

## TISSUE CULTURE PROPAGATION OF RUBBER (*HEVEA BRASILIENSIS* (WILLD. EX ADR. DE JUSS.) MUELL. ARG.) CLONE GT (GONDANG TAPEN) 1.

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An *in vitro* propagation system for clone GT 1 is reported. The optimal growth regulator range for shoot and root development was 1.5 - 3.0 mg l<sup>-1</sup> indoleacetic acid (IAA) with 0.5 - 1.5 mg l<sup>-1</sup> kinetin. Rooted plantlets were successfully transplanted in the field.

*Key words* - *Hevea brasiliensis*, Tissue culture, *In vitro* propagation.

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### INTRODUCTION

*Hevea brasiliensis* (Willd. ex ADR. de Juss.) Muell. Arg., the commercial source of natural rubber can be propagated generatively and vegetatively. Traditionally clonal materials are multiplied by budgrafting. Natural rubber producing countries have been experiencing the need, for quite some time, for a tissue culture propagation system for rubber clones. It is reasonably assumed that tissue culture derived rubber clones would have the possibility of bigger trunk-girth causing earlier tapping and that they would be devoid of the disadvantages, usually associated with the traditional propagation system, such as stock-scion interaction resulting in high coefficient of variation among trees. Paranjothy and Ghandimathi (1975 a and b) could grow shoot tips from 2-4 weeks old seedlings. They could induce rooting also among some of the seedling-derived cultures but failed to do so with clonal materials. Shoots have been regenerated from auxiliary bud explants of a few *Hevea* clones by Sinha *et*

*al.* (1985) but failed to obtain rooting. However, shoot and root development were successfully obtained from seedlings by Carron *et al.* (1988).

### MATERIALS AND METHODS

Shoot apices were excised from GT 1 clonal trees and were surface sterilized for 5 min in 70 per cent alcohol with 1.0 per cent Tween 20 followed by immersion in 1.0 per cent sodium hypochlorite for 8 min and thorough rinsing in sterile double distilled water. With the aid of a dissection microscope, 3-5 mm shoot apices were excised and placed in the culture tubes (15 x 2.5 cm) containing 10 ml of AH-I medium (medium standardised in this laboratory) per tube. The concentration of Bacto Agar (BA) was 8.0 g l<sup>-1</sup>. The pH was adjusted to 5.7 prior to autoclaving for 15 min at 1.01 kg cm<sup>-2</sup> and 121°C. Kinetin and indole acetic acid (IAA) were added in the medium at the following concentrations : 0 - 5 mg l<sup>-1</sup> IAA in combination with 0 - 5 mg l<sup>-1</sup> kinetin, both in increments of 0.5 mg l<sup>-1</sup>

(all possible combinations). The IAA plus kinetin combinations had 121 x 3 explants in 3 replications. Three tests were conducted. Cultures were maintained at 23°C ( $\pm 2$ ) for 3 months under a light regime of 16 h light (1.5 klx) using cool white fluorescent bulbs.

## RESULTS AND DISCUSSION

Explants displayed marked differences in their response to different growth regulator combinations. The first visible sign of explant enlargement was observed 3–5 days after inoculation followed by leaf and shoot elongation during subsequent weeks. Rhizogenesis was generally observed 6–8 weeks after inoculation. Tap root emerged first (Fig. 1). Secondary roots developed only 4–5 weeks after tap root emergence. Shoot development was observed in almost all cultures irrespective of the range of growth regulators, except at 0 level of kinetin. This indicates the adequacy of endogenous/exogenous growth regulators for shoot development. However, rhizogenesis was limited to specific hormonal combinations. The maximum rooting of shoots was observed within the growth regulator range of 1.5–3.0 mg l<sup>-1</sup> IAA with 0.5 – 1.5 mg l<sup>-1</sup> kinetin (Table 1). IAA in the range of 3.5 – 4.0 mg l<sup>-1</sup> displayed only a very few cultures



Fig. 1. Rhizogenesis of *Hevea* clone, GT1

having roots. But no rooting was observed beyond 4.0 mg l<sup>-1</sup> IAA level, presumably due to the inhibitory effect of higher concentrations. The absence of IAA (0 level) alone had no effect on leaf and shoot development. The absence of IAA and

Table 1. Effects of kinetin and IAA on rhizogenesis of shoots of rubber clone, GT 1.

Kinetin (mg l <sup>-1</sup> )	IAA (mg l <sup>-1</sup> )				
	0	1.5	2.0	2.5	3.0
0					
0.5	3	31 ( $\pm 26.2$ )		42 ( $\pm 28.2$ )	
1.0			52 ( $\pm 44.3$ )	71 ( $\pm 24.4$ )	68 ( $\pm 21.8$ )
1.5				42 ( $\pm 64.2$ )	

Note:— The means ( $\pm$  SD) of number of rooted shoots are from pooled data of three tests (figures were rounded off to eliminate decimals). Only means ( $\pm$  SD) above 30 are recorded in the table.

BA (0 levels) resulted in the lack of growth and eventual death of the cultures. The absence of BA (0 level) also caused similar results. The hardening process of tissue culture derived *Hevea* plant is very critical (Leconte and Carron, 1988). Humidity, temperature and composition of the transplanting medium were found very crucial to the successful hardening process. Humidity was gradually reduced and temperature increased during 3 to 4 weeks of this process. Potting mixture was composed of equal sand: soil (v/v) mixture.

Reports on *in vitro* propagation systems for tree crops are fewer than those for horticultural crops. The results of this study demonstrated that *in vitro* propagation of clone GT 1 is possible. A few other *in vitro* systems also have been developed by us for the propagation of other commercial clones (unpublished).

The successfully hardened plants were transplanted in the field (Fig. 2). The rate of survival of such plants was 93.0 per cent.



Fig. 2. Tissue culture derived plants growing in the field.

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