

Types and process of TISSUE CULTURE

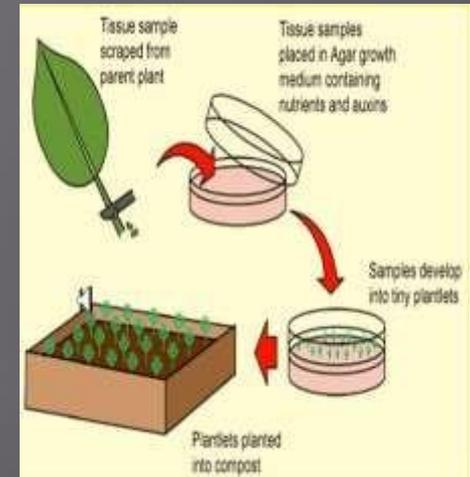


INTRODUCTION

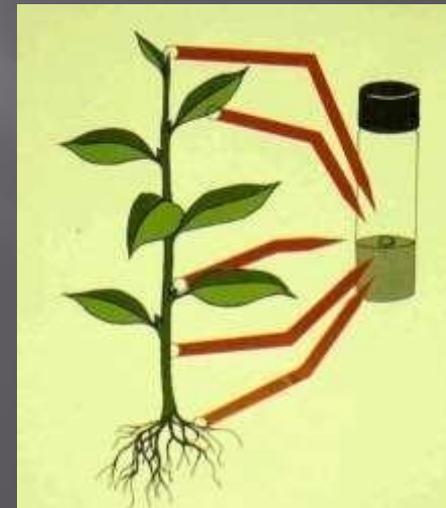
- **Plant tissue culture** is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition.
M Razdan (2003)
- Plant tissue culture refers to growing and multiplication of cells, tissues and organs of plants on solid or liquid media with nutrients under **aseptic and controlled environment**.
- Plant tissue culture is genetic description which embraces plant protoplast ,plant cell and tissue of plant. Plant tissue culture comprises a set of *in- vitro* techniques, methods, strategies. Tissue culture had been exploited to create **genetic variability, to improve the health of plant material**, increase the number of desired germplasms.

RELATED TERMS

- **Totipotency** :- The potential or inherent capacity of a plant cell to develop into an entire plant . It implies that all the information necessary for growth and reproduction of the organism is contained in the cell.
- **Explant** :- The plant tissue or organ excised and use for invitro culture is know as explant.



Source:- U kumar (2008)



IMPORTANT CONTRIBUTORS TO PLANT TISSUE CULTURE

- **Haberlandt** German botanist
Gottlieb Haberlandt (1902) developed the concept of *in vitro* cell culture.
- **Folke Skoog** in 1955 discovered cytokinins e.g. kinetin as potent cell division factor. In 1957, Skoog and Miller predicted shoot and root initiation.
- **Toshio Murashige** student of Skoog developed standard methods of propagation of species *in vitro* in fruit plants. Name known as Murashige and Skoog media.



A GLANCE IN HISTORY

- In 1902, Mr. **Haberlandt**; father of plant tissue culture(He proposed that plant cells could be cultured.)
- In 1930- Mr. **White** cultured tomato root tip and subcultured to fresh medium containing salts, yeast extract and sucrose and vit B.
- During this period ,some plant growth regulators, additives and vitamins was discovered for the plant micro-propagation.(discovery of PGR-Indole Acetic Acid, in 1937)
- In 1962, Murashige and Skoog published a recipe for MS media.
- In 1972, protoplast fusion has been done in tobacco.

WHY TISSUE CULTURE

- A single explant can be multiplied into several thousand plants in less than a year.
- Once established, It can give a continuous supply of young plants throughout the year.
- Disease free plants ready by the tissue culture techniques.
- This technique is rapid continuous and efficient.
- Breeding cycle is reduced.
- This technique useful in hard to propagate plant.
- Germplasm preservation also possible.
- Clones through this method are 'true to type' as compared with seedlings, which show greater variability.

MICROPROPAGATION

- Tissue culture is a term used for the growth of plants or more commonly plant parts in sterile culture. Micro propagation is a method of propagating plants using very small parts of plants that are grown in sterile culture.
- A whole plant can be regenerated from a small tissue or plant cells in a suitable culture medium under controlled environment. The plantlet so produced are called tissue culture raised plant.

ADVANTAGES OF TISSUE CULTURE

- Plant production is reliable and consistent. Multiplication rates also high.
- Plant produced via tissue culture are usually true-to-type and uniform.
- Produce mature plant quickly.
- Disease resistant plants are produced by tissue culture.
- High rate of fecundity is obtained.

DISADVANTAGE

- the experiment involved in tissue culture are expensive.
- Handled by highly trained people and careful observation.
- As all the plants are genetically similar, there is reduction in genetic diversity.
- if precautions are not taken the whole stock, may be contaminated or infected.
- The technique is a complex procedure and it is has varied procedure requires special and careful observation.

