
PLANT TISSUE CULTURE
PLANT TISSUE CULTURE
ITS METHOD AND
APPLICATIONS
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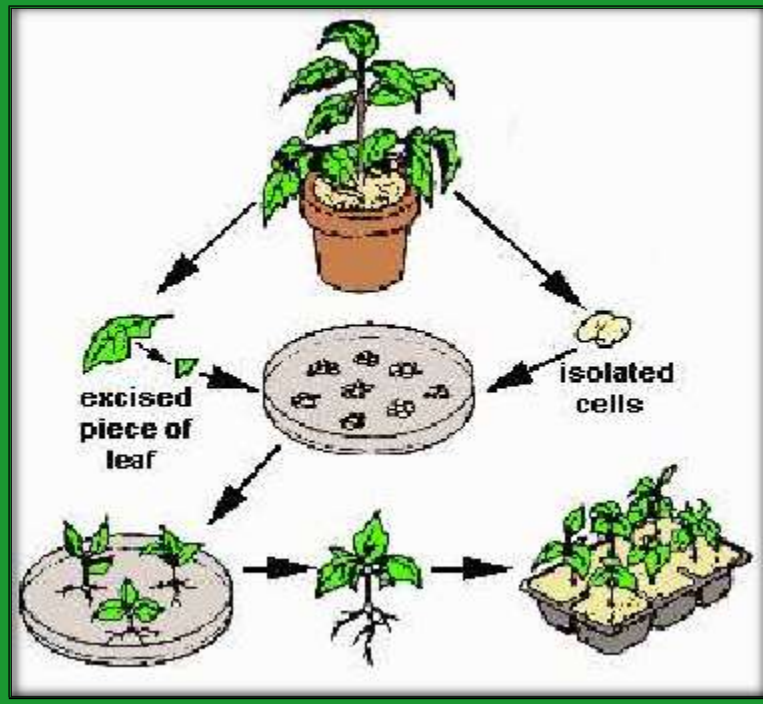
WHAT IS TISSUE CULTURE?

Origin : Early 20th century with the work of **Gottleib Haberlandt** (plants).

TISSUE CULTURE is the term used for "the process of growing cells artificially in the laboratory.

PLANT TISSUE CULTURE- Technique of growing plant cells, tissues, and organs in an artificially prepared nutrient medium under aseptic condition.

Plant cells are ***totipotent***.



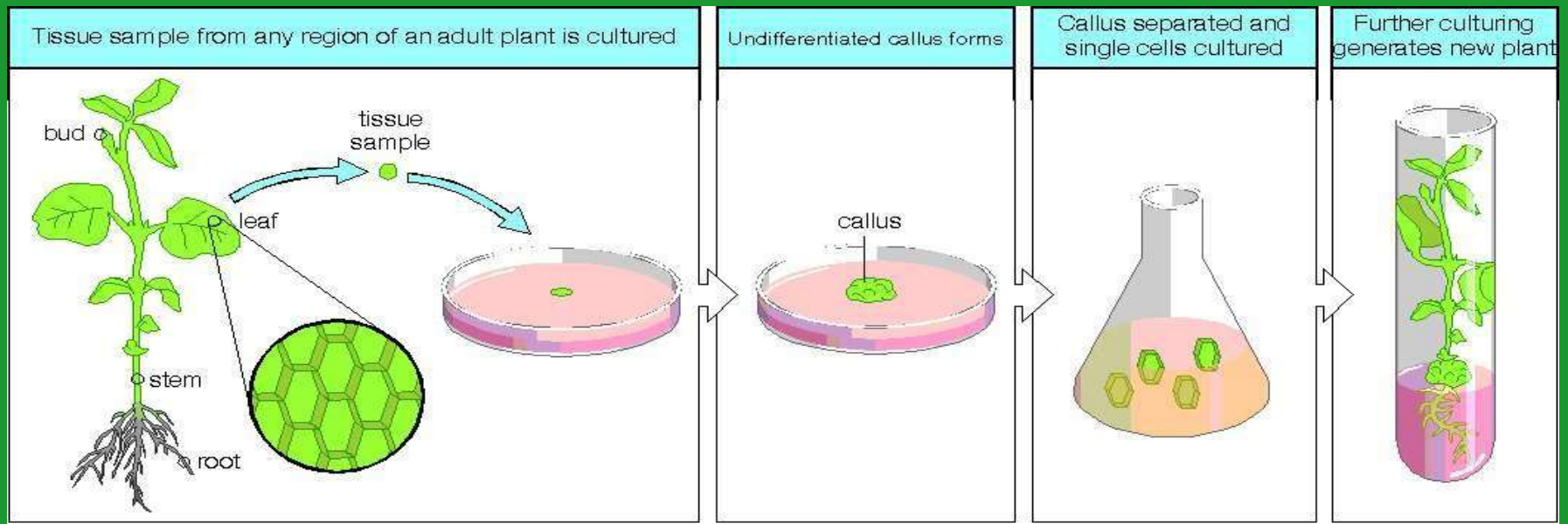
REQUIREMENT OF TISSUE CULTURE:

