FACULTY OF MEDICAL SCIENCES

NUTRIENT MEDIA

Culture media are largely responsible for the in vitro growth and morphogenesis of plant tissues.

The success of the plant tissue culture depends on the choice of the nutrient medium. In fact, the cells of most plant cells can be grown in culture media

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FUNCTIONS OF NUTRIENT MEDIA

- Provide water
- Provide mineral nutritional needs
- Provide vitamins
- Provide growth regulators
- Access to atmosphere for gas exchange
- Removal of plant metabolite waste

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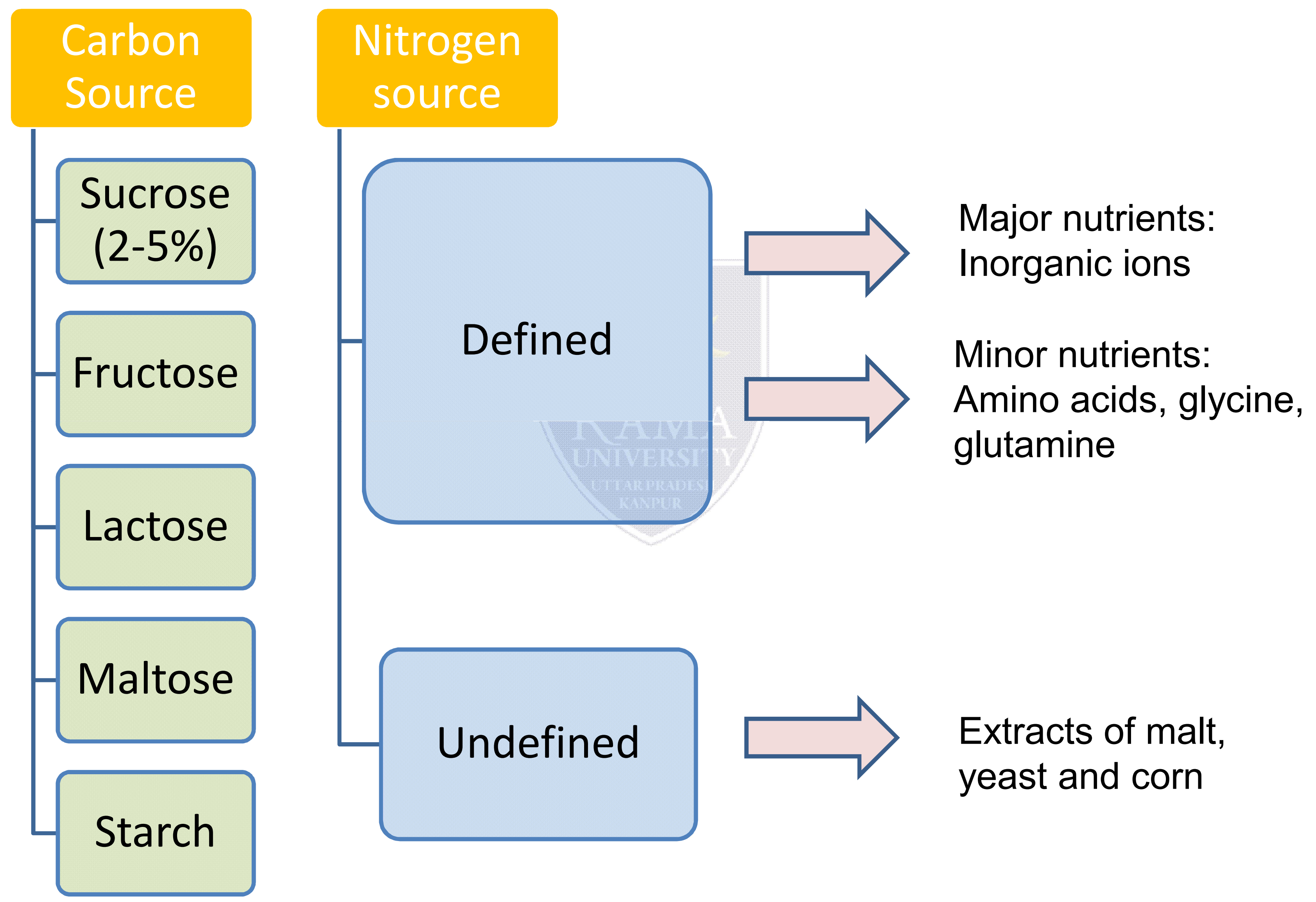
SOURCES OF ENERGY



Composition of Media:

The composition of the culture media is primarily dependent on two parameters:

1. The particular species of the plant.
2. The type of material used for culture i.e. cells, tissues, organs, protoplasts.

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Thus, the composition of a medium is formulated Considering the specific requirements of a given culture system. The media used may be solid (solid medium) or liquid (liquid medium) in nature. The selection of solid or liquid medium is dependent on the better response of a culture.

