PLANT
TISSUE CULTURE

DEFINITION

Tissue culture is *in vitro* cultivation of plant cell or tissue under asceptic and controlled environmental conditions, in liquid or on semi solid well defined nutrient medium.

- To quickly produce mature plants.
- The production of multiples of plants in the absence of seeds or necessary pollinators to produce seeds.
- The regeneration of whole plants from plant cells that have been genetically modified.

- The production of plants in sterile containers that allows them to be moved with greatly reduced chances of transmitting diseases, pests, and pathogens.
- The production of plants from seeds that otherwise have very low chances of germinating and growing, i.e.: orchids and *Nepenthes*.

- To clean particular plants of viral and other infections and to quickly multiply these plants as 'cleaned stock' for horticulture and agriculture.
- Plant tissue culture relies on the fact that many plant cells have the ability to regenerate a whole plant (totipotency). Single cells, plant cells without cell walls (protoplasts), pieces of leaves, stems or roots can often be used to generate a new plant on culture media given the required nutrients and plant hormones.

Techniques

- Modern plant tissue culture is performed under aseptic conditions under HEPA filtered air provided by a laminar flow cabinet.
- Living plant materials from the environment are naturally contaminated on their surfaces (and sometimes interiors) with microorganisms, so surface sterilization of starting material (explants) in chemical solutions (usually alcohol and sodium or calcium hypochlorite)[1] is required.

- Explants are then usually placed on the surface of a solid culture medium, but are sometimes placed directly into a liquid medium, particularly when cell suspension cultures are desired.
- Solid and liquid media are generally composed of inorganic salts plus a few organic nutrients, vitamins and plant hormones. Solid media are prepared from liquid media with the addition of a gelling agent, usually purified agar.

REQURIMENTS

- Well equipped culture laboratory.
- Nutrient medium containing organic and inorganic salts, amino acids, supplemented vitamins and growth hormones.
- Sterilization of explants [source of plant tissue]
- Glass wares, Inoculation and incubation.

ADVANTAGES

- **Availability of Raw material** : Difficult in cultivation.
- **TC- Regular and uniform supply** Medicinal plant industry
- **Fluctuations in supplies and quality** Due to changes in climate, crop disease and seasons – collection, drying,& storing- quality of crude drugs are maintained in TC.

- **Patent rights** :- natural plants or their metabolites- individually R&D- production of biochemical compounds, constant supply, newer for the isolation and improvement of product(TC).
- **Political reasons**: “Rauwolfia serpentina, Dioscorea etc.
- **Easy purification of the compound**

- **Modifications in chemical structures**: Specific chemical compounds can be achieved.
- **Disease free and desired propagation:** Large scale production in limited space.
- **Crop improvement**: Production of hybrids.

- **Bio-synthetic pathway**:For tracing the bio-synthetic pathways of the secondary metabolites using labeled precursor in the culture medium.
- **Immobilization of cells** preservation cells facilitates transportation & bio-transformation

HISTORICAL DEVELOPMENTS

Principles of tissue culture in cell theory- Schleider & schwan (1839-1888).

- 1892 –First attempts to isolate protoplast mechanically – Klercker.
- 1902 – 1st cultivation experiment with isolated plant cells – "*Tradescantia* species".
- 1904 – 1st establishment of embryo cultures – "*Cochleria Raphanu*".
- 1909 – 1st observation of fusing cells – "Kuster".

- 1922 – *Invitro* cultivation of root tips, no permanent cultures obtained – “zea pirua”.
- 1934 – 1st permanent root cultures – white - “*Lycopersicum*”.
- 1934 – 1st permanent callus cultures using B vitamins and auxins
- (nicotiana glauca species) – “Gautheret”.
- 1942 – Observation of secondary metabolites in plant callus culture - “Gautheret”.
- 1983 – 1st industrial production of sec.plant products by suspension culture.

BASIC REQUIREMENTS FOR A TC LABORATORY

- Scissors,scalpels &forceps - explant preparation and transfer.
- Spirit burner or gas micro burner - sterilization of the instruments.
- Autoclave to sterilize the media,pressure cooker - small amount media.
- Hot air oven - sterilization of glass wares etc.
- Ph meter - to adjust the Ph of the media.

- A shaker - to maintain the cell suspension culture.
- Weighing balance - to weigh various nutrients for the preparation of the medium.
- Incubating chamber or laminar flow with UV light fitting for aseptic transfer of explants to the medium – subculturing.
- Incubator-maintaining temp for the growth of culture .

Washing & storage facilities

- Fridge freezer-to maintain culture media &stock solutions.
- Fresh water supply &disposal of waste water.
- Distillation unit - distilled , double distilled & de ionised water.

