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ACCLIMATIZATION OF MICROPROPAGATED PLANTS OF HEVEA

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ABSTRACT

In vitro produced *Hevea* shoots were successfully acclimatized to outdoor conditions by controlling the ambient conditions, mainly the humidity. Shoots, longer than 5 cm and with some leaves attached performed better. Shoots without roots produced roots soon after their transfer to soil and the root development and the acclimatization success was better than in the rooted shoots. Use of a concentrated auxin solution, to soak the bases of the shoots or to incorporate into soil improved root induction. The growth of the aerial part and the root system of the micropropagated plants were comparable to those of embryo cultured plants, up to one year of growth, but the field performance is yet to be monitored.

Key words: Micropropagation, Acclimatization, *Hevea, in vitro*

INTRODUCTION

Although *Hevea* shoots do not produce roots easily under *in vivo* conditions, rooting was rather easy on shoots of micropropagated plants. As the *in vitro* shoot multiplication is easier with juvenile origin materials, reports are available on rooting and acclimatization of juvenile origin plants (Carron *et al*, 1984; Carron *et al*,1985). When the technique of micropropagation was first established, even for juvenile origin materials, rooting was done in liquid media under *in vitro* condition and acclimatization needed about 2 months under controlled humidity and temperature (Carron *et al,* 1984). But later, higher success rates and less time for this process was reported mainly by inducing roots under *in vivo* conditions with the use of rooting powders (Carron *et al*, 1985 and 1989).

Moreover, root induction on shoots of clone GT 1 on media containing 1.5 – 3 ppm IBA and 0.5 – 1.5 ppm kinetin followed by hardening period of 3–4 weeks under controlled humidity and temperature has been reported (Asokan, 1988).

The primary aim of this work was, acclimatization of plantlets while monitoring the behaviour of the root system produced on the shoots, as the micropropagated plants produced by axillary shoot multiplication too, do not produce tap roots.

MATERIALS AND METHODS

Plant Material

The shoot material used for rooting and acclimatizing were of juvenile origin and were obtained from either micropropagation experiments or from embryo cultured plants.

Media Preparation and Culture Incubation

Murashige and Skoog (1962) medium in packets supplied by Flow Laboratories, U.K. was used as the basic medium. Activated Charcoal, Sucrose and agar were supplied by BDH Chemicals, UK and IBA was from Sigma Chemicals, USA.

All media were prepared in boiling tubes. A fluted filter paper was inserted prior to autoclaving at 121 °C and 15 lb/inch^2 for 20 minutes when using liquid media. Cultures were incubated at 25 ± 2 °C with 16 hour photoperiod.

Acclimatization

For the acclimatization of plantlets, propagators with vents on the lid supplied by BDH were used. Ordinary top soil was used as the potting mixture. Humidity was controlled first by adjusting the vents of the propagator lid and then by removing the lid for increasing time periods. Light intensity was controlled by keeping the plants inside the Laboratory, inside the glass house and then out door in succession.

Statistics

Kruskal–Wallis Test (Chisquare approximation) was used to find differences between media, and each treatment combination was compared by the Wilcoxon Rank Sums test.

RESULTS

1. The effect of the medium composition on rooting.

Five media, labelled R_1 to R_5, were tested; their compositions were as follows;

R_1 – 1/2 MS + 1BA 2ppm, solid
R_2 – 1/2 MS + 1BA 2ppm, liquid
R_3 – 1/2 MS + 1BA 2ppm + 0.5% charcoal, solid
R_4 – 1/2 MS + 1BA 2ppm + 0.5% charcoal, liquid
R_5 – 1/2 MS + solid (no hormones)

Data on shoot and root growth and quality of roots, collected 4 weeks after culture are given in Table 1.

Table 1. *The effect of the medium on root induction of shoots (n = 10).*

Medium	R 1	R 2	R 3	R 4	R 5
% rooting	100	30	40	10	0
Mean no. of roots	3.9±0.8	7.0±0.6	1.5±0.3	1	0
Mean length of roots(cm)	1.8	1.3	1	1	0
Presence of laterals	80%	0%	0%	0%	0%
Root quality	good	poor	poor	poor	–
Apical growth	v.good	good	good	good	poor

The percentage rooting were, 100%, 30%, 40%, 10% and 0% in the media R_1, R_2, R_3, R_4, R_5 respectively though the number of roots and their lengths were different in different media. However, it was found that there were significant differences in growth when grown in different media (X^2=25.68, P=0.0001). It was evident that the growth of cultures in medium R_1 was different from that in media R_3, R_4 and R_5 ($p< 0.05$).

