

Main applications of plant tissue culture:

1. Clonal Propagation of elite varieties.
2. Conservation of endangered plants.
3. Production of virus-free plants.
4. Germ plasm preservation.
5. Production of secondary metabolites.

Gottlieb Haberlandt ---- Totipotency-----

Mesophyll cells of *Lamium purpureum* --- Father of tissue culture.

Totipotency:

The property of live plant cells when cultured in nutrient medium to give rise to a complete Individual plant.

Dedifferentiation:

The Reversion of mature cells to the meristematic state leads to the callus formation.

Differentiation:

Cells become specialized in form and function.

Redifferentiation:

Already differentiated cell into another type of cell.

eg: Callus → whole plant

Fundamental principles of PTC:

1. Explant Isolation.
2. Explant must be maintained in controlled conditions.

Explant : Tissue from a plant.

Basic Concepts of Tissue Culture:

1. Totipotency
2. differentiation
3. Dedifferentiation
4. Redifferentiation

Laboratory Facilities for PTC:

Washing facility, Ovens.

P^H meter, autoclave, electronic balance.

Laminar air- flow bench.

(HEPA) High efficiency particulate Air.

Culture Facility: Growing explant inoculated into culture tubes. at [22-28°C, 2400 lux, 8-16 hours of Photoperiod]

Aseptic condition:

Invitro cultures free from microbes.

Cell culture:

Culture of single cells invitro in a liquid medium.

Cybrid: Fusion of cytoplasm of cells of different parental sources.

Chemically defined medium:

A nature medium, where each chemical of this medium is known as defined.

Knop's Solution:

Calcium Nitrate	-	3.0g
Potassium Nitrate	-	1.0g
Sucrose	-	50.0g
Dibasic potassium phosphate-		1.0g
Deionized water	-	1000.0ml

Haberlandt - Proposed concept of totipotency.

P.R. White - Developed root cultures used Knop's Solution.

F.C. Steward - used coconut water for cell proliferation of carrot explants.

Morel and Martin- Developed virus free Dahlia.

Murasbige and skoog:

Most frequently used medium.

Formulated tissue culture medium.

kanta et al:

Produced test tube fertilization in flowering plants.

Yamada et al:

produced calli in *Tradescantia reflexa*.

Guha and Maheswari:

Inviro production of haploid embryos (anthers of *Datura*).

Vasil and Hildbrandt:

Regenerated tobacco plants.

Carlson:

Obtained protoplast fusion between. *Nicotiana glauca*.

Nicotiana longsdorffi.

Developed first Inter specific somatic hybrid.

Melchess and co-workers:

Developed Intergenic hybrid between Potato and Tomato called pomatio.

Chilton:

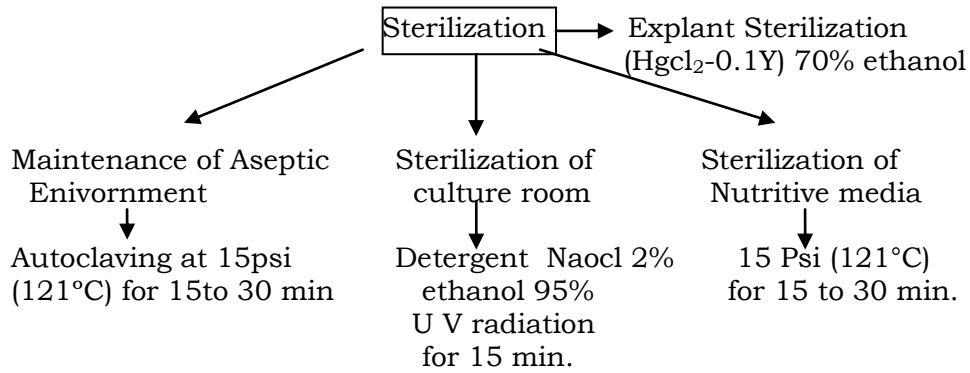
Produced transformed tobacco plants.