STEPS INVOLVED IN THE MICROPROPAGATION

Cleaning of glassware



Preparation of nutrient medium



Selection and sterilization of explant



Inoculation of aseptic explant into nutrient medium



Proliferation of shoots on a multiplication medium



Transfer of shoots for Sub-culturing



Rooting and hardening of plantlets

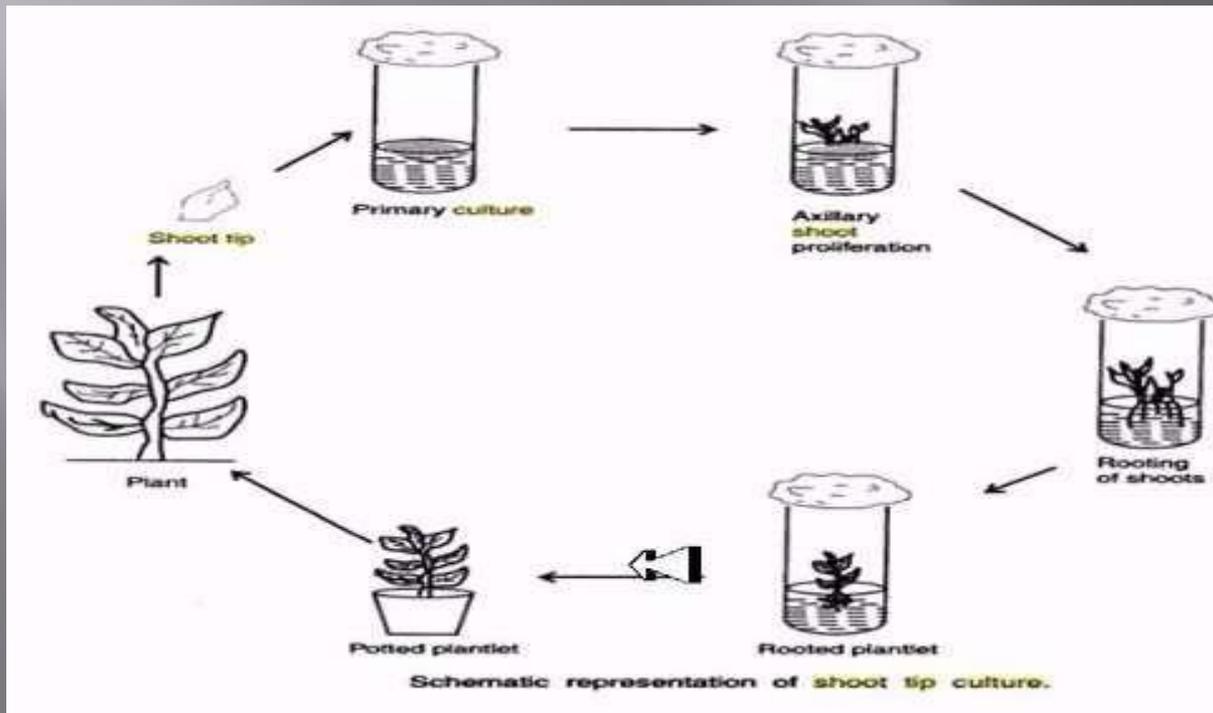


Field Trials

EXPLANTS SOURCE

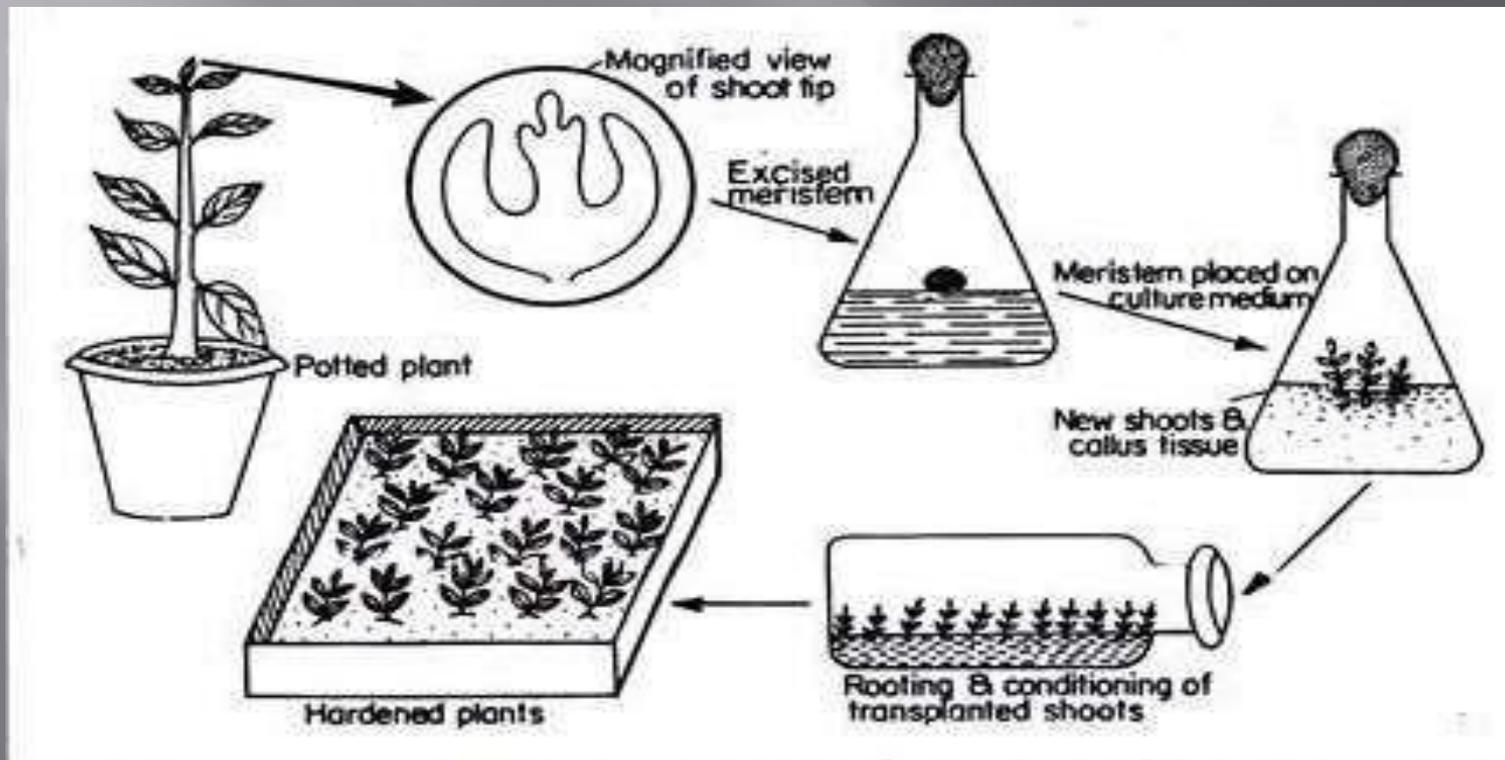
1. Shoot –tip culture

Shoots develop from a small of cells known as shoot apical meristem, described as the culture of terminal (0.1-1.0 mm) portion of a shoot.



○ 2. Meristem-tip culture

“Meristem-tip culture” is the invitro culture of a generally shiny special dome-like structure measuring less than 0.1 mm in length.



○ 3. Axillary Bud Culture

The nodal or axillary bud consists of a piece of stem with axillary bud with or without a portion of shoot. when only the axillary bud is cultured it is designated as “Axillary bud” culture.

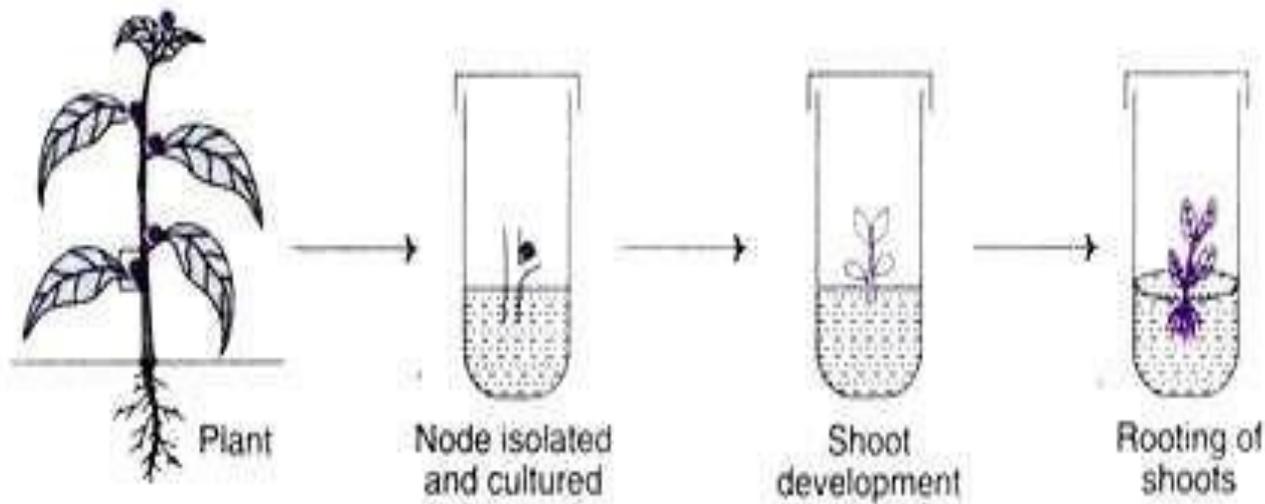
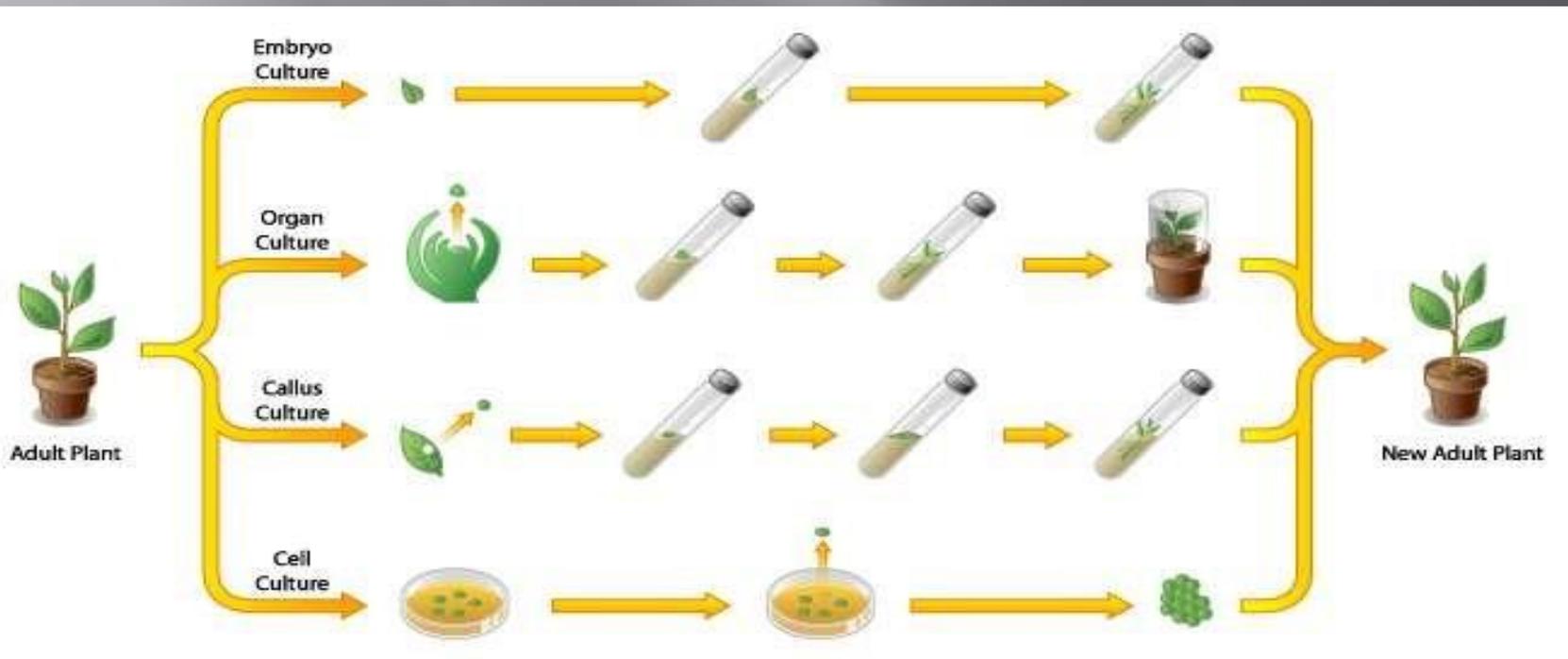


