APPROACHES TO MICROPROPAGATION

Approaches for conservation and improvement of plant genetic resources

Micropropagation as a method for sustainable resource generation

Micropropagation or in vitro propagation of threatened plants has been a time-tested and effective method of ex situ conservation. The method exploits the totipotent nature of a plant cell to produce new individuals from either protoplast, cells, undifferentiated masses of cells (callus), small pieces of tissue, and/or excised organs. After the usefulness of the method was first established, micropropagation has been extensively used in agriculture, forestry, and horticulture for the large-scale production of disease-free planting materials of high quality. It is also the most effective method of eliminating viruses from valuable horticulture crops, rescuing somatic hybrids and hybrids generated through conventional breeding, developing transgenic plants for desired traits, and gene pyramiding. The method has tremendous industrial applications. It can be used as a non-destructive approach for producing secondary metabolites from medicinally important plants in vitro for their upscaling in bioreactors. Micropropagation has proven particularly effective in the ex situ conservation of a number of endangered plants. Particularly, in plants where seeds or other propagules are not easily available, micropropagation offers an alternative route for either the replenishment of rapidly dwindling populations or the generation of alternative resources of medicinally important secondary metabolites.

Applications and merits of micropropagation over conventional plant breeding.

The various applications of micropropagation are:

• 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.

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

• 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%.

• Large amounts of plants can be maintained in small spaces. This helps to save endangered species and the storage of germplasm.

• The micropropagation method produces plants free of diseases. Hence, disease-free varieties are obtained through this technique by using meristem tip culture.

• 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.

• Increased yield of plants and increased vigor in floriculture species are achieved.

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

• 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.

• 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.

Micro propagation mostly involves in vitro clonal propagation by two approaches:

1. Multiplication by axillary buds/apical shoots.
2. Multiplication by adventitious shoots.

Besides the above two approaches, the plant regeneration processes namely organogenesis and somatic embryogenesis may also be treated as micro propagation.

3. Organogenesis: The formation of individual organs such as shoots, roots, directly from an explant (lacking preformed meristem) or from the callus and cell culture induced from the explant.
4. Somatic embryogenesis: The regeneration of embryos from somatic cells, tissues or organs.

1. Multiplication by Axillary Buds and Apical Shoots:

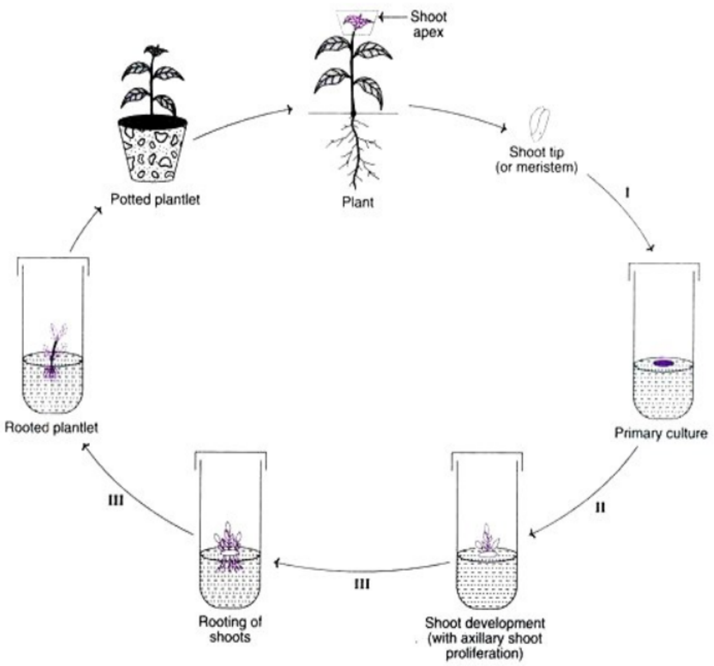
Quiescent or actively dividing meristems are present at the axillary and apical shoots (shoot tips). The axillary buds located in the axils of leaves are capable of developing into shoots. In the in vivo state, however only a limited number of axillary meristems can form shoots. By means of induced in vitro multiplication in micro propagation, it is possible to develop plants from meristem and shoot tip cultures and from bud cultures.

