Plant Tissue Culture

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Plant Tissue Culture Terminology

- Adventitious---Developing from unusual points of origin, such as shoot or root tissues, from callus or embryos, from sources other than zygotes.
- Agar---a polysaccharide powder derived from algae used to gel a medium. Agar is generally used at a concentration of 6-12 g/liter.
- Aseptic---Free of microorganisms.
- Aseptic Technique---Procedures used to prevent the introduction of fungi, bacteria, viruses, mycoplasma or other microorganisms into cultures.
- Autoclave---A machine capable of sterilizing wet or dry items with steam under pressure. Pressure cookers are a type of autoclaves.
- Chemically Defined Medium---A nutritive solution for culturing cells in which each component is specifiable and ideally of known chemical structure.
- Clone---Plants produced asexually from a single source plant.
- Clonal Propagation---Asexual reproduction of plants that are considered to be genetically uniform and originated from a single individual or explant.
- Coconut milk---The liquid endosperm of coconut contain the cytokinin *zeatin* and will support the continued cell division of mature cells, leading to the formation of callus.
- Contamination---Being infested with unwanted microorganisms such as bacteria or fungi.
- Culture--- plant growing in vitro.
- Detergent---Increasing the efficiency of sterilization.

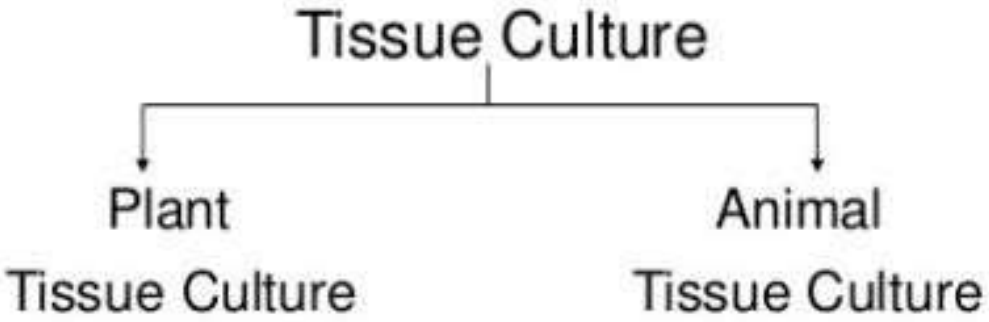
Plant Tissue Culture Terminology

- Plant Tissue Culture---The growth or maintenance of plant cells, tissues, organs or whole plants *in vitro*.
- Regeneration---In plant cultures, a morphogenetic response to a stimulus that results in the products of organs, embryos, or whole plants.
- Somaclonal Variation---Phenotypic variation, either genetic or epigenetic in origin, displayed among somaclones.
- Somaclones---Plants derived from any form of cell culture involving the use of somatic plant cells.
- Sterile--- (A) Without life. (B) Inability of an organism to produce functional gametes. (C) A culture that is free of viable microorganisms.
- Sterile Techniques---The practice of working with cultures in an environment free from microorganisms.
- Subculture---See "Passage". With plant cultures, this is the process by which the tissue or explant is first subdivide, then transferred into fresh culture medium.
- Tissue Culture---The maintenance or growth of tissue, *in vitro*, in a way that may allow differentiation and preservation of their function.
1. Totipotency---A cell characteristic in which the potential for forming all the cell types in the adult organism are retained.
- Undifferentiated---With plant cells, existing in a state of cell development characterized by isodiametric cell shape, very little or no vacuole, a large nucleus, and exemplified by cells comprising an apical meristem or embryo

History of Plant Tissue Culture

- A German plant physiologist Gottlieb Haberlandt (1902) cultured isolated single palisade cells from leaves in Knop's salt solution enriched with sucrose. **Haberlandt is regarded as the father of plant tissue culture.**
- Hanning(1904) **Embryo culture of selected crucifers**.
- Snow, Gautheret (1934-1939) **Importance of auxin** as growth regulator
- Morel & Martin 1952 used Meristem-tip culture for disease elimination. They recovered for the first time, **virus- free Dahlia plants**.
- Skoog and Miller (1957) **Discovery of principle of hormonal control of the organ formation in tissue culture**
- Reinert and Steward (1958-59) First report on **somatic embryogenesis.**
- Cocking (1960) Isolation of protoplasts by **enzymatic degradation method**.
- **Murashigeand Skoog** (1962) Developeda universallyused high salt medium containing mineral salts, vitamins, an energy source and growth hormone (**MS medium**).
- Guha and Maheshwawari (1964) Production of **First haploid plant by anther culture**.
- Power et al (1970) **Protoplast fusion**.
- Takabe et al (1971) **Regeneration of first plant from protoplast**.
- Carlson et al (1972) First report on inter-specific hybridization through protoplast fusion.

Plant Tissue Culture



- **Defination:**
- Plant-tissue culture is *in-vitro* cultivation of plant cell or tissue under aseptic and controlled environment conditions, in liquid or on semisolid well defined nutrient medium for the production of primary and secondary metabolites or to regenerate plant.
- In other words it is an experimental technique through which a mass of cells (callus) is produced from an explant tissue.
- The callus produced through this process can be utilized directly to regenerate palntlets or to extract or manipulate some primary and secondary metabolites.

- The plant tissue culture refers to the cultivation of a plant cell which normally forms a multicellular tissue.
- When grown on agar medium, the tisse forms a callus or a mass of undifferentiated cells. The technique of cell culture is convinient for starting and maintaining cell lines, as well as, for studies pertaining to organogensis and meristem culture.

- The technique of *in-vitro* cultivation of plant cells or organs is primarily devoted to solve two basic problems:

1. To keep the plant cells or organs free from microbes

2. To ensure the desired development in cells and organs by providing suitable nutrient media and other environmental condition.

Advantages of tissue culture

1. Availability of raw material

Some plants are difficult to cultivate and are also not available in abundance and tissue culture technique is considered a better source for regular and uniform supply of raw material for medicinal plant industry for production of phytopharmaceuticals.

2. Fluctuation in supplies and quality

The method of production of crude drugs is variable in quality due to changes in climate, crop diseases and seasons. All these problems can be overcome by tissue culture.

3. New methods for isolation

It is possible to obtain new methods for isolation and newer compounds from plant by this technique and for which Patent rights can be obtained.

4. Biotransformation (Process through which the functional group of organic compound are modified by living cells) reactions are feasible using plant-cell cultures.