Appropriate tissue (explant)

- A suitable growth medium (Some of the common media are MS, LS, Gamborg B5, White's, Hellar for haploids etc)
- Growth regulators
- Aseptic (sterile) conditions
- Frequent subculturing :

TYPES OF PLANT TISSUE CULTURE

- SEED CULTURE
- EMBRYO CULTURE
- MERISTEM CULTURE
- BUD CULTUR
- CALLUS CULTURE
- CELL SUSPENSION CULTURE
- ANTHER CULTURE/OVARY CULTURE
- PROTOPLAST CULTURE



PROCESS OF PLANT TISSUE CULTURE

Parent plant

Selection of explant from mother plant

Inoculation

Light &warmth for shoot initiation

Callus induction

Shoot formation

Shoots are transferred to rootingmedia(hig her auxin conc)

Plants are kept for hardening in green house ,sold to a nursery and then pottedup.

The rooted shoots are potted up (deflasked) and 'hardened off'

This is necessary as many young tissue culture plants have no waxy cuticle to prevent water loss.

Tissue culture plants sold to a nursery & then potted up

FACTORS AFFECTING TISSUE CULTURE

- Growth Media
  - Minerals, Growth factors, Carbon source, Hormones
- Environmental Factors
  - Light, Temperature, Photoperiod, Sterility, Media




- Explant Source
  - Usually, the younger, less differentiated explant, the better for tissue culture
  - Different species show differences in amenability to tissue culture

TISSUE CULTURE APPLICATIONS

- ✓ Micropropagation
- ✓ Germplasm preservation
- ✓ Somaclonal variation
- ✓ Haploid & dihaploid production
- ✓ *In vitro* hybridization – protoplast fusion
- ✓ Embryo rescue
- ✓ Synthetic seed production

MICRO-PROPAGATION

Large scale production of genetically identical clones in vitro is called **Micro-propagation.**

ADVANTAGES:

- Millions of shoot tips from a small piece of plant tissue in a short period of time and space.
- Maintain Genetically uniform progeny.
- Can produce exact copies of plants required that have desirable traits.
- Supplies plant throughout the year irrespective of seasonal variation

COMMERCIAL APPLICATION OF MICROPROPAGATION

- Micro propagation of Orchids demonstrated profit all over the world.
- Many other crops like banana, papaya, potato, gerbera, carnation, Anthurium, apple, pear,cherry etc have been grown through micro propagation are commercially exploited.
- Development of disease resistant crops .Some valuable clones recovered from virus are
- Tobacco- TMV, Sugarcane-Mosaic virus etc.