Meristem and Shoot Tip Cultures:

Apical meristem is a dome of tissue located at the extreme tip of a shoot. The apical meristem along with the young leaf primordia constitutes the shoot apex. For the development of disease-free plants, meristem tips should be cultured.

Meristem or shoot tip is isolated from a stem by a V-shaped cut. The size (frequently 0.2 to 0.5 mm) of the tip is critical for culture. In general, the larger the explant (shoot tip), the better are the chances for culture survival. For good results of micro propagation, explants should be taken from the actively growing shoot tips, and the ideal timing is at the end of the plants dormancy period.

The most widely used media for meristem culture are MS medium and White's medium. A diagrammatic representation of shoot tip (or meristem) culture in micro propagation is given in Fig 47.2, and briefly described hereunder.



Diagrammatic representation of shoot tip (or meristem) culture in micropropagation; I, II, III are stages.

In stage I, the culture of meristem is established. Addition of growth regulators namely cytokinins (kinetin, BA) and auxins (NAA or IBA) will support the growth and development.

In stage II, shoot development along with axillary shoot proliferation occurs. High levels of cytokinins are required for this purpose.

Stage III is associated with rooting of shoots and further growth of plantlet. The root formation is facilitated by low cytokinin and high auxin concentration. This is opposite to shoot formation since high level of cytokinins is required (in stage II). Consequently, stage II medium and stage III medium should be different in composition. The optimal temperature for culture is in the range of 20-28°C (for majority 24-26°C). Lower light intensity is more appropriate for good micro propagation.

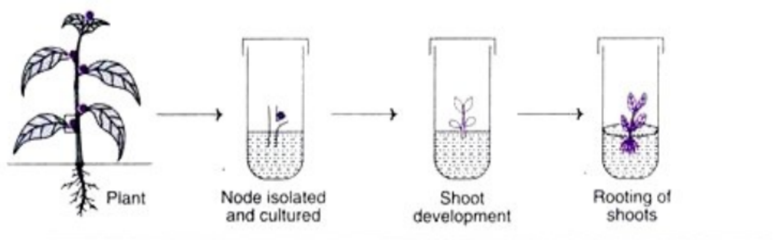
Bud Cultures:

The plant buds possess quiescent or active meristems depending on the physiological state of the plant. Two types of bud cultures are used— single node culture and axillary bud culture.

Single node culture:

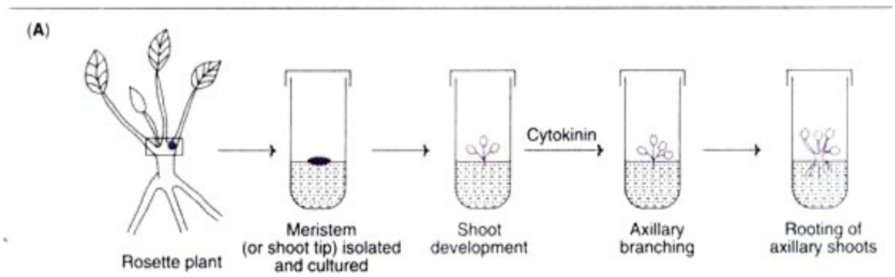
This is a natural method for vegetative propagation of plants both in vivo and in vitro conditions. The bud found in the axil of leaf is comparable to the stem tip, for its ability in micro propagation. A bud along with a piece of stem is isolated and cultured to develop into a plantlet. Closed buds are used to reduce the chances of infections.