6. Disease free and desired propagule

Large scale production of plant with disease free and desired propagule could be stored and maintained without any damage during transportation for subsequent plantation.

7. Biosynthetic pathway

Tissue culture can be used for tracing the biosynthetic pathways of secondary metabolites using labelled precursor in the culture medium.

8. Immobilization of cells

Tissue culture can be used for plants preservation by immobilization (entrapment)of cell further facilitating transportation and biotransformation.

9 Continuous, uniform biomass is obtained.

10. Medicinally important compound can be synthesized, which can't be synthesized chemically.

11. Useful natural compounds can be produced, independent of soil condition & change in climatic conditions.

12. Improvement of medicinal plant species.

13. Propogation of plant without seeds in defined and controlled condition.

Disadvantages of tissue culture

1. High level of expertise is required.
2. A small error may lead to complete collapse of product/plant.
3. Lots of chemicals are required for plant tissue culture which must contain high purity.
4. There is no chance for evaluation of mutation.
5. Culture on artificial medium may lead to the depression of unusual metabolic pathways, which may not be beneficial to biotechnologist.
6. In majority cases amount of secondary metabolites produced is negligible.
7. The protocols for individual plants differ very widely and Change in the medium constitution & environmental parameters affect the rate of cell growth & accumulation of secondary metabolites.
8. To maximize on the cell mass produced the cell suspension culture eventually becomes very dense and these presents problems of even aeration.
9. Instability
10. Slow growth
11. Expensive process
12. Aseptic conditions are to be maintained through out the growth of plant.

Basic requirement for tissue culture

- Plant material
- Equipments and Glasswares
- Aseptic Condition
- Washing and storage facilities
- Media preparation room
- Sterilization room
- Nutrient medium
- Transfer room
- Culture room or incubators
- Proper and optimum aeration
- Well equipped observation or recording area

Equipments and Glassware

- Incubating chamber or laminar airflow cabinet with UV light fitting for aseptic transfer
- Incubator with temperature control ± 0.5°C generally temperature recommended for most tissue culture studies is 36°C.
- Autoclave-for sterilization of glassware, media etc.
- Refrigerators and freezers-For storage of reagents, tissue culture stock solutions, chemicals etc.
- Hot air oven-for dry sterilization of glassware, media etc.
- Microscope-Simple and special microscope with a provision to take camera are required. The stage of this microscope should be large enough to accommodate large roller bottles in specific cases.
- pH meter- for adjusting the pH of the medium
- A spirit burner or gas micro burner for flame sterilization of instruments
- Washing up equipments- Washing facilities for glassware, pipette etc. in deep soaking baths or washing sinks of stainless steel or polypropylene are suitable for manual washing and rinsing of almost all types of glassware except pipettes.

Standard siphon type pipette washers are suitable for washing the pipettes soaked in detergent for overnight. The washed pipettes should be rinsed with deionised water and dried in a stainless steel pippette dryer.

- Water purifier- Pure water is required at most of the plant tissue culture study.
- Centrifuge- To increase the concentration of cell suspension culture

Equipments and Glassware

•Shakers- To maintain cell suspension culture
•Balance- To weigh various nutrients of the preparation of the medium
•Shelves- Build from rigid wire mesh to allow maximum air movement and minimum shading should be used in the culture room.
•Scissors, scalpels and forceps- For explant preparation from excies plant parts are for their transfer
•Culture vessels- Usually borosilicate glass vessels are preferred, it includes test tubes, conical flasks, bottles, special flat tubes etc.
Now, the common vessels are 100 ml conical flasks or large test tubes of 25 × 150 mm size.
•Glasswares- Like measuring cylinders, beakers, funnels, petri dishes, graduated pipette, conical flask etc. Are required for preparation of nutrient media.
•Miscellaneous-Non absorbent cotton plug, screw cap or polyurethane foam is required to close the mouth of the culture vessel. Aluminium foil is required to cover the exposed part of plug from becoming wet when autoclaved. Labels, marking pencils, hand lens, plastic disposables like syringes, plastic bottles, hot plate, stirrer etc.
•Microwave- not essential but it melts the solidified media for pouring in culture vessels like petri dishes etc.

Aseptic Condition

- The plant materials (tissues), equipments, culture media and the room should be free from microorganisms.
- Usually dry heat, wet heat, ultrafiltration and chemicals are used for the sterilisation process.
- Surface sterilisation of plant materials such as seed, fruit, stem, leaf etc. by agents like
  - 9-10% calcium hypochlorite for 5-30 minutes
  - 2% sodium hypochlorite solution for 5-30 minutes. The materials need to be washed thoroughly in double-distilled water, after sterilising in these solutions.
  - 10-12% of hydrogen peroxide solution for 5-15 minutes.
  - 1-2% bromine water, for 2-10 minutes
  - 1% solution of chlorine water, mercuric chloride, silver nitrate or antibiotics etc. can also be used.
  - Absolute alcohol is used for hard tissues

- Dry heat method is used for sterilisation of equipments in hot air oven.
- Sterilisation of equipment with chromic acid-sulphuric acid mixture, hydrochloric acid, nitric acid strong detergent solution, alcohol, incubator or autoclaves etc. are use for this purpose.
- Wet heat method is used for sterilisation of glassware, culture media in autoclave at 121°C and 15 lb pressure for 15 minutes.
- Ultrafiltration is used for sterilisation of liquid media which are unstable at high temperature.
- Antibiotics are added to medium to prevent the growth of the microorganisms e.g. Potassium benzyl penicillin, strptomycin sulphate, gentamycin etc.
- Chemicals like alcohol are used for sterilisation of working area and the instruments.
- Sterilisation of the environment is done by fumigation method, the inoculation chamber is generally laminar airflow cabinet is widely used these days.

Transfer room

•It is provided with the laminar flow hood where most of the work of culture initiation and subsequent sub culturing is performed. Culture re-plantation, transfer or re-initiation in a clean media, harvesting of 'ripe' cultures is also performed in this area.

Culture room or incubator

- Cultures are incubated on shelves or in incubators under specific condition of temperature, humidity, air circulation and light.
- Incubation chamber or area should have both light and temperature controlled devices managed for 24 hours period.
- Generally high output, cool, white fluorescent light is preferred for a photo-period duration (specified period for total darkness as well as for higher intenesity light) with a temperature range of 25 ± 2°C (range 18-25°C).
- The rooms are required to be maintined at a relative humidity upto 70-75% (range of 20-90% controllable to ±3%) and uniform forced air circulation.