Fig. 47.3 : A diagrammatic representation of micropropagation by single node technique.

○ 4. Cell suspension and callus cultures

Plant parts such as leaf discs, intercalary meristems, stem pieces, immature embryos, anthers, pollen, microspores and ovule have cultured to initiate callus.



TYPES OF MICRO-PROPAGATION

1. Callus culture
2. Suspension culture
3. Pollen culture
4. Ovule culture
5. Root tip culture
6. Shoot tip culture
7. protoplast culture
8. Leaf primordial culture

1. CALLUS CULTURE

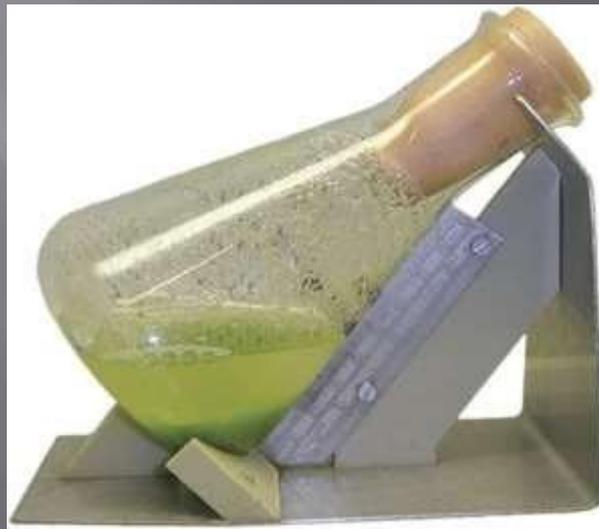
Callus culture concerns the initiation and continued proliferation of undifferentiated parenchyma cells from parent tissue or clearly defined semi solid media.



2.SUSPENSION CULTURE

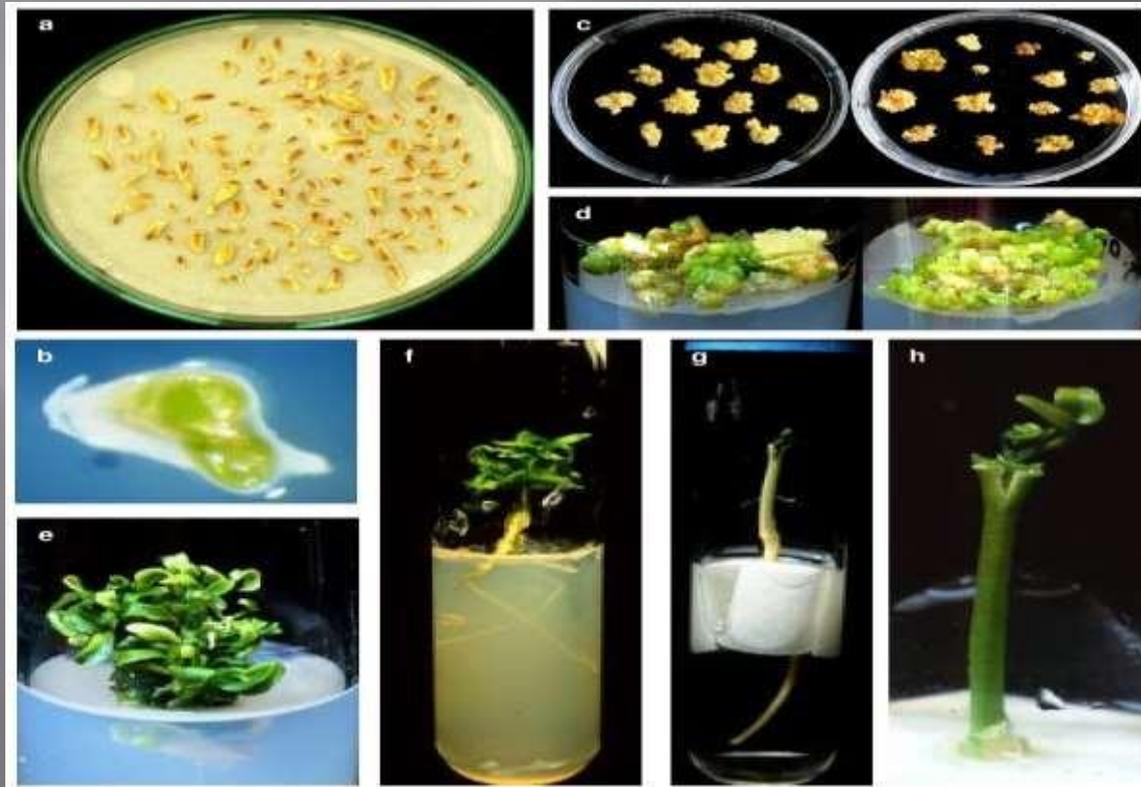
A suspension culture refers to cell or groups of cells dispersed and growing in an aerated liquid culture medium is placed in a liquid medium and shaken vigorously and balanced dose of hormones.

Cytokinin induced adventitious buds in kiwi fruit in a suspension culture, sub-culture for about a week.



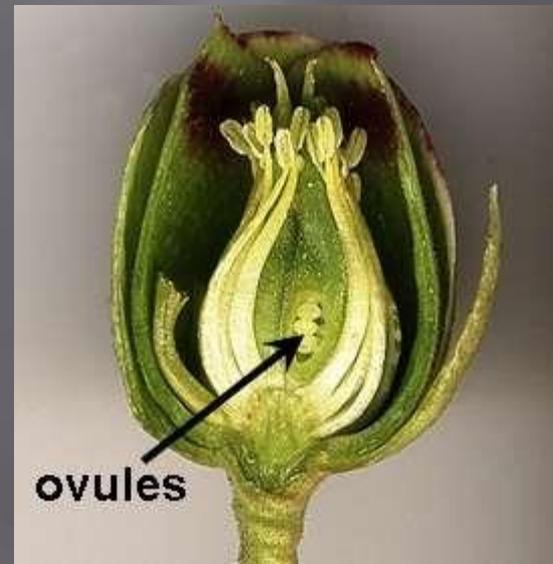
3.POLLEN CULTURE

- The culture of pollen grains which germinate *invitro*. Such cultures may eventually form monoploid callus, from which shoots embryoids develop into monoploid plants.



4.OVULE CULTURE

- Female gametophyte cells are also a source for haploid production. For haploid production for female gametophyte it is necessary to know : (1) the events related to the induction of haploidy in these tissues, (2) Factors that control *invitro* development of proembryo into the fully organized plants, and (3) major differences in the growth patterns of *invitro* development of unfertilized ovule cells (female gametophyte) and in pollen cells (male gametophyte).



