**Unit 9. Plant tissue culture**

## Micropropagation

Micropropagation technique is essentially established now a days and could overcome the genetic segregation of the plants germinating from seeds; field-selected elite strains could be efficiently propagated with micropropagation techniques.

Micropropagation techniques are of three types based on the way of propogation:

(i) the propagation from shoots with cytokinin like benzyladenine or kinetin;

(ii) multiple shoot differentiation from dedifferentiating tissue, callus, with an auxin like indole acetic acid (IAA); and

(iii) the embryo differentiation from callus.

The former two methods need the rooting process with an auxin like indole acetic acid and with naphthaleneacetic acid (NAA) thereafter.

Nowadays, the method of propagation from shoots is the most preferred one, because the latter two methods present the possibility of genetic variation owing to the dedifferentiated phase, callus.

## Applications and merits of micropropagation over conventional plant breeding

The various applications of micropropagation are:

(A) Plant tissue in small amounts is sufficient for the production of millions of clones in a year using micropropagation. It would take a great deal of time to produce an equal number of plants using conventional methods.

(B) The technique of micropropagation provides a good alternative for those plant species that show resistance to practices of conventional bulk propagation.

(C) An alternative method of vegetative propagation for mass propagation is offered through micropropagation. Plants in large numbers can be produced in a short period. Any particular variety may be produced in large quantities and the time to develop new varieties is reduced by 50%.

(D) Large amounts of plants can be maintained in small spaces. This helps to save endangered species and the storage of germplasm.
( E) The micropropagation method produces plants free of diseases. Hence, disease-free varieties are obtained through this technique by using meristem tip culture.

(F) Proliferation of *in vitro* stocks can be done at any time of the year. Also, a nursery can produce fruit, ornamental, and tree species throughout the year.
(G) Increased yield of plants and increased vigor in floriculture species are achieved.

(H) Fast international exchange of plant material without the risk of disease introduction is provided. The time required for quarantine is lessened by this method.

(I) The micropropagation technique is also useful for seed production in certain crops as the requirement of genetic conservation to a high degree is important for seed production.

(J) Through somatic embryogenesis production of synthetic artificial seeds is becoming popular nowadays.

With micropropagation having various advantages over conventional methods of propagation, this method holds better scope and future for production of important plant-based phytopharmaceuticals. Independent of availability of plants, micropropagation offers a lucrative alternative approach to conventional methods in producing controlled amounts of biochemicals. Therefore, intense and continuous efforts in this field will direct controlled and successful production of valuable, specific, and yet undiscovered plant chemicals.

**Micropropagation is the propagation of plants through tissue culture.**

There are four stages to micro-propagation. These stages are:

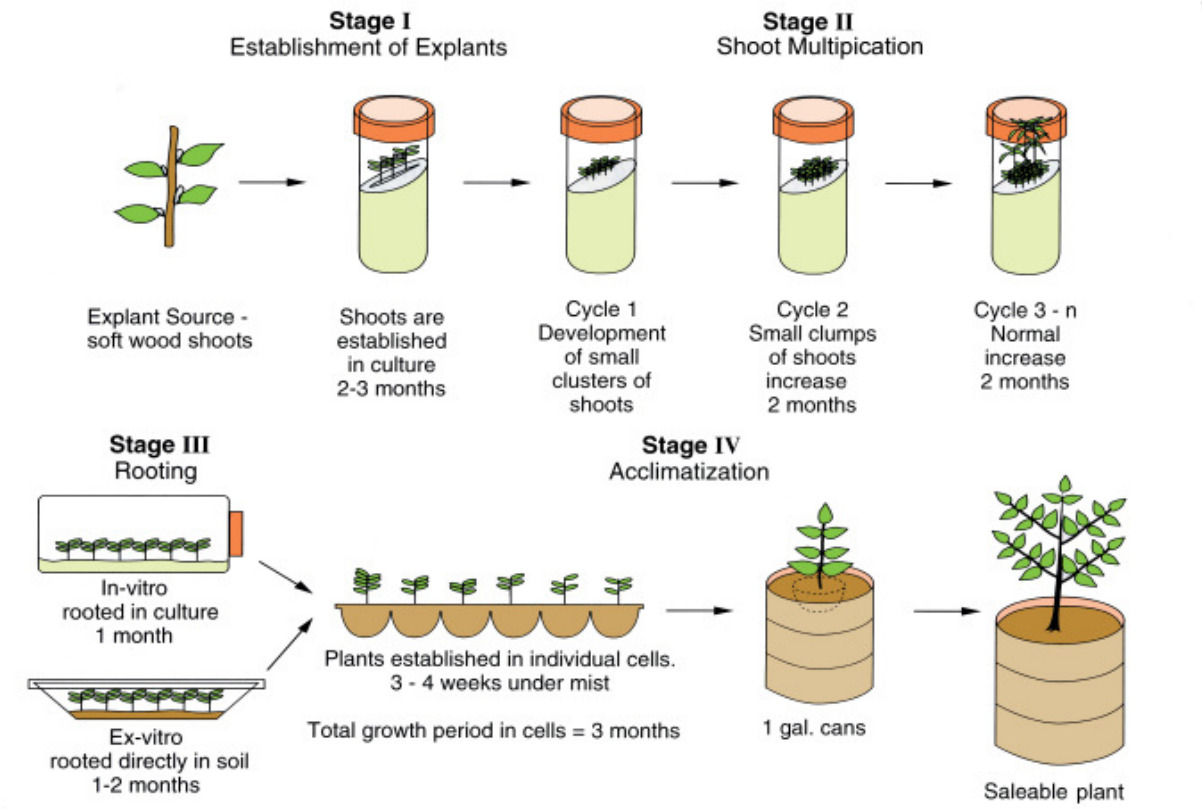
1. Stage I Establishment
2. Stage II Multiplication
3. Stage III Rooting
4. Stage IV Acclimatization

