

DEPARTMENT OF BIOTECHNOLOGY

MICROPROPAGATION

Ms. Payal Talekar



DEFINITION

"... the art and science of multiplying plants in vitro."

Production of whole plant from a section of plant such as stem tip, node, meristem, embryo or even a seed.

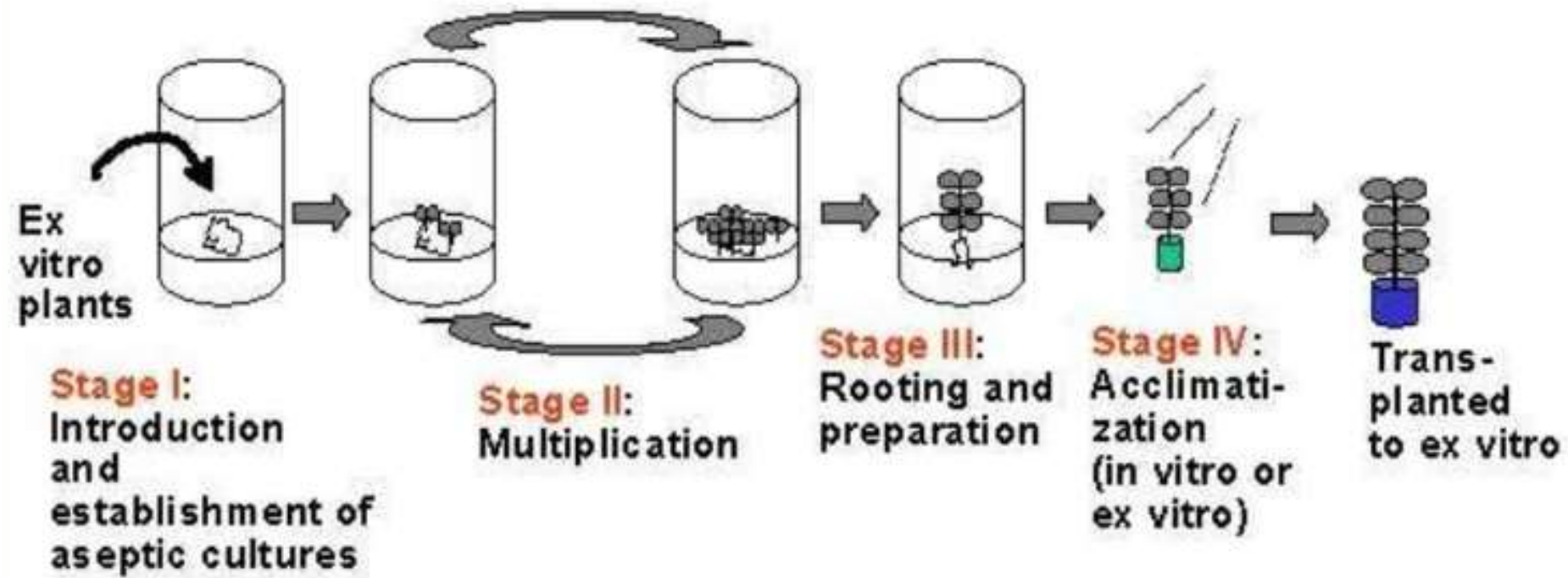
BASIS FOR MICROPROPAGATION

- Two Hormones Affect Plant Differentiation:
 - **Auxin:** Stimulates Root Development
 - **Cytokinin:** Stimulates Shoot Development
- Generally, the ratio of these two hormones can determine plant development:
 - ↑ Auxin & ↓ Cytokinin = Root Development
 - ↑ Cytokinin & ↓ Auxin = Shoot Development
 - Auxin = Cytokinin = Callus Development

