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STUDIES ON THE TISSUE CULTURE OF TEA (*CAMELLIA SINENSIS* (L.) O. KUNTZE)

4. Somatic embryogenesis in stem and leaf callus cultures

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Media suitable for the induction of callus on nodal explants and juvenile leaves have been investigated in this study.

Callus formed at the basal ends of nodal explants when cultured in Vacin and Went medium amended with green coconut water and in MS medium supplemented with 0.1 mg l^{-1} IBA and 1.0 mg l^{-1} BAP and on the cut margins of leaf pieces when placed in a callusing medium; the pH of all three media was 5.7. While high sucrose content enhanced the rate of multiplication of callus in nodal explants better callus formation was seen when whole leaves were employed. Though embryoid-like structures formed in nodal explants after 8 weeks this was not so with leaf callus cultures. Attempts are now being made to regenerate plants from these cultures.

INTRODUCTION

Only a few papers have been published on cotyledon and stem callus cultures of the tea plant (Wu, 1976; Wu *et al*, 1981; Kato, 1982, 1985; Sarwar, 1985; Arulpragasam, Latiff and Seneviratne, 1988). However there are no reports on callus formation or somatic embryogenesis using tea leaf tissue.

In preliminary experiments using stem, leaf and shoot tips, callus formation was found to occur but embryogenesis from these organs has not been observed although organogenesis in the form of root formation occurred very frequently in the stem tissues. This study was undertaken to obtain information on the response of different tissue types and explants and on the growth media to be used for the successful regeneration of plants from stem and leaf callus tissues of the tea plant. Information on this aspect would be beneficial in view of the fact that some high yielding clones have been found to be difficult to propagate by shoot tip cultures due to clonal variation (Arulpragasam and Latiff, 1986).

MATERIALS AND METHODS

Nodal cuttings and juvenile leaves were selected as explants for culture. Nodal cuttings obtained from field grown tea plants of clone TRI 2025 were initially surface sterilized by immersing in 10% Clorox with 0.01% Tween 20 for 15 min and then by dipping in 0.15% $HgCl_2$ for 10 min followed by washing in three changes of sterile distilled water and prepared for culture. Nodal explants were trimmed

to 1.5 - 2.5 cm and the leaves with the petioles were removed without damaging the axillary buds (Fig. 1). Juvenile leaves produced by the nodal cuttings after 3-4 months in culture, were aseptically removed and 4 cm^2 and 6 cm^2 leaf squares cut off from the centre of the leaf (Fig.2). Of the few media tried out, three amended media were found to be more suitable for callus induction. Media 1(Table1 1) and 2 (Table2) were used for nodal cuttings while medium 3 (Table 3) was used for leaf explants as well as for nodal cuttings. The cut leaf squares as well as whole leaves were placed in medium 3. Medium 1 was modified Vacin and Went (1949) medium supplemented with 15% green coconut water. Medium 2 was MS salts (Murashige and Skoog, 1962) amended with IBA and BAP at 0.1 mg l^{-1} and 1.0 mg l^{-1} respectively. Medium 3, based on MS salts was supplemented with 60 g l^{-1} sucrose, 1.0 mg l^{-1} 2,4, - D and 1.0 mg l^{-1} kinetin.

The pH was adjusted to 5.7 in all three media and dispersed into small vials (1—2 oz) after adding Agar at 7.5 g l^{-1} and autoclaved for 20 min at 15 psi at 120° C.

Cultures were grown in the growth room over a 16-h photoperiod at 2000 Lux light intensity at 24° - 26° C. Calli were maintained by subculturing at regular intervals of 30 days in fresh medium. After 3 - 4 months callus formed in both nodal and leaf explants were subcultured on MS basal medium supplemented with vitamins and different hormone combinations to induce embryogenesis.