5. SHOOT TIP CULTURE

- The culture of terminal part of shoot to a plant in-vitro condition or in lab called shoot-tip culture. Mostly the shoot-tip culture used for obtain disease free plant without genetically changes. The shoot-tip plant are more efficient to cultivation of differentiation *in vitro* because cells of them newly generated and healthy comparison to other parts.



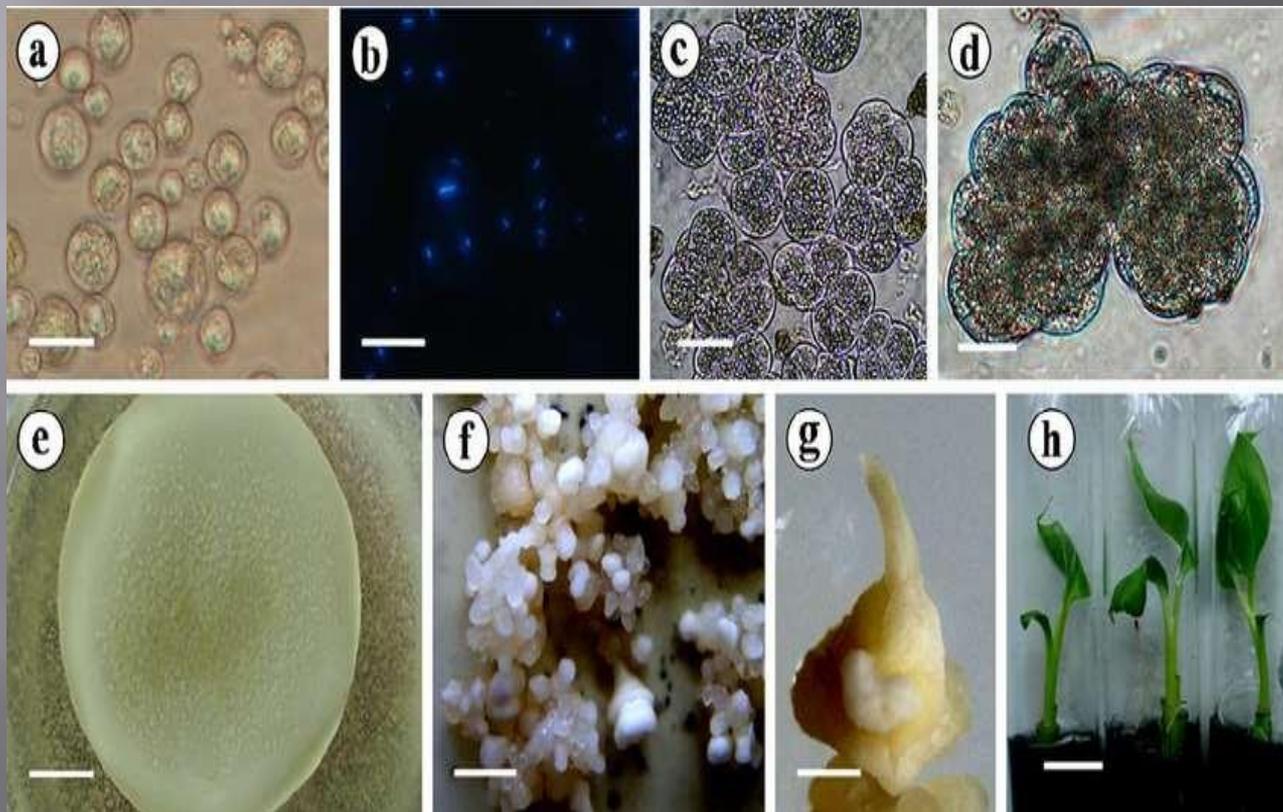
6. ROOT TIP CULTURE

- Isolated root tips of apical produce *invitro* root systems with indeterminate growth habits. These were among the first kinds of plant tissue cultures (white, 1934) and remain important research tools in the study of development phenomena.



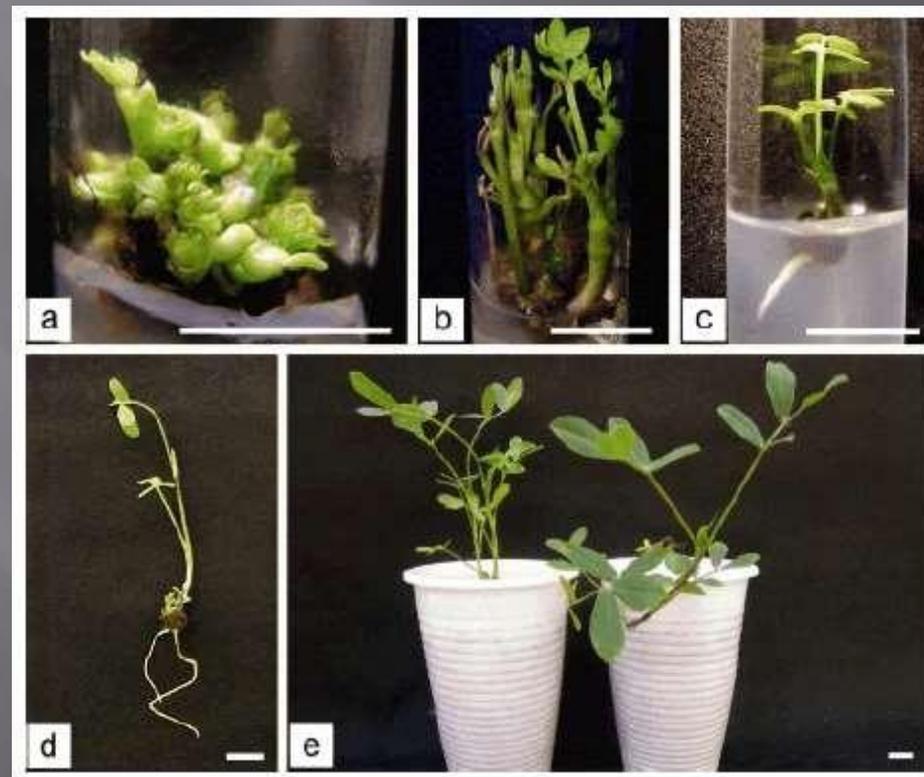
7. PROTOPLAST CULTURE

- The first step in protoplast culture involves the regeneration of the cell wall around the protoplast membrane. Once the cell wall has formed, cell division must be induced in the new cell.



8. LEAF PRIMORDIAL CULTURE

- Leaf culture a form of tissue culture in which excised leaves, leaf material, leaf primordia are grown on a sterile growth medium. Mature leaves can be kept healthy under culture conditions for considerable periods. Leaf primordia have been used to study growth and differentiation processes.



STAGES OF MICRO PROPAGATION

- **Micro propagation** is now typically divided into 5 stages. Stages 1-4 were originally proposed by **Murashige**; **Debergh** and **Maene** added Stage 0.
- **Stage 0:- Donor Plant Selection and Preparation**

Explant quality and responsiveness is influenced the physiological phytosanitary condition of the donor plants.

- **Stage 1:- Establishment of Explant in culture**

Surface-sterilization- disinfestations: Must free explant tissues of all contaminating microorganisms.



Stage 2: Multi plication

Repeated enhanced axillary shoot production. Encouraged by cytokinin in the medium ,alone or with a smaller amount of auxin. Amount of cytokinin and presence and amount of auxin may determined empirically.

Stage 3:- Rooting (pre transplant stage)

Adventitious rooting of shoot cluster invitro.

For root initiation in vitro ,IBA are important.

Stage 4:- Acclimatization

Process by which an organism or plant physiologically and anatomically adjust from in vitro to ex vitro means transfer to natural Environment.



APPLICATIONS OF TISSUE CULTURE

1. Clonal Propagation
2. Somaclonal Variation
3. Production of Virus free plants
4. Production of Synthetic seeds
5. Somatic Hybridization
6. In Vitro Plant Germplasm Conservation
7. Mutation Breeding
8. Molecular farming
9. Genetic Engineering
10. Production of secondary metabolites.

1. CLONAL PROPAGATION

- Clonal Propagation refers to the process of asexual reproduction by multiplication of genetically identical copies of individual plants.
- The clonal propagation is rapid and has been adopted for commercialization of important plants such as
 banana, apple, pear ,strawberry, cardamom,
 many ornamental plants.

