

TISSUE CULTURE FOR RUBBER

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In simple terms, plant tissue culture is the technique of growing plant cells, tissues or organs in artificially prepared media, under sterile conditions. The Latin term "*in vitro*" has the meaning "in glass" and *in vitro* is used instead of tissue culture. Though the general term is "tissue culture" depending on the type of explant, or the starting tissue used, more specific terms are used such as, embryo culture, leaf culture, anther culture, axillary bud culture etc.

The *in vitro* methods used for propagation of a plant is also called micropropagation, as the size of the starting plant materials here is very small compared with those used for *in vivo* methods.

Why *in vitro* methods?

In vitro propagation methods have several advantages over conventional methods of plant propagation. Among them mass propagation and true-to-type propagation methods have developed in to very powerful and useful tools.

Also, some key reasons are available to answer, why tissue culture techniques should be applied for a certain plant species.

- (a). When *in vivo* vegetative propagation is not possible.
- (b). When the existing *in vivo* methods of vegetative propagation are too slow.
- (c). When production of plant material free from pathogens is necessary.

For rubber, what is given under (c) is least important. Yet, (b) is important as the short supply of quality planting materials is a common problem. Regarding (a), for rubber, rooted cuttings are the only fully vegetative propagation method available, which is not a satisfactory method. Up to date, rooting of clonal cuttings has been possible after 2-3 months under a permanent mist spray with root inducing chemicals. Nevertheless, success rate remains at a low level and therefore this method is not practised commercially. Furthermore, true-to-type propagation should be started with a single mother tree, and hence multiplication of source bush plants should also require a substantial length of time. Also, the root system produced on cuttings is fibrous or adventitious and therefore, whether they can give enough anchorage to the

tree when it is fully grown is also questionable.

Therefore, the conventional method of propagation for rubber is by grafting buds from selected clones on to unselected seedlings. This is a partial vegetative propagation method, and the major short coming of this method is the intracloonal variation that is partially due to inevitable root stock/scion interaction.

It has been reported that some trees identified by selection, yielded 15-20 kg of dry rubber per tree per year while the grafted clones today give only average of about 4-6 kg per tree per year. This suggests a potential yield increase by more than 200%, which requires the help of a proper vegetative propagation method.

What technique to be used ?

For true-to-type propagation, axillary bud proliferation is the most suitable method. In case of rubber, field grown plants cannot be used to harvest explants due to high phenolic content and difficulties in surface sterilizing such materials. As a solution for this, plants are grown in a glasshouse and shoots are harvested from glass-house-grown plants (Fig.1).

The size of the explants used is about 2-4 cm and they generally contain 1-2 axillary buds on them. (Fig.2). First 2-3 weeks of culture is called the establishment period. The medium used for this passage is generally free of plant growth regulators as no growth is expected during this period. Cultures are regularly monitored for any bacterial or fungal contaminations and contaminated cultures are immediately discarded after autoclaving.

After the establishment period, the cultures are transferred onto a new medium containing plant growth regulators. After 4-6 weeks on this medium, the axillary buds burst out and axillary shoots are produced (Fig.3). The number and the type of axillary buds produce will mainly depend on the type and the quantity of plant growth regulators. This phase is called the multiplication phase as the main concern here is shoot multiplication. The length of the multiplication period depends on various factors such as, the growth rate of the cultures, number of propagules required etc.

However, the *in vitro* produced axillary shoots, are then transferred on to rooting medium. Rooting can be done under *in vivo* condition also. Rooting of micropropagated shoots is easy compared to shoot multiplication phase. Both clonal and seedling shoots have successfully rooted under *in vitro* (Fig.4) and *in vivo*.

Nevertheless, shoot multiplication rate of clonal materials is extremely slow compared with that of seedling materials.

Acclimatization of *in vitro* plants into *in vivo* conditions is the last stage of *in vitro* procedure. If rooting is induced under *in vivo* conditions, acclimatization also

takes place simultaneously. Main concern here is the relative humidity that requires gradual decrease up to atmospheric levels. Light intensity, on the other hand, should be increased gradually.

