Journal of the Rubber Research Institute of Sri Lanka, (1996) **78**, 69-78

THE POSITIONAL EFFECT OF THE EXPLANT ON *IN VITRO* GROWTH OF AXILLARY BUDS OF *HEVEA BRASILIENSIS*

Priyani Seneviratne, A W Flegmann and G A S Wijesekera

(Accepted 7 February 1996)

ABSTRACT

Apart from the high apical dominance showed in shoot explants of Hevea, the location of the node in the shoot, seems to play a vital role in in in vitro axillary bud proliferation. Better performance was observed when the axillary buds harvested as nodes, were not too close and also not too far from the apex as measured by the primary axillary shoot growth. This was true for both juvenile and mature origin materials. Furthermore, "active" nodes were superior to "dormant" nodes.

Key words: axillary buds, *Hevea*, *in vitro*, positional effect

INTRODUCTION

Clonal propagation of *Hevea* by tissue culture techniques can serve the rubber industry to a greater extent, if a reliable technique is developed for selected varieties, as the conventional method of propagation which is by bud grafting is not producing true-to-type plants. The yield of each tree in a plantation is becoming an important parameter in order to use the land efficiently, to increase the tapper productivity and also to realize the full capacity of the improved cultivars. Combe (1975) estimated the variation in eight year old trees of clones RRIM 607, PB 86 and PR 107 and reported that 25%, 15% and 10% of the trees respectively, never reached the tappable girth. Also the yield during the first two years of tapping showed that, 50% of the total yield was harvested from 34%, 36% and 39% respectively, from the trees of clones RRIM 607, PB 86 & PR 107.

Micropropagation via axillary shoot proliferation has been demonstrated by many workers with varying degrees of success.

Production of 30 $\pm$ 2 shoots per explant after 165 days of incubation has been reported for seedlings by Gunatilake & Samaranayake (1988). Carron *et al* (1989) reported of monthly multiplication coefficient of 2-3 shoots for juvenile explants. Multiplication rate of 4-5 propagules per explant has been reported by Chandrakanthi (1991). Seneviratne (1991) reported of shoot doubling time of 28 days for juvenile origin materials. Reports are available on plantlet production of clonal materials with no indication on shoot proliferation rate (Asokan *et al.*, 1988; Carron *et al.*, 1989).

The present report discusses the differences in behaviour of the nodes harvested from different portions of the shoot to emphasize the fact that the endogenous content of compounds

are as important as the other factors such as the physiological growth state, culture medium *etc.* at least in the initial stages of the growth.

MATERIALS AND METHODS

Both juvenile and mature origin plants were used as explants. Juvenile materials were those originated from seeds and mature materials were clonal materials grafted onto seedling plants. Budded plants of clones PB 86, RRIC 100, RRIC 110, RRIC 117 & RRIC 121 were used. The age of the two types of plants were the same, in fact juvenile materials were harvested from plants where the budgrafting had been unsuccessful. All were grown in black polythene bags in the glass house.

Terminal shoots of all plants were cut at the second whorl of leaves in order to enhance the development of lateral shoots and explants were harvested from these shoots.

Murashige and Skoog medium supplied in packets by flow laboratories was used as the basic medium. Hormones used were from Sigma Chemicals, USA. All other chemicals including agar, sucrose, vitamins etc. were of analytical grade supplied by BDH chemicals, England.

Four combinations of hormones were used in this study namely S-0, S-1, S-2, S-3, S-0 being the control containing no hormones. Hormone composition of each medium is given below.

S-0 - no hormones (control medium)
S-1 - kinetin 2 ppm + BAP 1 ppm + NAA 0.2 ppm
S-2 - Kinetin 7.5 ppm + BAP 3.75 pm + NAA 0.2 ppm
S-3 - Kinetin 10 ppm + BAP 5 pm + NAA 0.2 ppm

Agar was used at 0.6% and sucrose at 4% unless otherwise stated. Solid media prepared in 9 cm diameter glass petri dishes were used. Explants were sterilized with 0.2% $Hgcl_2$ for 10 min with a prewash in 70% EtOH for 1 min. Media, glassware and instruments were sterilized in an autoclave at 121 °C under 15 lb/inch^2 for 20 min.

