Micropropagation: Concept, Principle and Types

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What is Micropropagation?

- Micropropagation is the artificial process of producing plants vegetatively through tissue culture techniques.
- Micropropagation is the production of new plants under the ultra controlled environment within the culture vessel *i.e.*, test tube.
- *In vitro* propagation of plants vegetatively by tissue culture to produce genetically similar copies of a new plants is referred to as micropropagation.
- The first tissue culture Laboratory in India was established at Delhi University in **1950**.

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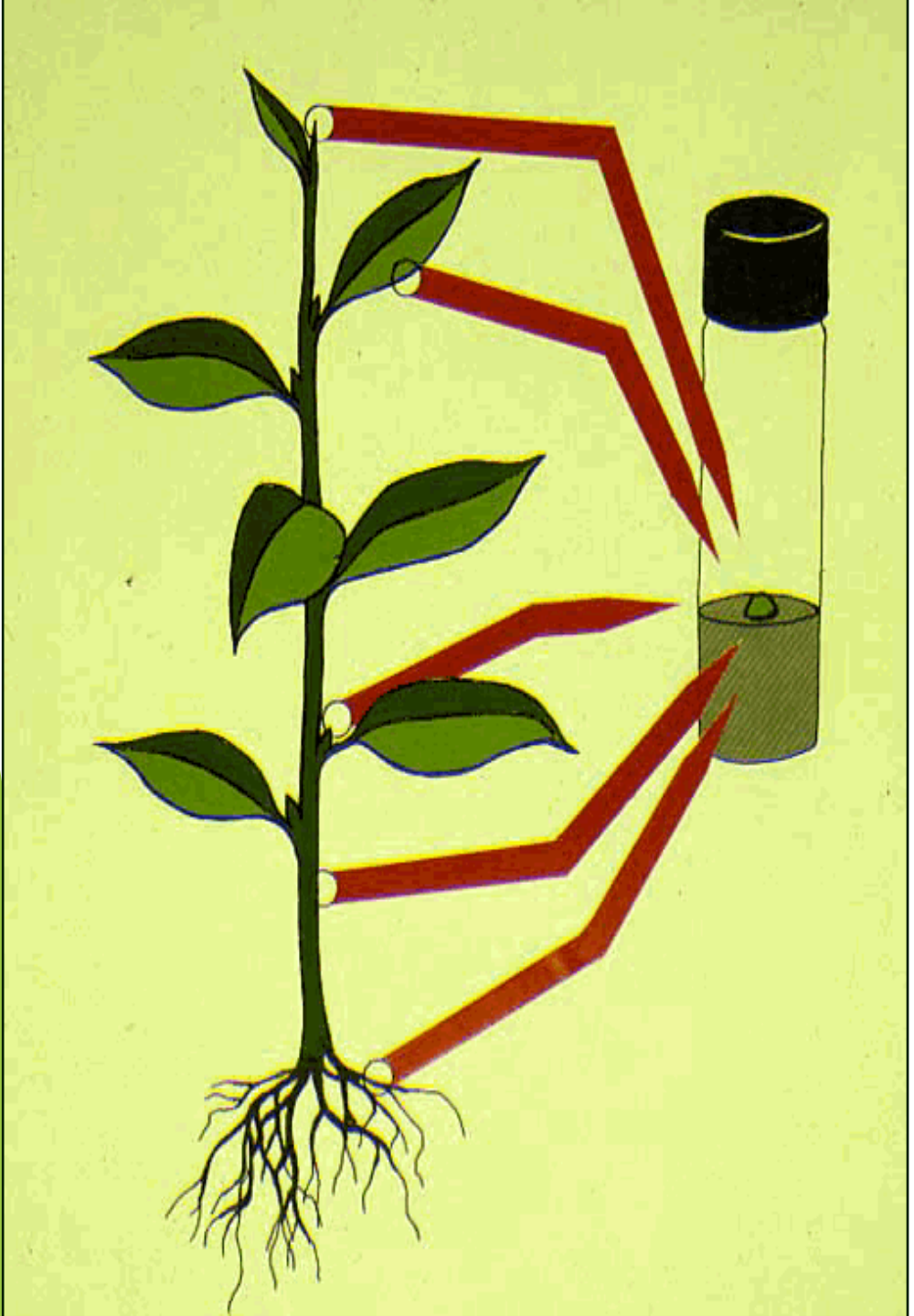
- At present most of the large commercial tissue culture laboratories are operative in states like; **Maharashtra**, **Karnataka**, **Kerala** and **Andhra Pradesh**.
- In **1902,** a German physiologist, ***Gottlieb Haberlandt*** for the first time attempted to culture isolated single cells from leaves in salt solution enriched with sucrose.
- Commercial micropropagation began in the ***United States*** in **1965** with orchids production.
- **Banana** is the largest sold micro-propagated fruit crop in **India** and abroad.
- ***Strawberry*** is also catching up in our country.
- The part which is cultured is called ***Explant***, *i.e.*, any part of a plant taken out and grown in a test tube, under sterile conditions in special nutrient media.



