

#### Regeneration Ability of an Explant 1. Histogenesis (Micropropagation) (cont) depends on: -collection prior to flowering 1. Organ from which it is derived Stage I: 1. Explant isolated is surface sterilized and transferred Initiation into nutrient medium 2. The physiological state of explant/ Differences in the stage of the stage cells in the cell cycle 2. Combined application of bactericide and fungicide 3. Size of the explant (generally) 4. Orientation of the explant on the medium 3. Cultures are incubated in growth chamber either 5. Inoculation density under light or dark conditions according to the method 6. Availability or ability to transport endogenous growth regulators of propagation 7. Metabolic capabilities of the cells \*disinfectants: sodium hypochlorite, calcium hypochlorite, ethanol, mercuric chloride (HgCl2) Plant Regeneration Pathways Stage II: -aim: increase the number of propagules 1. Histogenesis Uses meristematic cells to regenerate whole Multiplic-(Micropropagation; plant (shoot culture/nodal culture) ation stage Pre-existing -number of propagules is multiplied by repeated Meristems) subcultures until the desired (or planned) number of 2. Organogenesis Relies on the production of organs either plants is attained directly from an explant or callus structure -repeated enhanced formation of axillary shoots from 3. Somatic Embryo-like structures which can develop into shoot tips or lateral buds

1. Histogenesis	(Micropropagation)
-----------------	--------------------

- -most commonly used tissue explants are the meristematic ends of the plants like the stem tip, auxillary bud tip & root tip
- -these tissues have high rates of cell division & produce required growth regulating substances including auxins & cytokinin

Stage 0: Preparation of donor

Embryogenesis

-if possible, mother plant should be ex vitro cultivated under optimal conditions to minimize

contamination in the in vitro culture

plant

-explant should be selected from young and healthy part that actively grow

whole plants in a way that is similar to zygotic

embryos are formed from somatic cells



By woozing cheatography.com/woozing/ Not published yet. Last updated 28th April, 2022. Page 1 of 8.

Sponsored by ApolloPad.com Everyone has a novel in them. Finish Yours! https://apollopad.com

-4-8 weeks subculturing intervals (1 cycle)

1. Higher concentration of cytokinin provided 2. Lower concentration of auxin provided

-multiplication is very labor-intensive



#### 1. Histogenesis (Micropropagation) (cont)

3. Gibberellins (GA's) may be added to promote etiolation, especially in species that form rosettes.

Stage III:

- 1. Plants must be rooted by using media containing auxin or by dipping explant bases in auxin solutions.
- Rooting stage
- a) may use the same culture media used in multiplication stage
- b) sometimes, it is necessary to change media, including nutritional modification and growth regulator composition to induce rooting and the development of strong root growth
- 2. Higher concentration of auxin provided
- 3. Lower concentration of cytokinin provided

Stage IV: -aim: in vitro plants need to be weaned and hardened by undergoing acclimatization

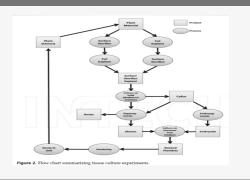
Acclimatization Stage

- 1. Microshoots are moved from sucrose in jar (heterotrophic stage) to photosynthesis (photoautotrophic stage)
- 2. Increasing the light intensity (to harden the plants)
- 3. reducing sugar, inorganic salts and humidity (to harden the plants)

#### 1. Histogenesis (Micropropagation) (cont)

- 4. The plants are then transferred to an appropriate substrate (sand, peat, compost etc.) and gradually hardened under greenhouse
- \*Medium must be removed prior to transplantation to prevent contamination

### Micropropagation Flow Chart



#### Conventional propagation vs Micropropagation

	Conventional propagation	Micropropagation
	Cuttings, budding, grafting, layering	Tissue culture using axillary buds and meristems
Advantages	Equipment costs minimal.     Little experience or technical expertise needed     Inexpensive     Specialized techniques for good ownering not dwarfing not stocky.	From one to many propagate regardly.     Multiplication in centralized laboratorisms & Seduce shock plant conditions & Seduce shock plant.     Precise crop production scheduling.     Long-term germpleam stronge.     Production of difficult-to-Continuous Exoducitop.