At the end of 4 weeks, all non rooted cultures from media R_2, R_3 and half of the non rooted cultures from each media R_4 and R_5 were transferred to R_1 medium. The remaining two halves from media R_4 and R_5 were transferred to R_2 medium.

Results observed at 4 weeks and 8 weeks on new media are given in Table 2.

Table 2. *Root formation on shoots in R_1 and R_2 media.*

Original Medium	R 1	R 2	R 3	R 4		R 5	
New medium	R 1	R 1	R 1	R 1	R 2	R 1	R 2
After 4 weeks							
% rooing	100	60	100	80	100	0	0
Mean length(cm)	6.1	6.7	3.1	5.7	4.3	–	–
% Lateral roots	80	0	40	60	50	–	–
After 8 weeks							
% rooting	100	80	100	100	100	–	–
% Lateral roots	77	25	43	80	50	–	–
Shoot elongation	100	90	85	100	100	70	70

Roots produced on R_1 medium during the first 4 weeks were better than those produced in all other media. However, the shoots cultured on other media also produced roots on their transfer on to R_1 or R_2 medium. Nevertheless, as far as the root quality, *ie*, presence of lateral roots, root length and the apical growth, is concerned, R_1 medium was superior to all other media.

2. The effect of the size of the explant on rooting.

Three sizes of shoots, 3 cm, 5 cm and 7 cm were tested in this experiment. R_1 medium was used in test tubes and there were 10 replicates from each shoot size. The percentage rooting of the 3 shoot sizes are shown in Figure 1.

Results up to 3 weeks showed that the percentage rooting of 3 cm long shoots were lower than other two sizes. But from the statistical analysis done (Wilcoxon Rank Sum Test) there is no evidence for significant difference in rooting among the three sizes of shoots (X_R = 0.089, Prob Chisq = 0.965).

At the end of 3 weeks all shoots (rooted and non rooted) were transferred to soil. At this stage, all shoots carried leaves on them and there was no observed difference in leaf growth among the 3 sizes of shoots. But the number of roots and their lengths were different in three sizes of shoots. The mean number of roots/shoot were 3.5, 2.6 and 2.8 and the mean lengths of the roots were 1.9, 2.6 and 2.6 cm for 3,5 and 7 cm shoots respectively.

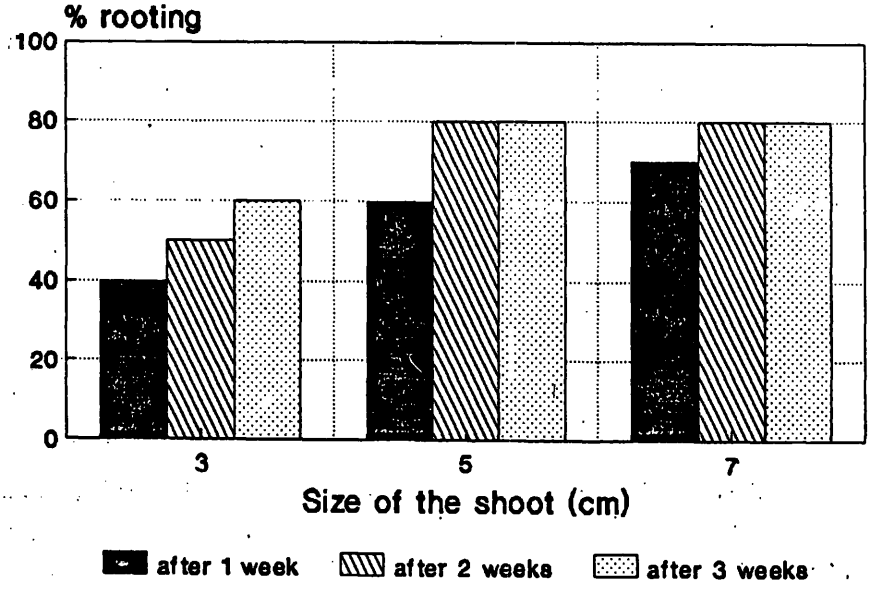

Figure 1. Root formation on shoots of three different sizes.

After 8 weeks, plants were pulled out carefully and soil was washed off to see the root growth. As far as the root length and the morphology was concerned, there was no difference of the root systems of three sizes of shoots. All roots were positively geotropic and contained lateral roots. Apical growth too was observed at this stage and mean heights of 4.5, 7.3 and 8.7 cm were observed for 3 cm, 5 cm and 7 cm shoots. Plantlets were transferred to small polythene bags individually, and kept in the laboratory.

