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PROGRESS TOWARDS THE COMMERCIAL PROPAGATION OF TEA BY TISSUE CULTURE TECHNIQUES

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A tissue culture method is described for micropropagation of tea using single node explants of field grown plants of clone TRI 2025. Pre-treatment with PVP (polyvinyl pyrolidone) reduced browning of explants and prevention of contamination was successful with 95% ethanol and mercuric chloride. Nodal explants showed best establishment and growth response on modified MS medium containing BAP 2.5 mg l^{-1} and IBA 0.1 mg l^{-1}. Shoots and nodes excised from 8-week-old established cultures showed about 2-3 fold multiplication every 6 weeks on modified MS medium containing BAP 2.5 mg l^{-1} and IBA 0.01 mg l^{-1}. From 50 established cultures 36153 shoots were obtained after 12 months. Further attempts are being made to improve the multiplication ability of the shoots of various clones and to induce root formation.

INTRODUCTION

Considerable progress has been achieved in the recent past in the micropropagation of many herbaceous plants (Longer, 1981) and fruit trees. However, similar advances have not been achieved in the propagation of woody plants of economic value. Woody plants were assumed to be intractable in culture and research workers therefore paid only scant attention to them (Abbot, 1977).

Although there have been a few reports on the regeneration of *Camellia sinensis* from cotyledon, embryo, stem and leaf callus, very little work has been done on micropropagation of tea using nodal explants and shoot tips. The possibility of regeneration of shoots from nodal explants of tea has been reported by Phukan and Mitra (1984). *In vitro* proliferation of shoots of tea has been reported by Arulpragasam and Latiff (1986).

The aim of this study was to develop a successful method for establishment of nodal explants of *Camellia sinensis in vitro* without browning and contamination and for the mass propagation of shoots through axillary branching.

MATERIALS AND METHODS

Plant material (clone TRI 2025) was collected directly from the field. Single node explants were prepared for culture by removing the leaves with the petioles. The nodal explants 2-3 cm in size, were dipped in 1.5 % PVP (polyvynyl pyrolidone) for 30 min. The initial surface sterilization was done by immersing them in 95 % ethanol for 45 seconds, followed by a second sterilization in 0.1 % mercuric chloride solution for 15 min and then rinsed 3 times in sterile distilled water. Establishment of explants *in vitro*

was done on modified MS (Murashige and Skoog, 1962) medium (Table 1) containing $^1/_2$ strength MS salts, 0.4 mg l^{-1} Thiamine HCl, 2.0 mg l^{-1} ascorbic acid, 10 mg l^{-1} myo-Inositol, 30 g l^{-1} sucrose, 0.1 mg l^{-1} IBA and 2.5 mg l^{-1} BAP. The pH was adjusted to 5.3 before autoclaving.

After 8 weeks in culture 50 established nodal explants were selected for further multiplication (Fig. 1). Shoots produced by these explants were aseptically removed, leaves trimmed and each node separated and placed on MS 2 medium (Table 2) consisting of $^1/_2$ strength MS salts, 0.4 mg l^{-1} Thiamine HCl, 2.0 mg l^{-1} ascorbic acid, 10 mg l^{-1} myo-Inositol, 30 g l^{-1} sucrose, 0.01 mg l^{-1} IBA and 2.5 mg l^{-1} BAP and the pH was adjusted to 5.3 before autoclaving. All cultures were given 2000 LUX light intensity and temperature was maintained at 24 ± 2 °C in a growth room with 16 h photoperiod.

Subsequently, for further shoot proliferation, single nodes with axillary buds without leaves were separated from solitary shoots and transferred at 6 week intervals.

TABLE 1 - *The composition of medium used for establishment of nodal explants of tea*

Component	*Concentration mg l^{-1}*	
Major elements	MS *	half strength
Minor elements	MS *	half strength
myo-Inositol	100	
Thiamine - HCl	0.4	
Ascorbic acid	2.0	
Sucrose	30 g	
IBA	0.1	
BAP	2.5	
Agar	5 g	

pH 5.3

* Murashige and Skoog (1962) mineral salts

TABLE 2 - *The composition of medium used for proliferation*

Component	*Concentration mg l^{-1}*	
Major elements	MS *	half strength
Minor elements	MS *	half strength
myo-Inositol	100	
Thiamine - HCl	0.4	
Ascorbic acid	2.0	
Sucrose	30 g	
IBA	0.01	
BAP	2.5	
Agar	5 g	

