CULTURE TECHNIQUES
PLANT TISSUE CULTURE MEDIA

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Learning objectives:

You will be able to understand

- Composition of plant tissue culture media
- Basic media used in Plant tissue Culture
- MS media
- Preparation of stock solutions
- Directions for making media

Selection of right culture medium is important for the success of a plant tissue culture experiment.

Basic culture medium were developed based on the nutritional requirements of plants growing in soil.

Types of media

Based on the consistency, culture media are three types;

1. Liquid media- water based media, do not have agar , also called broth
2. Semi solid media- Contain low % of agar (<1%)
3. Solid media- high % of agar (1.5-2.0%)

Synthetic or Natural Media

- When a medium is composed of chemically defined components, it is referred to as synthetic or defined medium.
- If a medium contains chemically undefined components(eg. Vegetable extracts, fruit juice, plant extract), it is regarded as natural or undefined medium.

Knop's Nutrient Solution

Wilhelm Knop with Julius von Sachs identified Nitrogen, Phosphorus, Sulfur, Potassium, Calcium, Magnesium and Iron as essential elements of plant nutrition.

Sachs and Knop were the first to use standardized nutrient solutions.

The Knop's nutrient solution developed for the growth of higher plants included four salts.

Macronutrient salts	Quantities in solution
	g/L
KNO_3	0.25
$Ca(NO_3)_2$	1.00
$MgSO_4 \cdot 7H_2O$	0.25
KH_2PO_4	0.25

Earliest plant tissue culture media

Callus culture medium of Gautheret (1939) and root culture medium of White (1943), were based on Knop's (1865) salt solution and Uspenski and Uspenskaia medium (1925) for algae, respectively.

Components of Plant Tissue Culture Media:

1. Macronutrients
2. Micronutrients
3. Vitamins
4. Amino acids or nitrogen supplements
5. Carbon source
6. Growth regulators
7. Activated charcoal
8. Antibiotics
9. Solidifying/ gelling agents

Essential elements are needed for plant growth, development, or reproduction.

Essential mineral elements are usually classified as macronutrients or micronutrients according to their relative concentrations in plant tissue.

1. Macronutrients : C ,H, O , N, P, K, Ca, Mg and S

Elements required in concentrations greater than 0.5mM / L

N- Constituent of amino acids, proteins, nucleic acids and coenzymes.

P- component of sugar phosphates, nucleic acids, coenzymes, phospholipids and has a key role in reactions that involve ATP.

K- required as cofactor for more than 40 enzymes, important in establishing cell turgor.

1. Macronutrients

Ca- constituent of middle lamella of cell walls, cofactor for some enzymes, required for hydrolysis of ATP and phospholipids, acts as second messenger in metabolic regulation.

Mg- Cofactor for many enzymes involved in phosphate transfer, constituent of chlorophyll molecule.

S- Component of cysteine, cystine and methionine amino acids, constituent of lipoic acid,coenzyme A, thiamine pyrophosphate, glutathione, biotin-5-adenylyl sulphate and 3-phospho adenosine.

2. Micronutrients : Fe, Mn, Zn, B, Cu and Mo

Elements required in concentrations less than 0.05 mM/L

Fe-constituent of cytochromes and non heme iron proteins involved in photosynthesis, N- fixation and respiration.

Mn- required for activity of some dehydrogenases, decarboxylases, kinases, oxidases and peroxidases. Involved with other cation activated enzymes and photosynthetic O_2 evolution.

Zn- constituent of alcohol dehydrogenase, glutamic dehydrogenase and carbonic anhydrase etc.

B- Complexes with mannitol, mannan and poly mannuronic acid and other constituents of cell walls. Involved in cell elongation and nucleic acid metabolism.

Cu- Component of ascorbic acid oxidase, tyrosinase, monoamine oxidase, uricase, cytochrome oxidase, phenolase, laccase and plastocyanin.