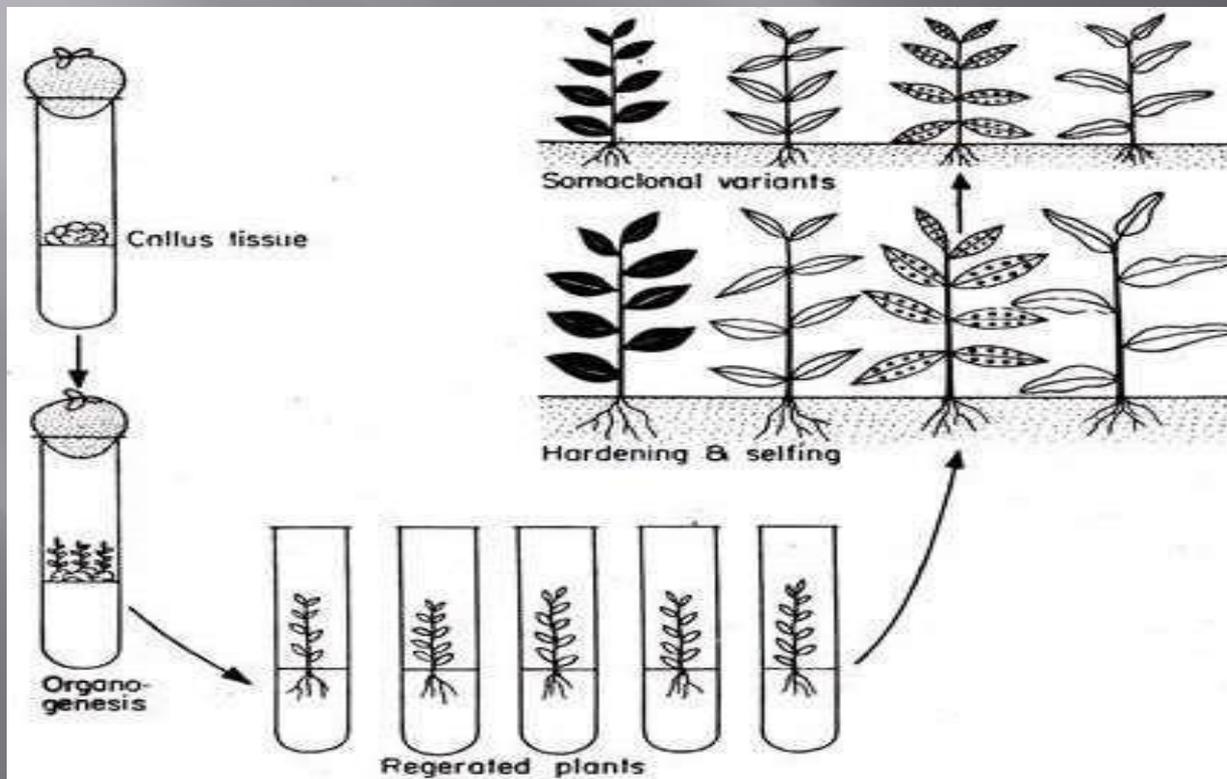


BENEFITS

- Rapid multiplication of superior clones can be carried out throughout the year, irrespective of seasonal variations.
- Multiplication of disease-free plants, e.g. virus-free plants of apple, strawberry, banana, pear etc.
- Multiplication of sexually derived sterile hybrids.
- It is a cost-effective process as it requires minimum growing space.

2.SOMACLONAL VARIATION

- The **genetic variation** found in the invitro cultured cells are collectively referred to as somaclonal variation and the plants derived from such cells are called as 'somaclones'



Larkin and Scowkraft in 1981 coined a general term “Somaclonal variation”

Advantages:- Helps in crop improvement.

- Creates additional genetic variants.
- Plants with resistant and tolerant to toxins, herbicides, high salt and even mineral toxicity.

SOMACLONAL VARIATION IN FRUIT CROPS

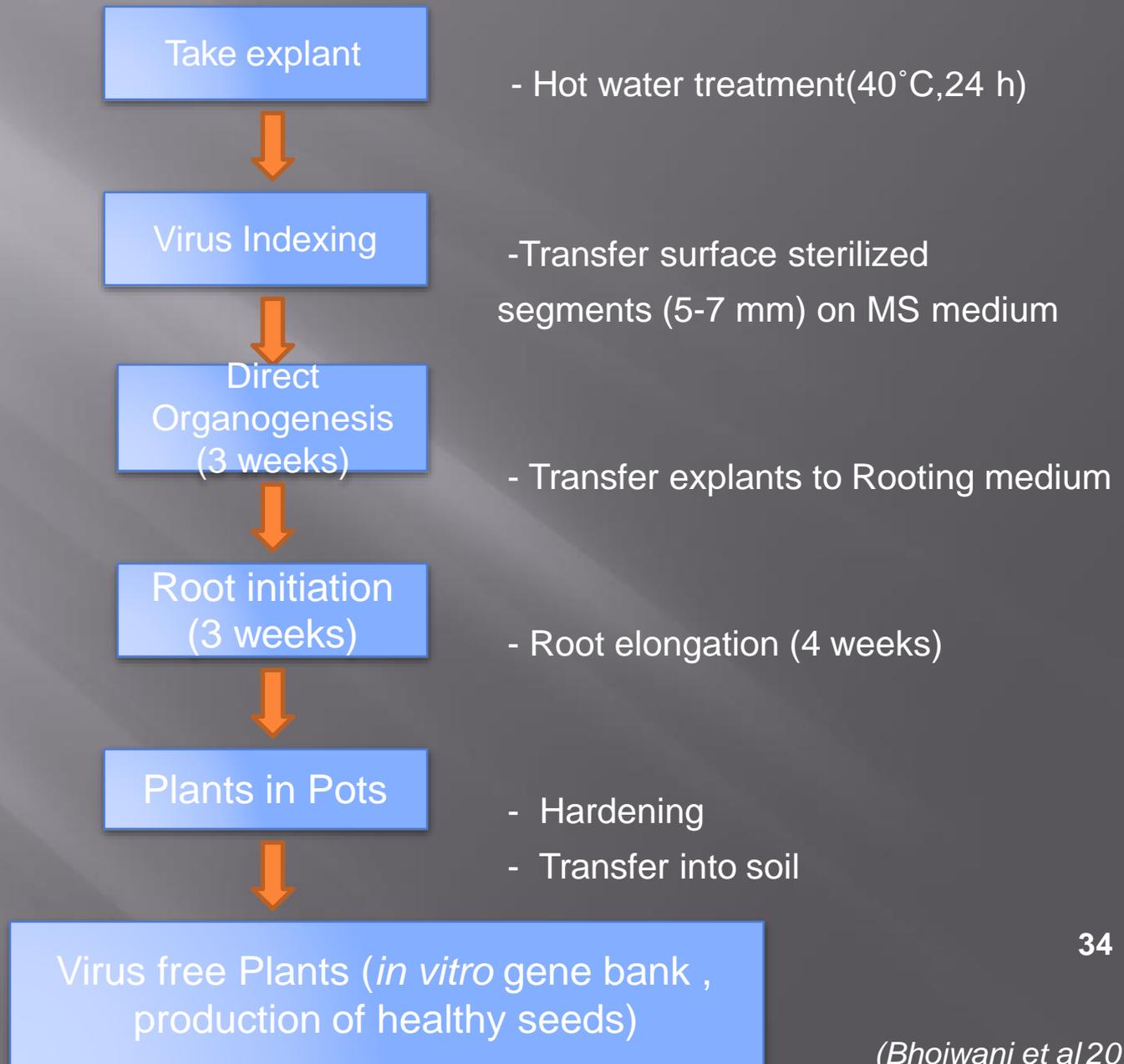
Table 1:- Somaclonal variation in different fruit crops.

