Tropical Agricultural Research and Extension. 1(2): 98-102, 1998

The effect of the origin of the explant on *in vitro* growth of axillary buds of *Hevea brasiliensis*

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Accepted 8 September 1998

ABSTRACT

The effect of the origin of explants from different zones of the source plants on *in vitro* axillary bud growth in rubber (*Hevea brasiliensis* Muell Arg.) was investigated. Both juvenile (seedling) and mature (clonal) plants were used. Explants were harvested from shoots originated at different heights from 10 cm up to 180 cm. Mean lengths of the axillary shoots produced by the first nodal explants harvested from juvenile origin plants cut at 10, 60 and 120 cm were 38.2, 37.1 and 11.5 cm respectively at 10 weeks of culture. However, with clonal materials, mean lengths of 25 and 7.5 cm were obtained after 9 weeks of culture for the first nodal explants produced from plants cut at 30 and 180 cm heights. The results confirmed that juvenile materials are more responsive to *in vitro* culture. It was also revealed that the juvenile characters are more in the tissues close to the root system in mature rubber plants. Better understanding and manipulation of explants seems to be important for developing a successful protocol for micropropagation of clonal *Hevea* of mature origin.

Key words: *Hevea brasiliensis, in vitro* culture, juvenility, micropropagation, rubber.

INTRODUCTION

Micropropagation of rubber (*Hevea brasiliensis* Muell Arg.) can be an important tool towards realising the full agronomic potential of the plant. However, the phase change of the plant from juvenile to mature and the difficulties in reversing this change or rejuvenating the plant material are the main obstacles in the development of an *in vitro* protocol for elite material of *Hevea.*

The phase change and the differences in propagation behavior of various materials is known and has been discussed periodically (Baptist 1939, Songquan *et al.* 1990). Both juvenile and mature developmental stages exist in seedlings as well as buddings. The juvenile phase of *Hevea* is regarded as being from 0-4 years, followed by the transition phase (5 years of age) and the mature phase (6 years of age) (Songquan *et al.* 1990).

Differences between axillary bud proliferation of juvenile and clonal material have been reported (Carron *et al.* 1984; 1989). High shoot proliferation rates have been reported for juvenile material (Gunatilake and Samaranayake 1988; Carron *et al.* 1989; Chandrakanthi 1991; Seneviratne 1991). Plants have been produced through *in vitro* culture from clonal materials since 1985 (Carron *et al.* 1985), although the rate of proliferation from these materials has not been reported.

Various rejuvenating techniques such as the application of sprays or plant growth regulators (Robbins 1957; Franclet *et al.* 1987; Pierik 1990), severe pruning (Hackett 1985, Howard *et al.* 1989) and multiple grafting (Cresswell *et al.* 1982; Zimmerman 1985) may be successfully applied to *Hevea*. As most trees have organs that retain juvenility (for example the roots) and thus a greater capacity for vegetative propagation (Bonga 1982), these may be used successfully in micropropagation. Similarly, the closer a shoot apical meristem is located to the base of the trunk, the better are the chance of successful vegetative propagation.

It is possible that the difficulties previously experienced in the clonal propagation of *Hevea* (Seneviratne 1991) are related to the use of mature plant material and the objective of the study reported here was to determine the effects of using explants originating closer to the root system (the juvenile phase) on *in vitro* shoot proliferation in *Hevea brasiliensis*.

MATERIALS AND METHODS

Plant material

Two types of *Hevea* plant material were used in this study. Juvenile or seedling explants were harvested from plants grown in polybags in the glass house. Plants were cut at either 10, 60 or 120 cm from the root collar and sprayed with a solution of Thiourea (2% thiourea, 1% KNO_3 and 100 mg l^{-1} Gibberillic acid). Thiovit (80% sulphur-wettable powder) and

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Abbreviations: PVP- Polyvinyl polypirrolidin; NAA- Napthalene acetic acid; BA- Benzylaminopurine

Benlate (Methyl-1(butacarbamoyl) 2-Benzimidazol carbamate -50% wettable powder) were also sprayed when the plants produced some foliage.

Clonal explants were harvested from source bush plants of the clone RRIC 100 from a budwood nursery aged about 12 years. Trees were pollarded either at 30 or 180 cm above the bud union and covered with a polythene sheet about 1.5 m above the cut plants to protect from rain water. General agro-management practices (manuring and weeding *etc.*) applicable to rubber budwood nurseries were adopted throughout. Shoots about 30 cm long could be harvested after about 4-5 weeks of cut back. Spraying of fungicides, Thiovit and Benlate, and thiourea solution was done as for glass house grown plants.