Mo- Constituent of nitrogenase, nitrate reductase and xanthine dehydrogenase.

- Plants assimilate **N & S** and create organic compounds (e.g., amino acids, nucleic acids, and proteins).
- **P, Si and B** are important in energy storage reactions or in maintaining structural integrity.
- **K, Ca, Mg, Cl, Zn & Na** have important roles as enzyme cofactors, in the regulation of osmotic potentials, and in controlling membrane permeability.
- **Fe, Mn, Cu, Ni & Mo** are involved in redox reactions.

3. Vitamins :

Thiamine (B1), Nicotinic acid and Pyridoxine (B6)

4. Amino acids :

Amino acid mixture such as Casein hydrolysate,L glycine, L glutamine, L asparagine, L arginine, L cysteine and L tyrosine

5. Carbon and Energy source

Plant cells and tissues in the culture medium lack autotrophic ability and need external carbon source for energy.

Sucrose **(**2-5%) is the most preferred carbon source in plant tissue culture.

Sucrose enhances proliferation of cells and regeneration of green shoots. Sucrose acts as morphogenetic trigger in the formation of axillary buds and branching of adventitious roots.

6. Growth regulators :

- Proportions of auxins and cytokinins determine the type and extent of organogenesis in plant cell cultures.
- High cytokinin to auxin ratio promotes shoot differentiation.

- Low cytokinin to auxin ratio promotes root differentiation.

Auxins

An auxin (IAA, NAA, 2,4-D, or IBA) is required by most plant cells for division and root initiation.

High concentrations of auxin can suppress morphogenesis.

2,4-D is widely used for callus induction.

IAA, IBA, and NAA are used for root induction.

Auxins

Preparation of auxin stocks- 10 mg of auxin + several drops of 1 N NaOH or KOH until the crystals are dissolved (not more than 0.3 ml), add 90 ml of double-distilled water, and make the volume to 100 ml.

Make IAA stocks fresh weekly; IAA is degraded within a few days by light .

Auxins are thermostable at 110–120°C for up to 1 h.

NAA and 2,4-D, which are synthetic auxins, are more stable than IAA, which is the naturally occurring auxin.

Cytokinins

Cytokinins (kinetin, BA, zeatin, and 2iP) promote cell division, shoot proliferation, and shoot morphogenesis.

Thidiazuron (TDZ; N-phenyl-N1-1,2,3-thiadiazol-5-yl urea) has cytokinin activity effective in low concentrations to stimulate shoot formation.

Cytokinins

Preparation of Cytokinin stocks-

10 mg of cytokinin + drops of 1 N HCl and few drops of water to dissolve the crystals , add 90 ml of double-distilled water, and make the volume to 100 ml. Gentle heating is usually required to completely dissolve crystals.

Cytokinin stocks can be stored for several months in the refrigerator.

Cytokinins (kinetin and zeatin) are thermostable.

Gibberellins

Gibberellins can inhibit callus growth and auxin-induced adventitious root formation, therefore not much used in plant cell culture .

GA is useful in studies on morphogenesis.

Preparation Gibberellin stocks-

Dissolve 10 mg GA3 crystals in 100 ml water and adjust the pH to 5.7.

Solutions of GA3 are not thermostable, and should be made up fresh before addition to the medium by filter sterilization.

Abscisic acid (ABA)

Abscisic acid (ABA), is useful in embryo culture.

Abscisic acid is heat stable but light sensitive.

Stock solutions can be prepared in water.

7. Activated Charcoal(AC)

Ac is generally acid washed and neutralised before its addition in concentrations of 0.5- 3% to the culture medium.

Activated charcoal helps to reduce toxicity by removing toxic compounds (eg. Phenols) produced during the culture and permits unhindered cell growth.

8. Antibiotics/ Antifungal

Sometimes media is supplemented with low concentration of antibiotics (eg. Streptomycin, Kanamycin) to control systemic infection of microorganisms in plant cells. Benomyl is a fungicide used to control fungal infection.