MAJOR TYPES OF MEDIA

White's medium - is one of the earliest plant tissue culture media –

MS medium - formulated by Murashige and Skoog (MS) is most widely used for many types of culture systems
Murashige and Skoog (MS) originally formulated a medium
to induce organogenesis, and regeneration of plants in cultured tissues. These days, MS medium is widely used for
many types of culture systems.

B5 medium - developed by Gamborg for cell suspension and callus cultures and at present it's modified form used for protoplast culture
Developed by Gamborg, B5 medium was originally designed
for cell suspension and callus cultures. At present with
certain modifications, this medium is used for protoplast
culture.

N6 medium - formulated by Chu and used for cereal anther culture

Nitsch's medium developed by Nitsch and Nitsch and used for anther culture

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Synthetic and natural media:

When a medium is composed of chemically defined components, it is referred to as a synthetic medium. On the other hand, if a medium contains chemically undefined compounds (e.g., vegetable extract, fruit juice, plant extract), it is regarded as a natural medium. Synthetic media have almost replaced the natural media for tissue culture.

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NUTRIENT MEDIA

Plant Tissue Culture refers to the technique of growing plant cells, tissues, organs, seeds or other plant parts in a sterile environment on a nutrient medium.

Culture media used for in vitro cultivation of plant cells are composed of following basic components:

1. Complex mixture of salts: Essential elements, or mineral ions.
2. Organic supplement: vitamins and/or amino acids.
3. Carbon source: usually sugar sucrose.
4. Gelling agents
5. Plant Growth Regulators
6. Antibiotics

1. Complex mixture of salts:

These include essential elements or mineral ions important for plant nutrition and their physiological function.

The essential elements can further be divided into the following categories:

a. Macroelements (or macronutrients)
b. Microelements (or micronutrients)
c. Iron Source

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Macroelements:

Required in large amounts for plant growth and development.
For e.g.: Nitrogen, phosphorus, potassium, magnesium, calcium and sulphur

Microelements:

Required in trace amounts for plant growth and development.
For e.g.: Manganese, iodine, copper, cobalt, boron, molybdenum, iron and zinc are regarded as microelements, although other elements like aluminium and nickel are frequently found in some formulations.

Iron Source:

Iron is usually added in the medium as iron sulphate,
Ethylenediaminetetraacetic acid (EDTA) is usually used in conjunction with the iron sulphate.

2. Organic supplements:

These include vitamins and amino acids. Two vitamins, i.e., thiamine (vitamin B1) and myoinositol (a vitamin B) are essential for the culture of plant cells in vitro. The most commonly used amino acid is glycine.

3. Carbon source:

The most commonly used carbon source is sucrose. It is readily assimilated and relatively stable. Other carbohydrates like glucose, maltose, galactose and sorbitol can also be used and may prove better than sucrose in specialized circumstances.

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4. Gelling agents:

It can be used in either liquid or 'solid' forms, depending on the type of culture being grown.

Purified agar or agarose can be used, as can a variety of gellan gums.

Using Agar as a gelling agent main advantage is that agar does not react with any components of the medium and is not digested by enzymes from the plant tissue. If necessary, agar can be washed to remove inhibitory impurities.

Agarose is a purified extract of agar used for more demanding procedures like culturing protoplasts.

Gellan gum, used primarily as a gelling agent, in microbiological culture is able to withstand 120 °C heat, making it especially useful in culturing thermophilic organisms.

5. Plant growth regulators:

Plant growth regulators are the critical media components in determining the developmental pathway of the plant cells.

There are five main classes of plant growth regulator used in plant cell culture, namely:

a. Auxins

b. Cytokinins

c. Gibberellins

d. Abscisic acid

e. Ethylene

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Activated charcoal:

Supplementation of the medium with activated charcoal stimulates the growth and differentiation of certain plant cells (carrot, tomato, orchids).

Some toxic/inhibitory compounds (e.g. phenols) produced by cultured plants are removed (by adsorption) by activated charcoal, and this facilitates efficient cell growth in cultures.

Addition of activated charcoal to certain cultures (tobacco, soybean) is found to be inhibitory, probably due to adsorption of growth stimulants such as phytohormones.

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6. Antibiotics:

Antibiotics are substances produced by certain microorganisms that suppress the growth of other microorganisms and eventually destroy them.

Their applications include:

a. Suppresses bacterial infections in plant cell and tissue culture.
b. Suppresses mould and yeast infections in cell cultures.
c. Eliminates Agrobacterium species after the transformation of plant tissue.
d. As a selective agent in plant transformation experiments.