Horsh et al:

Developed transgenic tobacco by *Agrobacterium*.

Technique Involved in PTC:

1. Sterlization.
2. Media preparation.
3. Culture condition.
4. Induction of Callus.
5. Embryogenesis.
6. hardening.



Agar: Mucilaginous Polysaccharide obtained from marine algae. It is a non nutritive solidifying agent.

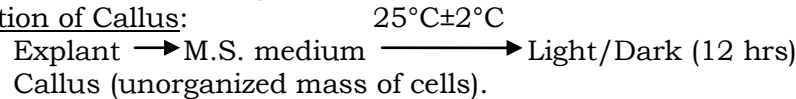
Medium in PTC: Ms, B5, white medium Nitsch.

Culture Condition:

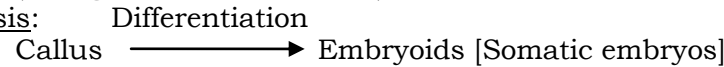
PH	-	5.6 to 6.0
Temperature	-	25°C± 2°C
Humidity	-	50-60% Relativity humidity.
Photoperiod	-	16 hours

Aeration: Shaking flasks with automatic shaker.

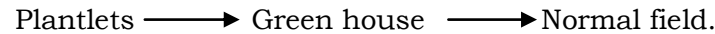
Induction of Callus:



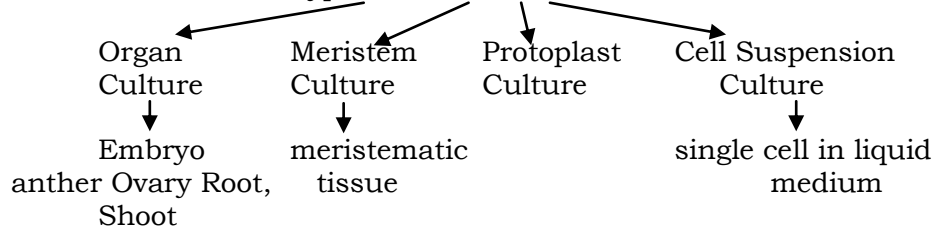
Embryogenesis:



Hardening:



