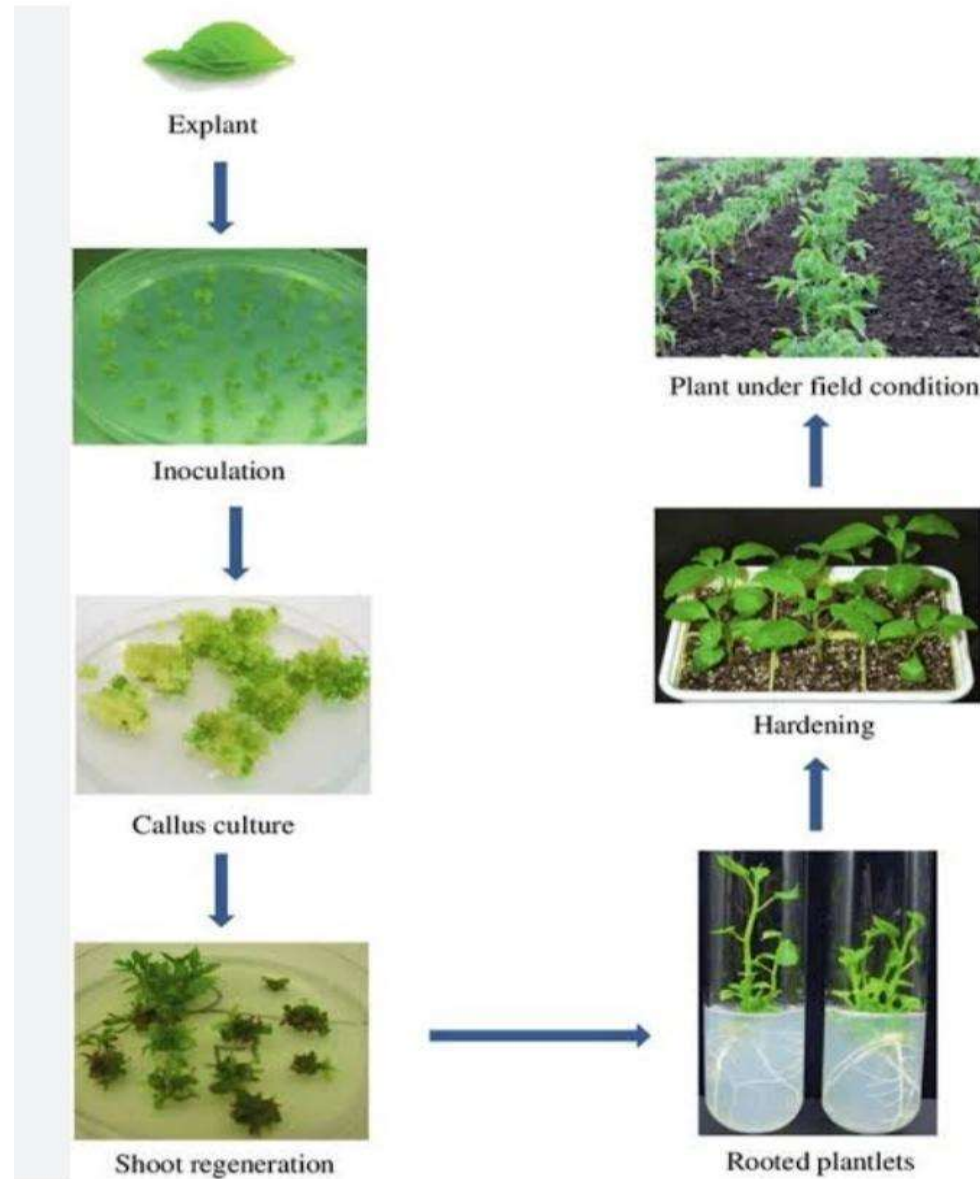


Micropropagation.



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Introduction

- ▶ Micropropagation is the artificial process of vegetative production of plants through tissue culture or cell culture technique.
- ▶ In this artificial propagation process plants are produced invitro by asexual reproduction or vegetative propagation.
- ▶ Plants can be produced both asexually i.e by propagating vegetative parts and sexually i.e.by seed production.
- ▶ One means of asexual reproduction is the propagation of genetic replicas of plants termed as clonal propagation,where by plants can be colonized by single individual through asexual reproductive means.
- ▶ Cloning in plants usually refers to asexual multiplication of plants from the somatic tissue.It is often a common practice followed by horticultures for preserving the unique required characters of particular genotype.
- ▶ Conventionally this can be attained by cutting, grafting.