TABLE 1—*The composition of medium used for callus induction on tea nodal cuttings (modified Vacin and Went medium)*

Component	*Concentration*
Tricalcium phosphate	200 mg l^{-1}
Potassium nitrate	525 mg l^{-1}
Potassium phosphate	250 mg l^{-1}
Magnesium sulphate	250 mg l^{-1}
Ammonium sulphate	500 mg l^{-1}
Ferrous sulphate	28 mg l^{-1}
Manganese sulphate	7.5 mg l^{-1}
Sucrose	20 g l^{-1}
Agar	7.5 g l^{-1}
Green coconut water	150 ml l^{-1}
pH	5.7

TABLE 2—*The composition of medium used for callus induction on tea nodal cuttings*

Component	*Concentration mg-l-¹*	
Major elements	MS *	Full strength
Minor elements	MS *	
myo-Inositol	100	
Thiamin-HCl	0.4	
Ascorbic acid	2.0	
Sucrose	30 g	
IBA	0.1	
BAP	1.0	
Agar	7.5 g	
pH	5.7	

*Murashige and Skoog (1962) mineral salts

TABLE 3—*The composition of medium used for callus induction on nodal cuttings and leaf explants*

Component	*Concentration mg l-¹*	
Major element	MS *	Full strength
Minor element	MS *	
myo-Inositol	100	
Thiamin HCl	0.4	
Ascorbic acid	2.0	
Sucrose	60.0 g	
2,4-D	1.0	
Kinetin	1.0	
Agar	7.5 g	
pH	5.7	

*Murashige and Skoog (1962) mineral salts

RESULTS AND DISCUSSION

In media 1 and 2 initially only a rim of callus tissue was formed around the cut basal end of the nodal explants. The callus developed into a light green, hard and non friable globular structure after 8 weeks. In fresh medium it continued to grow and multiply. A rapid formation and multiplication of callus was observed on nodal cuttings cultured in medium 3, with high concentration of sucrose and the presence of kinetin and 2,4-D (Fig. 3).

Leaf explants started to form a callus at the margin of the leaf, after 6 weeks in culture (Fig. 4). The texture of the callus formed on the leaf tissues in medium 3, was different to that formed on nodal cuttings being greenish yellow, soft and friable.

Both IBA and 2,4-D individually induced callus at the cut ends of nodal explants and leaf margins. Callus formation was vigorous in 2,4-D with a high concentration of sucrose as compared with IBA alone. Callus formation was better when whole leaves were placed in medium 3, since browning at the cut ends of the leaf squares retarded the callus growth in some instances. Although embryoid-like structures were formed in the leaf callus, regeneration did not occur in any of the callus tissues in the various hormonal combinations tested (Fig. 5). Organcgenesis in the form of root formation was observed on callus tissue subcultured in a medium containing MS salts supplemented with 2 mg l^{-1} IBA and 10 mg l^{-1} BAP.

It was reported that by altering the kinetin/2, 4-D ratios leaf callus cultures could be induced to form somatic embryos (R.E. Litz, pers. comm.). Work is now being carried out on these lines to induce somatic embryogenesis on both stem and leaf calli.

These studies will be useful for the successful develcpment of a micropropagation system in tea clones that are difficult to propagate by shoot tip culture. Any somaclonal variations in the plants regenerated will have to be evaluated.

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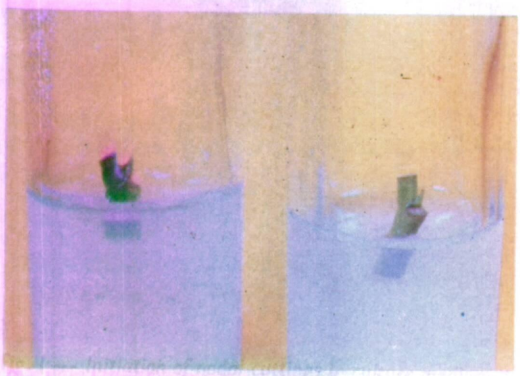

Fig. 1 — Initiation of nodal cuttings in culture medium.

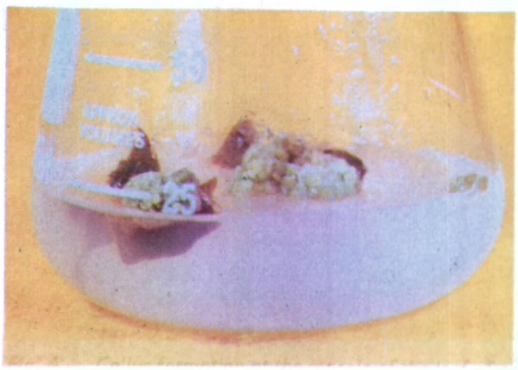

Fig. 4 — Callus formation at the margin of the leaf after 6 weeks in culture.

Fig. 2 — Source of explants for leaf callus culture.

Fig. 5 — Formation of embryoid - like structures in the leaf callus.

Fig. 3 — Rapid formation and multiplication of callus on nodal cuttings cultured in medium 3

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