Plant Tissue Culture

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Plant biotechnology

Plant biotechnology is an field that apply technology on plant life.

Historical development of PTC:-

1902	C.Haberlant	First attempt to culture isolate plant cell in vitro on artificial medium.
1922	WJ Robbins& w kotte	Culture of isolate roots
1934	P R White	Demonstration the indefinite culture tomato roots
1939	RJ Gautheret & Nobecourt	First long term PTC of callus
1939	P R White	Callus culture of tobacco tissue from intersepcific hybrid of *Nicotina glaucum X N. longsdorffi*
1941	Overbeek	Use coconut milk containing cell division factor first time in datura.
1951	Skoog and Tsui	Discovery of kinetin
1952	Morel and Martin	Discovery of virus free plnant
1960	Jones et al	Drop method of cell division
1960	Bergmann	Bergmann cell culture
1962	Skoog	Development of MS media
1970	Power et al	Protoplast fusion
1971	Takabe et al	Regeneration of first plant from protoplast

Plant tissue culture

- A method of biological research in which fragment of tissue from plant are grow in vitro in artificial medium under aseptic condition to survive and function.
- PTC is an collection of technique used to maintain or grow plant cell, tissue or organ under sterile conditions on nutrient culture medium.

Type of culture:-

Callus culture

Suspension culture

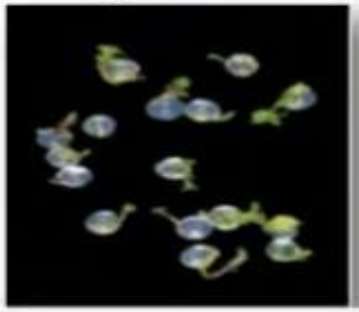
Pollen culture

Ovule culture

Root tip culture

Shoot tip culture

Protoplast culture



Leaf primordial culture

Flower culture

Nutritional requirement for PTC:-

- No single medium support growth of all tissue.
- The growth medium depends on the plant species being grown. The medium contains the following components.
- Medium is composed of inorganic salt, iron, vitamin, amino acids, plant hormones and carbohydrates
- All of the minerals and vitamins needed for plant growth.

- Energy/Carbon source such as sugar (sucrose) are used.
- Various growth regulator are also used.
- Agar is used to solidify the medium
- Minerals are supplied in the form of salts viz.N, Mg, K, Ca, P.
- Vitamins viz. Thiamin(B1) Nicotinic acid (B3) Pyridoxine(B6) etc.
- Growth hormones viz. cytokinens, Gibberline, Ethylene, Auxins, Abscisic acid etc.

Protoplast Tissue Culture:-

- Protoplast:- protoplast is a plant cell without cell wall. The cell wall is completely or partially removed by using either mechanical or enzymatic means.
- Protoplast= Cell- Cell wall

Isolation of protoplast:-

- Isolation of protoplast can be done by three methods:-

1) Mechanical (Non enzymatic)
2) Enzymatic (non mechanical)
3) Mixed enzymatic (simultaneous)

Mechanical method:-

- Mechanical method of protoplast isolation was first done by Klercher (1982)
- Cut the tissue which are first plasmolysed with sharp knife in small piece.
- Then these piece are deplasmolysed by using dilute solution to release the protoplast.

Sequential Enzymatic Method:-

- This method was first used byTekebe and other in 1968 in two step.
- The macerated tissue was first incubated in pectinase and then treated with cellulase for liberation of protoplast.

Mixed enzymatic method:-

- This is one step procedure in which both enzymes are used together to reduce the time. Power and Cocking (1968) used this method for isolation of protoplast.
- Protoplast can be isolated by treating cell with suitable cell wall degrading enzymes. The mixture of pectinase (0.1-1.9%) and cellulose (1-2%) are used.
- The pH value is adjusted between 4.7- 6.0 and kept at room temperature 25-30 ^{o}C.

Application of protoplast culture:-

- Monoclonal antibody production.
- Enhance the productivity of crops.
- Disease resistance or insect resistance plant production.
- Protoplast of sexually sterile plant can be fused to produced fertile diploid plant.
- Fusion of two related or unrelated species.