Stages of Micropropagation

▶ Micropropagation is well defined by five steps:-

▶ Stage 0 :- Preparative stage

successful establishment of aseptic culture, the mother plant should be in good hygienic conditions. Most often the explants that are obtained from the plants that are preserved in greenhouse providing higher percentage of aseptic culture. Stage 0 involves the manipulation of the temperature and light according to which the mother plants are preserved.

▶ Stage 1:- Initiation/Establishment of culture

The aim of this stage is to allow the aseptic growth of plant culture that are been micropropagated by the use of suitable explants i) Explant:- The most frequently used explant for micropropagation is the apical bud or the nodal segment with an axillary bud. ii) Sterilization :- Sterilization of explants with 70% ethanol, sodium hypochloride , distilled water.

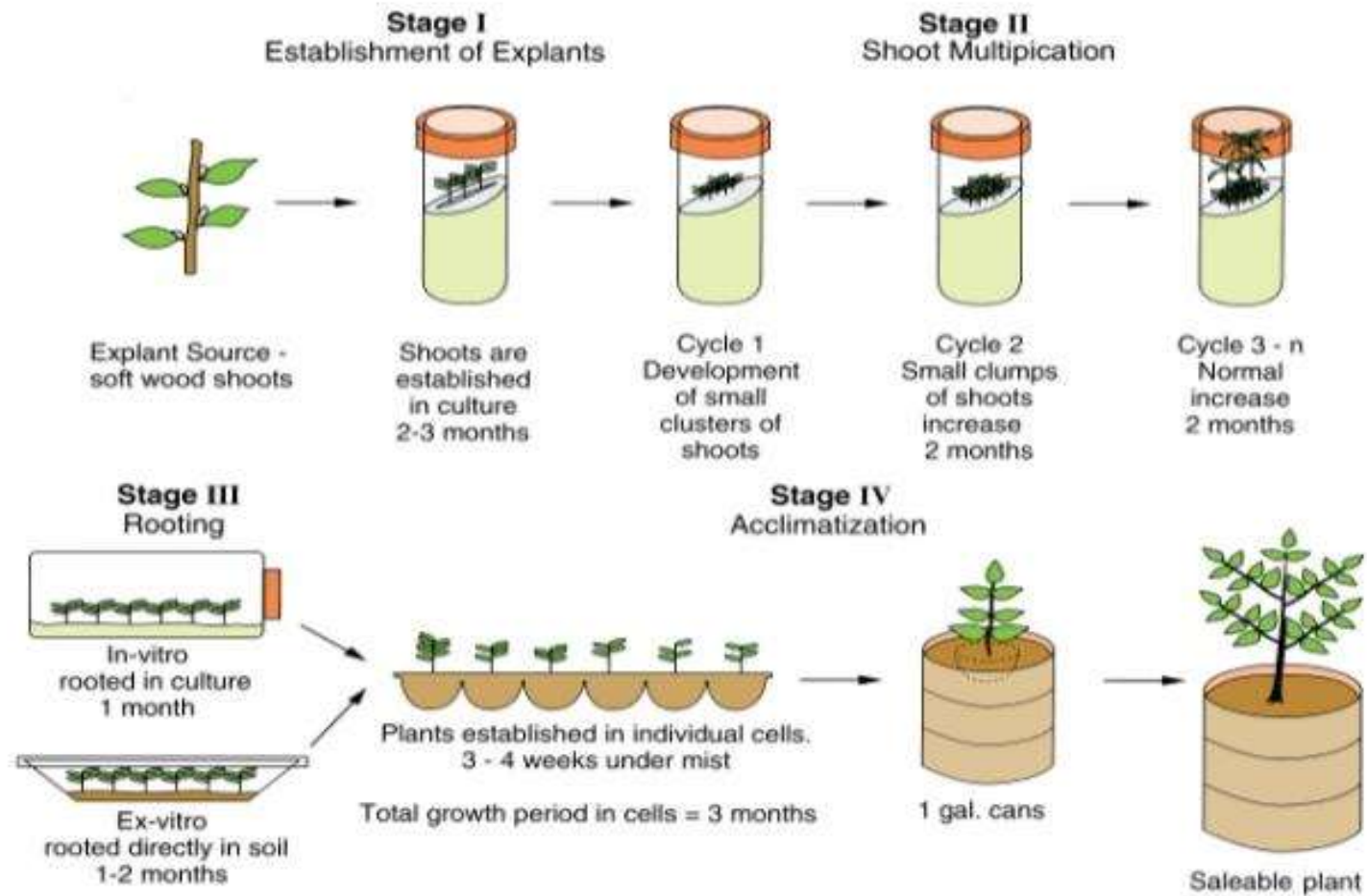
► Stage 2:- Multiplication

Regeneration from callus - various plant cells are accomplished to develop callus under most appropriate culture conditions on suitable media this callus can be made to regenerate shoots through organogenesis or to form somatic embryos.

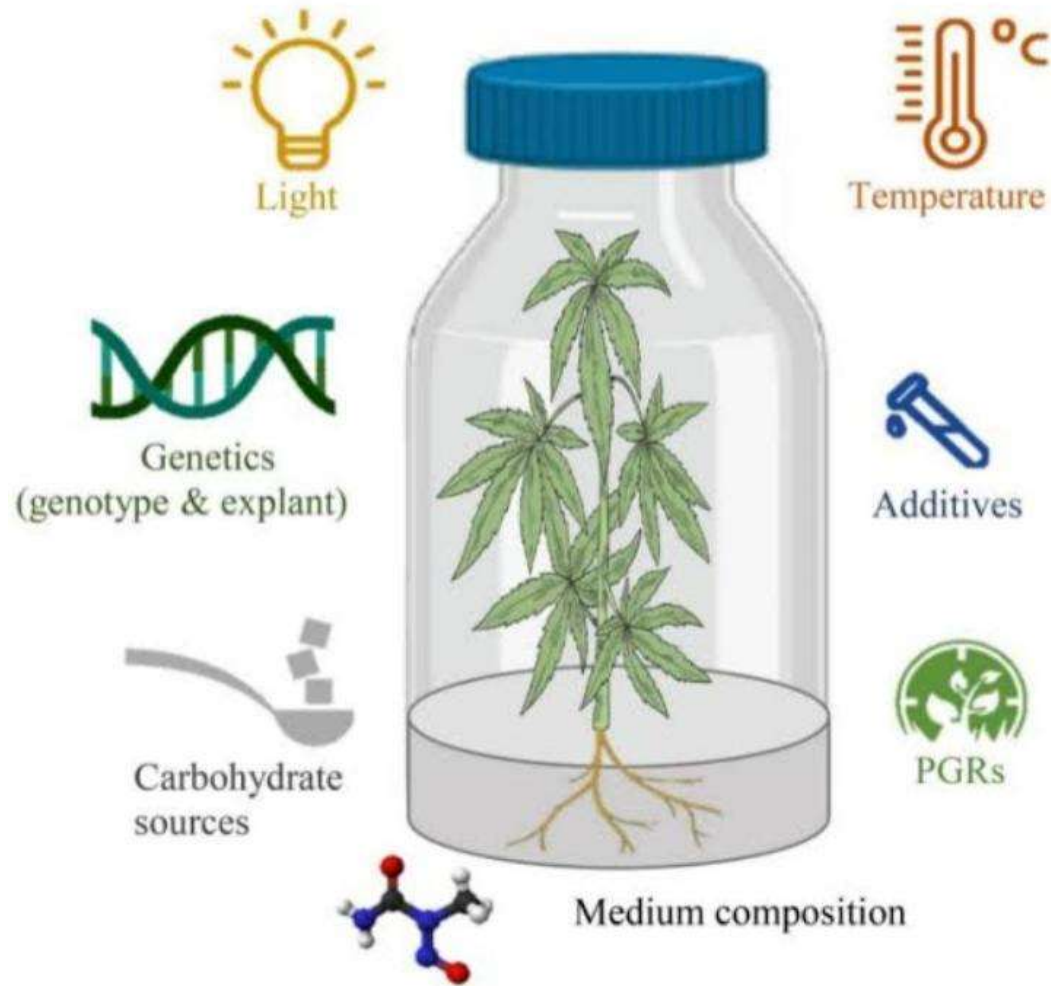
Stage 3 :- Rooting and elongation

Stage 4 :- Transplantation and accimatization

Final successful micropropagation usually depends on development of plants in the potting mix as well as in the soil .