Stage I Establishment

Stage II multiplication

Stage III Rooting

Stage IV Acclimatization

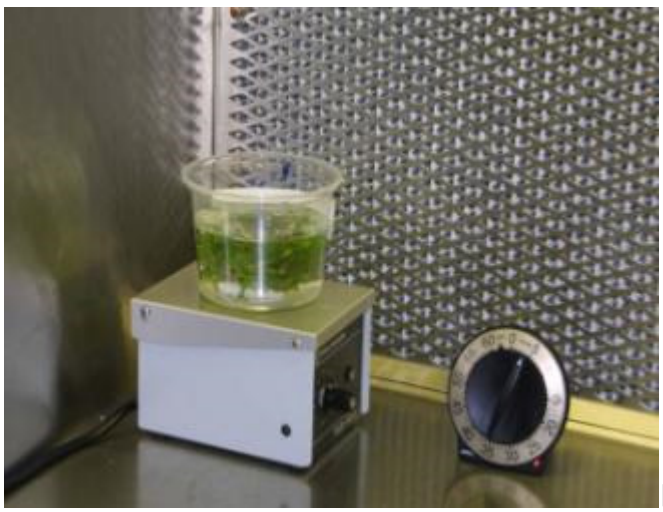


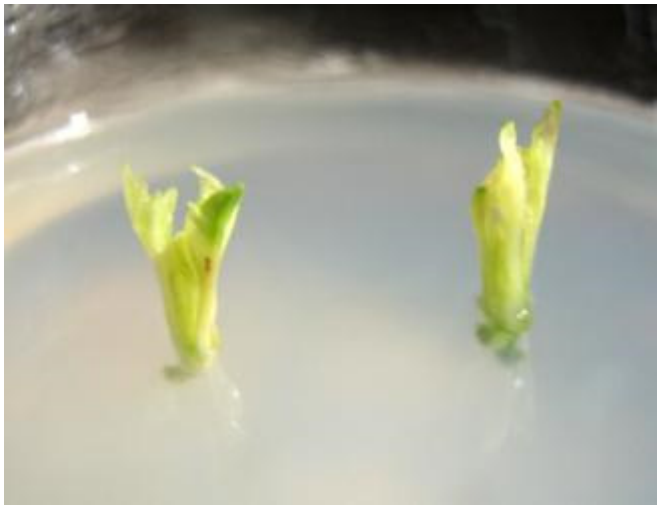

## Stage I - Establishment

During the establishment stage, the explant must be disinfested and stabilized.

The explant is usually sterilized with a combination of detergent and bleach. In difficult situations, alcohol or a fungicide may be used.

The objective of this stage is to get clean cultures that can begin the process of shoot multiplication.


Disinfesting explants in bleach



Initial explant

## Micropropagation Stage II - Shoot multiplication

The objective of the shoot multiplication stage is to increase the number of shoots produced by the original explant.

By subculturing these new shoots on to new medium, the number of shoots produced in culture increases dramatically.

The technician is preparing to subculture. The culture or a portion of the culture is removed from the jar and placed on a sterile paper towel.

