# APPLIED ASPECTS OF PLANT TISSUE CULTURE WITH SPECIAL REFERENCE TO TREE IMPROVEMENT

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ABSTRACT

The potential application of plant tissue culture for the improvement of agricultural crops has been very well established. Present-day advances in plant cell and tissue culture have reached new heights. Forest trees are important sources of wood and wood products. The world demand for wood is expected to double by the year 2000. The forest improvement programmes generally practised at present are not adequate to meet the growing demand and unless suitable techniques are evolved, there is going to be a shortage of wood. Tissue culture can offer a solution to this problem. In our laboratory, we have been interested for the past five years in the application of plant tissue culture for the improvement of economically important trees like sandalwood, eucalyptus and rubber. In sandalwood somatic embryogenesis was induced from shoot callus cultures derived from 20–25 year old trees. Embryoids have subsequently developed into well-established plants and planted in the forest area. The technique can be used for the propagation of selected superior varieties.

Plant tissue culture is an important technique with applications in several areas ranging from horticulture and agriculture to chemical and biochemical sciences. The idea of culturing plant cells was conceived as early as 1905 by Haberlandt. His idea was to develop a versatile tool for studying the metabolism of plants and to demonstrate "totipotency", *i.e.*, the regeneration of whole plants following the induction of embryos from somatic cells. However, successful experiments in the culturing of unorganized plant cells were not demonstrated until 1939. Although the technique was well established, its economic potential was not recognized until the 1960s.

The stimulus to the whole field of research on tissue culture came from the discovery[1] that the orchids could be propagated by sterilizing the surface of the orchid shoot followed by exposure and excision of the extreme shoot tip (meristem) and its transfer to sterile nutrient medium under aseptic condition. The cells of the small explant divided to give rise to protocorms, which, in turn, could further be subdivided and cultured to develop into orchid seedlings. By this method the individual orchid could rapidly be multiplied and the progeny brought to flowering in a short period. Further, as the original explant was very small, the progency was virus free. Orchid-growers were the first to take advantage of this technique and presently orchids are multiplied on a commercial scale, by using meristem culture technique. Presently the technique has been extended to several herbaceous crops for the multiplication of selected varieties of plants. These methods are currently used in the USA, UK and other European countries, to mass produce dahlias, carnations, chrysanthemums and many other plants.[2]

Tissue culture consists of growing single cells or cell aggregates under controlled conditions. For continuous growth of tissue, an adequate supply of nutrients and growth regulators is required. Any part of the plant can develop into a callus tissue, *i.e.*, an undifferentiated mass of cells. Depending on the purpose of the investigation, further experimental conditions can be manipulated. The major areas of application of plant tissue culture are (a) production of pharmaceuticals and other natural products (b) the recovery of disease-free clones and preservation of valuable germ plasm (c) rapid clonal multiplication of selected varieties in horticultural and agricultural crops and finally (d) genetic engineering, which can introduce into a species, new and valuable genetic material *i.e.*, to introduce directly new genes into a species. The possibility of obtaining newer varieties and newer hybrids (not possible by cross pollination) arises from the latest techniques of protoplast cultures.

The most substantial application of plant tissue culture so far has been in rapid clonal multiplication and is mostly confined to herbaceous plants[3]. Similar advances have not been made in tree crops[4], especially, the commercially important ones. Forest trees are the raw materials for wood and wood products. The world demand for wood and wood products is estimated[5] to double by the year 2000. The present-day tree improvement programmes are not adequate to meet the growing demand. Trees of improved quality and quantity are needed. Tissue culture seems to offer an

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The 10th Symposium of the International Measurement Confederation Technical Committee (IMEKO Technical Committee) on Photon-detectors will be held in Berlin (West) from 20 to 22 September 1982 and will deal with the following main topics: (a) Physical properties of photon-detectors. (b) Application of photon-detectors in light, colour, and radiation measurement. (c) Application of photon-detectors in production control. (d) Medical and photobiological application of photo-detectors.

Experts in the field of photon-detectors are welcome to deliver lectures at the Symposium. Abstracts (in English) not exceeding two typewritten pages, should be sent to the Organizing Committee before 1 May 1982.