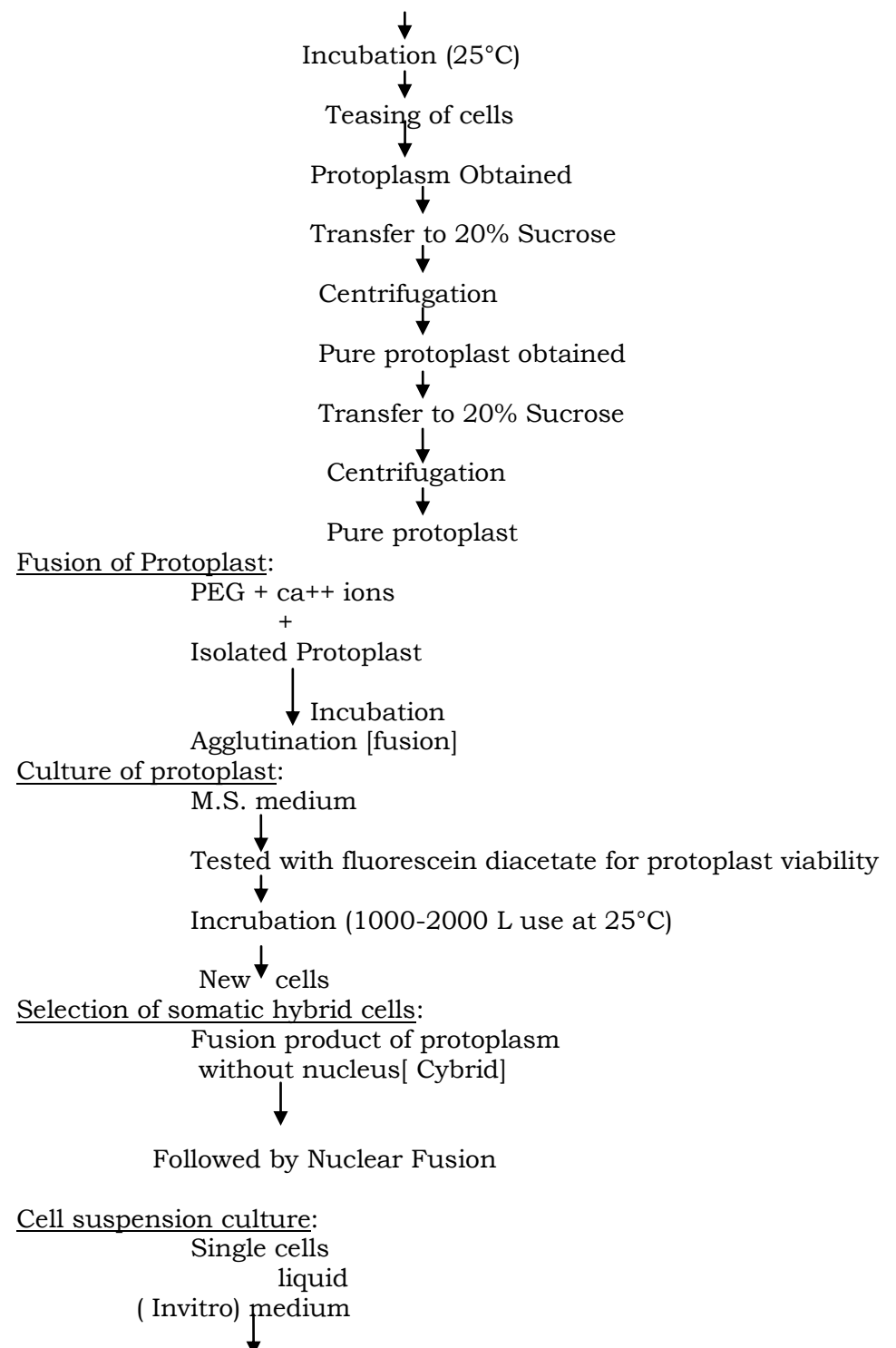
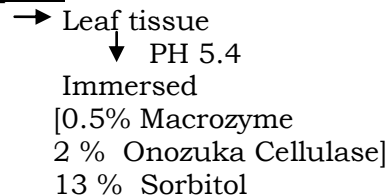
Types of Plant tissue Cultures



Protoplast Culture:

- Cells without a cell wall bounded by a plasma membrane.
- Single cells → Somatic hybrids.

Isolation of Protoplast:



Agitation by rotary shaker

↓
Seperation of cells

Production of Secondary Metabolites:

- Substances not required by the plant for normal growth. [By products]
- Efficient method for the production of Secondary metabolites.
 - i) Biotransformation.
 - ii) Elicitation.
 - iii) Immobilization

Secondary Metabolites:

	Plant source	uses
Digoxin	Digital purpuria	Cardiac Tonic
Codeine	papaver somiferum	Analgeric
Capsaicin	Capsicum annum	Rheumatic pain
Vincristine Indole	Cathoran roseus	Anti carcinogenic
Quinine	Cinchena officinalis	Anti material

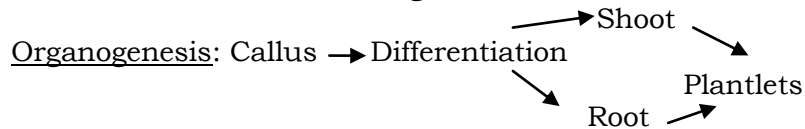
Somatic Embryogenesis:

Callus tissue → Embryo [Embryoids]

Application:

- Potential plantlets.
- Production of Synthetic seeds.
- Reported in Oryza sativa, Allium sativum.

Synthetic Seeds: Encapsulation of embryoids in agarose gel or Sodium, Calcium alginate.