TABLE : Composition of commonly used plant tissue culture media

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

Element	Function
Nitrogen (N)	Component of proteins, nucleic acids and some coenzymes Element required in greatest amount
Potassium (P)	Regulates osmotic potential, principal inorganic cation
Calcium (Ca)	Cell wall synthesis, membrane function, cell signalling
Magnesium (Mg)	Enzyme cofactor, component of chlorophyll
Phosphorus (P)	Component of nucleic acids, energy transfer, component of intermediates in respiration and photosynthesis
Sulphur (S)	Component of some amino acids (methionine, cysteine) and some cofactors
Chlorine (Cl)	Required for photosynthesis
Iron (Fe)	Electron transfer as a component of cytochromes
Manganese (Mn)	Enzyme cofactor
Cobalt (Co)	Component of some vitamins
Copper (Cu)	Enzyme cofactor, electron-transfer reactions
Zinc (Zn)	Enzyme cofactor, chlorophyll biosynthesis
Molybdenum (Mo)	Enzyme cofactor, component of nitrate reductase

https://www.slideshare.net/SudheerKandibanda/planttissueculture-56810101

Murashige & Skoog Medium (MS) APM1005/APM5005

Use

Murashige & Skoog Medium (MS) is used for micropropagation, organ culture, callus culture and cell suspension culture..

Summary

Murashige & Skoog Medium (MS) is established by Murashige & Skoog (1962) for in vitro callus culture of Nicotiana tabacum (family- Solanaceae).

Principle

Murashige & Skoog Medium (MS) provides all essential Macroelements, Microelements, & Vitamins for the growth of plant cell, tissue and organ culture in vitro. Medium with high concentration of salts is used for cultivating plant cell, tissue and organ culture.

Macroelements :

In this medium nitrogen is supplied as ammonium and nitrate ions. This mixture of cation and anion balances the pH of the medium. Also plays a important role in plant growth. Potassium dihydrogen phosphate serves as a source of phosphate in medium.

Microelements:

Boron, Managanese, molybdenum, copper, iron and zinc plays a vital catalytic role in plant metabolism. Boron plays a key role in carbohydrate metabolism in plant cells.

Vitamins:

Thiamine, pyridoxin and nicotinic acid content had been increased in the medium which have a stimulatory effect.

Amino acid:

The medium contains increased concentration of glycine.

Formula

Ingredients in mg per liter

Ingredient	mg/L
Macroelements	
Potassium nitrate	1900.00
Ammonium sulphate	1650.00
Calcium chloride anhydrous	332.16
Magnesium sulphate	180..69
Potassium phosphate monobasic	170.00
Microelements	
Manganese sulphate.H_2O	16.90
Boric Acid	6.20
Potassium iodide	0.83
Molybdic acid (sodium salt).$2H_2O$	0.25
Zinc sulphate.$7H_2O$	8.60
Copper sulphate.$5H_2O$	0.03
Cobalt chloride.$6H_2O$	0.03
Ferrous sulphate.$7H_2O$	27.80
Na_2.EDTA	37.30
Vitamins	
Myo-Inositol	100.00
Thiamine HCL	0.10
Pyridoxine HCL	0 .50
Nicotinic acid (Free acid)	0 .50
Amino Acid	
Glycine (Free base)	2.00
Carbohydrate	
Sucrose	30000.00
Buffering Agent	
MES (Free acid)	500.00
Plant Growth Regulators	
6-Benzyl amino purine(BAP)	5.00
Gelling Agent	
Agar	8000.00

Storage

Store at 2-8°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Reference:

Murashige T. and Skoog F., 1962. Physio. Plant., 15, 473-497

BM Medium APM1002/APM5002

USE

BM Media is used for seed culture and micropropagation of orchids.

Summary

Van waes, (1986) has developed BM medium for in vitro cultivation of Protocorms from orchid seeds.

Principle

BM medium provides all essential Macroelements, Microelements, Vitamins, Amino acid & Plant growth regulators for the growth of Orchid in vitro. This medium is especially suitable for terrestrial orchids.

Macroelements :

Potassium dihydrogen phosphate serves as a source of phosphate. This medium lacks in inorganic nitrogen.

Microelements:

Zinc and boron content in the medium is increased to provide proper nourishment to developing protocomes.

Vitamins:

Thiamine content had been increased (0.5mg/l) in the medium. It is a most important element in carbohydrte metabolism and some amino acids biosynthesis. Biotin and folic acid along with other vitamins facilitates in vitro development of Protocorm.

Amino acid:

Glycine and glutamine serve as reduced nitrogen source.

Carbohydrte:

Sucrose serves as a carbon source.

Organic supplements:

Casein hydrolysate used as a supplement, which is a sources of free amino acid.

Plant growth regulators:

6-Benzyl amino purine(BAP) induces shoot proliferation.

Formula

Ingredients in Grams/Litre	
Macroelements	
Potassium nitrate	2830.00
Ammonium sulphate	463.00
Calcium chloride anhydrous	125.33
Magnesium sulphate	90.37
Potassium phosphate monobasic	400.00
Microelements	
Manganese sulphate.H_2O	3.33
Boric Acid	1.60
Potassium iodide	0.80
Zinc sulphate.7H_2O	1.50
Ferrous sulphate.7H_2O	27.80
Na_2.EDTA	37.26
Vitamins	
Thiamine HCL	1.00
Pyridoxine HCL	0.50
Nicotinic acid (Free acid)	0.50
Amino acid	
Glycine (free base)	2.00

Storage

Store at 2-8°C and prepared medium at 2-8°C.

