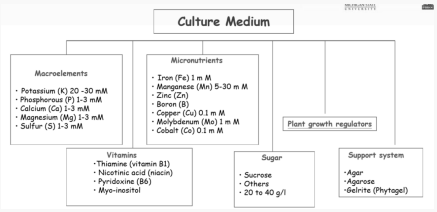
Culture Medium Components



Media for Plant Cultures

1. Macronutrients (mM concentrations)	N, P, K, Ca, Mg, S
2. Micronutrients (µm concentrations)	Fe, B, Cu, Mn, Zn, Mo, I, Co
3. Carbon Source	sucrose, glucose, other sugar
4. Vitamins	thiamine, biotin, pantothenic acid, nicotinic acid, pyridoxine, folic acid, ascorbic acid, tocopherol, myo-inositol
5. Complex Organic Supplements	coconut water, banana powder, yeast extract, peptone, potato homogenate

Macronutrients

Table 1: Some of the essential elements important for plant nutrition and their physiological function. These elements have to supplied by the culture medium in order to support the growth of healthy cultures *in vitro*

Element	Function
Nitrogen	Component of proteins, nucleic acids and some coenzymes Element required in greatest amount
Potassium	Regulates osmotic potential, principal inorganic cation
Calcium	Cell wall synthesis, membrane function, cell signalling
Magnesium	Enzyme cofactor, component of chlorophyll
Phosphorus	Component of nucleic acids, energy transfer, component of intermediates in respiration and photosynthesis
Sulphur	Component of some amino acids (methionine, cysteine) and some cofactors

Micronutrients

Table2: Micronutrients required in trace amount

Element	Function
Chlorine	Required for photosynthesis
Iron	Electron transfer as a component of cytochromes Required in the construction of chlorophyll
Manganese	Enzyme cofactor
Cobalt	Component of some vitamins
Copper	Enzyme cofactor, electron-transfer reactions
Zinc	Enzyme cofactor, chlorophyll biosynthesis
Molybdenum	Enzyme cofactor, component of nitrate reductase

Carbon and Energy Source

Every living organism needs to have a source of energy in order to complete all the vital processes within the organism, and therefore each medium needs sugars as a source of carbon and energy.

The preferred carbohydrate in plant cell culture media is sucrose.

Vitamins

Vitamins work as an assistant in enzymatic systems.

They are required in very small amounts.

Thiamine (B1), is more commonly used in plant tissue cultures and other vitamins such as nicotinic acid, pyridoxine (B6) etc.

Plant Hormones / Growth Regulators

-involved in the regulation of growth and organized organ development of plant tissues directly or indirectly

-interactions of auxin and cytokinin are considered to be the most important regulation to induce organ development in the cultured tissues

-requirement of hormones (gibberellin, abscisic acid, ethylene) which will help to induce the developmental response in cultures depends on the type of explant and species to be cultured

-addition of growth regulators in the culture media also depends on the goal of the culturing process

-i.e. gibberellin, ethylene, and abscisic acid are not required for organ development or cell proliferation in culture

Five Classes of Plant Hormones/Growth Regulators

1. Auxin
2. Cytokinin
3. Gibberellin
4. Ethylene
5. Abscisic acid

1. Auxins

-Naturally occurring: indole-3-acetic acid (IAA)

-Synthetic: 2,4-dichlorophenoxyacetic acid (2,4-D), 1-naphthaleneacetic acid (NAA), idole-3-butyric acid (IBA)

-Functions:
a) Cell division and differentiation (with cytokinin)
b) Shoot and root apical dominance
c) Parthenocarpy in some species
d) Abscission of fruits in other species
e) At high concentration will kill plant as herbicide

1. Auxins (cont)

*Parthenocarpy is the natural or artificially induced production of fruit without fertilization of ovules, which makes the fruit seedless

*Abscission is the shedding of various parts of an organism, such as a plant dropping a leaf, fruit, flower

2. Cytokinin

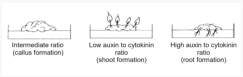
-Naturally occurring: zeatin

-Synthetic: kinetin, benzylaminopurine (BAP) and adenine

-Synthesis from adenine in root tips, embryos, young fruits, leaves in all plants

-Functions:
a) Growth and development (in combination with auxin)
b) Delay senescence (Plant senescence is the process of aging in plants)
c) Break apical dominance

Collaboration of Auxin and Cytokinin



3. Gibberellins

-Naturally occurring: gibberellic acid-3 (GA3), GA4, GA7 (more than 90 different GA recognized)

-Synthesized from mevalonate shoot and root apices, embryos, cotyledons, fruits, tubers

- Function:
a) stem elongation and flowering
b) effects on seed germination (breaking seed dormancy)
c) promotes cell division in combination with IAA
d) Improves fruit set, fruit growth, fruit maturation and fruit ripening

In tissue culture, GA's (gibberellic acids) are supplemented in some operations and avoided in others.