9. SOLIDIFYING/ GELLING AGENT :

Solidifying agents support the tissues growing in static conditions.

Agar is the most commonly used gelling agent obtained from red algae .

Other forms of agar used as gelling agents are – agarose, phytoagar, flow agar etc.

Other gelling agents used are-

Alginate -for plant protoplast culture
Gelrite
Synthetic polymer biogel P200 (polyacrylamide pellets)
Agargel , a mixture of agar and synthetic gel

pH of the medium

Optimum pH for most tissue culture medium is set in the Range of 5.0-6.0.

pH above 6.0 makes the medium hard and pH below 5.0 does not allow gelling of the medium

Basic Media

Basic media that are frequently used in plant tissue culture are -

- To induce organogenesis and regeneration of plants in cultured tissues Murashige and Skoog (MS) media,1962 and Linsmaier and Skoog (LS) media contain the desired salt composition and are widely used.

- Gamborg (B5) medium,1968 originally designed for cell suspension or callus cultures, with modifications proved valuable for protoplast culture and regeneration of protoplast –derived plants.

- White”s (W) medium, 1953 was developed for root culture.
- Nitsch and Nitsch (NN) medium,1969 is frequently used for anther culture.
- N6 media was formulated by Chu, 1978 and is used for cereal anther culture.

Components	Amount (mg l^{-1})				
	White's	Murashige and Skoog (MS)	Gamborg (B5)	Chu(N6)	Nitsch's
Macronutrients					
$MgSO_4.7H_2O$	750	370	250	185	185
KH_2PO_4	—	170	—	400	68
$NaH_2PO_4.H_2O$	19	—	150	—	—
KNO_3	80	1900	2500	2830	950
NH_4NO_3	—	1650	—	—	720
$CaCl_2.2H_2O$	—	440	150	166	—
$(NH_4)_2.SO_4$	—	—	134	463	—
Micronutrients					
H_3BO_3	1.5	6.2	3	1.6	—
$MnSO_4.4H_2O$	5	22.3	—	4.4	25
$MnSO_4.H_2O$	—	—	10	3.3	—
$ZnSO_4.7H_2O$	3	8.6	2	1.5	10
$Na_2MoO_4.2H_2O$	—	0.25	0.25	—	0.25
$CuSO_4.5H_2O$	0.01	0.025	0.025	—	0.025
$CoCl_2.6H_2O$	—	0.025	0.025	—	0.025
KI	0.75	0.83	0.75	0.8	—
$FeSO_4.7H_2O$	—	27.8	—	27.8	27.8
$Na_2EDTA.2H_2O$	—	37.3	—	37.3	37.3
Sucrose (g)	20	30	20	50	20
Organic supplements					
Vitamins					
Thlamine HCl	0.01	0.5	10	1	0.5
Pyridoxine (HCl)	0.01	0.5	1	0.5	0.5
Nicotinic acid	0.05	0.5	1	0.5	5
Myoinositol	—	100	100	—	100
Others					
Glycine	3	2	—	—	2
Folic acid	—	—	—	—	0.5
Biotin	—	—	—	—	0.05
pH	5.8	5.8	5.5	5.8	5.8

Murashige and Skoog Medium

Murashige and Skoog Medium (MS) was originally formulated for tobacco callus culture.

It is widely used for micro propagation, organ culture, callus culture and suspension culture.

MS medium is a nutrient blend of inorganic salts, vitamins and amino acid.

Murashige and Skoog Medium (MS) provides all the essential macroelements and microelements.

Preparation of Stock Solution

To measure each component of a basal medium is time consuming, therefore, concentrated solutions of the desired composition of a medium are used.