Culture media

The basal medium of Lloyd and Mc Cown (1980) was used with 0.75% agar (Lab M type), 4% sucrose and 100 mg l^{-1} PVP. The medium was supplied with Naphthalene Acetic Acid (NAA), Benzylaminopurine (BA) and Kinetin at 0.2, 1 and 2 mg l^{-1}, respectively. The pH was adjusted to 5.7 prior to autoclaving. Solid media prepared in boiling tubes were allowed to solidify as slants after autoclaving. All chemicals including sucrose and agar were of analytical grade supplied by BDH Chemicals, UK, except the plant growth regulators which were cell culture tested types supplied from Sigma Chemicals, USA. Polyvinyl polypirrolidin (PVP) used was water soluble type with MW 10,000.

Sterilization

Green shoots of about 3-4 weeks age were harvested and washed thoroughly under running tap water for about ½ hour after removing all expanded leaves. Explants of about 10 cm long with small pieces of petioles attached were sterilized by dipping in 70% ethanol for 3 min followed by 10 min soak in a 0.2% $HgCl_2$ solution into which 2-3 drops of surfactant (Tween-20) were added. The explants were shaken throughout and washed 6-7 times with sterilized distilled water, after the $HgCl_2$ treatment. Culture media, glassware and instruments were sterilized in an autoclave at 121°C under 1.1 kg m^{-2} pressure for 20 min.

Culture procedure and culture conditions

Remaining petioles were further trimmed and two 1 cm pieces from either sides of the nodes were removed. Explants were then cut into two, pieces of 4 cm length and placed horizontally on the solid slant medium in tubes. When the explant was an apical shoot, 1 cm long apex was removed.

Cultures were incubated horizontally in the growth room maintained at 26$\pm$2 °C under 12 h photoperiod at irradiance of about 100 µ mol m^{-2} s^{-1} supplied by cool white fluorescent tubes.

Assessment of growth

Number of axillary buds produced and their length were the main criteria used to compare the response of different types of explants. Leaf growth, root formation etc. were also recorded for each culture over a 10 weeks period. Experiment was repeated three times and replicated 12-15 times on each occation. Mean values and standard errors were calculated for comparison of treatments.

RESULTS

Growth of plants pollarded at different heights

The survival was significantly affected by the pollarding height in plants of juvenile origin. Three plants out of eight died without producing any shoots. Plants cut at 60 cm produced 1.4 shoots per plant and those cut at 120 cm produced 2 shoots per plant. Contrary to this, clonal plants cut at 30 cm height yielded 5-6 shoots per plant while those cut at 180 cm produced only 3-4 shoots per plant.

The length of axillary shoots

Mean axillary shoot length of *in vitro* plantlets produced by explants harvested from juvenile (seedling) plants pollarded at different heights are shown in Fig.1. Since the position of the explant on the shoot is also important for the growth of the axillary buds, the results for the first and the second nodal explants are given separately in Fig 1.

The pollarded height affected the *in vitro* growth of axillary buds. However, differences also exist between *in vitro* response of the first node and the second node suggesting an effect of position of the node as well. When the explant is the first node, axillary shoots produced by plants cut at 120 cm are significantly shorter than those harvested from trees pollarded at 10 and 60 cm heights (Fig 1a). However, with second nodal explants, the pollarding height did not show any significant effect and explants from all three pollarding heights produced 35-40 mm long axillary shoots within a period of 10 weeks.

When the leaf growth of the axillary shoots produced *in vitro* was compared, the differences

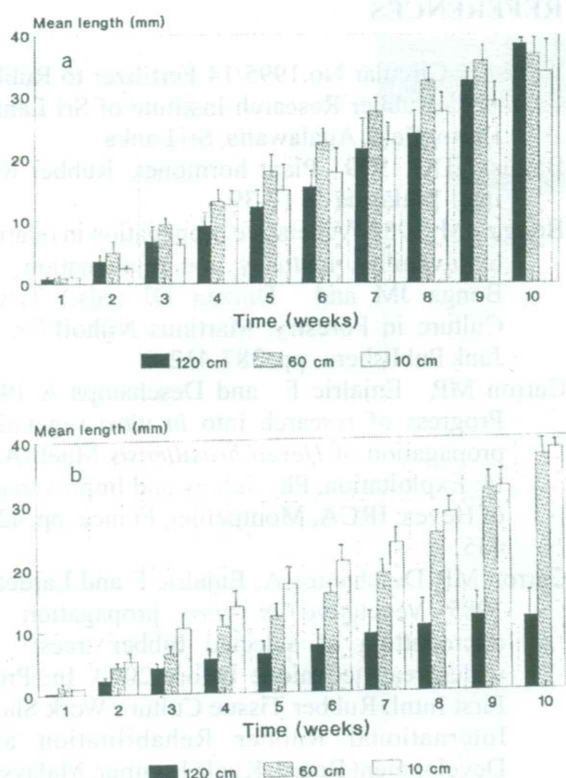

Fig. 1 The mean length of axillary shoots of the first (a) & second (b) nodal explants.