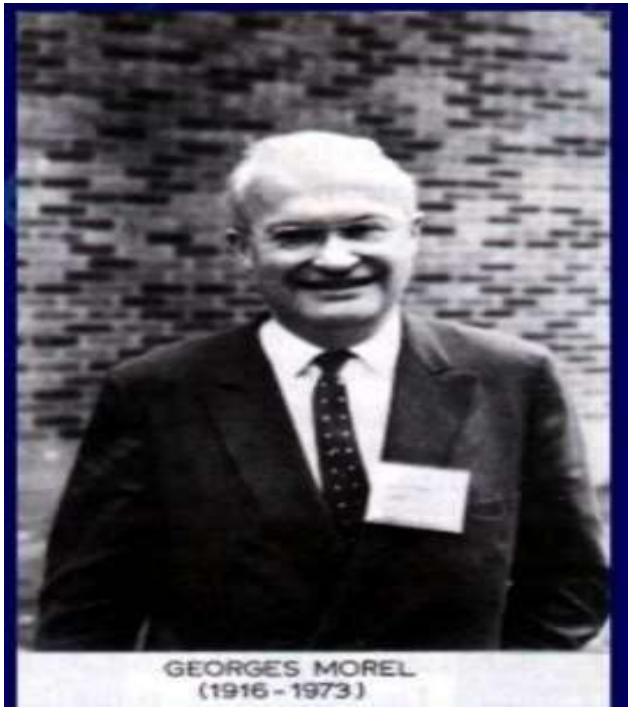
Techniques for plant tissue culture

The general technique used in the isolation and growth of culture is described as follows:

1. Preparation of suitable nutrient medium: As per the selection of plant medium is autoclaved.
2. Selection of explant: Any excised part of health plant to be used e.g. Bud, leaf, root, seed etc.
3. Sterilisation of explants: by sodium hypochlorite, mercuric chloride etc. and washed asceptically for 6-10 times with sterilised water.
4. Inoculation (Transfer): The sterile explant is inoculated on solidified nutrient medium under asceptic condition.
5. Incubation: Cultures are incubated at of 25±2°C and at a relative humidity upto 50-70% fro 16 hrs of photo period.
6. Regeneration: Plantlets regenerated after transferring a portion of callus into another medium and induction of roots and shoots or directly from explants.
7. Hardening: Is the gradual exposure of plantlets for acclimatisation to environment condition.
8. Plantlet transfer: Plantlet are transferred to green house or field conditions.

HISTORY 0F VIRUS FREE PLANT CULTURE

1952 Morel & Martin:
First virus-free plant through shoot tips culture (*Dahlia*)
1960 Shoot tip culture of *Cymbidium*



- Morel and Martin (1952) developed meristem culture technique and recovered Dahlia shoots, free from viruses, by meristem tip culture. In 1955, they recovered virus free potato. This attained wide application of plant tissue culture to raise virus free plants in agriculture.

Callus culture

Callus is **defined** as an unorganized **tissue** mass growing on solid substrate. **Callus** forms naturally on plants in response to wounding, infestations, or at graft unions (Bottino, 1981). **Callus** formation is central to many investigative and applied **tissue culture** procedures.

- It may initiate from explants of any multi-cellular plant.
- The organs such as root, stem tips, leaves, flowers and fruit are grown on solid media.
- The cell groups are initiated from:
  - Explant/Segments of root, stem or leaf either from the mature or embryogenic plant
  - Explant/Excised fragments of parenchyma or mixed tissue containing cambium or endosperm
- The longer the tissue explant the more complex the range of cell types & greater the possibilities of initiating a culture of mixed cells.
- Callus can be induced to undergo organogenesis and/or embryogenesis and eventually whole plant by providing suitable nutrient medium.
- To study the biosynthetic pathway of various metabolic processes by using tracer elements in callus culture.

Callus

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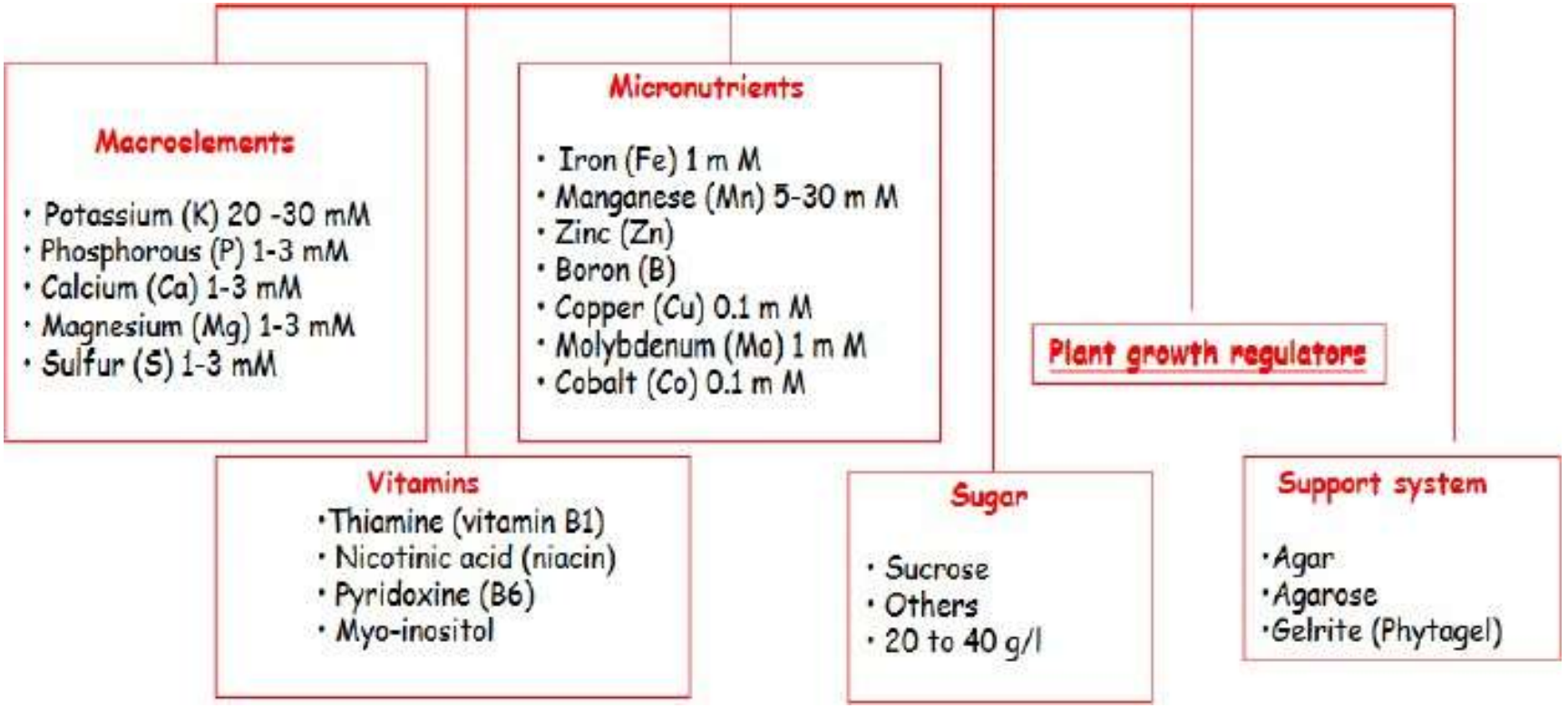
Nutrient medium