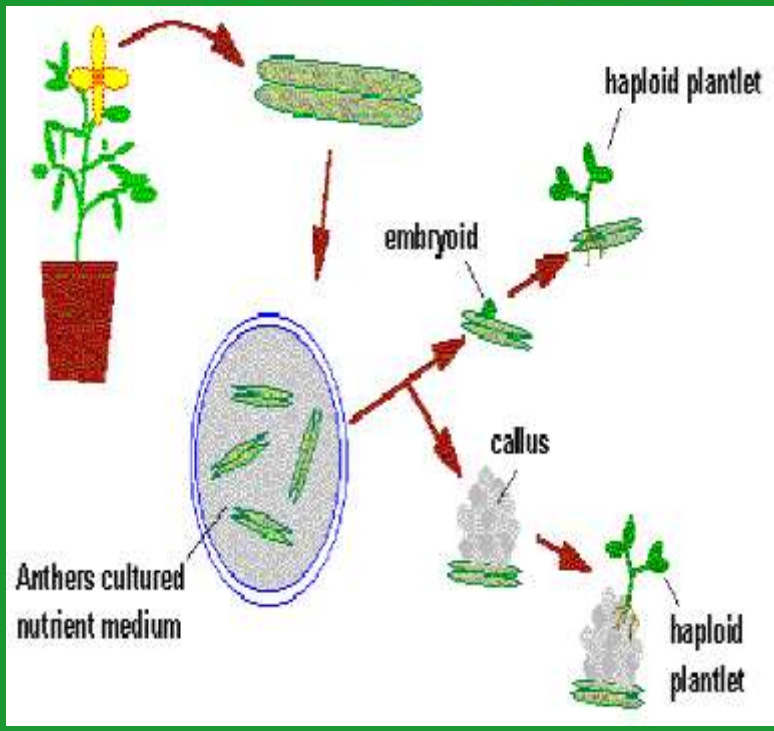

- Among the innovative techniques of micro propagation, the concept of somatic embryogenesis with synthetic seed production or artificial seed technology is very promising

GERMPLASM CONSERVATION

- Maintenance of germplasm of plants vulnerable to pathogen, insects and various climatic hazards and plants with early embryo degeneration, are difficult to maintain.
- Plant species which are endangered, rare threatened with extinction are needed to be conserved by ex-citu method of germplasm conservation.
- Provides cost effective alternative to growing plants under field condition , nurseries or green houses.
- Cryopreservation of germplasm is really effective in conservation biotechnology.