What is explant?

Any part of a plant taken out and grown in a test tube or *In vitro*, under sterile conditions in special nutrient media.

Totipotency-

The plants have a capacity to generate a whole plant from any explant.

Principle of micropropagation

All the biological principles of micropropagation techniques are based on the totipotency of cell, which implies that a plant cell has a capacity to generate into a whole plant having different organs.

Cryopreservation plays a vital role in the long-term *in vitro* conservation of essential biological material or any genetic materials. It involves the storage of *in vitro* cells or tissues in liquid nitrogen (-196 ^{0}C) and regeneration of cells or tissue have more success.

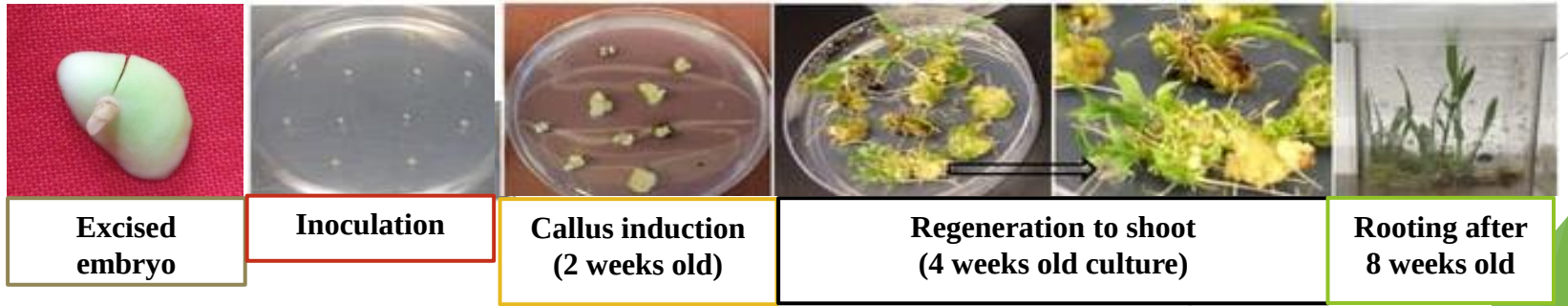
Types of Micropropagation

Seed Culture:

- Seeds may be cultured ***in vitro*** to generate seedlings or plants. It is the best method for **raising the sterile seedling**.
- The seed culture is done to get the different kinds of explants from aseptically grown plants which help in better maintenance of aseptic tissue.

Embryo Culture:

- Embryo culture is the sterile isolation and growth of an immature or mature embryo in *in-vitro* with the goal of obtaining a viable plant.
- In some plants seed dormancy may be due to chemical inhibitors or mechanical resistance, structures covering the embryo. Excision of embryos and culturing them in nutrient media help in developing viable seedlings.



Meristem culture:

- The apical meristem of shoots can be cultured to get the *virus-free plants*. The size of explant may vary from as small as 1.0-5.0 mm long meristem tip for meristem culture to a piece of shoot several centimeter long.
- This method is more successful in case of *herbaceous plants* than woody plants. In case of woody plants, the success is obtained when the explant is taken after the dormancy period is over. After the shoot tip proliferation, the rooting is done and then the rooted plantlet is potted in soil medium.