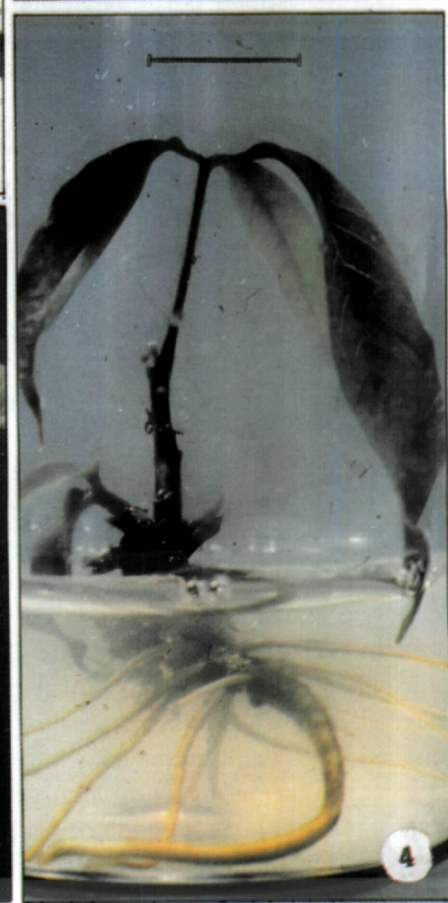
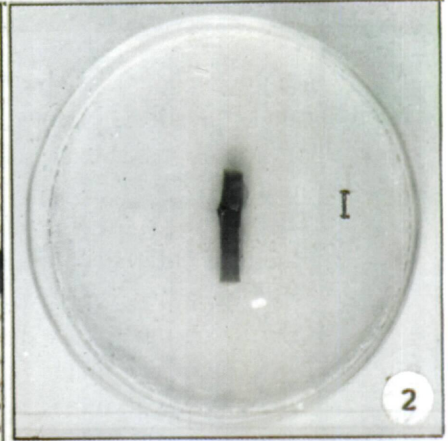
At the Rubber Research Institute of Sri Lanka tissue culture plants have been produced by axillary shoots of both juvenile and clonal origin materials. However, producing clonal origin plants is a very slow process due to the poor response of explants to culture media. Some juvenile origin plants were acclimatized and transferred to the field about four years ago. Though the plants are growing well in the field, investigation on uniformity, the performance of root systems etc., should be studied thoroughly before commenting on the technique. Reports are available on the uniformity of the micropropagated plants but no reports are available on the type or the performance of the root systems of micropropagated plants. Theoretically, micropropagated plants produced by axillary shoot proliferation should not contain a tap root system, but whether the root systems produced by these plants is sufficient for the tree is not yet fully known.

Culture medium and conditions

Explants require nutrients, light, temperature etc. in right quantities. All nutrients are supplied in the medium. Culture medium generally contains, macro elements, micro elements, iron salts, plant growth regulators vitamins and a carbon source. Normally, tissue culture media are prepared in solid form by incorporating agar in to the culture medium. When solid media are used, explants do not require an additional support as for liquid media. Apart from them, any chemical required by the plant can be supplied in the medium. Though tissue cultured plants are not expected to photosynthesize in culture, they are provided with a 12 or 16 hours light period supplied by cool white florescent tubes at $100 \mu\text{Em}^{-2}\text{s}^{-1}$ irradiance. The temperature should also be maintained at $26 \pm 2^\circ\text{C}$. These conditions are obtained by growing them either in incubators/growth chambers or growth rooms.

Culture vessels

Culture vessels can be of any shape of either disposable plastics or of autoclavable glass. Normally, petri dishes, tubes, jars, flasks or special containers made for tissue culture are used. If disposable containers are used then the culture medium is prepared in a glass container and autoclaved followed by pouring into the containers under sterile conditions i.e., under a Laminar Flow Cabinet. When autoclavable glass containers are used, the medium is poured into vessels and then autoclaved.



Figures 1. Glass house grown plant, 2. Explant (Nodal cutting), 3. Multiplication of axillary shoots, and 4. *In vitro* rooted culture.

Sterilization

Once the explants were prepared and taken into culture, the entire process is carried out under sterile conditions, until the micropropagated plants are transferred to out door conditions. Sterile conditions are achieved by adopting following sterilizing methods.

1. Disinfecting starting plant materials or explants by using chemicals such as. ethyl alcohol(EtOH), sodium hypochlorite (NaOCl), Hydrogen peroxide(H₂O₂) and mercuric chloride (HgCl₂).
2. Sterilizing the culture medium, culture vessels, water, tools etc. in an autoclave under 121°C temperature and 15 lb/min/sec pressure for 20 min.
3. Using a sterile environment, i.e., either a Laminar Flow Cabinet or a UV chamber for all operations such as pouring culture media, culturing explants and transferring cultures etc.

Most of the chemicals, equipment and culture procedures, used for tissue culture, are similar to those used by microbiologists and therefore well established and known. Laminar Flow Cabinet is an equipment that generates an aseptic air flow and thus providing an aseptic environment. UV chambers can also be used in place of Laminar Flow Cabinet. Nevertheless, their performance is not very satisfactory under tropical weather conditions since the normal microbial flora is very high.

Other uses of tissue culture for *Hevea*

Among the other uses of *in vitro* techniques for *Hevea*, techniques used for plant breeding is extremely useful. Because, it seems that plant breeders have reached a limit in the ability to introduce new genetic information into plants and to create new plant varieties through conventional plant breeding techniques. Isolation and culture of plant protoplasts and the development of genetic engineering techniques for transferring and cloning genes are the two major achievements accomplished toward introducing foreign genetic information into plants. Studies on genetic transformation have also shown promising results. Potential use of successful transforms, would be endless, and would never be achieved by conventional methods.

Culturing immature embryos is a simple way to increase the success rate of hand pollination. Production of haploid plants, which is impossible by conventional breeding techniques, has been successful by culturing anthers. However, the success rate of producing plants is very low.

Culture of seeds *in vitro* as an alternative to the present method of *Hevea*

germplasm preservation or exchange has the advantage as an additional precaution against the introduction of undesirable pathogens. Also as *Hevea* seeds are short lived, germplasm can potentially be stored through cultured embryos or seedlings.

However, it should be mentioned here that as for any other aspect on improvement and breeding etc., applications of *in vitro* techniques are difficult with higher plants such as perennial tree species. Furthermore, any research on biological aspects is always time consuming and painstaking. Nevertheless, research should be continued as alternative methods are hardly available.