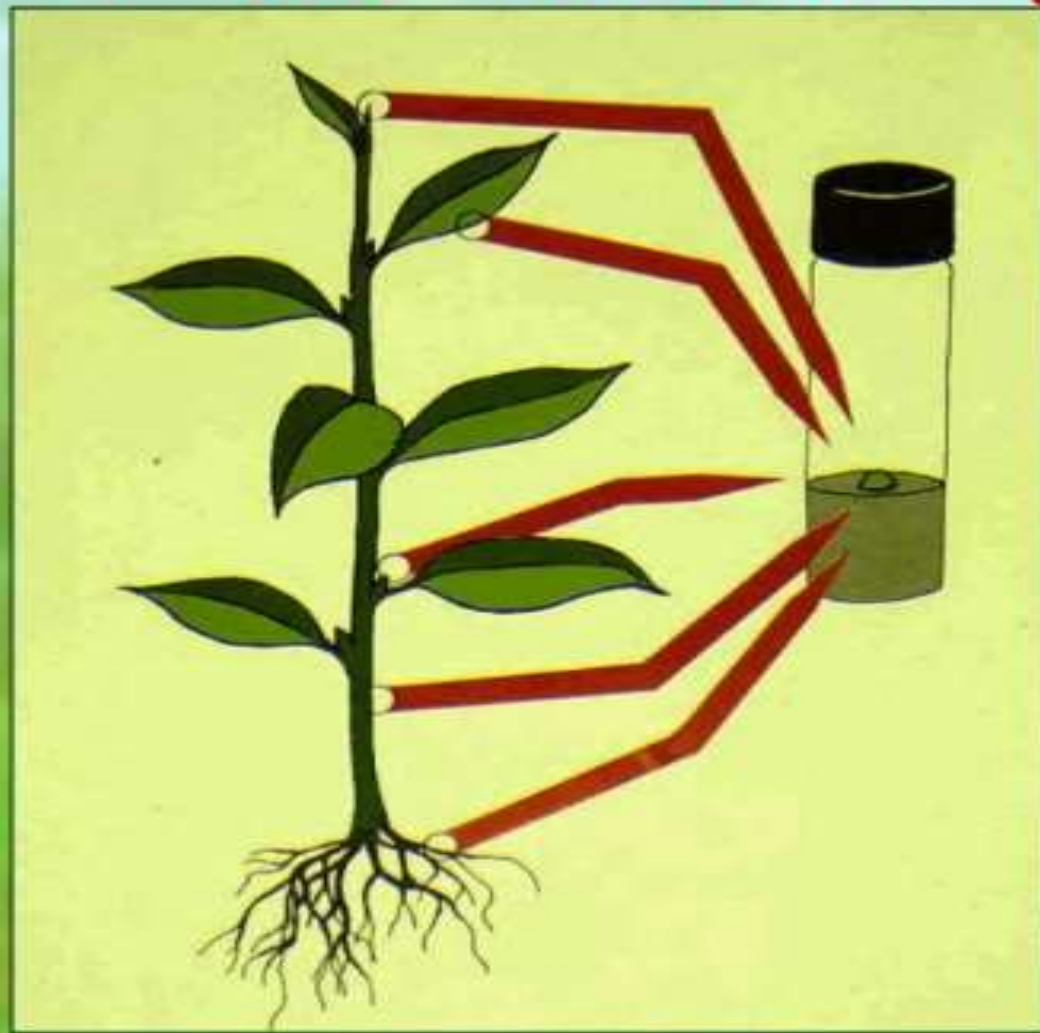
STEPS OF MICROPROPAGATION

- Stage 0 - Selection & preparation of the mother plant
 - sterilization of the plant tissue takes place
- Stage I - Initiation of culture
 - explant placed into growth media
- Stage II - Multiplication
 - explant transferred to shoot media; shoots can be constantly divided
- Stage III - Rooting
 - explant transferred to root media
- Stage IV - Transfer to soil
 - explant returned to soil; hardened off

Four stages in micropropagation



Stage 0 - Selection & preparation of the mother plant



- The plant tissues are removed from an intact plant in a sterile condition.
- Clean stock materials that are free of viruses and fungi are important in the production of the healthiest plants.
- Explants used can be stem tips, anthers, petals, pollen and others plant tissues.
- The explant material is then surface sterilized, usually in multiple courses of bleach and alcohol washes, and finally rinsed in sterilized water.

Stage I - Initiation of culture

- A small portion of plant tissue, sometimes only a single cell, is placed on a growth medium, typically containing sucrose as an energy source and one or more plant growth regulators (plant hormones).
- Usually the medium is thickened with agar to create a gel which supports the explant during growth.

Stage II - Multiplication

❑ Multiplication is the taking of tissue samples produced during the first stage and increasing their number.

Establishment stage is followed by multiplication.

❑ Through repeated cycles of this process, a single explant sample may be increased from one to hundreds and thousands of plants.

❑ Depending on the type of tissue grown, multiplication can involve different methods and media. If the plant material grown is callus tissue, it can be placed in a blender and cut into smaller pieces and recultured on the same type of culture medium to grow more callus tissue.

❑ If the tissue is grown as small plants called plantlets, hormones are often added that cause the plantlets to produce many small offshoots.

Stage III - Rooting

After the formation of multiple shoots, these shoots are transferred to rooting medium with a high auxin\cytokinin ratio. After the development of roots, plantlets can be used for hardening.

Stage IV - Transfer to soil

"Hardening" refers to the preparation of the plants for a natural growth environment. Hardening typically involves slowly **weaning** the plantlets from a high-humidity, low light, warm environment to what would be considered a normal growth environment for the species in question.