### \*\*2. Organogenesis

- -refers to the production of adventitious plant organs i.e. roots, shoots and leaves that may arise directly from the meristem or indirectly from the undifferentiated cell masses (callus)
- -ability of non-meristematic plant tissues to form various organs
- -production of roots, shoots or leaves
- -organs may arise out of pre-existing meristems or out of differentiated cells
- -may involve a callus intermediate but often occurs without callus
- -involves the callus production and differentiation of adventitious meristems into organs by altering the concentration of plant growth hormones in nutrient medium

Type of Organogenesis

1. Direct Organogenesis



By woozing cheatography.com/woozing/

Not published yet. Last updated 28th April, 2022. Page 2 of 8. Sponsored by **ApolloPad.com**Everyone has a novel in them. Finish
Yours!

https://apollopad.com



#### \*\*2. Organogenesis (cont)

- a) Directly from an explant
- b) Axillary bud formation and growth
- 2. Indirect organogenesis

a) Callus culture	Dedifferentiation - less committed, more plastic developmental state
	2) Induction - Cells become organogenically competent and fully determined for primordia production
	3) Differentiation

#### Characteristics

-relies on the inherent plasticity of plant tissues, and is regulated by altering the components of the medium

-auxin to cytokinin ratio determines which developmental pathway

-induce shoot formation by increasing the cytokinin to auxin ratio of the culture medium.

-these shoots can then be rooted relatively simply

- Auxin = Cytokinin = Callus Development

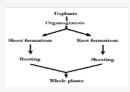
#### Control of Organogenesis

1. Auxin:	-↑ Auxin ↓ Cytokinin = Root Development
Stimulates Root	
Development	
2. Cytokinin:	-↑ Cytokinin ↓Auxin = Shoot Development
Stimulates Shoot	
Development	

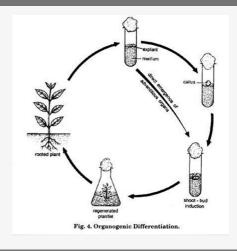
## Advantage

- 1. Mass multiplication of elite germplasm.
- 2. Source material for protoplast work or genetic transformation
- 3. Conservation of endangered genotypes
- \*Organogenesis may not produce clones!

#### Organogenesis Flow Chart



## Organogenesis Process



#### 3. Somatic Embryogenesis

- -in vitro method of plant regeneration widely used as an important biotechnological tool for sustained clonal propagation
- -process by which somatic cells or tissues develop into differentiated embryos, then develop into whole plants without undergoing the process of sexual fertilization
- -Plant growth regulators play an important role in the regeneration and proliferation of somatic embryos
- -usually involves a callus intermediate stage which can result in variation among seedlings
- A) Plant regeneration via somatic embryogenesis occurs by the induction of embryogenic cultures from zygotic seed, leaf or stem segment and further multiplication of embryos
- B) Mature embryos are then cultured for germination and plantlet development, and finally transferred to soil
- Direct Somatic
   Embryogenesis

-Embryos initiate directly from explant in the absence of callus formation.



By woozing

cheatography.com/woozing/

Not published yet. Last updated 28th April, 2022. Page 3 of 8.



### 3. Somatic Embryogenesis (cont) -Though common from some tissues (usually reproductive tissues such as the nucellus, styles or pollen), direct somatic embryogenesis is generally rare 2. Indirect -Embryos initiate from callus developed from explant Somatic Embryogenesis Explant → Callus induction → Callus Embryogenic development → Maturation → Germination 1) Initial high concentration of 2,4-Dichlorophestage noxyacetic acid (selective herbicide) is (embryo used initiation) 2) Second embryos are produced in a medium with no or very low levels of 2,4-D stage (embryo production) \*supplying a source of reduced nitrogen (specific amino acids/casein hydrolysate) can also improve

- also regarded as a valuable tool for genetic manipulation
- -The process can also be used to develop the plants that are resistant to various kinds of stresses and to introduce the genes by genetic transformation. adventitious

## 3. Somatic Embryogenesis (cont)

Various terms for non-zygotic embryos

- Adventitious embryos
   Somatic embryos arising directly from other organs or embryos.
- 2. Parthenogenetic embryos Somatic embryos are formed by the unfertilized egg.
- 3. Androgenetic embryos Somatic embryos are formed by the

male gametophyte.

