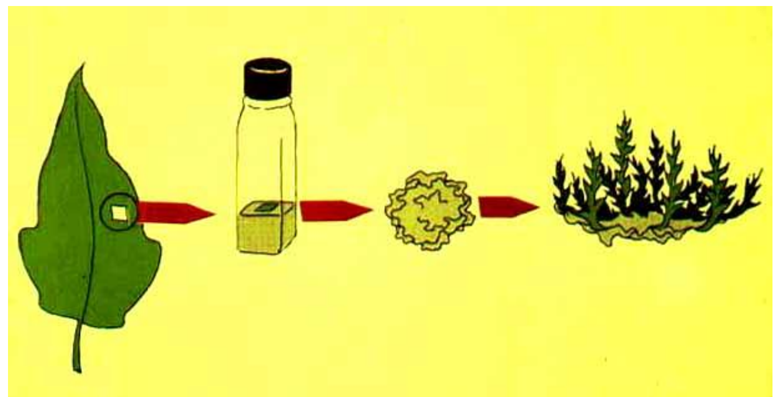
Micropropagation

Multiplication of genetically identical copies of a cultivar by asexual reproduction is called *clonal propagation*. In nature, clonal propagation occurs by apomixis (seed development without meiosis and fertilization) and/or vegetative propagation (regeneration of new plants from vegetative parts). Tissue culture has become popular method for vegetative propagation of plants. Aseptic method of clonal propagation is called as *Micropropagation* and it offer the advantage of large number of true-to-type plantlets can be produced with relatively short time and space from a single individual. It is the fact that micropropagation is the only commercially viable method of clonal propagation of most of the horticultural crops. E.g. Orchids.

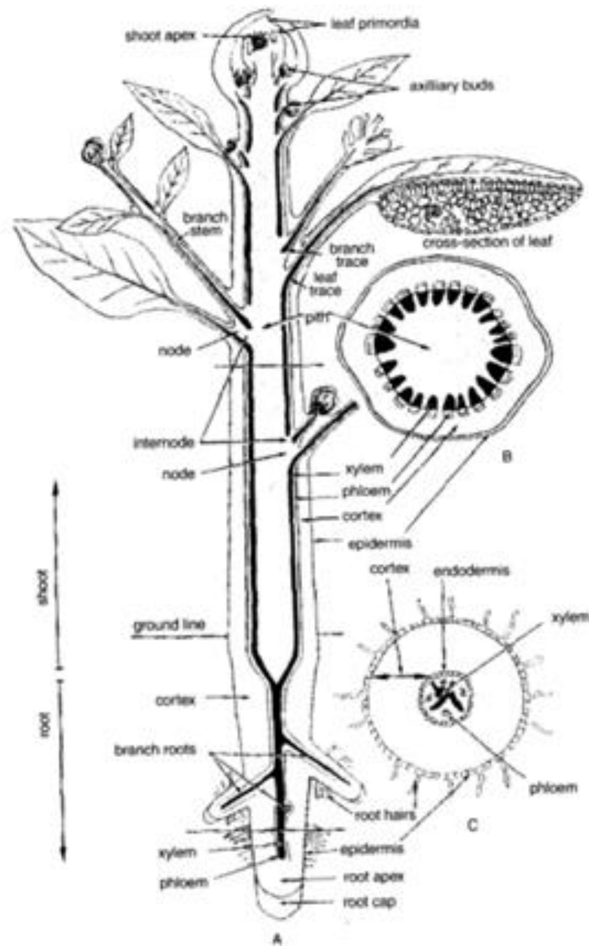
Explants used in micropropagation

Different kinds of explants were used in micropropagation. For example, in case of orchids, shoot tip (*Anacamptis pyramidalis, Aranthera, Calanthe, Dendrobium),* axillary bud (*Aranda, Brassocattleya, Cattleya, Laelia)*, inflorescence segment (*Aranda, Ascofinetia, Neostylis, Vascostylis)*, lateral bud (*Cattleya, Rhynocostylis gigantean)*, leaf base (*Cattleya)*, leaf tip (*Cattleya, Epidendrum),* shoot tip (*Cymbidium, Dendrobium, Odontioda, Odontonia),* nodal segment (*Dendrobium),* flower stalk segment (*Dendrobium, Phalaenopsis)* and root tips (*Neottia, Vanilla)* are being used in micropropagation.