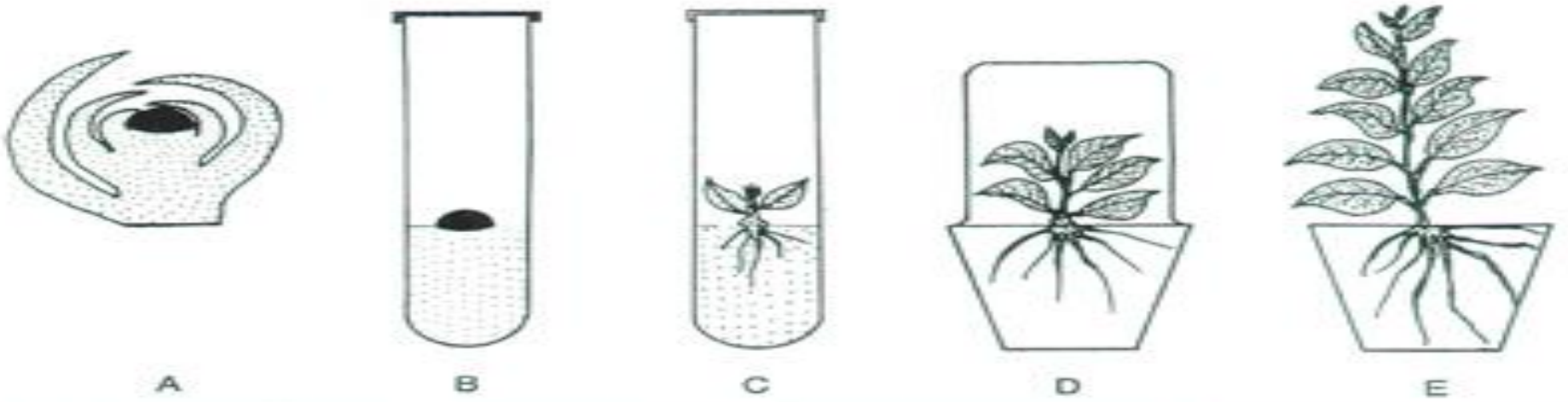
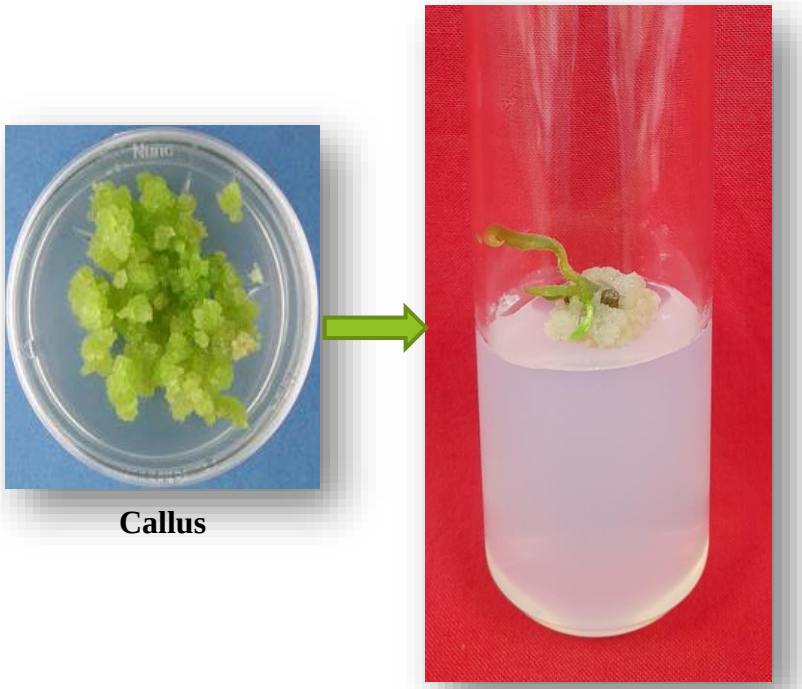


Fig. **Meristem tip culture- A.** Apical meristem showing section to be excised, **B**. Excised meristem tip cultured on agar medium, **C**. Plantlet regenerated from excised meristem tip, **D.** Plantlet transferred to sterile soil, E. Virus free plant growing in soil

Callus Culture:

- Callus is basically more or less un-organized dedifferentiated mass of cells arising from any kind of explant under in vitro cultural conditions.
- The cells in callus are parenchymatous in nature, but may or may not be homogenous mass of cells.
- After callus induction it can be sub-cultured regularly with appropriate new medium for growth and maintenance.