- Media is composed of
  - **Inorganic nutrients** which includes macronutrients like nitrogen, phosphorous, potassium, calcium etc. and micronutrients like boron, copper, iron, manganese, zinc etc.
  - **Organic nutrients** includes Vitamins like Vitamin B_1, B_6, B_3, B_5 etc. Amino acids like L-arginine, L-asparagine, L-cysteine HCL, L-glutamine etc, Carbon source like glucose or maltose, Growth hormones/regulators like auxin, cytokinins and gibberellins, ethylene, abscisic acid.
  - **Others media substances** like protein hydrolysates, yeast extaracts, fruit (e.g. banana) extracts, coconut milk, solidifying agents like agar, alginate, gelatin etc., Iron source e.g.EDTA, Antibiotics.
  - **pH** of the medium should be in a range of 5.6-6.0 before autoclaving the culture medium

Culture Medium



Essential elements for plant growth

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

Plant growth regulators used in plant tissue culture media
Normal concentration range is 10^{-7} ~ 10^{-5}M

Class	Name	Abbreviation	MW	Stock solution
Auxin	p-chlorophenoxyacetic acid	pCPA	186.6	All auxins dissolved in dilute NaOH or aqueous ethanol
	2,4-Dichlorophenoxyacetic acid	2,4-D	221.0	
	Indole-3-acetic acid	IAA	175.2	
	Indole-3-butyric acid	IBA	203.2	
	1-Naphthaleneacetic acid	NAA	186.2	
	2-Napthoxyacetic acid	NOA	202.2	
Cytokinin	6-Benzylaminopurine	BAP	225.2	All cytokinins dissolved in dilute NaOH or aqueous ethanol
	N-Isopenteylaminopurine	2iP	203.3	
	6-Furfurylaminopurine (Kinetin)	K	215.2	
	Zeatin***	Zea	219.2	
Gibberellin	Gibberellic acid***	GA_3	346.4	Dissolved in water
Abscisic acid	Abscisic acid	ABA	264	Dissolved in aqueous ethanol

Murashige and Skoog (MS) Medium (1962)

Macroelement (10x)		Microelement (1000x)		Organics	
NH_4NO_3	1.65 g/L	H_3BO	6.2 mg/L	Nicotinic acid	0.5mg/L
$MgSO_4$	370 mg/L	$CoCl_2$	0.025 mg/L	Pyridoxin-HCl	0.5 mg/L
$CaCl_2$	440 mg/L	$CuSO_4$	0.025 mg/L	Thiamine-HCl	0.1 mg/L
KPH_2O_4	170 mg/L	$ZnSO_4$ ($7H_2O$)	8.6 mg/L	*myo*-Inositol	100 mg/L
KNO_3	1.9 g/L	$NaMoO_4$ ($2H_2O$)	0.25 mg/L	Glycine	2 g/L
		$MnSO_4$ ($4H_2O$)	22.3 mg/L	Sucrose	30 g/L
		KI	0.83 mg/L		
		*$FeSO_4$ ($7H_2O$)	27.8 mg/L		
		*Na_2EDTA	37.3 mg/L		

Composition of nutrient medium

Table 1. Inorganic salt composition of Murashige and Skoog (13), Hoagland and Arnon (7) and White's (20) media.

Ingredients	Media		
	Murashige and Skoog	Hoagland and Arnon	White
	Macronutrients (µmoles/liter)		
Nitrogen	60.0	15.0	2.0
NH^+_4	20.6	-	-
NO^-_3	39.4	15.0	2.0
Phosphorus	20.0	1.0	0.1
Potassium	1.3	6.0	1.7
Calcium	3.0	5.0	1.2
Magnesium	3.0	2.0	3.0
Sulfur	3.2	2.0	4.5
	Micronutrients (µmoles/liter)		
Boron	100.0	46.3	-
Chlorine	2,993.0	10.9	870.0
Cobalt	0.1	-	-
Cooper	0.2	0.3	-
Iodine	5.0	-	4.5
Iron	10.0	9.0	10.0
Manganese	103.0	10.9	30.0
Molybdenum	1.1	0.1	-
Sodium	3.2	-	-
Zinc	3.0	0.8	9.0

Composition of nutrient medium

- **Salts** are supplied in the form of macronutrients viz. N, Mg, K, Ca, P
- **Micronutrients** Cu, Ni, Mn, Co, etc.
- **Iron** is supplied in the chelated, Fe-EDTA (Ferric-Sodium Ethylene-Amine Tetra Acetate) form.
- **Vitamins** viz. meso-inositol, thiamin (B1), nicotinic acid (B3), pyridoxine (B6), etc.
- **Aminoacids**, mostly glycine is used.
- **Carbohydrate** is supplied usually in the form of sucrose.
- **Phytohormones** (auxins and cytokinins), their chemical form, concentration and ratio may vary from plant to plant.
- In general Auxins, such as IAA (Indole Acetic Acid) NAA (Naphthalene Acetic Acid), IBA (Indole Butyric acid); Cytokinins viz. Kinetin (6-furfuryl amino purine) 6-BAP (6, Benzyl Amino Purine) and Zeatin are used in nutrient medium.

