

THE ROLE OF TISSUE CULTURE IN THE AVOCADO PLANT IMPROVEMENT SCHEME

Review



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INTRODUCTION

The aim of a plant improvement scheme is the production of **selected, healthy** plants which can reach the ultimate destination namely the growers, in a good and acceptable condition and in **adequate quantities** to meet their demands. This article deals with the *in vitro* methods in obtaining healthy clones and the rapid propagation of avocado plant material.

1. Tissue culture and the production of disease-free avocado trees

Some of the main diseases of avocado trees include *Phytophthora* root rot, Bacterial canker and Sunblotch — caused by a viroid. There are other diseases which are unimportant to our industry at this stage. Fruit diseases are not considered. (To date the presence of four viruses has been detected: AV 1, AV 2, AV 3, and AV 4.) A combination of these viruses can occur and it was speculated that AV 2 + AV 3 may be the cause of avocado black streak (Allen, 1983). However, the cause of black streak has not yet been established.

Diseases of viroidal and viral origin are systemic and are not located in one area or organ but spread throughout the whole plant and are extremely difficult to eliminate. A further disadvantage of these diseases is the fact that vegetative propagation from an

infected mother plant produces infected progeny. The fact that some avocado viruses are also seed transmitted heightens the problem.

Among the methods used for obtaining pathogen-free plants are the following:

(a) Selection

A symptomless tree is indexed for viruses, viroids and other systemic diseases and may be found free from the pathogens. Such a plant can then be used as the selected mother plant. It is often difficult and sometimes totally impossible to find a disease-free plant.

(b) Chemotherapy

According to Dr Fredrika Quak (1977) virus multiplication is so intimately associated with the normal metabolic processes of the plant that chemotherapy has not been successful and although there are many virus inhibitors they have often proved toxic to the plant.

(c) Thermo-therapy

Some plant viruses can be eliminated by means of heat therapy. The plant is subjected to a temperature that allows the plant to survive but which inhibits virus multiplication. Sometimes a whole plant is cured in this way. In most cases, however, tip cuttings of treated plants are used to obtain virus-free progeny.

In the case of the avocado, attempts to eliminate sunblotch by thermo-therapy failed. Hot water treatment eliminated AV 1 but not AV 3 from infected seed (Mass and Fuerte). Hot air in a controlled thermo-therapy chamber may eradicate AV 1 and AV 3 but not AV 2. No work has as yet been done on AV 4. Heat treatment alone is therefore not successful in producing virus-free avocado plants.

(d) The only other method of virus-elimination is tissue culture or more commonly called meristem tip culture.

In systemically infected plants the concentration of virus particles decreases towards the apical meristem and viruses are seldom found in the meristematic dome. The meristem dome is difficult to culture *in vitro* but if one or two leaf primordia are excised with the dome, the chance of growth is greatly enhanced although the percentage of virus-free plants is lowered. By combining heat therapy and meristem tip culture a larger, more viable explant can often be taken with success. Even in this case the tip is extremely small — from 0,2 to 2 mm. Viruses and other pathogens have been eliminated from a large number of crops by means of meristem tip culture.

Sometimes, however, as in the case of certain citrus varieties and the avocado, the excised tip fails to grow if placed directly onto the nutrient medium and a second method known as tip grafting is used. This process involves the following procedure.

An aseptic seedling is grown *in vitro* by planting a seed or embryo on a nutrient medium. When the young seedling is well established it is decapitated, the stem is slit and the tiny meristem tip that has been excised from a tip or lateral bud of the

infected tree is implanted into this opening. If the graft is successful the tip grows out and a small grafted tree is produced which may be virus-free.

This is the method that we are at present trying to apply to the avocado. Unfortunately the avocado is a much more difficult plant to grow *in vitro* than citrus where the technique is applied successfully. Compare for instance the size of the orange seed to that of the avocado. Whereas the whole citrus seed is easy to plant and grow *in vitro*, the avocado embryo (plumule and radicle axis) has to be excised and a more complicated medium is required.

This is the first year that we have concentrated on this method and we have planted approximately 300 avocado embryos *in vitro*. Edranol embryos were small and difficult to germinate but the results with Ryan were better. We have not started grafting onto these as the seedlings are still too small. In the previous year we grafted a few seedlings with very limited success.