- Acid & alkali resistant sink or wash basin working table.
- Dust proof cupboards or cabinet for storing dried glass wares.
- Maintain cleanliness in the area of washing,drying &storage.

Media preparation room

- Supply space for chemicals, glass wares, culture vessels and equipments.
- Ph meter, water bath, Bunsen burner with gas supply , autoclave, microwave oven, pressure cooker,fridge,freezer-storing preparation.

Sterilization room

(for sterilizing the culture media)

- For high amount – good quality ISI mark autoclave.
- Small amount-pressure cooker.
- For glass wares and metallic equipments-hot air oven with adjustable.

Aseptic chamber/area for transfer of cultures

- For transfer of culture into sterilized media ,small wooden are with glass or plastic door with UV tube –in a corner of lab.
- Laminar air flow-air filter(0.3micro.m HEPA filter)-free from fungi and bacterial contaminant.
- Bunsen burner and UV tube fitted on the ceiling of the inside cabinet .

Incubator or incubation room

- Light and temp controlled devices , uniform air circulation for storing the culture vessels(humidity - 20-90%,temp 2-40℃) Label-date of inoculation ,name of explant medium-identity for maintaining the data of experiment.

Data collection and recording of the observation

- Growth and the maintenance of the tissue culture observed under aseptic conditions and recorded at regular intervals.
- Observe under laminar air flow-feed to computer .

GENERAL PROCEDURE INVOLVED IN PLANT TISSUE CULTURE

- Sterilization of glassware tools or vessels.
- Preparation and sterilization of explants.
- Preparation of callus from explants.
- Proliferation of cultured callus
- Sub culturing of callus
- Suspension culture

Sterilization of glassware tools or vessels

- Dipped in sodium dichromate-sulphuric acid soln.-kept for overnight washed with fresh water and distilled water – inverted position , keep in tray.
- Dried in hot air oven ,120 degree centigrade for 30 min to 1 hr.

- For sterilization of explants, chromic acid , mercuric chloride (0.11%) calcium hypo chloride , sodium hypo chlorite (1-2%) & alcohol (70%) are used.
- Tissue is immersed in the solution of sterilizing agent→10-20min →washed with water→sodium hypo chlorite→20min→inoculated after washing with water

SEEDS

- Dip in ethyl alcohol for 10 seconds.
- Rinse with water add 10% w/v aq.Ca.hypochloride after 20-30minutes add 1% bromine water after 5 min.
- Wash with sterile water – Germinated on damp sterile filter Paper.

FRUITS - Rinse the fruit with absolute alcohol.

Dip in 2% w/v sol of sodium hypochlorite for 10 minutes and Washed with water

**STEM - ** Rinse with pure alcohol. Submerge in 2% w/v sodium hypochlorite for 15-30minutes and Wash with sterile water

LEAVES

- Purified with water & absolute ethyl alcohol.
- Dip in 0.1% w/v mercuric chloride solution and Wash with water to make it free from water.
- Dry on sterile tissue paper.

Production of callus from explant

Sterilized explants→medium in flask→Incubated at 25 degree centigrade→with light→ 3-8 days of incubation→Production of callus.

Proliferation of callus

Well developed callus →cut in to small pieces → fresh medium with growth hormones→ production of more amount of callus

Sub culturing of callus

Callus→ fresh medium→to maintain viability of cells→ 4-6 weeks interval.

Suspension culture (uniform suspension of separate cells in liquid medium)

Callus→liquid medium→ agitated to separate the cells→50-150rp/m

(Rotary shake system)→incubator →ppn of sub culture.

CULTURE MEDIA

1942---- Gautheret
1943---- White
1946---- Haberblandt
1953---- Haller (Hellers)
1956---- Nitsch &Nitsch
1962---- Murashige & skoog (MS)
1965---- Eriksson (ER)
1968---- Gamberg et al (B)

MEDIA COMPOSITION

(INORGANIC NUTRIENTS)

Macro nutrients

(Includes 6 major elements)

N — Nitrogen

P— Phosphorus

K— Potassium

Ca — Calcium

Mg— Magnesium

S— Sulphur

Micro nutrients

Cu— 0.1 µmol/lit——— copper

Fe—1 µmol/lit——— iron

Mo—5 µmol/lit——— molybdenum

Zn—1.5-30 µmol/lit——— zinc

Mn—20-90 µmol/lit——— manganese

B—25-100 µmol/lit——— boron

ORGANIC NUTRIENTS

Nitrogenous substances

- Essential vitamins Thiamine (vitamin B_1)
- Pyridoxine (vitamin B_6)
- Nicotinic acid (vitamin B_3)
- Ca pentothenate (vitamin B_5)
- Ionisitol