Correspondence related to the Symposium should be sent to the Organizing Committee, Institut fur Lichttechnik der, Technischen Universtat Berlin Prof. Dr. Ing. Krochmann, Einsteinufer 19, D-1000 Berlin (West) 10.


**Figures 1-5.** Stages of Development. **1.** Undifferentiated callus. **2.** Differentiated embryoids showing well-developed shoot and root ends. **3.** Fully developed plantlet with a well-developed tap root system on a filter paper bridge. **4.** Potted plant. **5.** 2-year old plant in the ground.

Several laboratories in the world are trying to induce somatic embryogenesis especially in plants like oil palm, coconut and coniferous trees. To date, it has been possible only in sandalwood and oil palm. M/s Unilever, UK have started plantations of oil palm in Malaysia. Bapat and Rao[18] also observed somatic embryogenesis in seedling callus of sandalwood. In trees like coffee and rubber also, somatic embryogenesis has been reported, but its frequencies are low.

In addition to obtaining diploid plants in sandalwood, we have also produced triploids by inducing embryogenesis from endosperm callus. Although the triploid plants are seed sterile and consequently undesirable, where seeds are of commercial importance, there are instances where seedlessness caused by triploids is of no serious consequence. Rather, at times it is of great advantage as in the case of seedless fruits and also in forest plants where wood quality and yield are of importance as in quacking aspen[19]. Normally it would be difficult if a triploid plant cannot be multiplied vegetatively. But in sandalwood, we assume that the triploids could be multiplied by inducing embryogenesis from shoot callus as in the diploid sandalwood.

The immediate problem faced by the forest researcher today is to develop an economically efficient technique of propagation by cell and tissue cultures. The ultimate goal is to develop a viable method for mass producing identical copies of superior trees by the millions. The immediate advantage of this technique is the drastic reduction in time required to produce a second generation of trees. Somatic embryogenesis can play an important role in large scale production of superior trees whose desirability is established. Success achieved in sandalwood and oil palm gives a new impetus to research on trees of economic importance.

With the increasing volume of relevant publications[20-24], geneticists and plant breeders are evincing great interest in the potential, practical applications of tissue and cell culture, to plant breeding. Already important work has been done with agriculturally important crops. Despite the success achieved with tissue culture, there are some limitations and problems such as lack of stability in many cultures, limited knowledge of important physiological and biochemical processes in trees and mechanisms governing them. Closer links between tree physiologists, biochemists, geneticists and breeders with an interdisciplinary approach will solve some of the problems and help develop superior quality trees.

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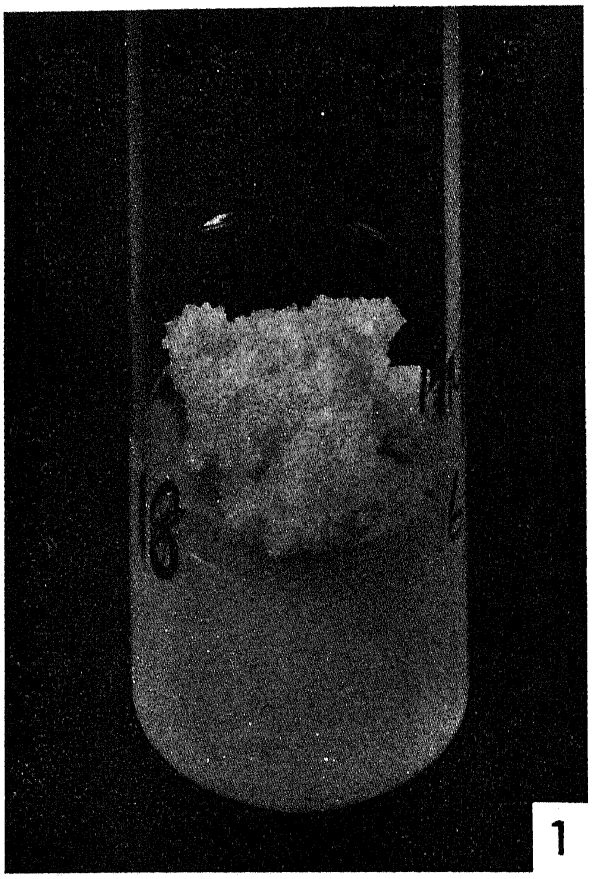

