Strategies for Enhancement in food production

TISSUE CULTURE

TISSUE CULTURE :

- It is the technique of maintaining and growing plant cells, tissues or organs on artificial medium in suitable containers in the presence of controlled environmental conditions.
- **Gottlieb haberlandt (1902)** firstly described tissue culture.
- The plant part that is cultured is called **explant**. The ability of any cell/explant to generate whole plant under sterile conditions in special nutrient media is called **cellular totipotency**.
- **Nutrient Media:** It is prepared by the addition of inorganic salts (both micro and macro elements), a carbon source (usually **sucrose**), vitamins, amino acids, **growth regulators – auxins like 2, 4–D, cytokinins such as BAP (Benzyl Amino Purine), gibberellins**. Other compounds like Casein hydrolysate, Coconut milk, malt extract, yeast extract, tomato juice, etc. Culture medium may be liquid, semi solid or solid.
- Both explant and Nutrient media are sterilized before culturing. Explants are sterilized by the use of disinfectants like **Sodium hypochlorite**. It is called **surface sterilization**. Containers, nutrient medium and instruments are thoroughly sterilised in **Autoclave (at 120º C) for 15–30 minutes** or by dry heat, alcohol filtration.

Types of Plant Tissue Culture:

Callus Culture:

Explant undergoes cell division in culture medium and forms irregular unorganised and undifferentiated mass of actively dividing cells called **Callus**. Growth regulators **2, 4-D and BAP** are added in the medium that stimulates cell division in explant. The Callus is obtained within 2–3 weeks.

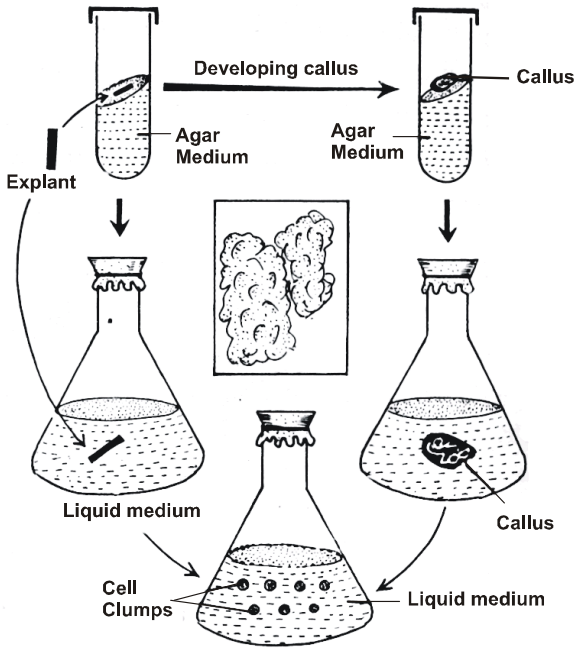

Fig :- Initiation of callus and suspension cultures.

Suspension Culture:

In this culture single cells or small groups of cells suspended in a liquid medium containing **auxin 2, 4–D**. Now this culture is constantly agitated at 100–250 rpm (revolutions per minute). Agitation serves following three purposes.

(i) **Aeration of culture**

(ii) **Constant mixing of medium**

(iii) **Breakage of cell aggregates into smaller groups.**

- Suspension cultures grow much faster than callus culture. Cell / tissues are regularly transferred into new culture vessels containing fresh media to avoid dryness & death of cell / tissue. This process is called **subculturing.**

Meristem Culture:

It involves Cultivation of axillary or apical shoot meristems.

- **Multiple shoot culture :** A shoot tip or bud with 1– 4 leaf primordia is sterilised and introduced over culture medium with high salt content and **Benzyl Amino Purine (BAP)** after 4–6 weeks the shoot tip is given cuts or shaken to form more buds. They are excised and transferred to medium rich in auxin for induction of rooting. Plantlets are first subjected to hardening and then established in fields.
- **The meristem (apical and axillary) is free of virus. Hence, one can remove the meristem and grow it in vitro to obtain virus-free plants.**
  The shoot apical meristem with 1–2 leaf primordia is taken and sterilised. After that it is introduced in the aseptic culture medium.

Anther and Pollen Culture or Production of Haploid Plants:

- **Guha and Maheshwari (1964)** developed this technique by using **young anthers of Datura**.
- Firstly **clorox** is used to sterilise unopened floral buds for 10–20 minutes. After that anthers are cut and introduced over culture medium. Each anther develops into number of **haploid embryoids** with in 4–6 weeks by which haploid callus and then shoots regenerate. The **young haploid plants (Androgenic haploid)** are sterile. **Colchicine treatment** changes **haploids** into **homozygous diploids** that are pure for every trait. **e.g. Jinghua-1 variety of winter wheat, Guan - 18 variety of rice** are produced by this technique.
- Androgenic haploids are pure for their characters and useful in mutation breedings.
- **Gynogenic haploids** can be formed by using **unfertilized ovules**.