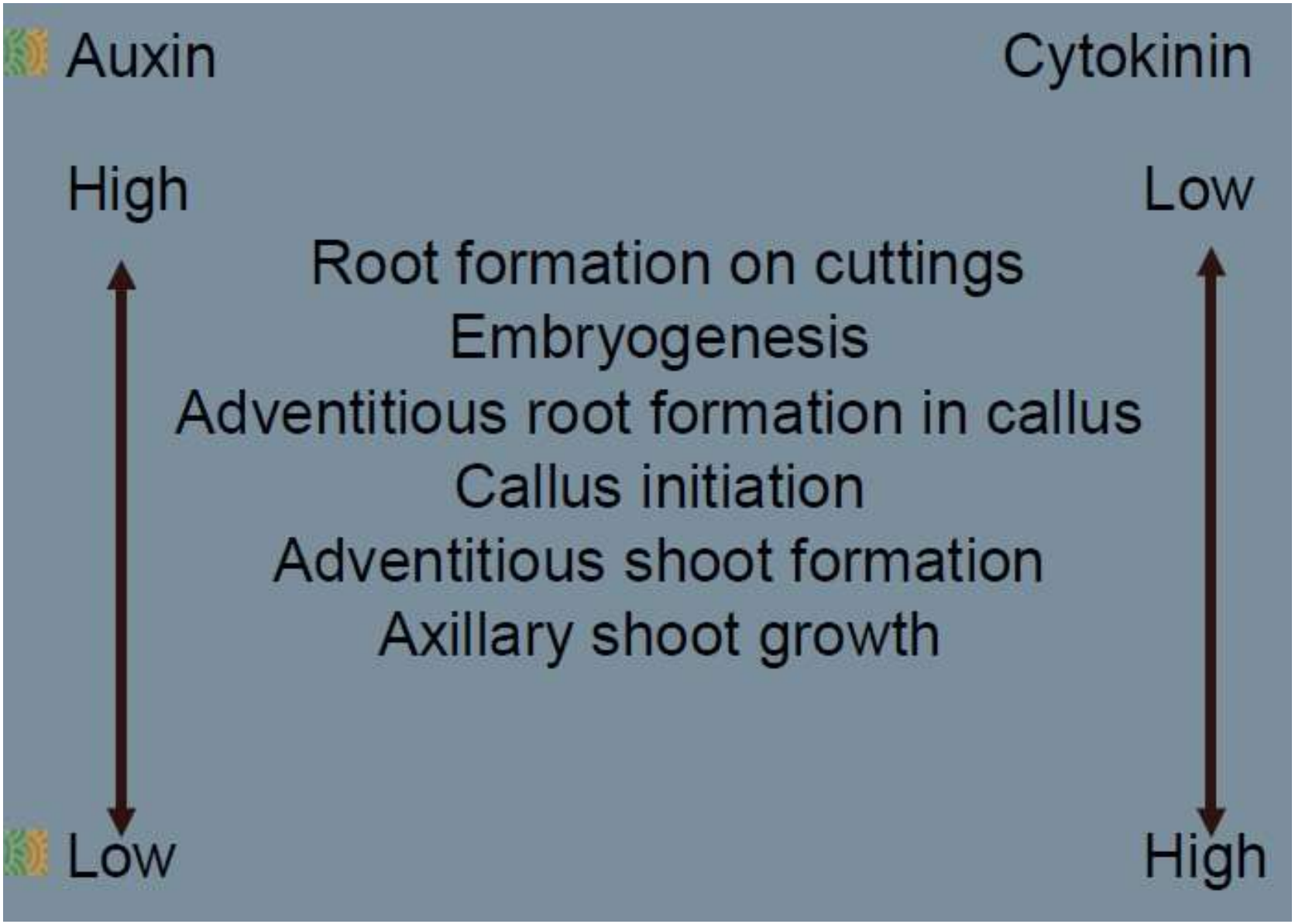
Plant growth regulators

Two major hormones affect Plant Differentiation:

- **Auxins:** Stimulates Root Development &
- **Cytokinin:** Stimulates Shoot Development

- Generally, the ratio of these two hormones can determine plant development:
  - ↑ Auxin ↓Cytokinin = Root Development.
  - ↑ Cytokinin ↓Auxin = Shoot Development.
  - Auxin = Cytokinin = Callus.

Hormonal balance



Types of medium

- **Chemically defined nutrient medium**
  - Chemical composition and structure is known
- **Chemically undefined nutrient medium:**
  - Complex additives viz. coconut milk, Casein hydrolysate, yeast extract, water melon juice, etc. are added in the medium.

1. **Solid medium**: 6-8% agar-agar
2. **Semi solid medium**: Less amount of agar
3. **Liquid medium:** Agar is not added. It is used for cell suspension culture.

Tissue culture

"... A method of biological research in which fragments of tissues from an animal or plant are grown in vitro in artificial medium under aseptic conditions and continue to survive and function."

"... the aseptic culture of plant protoplasts, cells, tissues or organs under aseptic conditions which lead to cell multiplication or regeneration of organs or whole plants."

Basic concepts of plant tissue culture(PTC)

Two concepts, are central to understanding plant cell, tissue, organ culture and regeneration.

Plasticity:

- -ability to initiate cell division from almost any tissue of the plant.
- -ability to regenerate lost organs or undergo developmental pathways in response to particular stimuli.