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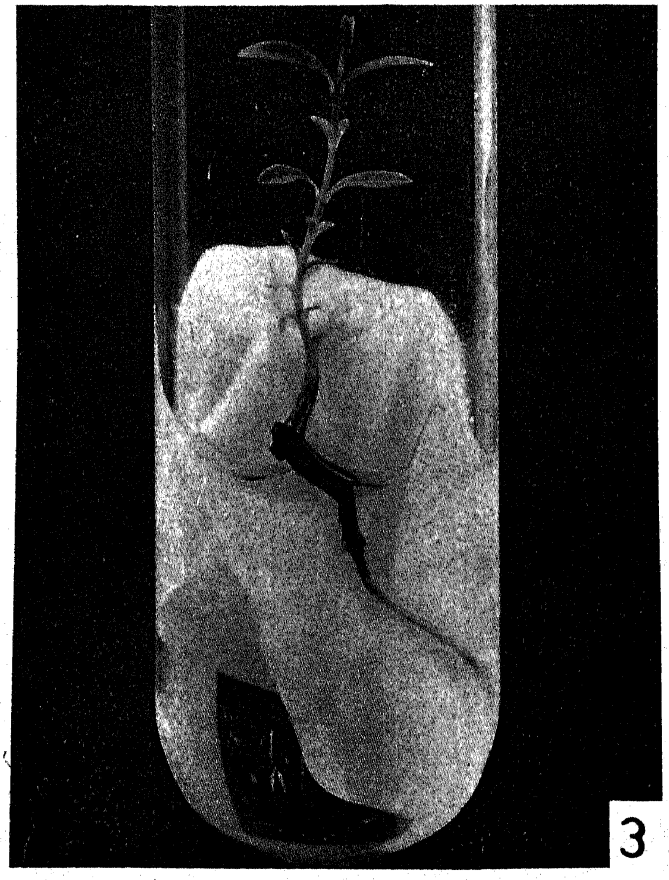

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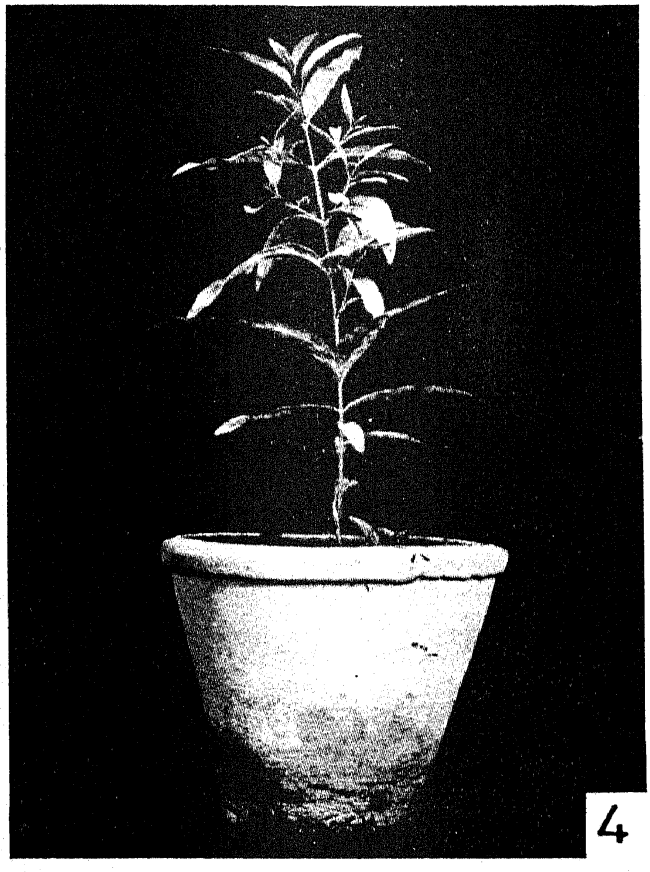