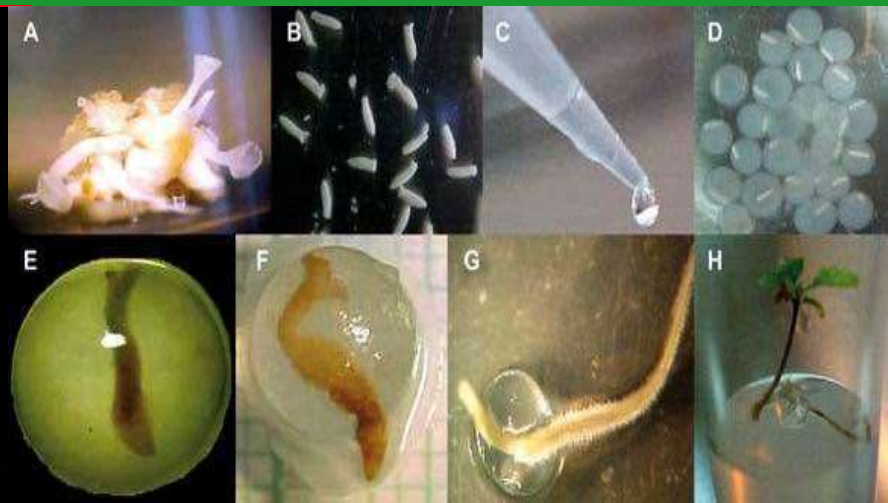
HAPLOID&DIHAPLOID PRODUCTION

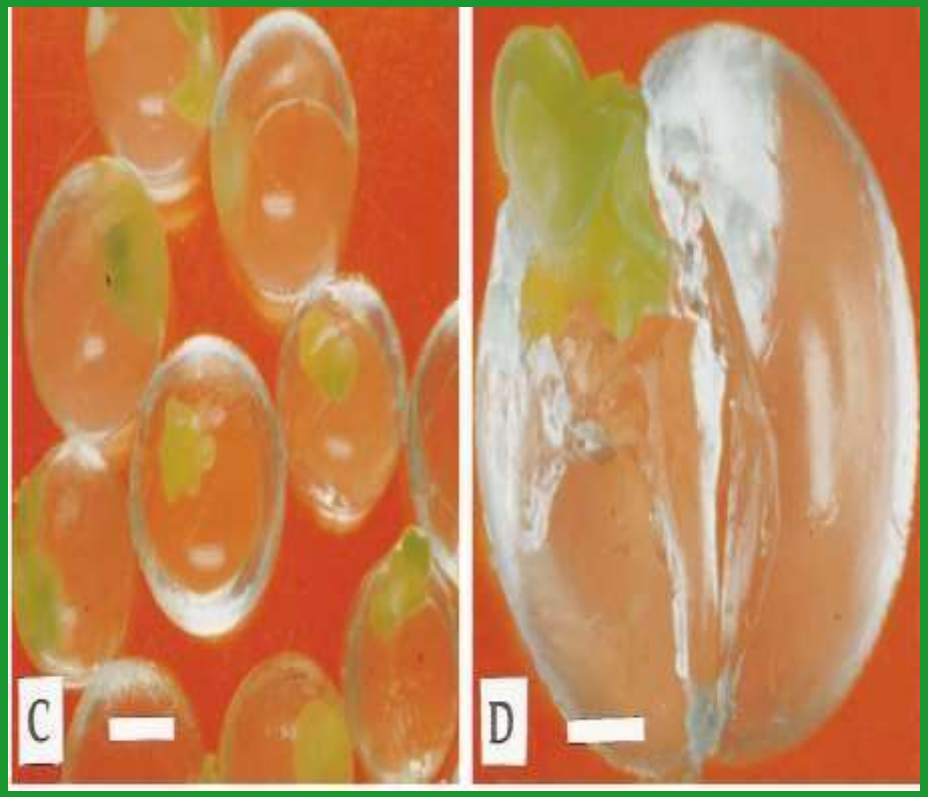
- Anther or Ovary/ovule culture
- Production of pure homozygous line in leser time period.
- Androgenic haploids are used to produce different lines of aneuploids like monosomic,nullisomic,trisomic etc.
- Induction of mutagenesis
- Recessive traits(e.g low glucosinolate in Brassica) can be made expressed.
- Use in hybrid development and early release of varieties.



ARTIFICIAL SEED PRODUCTION

Synthetic or Artificial Seeds (living seed-like structure) are any totipotent cells(somatic embryos or meristem tips etc)which is artificially encapsulated by chemicals (Hydrogels) which behave like true seeds if grown in soil and can be used as substitutes of true seeds and possess the ability to convert into a plant ex vitro or invitro.





GERMINATED SYNTHETIC SEEDS



EMBRYO CULTURE

- Using embryo culture seed dormancy can be overcome.
- Embryo rescue which can circumvent post zygotic barriers.
- To raise the rare hybrids by rescuing embryo from incompatible crosses is the most popular application. The production of embryos from somatic or "non-germ" cells.
- Usually involves a callus intermediate stage which can result in variation among seedlings

PROTOPLAST FUSION




- Production of somatic hybrids.
- Production of CMS line.
- Somatic Hybrids with specific alien gene.
- Production of Unique Nuclear Cytoplasmic Combination.
- Production of Autotetraploid or Allotetraploid.
- Production of Heterozygous line.