Variation Presence			Variation Absence		
Fruit	Explant Source	Reference	Fruit	Explant Source	Reference
Kiwifruit	Leaf blade and Petiole	Prado <i>et.al</i> (2008)	Almond	Axillary branching	Martins <i>et.al</i> (2004)
Oil palm	Zygotic embryo	Rival <i>et.al</i> (2013)	Banana	Shoot tip	Ray <i>et.al</i> (2006)
Papaya	Axillary Shoot tip	Kaity <i>et.al</i> (2009)	Vitis Sp.	Nodal Segment	Alizadeh <i>et.al</i> (2008)

3.PRODUCTION OF VIRUS FREE PLANTS

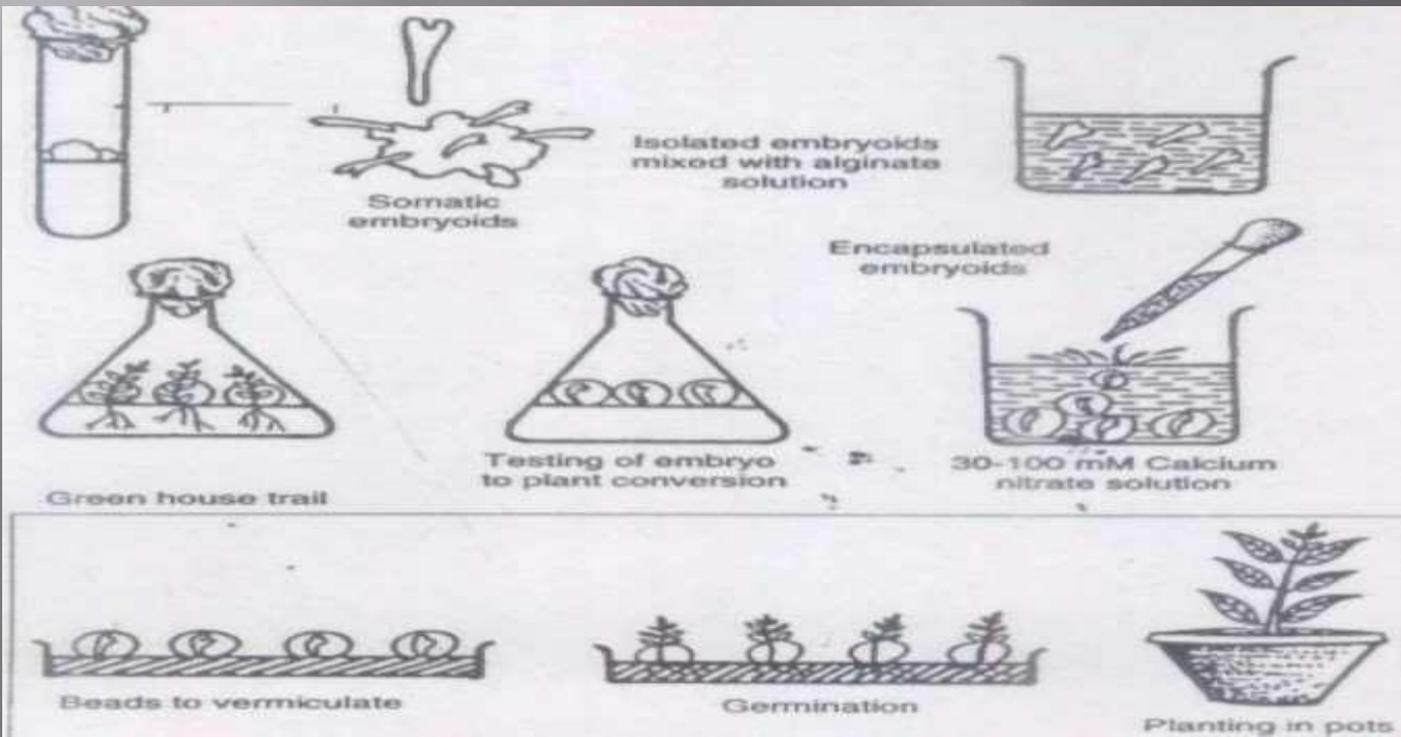
- In tissue culture application produced virus free plants. The Viral diseases in plants transfer easily and lower the quality and yield of the plants. It is very difficult to treat and cure the virus infected plants therefore the plant breeders are always interested in developing and growing virus free plant.
- In some crops like ornamental plants, it has become possible to produce **virus free plants through tissue culture at the commercial level.**

PROCEDURE TO PRODUCE VIRUS FREE PLANT



4. PRODUCTION OF SYNTHETIC SEEDS

- In Synthetic seeds the somatic embryos are encapsulated in a suitable matrix (e.g sodium alginate), along with substances like insecticides, fungicides, and herbicides. These artificial seeds can be utilized for the rapid and mass propagation of desired plant species as well as herbicides varieties.



SYNTHETIC SEED PRODUCTION

Explant selected from healthy plant



Induced callus in explant



Somatic embryo induced in callus



Somatic embryo proliferated



Maturation of somatic embryo



Encapsulation of somatic embryo



Invitro germination



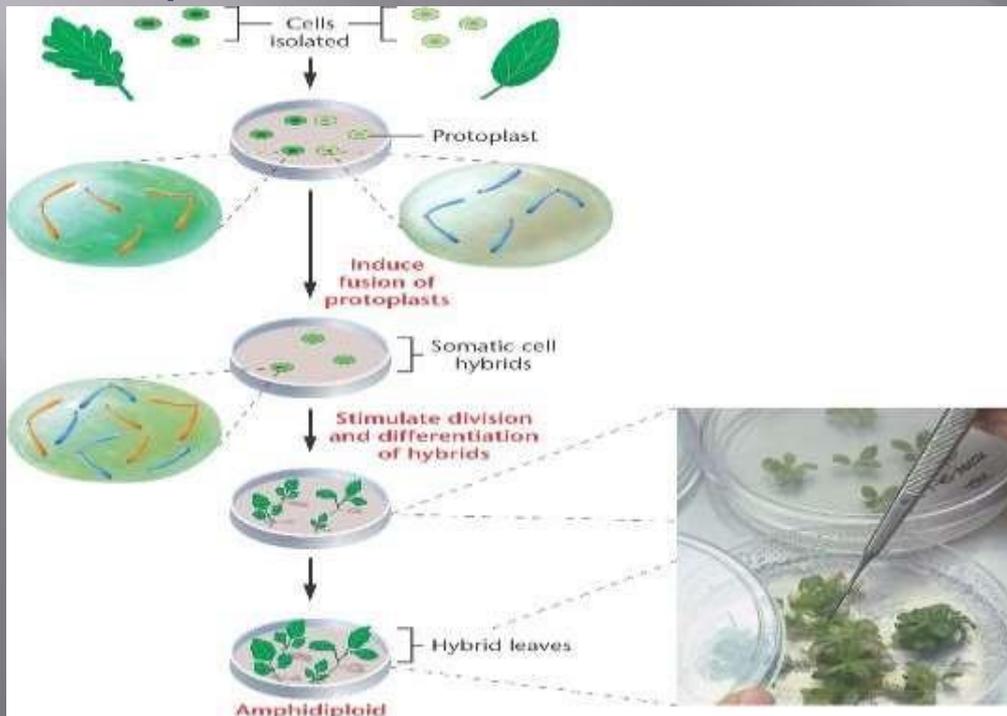
Acclimatization, induce fruit



Produce Synthetic Seed

5.SOMATIC HYBRIDIZATION

- Somatic hybridization broadly involves in vitro fusion of isolated protoplasts to form a hybrid cell and its subsequent development to form a hybrid plant.
- Development of hybrid plants through the fusion of somatic protoplasts of two different plant species/varieties is called somatic hybridization.



SOMATIC HYBRIDIZATION TECHNIQUES

Isolation of protoplast



Fusion of the protoplasts of desired species/varieties



Identification and Selection of Somatic hybrid cells



Culture of the hybrid cells



Regeneration of hybrid plants

6. IN VITRO GERmplasm APPLICATION

- Germplasm refers to the sum total of genes present in a crop and its related species.
- The conservation of germplasm involves the preservation of the genetic diversity of a particular plant.
- This will ensure the availability of valuable germplasm to breeder to develop new and improved varieties.
- Germplasm conservation depending upon the crop species and method of preservation of genetic resources from 1 to 15 years.
- Important **method** of conservation of germplasm is **Cryopreservation**.