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alternate approach to these problems. Any exercise which promises an increase in the yield of even 2–5% is well worth the cost, according to some economic surveys[6]. Horticulturists use techniques of vegetative propagation to produce selected plants. Conventional methods of propagation such as cuttings and grafts have great advantages, especially when the characteristics of plants grown from the seeds of a single plant are found to vary, or, in the case of many hybrids where no viable seed is produced. It is possible to produce only small numbers of plants by using conventional methods of cuttings and graftings. If we could find a means of producing large number of plants from one selected plant, it would pave the way for improving the yield and quality of many crops, especially in the tree crops where breeding cycles are long and vegetative propagation is difficult. The tissue culture technique is a possible answer to this problem. It is essentially a method of vegetative propagation by the use of which a small piece of plant tissue can be made to grow, if the right conditions are given, into many new plants each identical to the selected variety.

Although the technique sounds simple, a systematic study is needed to develop a commercially practicable procedure for any crop. The best medium for the culture needs to be worked out for each new species. Nutritional requirements vary considerably from species to species. The method usually proceeds through a sequence of 2–3 stages *in vitro*. Each step will have specific requrements. The first stage concerns the establishment of aseptic cultures of freshly excised tissues; the second stage is directed towards the multiplication of tissues, organs, embryos, and other structures that could be used as propagula; finally the third stage leads to the individual plant development and establishment into pots or the ground. With some plants, it may be possible to proceed through the first two stages by using the same or different culture conditions as in the case of sandalwood[7].

The potential application of the various tissue culture methods and their significance for crop improvement and their advantages over conventional breeding programmes have been discussed by Johri *et al*[8].

The potential value of tissue culture to tree breeding is of more than just academic interest. Three major areas in which cell and tissue culture and related techniques are of potential value are: (a) shoot tip culture which allows rapid clonal multiplication, in those plants which are difficult and time-consuming to propagate by traditional methods; (b) embryo culture of hybrids of desirable crosses that cannot develop normally due to incompatibility between the embryo and the maternal tissues, (c) Manipulation at cellular level in order to use them in asexual plant improvement in addition to conventional sexual breeding programmes. There are several approaches at cellular level which can aid in increasing genetic diversity, such as variation in tissue culture leading to polyploidy, haploid plant production from pollen and anther culture leading to homozygous diploid production, fusion of cells by protoplast cultures enabling interspecific and intergeneric crosses and, finally, transgenosis, leading to transfer of desirable genes from one species to the other.[9]

For the past few years we have been interested in the application of these techniques for the improvement of commercially important trees like sandalwood[7,10,11], eucalyptus[12,13], rubber and others. Sandalwood is one of the important commercial crops of Karnataka State with an annual turnover of nearly two crores of rupees. Since 1974 the production has gone down considerably due to several factors[14–16]. One of the main problems is the spike disease caused by mycoplasma. Plants are susceptible to infection from an early age and die soon. However, apparently healthy trees which are about 20–25 years old are found in certain areas of Karnataka which normally abound in spiked trees. We have taken up tissue culture to study the possibility of using the technique for large scale multiplication of such disease-free varieties. Callus was isolated from shoot segments of (0·5–0·8 cm) mature sandal trees of 20–25 years. These segments have callused on Murashige and Skoog's[17] basal medium supplemented with 2,4 dichlorophenoxyacetic acid (2,4, D), an auxin. This will correspond to stage 1 mentioned earlier. The second stage consists of experiments for regeneration. Regeneration can be of many types. One is the spontaneous development of shoot buds and roots. Shoot buds can be separated and rooted on a different medium. In callus cultures producing both buds and roots, their origin is from different centres, and are not interconnected; so complete plants are not formed. Far more exciting is the approach *via* embryo-like structures, that come from groups of cells, in callus or suspension cultures. These (so-called embryoids) develop along the same path as the normal seed embryos and germinate into perfectly formed plantlets with a well-developed tap root system. In sandal tissue cultures, we have induced somatic embryogenesis, *i.e.*, development of embryo-like structures from callus produced from vegetative parts. These embryoids have developed into complete plantlets. Figures 1–5 show the stages of development. Even where traditional methods are available '*somatic embryogenesis*' will have a special value as the plants produced will have tap root system necessary for thrifty growth to stand for years. With regard to plants like rubber, even though they can be propagated vegetatively, rooting is not satisfactory, since the adventitious root system is inferior to the taproot system.