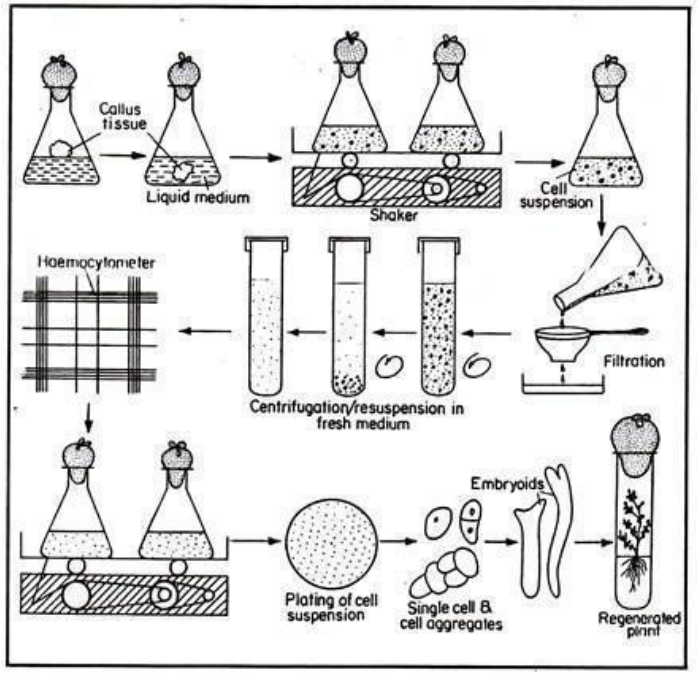


Callus

Initiation of shoot from callus

Cell Suspension Culture:

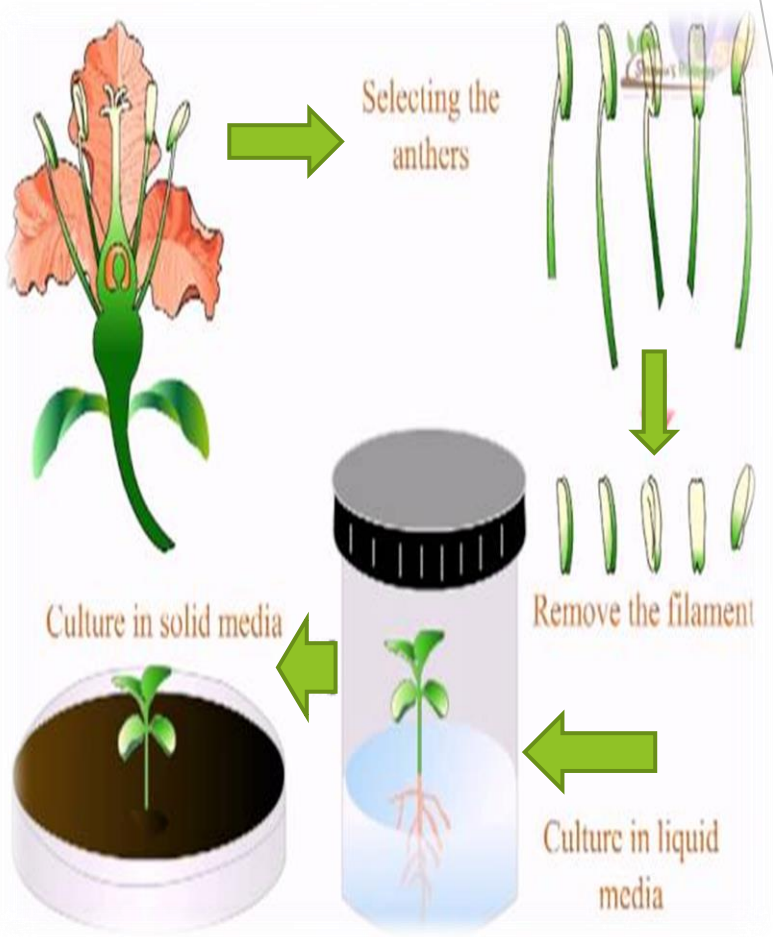
- The growing of individual cells that have been obtained from any kind of explant tissue or callus referred to as cell suspension culture.
- These are initiated by transferring pieces of tissue/ explant/callus into liquid medium (without agar) and then placed them on a shaker to provide both aeration and dispersion of cells.
- Cell suspension cultures may be done in batch culture system.
- In the later system, the culture is continuously supplied with nutrients by the inflow of fresh medium with subsequent draining out of used medium but the culture volume is constant.