Factors affecting micropropagation:-



Applications of Micropropagation

Micropropagation helps in bulking of rapidly new cultivators of important trees that would otherwise take many years to bulk up by conventional methods

The invitro stocks can be quickly proliferated at any time of the year.

Meristem tip culture is generally employed in cases where the aim is to produce disease free plants.

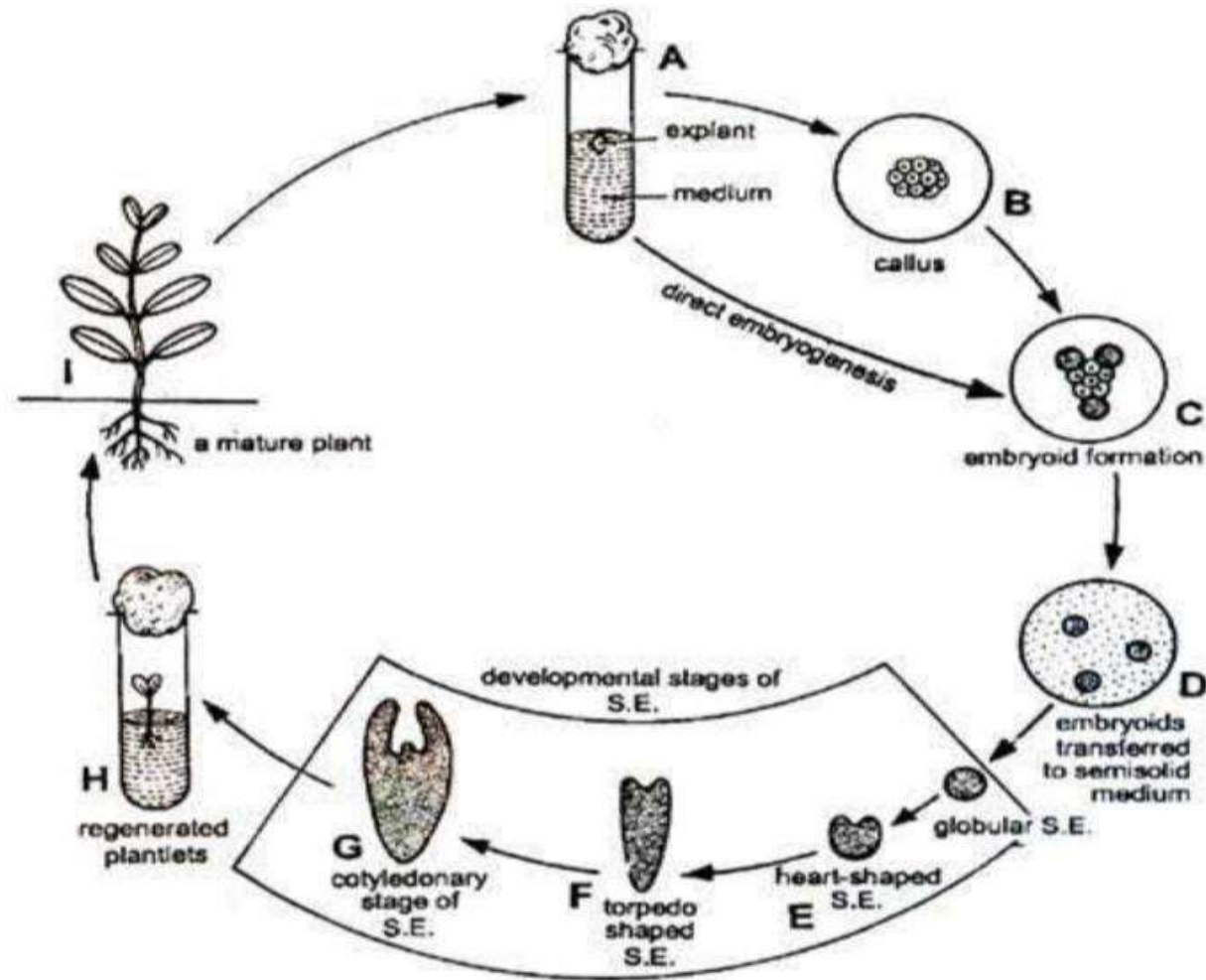
Preservation of germplasm is a means to assure the availability of genetic materials as the need arises.