Complex nutritive mixture casein hydrolysate, coconut milk, corn milk, malt extract, tomato juice, yeast extract

Carbon source : sucrose 2-5 % ,glucose& fructose, maltose, galactose, mannose, lactose,& sorbital

Growth hormones

- **Auxins** - induces cell division,stimulates root formation.
- **Cytokinin -** chemically it is adenine derivative to promote the formation of adventious buds & shoots . kinetin is 30,000 times more potent than adenine.
- **Zeatin -** Isolated from maize embryos at milky stage.
- **Gibberellins** - To increase shoot elongation in TC (very rarely used).

Solidifying agents

- Agar, alginate, gelatin, carrageenan, silica gel, starch, hydroxyethyl cellulose, polyacrylamide

PH

- Culture medium 5-6 before sterilization.
- Heigher PH than 6- Hard medium.
- Lower PH than 5—Gelling of agar does not takes place.

Media preparation

- Required quantity of Culture media packed in glass or vessels are sealed with cotton or with aluminium foil and autoclaved at 121^0c For 15-40 min

STEPS OF PLANT TISSUE CULTURE

- Root tip culture
- Leaves or leaf primordia culture
- Shoot tip culture
- Complete flower culture
- Anther and pollen culture
- Ovule and embryo culture
- Protoplast culture
- Hairy root culture

Root tip culture: (clone of isolated roots)

- Tips of the lateral roots are sterilised,excised & transferred to fresh medium several roots develop after 7 day stock or explant culture.

Leaves or leaf primordia culture

- (800µm)leaves(shoots) solidified medium young leaves have more growth potential

Shoot tip culture

- Excised shoot tips(100-1000µm long) - cultured in nutrient media & growth hormones - development of whole plants.

Complete flower culture

(Nitsch in 1951)

- Flower(2days after pollination) ---- excised --- - sterilized by immersion ---- 5% Ca hypochloride --- - washed with sterilised water ---- culture tube(In agar medium).

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Anther and pollen culture

- Young flower buds are sterilized - anthers separated -nutrient medium.

Ovule and embryo culture

- Matured embryo of the ovule culture media seeds 70% alcohol 2min washed with sterile H_20

Protoplast culture

- Naked cells without cell walls. Cultivated in liquid and solid media.