Cell or suspension culture

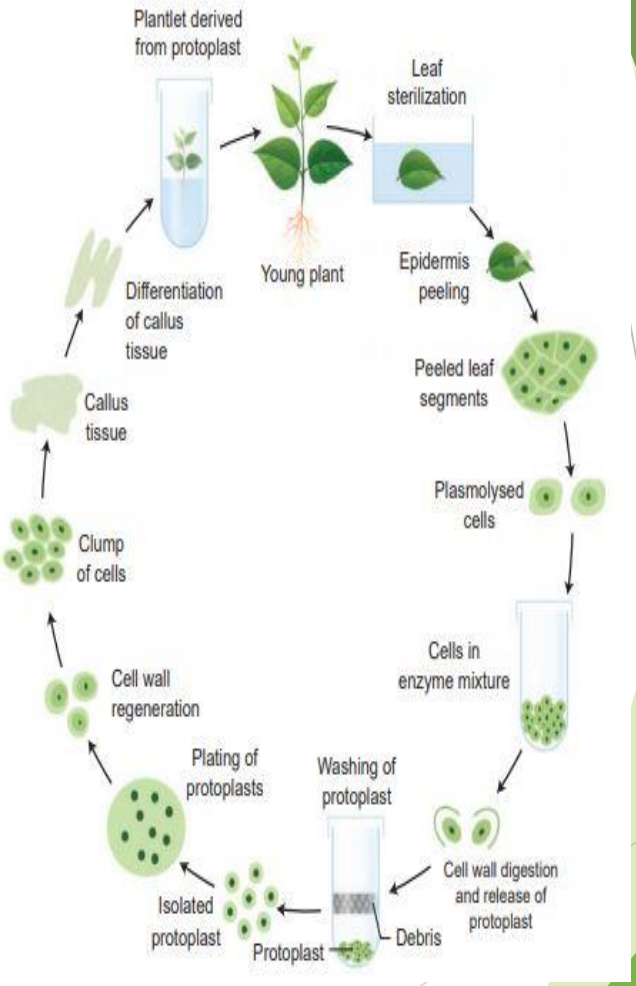
Anther or pollen Culture:

- An important aspect of plant tissue culture is the haploid production by anther culture, also known as pollen culture.
- First established by Guha and Maheswari (1964, 1966) in Datura.
- The anther culture can be done with the isolated anthers on liquid media than transferred into solid media where anther wall break open and the androgenic calli will be formed from the pollen.



Protoplast Culture:

- It is the culture of plant protoplasts i.e., culture of cells devoid of cell wall.
- Isolated protoplasts are usually cultured in either liquid or semisolid agar media plates.
- Protoplasts are isolated from soft parenchymatous tissue by enzymatic method and then viable protoplasts are purified and cultured.
- The protoplast culture is aimed mainly to develop genetically transformed plant, where the transgenic is put successfully within the plant protoplast and the transgenic plant is regenerated from that transformed protoplast.
- Another aspect of protoplast culture is somatic hybridization of two plant species through protoplast fusion.



The following factors are essential in the success of tissue culture:

- Sterilization of equipment, glassware and the media-

  Hot air oven, media sterilization by autoclaving

- Collection of explant- fresh material
- Sterilization of explant- by 0.1 % $HgCl_2$ or 4 -10 % Sodium hypochlorite
- Media composition- **MS**, B-5, Nistch, Knudson, WPM
- Culture of explant- Test tube, Jam bottle, magenta box etc.
- Incubation of culture- humidity, temperature, light(16 and 8 hr.)

Basic requirements of micropropagation

In micropropagation techniques, there are some basic requirement, *viz.*

1- Aseptic condition-

Laboratory should be well sterilized against pathogen and explant and glassware should also be properly sterilized before their entry into the laboratory.

2- Control of temperature

Air conditioning of the laboratory is essential, generally temperature between 23-25±2 °C is used. However temperature varies from species to species.

