

Plant Tissue Culture medium

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Plant Tissue Culture medium

- Media used in plant tissue culture contain nutritional components.
- Nutritional components are essential for growth and development of cultured tissue
- The success of the tissue culture depends very much on the type of culture media used.

- Each plant tissue culture medium must contain the following essential components to support invitro plant growth.
- These are as follows.
 - 1) Macro inorganic nutrients.
 - 2) Micro inorganic nutrients
 - 3) Iron (as chelating agent)
 - 4) Vitamins
 - 5) Carbon sources
 - 6) Organic nitrogen
 - 7) Plant growth regulators
 - 8) Agar (as gelling substance)

Nutrients

Inorganic nutrients

macro nutrients

micro nutrients

Organic nutrients

1. vitamins
2. hexitols
3. amino acids
4. carbohydrate

Nitrogen

Phosphorus

1. Macro nutrients

- Need of macro nutrients is higher.
- It is present in milli molar (mM) quantities (more than 30 ppm/l or mg/l)
- Macro nutrients provide both anions and cations for the plant cells.

- Macro nutrients are nitrogen (as NO_3 and NH_4), phosphorus (PO_4), potassium (K), sulphur (as SO_4), magnesium (Mg), and calcium (Ca).
- Macro nutrients have structural and functional role in protein synthesis, cell wall synthesis enzyme Co-factors and membrane integrity.

Nitrogen

- In organic form used as amino acids, different organic acids and casein hydrolysate.
- In inorganic form used as Nitrate or ammonia.
- Nitrogen is major component of all plant tissue culture media.
- Nitrogen helps to synthesis complex organic molecule.

Potassium

- K ion is present in high concentration in the cytoplasm (100-200 mM) and in chloroplast(20-200 mM).
- K^+ is essential for maintaining the ion balancing, activation of many enzymes. Maintaining osmotic pressure and osmotic regulation of cells.

Calcium

- Calcium functions with different enzymes as Co-factor and bound to the cell wall and cell membrane.
- It gives strength to cell wall.
- It helps in the regulation of the the cell membrane structure.
- Deficiency causes disintegration of the membrane and shoot tip necrosis.
- Important in cell and root multiplication.
- Supplied as calcium chloride and calcium nitrate.

Phosphorus

- Phosphorus
- Very important for energy metabolism.
- Essential element for DNA & RNA.
- Deficiency may cause delayed growth and dark green colour of leaves.
- Supplied as sodium hydrogen phosphate or potassium hydrogen phosphate.

Magnesium

- Essential for enzymatic reactions, energy metabolism(ATP synthesis).
- Supplied as magnesium sulphate.

- Sulphur
- Important substance.
- Deficiency of Sulphur inhibits protein synthesis and decreases Chlorophyll in leaves.
- Supplied as magnesium Sulphate and Potassium Sulphate.

- **Micro Nutrients**

- Boron(B),

Manganese(Mn), Zinc(Zn), Molybdenum(Mo), Copper(Cu), Cobalt(Co).

- Used in less amount less than 30ppm.(mg/l).

- Concentration is always in uM.

- Zinc
- Zn plays an active role in protein synthesis and in the synthesis of tryptophan.
- Supplied as Zinc Sulphate.

Manganes

- Plays an important role in the Hill reaction of photosynthesis.
- Required in many enzymatic activities.
- Supplied as Manganese Sulphate.

Copper

- Copper plays important role in photosynthesis.
- Intermediate of the electron transport chain between photo system 1 & 2
- Deficiency leads to decrease in photosynthesis.
- Supplied as Copper Sulphate.

Molybdenum

- Essential for conversion of Nitrate to Ammonium.
- Supplied as Sodium molybdate.

Boron

- Involves in different enzymatic activities.
- Supplied as Boric acid.

Iron

- Important Enzyme Co-factor
- Supplied in μM quantities.
- It is supplemented with chelators and Complex compounds due to its solubility problem.
- Supplied as Na_2FeEDTA
- Iron deficiency have severe effects on the growth and development plant cells.

Organic Nutrients

1. Vitamins

- Plant synthesis required vitamins.
- Essential for many biochemical reaction.
- Cultured cell are capable to produde vitamins at some level.
- They require an exogenous supply of different vitamins for optimum growth.
- Most usable vitamins are Thiamine, Pyridoxine nicotinic acid Vitamin B Complex.

Hexitols

- Most tissue culture media have this compound.
- Essential for seed germination, sugar transport, carbohydrate metabolism, membrane structure and cell wall formation.
- Mannitol and sorbitol are hexitols.

Amino Acids

- Glycine is the most common Amino Acid used in different culture media.
- It is not essential but Nitrogen containing Amino Acid enhance growth and plant regeneration.

Carbohydrate

- Cells and tissue requires exogeneous supply of carbohydrates to replace the carbon

which the plant normally fixes from the atmosphere by photosynthesis.

- Supplied by adding sucrose.
- Concentration is 20-30 gm/l.

Gelling Agent

- Agar – Agar
- Agar is a natural product of seaweeds.
- Since 1658 agar-agar is obtain from red algae (Gelidium Gracilaria)
- With water it melts at 100°C and solidify at 45°C

Agarose

- It is highly purified agar prepared from *Gelidium* sp. Of seaweed.
- Agarose melt and gel at temperatures below 30°C and dissolve through boiling.
- Agarose is much more expensive agar-agar

Gelrite or Phytigel

- Gelrite is a naturally derived polymer and produced by the microbial fermentation of a bacterium *Pseudomonas elodea*.
- It is low cost gelling agent.
- 0.1-0.2 % concentration per litre required.

Natural Media

- Endosperm fluid / coconut milk
- Fruit materials
 - Coconut fruit
 - Orange juice, Tomato juice, Banana pulp
 - potato
- Potato extract
- Extracts of malts, yeast
- Malt, Yeast
- Animal extracts
- Fish emulsion
- Protein hydrolysates
- Casein hydrolysate
- Peptone

Plant growth regulators

- A plant hormone can be defined as a small organic molecule that elicits a physiological response at very low conc.
- PGS plays an important role in the phenotype.
- Act as messenger between environment and the genome.

Auxins

- Essential for cell division, cell elongation, cell differentiation, organogenesis and embryogenesis, callus formation
- Natural form auxins are IAA, IBA, PAA
- Synthetic form of auxins are NAA, 2, 4-D.

Cytokinins

- Cytokinins promote cell division, shoot proliferation and influence the cell cycle.
- Embryogenesis and inhibit root formation.
- Synthetic form is 2-ip which is most active cytokinins.
- Natural forms are BAP and kinetin.

Gibberellins

- It promotes stem elongation, bulb corm formation and embryo maturation but can inhibit callus growth and root induction.
- GA3 is most common gibberellins.

Abscisic acid

- It inhibits shoot growth and germination of embryo.
- It is thermostable but light sensitive

Ph of tissue culture media

- Ph is adjusted between 5 & 5.8 before gelling and sterilization with the help of dilute NaOH, KOH or HCL.
- Ph below 5 will not gel properly.
- Ph above 6 may be too hard.