Root growth was monitored again after 12 weeks but only 3 plants from each size were used for this and no difference was observed. But however, the root growth was satisfactory in all plants. The mean heights of the shoots, started at different heights, are shown in Figure 2.

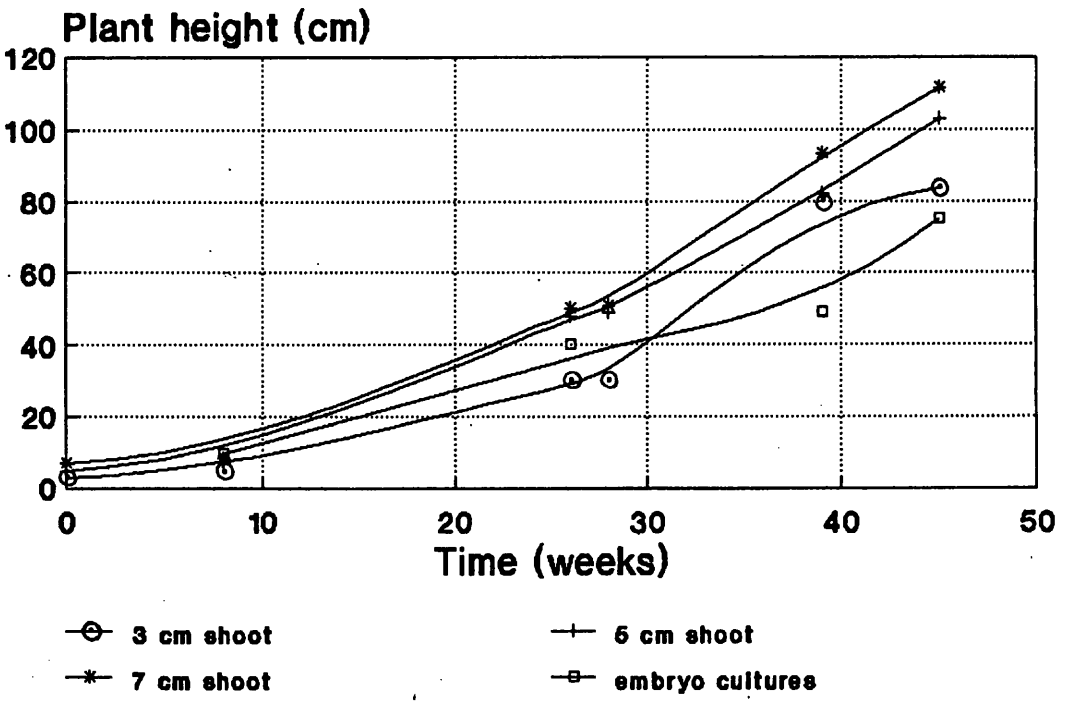

Figure 2. The shoot growth of the plants started at three sizes.

In a separate trial, the growth of the micropropagated plants was compared with that of embryo cultured plants. Acclimatized shoots (from micropropagation experiments) with no roots on them were soaked in a solution of 100 ppm IBA for 1 hour prior to transferring to soil (Plate 1). After 4 weeks in soil, they all produced good roots. The embryo cultured plants which contained a main tap root at the time of transferring to soil too had lateral roots, but clearly, the number of roots and the lengths were higher with micropropagated shoots.

Plate 2 shows the root growth of micropropagated plants and embryo cultured plants, after about 12 weeks in soil.

The growth of the plants in polythene bags after 10 months is shown in Plate 3.