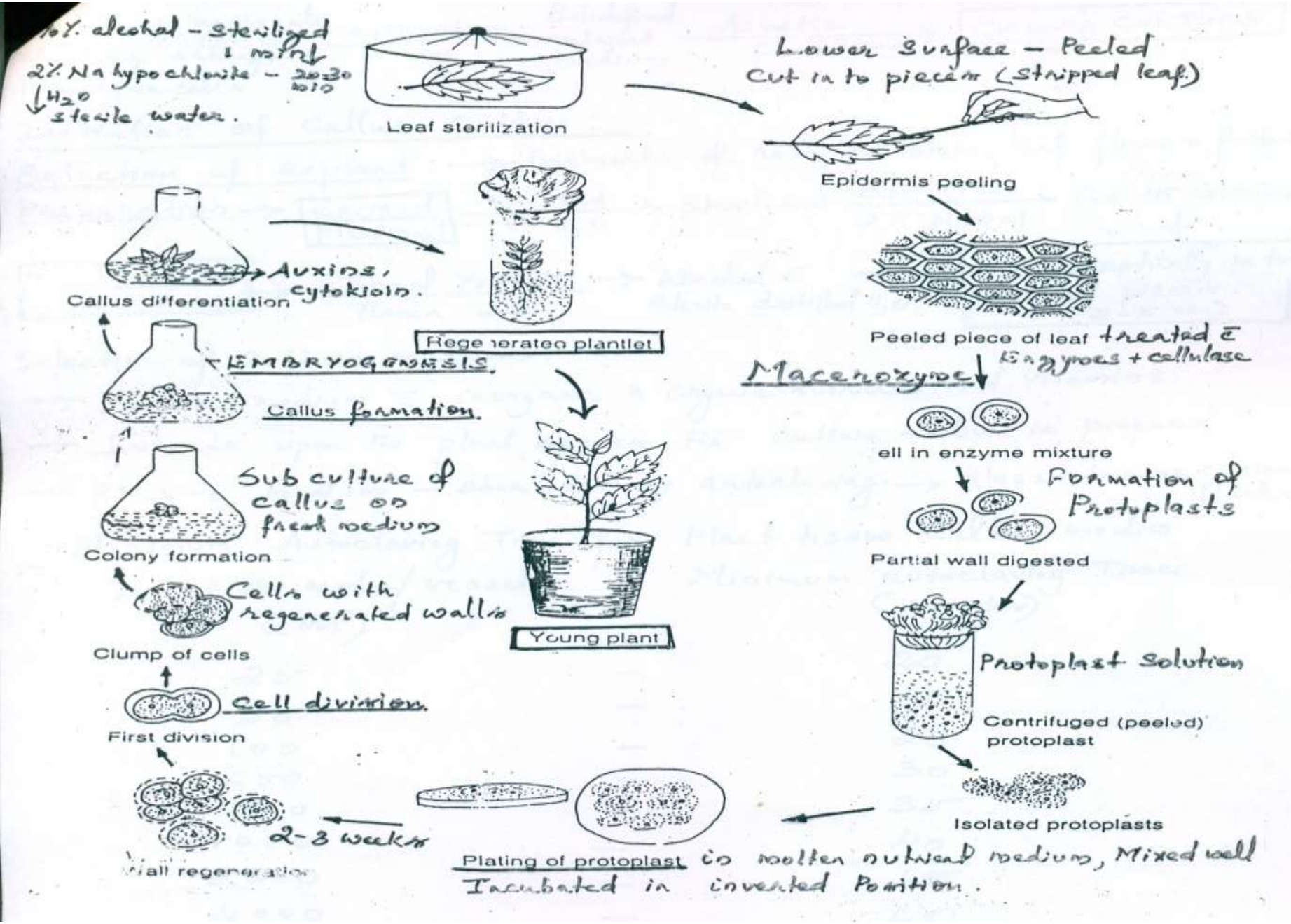
Hairy root culture

- “Hairy roots”- STEWARD et al(1900)
- Large no of small fine hairy roots covered with root hairs originate from explants *Agrobacterium rhizogenes* infection- HAIRY ROOTS.

MAINTENANCE OF CULTURES

- Three main culture systems, selected on the basis of the objective:
- Growth of callus masses on solidified media (callus cultures also known as static culture).
- Growth in liquid media (suspension culture) consists of mixture of single cells or cell aggregates.
- **Protoplast cultures** – can be grown as callus cultures (static culture) or suspension cultures.

PROTO PLAST CULTURE



- Protoplast culture is the process in which isolated protoplasts are cultured in a liquid or semi solid agar medium contained in a petri dish either as a thin layer or as small drops.
- Proto plasts are naked plant cells lacking the cell wall, but possessing the plasma membrane and other components.
- The medium components for protoplast culture are the same as used for callus or suspension culture.

Stages of protoplast culture

It has 4 developmental stages.

- The viable protoplasts develops its own cell wall in culture and then is prepared for sequential cell division, which forms callus.
- The callus through organo genetic or embryo genetic differentiation gives rise to plantlets or embryos.
- Since these plant lets or embryos develop from a single protoplast, their genetic constituent is same.

- The first requirement for cell division is the regeneration of cell wall, after which the walled cells expand and divide in to 8 shaped daughter cells.
- Each daughter cell divides in to 2 cells after first cell division.
- Repeated division forms cell clump or cell aggregates. Not all the cells derived from protoplasts undergo division.
- The genotype of donar plant culture medium, hormones and physical factors play important roles in protoplast division and callus formation.

HAIRY ROOT CULTURE

- **Steward et al,**mentioned the term hairy root in one of his literatuers.
- A large number of small fine hairy roots are found to be covering the roots.
- Hairy roots originate from the explant infected with ***Agrobacterium rhizogenus***.
- These are fast growing, highly branched, adventitious roots which grow at the infection site and even on a culture medium without hormone.

- Investigations are carried out by using hairy root culture methods on those plant cell culture systems which failed to produce an adequate amount of desired compounds.
- With the use of various bacterial strains, a diversified range of plant species has been transformed.
- The transformed roots are capable of synthesizing secondary metabolites, which are specific to the plant species from which they have originated.

Callus culture

- Amorphous aggregates of loosely arranged parenchyma cells cultivated on solidified nutrient medium under aseptic conditions.

INITIATION OF CALLUS CULTURE

- Selection of explant
- Segments of root or stem, leaves, flowers, fruits etc

Preparation of callus culture

- Excised plant parts are washed with water and sterilzed with 0.1% Hgcl2 and 2% Naocl. Then cut aseptically into small parts washed with sterile water and treated with ethanol 70 to 90 % and cultured in a medium.