Shelf Life

Use before expiry date as mentioned on the label.

Reference:

Chu C.C., *et. al.*, 1975. Scientia Sinic., 18, 659-668.

Selection of a Suitable Medium:

In order to select a suitable medium for a particular plant culture system, it is customary to start with a known medium (e.g. MS medium, B5 medium) and then develop a new medium with the desired characteristics. Among the constituents of a medium, growth regulators (auxins, cytokinins) are highly variable depending on the culture system.

In practice, 3-5 different concentrations of growth regulators in different combinations are used and the best among them are selected. For the selection of appropriate concentrations of minerals and organic constituents in the medium, similar approach referred above, can be employed.

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PLANT HORMONES

- Naturally occurring organic compounds other than nutrients produced by plants that control or regulate germination, growth, metabolism, or other physiological activities.
- Also called phytohormone and recently called growth bioregulators.
- Relatively low molecular weight.

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- Plant hormones, which are active in very low concentrations, are produced in certain parts of the plants and are usually transported to other parts where they elicit specific biochemical, physiological, or morphological responses.

- They are also active in tissues where they are produced.

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Plant hormones (or plant growth regulators, or PGRs) are internally secreted chemicals in plants that are used for regulating the plants' growth.

According to a standard definition, plant hormones are: Signal molecules produced within the plant at specific locations, that occur in extremely low concentrations, and cause altered processes in target cells at other locations.

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CHARACTERISTICS

The concentration of hormones required for the plant response is very low(10-6 to 10-5M), comparing with the requirement of mineral and vitamin for plants.

The synthesis of plant hormones is more diffuse and not always localized.

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Classes of Plant Hormones

There are two major classes of plant hormones:

Class	Action	Examples
Promoters	Cause faster growth	Auxins Cytokinins Gibberellins Brassinosteroids
Inhibitors	Reduce growth	Ethylene Abscisic acid Jasmonic acid

WHAT DO HORMONES CONTROL IN PLANTS?

Roots and shoots growth

Seed germination

Leaf fall

Disease resistance

Fruit formation and ripening

Flowering time

Bud formation

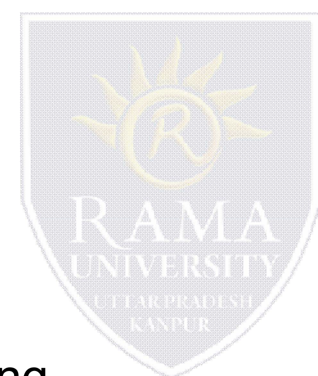
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Table 11.1 Plant Hormones (1 of 2)

Hormone		Where synthesized in plants	Major functions
Auxins (Example shown: IAA)	CH_2COOH	Embryos, meristems, buds, young leaves	Stimulates stem and root growth; promotes cell differentiation in tissue culture and in procambium; regulates development of fruit; apical dominance; causes phototropism and gravitropism
Cytokinins (Example shown: zeatin)	CH_2OH, CH_3	Roots	Promotes root growth and differentiation; stimulates cell division and growth in tissue culture; stimulates germination; retards aging
Gibberellins (Example shown: GA_3)	OH, CH_2, HO, CH_3, COOH	Meristems, young leaves, embryos	Promotes seed germination and bud growth; promotes stem elongation and leaf growth; stimulates flowering and fruit development

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Table 11.1 Plant Hormones (2 of 2)

Hormone		Where synthesized in plants	Major functions
Abscisic acid (ABA)	H_3C, CH_3, CH_3, OH, CH_3, COOH, O	Leaves, stems, roots, fruits	Inhibits growth; closes stomata during water stress; promotes dormancy
Ethylene	H, H, C=C, H, H	Ripening fruits, aging leaves and flowers	Promotes ripening of some fruits and thickening of stems and roots
Brassinosteroids (Example shown: brassinolide)	$C_7H_{13}(OH)_2$, HO, HO, O, O	Seeds, fruits, shoots, leaves, and flower buds	Auxin-like effects; inhibits root growth; retards leaf abscission; promotes xylem differentiation