Different explants



Differentiation of shoots directly from callus



A. The principle organs and tissues of the body of a seed plant; B. Cross-section of stem; C. Cross-section of root. Apical or axillary buds are good sources of explants

Stages in micropropagation

Micropropagation generally involves five stages. Each stage has its own requirements.

Stage 0: Preparative stage

This stage involves the preparation of mother plants to provide quality explants for better establishment of aseptic cultures in stage 1. To reduce the contamination problem in the subsequent stages, mother plant should be grown in a glasshouse and watered so as to avoid overhead irrigation. This will also reduce the need for a harsh sterilization treatment. Stage 0

also includes exposing the stock plants to suitable light, temperature and growth regulator treatments to improve the quality of explants. In the case of photosensitive plants it may be possible to obtain suitable explants throughout the year by controlling photoperiod in the glasshouse. For example, red-light treated plants of *Petunia* provided leaf explants which produced up to three times as many shoots as did the explants from untreated plants.

Stage 1. Initiation of culture

1. **Explant:** The nature of explant to be used for *in vitro* propagation is governed by the method of shoot multiplication. For enhanced axillary branching, only the explants which carry a pre-formed vegetative bud are suitable. When the objective is to produce virus-free plants from an infected individual it becomes necessary to start with sub-millimeter shoot tips. If the stock is virus-tested or virus eradication is not necessary, then the most suitable explant is nodal cuttings. Small shoot-tip explants have a low survival rate and show slow initial growth. Meristem tip culture may also result in the loss of certain horticultural characteristics which are controlled by the presence of virus, such as the clear-vein character of the Geranium cv. Crocodile. Generally, the clear vein character is transmitted in petiole-segment culture but not in shoot-tip culture.

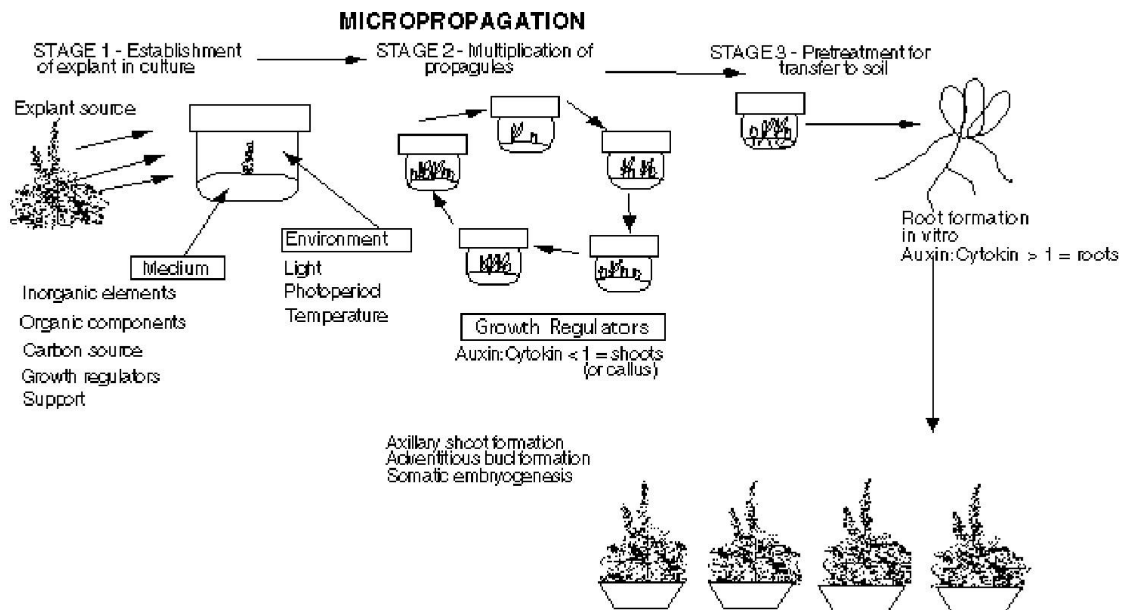
2. **Sterilization:** Special precautions need to be taken when explants are derived from field-grown materials, which is often necessary in cloning an elite tree. In such cases an ideal approach would be to take cuttings from the selected plant and grow them in greenhouse. Discarding the surface tissues from plant materials while preparing the explants also minimizes the loss of cultures due to microbial contamination.