Selection of culture medium

- Nutrient medium with inorganic and organic nutriants and vitamins
- Depends upon the plant species the culture media is prepared
- P^H 5-6 ,medium – sterilized by autoclaving-glass tubes or conical flasks.

Minimum autoclaving time for plant tissue culture media

Vol. of media /vessel (ml)	Min autoclaving time (in minutes)
25	20
50	25
100	28
250	30
500	35
1000	40
2000	45
4000	65

Transfer of explants

- Sterilized organs (explants) of stem, root or leaf etc are transferred aseptically into semi solid culture media called as inoculation process.

Incubation of culture

- Inoculated culture vessels are incubated at 25 to 28℃ for 12 hours. Then add auxin to media
- Cell division is induced after 3 – 4weeks. Formation of callus occur which is 5 times more than the explant.

Stages involved in the Formation of callus

(3 stages)

1. **INDUCTION** - Formation of no. of cells
2. **CELL DIVISION** - explants cells- individual cells
3. **CELL DIFFERENTIATION**

Sub culture of callus:

- Callus tissue (5-10mm in d.m 20-100 mg in wt) transfer aseptically into fresh medium

Colours of callus

- **White-** grown in dark due to absence of chlorophyll
- **Green**- grown in light
- **Yellow**- due to the presence of carotenoids pigment in large amount
- **Purple-** due to accumulation of anthocyanins
- **Brown-** due to excretion of phenolic substance and formation of quinones