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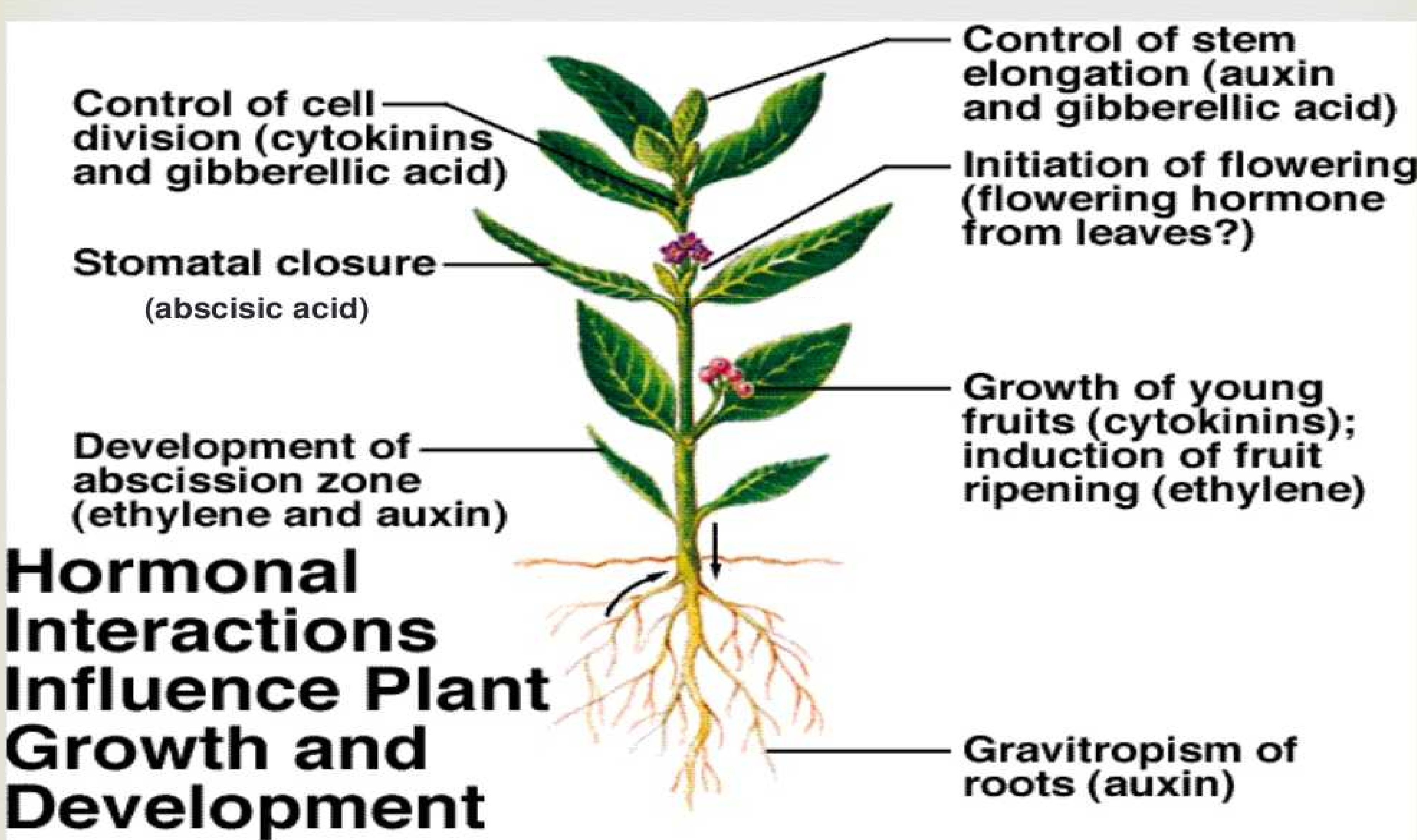
Plant Hormones & Growth



Naturally occurring (endogenous) auxins in plants include:

Indole-3-acetic acid

4-chloroindole-3-acetic acid

Phenylacetic acid

Indole-3-butyric acid

Indole-3-propionic acid

Synthetic auxin analogs include:

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1-naphthaleneacetic acid

2,4-dichlorophenoxyacetic acid (2,4-D)