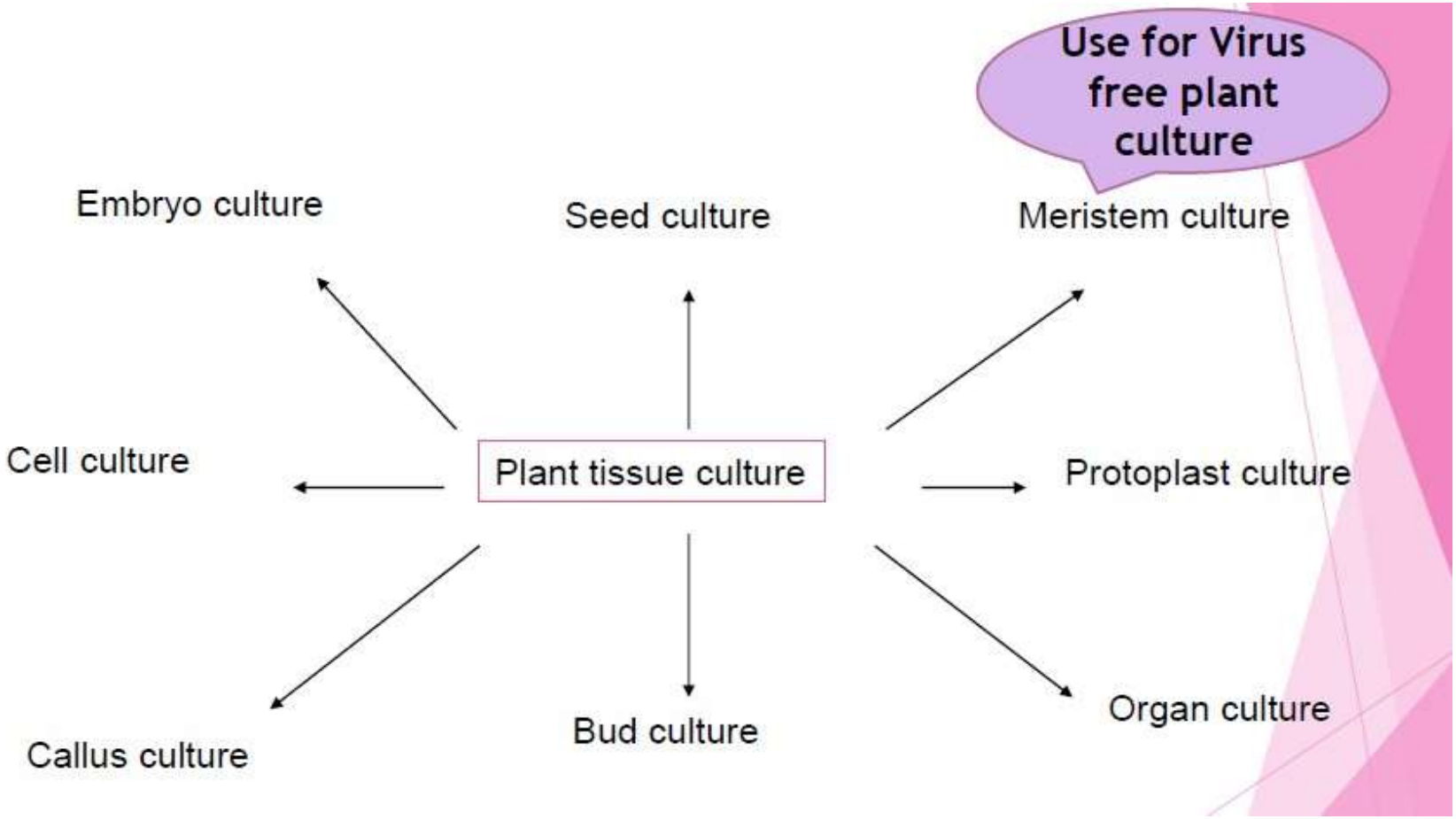
Totipotency:

- -each cell has the capacity to regenerate the entire plant.

Basic concepts of plant tissue culture(PTC)

- Cells lines differentiate to form specialized tissues and organs.
- Unlike animal cells, living plant cells re-differentiate.
- Therefore, tissue can be regenerated from explants such as cotyledons, hypocotyls, leaf, ovary, protoplast, petiole, root, anthers, etc.

Types of culture

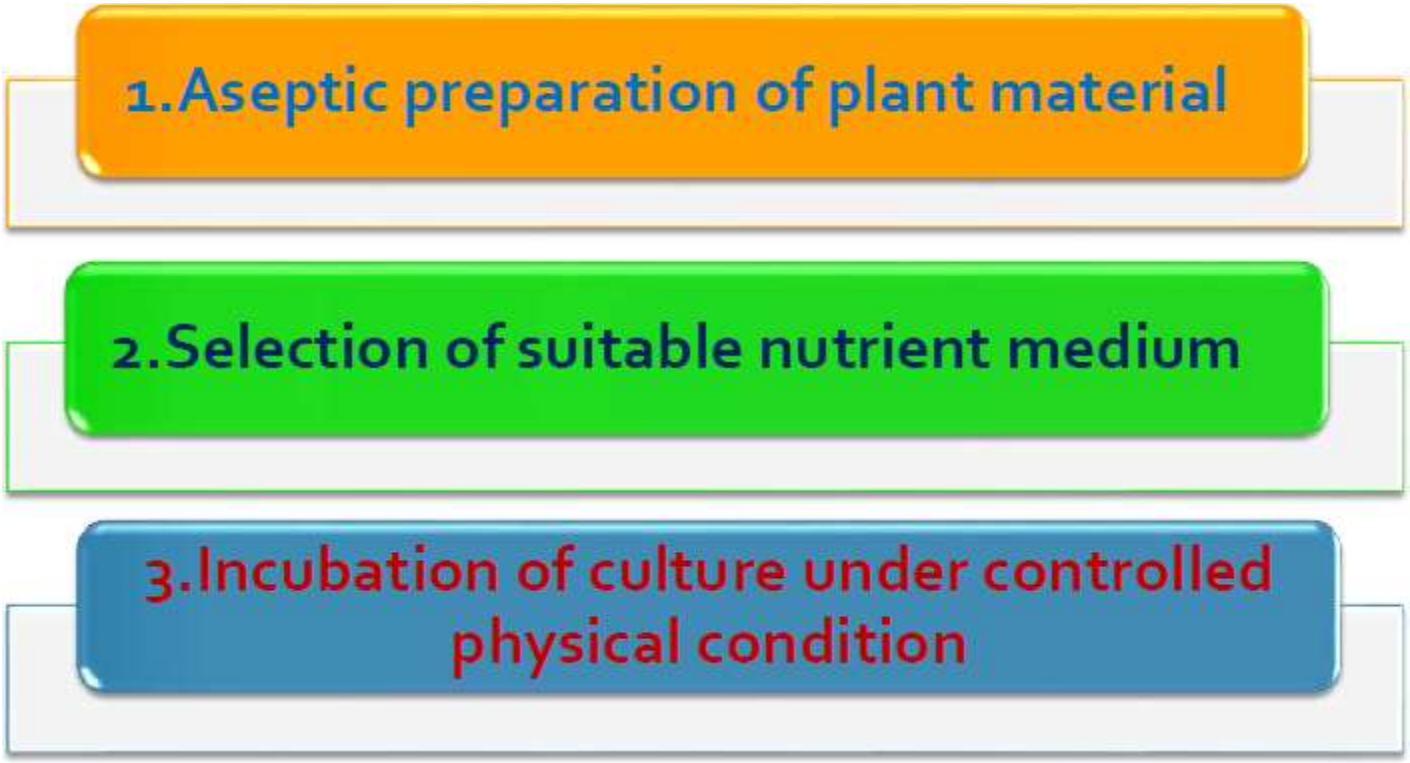


Callus

- It is an unspecialized , unorganized, growing and dividing mass of cells.
- It produced when explants are cultured on the appropriate solid medium, with both an auxin and a cytokinin in a correct conditions. 2,4-D are commonly used.
- During callus formation there is some degree of dedifferentiation both in morphology and metabolism, resulting in the lose the ability to photosynthesis.
- Callus cultures may be compact or friable.
  - Compact callus shows densely aggregated cells .
  - Friable callus shows loosely associated cells and the callus becomes soft and breaks apart easily.
- **Habituation**: it reduce the requirement of auxin and/or cytokinin by the culture during long-term culture.