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3- Proper culture medium:

Culture media have been developed by verious workers for different crop species. The culture media developed by Murashige and Skoog (1962) and Gamberg, *et al.* (1968) are used with some modifications in various crop species.

4- Sub-culturing:

Transfer of tissue or callus from old culture media to fresh culture media is called sub-culturing. This is essential to maintain good health of the callus or tissues, because after some period, some nutrients are depleted in the culture media.

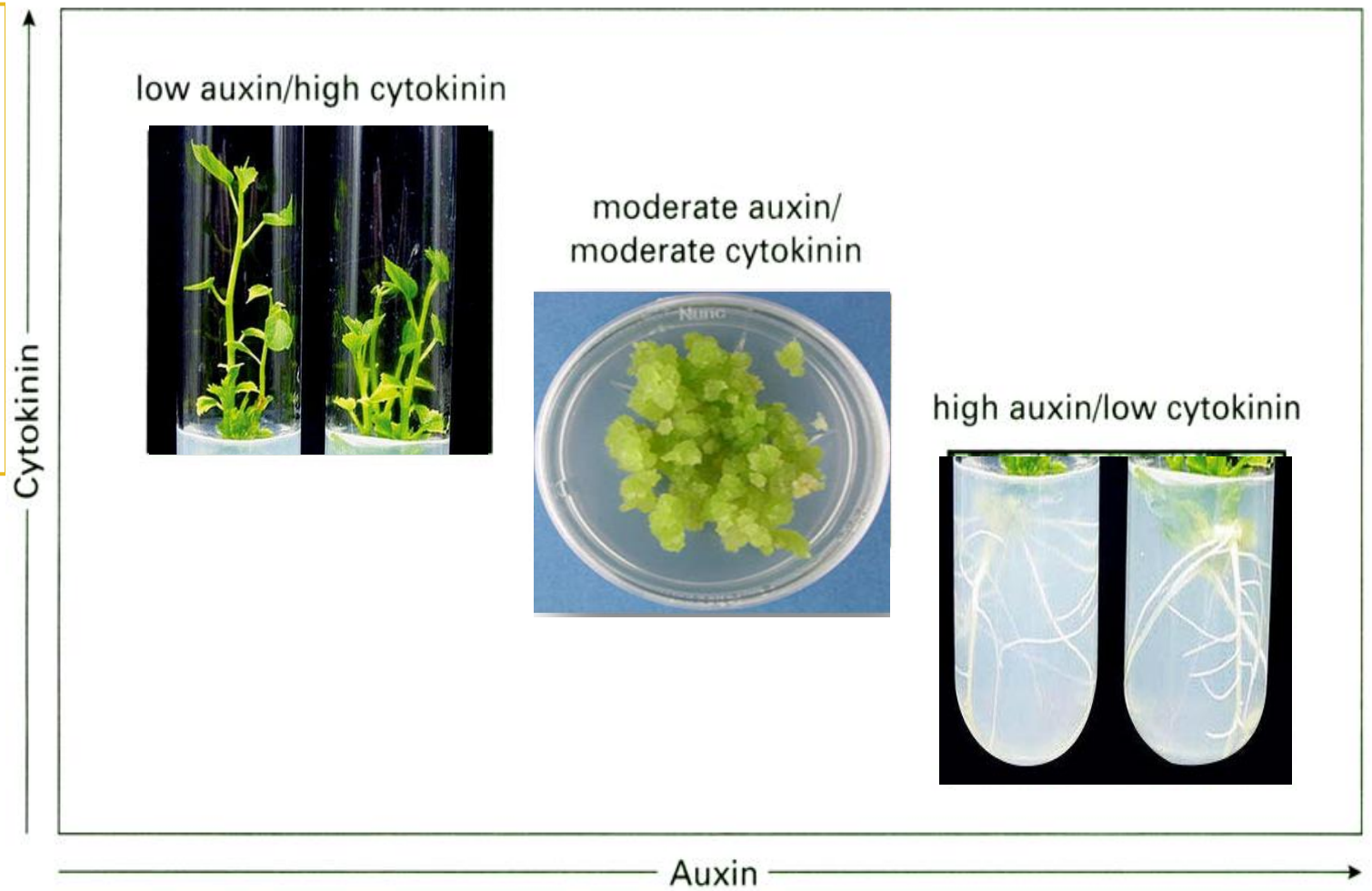
Use of Growth regulators in plant tissue culture