AUXIN (IAA)
Indole-3-acetic acid

- Synthesised from the amino acid L-tryptophane

-leaf primordia
- Young leaves
- Developing seeds

- Moves from cell to cell from tip to base

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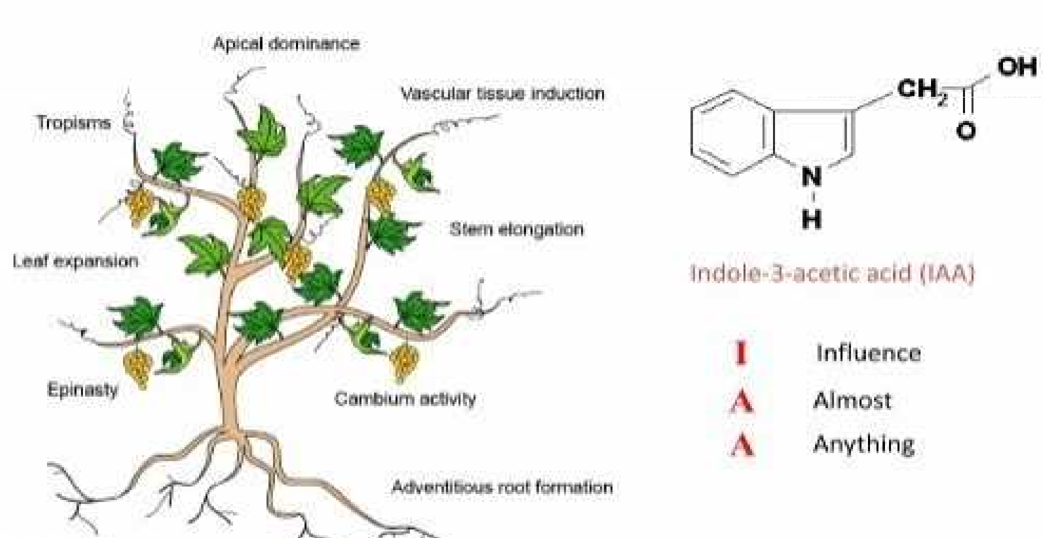
1-naphthaleneacetic acid

2,4-dichlorophenoxyacetic acid

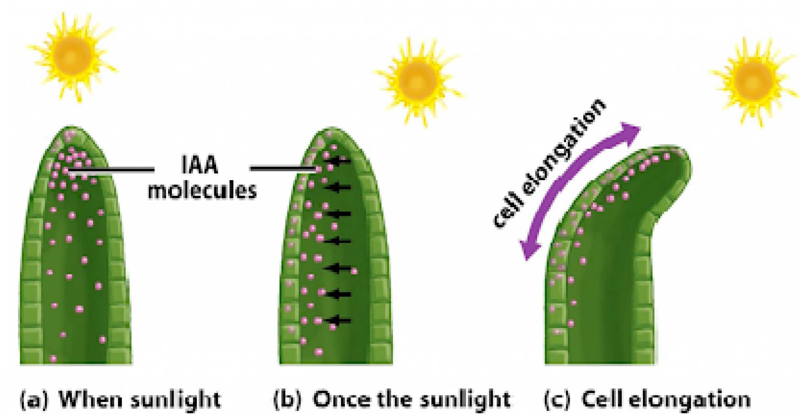
AUXIN (IAA)
Indole-3-acetic acid

1. Coleptile bending towards light
   (cell elongation)

2. Inhibition of lateral buds by terminal buds
   (apical dominance)



https://www.yumpu.com/en/document/view/13774692/indole-3-acetic-acid-iaa-influence-a-almost-a-anything