3. **Browning of medium:** A serious problem with the culture of some plant species is the oxidation of phenolic compounds leached out from the cut surface of the explant. It turns the medium dark brown and is often toxic to the tissues. This problem is common with the adult tissues from woody species.

3. Stage 2. Multiplication

This is the most crucial stage since it is the point at which most of failures in micropropagation occur. Broadly three approaches have been followed to achieve *in vitro* multiplication.

1. **Through callusing:** The potentiality of plant cells to multiply indefinitely in cultures and their totipotent nature permit a very rapid multiplication of several plant types. Differentiation of plants from cultured cells may occur via shoot-root formation (organogenesis) or somatic embryogenesis. Somatic embryogenesis is most appealing from a commercial angle. A somatic embryogenesis system once established lends itself to better control than organogenesis. Since somatic embryos are bipolar structures, with defined root and shoot meristems, the rooting stage required for microshoots gets eliminated. Above all, somatic embryos being small, uniform and bipolar are more amenable to automation at the multiplication stage and for field planting as synthetic seeds, offering cost advantages from labour savings, can also be stored through cold storage, cryopreservation or desiccation for prolonged periods. These characteristics make somatic embryogenesis potentially a less expensive and flexible system for micropropagation. The most serious objection against the use of callus cultures for shoot multiplication is the genetic instability of their cells.



2. **Adventitious bud formation:** Buds arising from any place other than leaf axil or the shoot apex are termed adventitious buds. The shoots differentiated from calli should also be treated as adventitious buds. In many crops, vegetative propagation through adventitious bud formation from root (blackberry, raspberry) and leaf (*Begonia, Crassula)* cuttings is standard horticultural practice. In such cases the rate of adventitious bud development can be considerably enhanced under culture conditions. For most bulbous plants (e.g. Lilley) adventitious bud formation is the most important mode of multiplication and the best

explants are obtained from bulb scales. A serious problem may arise when this method of propagation is applied to varieties which are genetic chimeras. Adventitious bud formation involves the risk of splitting the chimeras leading to pure type plants. For example, in variegated geranium cv. Mme Salleron, the chimera is perpetuated in meristem culture but broken down in petiole culture.

3. **Enhanced axillary branching:** In cultures the rate of shoot multiplication by enhanced axillary branching can be substantially enhanced by growing shoots in a medium containing a suitable cytokinin at an appropriate concentration with or without auxin. Due to continuous availability of cytokinin, the shoots formed by the bud, present on the explant, develops axillary buds which may grow directly into shoots. This process may be repeated several times and the initial explant transformed into a mass of branches.

4. Stage 3. Rooting of shoots

Somatic embryos carry a pre-formed radical and may develop directly into plantlet. However, these embryos often show very poor conversion into plantlets, especially under *in vitro* conditions. They require an additional step of maturation to acquire the capability for normal germination. Adventitious and axillary shoots developed in cultures in the presence of a cytokinin generally lack roots. To obtain full plants the shoots must be transferred to a rooting medium which is different from the shoot multiplication medium, particularly in its hormonal and salt compositions. For rooting, individual shoots measuring 2 cm in length are excised and transferred to the rooting medium.

5. Stage 4. Transplantation

The ultimate success of commercial propagation depends on the ability to transfer plants out of culture on a large scale, at low cost and with high survival rates. The plants multiplied *in vitro* are exposed to a unique set of growth conditions (high levels of inorganic and organic nutrients, growth regulators, sucrose as carbon source, high humidity, low light, poor gaseous exchange) which may support rapid growth and multiplication but also induce structural and physiological abnormalities in the plants, rendering them unfit for survival under *in vivo* conditions. The two main deficiencies of *in vitro* grown plants are – poor control of water loss and heterotrophic mode of nutrition. Therefore, gradual acclimatization is necessary for these plants to survive transition from culture to the greenhouse or field. During acclimatization the *in*

vitro formed leaves do not recover but the plant develops normal leaves and functional roots. While transferring out shoots/roots their lower part is gently washed to remove the medium sticking to them. The individual shoots or plantlets are then transferred to potting mix and irrigated with low concentration of inorganic nutrients. This probably recommissions the photosynthetic machinery of plants, enabling them to withstand the subsequent reduction in the ambient relative humidity and survive under field conditions. A variety of potting mixtures such as peat, perlite, polystyrene beads, vermiculate, fine bark, coarse sand etc. or their mixtures in different combinations are used for transplantation. For initial 10-15 days, it is essential to maintain high humidity (90-100%) around the plants, to which they got adapted during culture. The humidity is gradually reduced to ambient level over a period of 2-4 weeks.