Application of Plant tissue culture:

- Imported hybrids are produced.
- Syn Seeds are produced helps in conservation of plant biodiversity.
- Disease, Stress resistant, herbicide, heat tolerant plants are produced.
- With in a short span of time large number of plantlets are produced.
- Production of Secondary metabolites.

<u>Somoclonal variation</u>	<u>Gameto Clonal variation</u>
Somatic Variation	Gametophytic Variation
Found in Leaf, Stem, root, tuber.	Found in Gametes.

Micropropagation of Banana:

Invitro micro propagation

(Mrsa sp)

↓
1% Naocl 30min
(Sterilization)

↓
M S medium with Benzyl aminopesine, IAA

↓
Kinetin 2.0mg/1 NAA
↓
Root Induction

↓
Polyhouse

↓
Shade house

↓
Genetic uniformity

↓
Transfer to field

Advantages of Artificial Seeds:

- Produced at low cost
- Desirable traits
- Easy to test genotype of plants
- Can be stored for long time
- Growth is faster
- Produce Identical plants

Virus free plants:

Shoot meristem tip culture is used to produce Virus free plants.

Protocol for virus free meristem tip culture

Apical meristem
↓
Leaf primorida

↓
M S medium

↓
24 ± 1°C 2400L

↓
Organogenesis

↓
Transfer to field

Germplasm Conservation:

- Conservation of genetic resources (eg: Pollen, Seeds, Tissues)
- Also involve gene bank, DNA bank.

- Maintenance of biological diversity, food security
- Cryopreservation/Cryoconservation (-196°C)
 - Organs, Matrix, enzymes, cells, organelle.
 - Cryoprotectants → dimethyl sulphoxide, glycerol or sucrose.

IPR: Intellectual Property right

- Consists of copyrights, patents, trade marks, trade secrets, Publicity rights, moral rights.
 - The right of the discover must be protected.
 - IPR is protected by patents, copyrights, trade secrets, designs, geographical indications.

Patents:

- Special right to the discover/ inventor given by the government.
- Inventor rights to use, sell or make his invention.
- Guidance should be obtained from patent attorney.
- A patent is a personal property

Grant	Patent	Specification	Claim
→ Signed document	single document		Invention to be
→ Filled at patent office	Narrative subject matter		protected

Bio safety and Bio ethics:

- ELSI [Ethical legal and social Implications] covers the relationship between biotechnology and Society.
- Prevention of large scale loss of biological integrity, focus on ecology, human health.
- Protect from harmful incidents.

Potential risks and consideration For Safety aspects:

- Pathogenicity of living organisms
- Toxicity of allergy
- Antibiotic resistant micro organisms
- Problems associated with the disposal of spent microbial biomass and purification of effluent.
- Safety associated with contamination, infection, mutation of strains.

Bio Safety guidelines:

- IBSCS → Institutional Bio safety committee → Monitor research activity
- RCGM → Review Committee on Genetic manipulation → monitor risky research activity
- GEAC → Genetic Engineering → Permit use of Gmp modification Approved Committee (genetically organism)
 - Bioethics → ELSI(1990)
 - Moral discernment.

- Relates to medical policy.
- Scope of bioethics related to biotechnology, gene therapy, Cloning, life inspace.
- Integral part of Human genome project..
- ELSI used to address the issues raised by genomic research.

Ethical Issues in Genomic Research:

- Potential for genetic discrimination in employment.
- Genetic testing into practice of clinical medicine.
- Conduct of genetic research with people.

GEAC: Genetic Engineering Appraisal Committee

- Under ministry of Environment forests, climate change.

- Storage of GMO and hazardous microbes.
- Release of Genetically engineered organisms including field trials (BRL-I,II) Bio safety Research Level trial- I&II.

Future OF Biotechnology:

New scientific Revolution that would change the lives.

with in a short time with the sequencing of human genome and genome of some Important Organisms.