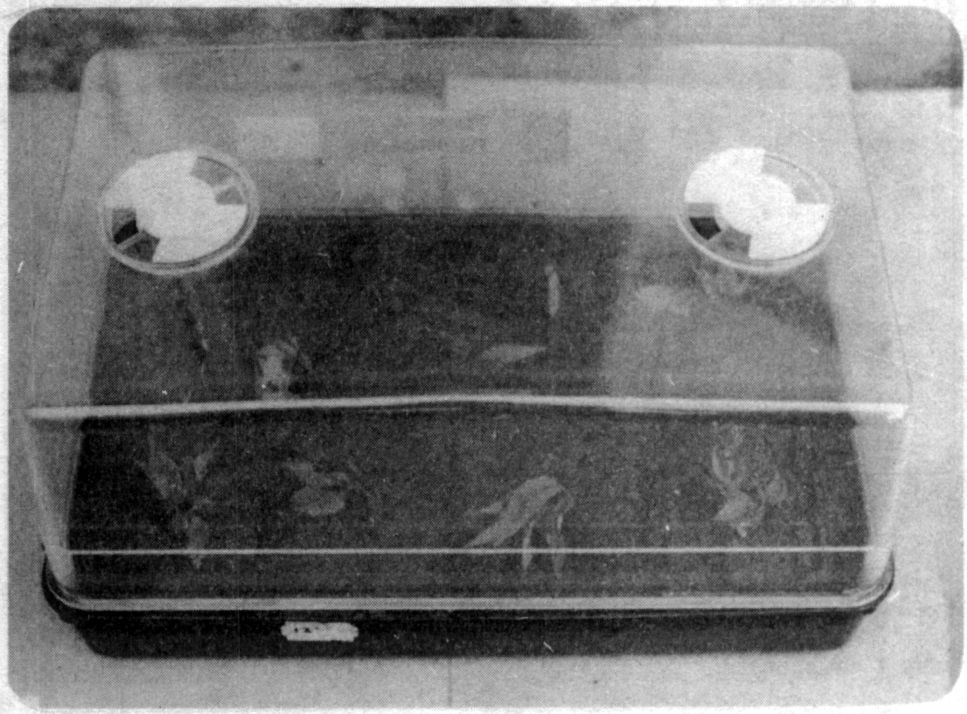

Plate 1. Rooting and acclimatization of shoots in the propagator.

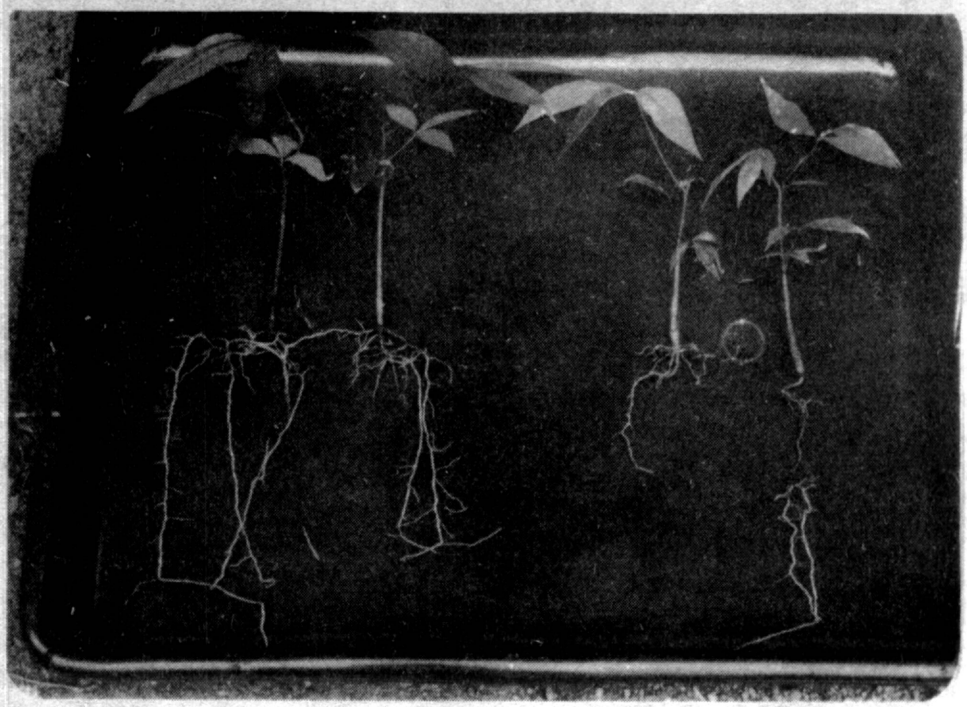

Plate 2. Root growth of micropropagated (left) and embryo cultured (right) plants. (Note the root collar and the main root in embryo cultured plants).