In vitro regenerative protocol of _V. reitzii_

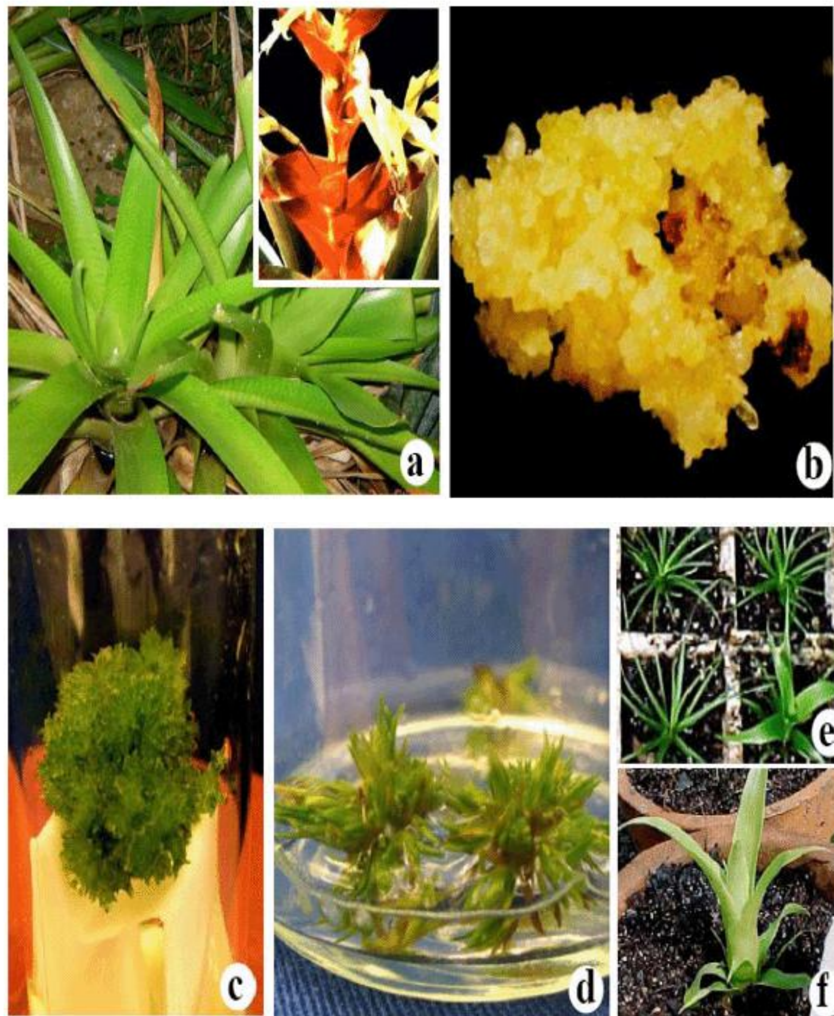
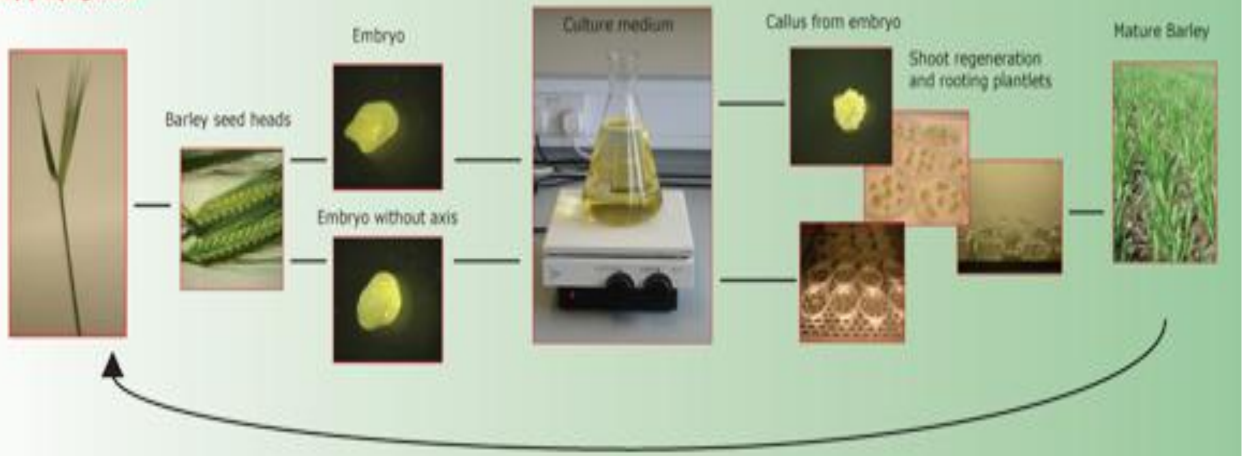
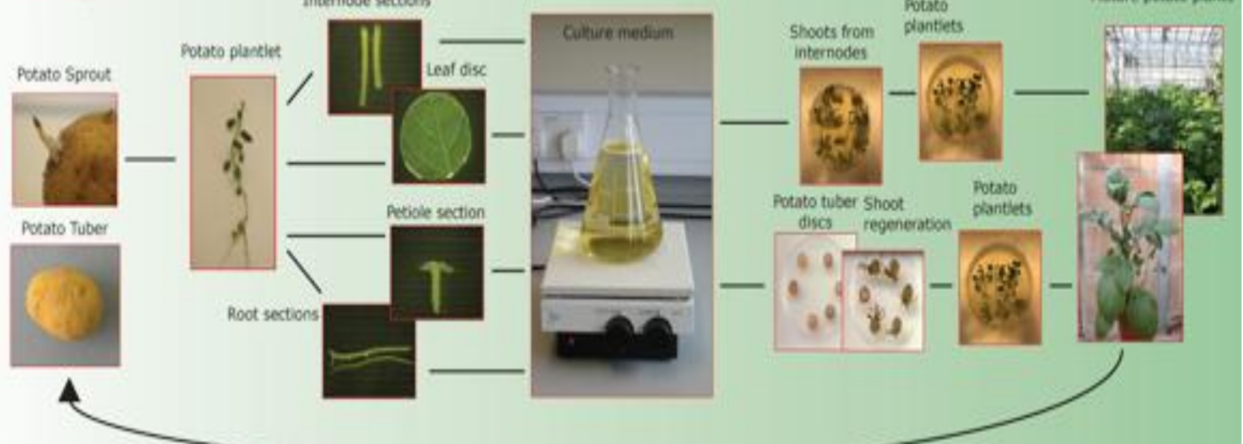