#### Somatic Embryo Development

- -Auxin must be removed for Continued use of auxin inhibits embryo development embryogenesis
- -Polarity is established early in embryo development.
- -Signs of tissue differentiation become apparent at the globular stage and apical meristems are apparent in heart-stage embryos.

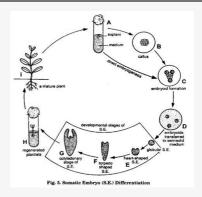
#### **Development Stages**

- 1. Zygote 4. Torpedo
- 2. Globular 5. Cotyledonary
- 3. Heart 6. Germination

#### Characteristics

- 1. Bipolar structure shoot and root pole
- 2. Source of protoplasts and suspension cultures.
- 3. Clonal propagation

#### Somatic Embryogenesis Process





By woozing cheatography.com/woozing/

Not published yet. Last updated 28th April, 2022. Page 4 of 8.



### Somatic Embryo Development Stages



#### Embryogenesis, Organogenesis, Micropropagation

- -Both of these technologies can be used as methods of micropropagation
- -It is not always desirable because both of them may not always result in populations of identical plants which is needed for micropropagtion.
- -The most beneficial use of somatic embryogenesis and organogenesis is in the production of whole plants from a single cell (or a few cells).
- a) High probability of mutations
- b) The method is usually rather difficult.
- c) Losing regenerative capacity become greater with repeated subculture
- d) Induction of embryogenesis is very difficult with many plant species
- e) A deep dormancy often occurs with somatic embryogenesis

Difference of S. Embryogenesis and Organogenesis				
Organogenesis	Somatic Embryogenesis			
-monopolar structure	-bipolar structure with a closed radicular end			
-has vascular connection with	-has no vascular connection with			
the mother tissue	the mother tissue			

Compare Organogenesis and Embryogenesis				
Organogenesis	Embryogenesis			
-Explant or callus is	-Explant or callus is subcultured on			
subcultured on shooting	embryogenesis medium to induce			
medium to induce shoot	formation of pro-embryogenic cell			
formation	masses (PEMs)			

### Compare Organogenesis and Embryogenesis (cont)

-Group of cells differentiate to form shoots (5,000-10,000)

-PEMs are form from single cells and subcultured into the same medium for PEM proliferation (hundred thousands to million)

-Each shoot of appropriate size is identified and excised individually and subculture on rooting medium to induce rooting (labour intensive) -PEMs are split and subcultured onto medium with less auxin in batches (less labour) to grow and differentiate further

-Somatic embryos are subcultured on medium without hormone for germination

Different problems in Plant tissue culture
--

Different problems in Plant tissue culture			
Problem	Description	Way to Overcome	
1. Recalc- itrance	-inability of plant tissue culture to respond to culture manipu- lation	-Antioxidant Protection: Antioxidants are special compounds that have the capability of neutralizing reactive molecules and particles - so called free radicals	
	-loss of morphogenetic competence and totipotency capacity	-Juvenile tissue can be selected as explant	
	-Free radical-m- ediated stress has a role in tissue culture recalcitrance.	-Parts of the desired plant rejuvenated by treatments like cytokinin spray on selected branches	



By woozing

cheatography.com/woozing/

Not published yet. Last updated 28th April, 2022. Page 5 of 8.



Different	problems in Plant tissue culture (cont)		Different problems in Plant tissue culture	e (cont)
	-Free radicals and their reaction products react with macromolecules such as DNA, proteins and enzymes, causing cellular dysfunction and, as a result, the cultures become recalcitrant	-	-Many of the microorganisms that are likely to be present intercellular, in plant tissues will be capable of growth on the plant tissue culture medium, although some may be inhibited by the high salt or sucrose concentration and the pH	-Wipe down working surfaces with ethanol.
	-All aerobic organisms are totally - dependent upon redox reactions and the transfer of single electrons and many life	-		
· ·				-Use sterile equipment.
				-Inspect all equipment and media for visible contam-
2.	-source: a) carry over of microorganisms on the surface or in the tissues of explants; b) faulty procedures in the laboratory	-Wear gloves and a lab coat and keep long hair		ination before use.
ination b				-NO cross over - Do not pass your hands/arms over any open bottle, plate or tube.
	-Bacteria, fungi, mould and yeasts are	tied backWork in a		-Use proper antibiotics in your culture media.
	common contaminating microorganisms in tissue culture.	laminar flow hood when passaging cells.		-When finished, dispose of materials properly, wipe down working surfaces with ethanol, and turn on UV lamp within laminar flow hood for 10 minutes to sterilize the area.