Hairy root culture:-

- It is culture produced after the infection of the explant by gram negative soil bacterium ***Agrobacterium rhizogenes***.
- This process takes advantage of naturally occurring hairy root culture in dicotyledonous plants.
- These hairy root which retains the ability to synthesized natural products as normal roots, can be used by 'hairy root culture'.

Characteristics of hairy root culture:-

- High degree of lateral branching.
- A perfusion of root hairs.
- Absence of geotropism.
- Do not required conditioning of medium.
- High growth rate in culture due to extensive branching.

Hairy roots of Beta vulgaris on agar plate

Induction hairy root culture in vitro:-

- Explant are wounded and then incubated with A.rhizogenes
- Usually two or three day later, the explant can be transferred in solid media with antibiotics such as to kill the unwanted bacteria.
- The hairy root will be induced within a short period of time, which varies from one week to over month depending upon the plant species.

Advantage of HRC:-

- The hairy root culture system are genetically and biosynthetically stable.
- High production of secondary metabolite.
- The culture can grown under phyto hormone free condition.
- Culture show fast growth and is easy to handle.

Application of HRC:-

- Production of secondary metabolites.
- Culture may produced the compound which are not found in untransformed roots.
- Culture can be used to regenerate the whole plants.
- This culture may change the composition of the metabolites.
- Functional analysis of genes.

Cell suspension culture:-

- The cell suspension culture also called as plant cell culture.
- It can be defined as “The culture of tissue and cell culture in liquid nutrient medium” producing suspension of single cell.

Type of cell suspension culture:-

- There are two type of cell suspension cultures.
- 1) Batch culture
- 2) Continuous culture

Batch culture:-

- Batch culture type of cell suspension where the cell material grow in finite volume of agitated liquid medium.
- These culture are maintained continuously by subculturing.
- Batch culture are most commonly maintained in conical flask incubated in shaker platform at 80-120 rpm.

Growth curve in batch culture

Growth profile of plant tissue culture:-

- **Lag phase:-** tissue start to growth.
- **Exponential phase:-**This phase is characterised rapid cell growth.
- **Linear phase:-**the growth followed by linear pattern with respect to time.
- **Progressive phase:-** Cell division decrease.
- **Stationary phase:-**no further growth.

Callus tissue culture:-

- It is unspecialised, unorganized , growing and dividing of mass cell.
- It is product when explant are cultured on appropriate solid medium with both auxin and cytokinine in correct condition
- A callus is a unorganised tissue.
- A callus is naturally developed on plant as result of wound.

Stage of callus culture:-

- There are three stage of callus culture:-

- 1) **Induction:- C**ell in explant differentiated and starts dividing.
- 2) **Proliferative stage:-R**apid cell division.
- **3) Morphogenesis stage:-** Formation of organised structure and regeneration of plant from somatic cell.

