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STUDIES ON THE TISSUE CULTURE OF TEA (*CAMELLIA SINENSIS* (L.) O. KUNTZE)
2. Rooting of shoots produced in culture

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Following successful establishment and multiplication of tea in culture using shoot tips and nodal segments as explants the work carried out in rooting the shoots produced *in vitro* are reported in this paper.

Shoots of clones TRI 2025, CY9 and shoots regenerated directly from cotyledons in culture were successfully induced to produce roots. The basic MS medium was used with IBA at concentrations of 0·1, 0·2, 1.0 and 2·0 mg/l without any cytokinins.

Differences in genotypic responses to root initiation was observed in the clones that were studied, different clones requiring different concentrations of auxins for root initiation. We have been unsuccessful so far in inducing rooting of clones of the three thousand series.

The rooted plantlets were transferred into soil and were acclimatized in the humid chamber. This is the first record of the micropropagation of tea using shoot tips as explants.

INTRODUCTION

Arulpragasam and Latiff (1986) reported the successful establishment and multiplication of shoot tips and nodal segments of *Camellia sinensis* (L.) O. Kuntze in culture. The work carried out on the rooting of the shoots produced in culture is reported in this paper.

Auxins are regarded as the main factor promoting root initiation (Tizio, Moyano and Morales, 1968, Lee Chong, Mc Guire and Kitchin, 1969). Indole Butric Acid had been used in the culture medium by Kim, Patel and Thorpe in 1985 for the induction of roots of micro-cuttings of Mulberry. Shoot buds of tea regenerated from stem callus had been rooted by incorporating IBA into the culture medium (Kato, 1985).

These experiments were conducted to find out a suitable medium for the induction of roots in the micropropagated tea shoots.

MATERIALS AND METHODS

Micropropagated shoots (Fig. 1) belonging to clones TRI 2025, CY9, TRI 3011, TRI 3017 and shoots produced through direct regeneration from cotyledon pieces were used as materials for the experiments. The basic Murashige Skoog Salt medium (Murashige and Skoog, 1962) was used at half strength in all occasions (Table 1) but changing the concentration of auxins and sucrose in the medium.

TABLE I—*Composition of the basic medium used for rooting of micro-cuttings of tea*

Component	*Concentration mg/l*
Major elements	½MS *
Minor elements	½MS *
Thiamine HCl	0.5
Inositol	100
Pyridoxine - HCl	0.01
Nicotinic Acid	1.0
Calcium Pantothenate	1.0
Agar	8,000

* Murashige and Skoog (1962) Mineral Salts

Indole Buteric Acid at the concentrations of 0.1, 0.2, 0.5, 1,2 and 3 mg/l was used in the experiment. A control experiment was carried out without the addition of IBA to the medium. All concentrations of IBA were tested with 30, 20 and 15 g/l of sucrose in the medium. No cytokinins were added. The pH of the culture medium was adjusted to a value between 5.5 to 5.8 before autoclaving.

Shoots 2-3 cm in length were transferred aseptically into sterile culture media (Fig. 2). The cultures were kept upright in the growth room at 26—28 °C under 16h photoperiod of 2,000 lux light intensity (from fluorescent lamps) at the level of the cultures.

After the formation of roots the plantlets were removed from the culture vessels, their shoot system washed thoroughly to remove all traces of agar and nutrients and were transferred into autoclaved soil. Autoclaved sand was added to improve the texture of the soil. The plantlets were kept inside a humid chamber for 7 days for hardening and were gradually acclimatized to outdoor environmental conditions.

RESULTS

Growth of the shoots was observed within 2-3 weeks (Fig. 3) and formation of roots was observed about one month after transferring to the rooting medium (Fig. 4). Rooting occured only in microcuttings belonging to clones CY9, TRI 2025 and in shoots that had been regenerated directly from cotyledon pieces (Table 2).

TABLE 2 — *Effect of IBA in the culture medium on the rooting of micro-cuttings (Sucrose concentration 30 g/l)*

Clone	Control	Concentration of IBA in the medium mg/l 0.1	0.2	0.5	1.0	2.0	3.0
CY 9	—	—	—	—	X	—	—
TRI 2025	—	X	X	—	—	—	—
TRI 3011	—	—	—	—	—	—	—
TRI 3017	—	—	—	—	—	—	—
Shoots regenerated directly from cotyledon pieces	—	—	—	—	X	X	—

X - denotes formation of roots

Rooting did not occur in any of the explants in media containing sucrose at the lower concentrations of 20 and 15 g/l.

Callusing occurred at the base of shoots in media containing 1,2 and 3 mg/l of IBA.