Stock solution of MS basal medium

Constituent	Concentration in MS medium (mg/l)	Concentration in the stock solution (mg/l)	Volume to be taken/litre of medium
Macronutrients (10x) Stock solution I			
NH_4NO_3	1650	16500	100 ml
KNO_3	1900	19000	
$MgSO_4 . 7H_2O$	370	3700	
KH_2PO_4	170	1700	
Macronutrient (10x) Stock solution II			
$CaCl_2\ 2H_2O$	440	4400	100 ml
Micronutrients (100x) Stock solution III			
H_3BO_3	6.2	620	10 ml
$MnSO_4 . 4H_2O$	22.3	2230	
$ZnSO_4 . 7H_2O$	8.6	860	
KI	0.83	83	
$Na_2MoO_4.2H_2O$	0.25	25	
$CuSO_45H_2O$	0.025	2.5	
$CoCl_2 . 6H_2O$	0.025	2.5	
Iron source			
Fe EDTA Na salt	40	Added fresh	
Vitamins			
Nicotinic acid	0.5	50 mg/100 ml	1 ml
Thiamine HCl	0.1	50 mg/100 ml	0.2 ml
Pyridoxine HCl	0.5	50 mg/100 ml	1 ml
Myo-inositol	100	Added fresh	
Others			
Glycine	2.0	50 mg/100 ml	4 ml
Sucrose	30,000	Added fresh	
Agar	8000	Added fresh	
pH 5.8			

Preparation of Stock solution of Growth Regulators

To prepare 1 mg/ml stock solution, add 100 mg of plant growth regulator to a 100 ml volumetric flask. Add 2-5 ml of solvent to dissolve the powder. Add double distilled water to make up the volume 100ml.

Add 1.0 ml of the stock solution to 1 L of the medium to obtain a final concentration of 1.0 mg/L of the plant growth regulator in the culture medium.

volume of stock solution = (desired hormone conc. X medium volume)

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stock solution conc.

Directions for making media :

- Stock solutions are measured as directed in the basal media.
- Agar is separately dissolved in distilled water by heating on a water bath.
- Mix all the ingredients and agar and make up the final volume with distilled water.
- Adjust the pH of the medium to 5.75 ± 0.5 using 0.1N NaOH/ 0.1 N HCl.
- Dispense the medium in sterile flask or test tube (only one third volume should be filled). Plug the flask/ tube with cotton plugs.
- Sterilize the medium by autoclaving at 15 psi and 121°C for 15-20 min.

- Cool the autoclaved medium to about 45°C before adding heat labile supplements.

- Vitamins and growth regulators are sterilised by filtering through a microfilter of pore size 0.22-0.45 µm and added in the autoclaved media under laminar air flow cabinet (sterile conditions).

Some plant tissue culture media suppliers are :

HiMedia

Sigma-Aldrich

TM media

Source: Amazon.com

https://www.sigmaaldrich.com/US/en/product/sigma/m5519

Let's revise

Q.1 What do you understand by synthetic and natural media?

Q.2 What are the main constituents of plant tissue culture media?

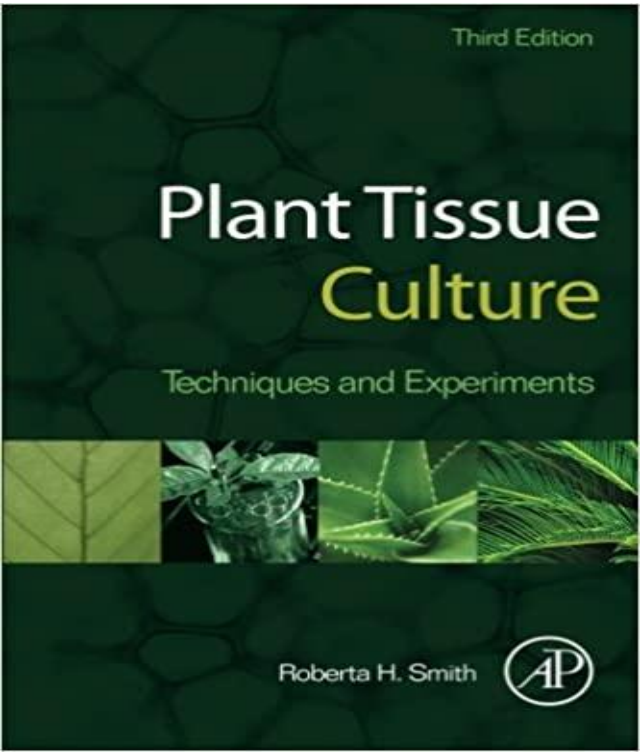
Q.3 Why plant cultures need a carbon source in the medium?