pH 5.3

* Murashige and Skoog (1962) mineral salts

RESULTS AND DISCUSSION

Pre-treatment with PVP before surface sterilization reduced the browning of explants during the preparation for culture. However, once established browning did not occur when leaves and basal ends were trimmed or nodes were separated for transfer. On the other hand, it has been observed that the presence of ascorbic acid in the medium greatly decreased oxidation of plant material in culture. Since higher salt concentration also caused browning and sometimes even killed plant tissues in previous experiments, reducing the salt concentration to half strength could also be a contributory factor in reducing the browning of tissues. Similar effect of low salt concentration in *Azaleas* was also reported by Anderson (1975).

The explants established well in modified MS medium. After 8 weeks, the first transfer was done to the same medium. Shoots with 2-4 leaves were produced from axillary buds of nodal explants after 6 weeks in culture. Well established explants in the initial medium showed rapid proliferation after the 2nd and 3rd transfer on the proliferation medium. However, if the explant bearing the original basal node was transferred to fresh medium, it also developed few additional shoots. The combination of auxins and cytokinins in the establishment medium has a vital effect on the initial establishment of the explants. The best combination was 0.1 mg l^{-1} IBA and 2.5 mg l^{-1} BAP. Calli formed at the basal end of some cultures were cut off before transference to the proliferation medium (MS 2) since the calli retarded axillary bud formation (Sarathchandra, Upali and Wijewardena, 1988). The tendency for calli formation at the basal end was reduced appreciably by decreasing the auxin concentration in MS 2. It seems that the presence of BAP in the medium and trimming of the leaf during the transfer, induced axillary bud formation and elongation; the axillary shoot, reached a maximum length of 3 cm after 6 weeks. From 50 established cultures 36153 shoots were obtained after 12 months (Fig. 2). In conclusion, *in vitro* propagation of *Camellia sinensis* is feasible through axillary bud proliferation. Further attempts are being made to improve the multiplication ability of the shoots of various clones and to induce their root formation. This work can be regarded as the first successful organized study towards the commercial micropropagation of tea using nodal explants.

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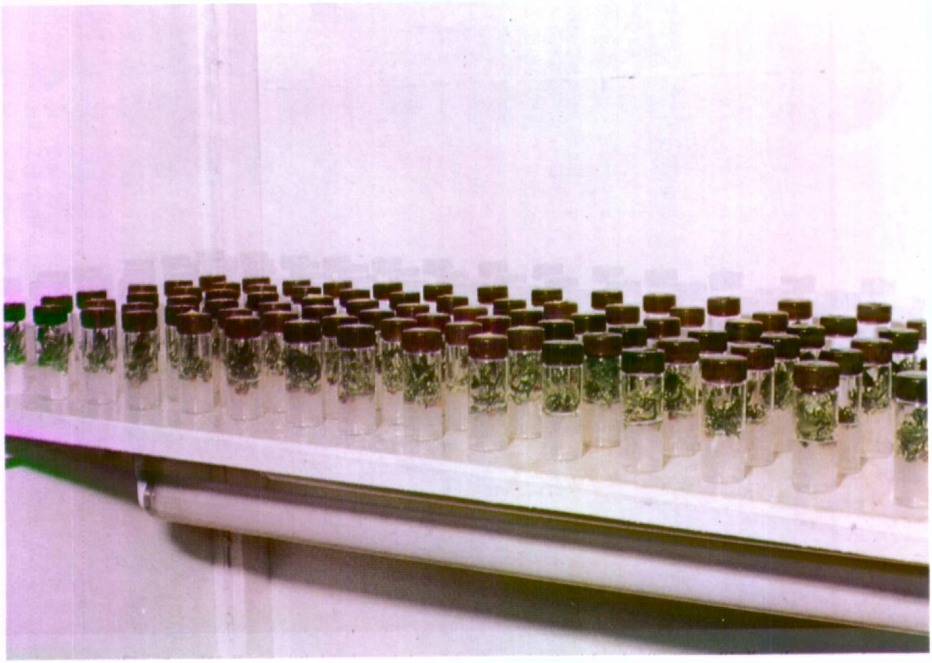

Fig. 1 – *Well established nodal explants selected for further multiplication.*

Fig. 2 – *Thousands of shoots obtained after 12 months.*

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