

MORPHOGENESIS, AND TISSUE CULTURE TECHNIQUES IN VEGETABLES

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MORPHOGENESIS

- Is the biological process that causes an organism to develop its shape.
- Morphogenesis can take place also in a mature organism, in cell culture or inside tumor cell masses. Morphogenesis also describes the development of unicellular life forms that do not have an embryonic stage in their life cycle.
- Morphogenetic responses may be induced in organisms by hormones, by environmental chemicals ranging from substances produced by other organisms to toxic chemicals or radionuclides

TISSUE CULTURE

- Producing a plantlet from isolated cells,tissues,or organs in a artificially prepared nutrient medium in the laboratory in aseptic conditions.
- Plant tissue culture, also called micro propagation.
- Tissue culture is based on principle of totipotency of cells.
- In India tissue culture was developed by S.C.Maheswari in 1964.
- He is called as Father of Tissue culture in India

BASIC NEEDS OF TISSUE CULTURE

1.NUTRIENT MEDIUM.

2.ASPETIC CONDITIONS.

3.AERATION.

NUTRIENT MEDIUM:-Nutrient medium is also called as culture medium or basal medium or growth medium.

Growth of plant tissues depend on the nutrient medium.

Nutrient medium contains sucrose glucose starch which supplies energy inorganic salts(major and minor elements)vitamins and hormones(auxins cytokininis).

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ASPETIC CONDITIONS:-

Utensils, glassware ,and nutrient medium culture room, inoculation chamber must be in aseptic conditions.

Contamination occurs through utensils so surface sterilization should be done sodium hypochloride or calcium hypochloride.

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AERATION:-

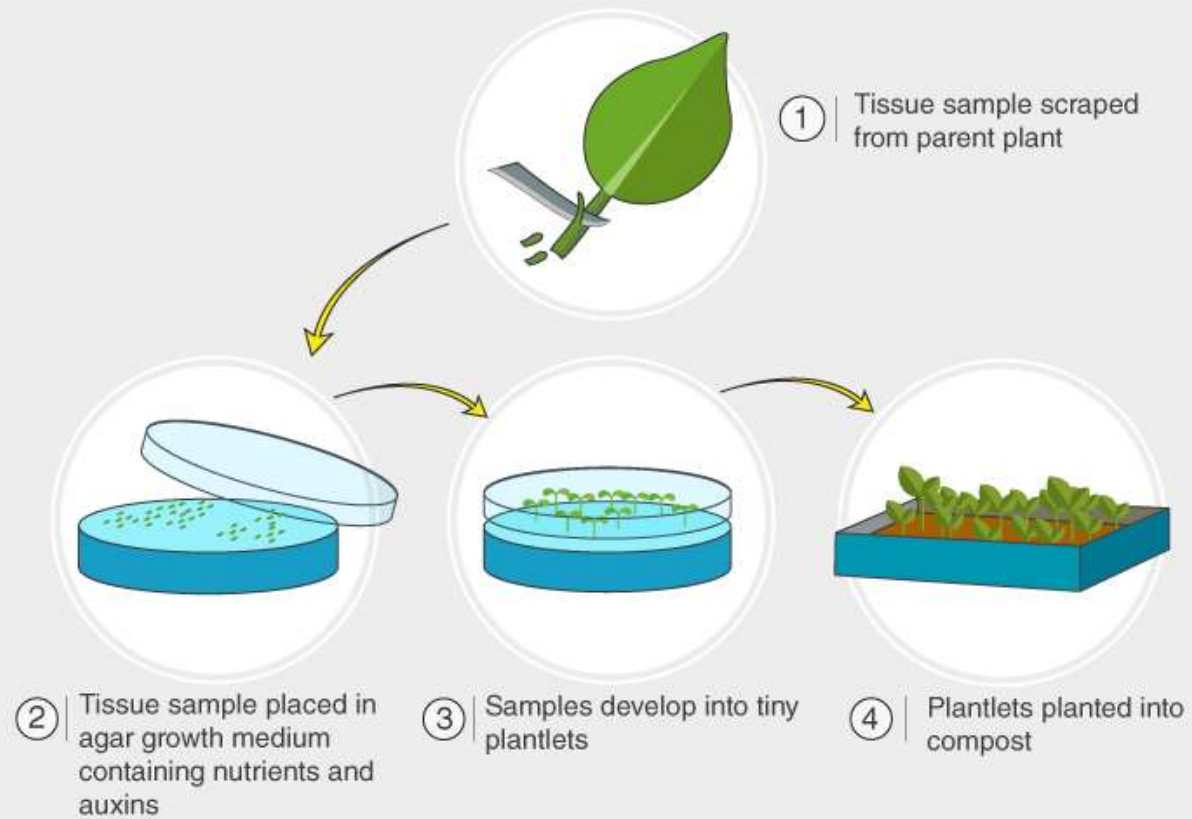
Growing tissues requires aeration.