Preservation of endangered species.

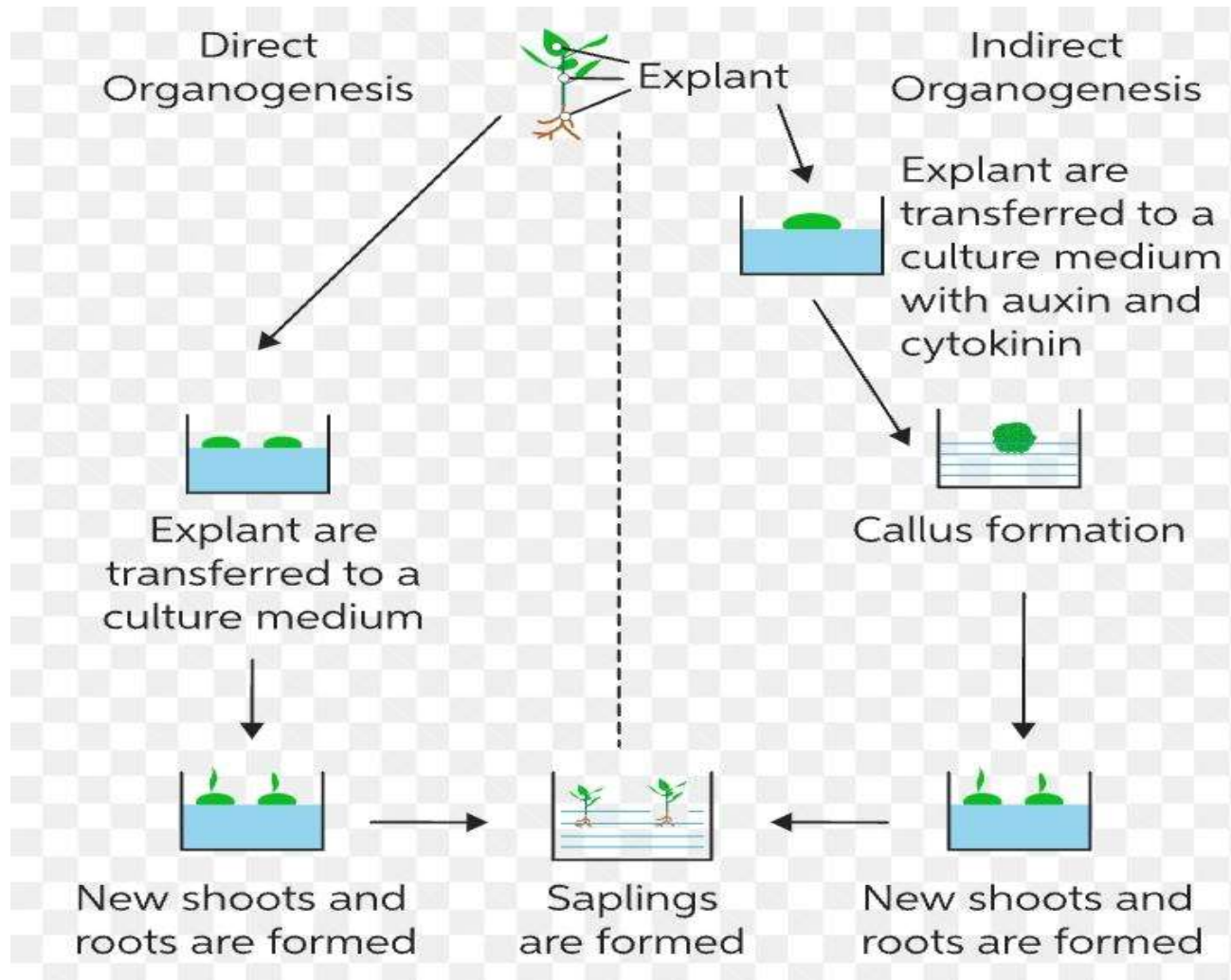
Pathways for Micropropagation

- ▶ There are various pathways for Micropropagation
- ▶ i) Somatic embryogenesis
- ▶ ii) Organogenesis
- ▶ iii) Meristem culture
- ▶ iv) axillary bud proliferation