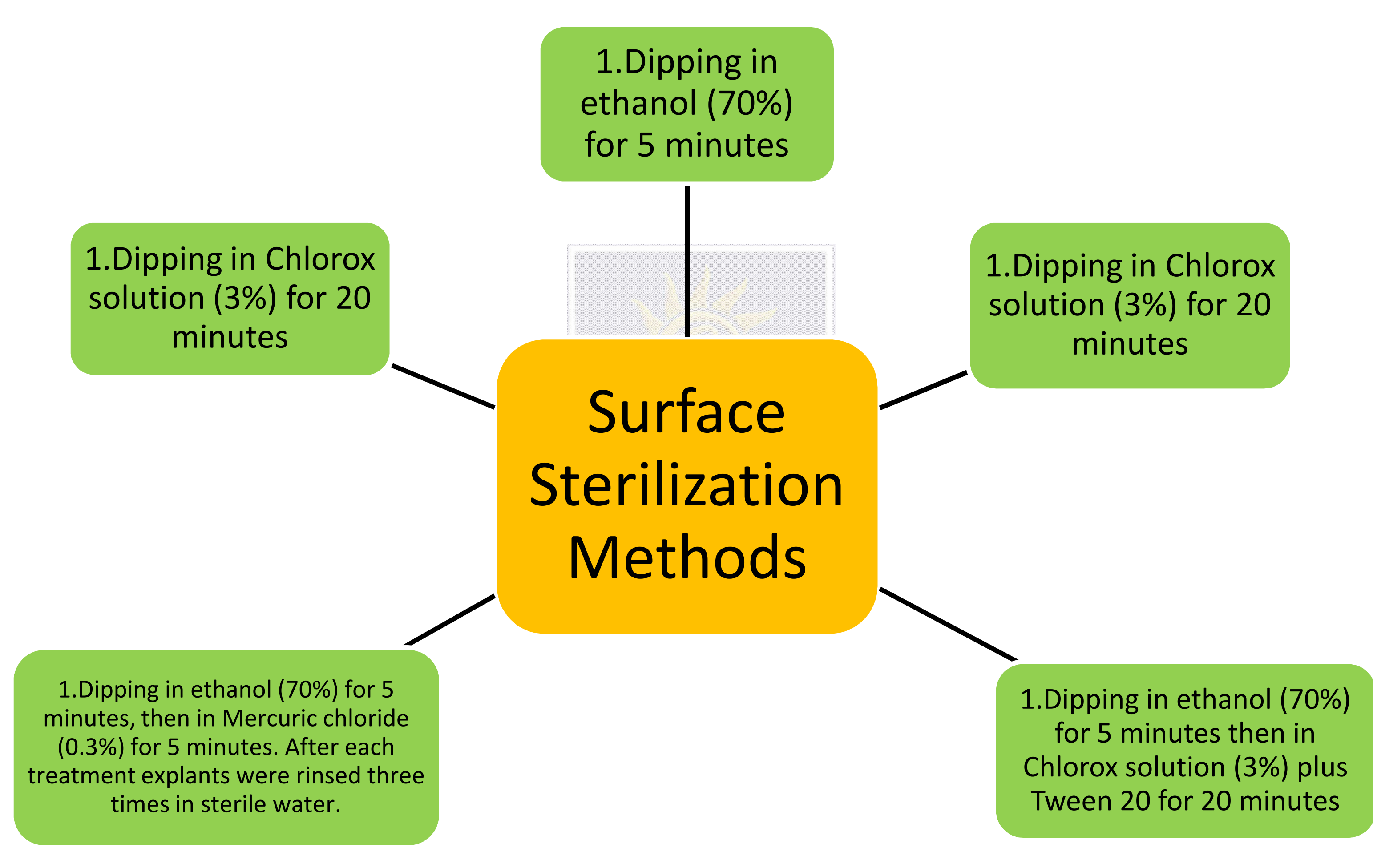


https://sites.google.com/a/aisr.org/mun-ib/biology/plant-biology/topic-9-3-growth-in-plants

3. Formation of abscission layer on leaves and fruit

4. Activation of cambial growth.

SURFACE STERILIZATION OF PLANT MATERIAL



SURFACE STERILIZATION OF EXPLANTS

Wash the explant with tap water to remove surface borne mico-organisms.

Transfer the washed explant into a glass beaker containing tap water; add few drops of liquid detergent – Tween 20 for 10-15 min.

Cover beaker mouth with muslin cloth with the rubber band and keep under running tap water for 1 hour to remove any waxy/ oily deposition on surface of explant.

Wash it thrice with distilled water.

Transfer the explant into laminar airflow hood for farther work to avoid contamination.

Wash the above explant with sterile distilled water for thrice each washing should be for 3-4 minutes.

Treat it with 0.1% $HgCl_2$ or 5-10% sodium hypochlorite solution for 60 sec. After treating it with disinfectant, wash it with sterile distill water for thrice, each washing should be for 3-4 minutes.

Wash with 70% alcohol for 30 seconds to remove water from the surface of the explant.

Transfer t**he** sterile explant to a sterile petriplate and cut the leaf into small pieces of about 1x1 cm with sterile blade.

Now the explant is ready for inoculation.