By **woozing** cheatography.com/woozing/

Not published yet. Last updated 28th April, 2022. Page 6 of 8.



Different problems in Plant tissue culture (cont)		Different problems in Plant tissue culture (cont)				
3. Phenolic browning	owning enolic compounds that		-germination of shoots and roots also delayed due to the seasonal variation	-		
	are commonly regarded as inhibitory agents.		5. Vitrif- ica- tion( hyperh ydr- icity)	rif- logical malformation due to ltu- excessive hydration, low lignif- ov- n( ication and reduced mechanical ica- perh strength of tissue culture generated plants.	-Culture are sub-cu- ltured frequently to overcome this vitrif- ication	
	-In most of the cases, when these plants are cultured in vitro, the culture medium turns brown.	-Addition of antioxidants (Polyvinylpyrrolidone, PVP- 40) to medium was more effective to reduce the browning.				
	-Phenolic browning caused by the accumu- lation and oxidation of phenolic compounds.	-inhibiting the activity of the phenylalanine ammonia lyase enzyme (PAL), thereby reducing the biosynthesis of phenolic compounds		Hyperhydricity in plant tissue cultures are those factors triggering oxidative stresses such as high salt concentration, low calcium content in culture	-Vitrification can be lessen by raising the agar and/or sugar concentration, addition of ethylene-inhibitors,	
4. Seasonal variation			medium, gas built up within the container, high relative humidity, low light intensity, gas accumulation in the atmosphere of the jar, length of time intervals	amino acid, phenolic glycosides phloridzin, naringin or esculin hydate, using two- phase media, bottom		
	-extreme moist climate such as poor tropical region, fungi is effected on media	-Use in vitro plantlets as explant			between subcultures.High ammonium concentration, culture bottles kept in same container.	cooling of the culture vessels,ventilation of the vessels, adding silver nitrate
	-dust in air is also a major source of bacterial contaminants	-Controlled environment				



By **woozing** cheatography.com/woozing/

Not published yet. Last updated 28th April, 2022. Page 7 of 8.



Different prob	olems in Plant tissue culture (cont)	
6. Somaclonal Variation	-genetic variations along with phenotypic changes found in the in vitro cultured cells	-Avoiding long term cultures
	-Somaclonal variations occur as a result of genetic heterogeneity (change in chromosome number and/or structure) in plant tissue cultures.	-Axillary shoot induction systems
	-cause: a) Expression of chromosomal mosaicism or genetic disorders; b) ii. Spontaneous mutations due to culture conditions	-Regularly reinitiating clones from new explants.
	-factors: a) Genotype and explant source; b)Duration of cell culture; c) Growth hormone effects	-Prevent usage of 2,4-D IN media

#### **Limitations of Somaclonal Variations**

- i. Most of the somaclonal variations may not be useful.
- ii. The variations occur in an unpredictable and uncontrolled manner.
- iii. Many a times the genetic traits obtained by somaclonal variations are not stable and heritable.
- iv. Somaclonal variations are cultivar-dependent which is frequently a time consuming process.
- v. Somaclones can be produced in only those species which regenerate to complete plants.
- vi. Many cell lines (calli) may not exhibit regeneration potential.

#### **Nodal Cutting**

Function: Removes the inhibitory effect of the shoot apex on bud outgrowth (Apical dominance)

#### **Nodal Cutting Image**



#### Gibberellins

Growth hormones that stimulate cell elongation and cause plants to grow taller.

#### Rosette

Circular arrangement of leaves or of structures resembling leaves

#### Etiolation

Etiolation is a process in flowering plants grown in partial or complete absence of light.

It is characterized by long, weak stems; smaller leaves due to longer internodes; and a pale yellow color.



By woozing

cheatography.com/woozing/

Not published yet. Last updated 28th April, 2022. Page 8 of 8.