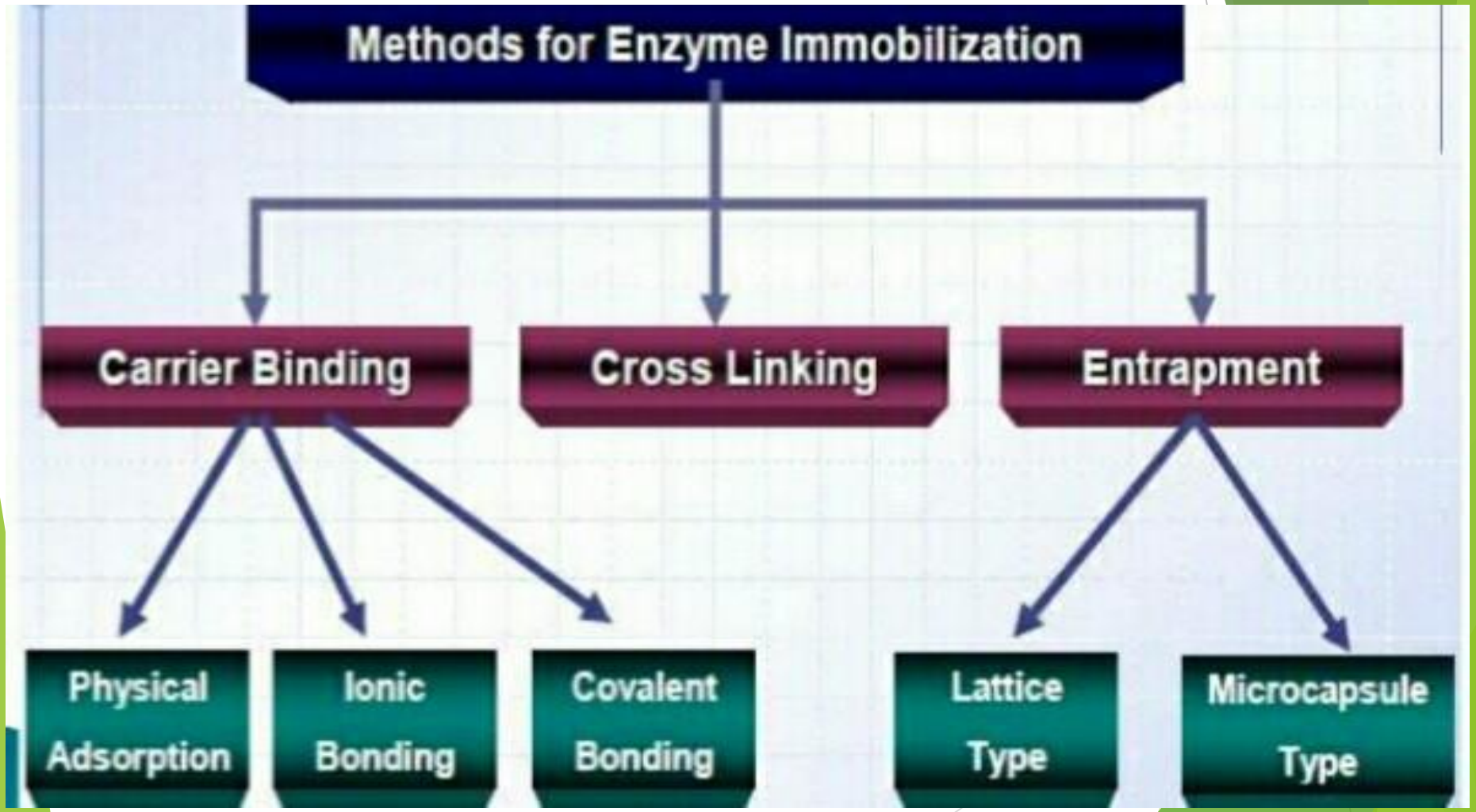
Biotransformation in plant:-

- Conversion of one chemicals into another by using biological system as biocatalyst is regarded as biotransformation.
- Conversion of less important substance to valuable product.
- Bioconversion involved many reaction. Eg. hydroxylation, reduction, glycosylation.
- Example. Production of cardiovdiascular drug digoxin from digitoxin in *Digitalis lanata*.

