PROPAGATION BY TISSUE CULTURE - WHAT IT'S ALL ABOUT

SEETHA KARUNARATNE
Head/Tissue Culture Division
Cocnut Research Institute
Lunuwila.

Tissue culture is a technique by which small pieces of actively growing parts removed from a donor plant are cultured aseptically in specially formulated nutrient media, under controlled enviornmental conditions. Depending on the requirements, growth and development of the cultured material is directed into different channels. One important channel is plant regeneration from the cultured material for mass propagation of plants.

What is the Basis for Plant Regeneration?

What makes plant tissues or organs removed from donor plants and incubated under appropriate culture conditions to produce plants? It is a problem in the differentiation of eukaryotic organisms. Answer is not known yet. This capacity to regenerate however, is one of the features which distinguishes plants from animals. In the case of the animal cell, differentiation is final and irreversible; that is, the zygote through a series of developmental stages gives rise to an adult animal. Similarly, plant cells differentiate; the zygote finally gives rise to an adult plant but the process is not final or irreversible. Like in the development of an animal from the zygote, the plant zygote also during its development **differentiates** into various specialised types to carry out specialised tasks in the plant. Phloem cells, xylem cells and parenchyma cells differentiated for photosynthesis and storage of food reserves are some of the specialised cells in the plant. Unlike the specialised animal cells, these plant cells are capable of **dedifferentiating**; that is, specialised plant cells could be made to divide and give rise to less specialised types. These dedifferentiated cells could then **redifferentiate** into other new paths of specialisation. Plant regeneration is one such specialised path. In other words, the specialised vegetative cells removed from the donor plant have the capacity to dedifferentiate backwards into the most embryonic level, that of the zygote and then through redifferentiation, to develop into new plants. This natural capability to reproduce themselves by asexual means is exploited in propagating plants from excised tissues, organs and isolated cells and protoplasts. This type of plant regeneration is the most spectacular demonstration of **totipotency** in higher plant cells.

Regeneration of Plants in the Test Tube

Freshly cut, tender plant organs such as shoot apices, axillary buds, leaves, root segments, zygotic embryos and floral buds are used for this purpose. In the laboratory, all manipulations are carried out under aseptic or microbe-free conditions to prevent spoilage of cultures. Firstly, plant materials are disinfected using common disinfectants such as ethyl alcohol, chlorox, bleaching powder, mercuric chloride etc. The type and concentration of the disinfectant and the exposure time are determined through experimentation. Traces of disinfectants left in plant materials are then removed by rinsing in pre-sterilised water by heating to 120'C under a pressure of 15 psi. This material is then cut into small pieces; less than 1 cm in length (called explants) and placed in sterile dishes or vials containing the pre-sterilised culture medium (details given below). Small buds and embryos are cultured intact. Culture containers are carefully closed with sterilised cotton wool plugs or lids (depending on the container type) and incubated under controlled environmental conditions for further growth and development.

The culture medium is usually rich and consists of macro and micro minerals, vitamins, plant growth hormones and a source of carbohydrates, mainly sucrose. If necessary, this medium is solidified using a gelatine-like substance called agar.

Depending on the requirements, other substances such as coconut water, yeast extract etc. are used in the culture medium. pH of the medium is adjusted to between

5.0-6.0. Aliquots of the medium are then dispensed into suitable containers, and disinfected by heating at 120^0C under pressure for 15 minutes.

Plant regeneration from the cultured material is a multi-step process. Each step requires different culture conditions and is determined by systematic manipulation of culture medium composition and environmental conditions. Transfer of resulting seedlings from the test tube to soil also involves tedious work and has to be done with utmost care.

Paths of Plant Regeneration

There exists a few paths of plant regeneration from cultured materials (please see figure 1 for details). It is determined by the plant species, type of explant and the culture conditions. The two pathways, **direct morphogenesis** and **indirect morphogenesis** are based on the totipotency of plant cells. In the former, the specialised cells of the explant dedifferentiate and then during the redifferentiation process either become embryogenic (produce embryos) or organogenic (produce shoots). The end result in both cases is a complete plant. In indirect morphogenesis, the specialised cells dedifferentiate and divide to form an undifferentiated mass of cells called the callus. These callus cells redifferentiate into embryos (embryogenesis) or shoots (organogenesis). They later develop into complete plants.

Propagation from axillary buds (figure 1) resembles conventional rooting of cuttings, but in the test tube, buds can be induced to proliferate rapidly to produce a large number of buds, within a relatively short period. These buds are then separated and rooted to produce plants.

It is clear from the above description that, during plant development through tissue culture there is no gamete formation or fusion. Hence it is not a sexual process. It is purely a vegetative or asexual process and as such the resulting plants usually resemble the explant-donor or the mother plant phenotypically and genetically. Therefore the technique has tremendous potential in rapid propagation (or cloning) of superior plants. If successfully developed, the technique will be very useful in cloning perennial trees that are difficult or impossible to propagate by conventional methods. So far, cloning by tissue culture had been most successful with plants responding to conventional vegetative propagation methods. These include some vegetable and fruit plants and many ornamental plants. Perennial trees such as forest and fruit trees and plantation crops are difficult to propagate by conventional methods. Explants from these trees are generally stubborn in culture also. Therefore, with a few exceptions, development of technologies for clonal propagation of selected tree species is still at the experimental stage.