1. **Auxins-** (IAA, **IBA,** NAA, 2, 4-D)

2. **Cytokines-** (6-BAP, **Kinetin**)

3. **Gibberellins-** (Gibberellic Acid)

4. **Abscisic Acid-**(ABA)



COMPOSITION OF MS Media

INGREDIENTS:	
MACRONUTRIENTS-	**mg/l**
Ammonium nitrate	1650
Calcium chloride	332
Magnesium sulphate	180
Potassium nitrate	1900
Potassium phosphate monobasic	170
MICRONUTRIENTS-	
Boric acid	6.200
Cobalt chloride hexahydrate	0.025
Copper sulphate pentahydrate	0.025
EDTA disodium salt dihydrate	37.300
Ferrous sulphate heptahydrate	27.800
Manganese sulphate monohydrate	16.900
Molybdic acid (sodium salt	0.213
Potassium Iodide	0.830
Zinc sulphate heptahydrate	8.600
VITAMINS:	
Myo-Inositol	100.00
Nicotinic acid (free acid)	0.500
Pyridoxine HCl	0.500
Thiamine hydrochloride	0.100
AMINO ACID:	
Glycine	2.00
TOTAL(g/l)	4.40

Other Material required-

- Autoclaved distilled water-
- Gelling agents like-

  Agar-Agar- 7-8% =7- 8 g/l

  CleriGel- 4%= 4 g/l

  Gelrite-2-3% =2-3 g/l
- Plant growth regulators-

(as per required concentrations)

- pH meter-
- Sucrose-30 g/l

Micropropagation Procedure:

There are basically four stages of micropropagation process, these are :

Stage I- Explant establishment:

- The establishment of explant depends on several factors such as the source of explant, type of explant such as leaf, root, stem from mature or immature plants, explant sterilization, *in vitro* culture conditions such as culture media, composition, temperature, humidity, light etc.
- The explants showing growth are considered established.

Stage II- Shoot multiplication:

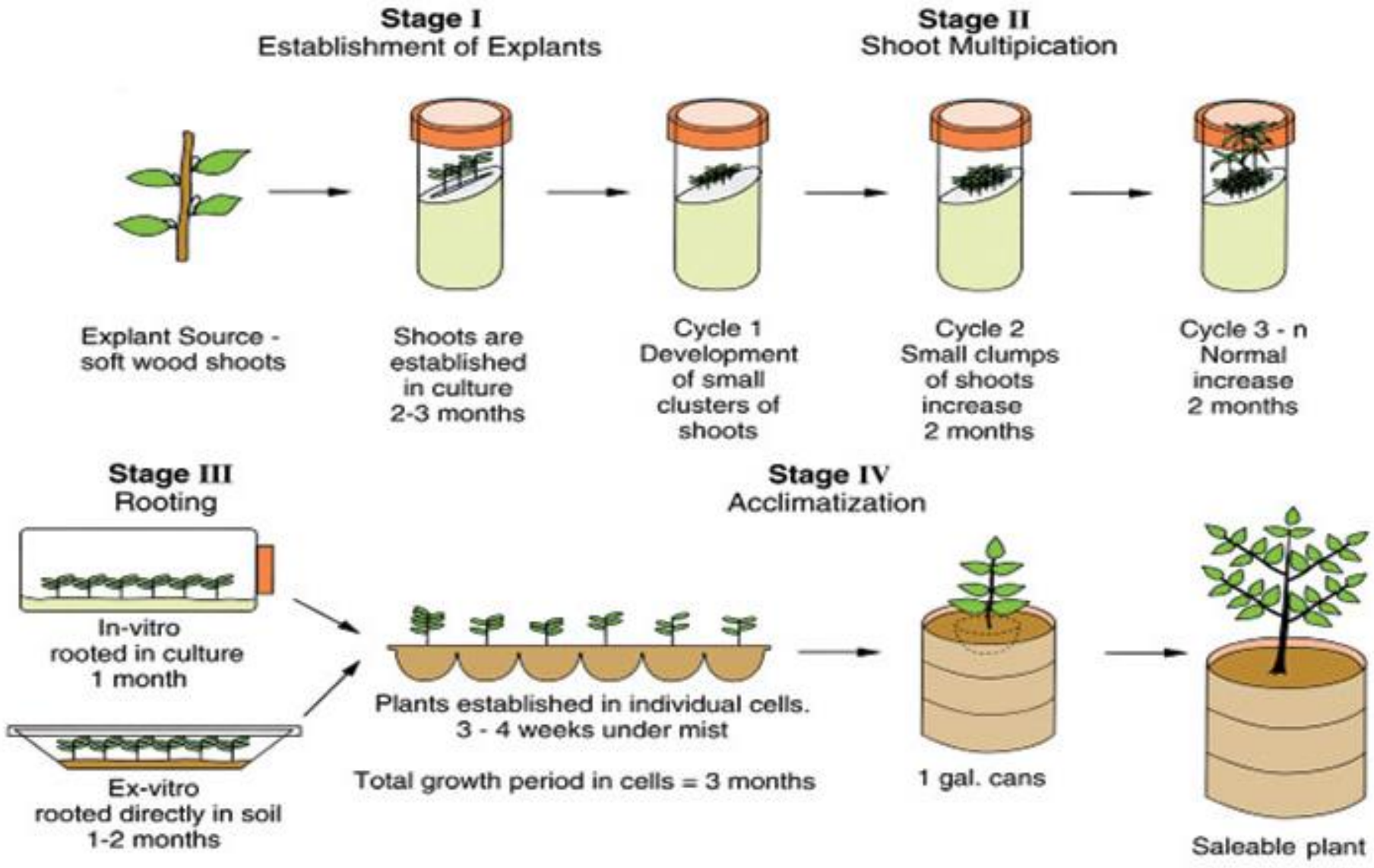
- The established explants are sub-cultured after 2-3 weeks, on shoot multiplication medium.
- Auxins like NAA, 2,4-D and cytokinins like BAP, Kinetin is used in culture medium.
- It is well-established fact that cytokinins enhance shoot multiplication.