Protoplast Culture and Somatic Hybridisation:

- It involves fusion of two somatic cells of two varieties or species, the product is called somatic hybrid and process of formation of **Somatic hybrids** is called **Somatic hybridisation**.
- First of all, the cell wall of plant cells are removed by the application of **pectinase** and **cellulase** enzymes as a result protoplasts are formed.
- Now protoplasts of the two plants are introduced in the culture medium and stimulated them for the fusion either by **electric currents** or by chemicals like **PEG (Polyethylene glycol) or Sodium nitrate**. It forms hybrid protoplasts which may bear single fusion nucleus (synkaryon) or two unfused nuclei (Heterokaryon).
- Sometimes one of the two nuclei degenerates and the hybrid protoplasts is called **cytoplasmic hybrid or Cybrid (heteroplasts)**.
- The first somatic hybrid was obtained by **Carlson et al (1972)** by the cross between two species of **Tobacco (Nicotiana glauca and N. langsdorfi)**. **Pomato** is somatic hybrid formed by the cross between **Potato and Tomato**. The former represents intergeneric somatic hybrids. **Bromato (Brinjal × Tomato)** is also intergeneric somatic hybrid.

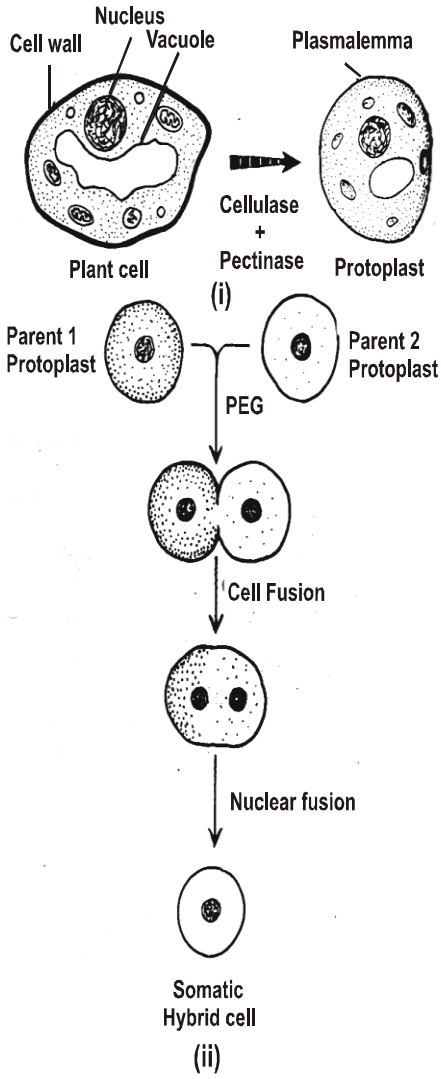

Fig:- Somatic hybridisation (i) Formation of protoplasts (ii) Protoplast fusion induced by PEG

Embryo Culture:

- Fragile or young or dormant embryos of developing seeds are separated and introduced in the culture medium to form seedlings and then young plants. Its applications are as follow.

(i) **Embryo Rescue:** It is useful technique particularly when embryo aborts at an early stage of development. The fragile embryos from fertilised ovules of interspecific crosses are taken before their abortion and culturing them to form viable hybrid seedlings **e.g. Jute (Corchorus olitorius × C. capsularis), Bean (Phaseolus vulgaris × P. angustissimus)**.

(ii) Stored food is absent in **Orchid** seeds. Embryo culture allows seedling development in the plants.

(iii) **Rare Plants:** The multiplication of some rare plants is performed by embryo culture **e.g. Makapuno Nut**.

MICROPROPAGATION:

- It is rapid vegetative multiplication of ornamental plants and fruit trees by using small sized explants through tissue culture technique. The propagation technique is named as micropropagation due to minute size of the propagules in the culture. Plants obtained by vegetative propagation of a single plant constitute a somaclone that have the same genotype.
- The method of micropropagation involves four steps.

(i) **Initiation of culture –** Introduction of explant like shoot tip on a suitable nutrient medium.

(ii) **Shoot formation –** Formation of multiple shoots by cultured explant.

(iii) **Rooting of shoots –** Development of roots in 'in vitro' developed shoots.

(iv) **Transplantation –** Plantlets are treated by hardening treatment and planted in the field.

APPLICATION OF TISSUE CULTURE:

(i) **Somaclonal Variation:** Genetic variations that occur during tissue culture are called somaclonal variations. Some of these are useful **e.g. high protein content & resistance to late blight in Potato, resistance to Tongro virus and Leaf Hopper in Rice short duration in sugarcane etc**.

(ii) **Rapid Clonal Propagation**

(iii) **Transgenic Plants**

(iv) **Resistance to Weedicides**

(v) **Induction and selection of Mutations**.