When should Tissue Culture be Utilised for Mass Propagation?

It is important to see whether tissue culture is the answer to the problem at hand. The advantages and disadvantages of tissue culture propagation in relation to the crop(s) to be produced must be considered. In many cases, classical propagation methodologies may be the fastest and least expensive means of overcoming the problem.

For tissue culture to be used sucessfully on a commercial scale:

1. Multiplication rate of plants from the explant must be rapid.
2. Resulting plants should be uniform not only phenotypically but genetically also. Under certain culture conditions, explants are known to produce abnormal plants in considerable numbers.
3. Large populations of plants should be produced in a relatively smaller growing space and in a reduced time frame (for low cost).
4. Product (plant) should come from superior or elite plants.
5. Establishment of seedlings in the soil should be highly satisfactory.

Finally, tissue culture technology if properly employed, will have a tremendous impact on agriculture and forestry. But development of technologies still seems to be difficult, specially with important tree species. Breakthroughs towards plant regeneration have been made but procedures still have a long way to go before they can collectively qualify as a routine tool.

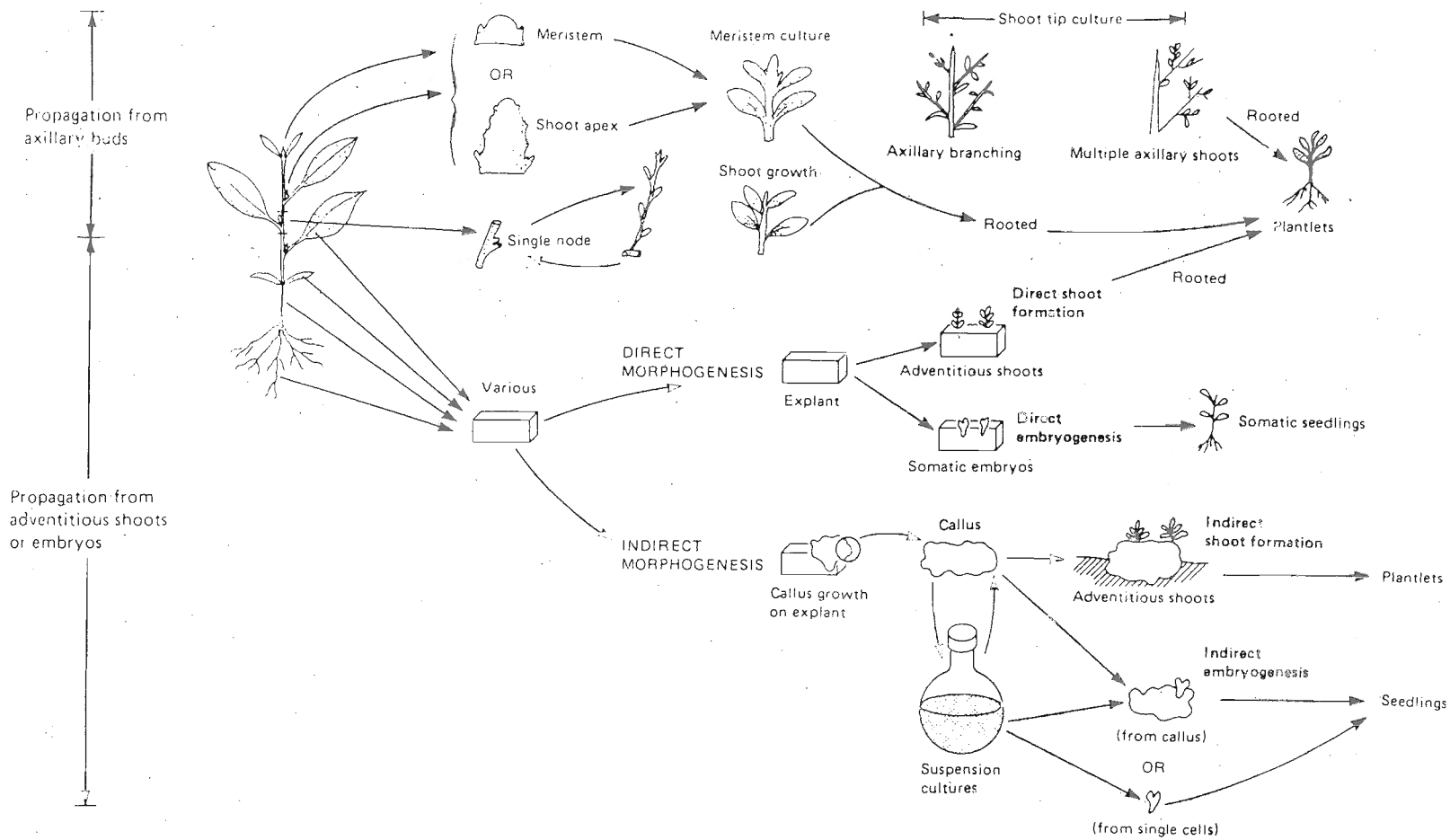

Figure 1 : The principal methods of micropropagation
Shoots and/or plantlets do not always originate in culture by a single method

Source : Lydiane Kyte (1983)