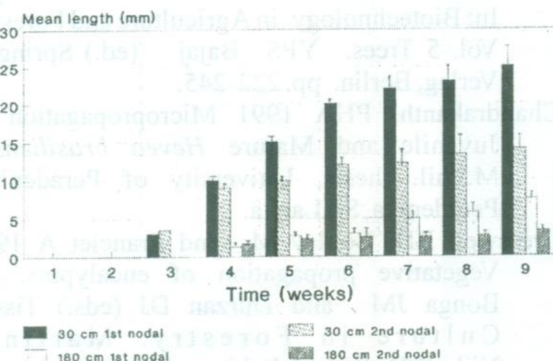

Fig. 2. Mean length of axillary shoots of first and the second node harvested from source bush plants pollarded at 30 cm and 180 cm.

were more marked between the first nodal explants and second nodal explants than those between the explants harvested from stock plants pollarded at different heights. Growth (number of leaves, shape and the colour) of the axillary shoots originated from the first nodal explants was better. Also, those produced from the nodes harvested from plants cut at 10 cm were better.

In vitro growth of axillary shoots from explants of clonal origin is shown in Fig 2.

Explants harvested from trees pollarded at 30 cm produced significantly longer axillary shoots compared with the explants harvested from trees cut at 180 cm. Both the first and the second node explants show a similar pattern, although there are differences between the heights of the axillary shoots. First nodal explants produced longer axillary shoots.

DISCUSSION

Problems associated with the phase change in micropropagation systems have often been reported in woody perennial trees (Bonga 1982; Cresswell *et al.* 1982; Howard *et al.* 1989). Rubber being a woody perennial, most of the problems associated with its micropropagation are common to those reported for other perennials. Extremely slow growth or the poor response to *in vitro* culture are the main problems which need to be solved in the micropropagation of clonal materials of *Hevea.*

The effect of the nodal position on the shoot in *Hevea in vitro* culture has also been studied earlier (Seneviratna 1991; Ekanayake 1994; Seneviratne *et al.* 1996). The behavior of the first and the second nodal explants of juvenile origin materials is different. This difference can partly be due to the nature of seedlings originated from heterozygous seeds which show a large variation among individuals. However, as far as the growth phase is concerned, these plants are in the juvenile phase, since they originated from seeds and also the plants are less than five years of age. Therefore, the pollarding height from where the shoots originated should not have any significant effect as far as the juvenility is concerned.

When the seedling plants were cut close to the root collar i.e. 10 cm above, some of them died without producing any shoots. One reason for this is the long inter- nodal distance which is characteristic of seedlings in the juvenile phase. Accordingly, the seedling stem below 10 cm may contain only a few dormant axillary buds to sprout when pollarded at 10 cm and the number of axillary shoots produced on plants cut at 10 cm is low when compared to those cut at 60 or 120 cm from the root collar.

With clonal materials, the number of shoots produced on plants cut at 30 cm was higher and also the shoots produced on these explants contained better foliage than the shoots produced on plants pollarded at 180 cm. The results obtained for clonal materials show some indication of variation in the juvenility in the different regions of the tree. The source bush trees used for the present study were about 12 years old and were propagated by bud grafting and thus in the mature phase of growth.

As it has been described by Bonga (1982), there

can be juvenile zones within mature trees. Also, there is a gradient in the degree of juvenility along the trunk. This degree of juvenility of an apical meristem is inversely proportional to the distance (along trunk and branches) between the base of the plant and the meristem. Accordingly the shoots originated close to the base of the plant should be more juvenile than those originated further away from the base.

Axillary shoots from nodes harvested from clonal trees pollarded at 30 cm showed some similarity to those harvested from seedling plants. This is also an indication of juvenility in the clonal explants when harvested close to the roots.

Chandrakanthi (1991) also reported better axillary shoot development when explants were harvested close to the root system of mature rubber trees. The trees used by Chandrakanthi (1991) may be more mature than those used in the present study. In her study, large trees were cut at about 30 cm. Normally, the number of shoots produced on large trees cut at the base is low. This may be due to the lack of dormant buds which are active enough for emergence and growth. The source bush trees used in the present study were about 12 years old, but they have been pollarded several times during this period. Generally, pollarding induces juvenility in plants (Hackett 1985). However, Chandrakanthi (1991) reported of difficulties in culture establishment of these explants due to high content of phenolics and higher rate of contaminations though there had been no control trees. In the present study also, the degree of contamination and phenolic browning remained high though the trees were covered with polythene film just after they were pruned and the resulting foliage was regularly sprayed with fungicides.

The main objective of applying micro-propagation techniques for *Hevea* is mass propagation of elite clonal materials, as the conventional method of bud grafting is only a partial vegetative propagation method. However, the results published so far indicate that response of clonal materials is very low, mainly due to the factors related to the growth phase. The results of the present study give some positive implication that pruning at the base of mature *Hevea* trees close to the root system will help in restoring juvenile characteristics, as evident from the increased length of the axillary shoots. Further studies on this aspect would be useful for the development of a successful micro-propagation technique for *Hevea*.

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