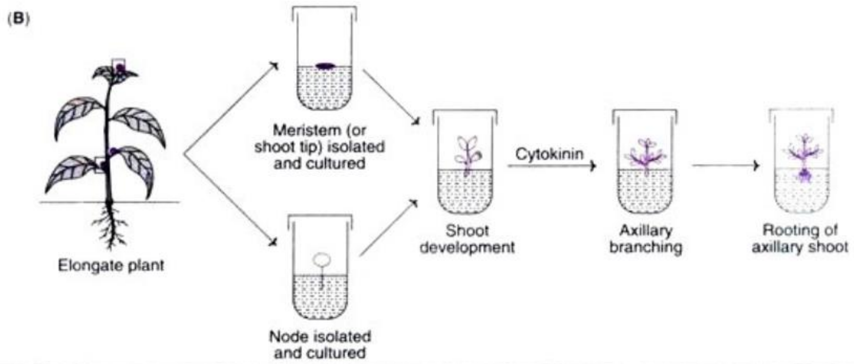
A diagrammatic representation of single node culture is below. In single node culture, no cytokinin is added.



Axillary bud culture:

In this method, a shoot tip along with axillary bud is isolated. The cultures are carried out with high cytokinin concentration. As a result of this, apical dominance stops and axillary buds develop. A schematic representation of axillary bud culture for a rosette plant and an elongate plant is given below





For a good axillary bud culture, the cytokinin/ auxin ratio is around 10: 1. This is however, variable and depends on the nature of the plant species and the developmental stage of the explant used. In general, juvenile explants require less cytokinin compared to adult explants.

Sometimes, the presence of apical meristem may interfere with axillary shoot development. In such a case, it has to be removed.

2. Multiplication by Adventitious Shoots:

The stem and leaf structures that are naturally formed on plant tissues located in sites other than the normal leaf axil regions are regarded as adventitious shoots. There are many adventitious shoots which include stems, bulbs, tubers and rhizomes. The adventitious shoots are useful for in vivo and in vitro clonal propagation. The meristematic regions of adventitious shoots can be induced in a suitable medium to regenerate to plants.

Micropropagation in summary

Just as every person is different and unique, so is each plant. Some have traits like better color, yield, or pest resistance. For years, scientists have looked for methods to allow them to make exact copies of these superior individuals. Plants usually reproduce by forming seeds through sexual reproduction. That is, egg cells in the flowers are fertilized by pollen from the stamens of the plants. Each of these sexual cells contains genetic material in the form of DNA. During sexual reproduction, DNA from both parents is combined in new and unpredictable ways, creating unique organisms. This unpredictability is a problem for plant breeders as it can take several years of careful greenhouse work to breed a plant with desirable characteristics. Many of us think that all plants grow from seeds but now, researchers have developed several methods of growing exact copies of plants without seeds. Tissue culture is the cultivation of plant cells, tissues, or organs on specially formulated nutrient media. Under the right conditions, an entire plant can be regenerated from a single cell. Plant tissue culture is a technique that has been around for more than 30 years. Tissue culture is seen as an important technology for developing countries for the production of disease-free, high quality planting material and the rapid production of many uniform plants.

Micropropagation, which is a form of tissue culture, increases the amount of planting material to facilitate distribution and large-scale planting. In this way, thousands of copies of a plant can be produced in a short time. Micro-propagated plants are observed to establish more quickly, grow more vigorously and taller, have a shorter and more uniform production cycle, and produce higher yields than conventional propagules. Plant tissue culture is a straight forward technique and many developing countries have already mastered it. Its application only requires a sterile workplace, nursery, and green house, and trained manpower. Unfortunately, tissue culture is labor intensive, time consuming, and can be costly.

Plants important to developing countries that have been grown in tissue culture are oil palm, plantain, pine, banana, date, eggplant, jojoba, pineapple, rubber tree, cassava, yam, sweet potato, and tomato. This application is the most commonly applied form of biotechnology in Africa. An example of the use of tissue culture in crop improvement in Africa include: A new rice plant type for West Africa (NERICA – New Rice for Africa) resulting from embryo rescue of wide crosses made between Asian rice (Oryza sativa) and African rice (Oryza glaberrima) followed by anther culture of the hybrids to stabilize breeding lines.