-For example, the presence of GA's in media can inhibit organ development (root and shoot formation) and somatic embryogenesis.

-However, gibberellic acids are necessary to induce normal callus growth. Similarly, gibberellic acids can inhibit the meristemoid initiation; the catch is the meristemoid initiation is required for the growth and development of organs that are already formed.

4. Ethylene

-naturally occurring gaseous hormone that plays a role in fruit ripening, senescence, and leaf abscission

- Function:
a) wound responses
b) causes thickening of stems and leaf abscission (aging)
c) reduces adventitious shoot formation
d) control fruit ripening in climacteric fruit
e) inhibits the growth and development of the plants in the culture at a higher concentration; but enhances the responses of plants towards auxin at lower concentrations

*Silver nitrate (AgNO3) has anti-ethylene activity

5. Abscisic acid

-maintains bud and seed dormancy, inhibits cell wall acidification and slows cell elongation

-promotes somatic embryogenesis at a lower concentration; but halts the developmental response of cultures at a higher concentration

-Used in agriculture where seed dormancy is important

-Function:
a) water stress response
b) seed protein synthesis
c) seed dormancy
d) seed germination (in combination with gibberellins)
e) enhance somatic embryogenesis

Support Matrices

1. Agar

-commonly used gelling agent in plant tissue culture

-mixture of polysaccharides derived from red algae

-Agarose is often used when the impurities in agar are not desired, such as in protoplast and anther culture

- contain:
a) 70% agarose (gelling component) (polymer of alternating D-galactose and 3,6anhydrogalactose)
b) 30% agaropectin (non-gelling fraction, polymer of sulphated D-galactose units)

2. Gellan Gums

-gelling agent used for plant tissue and cell culture

Support Matrices (cont)

-produces a high transparent gel, which allows better observation (inspection of contamination) of root growth compared with conventional agar gel

-polymers from glucose, glucoronic acid and rhamnose units

-not only easier for root inspection, but allow better root growth than agar

Antibiotics

-plants are sensitive to many antibiotics

-use of antibiotics to prevent contamination is uncommon in plant tissue culture

1. Cefotaxime	kill Agrobacterium but not the plant cell
2. Kanamycin	kill plant cell
	-Transgenic plant carrying the kanamycin selectable marker would survive on medium containing kanamycin
	-Non transgenic plant would not survive Plant Tissue Culture (PTC)

Step-By-Step Guide to Prepare Medium

Pre-Preparation

1. Prepare 1 L of MS agar medium with 1 mg/L 2,4-D	a) Prepare all the stock solutions
	b) Measure 800 mL of deionised water into a 2 L beaker
	c) Put in a magnetic stirrer and start stirring
	d) Add 50 mL of MS macro, 5 mL of MS micro, MS ferum and MS vitamin
	e) Weigh 30 g of sucrose
	f) Add into the mixture, stir until dissolve
	g) Add 200 µL of 2,4-D
	h) Adjust pH to 5.5
	i) Top up medium to 1 L
2. Weigh 5 g of agar	1a) Autoclave, then pour medium
	1b) Pour medium into a 2 L bottle.

Step-By-Step Guide to Prepare Medium (cont)

	1c) Add agar into medium in the bottle. Swirl to mix
	1d) Autoclave medium. Swirl after autoclaving
	1e) Allow medium to cool
	1f) Pour medium into containers in a laminar air flow cabinet
	2) Dispense, then autoclave
	2a) Add agar into medium in the 2 L beaker
	2b) Dissolve agar in a microwave oven
	2c) Dispense medium into containers
	2d) Autoclave all the containers with medium
	2e) Allow to cool
3. Allow medium to cool and incubate for at least 3 days to 5 days before use.	-if contaminants present, it would appear during this period