The roots formed resembled a tap root system with branching rootlets (Fig. 5). The roots turned green in colour as the culture tubes were kept in light. The rate of growth of the shoot system of the rooted plantlets was slow when compared to the growth in the shoot multiplication medium.

All rooted plantlets survived the outdoor environmental conditions after the acclimatization procedure (Fig. 6) and have been planted out in small field plots.

DISCUSSION

Most of the work that had been carried out on the tissue culture of Tea had been in connection with plant regeneration from callus cultures (Kate, 1985; Phukan and Mitra, 1984; Sarwar, 1985; Wu, 1976) and for the investigation of secondary product biosynthesis (Forrest, 1969; Ogutunga and Northcote, 1970). There are no reports to date, of work done on the micropropagation of tea by shoot multiplication.

The results of the experiment indicate that auxin concentration in the medium is a determining factor in root initiation. The concentration of sucrose in the culture medium is also important since lowering of the sucrose concentration had affected root formation.

Higher concentrations of IBA in the medium had resulted in the formation of a callus at the base of the shoot. This should be avoided since callus between shoot and root results in poor vascular connections which makes the field survival of plants difficult (Kim, Patel and Thorpe, 1985).

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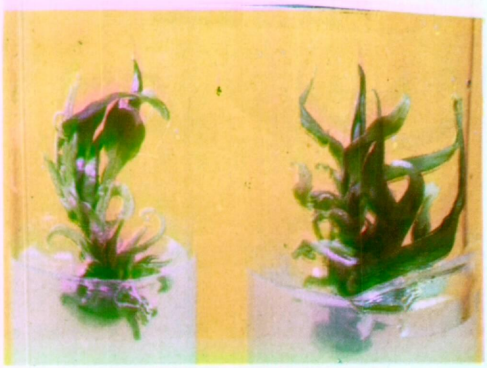

Fig. 1 — Proliferation of shoots in culture

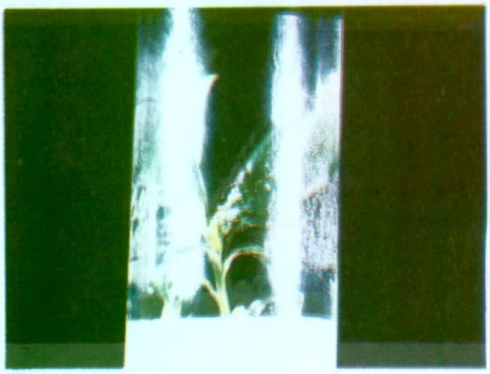

Fig. 4 — Formation of roots

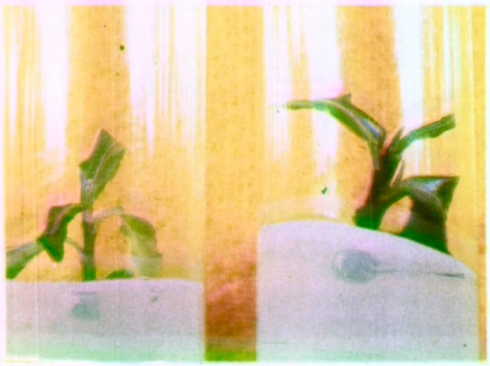

Fig. 2 — Micro-cuttings in rooting medium

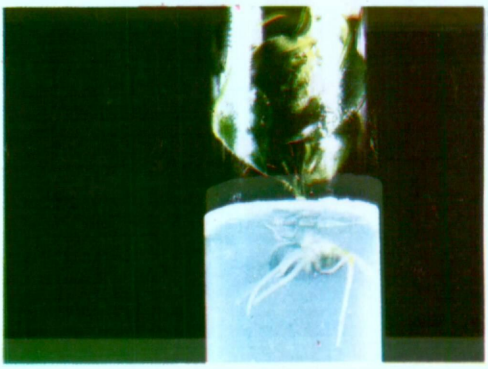

Fig. 5 — Further development of roots and formation of plantlet

Fig. 3 Growth of micro-cuttings in rooting medium

Fig. 6 — Acclimatized plantlets in plastic pots

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The clones of tea that had been studied required different concentrations of IBA for rooting (Table 2). This indicates that there is a clonal variation in the concentration of IBA required for rooting. A similar variation has been observed in the different cultivars of apple (Zimmerman and Broome, 1981).

This is the first ever record of plants being produced using shoot tips as explants in the micropropagation of tea.

The results achieved so far indicate that for tea, we have now reached the "how to" stage in micropropagation and we are now concentrating on finding out "how best to" propagate tea by using tissue culture techniques.

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