Somatic embryogenesis

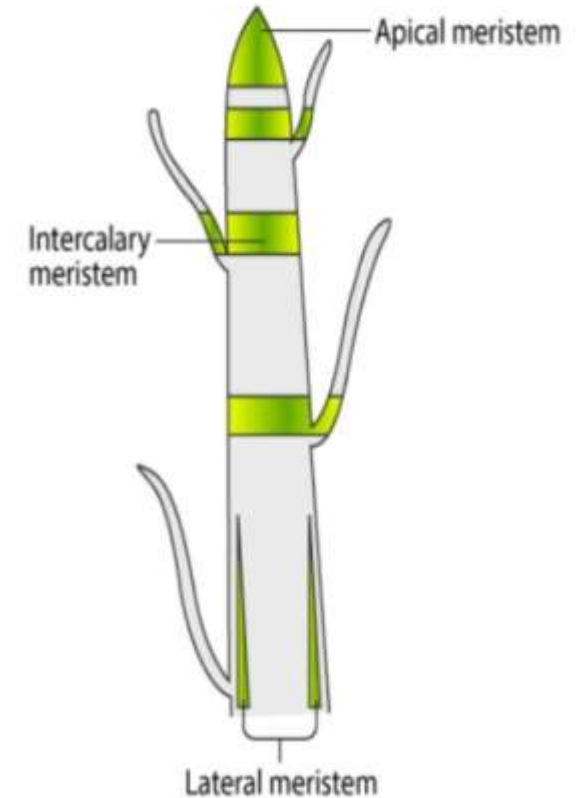


Organogenesis

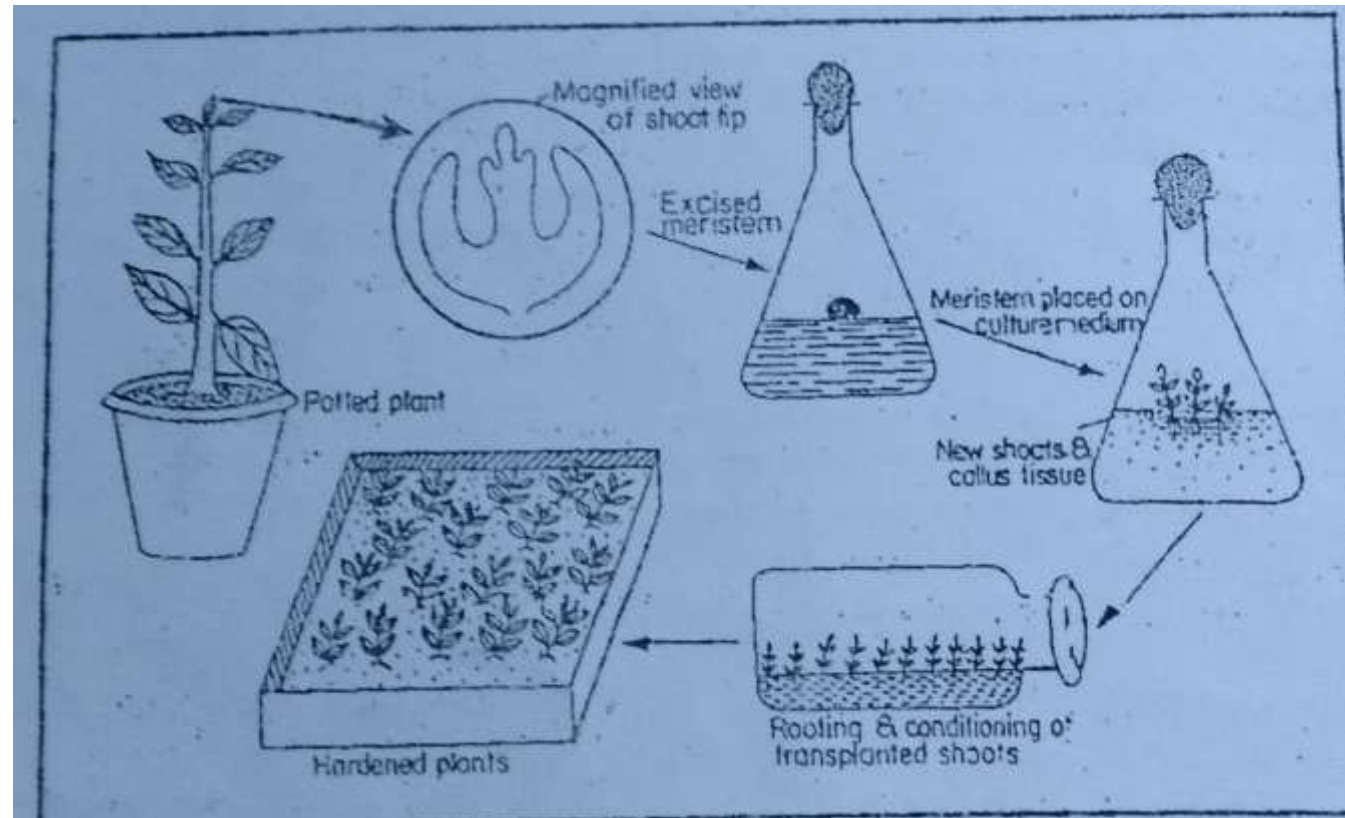


Meristem culture:-

- ▶ Meristem culture is the invitro culture of a generally shiny special dome like structure measuring less than 0.1mm in length and only one or two pairs of the youngest leaf primordia, most often excised from the shoot apex.
- ▶ In vitro clonal propagation of plants from various explant source including shoot tips, leaf sections and calli this is Known as meristemming.
- ▶ **Principle :-** The excised shoot tips and Meristem can be cultured aseptically on agar solidified simple nutrient medium, under the appropriate condition will grow out directly into a small leafy shoot or multiple shoots. Alternatively the meristem may form a small callus at it's cut case on which a large number of shoot primordia will develop.
- ▶ Exogenously supplied cytokinins in the nutrient medium plays a major role for the development of a leafy shoot or multiple shoots from meristem or shoot tip. Generally high cytokinins and low auxin are used in combination for the culture of shoot tip or meristem.