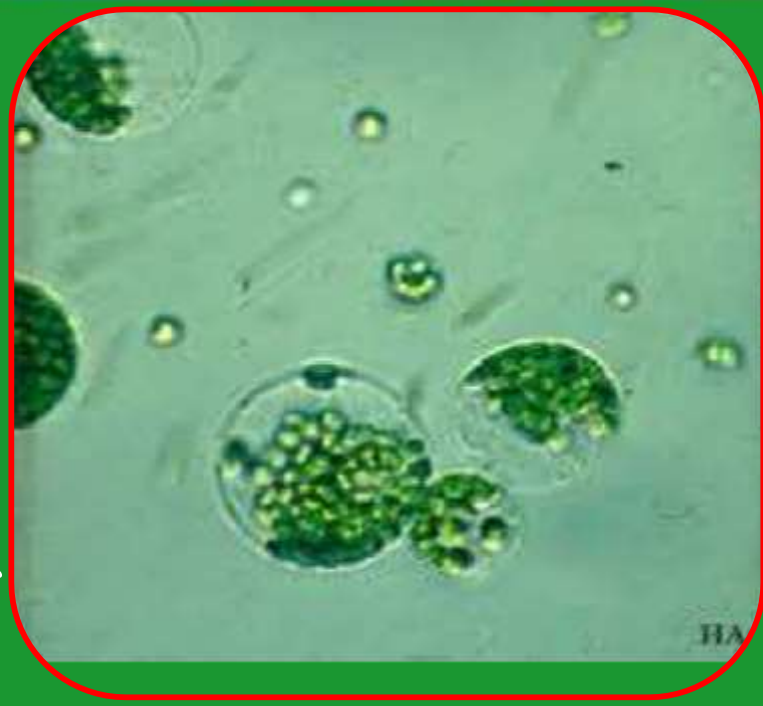
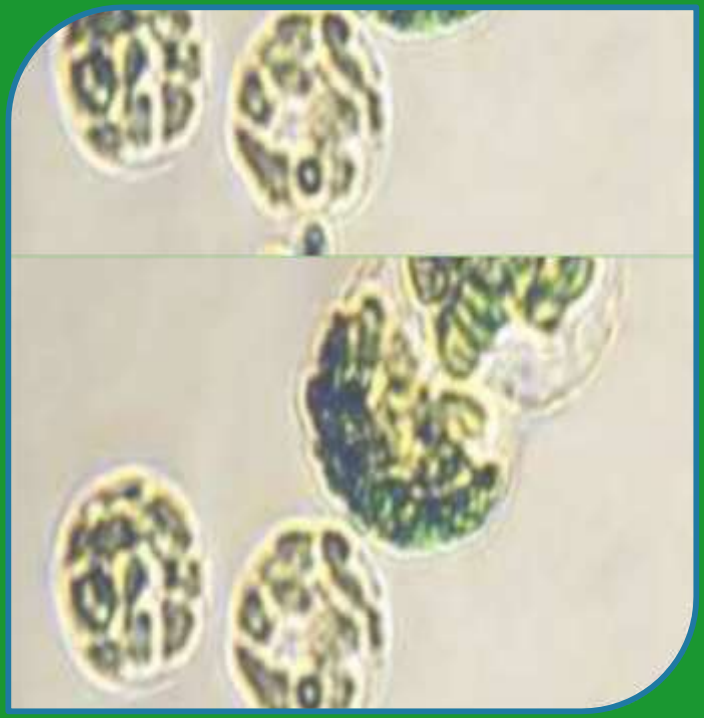


FIGURE 6.6a Two tobacco plant protoplasts, obtained by digesting away the cell wall, are fused (6a and 6b) to produce a cell that acquires some of the characteristics of both genetic backgrounds and can be regenerated into a plant with some traits from both parental plants.

SOMACLONAL VARIATION

- Play an important role in polyploidisation.
- Genetic variability can be raised which include resistance to a particular disease, herbicide resistance, stress tolerance etc



ADVANTAGES OF PLANT TISSUE CULTURE I

- fast commercial propagation of new cultivars.
- Prevention of starvation in third world countries with higher productivity if its genome is changed.
- Taking an explant does not usually destroy the mother plant, so rare and endangered plants can be cloned safely.
- Once established, a plant tissue culture line can give a continuous supply of young plants throughout the year.
- The production of transgenic plants that produce particularly good flowers, fruits, or have other desirable traits.

ADVANTAGES OF PLANT TISSUE CULTURE II

- The regeneration of whole plants from plant cells that have been genetically modified.
- Tissue culture clones are 'true to type' as compared with seedlings, which show greater variability.
- The production of plants in sterile containers reduces disease transmission. To clean particular plants of viral and other infections and to quickly multiply these plants as 'cleaned stock' for horticulture and agriculture.