In the final stage of plant micropropagation, the plantlets are removed from the plant media and transferred to soil or (more commonly) potting compost for continued growth by conventional methods.

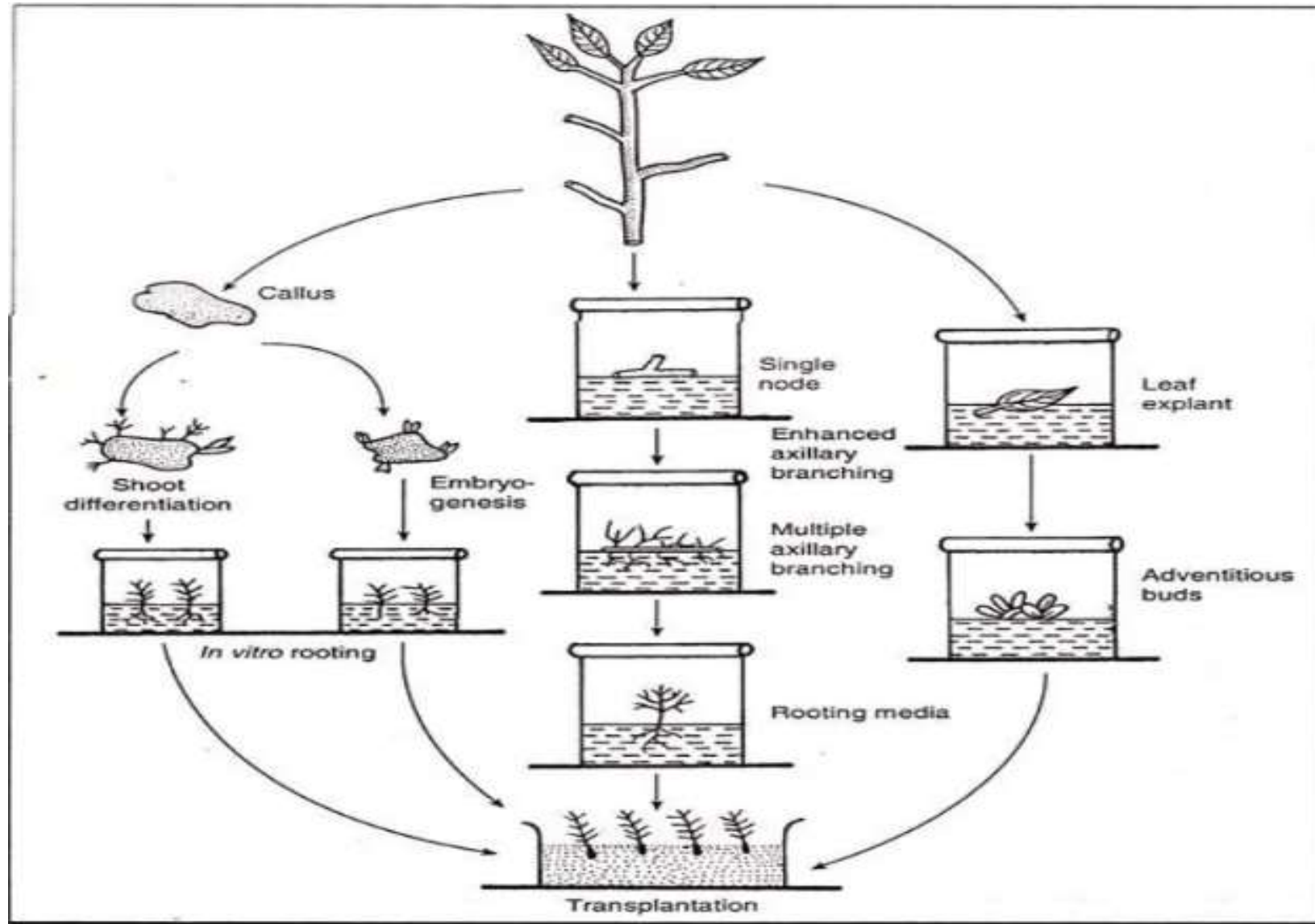


Fig 1: Overall technique of micropropagation

APPLICATIONS:

- Large scale multiplication in lesser time and space.
- It ensures true to type plants.
- A new plant can be regenerated from a miniature plant part.
- Facilitates long distance transport of propagation materials and long term storage of clonal materials.
- Production of female plants in dioecious plants is possible eg. papaya.
- It is independent of seasonal constraint.
- Production of virus free plants.
- It is the only viable method of regenerating genetically modified cells.
- This helps to save endangered species and the storage of germplasm.
- Rejuvenation of old fruit tree