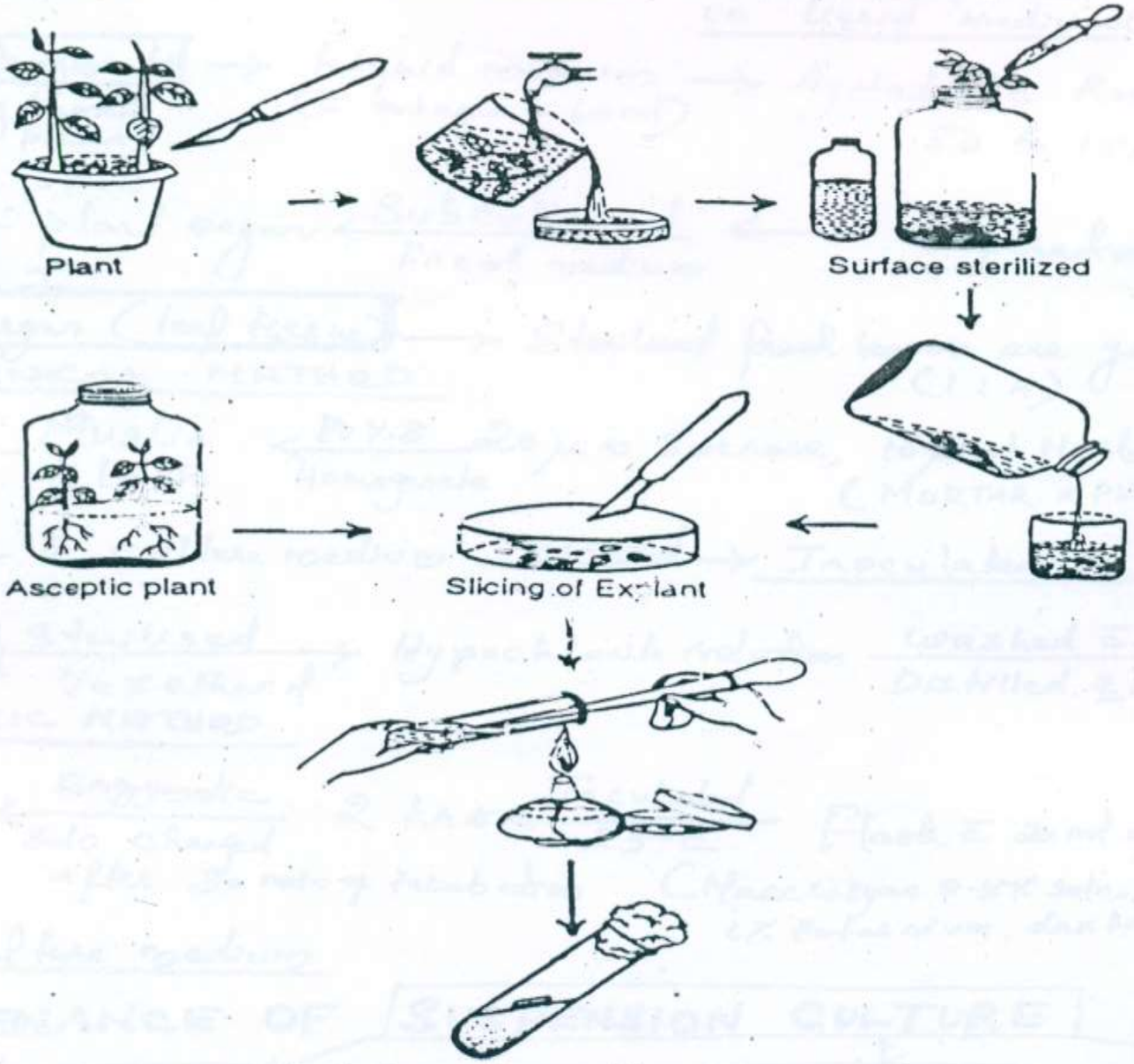


Fig. 24.3: Diagrammatic presentation of the procedure for surface sterilization of plant material and inoculation of explant for culture.

SUSPENSION CULTURE

- uniform suspension of separate cells in liquid medium (with out agar)
- Callus fragments makers (300-500mg small pieces) in liquid medium agitated with rotary (50-150r/min) separation of cells occurred sub cultured in fresh medium - Fresh plant organ .

Mechanical method

- Sterilized fresh leaves are grinded in a mortar and pestle with 20 µ gm of sucrose, 20 µ gm HCL buffer and 10 µ mol of $MgCl_2$ at 7.8 PH and homogenate. Then filtered in muslin cloth washed with water centrifuge with culture medium sieved and inoculated on the culture dish.

Enzymatic method

- Leaves are sterilized with 70 % ethanol and hypochlorite solution. Washed with distilled water.
- Peel of the lower surface cut into pieces. Put in a flask with 20 ml of sterilized enzyme solution (macerozyme 0.5 % solution 0.8 % mannitol , 1% potassium sulphate)
- Incubated at 25 c for 2 hours, wash the cells and inoculate in a fresh culture media.

Batch suspension culture

(closed type)

Cells are allowed to multiply- in liquid medium

Semi continuous suspension culture

(open type)

Periodical removal of culture and addition of fresh medium

Growth is maintained continuously

Continuous suspension culture

Closed type - cultured medium is added constantly to maintain fresh

Open type - equal vol. of culture maintained

Open type is divided into 2 types

Chemostats – desired rate of growth is maintained by adjusting concentration of nutrients in fresh medium.

Turbidostats – cell density is maintained.

Clonal propagation

- Cloning technology allows to generate a population of genetically identical molecules, cells, plants or animals
- In molecular cloning the word ‘clone’ refers to a gene or DNA fragment and also to the collection of cells or organisms such as bacteria containing the cloned piece of DNA.
- In cellular cloning a type of cell culture produces cell lines of identical cells.

- In mono-clonal antibody technology, scientist isolate one cell from an array of anti-body producing cells and then generate a clonal cell line from that cell.
- Plant clones are produced by rooting small pieces of fully developed plants.
- Animal cloning requires cells specialised for reproduction either eggs or embryonic cells at the vey early stages of development.

- In 1952, animals were cloned by transfering genetic material from the nucleus of frog embryonic cell to frog eggs.
- In 1997, **Dolly sheep** was cloned by animal cloning technology.
- **In 1980,** cattle was cloned and have now produced 100s of cattle, sheep and swine embryos.
- Dolly was considered a scientific break through not because she was a clone, but because the source of her genetic material was an adult animal cell not an embryonic one.

Molecular cloning

- It provides the foundation of the molecular biology revolution and is a fundamental and essential tool of biotechnology research, development and commercialization.
- All applications of recombinant DNA technology are from basic research to pharmaceutical production depends on molecular cloning.

Cellular cloning

- It is also a fundamental and essential tool of biotechnology research, development and commercialization.
- The regeneration of transgenic plants from single cells pharmaceutical manufacturing based on mammalian cell culture and generation of therapeutic cells and tissues depend on cellular cloning.

Animal cloning

- For studying genetic diseases, aging and cancer such as gene and cell theraphy.
- Finally in the feature , zoo researchers may help save endangered species with animal cloning.
- In Aug 1998, a rare breed of cow was successfully cloned.

Cell Totipotency

- A plant grows by increasing its cell population while the cells specialize their functions.
- Increasing cell population is done by cell division (also called mitosis).
- Before a mother cell divides into two daughter cells, it makes an exact copy of its genome first.

- As a result, the two daughter cells usually have exactly the same genetic makeup as their mother cell.
- Therefore, every living cell of a plant should contain all the genes the plant has and thus has the capacity to grow back to a full plant. This is called cell totipotency

Applications

- Plant tissue culture is used widely in the plant sciences, forestry, and in horticulture.
- The commercial production of plants used as potting, landscape, and florist subjects, which uses meristem and shoot culture to produce large numbers of identical individuals.