Q.4 Discuss the role of auxin and cytokinin in organogenesis.

Q.4 Name some basal media used for plant tissue culture.

Q.5 Write a note on MS medium.

References: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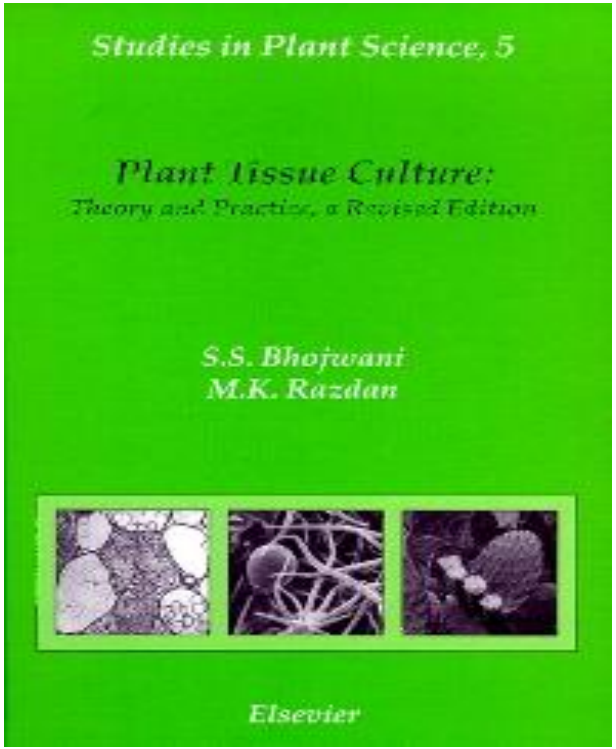
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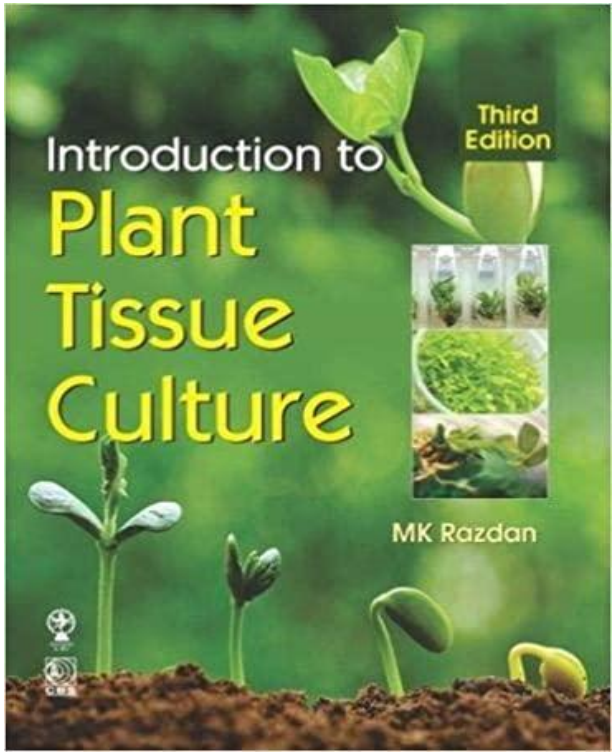
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