- ▶ BAP is the most effective cytokinin commonly used in shoot tip or meristem culture and NAA is the most effective auxin used in shoot tip culture
- ▶ Coconut milk and gibberellic acid are also equally effective for the growth of shoot apices in some cases.



Protocol



Importance of shoot tip/Meristem culture

- ▶ Virus eradication
- ▶ Micropropagation
- ▶ Storage of genetic resources

Axillary bud proliferation

- ▶ The axillary bud or lateral buds are formed in the angle between leaf and stem which lead to the formation of several branches.
- ▶ The shoot tips are isolated from the tip of the axil of leaves that the develop axillary bud under the effect of a high concentration of cytokinins The role of cytokinins is to suppress apical dominance and promote the development of axillary bud.
- ▶ Cytokinins: Auxin ratio is 10:1 stops apical dominance.
- ▶ Juvenile material require less cytokinins than adult.
- ▶ Single node culture -This is a natural method for vegetative propagation of plants both in vivo and in vitro.A bud along with a piece of stem isolated and cultured to develop into a plantlet

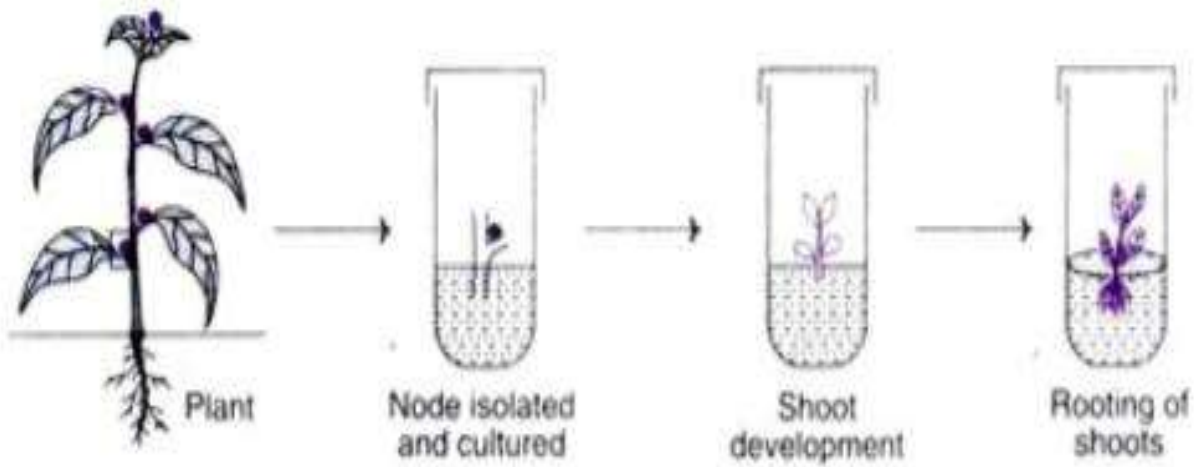


Fig. 47.3 : A diagrammatic representation of micropropagation by single node technique.