Figure 1 - Nodule cluster culture formation and shoot regeneration in *V. reitzii*. a) Donor plant, see in detail inflorescence of adult plant; b) Yellow nodular cluster cultures induced in MS medium free of PGR; c) Nodule cluster subcultured in MS culture medium supplemented with GA_3 (10μM) resulted in high proliferation rate and the subsequent development of adventitious microshoots; d) Elongation and growth of shoot in MS culture medium free of PGR; e) Acclimatization of plantlets; f) growth in greenhouse. CCA/UFSC, 2004





Advantages of micropropagation

1. Clonal mass propagation - extremely large numbers of plants can be produced. Rather than getting 10000 plants per year from an initial cutting in vegetative propagation, one can obtain more than 1,000,000 plants per year from one initial explant through micropropagation.
2. Culture is initialized from small parts of plants – so no need of much space: from 1 m^2 space in culture room, 20000 - 100000 plants can be produced per year.

3. Production of disease and virus free plantlets. This leads to simplification of international exchange of plants
4. Micropropagation enables growers to increase the production of plants that normally propagate very slowly such as *Narcissus* and other bulbous crops.
5. Introduction of disease free new cultivars is possible through micropropagation
6. Vegetative propagation of sterile hybrids can be used as parent plants for seed production. Eg. Cabbage
7. One of the rapid methods for cloning of disease free trees.
8. *In vitro* cultures can be stored for long time through cryopreservation.
9. Breeding cycle can be shortened.