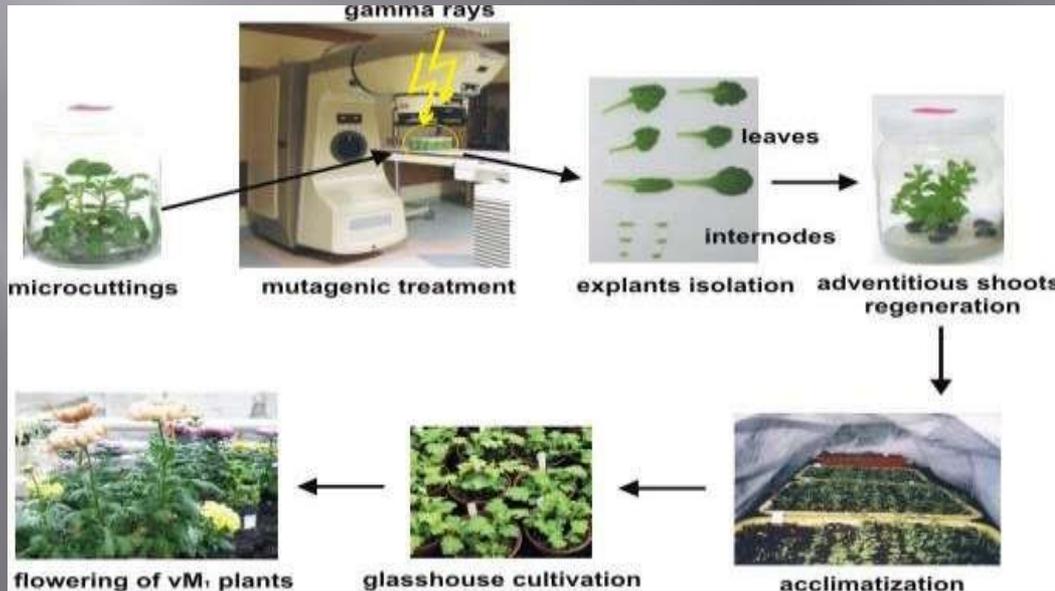


CRYOPRESERVATION

- The germplasm is stored as a very low temperature using solid carbon dioxide (at -79°C)
- Using low temperature deep freezers (at -80°C)
- Using Vapour nitrogen (at -150°C)
- Using Liquid nitrogen (at -180°C)
- Any tissue from a plant can be used for cryopreservation e.g, meristems, embryos, endosperms, ovules, seeds, cultured plant cells, protoplasts, calluses.

7. MUTATION BREEDING

- Mutagenic agents, such as radiation and certain chemicals, then can be used to induce mutations and generate genetic variations from which desired mutants may be selected.
- Mutation induction has become a proven way of creating variation within a crop variety.

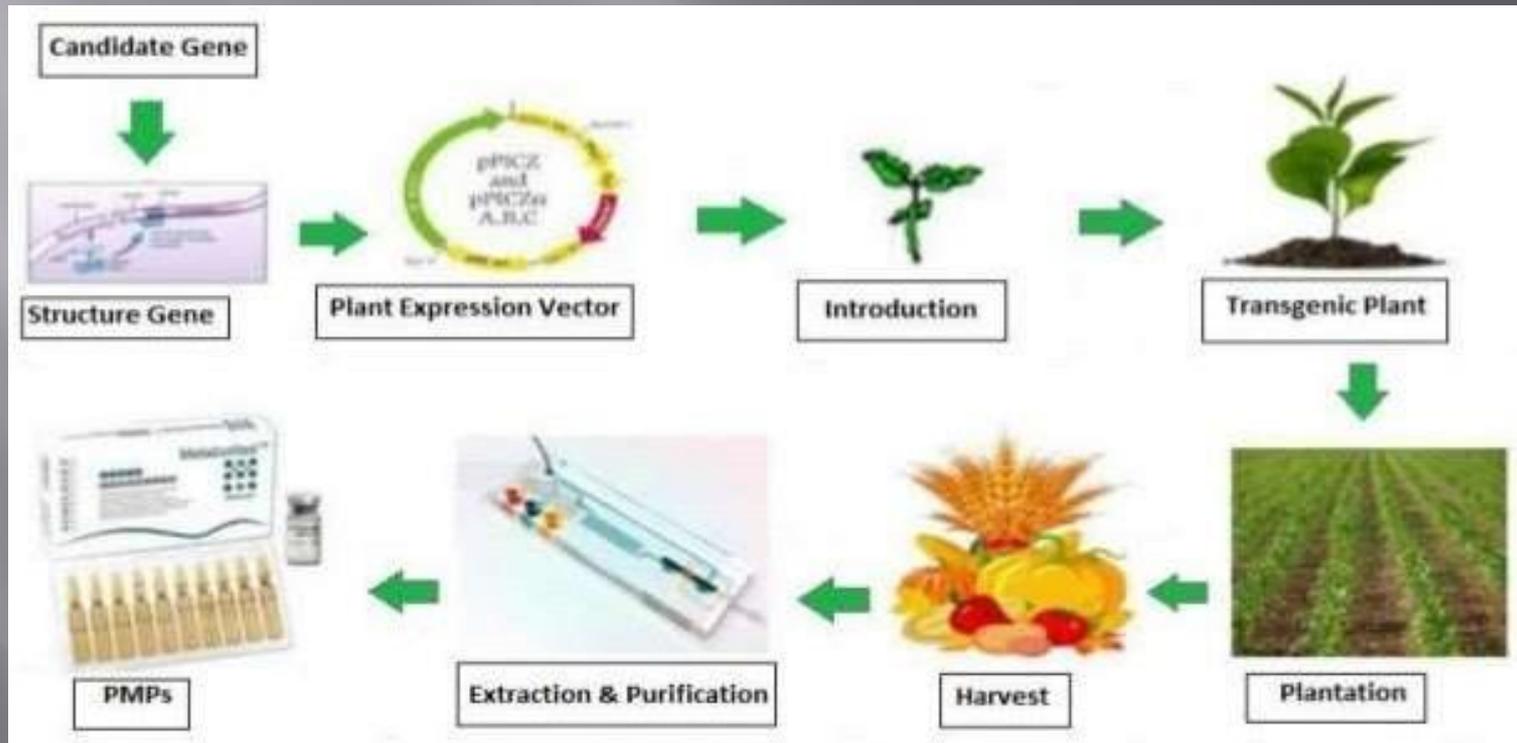


MUTATION BREEDING CHEMICALLY

1. Take shoot-tip area of explant (0.2 mm size)
2. Cultured on shoot induction medium
3. Stem segments incubated in growth chamber for 2 days
4. Activate the lateral vegetative buds
5. Transferred into 50 ml plastic tubes
6. 35–40 ml EMS(Ethyl MethaneSulphonate) solution and placed on a shaker .
7. 60–90 RPM for the desired time.
8. Explants were washed with sterile water 4–5 times
9. Shaken in sterile water for 1 h at 60–90 RPM
10. Treated and washed stem segments were cut into small pieces about 4–5 mm in length
11. Transferred to fresh SIM for incubation in the growth chamber set at 25 C (± 1), 16/8 light/dark with light intensity 1500–2500 LUX for 3–4 weeks.

8. MOLECULAR FARMING

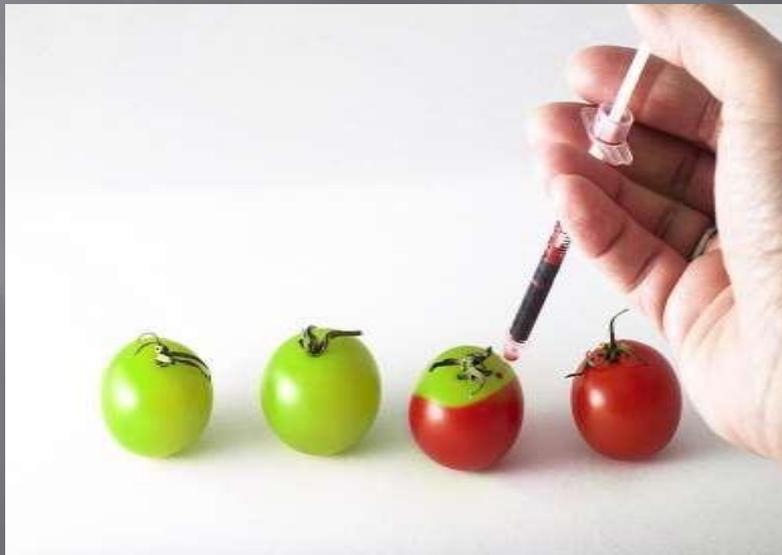
- Molecular farming is the use of whole plants or plant cells/tissues cultured in vitro for the production of valuable recombinant proteins.