All cultures were incubated at 26±2 °C at irradiance of about 100 $\mu E/S^{-1}/m^{-2}$ for 12 hour photoperiod in the growth room.

Observations on axillary bud break and their lengths were recorded every four week intervals. SEM values were calculated to compare the treatment means. Number of replicates for each experiment is given with the results.

RESULTS

The positional effect of juvenile origin materials

In this experiment, shoots of about 15-20 cm length were removed from stock plants. They were sterilized before they were cut in to pieces, numbered from 0-7, starting from the top, the top part being the shoot tip numbered as 0 (Fig. 1).

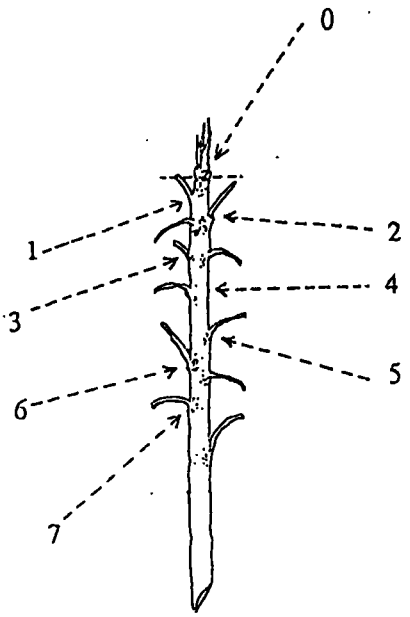

Fig. 1. Explants numbered according to their position.

Node size varied from 2-5 cm and there were 15 replicates. Nodes were cultured in hormone free medium. Mean lengths of the primary axillary shoots after culture are given in Fig. 2.

As expected, non of the axillary buds on shoot tips grew beyond 2-3 mm. The mean length of the axillary shoots increased from the shoot tip up to the node number 5 and then decreased from five to seven. After 16 weeks of culture, axillary shoots were divided into nodes and cultured onto S-1 medium. This medium contained kinetin 2 ppm, BAP 1 ppm and NAA 0.2 ppm. This medium had proved to give good axillary shoot growth in later passages of the previous experiments which are not reported here. The number of propagules produced by each group of nodes at 16 weeks is shown in Fig. 3.

As can be seen from Fig. 3, the number of propagules produced per explant has increased from the shoot tip up to the node number 5 and then decreased. In fact, this was directly related to the length of primary axillary shoot. The growth of the secondary axillary shoots was rather slow and for another 12 weeks no relationship of the growth and their original node number was observed.

This experiment was repeated twice and similar results were obtained with regard to the length of primary axillary shoots. In one of these two trials, the nodes harvested from *in vitro* grown primary axillary shoots were transferred on to a medium containing thidiazuron at 0.002 ppm. They produced multiple axillary buds but the elongation was not satisfactory.

Effect of the explant on *in vitro* growth

The effect of "active" and "dormant" nodes

The main difference between the two types of nodes was that "active" nodes contained a leaf attached to them while the "dormant" nodes contained only a scale leaf and a leaf scar. Mature origin materials of mixed clones were used.

Four media were used *i.e.* S-0, S-1, S-2 & S-3 compositions of which are given under materials and methods. Percentage axillary bud break and the mean length after 16 weeks are shown in figures 4 & 5.

The percentage axillary bud break of the two types of nodes shows that the active nodes are better explants. Up to 8 weeks, active nodes on S-0 & S-1 media gave 90% axillary bud break but at the end of 12 weeks all four media showed 100% axillary bud break. With dormant nodes, the maximum percentage axillary bud break observed was 60%, in the highest cytokinin containing medium. The maximum bud length observed with dormant nodes was 3 mm. But, with active nodes, maximum length of about 17 mm was obtained. The length increased with the amount of exogenous hormones but decreased at the highest level. No further growth was observed with dormant nodes and they turned brown after 8-12 weeks of culture. No leaf growth was observed on the axillary buds of dormant nodes, in fact, they were very small and had no shoot elongation. With active nodes, the axillary shoots produced on S-1, S-2 & S-3 media had leaf growth but leaves were absent on those produced on S-0 media.