Disadvantages of micropropagation

1. Expensive laboratory equipment and service
2. No possibility of using mechanization
3. Plants are not autotrophic
4. Poor Acclimatization to the field is a common problem (hyperhydricity)
5. Risk of genetic changes if 'de novo' regeneration is used
6. Mass propagation cannot be done with all crops to date. In cereals much less success is achieved
7. Regeneration is often not possible, especially with adult woody plant material.
8. More problems in inducing rooting
9. May not get uniform growth of original plant from tissue culture. Each explant has different *in vitro* growth rates and maturation. Thus cannot be used for floriculture crop production where uniformity is critical.

Horticultural uses for plant tissue culture

1. **Clonal mass propagation**. The important point here is that extremely large numbers of plants can be produced. Rather than getting 10000 plants per year from an initial cutting, one can obtain upwards of 1,000,000 plants per year from one initial explant.

2. **Difficult or slow to propagate plants**. Micropropagation enables growers to increase the production of plants that normally propagate very slowly such as narcissus and other bulbous crops.

3. **Introduction of new cultivars** eg. Dutch iris. Get 5 daughter bulbs annually. Takes 10 years for commercial quantities of new cultivars to be built up. Can get 100-1000 bulbs per stem section.

4. **Vegetative propagation of sterile hybrids** used as parent plants for seed production. Eg. cabbage.

5. **Pathology - Eliminate viruses, bacteria, fungi etc**. Use heat treatment and meristem culture. Used routinely for potatoes, carnation, mum, geranium, garlic, gypsophila

6**. Storage of germplasm**

Generally the only successful method to date is keeping them in refrigerator. Slows down, but does not eliminate, alterations in genotype.

Questions

1. Multiplication of genetically identical copies of a cultivar by asexual reproduction is called ………………

a) Clonal propagation b) Apomixis
c) Vegetative propagation d) None of the above

2. In nature, clonal propagation occurs by ………………

a) Vegetative propagation b) Apomixis
c) Both a & b d) None of the above

3. Apomixis is by ………………

a) Seed development without meiosis and fertilization b) Regeneration of new plants from vegetative parts
c) Both a & b d) None of the above

4. Vegetative propagation is by ………………

a) Seed development without meiosis and fertilization **b) Regeneration of new plants from vegetative parts**
c) Both a & b d) None of the above

5. Advantage of tissue culture is/are ………………

a) Production of large number of true-to-type plantlets from a single plant b) Less time requirement
c) Less space requirement **d) All the above**

6. Total number of stages in micro propagation is ………………

a) 3 b) 4
c) 5 d) 6

7. ………. stage involves the preparation of mother plants

a) Stage 0 b) Stage 1
c) Stage 2 d) Stage 3

8. ………. stage involves the preparation of mother plants

a) Stage 0 b) Stage 1
c) Stage 2 d) Stage 3

9. Stage 0 in tissue culture is

a) Preparative stage b) Initiation of culture
c) Multiplication d) Rooting of shoots

10. Stage 1 in tissue culture is

a) Preparative stage **b) Initiation of culture**
c) Multiplication d) Rooting of shoots

11. Stage 2 in tissue culture is

a) Preparative stage b) Initiation of culture
c) Multiplication d) Rooting of shoots

12. Stage 3 in tissue culture is

a) Preparative stage b) Initiation of culture
c) Multiplication **d) Rooting of shoots**

13. Stage 3 in tissue culture is

a) Preparative stage b) Initiation of culture
c) Multiplication **d) Transplanting**

14. Stage 3 in tissue culture is

a) Preparative stage b) Initiation of culture
c) Multiplication **d) Transplanting**

15. The suitable explant for producing virus free plant is …………..

a) Shoot tip b) Leaf bit
c) Stem bit d) None of the above

16. The oxidation of phenolic compounds leached out from the cut surface of the explant in tissue culture leads to …………..

a) Browning of the medium b) Blackening of the medium

c) Whitening of the medium | d) None of the above

17. The oxidation of phenolic compounds leached out from the cut surface of the explant in tissue culture leads to …………..

a) Browning of the medium b) Blackening of the medium
c) Whitening of the medium d) None of the above

18. Browning of medium is a common problem in …………..

a) Adult tissues from woody species b) Juvenile tissues from woody species
c) Both a and b d) None of the above

19. The crucial stage in tissue culture is

a) Preparative stage b) Initiation of culture
c) Multiplication d) Rooting of shoots

20. In tissue culture, the multiplication is through

a) Callusing b) Adventitious bud formation
c) Enhanced axillary branching **d) All the above**