ADVANTAGES OF PLANT TISSUE CULTURE III

- The production of multiples of plants in the absence of seeds or necessary pollinators to produce seeds.

- Allows production of plants from seeds that otherwise have very low chances of germinating and growing, i.e.: Orchids and Nepenthes.

DISADVANTAGES OF PLANT TISSUE CULTURE

- Chromosomal abnormalities appear as cultures age: undesirable changes.
- Expensive,labour intensive especially if less than 1000 plants are needed.
- The procedure is quite variable and also it depends on the type of the species so sometimes it needs trial-and-error type of experiments if there is no available review about that species.




Continued..

The procedure needs special attention and diligently done observation.

There may be error in the identity of the organisms after culture.

Infection may continue through generations easily if possible precautions are not taken

Decrease genetic variability.

ADVANTAGES OF TISSUE CULTURE IN CROP IMPROVEMENT I

- Tissue culture has been exploited to create genetic variability from which crop plants can be improved.
- to improve the state of health of the planted material and to increase the number of desirable germplasms available to the plant breeder
- The culture of single cells and meristems can be effectively used to eradicate pathogens from planting material and thereby dramatically improve the yield of established cultivars.

ADVANTAGES OF TISSUE CULTURE IN CROP IMPROVEMENT II

- Large-scale micropropagation laboratories are providing millions of plants for the commercial ornamental market and the agricultural, clonally-propagated crop market. With selected laboratory material typically taking one or two decades to reach the commercial market through plant breeding, this technology can be expected to have an ever increasing impact on crop improvement as we approach the new millenium.

Research on some horticulture crops

1.POTATO

Solanum tuberosum

Tissue culture plants are when grown In insect proof net houses produces mini tubers.

- Tissue culture of mini tubers give 20-30% higher yield.
- Protected against bacterial disease aphids(bacterial disease-crop failure).
- Uniform evenly shaped potatoes.

BANANA

- Disease & pest free planting material.
- Little space needed for multiplying large number of plants
- Plantlets more vigourous , uniform shorter harvest period, higher bunch weights.
- Allows for faster distribution of superior germplasm.

EMBRYO CULTURE OF WILD

FRUITS

PINEAPPLE

- Resistance to mealy bugs,nematodes,wilt disease,heart and root rot.
- Uniform yield,uniform fruit weighing.
- Faster growth and spineless darker leaves
- Sweeter pulp
- Ripens evenly and naturally

PAPAYA

Rapid and mass propagation of homogenous and uniform plants for both commercial and research purposes.

- As the sex of the plant is known,the required nummber of male to female plant species can be produced.
- To decrease mortality% and to increase gaermination%.
- To increase yield.
- To avoid papaya viral disease.
- To obtain export quality food.

FLOWERS

SUNFLOWER

- IMMATURE EMBRYO CULTURE- For the shortening of the entire lifecylce
- To overcome seed dormancy.
- In vitro tissue culture methods in sunflower accelerates the breeding process.
- Gene from sunflower known as **HAHB4**, which helps the plant endure water shortages. That gene is introduced into wheat, soybean and maize.

GERBERA

Gerbera – hybrid seed germination followed by axillary bud proliferation and separation

ORCHIDS

- Seeds are exceedingly small with small reserves,loose their viability very fast so they need to be germinated in a nutrient medium.
- Higher seed germination % even in rare to germinate species
- Exact clones are produced .
- Mass and rapid propagation.
- Diseased free plant
- Embryo rescue and culture in hybrid plant production.