- To conserve rare or endangered plant species.
- A plant breeder may use tissue culture to screen cells rather than plants for advantageous characters, e.g. herbicide resistance/tolerance.

- Large-scale growth of plant cells in liquid culture in bioreactors for production of valuable compounds, like plant-derived secondary metabolites and recombinant proteins used as biopharmaceuticals.
- To cross distantly related species by protoplast fusion and regeneration of the novel hybrid.
- To rapidly study the molecular basis for physiological, biochemical, and reproductive mechanisms in plants, for example in vitro selection for stress tolerant plants,and in vitro flowering studies.

- To cross-pollinate distantly related species and then tissue culture the resulting embryo which would otherwise normally die (Embryo Rescue).
- For chromosome doubling and induction of polyploidy,[9] for example doubled haploids, tetraploids, and other forms of polyploids. This is usually achieved by application of antimitotic agents such as colchicine or oryzalin.

- As a tissue for transformation, followed by either short-term testing of genetic constructs or regeneration of transgenic plants.
- Certain techniques such as meristem tip culture can be used to produce clean plant material from virused stock, such as potatoes and many species of soft fruit.
- Production of identical sterile hybrid species can be obtained.

APPLICATIONS OF TISSUE CULTURE IN PHARMACOGNOSY

1. Production of the phyto pharmaceuticals
2. Biochemical conversions
3. Clonal propagation (Micropropagation)
4. Production of immobilized plant cells

Production of Phytopharmaceuticals

- In 1959 wenstein et al - production of steroids by T.C
- In 1969 -kayl - production of steroids (*Dioscorea sylvatica)* 1.2% Diosgenin
- Several medical plants has been initiated by TC
- Compounds with high yield of sec. metabolites are produced

- (For Ex) Anthraquinone derivative 10 times more -“*Cassia tora”*- SC
- Anthraquinone derivative 20 times more - “*Morinda citrifolia*”- SC
- 1.5% dry wt of diosgenin- “*Dioscorea deltoidia”*
- 4 times more ajmalcine- “*catharanthus roseus”*

BIO TRANSFORMATION (OR)BIO CHEMICAL CONVERSION

- Conversion of small parts of chemical molecules by means of biological systems substrate can be modified.

  (eg)”podophyllum peltatum” produce anti cancer

CLONAL PROPOGATION (MICRO PROPAGATION)

Single individual cell entire plant

(CLONE)

To produce high yielding crops in a short period of time

(for eg) "*Foeniculum vulgare*"

equal composition of volatile oil, high yield of fennel with uniform characters

IMMOBILIZATION OF PLANT CELLS

- Plant cells are immobilized by using matrices such as alginates polyacrylamides, agarose and polyurethane fibres .
- Immobilized cell system are used for bioconversion such as
- (-)codeinone to (-) codeinine and digitoxin to digoxin.

- For biotransformation reactions such as glycosylations, hydroxylation, acetylation, demethylation etc.
- For tracing the biosynthetic pathways of secondary metabolites.
- For carrying out the biotransformation or biochemical reaction .

EDIBLE VACCINES

- In the last decade advancements in the field of medicine and health care,the newer safer and highly effective vaccines like re combinant vaccines, DNA vaccines, sub unit vaccines,peptide vaccines were introduced.
- Edible vaccines are transgenic plant and animal based production of those that contains agents that trigger an animals immune response.
- Edible vaccines are plant or animal made pharmaceuticals.

- Infectious diseases can be prevented by vaccines.
- 20% of infants not getting immunized and 2million/annum deaths occur in the remote and poor areas of the world.
- This happens due to limited production, distribution and delivary of vaccine.
- This can be prevented by making the un immunized populations in to immunized, safe areas.
- Immunisation for some infectious diseases is not available.

- This may be they have not discovered or they are unreliable or too expensive.
- (For ex) - immunization using DNA vaccines is a substitute but expensive,and also it has some undesirable immune responses.
- They have storage and transportation problems, most of them need to be stored in refrigerator.
- So easily storable vaccines can be produced from plants as a substitute for traditional vaccines.

- Edible vaccines are developed by introducing the selected genes in to the plants, and then allowing the production of encoded proteins by these altered plants(transgenic plants) the process is termed transformation.

- Similar to traditional sub unit vaccines the edible vaccines also have antigenic proteins and do not carry pathogenic genes.
- The traditional vaccines are un affordable and require technology, purification, refrigeration and give poor mucosal response.
- Edible vaccines do not require trained medical person for oral administration in children they are effectively produced and can be easily available.