Similar experiments were done with juvenile origin materials also. The results obtained were also similar to that of mature materials.

The positional effect of mature origin material

This was tested in two steps. In the first one, the shoots were cut into 4 cm size pieces, starting from the top, the first 4 cm being the shoot tip labelled as 0 (Fig.6). In this method, some nodes contained only one axillary bud, but most of the nodes contained more than one. The maximum number of explants per shoot was about five and they were cultured onto hormone free medium.

The mean length of axillary shoots of each group of nodes up to 16 weeks of culture are shown in Fig.7.

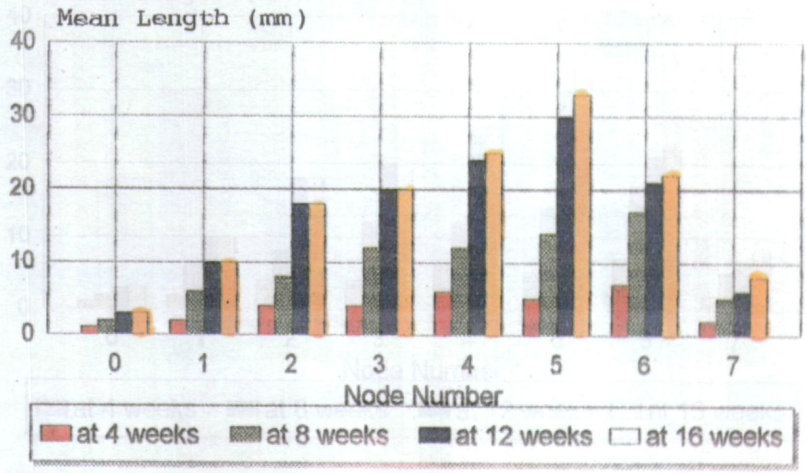

Fig. 2. Mean length of axillary shoots of nodal explants numbered from 0-7 (n=15, bars shaw SEM).

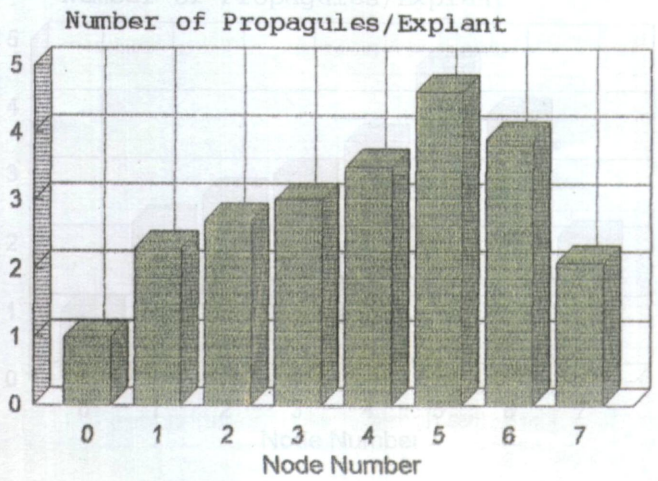

Fig.3. Number of propagules produced by each group of nodes after 16 weeks of culture. (n=15, bars shaw SEM).

Effect of the explant on *in vitro* growth

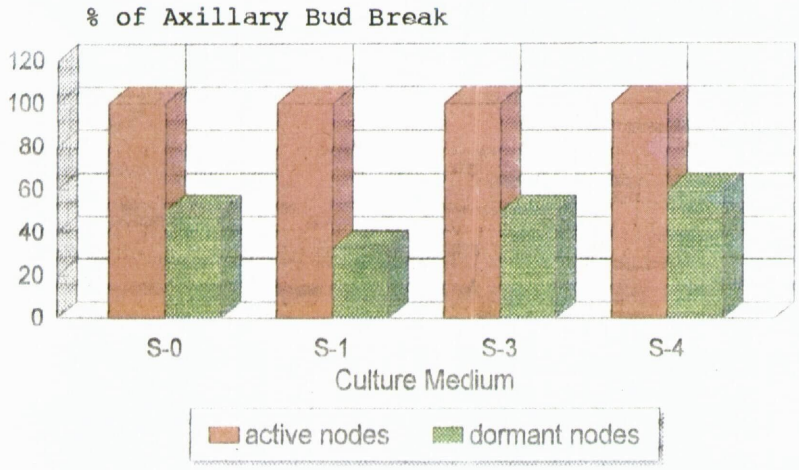

Fig.4. Percentage axillary bud break of "active" and "dormant" nodes (n=30).