For years, scientists dreamed of combining the ruggedness of the African rice species (Oryza glaberrima) with the productivity of the Asian species (Oryza sativa). But the two are so different, attempts to cross them failed as the resulting offspring were all sterile. In the 1990s, rice breeders from the West Africa Rice Development Association (WARDA) turned to biotechnology in an attempt to overcome the infertility problems. Key to the effort were gene banks that hold seeds of 1500 African rices — which had faced extinction as farmers abandoned them for higher-yielding Asian varieties. Advances in agricultural research helped scientists cross the two species — a breakthrough that is changing the lives of many rice farmers in West Africa. After cross-fertilization of the two species, embryos were removed and grown on artificial media in a process known as embryo-rescue. Because the resultant plants are frequently almost sterile, they are re-crossed with the sativa parent wherever possible (known as back-crossing). Once the fertility of the progeny was improved (often after several cycles of back-crossing), anther culture was used to double the gene complement of male sex cells (anthers) and thus produce true-breeding plants.

The first of the new rices dubbed ‘New Rice for Africa’ (or NERICA) was available for testing in 1994 and since then, the techniques have been refined and streamlined, so that many new lines are generated each year. The dream had come true. The new plants had the best of both worlds, some of them combined yield traits of the sativa parent with local adaptation traits from glaberrima. The NERICAs inherited wide, droopy leaves from their African parent, which smother weeds in early growth. That reduces labor, and allows farmers to work the same land longer, rather than having constantly to clear new land. The structure of the panicles, or grain heads, has also been changed. Panicles of the African species produce only 75-100 grains. The new rice inherited, from their Asian parent, longer panicles with ‘forked’ branches, and hold up to 400 grains.

Like their Asian parent, the new rices hold grains tightly, not allowing them to shatter. They produce more tillers than either parent, with strong stems to support the heavy grain heads. The new varieties outyield others with no inputs—but respond bountifully to even modest fertilization. During rice trials, yields as high as 2.5 tonnes per hectare at low inputs—and 5 tonnes or more with just minimum increase in fertilizer use, have been obtained, approximately 25% to 250% increase in production. The new rice mature 30 to 50 days earlier than current varieties, allowing farmers to grow extra crops of vegetables or legumes. They are taller than most rices, which makes harvesting easier—especially for women with babies strapped to their backs. They resist pests and tolerate drought better than the Asian rices— vitally important for rainfed-rice farmers. The new rices grow better on infertile, acid soils—which comprise 70% of West Africa's upland rice area. They also have about 2% more body-building protein than their African or Asian parents. Because of their success, NERICAs were quickly adopted by farmers.

In Kenya, as in many parts of the tropical and subtropical developing world, banana is a highly important food crop. In last 20 years, however, there was a rapid decline in banana production due to widespread soil degradation and the infestation of banana orchards with pests and diseases. These problems were further aggravated by the common practice of propagating new banana plants using infected suckers. The situation was threatening food security, employment and incomes in banana-producing areas. Tissue culture (tc) technology was considered an appropriate option to provide sufficient quality and quantity of such materials. With proper management and field hygiene, yield losses caused by pests and diseases at farm level have been reduced substantially. Tissue culture technology has made it possible for farmers to have access to the following:

- Large quantities of superior clean planting material that are early maturing (12-16 months compared to the conventional banana of 2-3 years)

- Bigger bunch weights (30-45 kg compared to the 10-15 kg from conventional material
- Higher annual yield per unit of land (40-60 tons per hectare against 15-20 tons previously realized with conventional material)

Moreover, uniformity in orchard establishment and simultaneous plantation development has made marketing easier to coordinate with the possibility of transforming banana growing from merely subsistence to a commercial enterprise. An encouraging finding from a cost-benefit analysis of the project is that tissue culture banana production is more remunerative as an enterprise than traditional banana production. The project has also benefited mainly women who tend the crop, thus helping to narrow the gender gap. Bananas propagated from apical meristem in Kenya have been shown to have increased vigour and suffer lower yield loss from weevils, nematodes, and fungal diseases.

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