PLANT TISSUE CULTURE MEDIA

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LEARNING OUTCOMES:

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

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.

Components of Plant Tissue Culture Media:

Principal components of plant tissue culture media are:

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 element 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 : Elements required in concentrations greater than 0.5mM / L

C ,H, O , N, P, K, Ca, Mg and S

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.

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 : Elements required in concentrations less than 0.05 mM/L

Fe, Mn, Zn, B, Cu and Mo

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.

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

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

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					
Thiamine 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 (MS) was originally formulated by Murashige and Skoog in 1962.

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

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	
Kl	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			

Solubility of different growth regulators

Name	Chemical formula	Molecular weight	Solubility
p-Chlorophenoxy acetic acid	$C_8H_7O_3Cl$	186.6	96% ethanol
2,4-Dichlorophenoxy acetic acid	$C_8H_6O_3Cl$	221.0	96% ethanol, heated lightly
Indole-3 acetic acid	$C_{10}H_9NO_2$	175.2	1N NaOH/96% ethanol
Indole-3 butyric acid	$C_{12}H_{13}NO_2$	203.2	1N NaOH/96% ethanol
α-Naphthalene acetic acid	$C_{12}H_{10}O_2$	186.2	1N NaOH/96% ethanol
β-Naphthoxy acetic acid	$C_{12}H_{10}O_3$	202.3	1N NaOH
Adenine	$C_5H_5N_5.3H_2O$	189.1	H_2O
Adenine sulphate	$(C_5H_5N_5)_2.H_2SO_4.2H_2O$	404.4	H_2O
Benzyl adenine 6 benzyl amino purine	$C_{12}H_{11}N_5$	225.2	1N NaOH
N-isopentenyladenine (2 iP)	$C_{10}H_{13}N_5$	203.3	1N NaOH
Kinetic	$C_{10}H_9N_5O$	215.2	1N NaOH
Zeatin	$C_{10}H_{13}N_5O$	219.2	1N NaOH/1N HCl, heated lightly
Gibberellic acid	$C_{19}H_{22}O_6$	346.4	Ethanol
Abscisic acid	$C_{15}H_20O_4$	264.3	1N NaOH
Colchicine	$C_{22}H_{25}NO_6$	399.4	H_2O

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.

Requirements for media preparation

Measuring cylinders and jars

Pipettes

Culture Vessel with Cotton plug

Storage bottles

Amber bottle

Millipore filter

Fridge

Ove
n

Electronic balance

Heating mantle

pH meter

Autoclave

Laminar air flow cabinet

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.

- pH of solutions of vitamins and growth regulators is adjusted using 0.1N NaOH or 0.1 N HCl.

- 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

Let’s revise

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

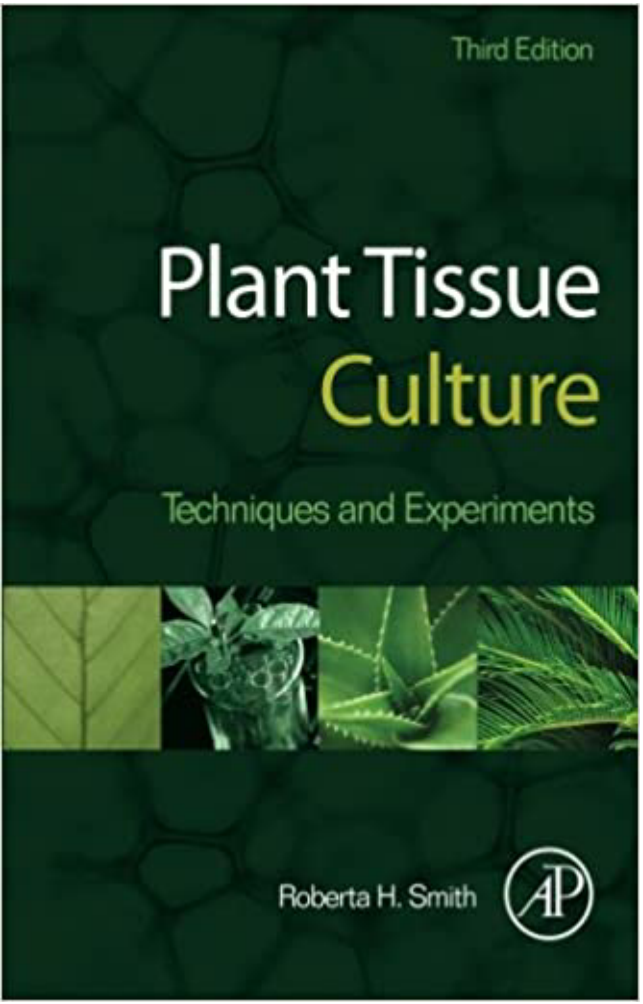
Q.2 What are the main constituents of plant tissue culture media?