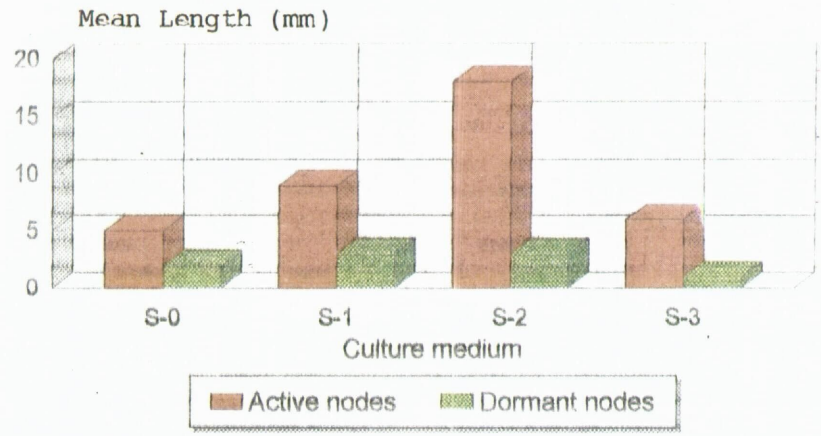

Fig.5. Mean length of axillary shoots of "active" and "dormant" nodes.

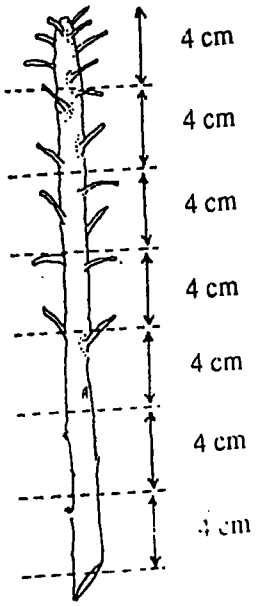

Fig.6. A shoot divided into 4 cm pieces.

As it is shown in Fig.7, the shoot tips did not show any axillary bud elongation. The nodal explants numbered as 2 were the best for axillary shoot elongation. From nodes numbered as 3, the elongation has started to decrease. Contamination rate also was high in nodes numbered as 4 & 5. In fact, most of the nodes in group 4 & 5 contained dormant buds. Leaf growth was observed in the axillary shoots produced by the nodes in group 2 & 3. Slight elongation & leaf expansion was observed in shoot tips.

For the second part, each axil was given a number as in the experiment with juvenile materials. As the internodal length are normally low in mature origin shoots, the nodes were small and sizes varied from 0.5 cm to 2 cm. Only active nodes were used in this experiment. The top 5 mm was separated as the shoot tip and numbered as 0. Nodes were cultured onto hormone free medium. The mean length of axillary shoot up to 16 weeks of culture are shown in Fig.8.

The length of axillary shoots increased up to the forth node and then decreased. In general, leaf growth was seen in axillary shoots which were more than 10 mm long. The elongated axillary shoots were cut into nodes and cultured in a medium containing kinetin 7.5 ppm, BAP 3.75 ppm and NAA 0.2 ppm. This combination of hormones was found to be better for axillary bud expansion of mature materials of *Hevea*.

These newly formed nodes carried at least 5-6 axillary buds in 1 cm size piece, as the internodal length of these *in vitro* grown primary axillary shoots was even lower. However, almost all axillary buds expanded but remained less than 4-5 mm in length.