Common method of aeration is by shaking the culture flasks on an automatic shaker.

STEPS OF TISSUE CULTURE

- ① 1. Inoculation and callus formation
- ② 2. Multiplication or sub culturing callus
- ③ 3. Development and differentiation of sub culture.
- ④ 4. Acclimatization (from test tube to field)

PLANT TISSUE CULTURE



INOCULATION AND CALLUS FORMATION:-

For inoculation explant may be taken from vegetative parts and reproductive parts. like shoots leaves and roots.

Now explant is transferred to sterilized culture medium kept in test tubes or flasks in culture chamber.

This chamber is also pre-sterilized with ultra violet ray emitting tubes.

callus formation is observed with in 2 weeks.

2.Multiplication or sub culturing callus:-

Callus produced in above stages is taken out and cut into pieces and each piece is transferred to fresh culture medium in separate tubes or flasks

After sometime these pieces develop into big masses of callus.

DEVELOPEMENT AND DIFFERENTIATION OF SUB CULTURE.

- The differentiation of organs occurs from callus this depends on auxin and cytokinin ratio.
- Appropriate proportions of auxin and cytokinin favours root and shoot formation.
- In this phase the entire plant is produced so it is called as regeneration phase.

ACCILIMATIZATION

- Cultured plants are taken out of test tubes and thoroughly washed under water for removing the agar molecules.
- Then plant is kept in low minimum salt solution for 24-48 hours and transferred to the soil (primary hardening) containing vermicompost and coco peat for maintaining growth and moisture retention. And after 1 month the plants are transferred to polythene bags contains sterilized soil (secondary hardening). and then directly sown in the soil

METHODS OF MICROPROPAGATION

- ◉ **MERISTEM TIP CULTURE.**
- ◉ **EMBRYO CULTURE.**
- ◉ **CALLUS CULTURE.**
- ◉ **ANTHER CULTURE.**
- ◉ **PROTOPLAST CULTURE.**
- ◉ **OVULE CULTURE.**

MERISTEM TIP CULTURE:-

- This technique mainly used for horticultural crops.
- In this method the meristem consisting of one or more leaf primordia are cultured in culture media
- After few weeks the plantlets are regenerated and after in vitro for hardening.
- Rapid multiplication of the plants which are otherwise not easily propagated by vegetative means, it is possible through meristem tip culture.
- Example:-Potato

EMBRYO CULTURE:-

- ⦿ Regeneration of plant from embryo.
- ⦿ The embryo is rescued while it is immature and before the degeneration
- ⦿ It is initiated the excised embryo is cultured on the semi solid or solid culture medium as the case may be embryo techniques are primarily useful in cases of making distant crosses in hybrid production.
- ⦿ It is also useful technique to overcome seed dormancy.

CALLUS CULTURE:-

- A callus is an unorganized mass of parenchymatous cells .
- Callus formation is basically a natural phenomenon essential for healing wounds graft and bud union and rooting's of cuttings.
- In callus culture the stimulation of cell division and multiplication is required.
- For callus culture auxins are required frequently followed by cytokinins.
- In callus culture organogenesis occurs in two types:-
- In first stage formation of meristems takes place and in second stage active growth of stem buds and roots takes place

ANTHER CULTURE:-

- Regeneration of plants by using anthers mainly used to produce haploid plants.
- Anther culture was 1st reported by **GUHA AND MAHESWARI** in *Datura*.
- Pollen grains pass from uninucleate to the binucleate condition as a result of cell division multicellular masses are developed which may develop into embryos or callus.
- Haploids plants are treated with colchicine for doubling of chromosomes and producing the true breeding diploid plants.
- Example:-Potato, Tomato.