- For producing edible vaccines plant products can be taken raw so that no degradation occurs during cooking.

Some antigens produced from plants;

- CT-B - Potato
- Foot and mouth virus – Arabidopsis
- Herpes virus B surface antigen –Tobacco
- Human cyto megalo virus gluco protein B- From tobacco.
- Rabies glycol protein – Tomato.

(CT-B=Cholera toxin , B – sub unit)

- The concept of edible vaccine was developed by **Arntzer** in the 1990`s.
- He is the head of the department of plant biology at the Arizona state university.
- The demonstration of edible vaccine was the expression of surface antigen from the bacterium streptococcus mutans in tobacco.
- As the bacterium causes dental cavities it was found that it prevents bacteria from damaging the teeth and therefore protect against tooth decay.

CURRENT STATUS

- Several plant derived vaccines for human use were introduced in the market but the first commercial plant derived vaccine is an veterinary vaccine.
- The first vaccine was developed by Dew Agro sciences in 2006.
- Edible vaccine contains DNA fragments of a protein that is usually a surface protein of the pathogen.
- This induces the bodys immune response.

EXAMPLES OF EDIBLE VACCINES

1.Transgenic potatoes for diarrhoea

In 1997,the first human trial for an edible vaccine was conducted which came out to be successful.

The volunteers were administered transgenic potatoes having the b-subunit of E.Coli heat labile toxin causing diarrhoea.

Rise in serum antibodies 1999 was studied in 10 volunteers out of 11.

- Another clinical trial at Boyce Thompson institute at cornell university, USA showed an immune response in 19 volunteers out of 20 who were administered potatoes having Norwalk virus (causing vomiting and diarrhoea).
- Potato based edible vaccines have to be ingested raw as cooking denatures the protein and makes the vaccine ineffective this is the major problem with vaccine potatoes.

2.Transgenic tomatoes against diarrhoea

- At cornell university,USA the transgenic tomatoes were discovered to be acting against Norwalk virus (causing severe diarrhoea).
- These tomatoes produce surface proteins that are specific to the virus.
- Even in mice,these transgenic tomatoes produced immune response for the virus.

ADVANTAGES

- They have better immunization action as they do not require subsidiary elements for stimulating immune response.
- They cause mucosal immunity.
- They are affordable since they do not require cold chain storage conditions like traditional vaccines.
- They have greater storage stability.
- They don’t require sophisticated machines or equipment's they can be grown on rich soils.

- They can be administered orally (Traditional vaccines are injected) so contamination risk is not there. And sterilization is not required.
- They are better in activity.
- Their production process is rapid.
- They are safe, they lack heat killed pathogens which removes the risk of protein reformation in to an infectious organism.

DIS ADVANTAGES

- Immune tolerance may develop in the individual for particular vaccine protein or peptide.
- Their dose changes on the basis of generation, plant, protein content, patients age and weight, ripeness of the fruit and food quantity intake.
- Their administration requires standardization methods for plant material.low doses produce lower antibodies and high doses cause immune tolerance.

- They depend on plant stability since some food cannot be eaten raw and needs to be cooked (potato)which may denature or weaken the protein present.
- Microbial attack can be caused. (for ex) vaccine potatoes can be stored at 4℃ for longer time but tomatoes cannot.
- Their function gets disturbed due to the variation in glycosylation pattern of plants and humans.
- Difference should be maintained between the vaccine fruit and normal fruit.otherwise vaccine tolerance will develop.

APPLICATIONS

1.Cancer therapy

Monoclonal antibody in soyabean (BR-96) which acts on doxorubicin causing breast cancer,ovarian cancer,colon cancer and lung tumours.

2.Birth control

Tobacco mosaic virus(TMV) administration produces a protein present in ***mousezona pellucida***(ZB3 protein)this protein inhibits egg fertilization in mice because of the antibodies formed.

3.Chloroplast transformation

The chloroplast genome transmission in to crops through usual cross pollination is not possible because of its nature.

4.Role in Auto immune diseases

Diseases under investigation are multiple sclerosis, rheumatoid arthritis etc. The results showed that the protein successfully suppressed the immune attack and delayed the on set of high level of blood sugar.

5.Re combinant drugs/proteins

- Many vaccines and antibodies are produced by re combination technique.
- The plant compositions are changed by engineered viral inoculations so that it gives enzymes: drugs
- (albumin,serum protease and interferon) hirudin an antithrombin anti viral protein capable of inhibiting the HIV Virus in vitro.