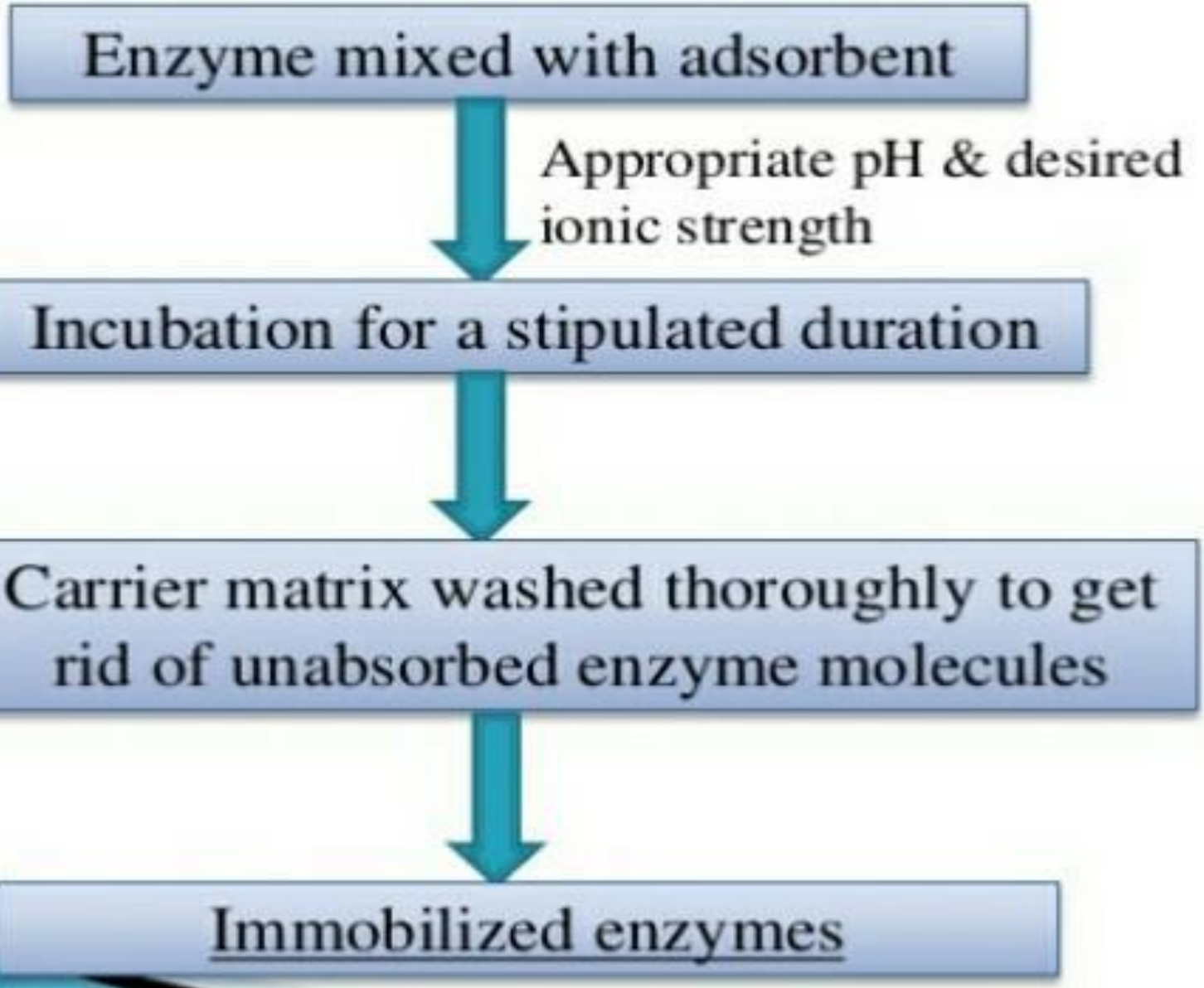
Immobilization :-

Immobilization can be defined as a process of confining the enzyme molecules to a solid support over which substrate is passed and converted to product.

Type of immobilization:-



PROCEDURE


Enzyme mixed with adsorbent
Appropriate pH & desired ionic strength
Incubation for a stipulated duration
Carrier matrix washed thoroughly to get rid of unabsorbed enzyme molecules
Immobilized enzymes

Gene transfer in plant:-

- Genetic transfer involved the removal of gene from one species and inserting them in another.
- The new DNA remain stable and can transmit the transferred gene to offspring result in development of transgenic plant.
- This technique of introduction of foreign gene is called **genetic transformation.**

Gene transfer requires transgene and targeted cell.

- **Transgene:-** isolated gene of plant
- **Targeted cell:-**culture cell

Method of gene transfer method:-

- 1)Vector mediated method
- 2)DNA mediated gene transfer method

Application of gene transfer:-

- Improvement of quality and quantity of crop
- Development of herbicidal resistance
- To produced valuable product
- Antigen and antigen production
- Vaccine production
- Protein production
- Enzyme production

Application of plant biotechnology:-

- Production of clones.
- Production of Artificial seeds
- Micropropogation.
- Transgenic plants i.ePlant modified by genetic variation.
- Cryopreservation

Application PTC:-

- Production of haploid plant.
- To obtain virus free plant.
- Plant tissue culture can be used for biotransformation
- Formation of secondary metabolite
- PTC are used in plant yield high fruit pulp and oil at large scale.

Acknowledgement

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Thank you……