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.

References: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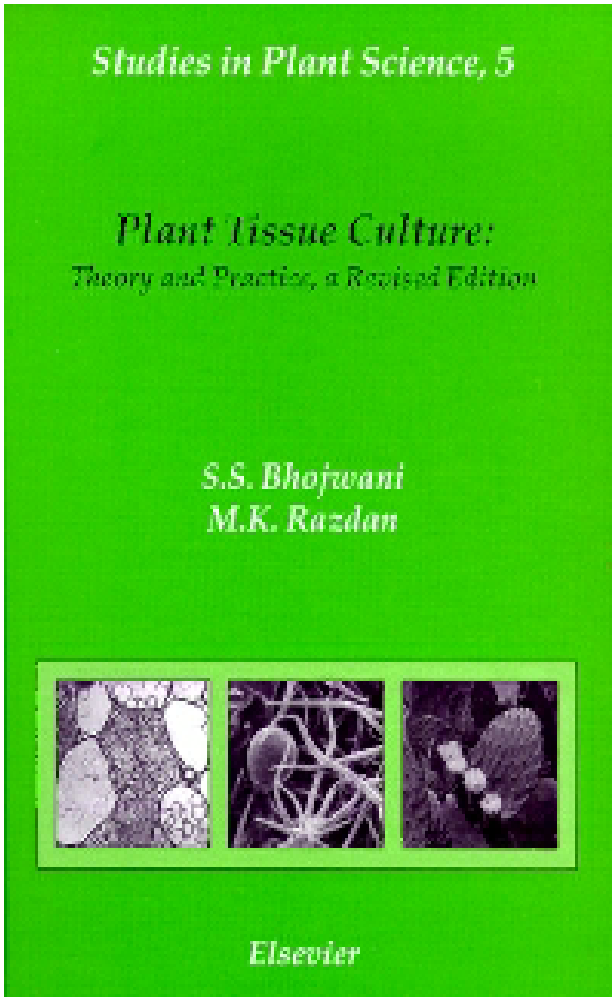
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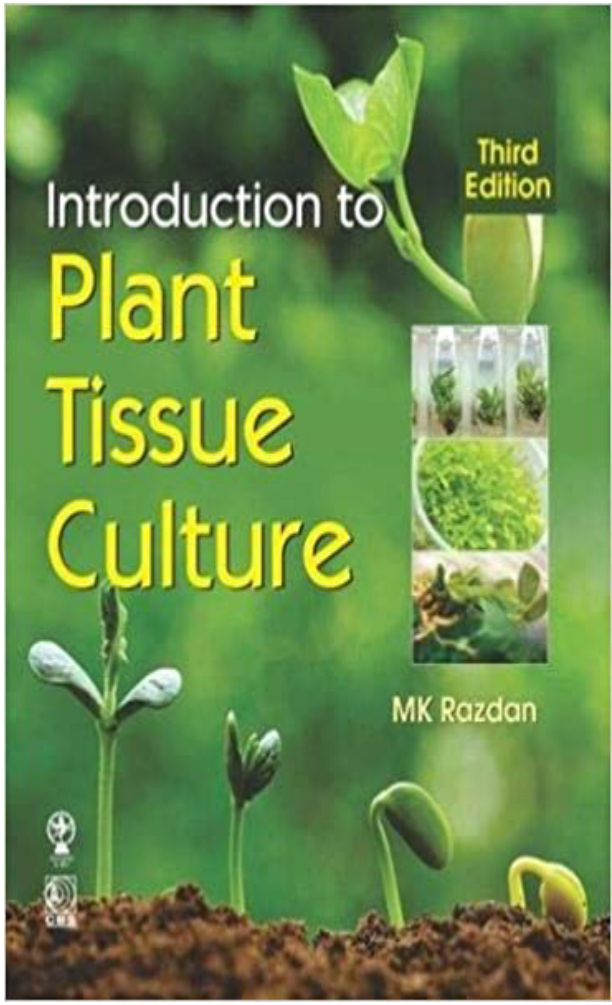
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Thank you all

Any questions ??

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