Micropropagation

Micropropagation is a method of propagating plants by culturing very small parts of it called explants. It is the *in vitro* propagation of plants vegetatively by tissue culture to produce genetically similar copies of a cultivar. It is also known as clonal propagation. Aitken-Christic and Connett (1992) defined Micropropagation as an *in vitro* method of producing clonal off-spring identical to a superior stock plant. Micropropagation is a complex multistep process involving different starting tissues and cell-types that can be induced to differentiate into plantlets, either through organogenesis or embryogenesis pathways.

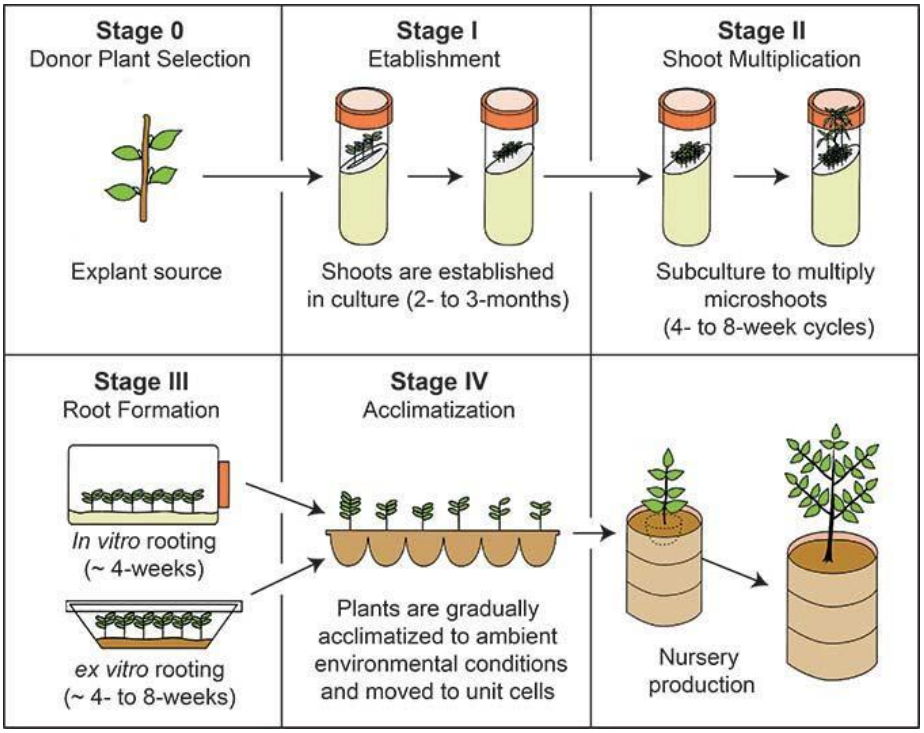
Micropropagation proves to be a useful tool for propagation of the following-

1. Sexually sterile species such as triploids, aneuploids etc. that cannot be propagated by seeds.
2. Cross bred perennials where heterozygocity has to be maintained.
3. Many mutant lines such as auxotroph that cannot be propagated *in vitro*.
4. Seedless plants like bananas, grapes etc.
5. Disease plants of fruit trees and ornamentals.

Micropropagation Methods:-

- Meristem culture- Meristems are characterised by a dome of totipotent cells situated at the uppermost portion of the plant body. Very small explants are taken from the apical or axillary buds for this purpose. Meristems in their early vegetative phase are considered to be the most suitable explant to obtain virus-free plants as these cells are the center of activity of different developmental programmes.
- Shoot-tip culture- For shoot-tip culture relatively large explants, consisting of the shoot apical meristem and a number of leaf primordial and small unexpanded leaves at different developmental stages are needed. A highly branched shoot system is developed from the explant by removing the apical dominance and stimulation of precocious axillary shoots by incorporation of growth regulators.
- Single node culture- Single node culture is utilised majorly in the species that produce elongated shoots. Lateral bud break is stimulated in these cases *in vitro* with the addition of cytokinin, which overcomes apical dominance and induces growth of hidden axillary buds.
- Seed culture- It is possible to obtain multiple shoots instead of one seedling from one seed through seed culture.

Stages of micropropagation



In vitro morphogenesis occurs through two different pathways- organogenesis and somatic embryogenesis.

Organogenesis- It is a process of development of adventitious organs or primordia (roots or shoots) from differentiated or undifferentiated tissue. It can again be of two types- adventive (direct) organogenesis and indirect organogenesis.

a) Adventive (direct) organogenesis- In this method adventitious shoots may arise directly from the tissue of the explant and do not need the formation of callus. In this instances, the immediate precursors of the new organs are cells in the explant itself. Exogenously supplied hormones can facilitate the process but are not absolutely required for organogenesis to occur.

The induction of direct shoot regeneration depends on the plant organ from which the explant is derived and mostly on the plant species. In some species, adventitious shoot arise *in vitro* on pieces of tissues derived from different organs like, leaves, stem, petals or roots, while in others they occur on a limited range of tissues. Plants produced through adventitious shoot formation are usually true-to type, especially in those species that propagate this way naturally, e.g.; Begonia from leaves, blackberry from roots and bulbous crops from leaf scales. This method is useful in species where axillary or apical meristem are difficult to disinfest or establish in culture.

b) Indirect organogenesis- this type of organogenesis is not adventive and involves a de-differentiation of the explant, resulting in formation of callus tissue along the cut edges of the explant and the induction of new organs from the newly formed callus tissue. Exogenously supplied phytohormones not only control the process but are required for organogenesis to occur.

Cell division, in callus cultures, usually occur haphazardly, producing disorganised masses of tissues without any form or polarity. But in some experimental conditions, root and shoot meristems and even embryoids may be formed, which often develop into plantlets and ultimately whole plants. The stimulation of root bud differentiation depends on many factors that vary from species to species. Generally, it is promoted by cytokinin and inhibited by auxin. Skoog and Millar (1957) demonstrated, in pith tissue of tobacco, that high level of cytokinin initiates bud formation, while high auxin concentration favours rooting. On the other hand, intermediate rates of the two hormones produced completely disorganised callus growth. However, some plant species do not follow this concept of auxin/cytokinin ratio. In most cereals, callus tissue exhibits organogenesis when the tissue is sub cultured from a medium containing 2,4-D to a medium containing IAA or NAA replacing 2,4-D. in general GA shows inhibitory effect on shoot buds, while many species exhibit enhanced shoot regeneration due to abscissic acid. Interacting effects of the hormones can be changed by the other constituents of the media also, like sugar, amino acid, casein hydrolysate and phosphate ions.

Size and source of the explants are the other factors affecting organogenesis. The larger the explant, more is likelihood of shoot bud formation. Genotypes of explants also affect shoot regeneration, as, explant taken from different varieties of same species show different frequencies of shoot bud differentiation.

Light has been shown to affect organogenesis by inhibiting differentiation. Even the quality of light has effect as blue light induces shoot formation, while root is initiated by red light in tobacco. The optimum temperature requirement may vary from species to species also.