Principle / procedure of callus culture

There are Three criteria for callus culture are



Aseptic preparation of plant material

- Surface sterilization :-

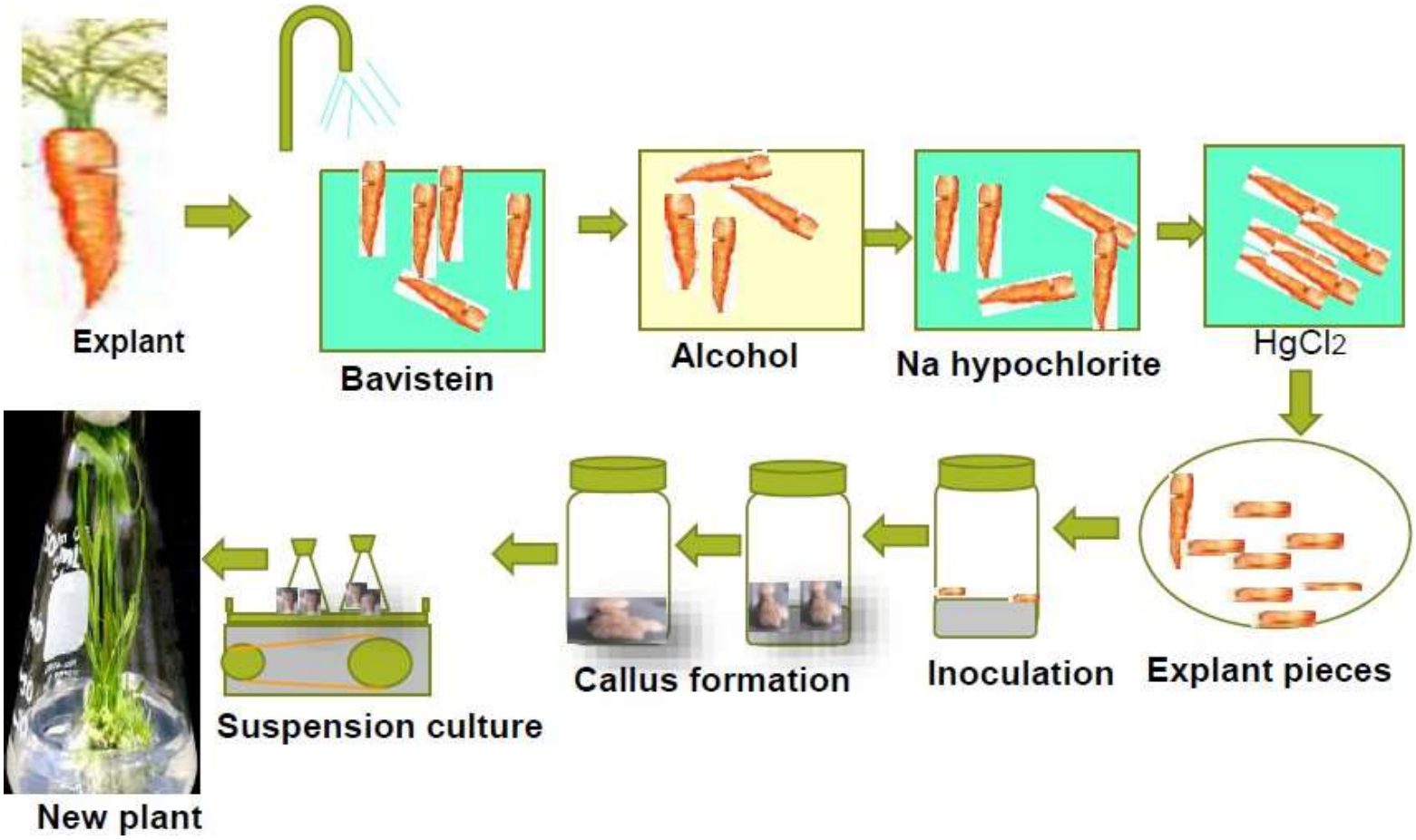
Selection of suitable nutrient medium

- Auxin/cytokinin –
  - 10:1-100:1 induces roots.
  - 1:10-1:100 induces shoots
  - Intermediate ratios around 1:1 favour callus growth .
- Agar solidified or semi – solid nutrient medium are used.
- That media are autoclaved at 15 psi pressure for 15 – 20 min at 121 0c.

Incubation of culture under controlled physical condition

- Temperature : 25 ± 2 oC
- Photoperiod : 16 hr Light , 8 hr Dark
- Light intensity : 2000 – 3000 lux
- Relative Humidity : 55 % - 60 %

STEPS INVOLVED IN CALLUS CULTURE



Three stages of callus culture

1. **Induction:** Cells in explant dedifferentiate and begin to divide
2. **Proliferative Stage:** Rapid cell division
3. **Morphogenesis stage:**
   - Organogenesis
   - Somatic embryogenesis