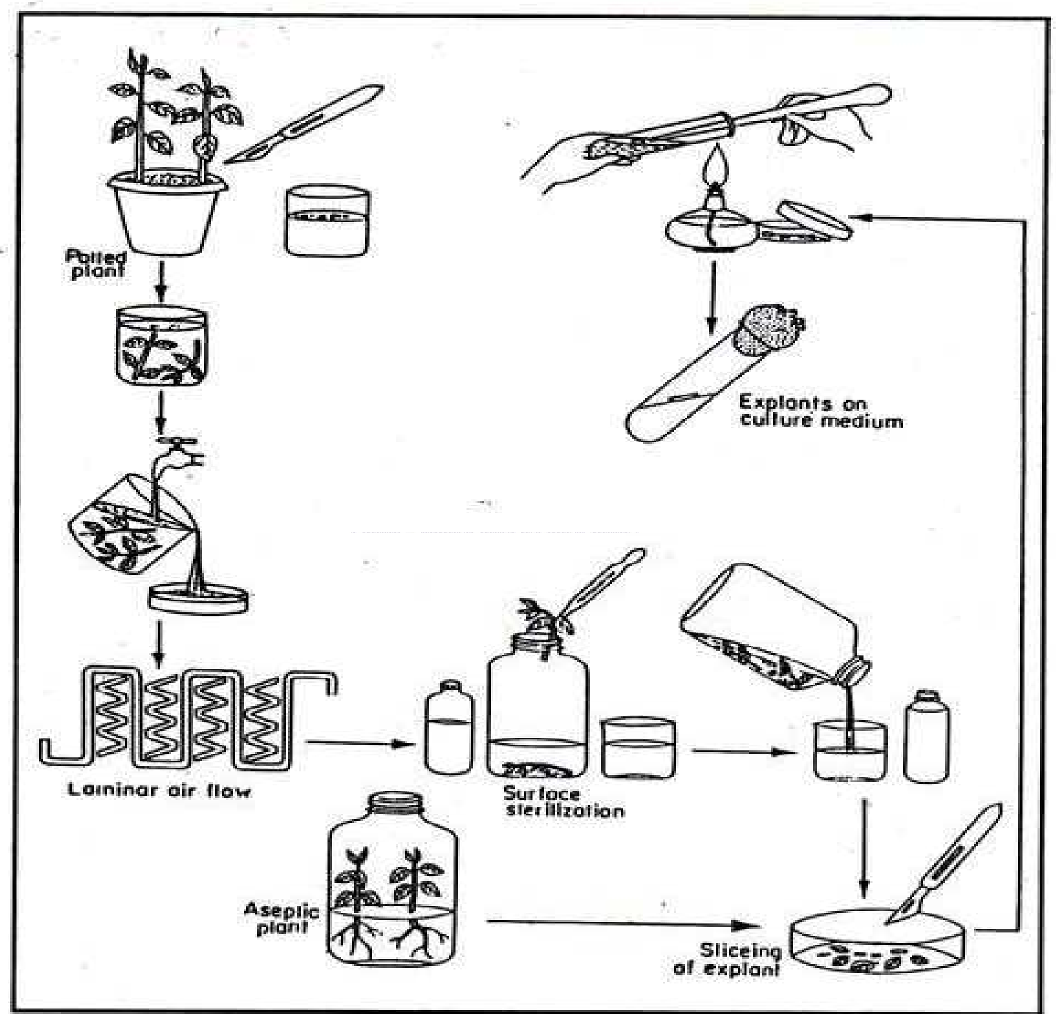


Fig 1.10

Flow diagram illustrating the procedure for surface sterilization of plant material and inoculation of explant for culture

https://www.biologydiscussion.com/plant-tissues/culture/techniques-used-in-plant-tissue-culture-with-diagram/14549

BASIC PROCEDURE FOR ASEPTIC TISSUE TRANSFER

- ✓ Put all the sterilized articles (media, instru-ments, glass goods etc.) for inoculation on the glass racks of the inoculation chamber. Alternatively, if laminar air flow is avail-able, keep all articles on the table of air flow cabinet. Laminar air flow blows bacteria- free air over the working surface.
- ✓ Put on the switch of UV lamps of inocula-tion chamber for one hour before work. In case of laminar air flow, the power switch is put on and allows the air flow to blow air for at least 15 minutes before work.
- ✓ Put off the UV lamp before entering inside the inoculation chamber. Do not put off laminar air flow. The working glass table top of the inoculation chamber or the table of laminar air flow is swabbed with alcohol before starting work.

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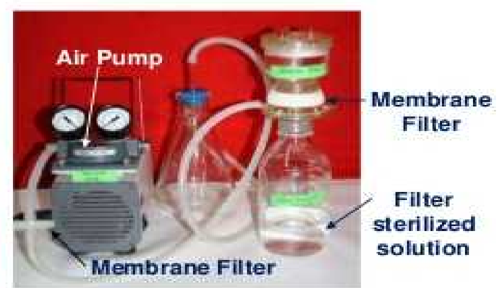
- ✓ Wear a clean apron and use a mask. Clean the hands with alcohol and dry it.
- ✓ Pour alcohol in a clean coupling jar and dip all instruments into it. Light the spirit lamp. Take the surface sterilized or aseptic plant material in a, sterile petri dish.
- ✓ Flame the neck of culture tube or flask and in quick succession remove the plug of glass vials. Transfer the tissue onto the medium and replace the closure. Each time, the in-struments are passed through the flame of the spirit lamp.

STERILIZATION OF MEDIA

- ✓ Sterilization of media is routinely achieved by autoclaving at the temperature 121 ^{o} C. (steam pressure of 1.05kg/cm2) for 15- 45 minutes
- ✓ Over autoclaving should be avoided
- ✓ Advantages of autoclaving are: the method is quick and simple, whereas disadvantages are the media pH changes and some components may decompose and so to loose their effectiveness.

- ✓ Filtration through microporus filters (0.22- 0.45) is also used for thermolabile organic constituents such as vitamins, growth regulators and amino acids.
- ✓ Filter assemblies of different sizes are available.
- ✓ Once the component is filter sterilized, it is collected in a sterile container which can be used immediately or dispensed in smaller amounts to be used later
- ✓ These filter sterilized components can be stored at 40C or -200C depending on the frequency of their usage



INCUBATION OF CULTURE

- ✓ Cultures are incubated in a culture room where light, temperature and humidity are controlled.
- ✓ For some tissues dark is essential while for some both dark and light conditions are required.
- ✓ Humidity has also some effect.
- ✓ The cultures are incubated on culture rack at constant temperature of 25-28°C. Culture tubes are placed at 30-45° inclined position.
- ✓ Illumination is provided by cool-white fluo-rescent light placed about 18 inches above the culture to give a light intensity of 4 – 10 x 10^3 lux for 16 hours.

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