Effect of the explant on *in vitro* growth

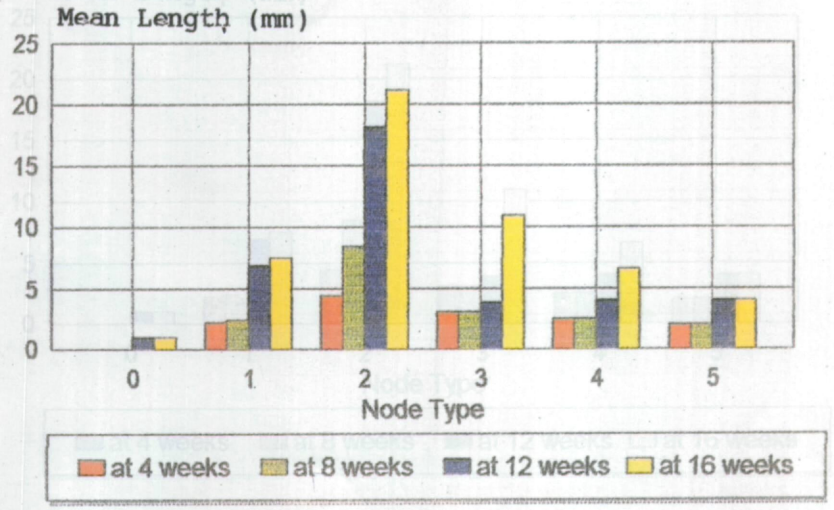

Fig.7. Mean length of axillary shoots of nodal explants labelled from 0-5 (n=15, bars show SEM).

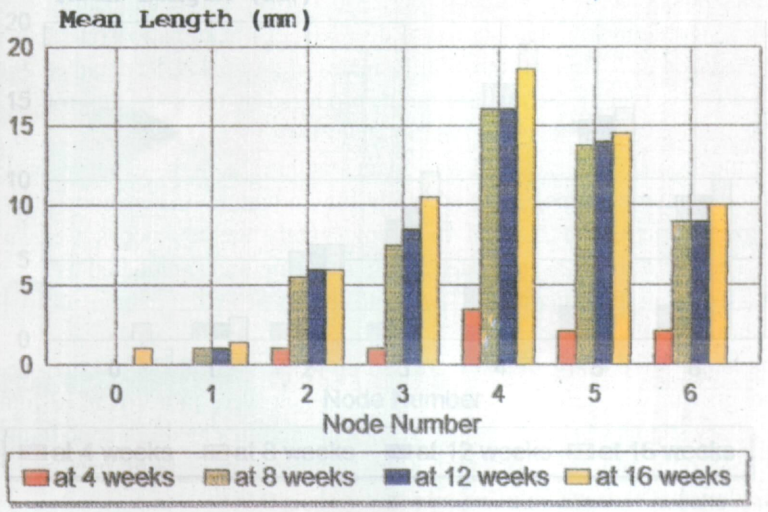

Fig.8. Mean length of axillary shoots produced by different nodes (n=12, bars show SEM)

DISCUSSION

The superior performance of nodal explants over shoot tip explants has not been reported by other workers, but it is evident since successful and sustainable proliferation methods have been reported only for nodal explants. Plantlets have been produced by shoot tips also, but with no proliferation. This behaviour of shoot tips is perhaps related to the high apical dominance seen in *Hevea*, irrespective of their origin i.e. juvenile or mature (Seneviratne & Wijesekara, 1994).

Also it has been reported that the role of exogenous hormones in the primary axillary shoot growth is relatively little in juvenile origin explants. Even for mature origin explants, axillary bud break is not dependent on the amount of exogenous hormones but the elongation improves when hormones are supplied in the medium (Seneviratne & Wijesekara, 1994).

The differences observed with "active" and "dormant" nodes are also important. From the four combinations of hormones tested active nodes proved to be superior to dormant nodes.

It is worth mentioning that for normal budgrafting purposes of *Hevea* only scale buds are used. Axillary buds are used if only budwood is in shortage. However, it has been reported by Seneviratne *et al* (1994) that the sprouting was earlier when axillary buds were used for grafting young plants.

Similar results have been reported by Samaranayake & Gunaratne (1977) on brown buddings. Furthermore, it has been reported that 30% and 10% of dormant and active buds failed to emerge at all suggesting that active buds are better for budgrafting also.

