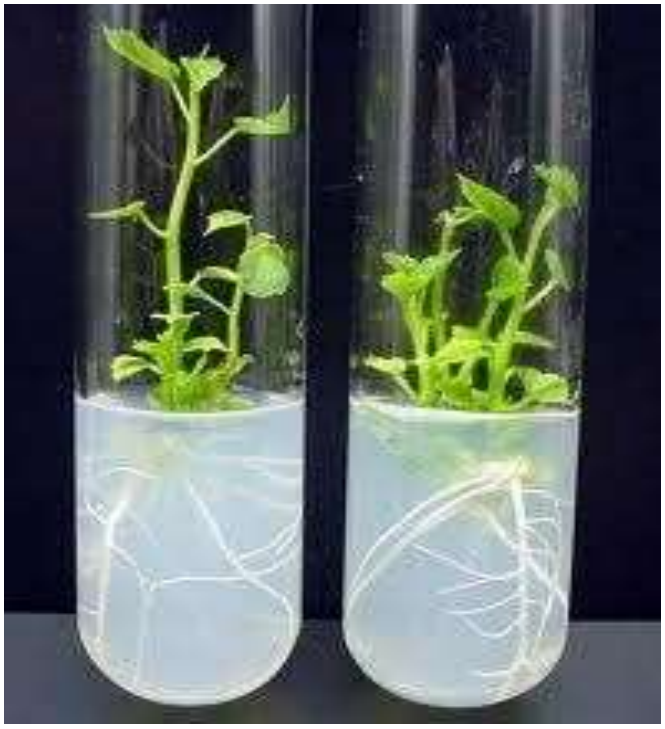
CULTURE MEDIA



Nutrient Medium – MS medium

- Medium depends upon the type of plant tissue or cell used for culture
- Generally nutrient consist of

➢ Inorganic salts (both micro & macro elements)

➢ a carbon source (usually sucrose)

➢ Vitamins (eg. nicotinic acid, thiamine, pyridoxine

➢ Amino acids (eg. arginine)

➢ Growth regulators (eg. auxins)

- An optimum pH (5.7) is also very important

Agar

- It is a polysaccharide.
- Obtained from red algae, such as **gracillaria** and **gellidium.**
- Gelling agent used to **soldify** liquid media.
- If liquid medium is used no agar is required.
- **8gm/L** is used.
- Provide solid surface for the growth of cells.
- If it is absent, tissues will submerge in the liquid medium and eventually die due to anoxia.

Organic constituents

- No autotrophic powers hence they require external source for carbon and energy.
- **20-30 gm/L** is required.
- **Sucrose** is the standard media
- Glucose also used
- Fructose, yeast extract, malt extract, coconut water ..etc rarely used.

Inorganic constituents

- Micronutrients
- Macronutrients
- EDTA

Growth hormones

- Cytokinin, auxin, gibberellins
- Cytokinin promote RNA synthesis, induce cell division and cell differentiation and regulate growth.
- Adenin , kinetin,zeatin & benzyl adenin are widely used cytokinins.
- Auxins promote cell division, callus formation and root differentiation and stimulate shoot elongation.
- IAA, 2,4-dichlorophenoxy acetic acid
- Gibberellins promote callus growth and elongation of dwarf plants.

- **Antibiotics :**

  Stertomycin,kanamycin

Activated charcoal

- **Other organic supplements** :

  Protein, coconut milk,yest,malt extract, orange juice, and tomato juice

- **Growth regulators :**

  Auxins,cytokinins

- **Water :**

  Demineralized or distilled water

- **Solidifying agents :**

  Agar, gelatin.

- **pH adjusters** :

  5 - 6 it is considered to be optimum.

Preparation of medium

- What is **stock solution**?
- Stock solution is the concentrated solution of selected constitutions of the culture medium, prepared & stocked in advance for the preparation of the medium.
- Usually it is **frozen or stored in a refrigerator** and a portion of it is used for medium preparation.

- Culture medium is prepared by mixing appropriate quantities of stock solutions.
- 4 stock solutions are prepared
    1. **Macronutrients**
    2. **Micronutrients**
    3. **Iron stock**
    4. **Organic nutrients except sucrose**
- Growth regulators are prepared as separate stock.
- pH adjusted to **5.5 to 6**
- Solution is mixed and made up to 1L

- **Sterilize** the medium before the addition of growth regulators.
- Keep the culture tubes in **dark and cool places** to minimize degradation.
- Many sensitive plants are cultured in freshly prepared medium.
- Glass wares , tools, and components of medium are heat sterilized.
- Transfer hood or laminar air flow chamber, should be set up.
- To provide protection against microbial contamination, **wiping with alcohol** and **use of UV light** are necessary.


Telstar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❖ PREPARATION OF CULTURE MEDIA:

✓Stock solution 1 : $MgSo_4$,KH_2Po_4,KNO_3,NH_4No_3,$CaCl_2$

✓Stock solution 2 : H_3Bo_3,$MnSo_4$,$ZnSo_4$,$CuSo_4$,$Cocl_2$

✓Stock solution 3 : $FeSo_4$,sodium EDTA

✓Stock solution 4 : ionositol,thiamine,pyridamine,nicotinic acid,glycine

To prepare 1 liter of medium:

Take 50 ml of stock solution 1 + 5ml of stock solution 2 & 4 in a beaker. The stock solution 3 prepared separately in a other 450ml flask by adding double distilled water and heat with constant stirring.
Mix two solutions and adjust PH to 5.5.

INORGANIC & ORGANIC SUPLLEMENTS

COUMPOUNDS	Mg/Ml
NH4NO3	1,650.00
KNO3	1,900.00
CaCl2 (anhyd)	332.20
MgSO4 (anhyd)	180.70
KH2PO4	170.00
Na_2EDTA	37.25
FeSO4.7H2O	27.80
H3BO3	6.20
MnSO4.H2O	16.90
ZnSO4.H2O	5.37
KI	0.83
Na2Mo4.2H2O	0.25
Sucrose	30,000.00
i-Inositol	100.00
Thiamine.HCl	0.40

Basic requirements of Plant Tissue Culture:

- Plant material
- Equipments and Glasswares
- Aseptic Condition
- Washing and storage facilities
- Media preparation room
- Sterilization room
- Nutrient medium
- Transfer room
- Culture room or incubators
- Proper and optimum aeration
- Well equipped observation or recording area