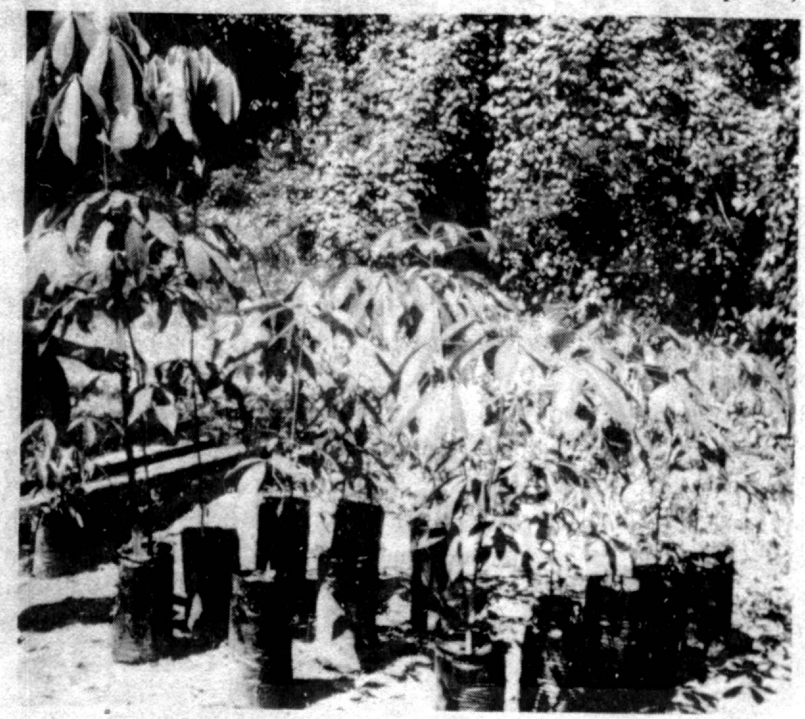

Plate 3. The growth of embryo cultured and micropropagated plants. (The five plants in the right hand side row are embryo cultured plants)

DISCUSSION

Acclimatization of micropropagated plants and their growth afterwards is satisfactory and comparable to those of embryo cultured plants.

The height of the shoot at acclimatizing, seems to have only a little effect on the growth of the plants as the aerial part starts increasing its height once the plants are established in soil. But, generally when the shoots are very small, the root induction is low and the casualties are high at acclimatizing. Therefore, 3 cm may be too small but 5 cm is as good as 7 cm, though the differences are not statistically different.

Generally, under *in vitro* conditions, absence of exogenous cytokining it-self, is sufficient for *Hevea* shoots to produce roots. Anyhow, presence of leaves on the shoot seems important for this. The 2 ppm IBA level was chosen on the experience gained over the years and good results were obtained. Roots were produced on other media also, but 2 ppm IBA alone seems ideal for root growth. Though the percentage rooting on solid and liquid media (with 2 ppm IBA) were 100% and 30% when the results were analysed by using of Wilcoxon Rank Sum Test, the mean scores for the solid and liquid media were 41.90 and 25.95 respectively, indicating no significant difference between the two media. Therefore, it seems that the physical state of the medium, either solid or liquid, affects root quality rather than root induction, because, the mean number of roots per rooted shoot was 3.9±.8 and 7±.6 in R_1 and R_2 media respectively.

The low percentage of rooting in the presence of activated charcoal can be attributed to the adsorption of IBA by charcoal and thereby IBA not been available in the medium for the shoot to absorb. Again, though statistically insignificant, the percentage rooting was different, when the physical state of the medium was different.

In this experiment, no root formation was observed when hormones were not supplied exogenously, indicating insufficient production of auxins by the shoot during the period monitored.

In the course of experiments carried out, it was evident that the shoots without roots are better for acclimatization and establishment in soil, than the rooted shoots (Data not published here). This is in agreement with the findings of Carron *et al* (1985 and 1989) where higher establishment was observed when the bases of non rooted shoots were dipped in rooting powder and acclimatized. In the present study, the shoots were dipped in 100 ppm solution of IBA for 1 hour before transferring them to soil and 100% rooting was observed.

One reason for the better establishment of non rooted shoots could be due to the fact that, only the shoot needs acclimatizing, whereas in the rooted shoots, both the shoot and the root require adjusting to the new environment. Another reason could be that there is a chance of root decay caused by soil born microbes as roots developed *in vitro* are more tender and the soil is unsterilized.

Washing the bases of the plants thoroughly under a running tap, prior to transferring them to soil is very important as any residual agar can encourage microbes growing on them.

Covering the plants with a polythene bag was also effective to maintain the high humidity but, use of proprietary propagators made control of humidity easy. However, high humidity was needed only for the first few days and extended periods under high humidity encouraged fungal growth. Therefore, reducing the humidity to normal levels as soon as possible is always preferable.

Established plants were supplied regularly with ordinary rubber fertilizer but in much smaller quantities.

The root systems, though without tap roots, contained good root systems elongating downwards and up to one year of experimental period. The growth of the plants were satisfactory, roots penetrating deep into the soil were present. The growth of the plants in the field were monitored up to 1 year and is satisfactory. It is reported else where that micropropagated plants perform well in the field (Asokan, 1988; Carron *et al,* 1989).

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