- The **advantages** of plant-based systems can be summarized as follows.
- Plants are less expensive to set up and maintain than cultured cells.
- Plant-based systems are extremely versatile.
- which has been established as an economically viable alternative to mainstream production system and cells cultivated in large-scale bioreactors.

9. GENETIC ENGINEERING

- Although genetic engineering and hybridization by conventional breeding can augment genetic variation in plants.
- In terms of quick returns, the time needed to produce a new genotype can be a critical factor for its commercial exploitation.



PRODUCTION OF GENE

The production of DNA fragments to be cloned



Insertion of the DNA fragments into a suitable vector



Introduction of the recombinant DNA into a suitable host



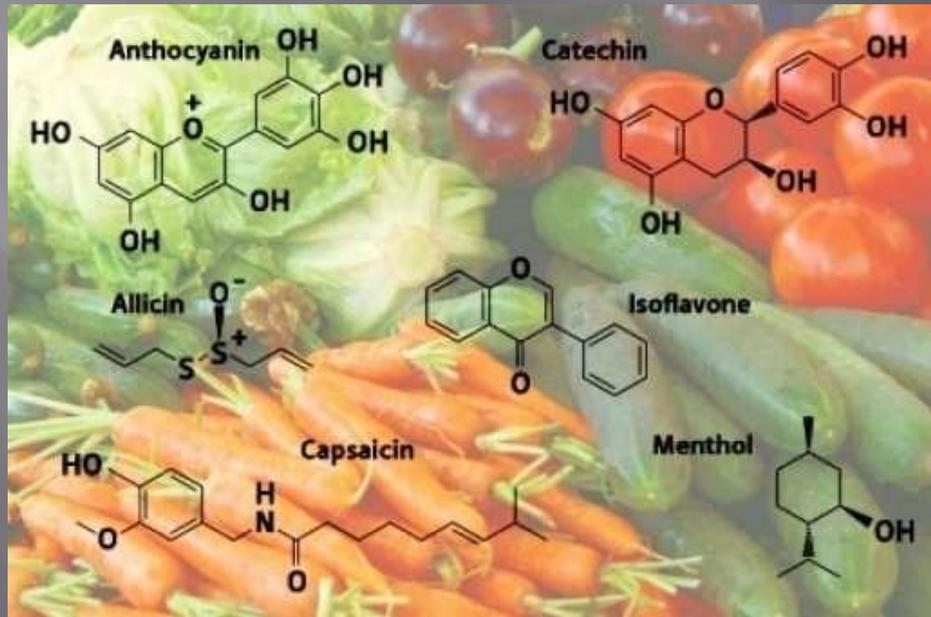
Selection of the host cell or clones carrying the desired DNA



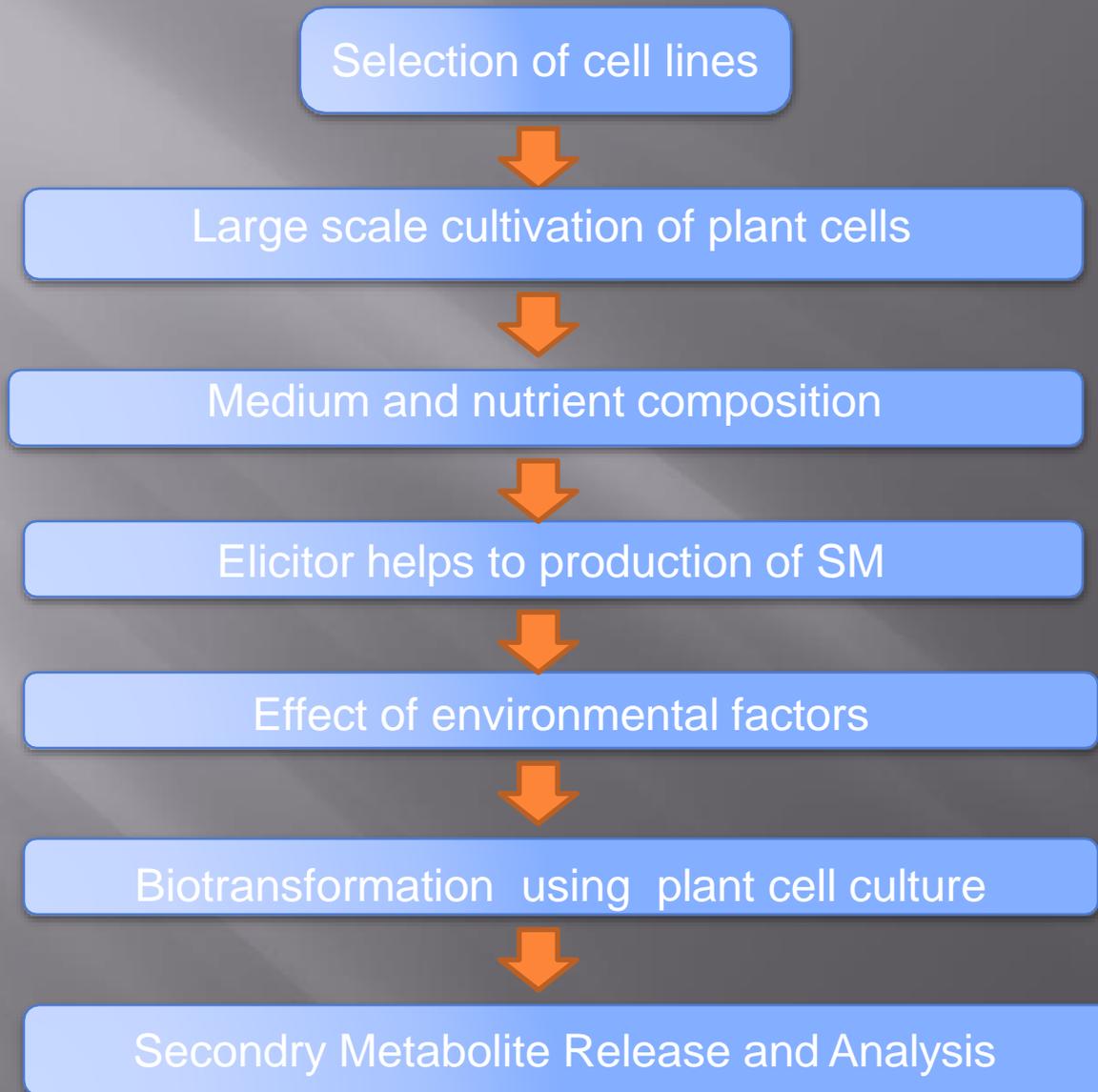
Using the DNA insert (gene) from recombinant DNA for Propagation 46

10. PRODUCTION OF SECONDARY METABOLITES

- Secondary metabolites can be produced by using different biotechnological approaches, such as callus cultures, cell suspension cultures and/or organ cultures.
- It was observed, that **production of secondary metabolites is generally higher in differentiated plant tissues**, there were attempts to cultivate whole plant organs, i.e. shoots or roots in *invitro* conditions with the aim to produce medicinally important compounds (Biondi et al., 2002).



ASPECTS OF SECONDARY METABOLITES



CONCLUSION

- Tissue culture is one of the most important part
 - ▣ of applied biotechnology.
- In the coming decades the world's population will increase more and accommodation space, agricultural lands will decrease significantly.
- Increase per capita availability of food by tissue culture process easily.
- Keeping these in mind we have to ensure a peaceful, healthy and hunger free greener world for our next generation. For doing this there is no alternate of plant tissue culture.

**THANK
YOU**