PROTOPLAST CULTURE:-

- In protoplast culture the rigid cell walls are first removed either mechanically or enzymatically to expose the protoplast.
- The exposed protoplast is then cultured on to a suitable culture medium.
- Individual protoplast of the plants has been regenerate into a full plant.
- Example:-Asparagus,brassica.

OVULE CULTURE:-

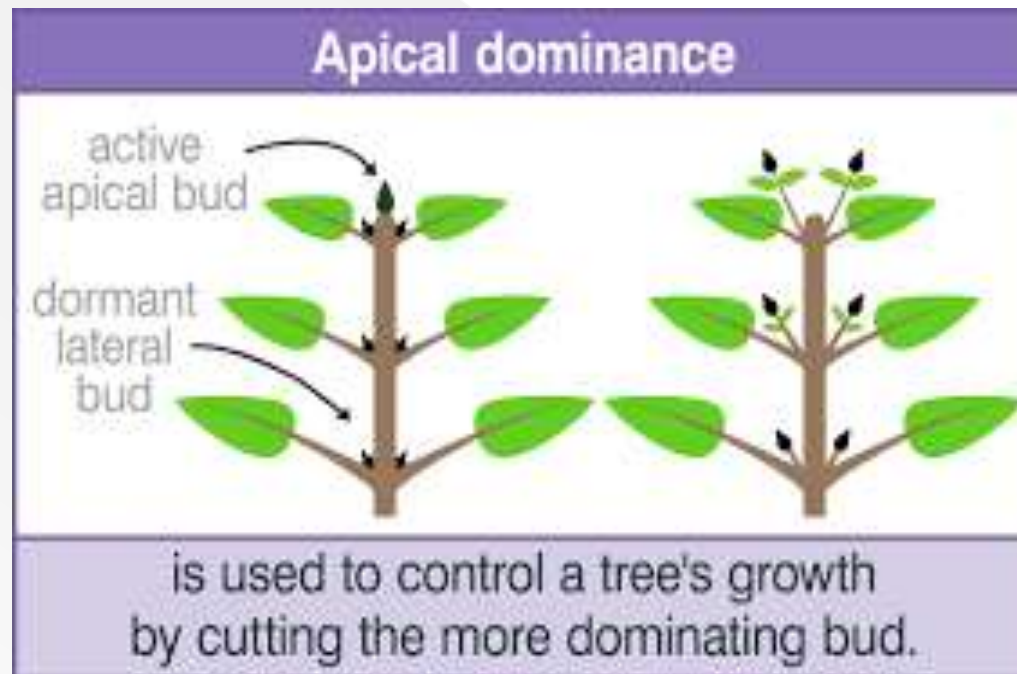
- In this method unfertilized ovules are excised and cultured into medium.
- This technique has potential application to produce hybrid seeds in wide genetic crosses.
- It is also an efficient method to obtain plants in self-incompatible species.
- One should have proper knowledge of the flowering and fertilization timing for isolation of the ovule at a proper time.

ADVANTAGES OF TISSUE CULTURE

- ⦿ Rapid multiplication of genetically uniform plants
- ⦿ (clones) that possess desirable traits.
- ⦿ A single explant can be multiplied into several thousand plants in a very short time.
- ⦿ Once established, actively dividing cultures
- ⦿ are a continuous source of micro cuttings which can result in plant production under greenhouse conditions without seasonal interruption.
- ⦿ Main advantage in tissue culture is producing viral free plants.

APICAL DOMINANCE

The phenomenon whereby the main, central stem of the plant is **dominant** over (i.e., grows more strongly than) other side stems.



ROLE OF AUXINS IN APICAL DOMINANCE

- Bangerth has recently proposed several innovative hypotheses focusing on the role of auxin transport in apical dominance
- His evidence for this is that when decapitation of the shoot apex occurs, the cytokinin concentration in the xylem greatly increases but when exogenous auxin is applied to the decapitated stump, the cytokinin content decreases

Although the concept of apical dominance control by the ratio of cytokinin to auxin is not new.

CONTD.....

- Recent experimentation with transgenic plants has given this concept renewed attention.
- In the present study, it has been demonstrated that cytokinin treatments can partially reverse the inhibitory effect of auxin on lateral bud outgrowth in intact shoots of Ipomoea.
- Auxin inhibited lateral bud outgrowth when applied either to the top of the stump of the decapitated shoot or directly to the bud itself

THANK YOU