Organogenesis

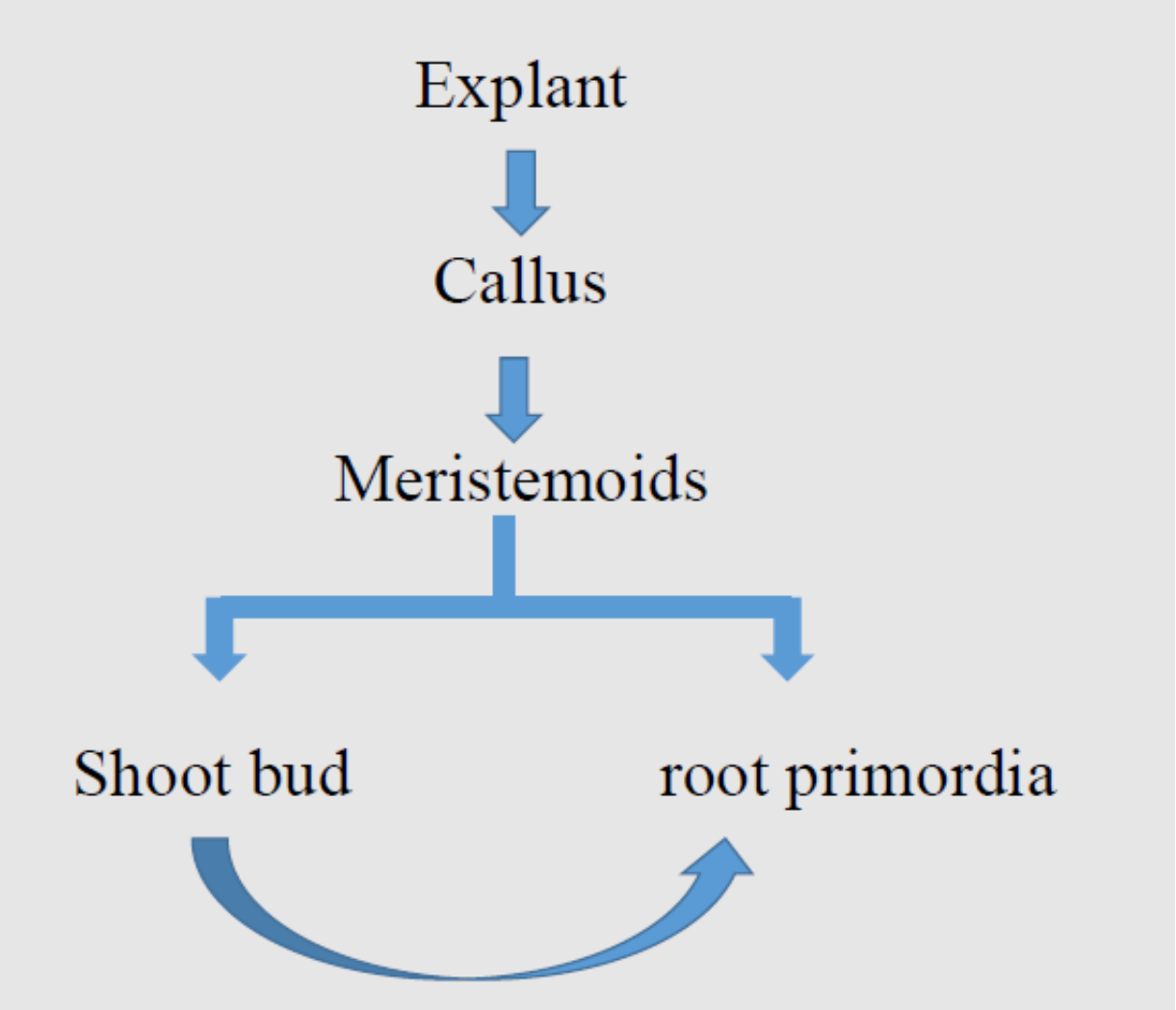
- The process of initiation and development of an organ is called organogenesis.
- In plant tissue culture, Organogenesis means the development of adventitious organs or primordia from undifferentiated cell mass in tissue culture by the process of differentiation.
- Organogenesis in plant tissue culture involves two distinct phases: dedifferentiation and redifferentiation.
- Dedifferentiation begins shortly after the isolation of the explant tissues with an acceleration of cell division and a consequent formation of a mass of undifferentiated cells (called callus).
- Redifferentiation, also called budding in plant tissue culture, may begin any time after the first callus cell forms. In this process of tissue called organ primordia is differentiated from a single or a group of callus cells. The organ primordia give rise to small meristems with cells densely filled with protoplasm and strikingly large nuclei.

Organogenesis

- Key factor of Organogenesis is the ratio of Endogenous Auxin – Cytokinin (T. A. Thorpe, 1980). The Explant develops into callus tissue in a medium containing either a particular concentration of Auxin or definite Auxin - Cytokinin ratio.

Organogenesis means the development of adventitious organs or primordia from undifferentiated cell mass in tissue culture by the process of differentiation.

Process



Factors effecting organogenesis

Genetic or a physiological change

- In a callus tissue the changes of chromosome structure or number such as aneuploidy, polyploidy, etc. Such chromosomal changes may lead to loss of totipotency of the cells.
- At the early stage of culture, the callus tissue exhibits maximum number of diploid cells. Eventually at the later stage of culture, the cells of callus tissue become mixaploid due to alteration of chromosome number and organogenesis could not be induced in such callus tissue, Occasionally, rooting occurs but shoot bud does not develop.

Factors effecting organogenesis

Phytoharmones

- For organogenesis the required balance of phytohormones by an exogenous supply of auxin, cytokinin or gibberellin either separately or in combination is essential .
- Generally high concentration of cytokinin brings about shoot bud initiation, whereas high levels of auxin favours rooting.
- Therefore, to obtain organogenesis, different permutation and combination of hormones as well as various concentrations of hormones are supplemented in the culture medium.

Factors effecting organogenesis

Other Chemicals

- Certain **phenolic compounds also induce shoot initiation in tobacco** callus- Phenolic compounds actually inactivate the auxins and consequently rise in the physiologically effective level of cytokinins which ultimately bring about the initiation of shoot buds.
- The use of **auxin inhibitor or auxin antagonist via culture medium also** causes the induction of shoot bud.
- Additions of **adenine in the culture medium also induce shoot bud in the** callus tissue.
- **Chelating agent like 1, 3 diamino-2- hydoxypropane-N.N.N'.N' tetraacetic acid initiates Shoot bud in haploid tobacco cultures.**
- **Abscisic acid in place of cytokinin also induces shoot bud formation in** root tuber tissue of sweet potato and stem tuber tissue of potato.

Factors effecting organogenesis

Enzymes

- **Peroxidase-One of the most important functions of peroxidase is** involvement in the metabolism of auxin.
- **Enzymes involving in carbohydrate metabolism- Gibberellic acid,** which represses starch accumulation by mobilising high amylase synthesis/activity, also inhibits shoot formation.
- **Embden Meyerhof-Parnas (EMP) and Pentose Phosphate (PP)** Pathway enzymes namely phosphoglucose isomerase, aldolase, pyruvate kinase, glucose-6- phosphate dehydrogenase, 6-phosphogluconate dehydrogenase etc. also involving in the shoot formation.

Thank You