Shoot tip grafting is an extremely delicate operation but if it is the only way of obtaining a virus-free plant we must endeavor to overcome the obstacles.

Meristem tip plants and those obtained from grafting must be thoroughly indexed before they can be used as a pathogen-free mother plant. From one such plant, however, multiplication can be undertaken by conventional means or by tissue culture.

2. Rapid propagation of the avocado by means of tissue culture

Normal processes of clonal propagation may be too slow to meet the growing demands and we are investigating the possibility of rapid multiplication of the avocado tree through tissue culture methods. Although it is fairly easy to propagate herbaceous plants by *in vitro* culture —

claims of 10 000 to 1-million plantlets from one tip or leaf cutting within a year are quite common — woody plants pose a much greater problem and it is often in rooting the excised tip that the greatest difficulty lies. Diligent research has resulted in overcoming the problems of a large number of woody plants eg *Malus*, *Prunus*, *Pinus*, *Citrus* and *Eucalyptus* spp.

Many factors determine the success in *in vitro* propagation of avocados eg nutrient medium, the incubation regime (temperature, light and humidity), seasonal changes and in the case of some woody plants the juvenile phase of the mother plant is often the only stage where growth and rooting *in vitro* will take place. Thus it is easy to root avocado explants taken from young seedlings but once the tree is mature — and it is only then that the quality of the tree can be assessed — rooting *in vitro* becomes extremely difficult.

There are a few ways in which this problem can be overcome. Fernando Pliego-Alfaro (1981) found that lateral buds of an adult avocado tree grafted onto one month old seedlings *in vitro*, developed into shoots and when these were cut off and sub cultured onto a rooting medium, roots developed with a 50% frequency. Moreover, subsections of these rooted shoots continued to root *in vitro*. It appears and the juvenile phase of the

seedling is carried over to the mature scion.

To obtain a certain form of juvenility our own approach has been to place trees in a dark room at a temperature of 27 °C with the hope of getting etiolated shoots. Very little growth however, took place in the cabinet but when the plants were taken out and cut back young, shoots appeared within a week and these shoots have given the best results for *in vitro* growth and have been the only Duke 7 material that has rooted thus far. The percentage of success is at present too low to make it a commercially viable proposition and further research is urgent.

Besides trying to solve the rooting problem we are also investigating methods of multiplication of the avocado. Rapid propagation *in vitro* can be accomplished by the following means.

- (a) The rapid elongation of a tip cutting or a lateral bud *in vitro*. The resulting stem can be cut up into one-node sections and the process can be repeated.
- (b) Under the influence of a plant growth substance known as a cytokinin, the apical dominance of the cultured tip can be suppressed and the lateral buds develop into shoots. The apical dominance is broken and side buds grow out, until there is a cluster of small shoots. These can be individually sub cultured onto either the multiplication medium for further propagation or a rooting medium for producing a plantlet.

The second method yields far more plants than the first. With the avocado we have been able to take up to 20 plantlets from a single *Persea indica* tip but only four or five from Duke 7.

- (c) By using a certain type of nutrient medium, callus growth can be obtained. Callus is a mass of undifferentiated cells which can multiply very quickly. Under the influence of plant growth substances some of these cells can be induced to form what is known as pro-embryos or somatic embryos. These structures are very similar to zygotic embryos and can develop into plantlets. This method is at present being exploited at Nelspruit for citrus multiplication and they have succeeded in transplanting their first plantlets ex citrus callus.

Although callus readily forms on avocado tissue, plant regeneration has not yet been encountered. In his thesis which includes avocado tissue culture, Fernando Pliego-Alfaro claims that he achieved somatic embryogenesis from avocado callus obtained from young embryos taken from immature fruit. Again, however, the initial plant material is of unknown quality as it is obtained from a zygotic embryo.

We have not tried avocado callus cultures but it is a method that can be resorted to if all else fails.

The avocado plant is not an easy plant to handle *in vitro*. Limited success should not deter us from continuing the research but should rather spur us on to accept the challenge to solve the tough problems. The establishment of a plant improvement scheme is a long term project and the role of tissue culture can be of vital importance to the success of such a project.

LITERATURE

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