Stage III- Rooting of shoots:

- The *in vitro* regenerated shoots are rooted in the medium containing auxins like; NAA, IBA.
- The rooting can also be induced when *in vitro* shoots are exposed to stress conditions.

Stage IV- Acclimatization/hardening-

- The *in vitro* plantlets thus obtained are hardened/acclimatized before transfer to the field.
- The hardening is necessary as the tissue culture derived plants grow under high humidity conditions, have open stomata, lower epicuticular wax, thus leading to increased transpiration losses and resulting in mortality of plants.



Stages of micropropagation

Advantages of Micropropagation:

- ✓ Plant tissue in small amounts is sufficient for the production of millions of clones in a year using micropropagation.
- ✓ An alternative method of vegetative propagation for mass propagation is offered through micropropagation. Plants in large numbers can be produced in a short period.
- ✓ Large amounts of plants can be maintained in small spaces. This helps to save endangered species and the storage of germplasm.
- ✓ The micropropagation method produces plants free of diseases. Hence, disease-free varieties are obtained through this technique by using meristem tip culture.
- ✓ The proliferation of in vitro stocks can be done at any time of the year. Also, a nursery can produce fruit species throughout the year.
- ✓ Fast international exchange of plant material without the risk of disease introduction is provided.

Disadvantages of Micropropagation

- The facilities required are very ***costly.***
- **Technical skill** is required to carry out different micro propagation procedures.
- Pathogens once appeared in the system, they also multiply at a very ***faster rate in a short time.***
- Plants having **high levels of phenols** (mango, date palm, coconut etc.), usually **do not respond** to micropropagation techniques.
- Establishment of laboratory-raised plants in the field is a very difficult task

CONCLUSION

- Plant tissue culture represents the most promising areas of application at present time and giving an out look into the future.
- The rapid production of high quality, disease free and uniform planting stock is only possible through micropropagation.
- New opportunities has been created for producers, farmers and nursery owners for high quality planting materials of fruits crops.
- Plant production can be carried out throughout the year irrespective of season and weather.
- However micropropagation technology is expensive as compared to conventional methods of propagation by means of seed, cuttings and grafting etc.
- Therefore it is essential to adopt measures to reduce cost of production. Low cost production of plants requires cost effective practices and optimal use of equipment to reduce the unit cost of plant production.


Thank You