Although *Hevea* shoot tips were inferior to nodes in the axillary bud proliferation, it has been reported by Rahaman & Blake (1988) that shoot tips of jackfruit were superior to nodes for shoot proliferation. Kito & Young (1981) also compared shoot tips & nodes of sour orange (*Carrizo citrange*) and found that shoot tips proliferated better than nodes.

This behaviour of shoot tips could be at least partially due to the strong apical dominance present in the apex. When an apex of about 10 mm size was removed from a shoot tip, the very first axillary bud of the resulting node started to grow suppressing the growth of the axillary buds below that.

As noticed in the present work, the increase in the axillary shoot length with increasing distance of the explant from the short apex could also be due to the gradual decrease of the extent of apical dominance which is present in the apex.

The *in vitro* growth can be associated with the endogenous content of plant hormones and other compounds. The decrease in the axillary shoot length after showing the maximum length could possibly be due to the dormancy with their age coupled with lack of necessary endogenous compounds which cannot be provided in the medium in correct quantities.

A similar situation has been noticed with tea by Rajasekaran & Raman (1993). They have reported that 7th & 8th axils were superior to all other axils above or below to them. High phenolic exudates in the area close to shoot tip has been an added disadvantage for tea and this is true for *Hevea* also, when the explants are of mature origin.

Effect of the explant on *in vitro* growth

It is evident that the differences in the explants due to their position in the node masks or makes less the differences between the other variables such as growth hormones. The present study concludes that the positional effect should always be taken into consideration when the effect of other factors are looked into.

REFERENCES

Asokan M P, Sobhana, P, Sushama Kuari and Sethuraj, M R (1988). Tissue culture propagation of rubber (*Hevea brasiliensis* wild Ex ADR De Jusse Muell. Arg.). Clone GT 1 (Goudang Tupen -1). *Indian Journal of Natural Rubber Research* **1**, 10-12.

Carron, M P, Enjalric, F, Lardet, L, and Deschamps, A (1989). Rubber (*Hevea brasiliensis* Mull. Arg.) In: *Biotechnology in Agriculture & Forestry, Vol.5, Trees 11*, pp. 222-245 (Ed. Y P S Bajaj), Springer-Verlag Berling.

Chandrakanthi (1991). Micropropagation of juvenile and mature *Hevea brasiliensis. Msc Thesis*, University of Peradeniya, Sri Lanka.

Combe, J C (1975). Mise en evidence de la variabilite intraclonale sur jeunes greffes. (Demonstration of intraclonal variability in young Grafted trees) *Reveu General Caoutchous et Plastiques* **52**, 91-94.

Gunatilake, I D and Samaranayake, C (1988). Shoot tip culture as a method of micropropagation of *Hevea. Journal of the Rubber Research Institute of Sri Lanka* **68**, 33-44.

Kito, S L and Young M J (1981). *In vitro* propagation of *Carrizo citrange. Hort Science* **16**, 305-306.

Rahaman, M A and Blake, J (1988). Factors affecting *in vitro* Proliferation and rooting shoots of Jackfruit (*Artocarpus heterophyllus lam*). *Plant Cell Tissue & Organ Culture* **13**, 179-187.

Rajasekaran, P and Raman, K (1993). Propagation of tea by in vitro culture of nodal explants. *Journal of Plantation Crops* **21**, 8-14.

Samaranayake, C, Gunaratne, R B (1977). The use of "Leaf buds" and "scale buds" in the vegetative propagation of *Hevea. Journal of the Rubber Research Institute of Sri Lanka* **54**, 65-69.

Seneviratne, P (1991). Micropropagation of juvenile and mature *Hevea brasiliensis. PhD Thesis*, University of Bath, England.

Seneviratne, P, Nugawela, A and Samarakoon, S M A (1994). Factors affecting the budgrafting success and the scion growth of young budding of *Hevea. Journal of the Rubber Research Institute of Sri Lanka* **74**, 24-41

Seneviratne, P and Wijesekara, G A S (1994). The growth phase and its effect on *in vitro* culture of *Hevea brasiliensis. Journal of the National Science Council of Sri Lanka* **22**(4), 313-324

(Received 15 November 1994)