ORCHIDS

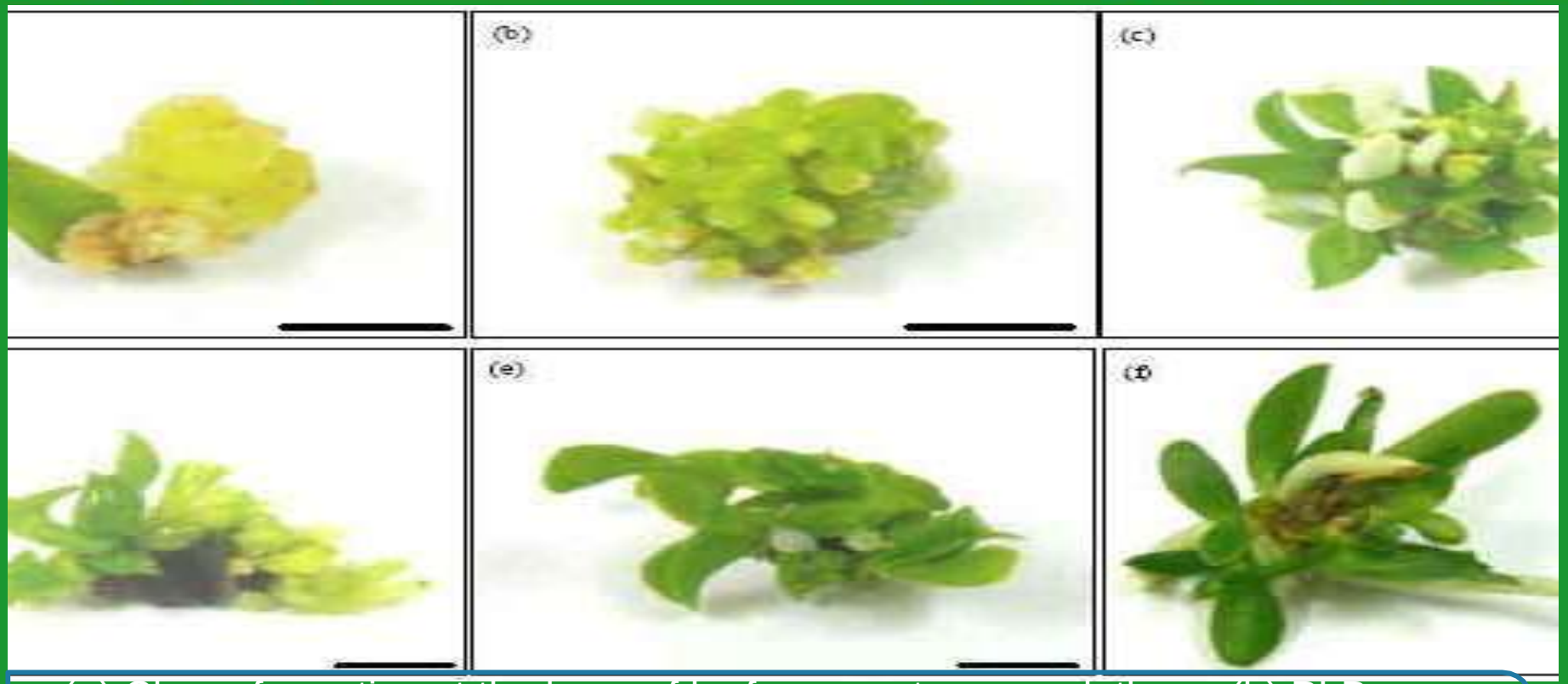
CAPSULE OF ORCHID

MATURE CAPSULE BEARING SEEDS

CALLUS (OR PLB)OF ORCHID

INVITRO GERMINATION BY SEEDS

Callus culture of Orchids



(a) Clump formation at the base of leaf segments on week three, (b) PLB initiation, (c) PLB development into shoots and roots, (d) direct shoot formation (e) shoot induced roots on week six and (f) 3-month-old plantlets

Conclusion

- Plant tissue culture represents most promising areas of application and giving an outlook into the future.
- While tissue-culture offers tremendous benefits, it could be a bane if undertaken improperly.
- It could be the fastest means of spreading disease in any crop. If the source of the material to be tissue-cultured is disease-infected, the resulting tissue-cultured plantlets will carry the disease.
- Since tissue culture could produce thousands if not millions of plantlets tremendously fast, the disease could spread super fast. Thus conducting tissue culture we must be sure that the parent plant is free from infection or disease.




THANK YOU









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