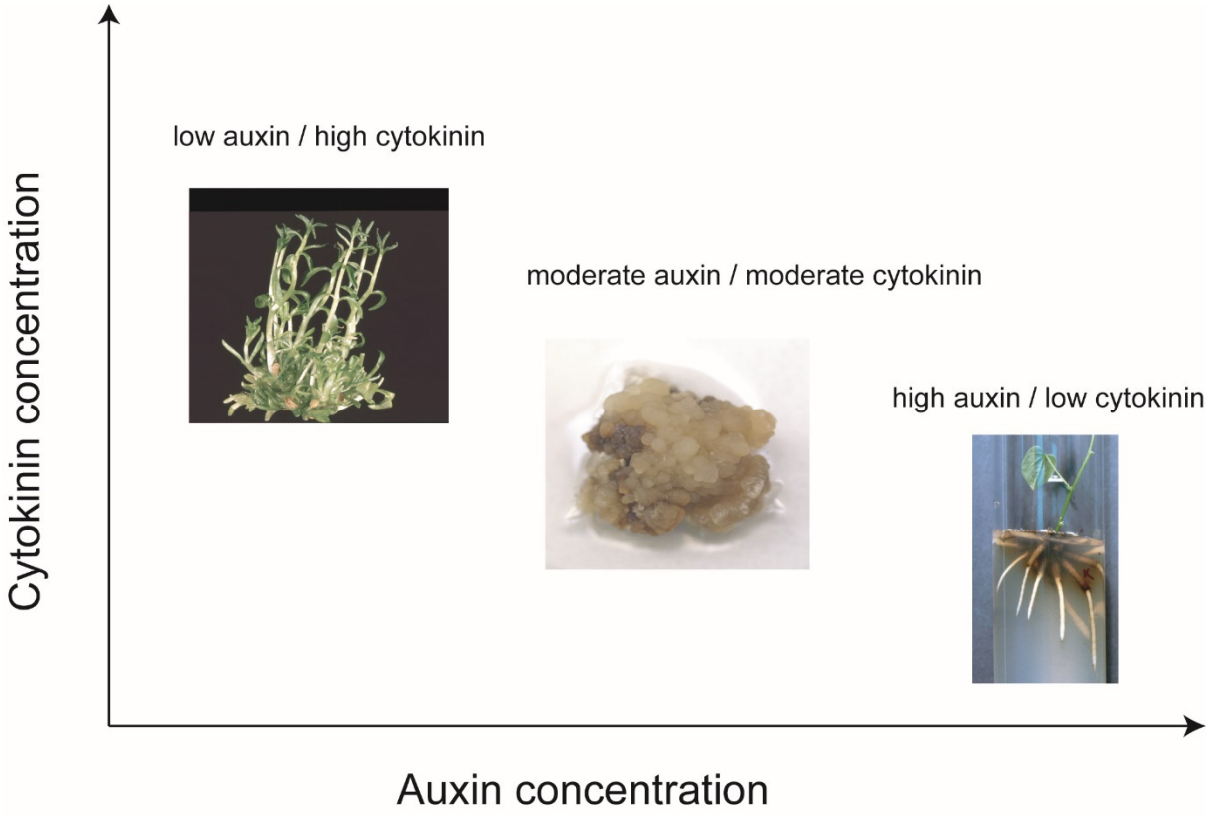
A scalpel and forceps are used to cut and separate the larger culture into smaller pieces for transfer to a new jar to complete the subculturing procedure.

A high cytokinin to auxin ratio is used during the multiplication stage to induce axillary or adventitious shoot formation.

This ratio is decided upon by preliminary research.

Too high a concentration of cytokinin will result in a high number of adventitious shoots that do not elongate.

Common cytokinins used in culture are benzyladenine and kinetin.




## Micropropagation Stage III - Root formation

Shoots multiplied in culture must be rooted in Stage III in order to create a new plantlet.

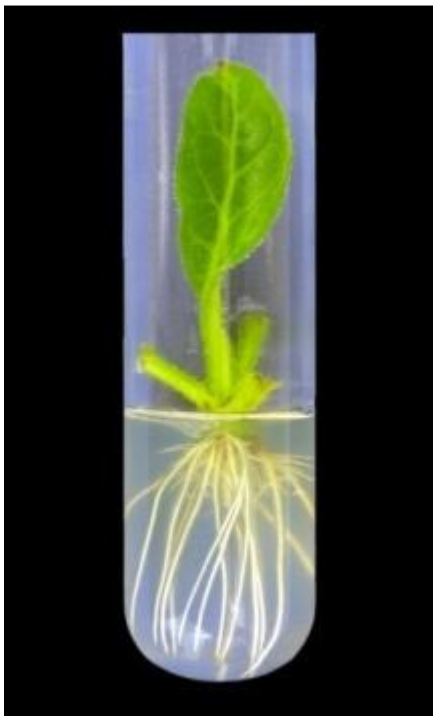
In the rooting stage, microcuttings are induced to form roots - usually by application of auxin.

In general, species root easier in tissue culture than they do from conventional cuttings.

Microcuttings can either be rooted *in vitro* or *ex vitro*.

In general, microcuttings rooted *ex vitro* have a more normal root system and acclimatize to a normal growing environment better than cuttings rooted *in vitro*.

However, the propagator has more control over the rooting environment *in vitro* and this method may fit their production scheme better.



*in vitro*

*ex vitro*

Microcuttings are inserted directly into the rooting substrate often using forceps to handle the small cuttings.

At each work station the technicians have their rooting flats, a syringe bottle to spray microcuttings periodically to keep them from drying out.

Technicians sticking microcuttings at a workstation.

Microcuttings after the agar has been washed off.

Sticking microcuttings.

## Micropropagation Stage IV - Acclimatization

Finally, after roots have become well established on the microcutting, plantlets must be acclimatized to a normal growing environment in Stage IV.

This involves gradually moving to open-air conditions where the humidity is reduced and the light levels increased.

This is a vulnerable stage for plantlet survival where the propagator can see large losses without proper acclimatization.

Rooted microcuttings being acclimatized using intermittent mist in a closed polyethylene tent.