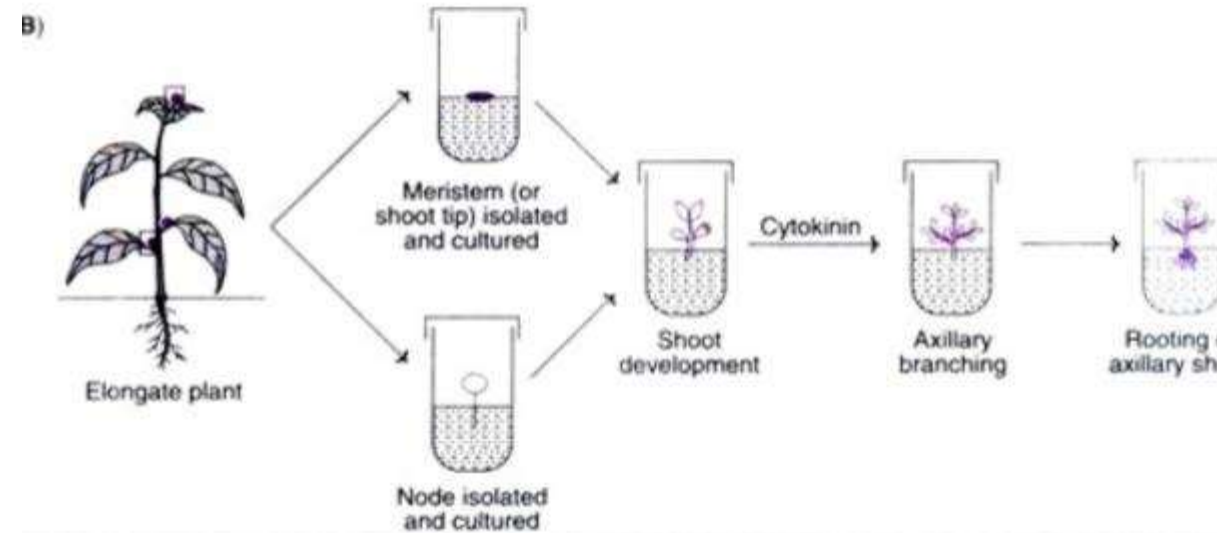
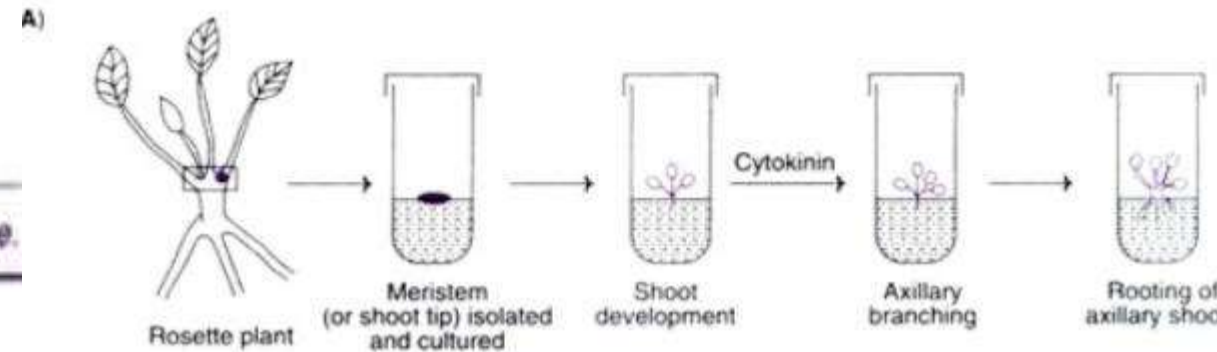


Fig. 47.4 : A diagrammatic representation of micropropagation of plants by axillary bud (or shoot tip) method (A) Rosette plant (B) Elongate plant.