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AN *IN VITRO* AND *EX VITRO* ROOTING OF MICROPROPAGATED SHOOTS OF TEA *(CAMELLIA* SPP.)

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Tea microcuttings, prepared from shoots proliferated *in vitro*, initiated roots readily when immersed for 30 min. in an indole butyric acid (IBA) solution (1000 mgl^{-1} w/v) and transferred either to modified Murashige and Skoog's medium or unsterile soil medium. IBA was the most effective out of the tested auxins and napthalene acetic acid (NAA) the least effective. Incorporation of auxins (IBA and NAA) individually in the rooting medium did not induce rooting. Maintaining the cultures in dark for more than 7 days improved rooting and after 9 days of incubation shoot tip necrosis was observed in 12% of cultures. Roots that were initiated in *in vitro* condition after 7–10 days of incubation, elongated within 20–25 days after cuttings were transplanted to suitable soil medium. After acclimatization these plants were transferred to the soil where 97% plantlets survived and grew vigorously.

INTRODUCTION

In the past, several tea clones have been selected from seedling populations and released by various centres with proven productivity, quality and other characteristics like drought tolerance and resistance to some of the common diseases and pests. In the overall selection programme, among the various attributes, the extent to which cuttings can be rooted is one of the characters that is taken into consideration before a bush is finally chosen. This is because, the probability exists that a particular bush may be excellent in many other important characters except in rooting efficiency, which precludes its selection. To overcome such problems, *in vitro* culture technologies could serve as a technique for rapid multiplication and rooting of "difficult-to-root" cuttings in a relatively shorter period at any season of the year.

From mid-eighties *in vitro* propagation of tea has been reported (Agarwal, Singh and Banerjee, 1992; Arulpragasam and Latiff, 1986; Jha and Sen, 1992; Nakamura, 1988; Rajasekaran, 1990; Rajasekaran and Raman, 1993; Seneviratne, Latiff and Arulpragasam, 1988). However rooting and hardening of *in vitro* propagated tea poses many difficulties which so far has not been dealt in detail. A successful tissue cultured method of propagation must result in re-establishment in soil of the tissue culture derived plants. This paper describes experiments to evaluate several factors and to find out optimum conditions for easy rooting in *in vitro* and *ex vitro* conditions of micropropagated tea shoots, thereby maximising the overall efficiency of *in vitro* propagation of tea.

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MATERIALS AND METHODS

Shoot bud cultures were initiated from actively growing shoot tips and from dormant axillary buds (Figs. 1–4). Culture establishment procedures were according to Rajasekaran (1990) and Rajasekaran and Raman (1993). Shoots were proliferated on medium based on MS, Murashige and Skoog (1962) salts supplemented with benzyl-aminopurine (BAP) at 5 mg l^{-1}. The basal medium for rooting contained half-strength macronutrients and full strength micronutrients of MS supplemented without or with various concentrations: 0.1, 0.5, 1.0, 1.5 and 2.0 mg l^{-1} of IBA and NAA individually. Experiments were carried out to determine the suitable auxin, concentration, the optimum duration of dipping the basal cut ends of shoots in the concentrated (1000 mg l^{-1} IBA or NAA) auxin solutions (chronic auxin treatment) and the effects of darkness during rooting.

Terminal shoot cuttings (>3 cm long) were prepared from *in vitro* proliferating cultures of south Indian clones viz, UPASI-3, UPASI-9, UPASI-10 and UPASI-17 (released by United Planters Association of Southern India (UPASI). Harvested shoots, retaining the leaves only at the top 2 cm of the cuttings, were placed for 20–30 min. in an upright position in 60 x 100 mm autoclavable culture bottles containing auxin solutions. At the end of the treatment, the cuttings were transferred to either *in vitro* or *ex vitro* rooting media. While *in vitro* condition needed induction of rooting in aseptic conditions, the *ex vitro* technique had the advantage of inducing rooting of microcuttings directly in soil.

For shoot proliferation, cultures were grown at 25±2°C, 24 hours photoperiod provided by fluorescent light (about 32 $\mu Mol^{-2}s^{-1}$) and a relative humidity of 80±10%. The same conditions were used for rooting in light. Dark treatment at 25°C was obtained by covering culture bottles in black cloth and placing them in the growth room; diffused light treatment at the same temperature was obtained by placing cultures in reduced light.

In vitro rooted plants were transferred to polythene sleeves containing a mixture of peat moss, black soil, sand at a ratio of 1 : 1 : 2. Hardening was done by keeping the plantlets under the cover of polythene sheets (relative humidity = 100%) with overhead shade by a coir mat. In *ex vitro* rooting after dipping in 1000 mg l^{-1} IBA or NAA solutions the shoots were transferred to 10 x 30 cm polythene sleeves filled with a mixture of black soil and sand at a ratio 2 : 1 (root elongation medium) in the bottom 20 cm and red soil (sub soil) (root induction medium) in the top 10 cm or a mixture of peat moss, black soil and sand as used for *in vitro* rooted plants. The pH of the soil was previously adjusted to 4.8±0.2 by using 0.5% (W/V) solution of aluminium sulphate. Hardening was done as discussed earlier. Acclimatized plants were planted in the field during June 1992. All the experiments had 50 – 100 replications each and were conducted three to five times.

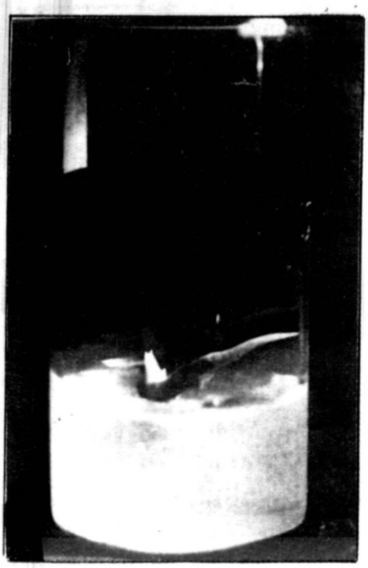

Fig. 1 – *Response of shoot tip explants of tea in shoot multiplication medium after 30 days*

Fig. 2 – *Response of nodal explants of tea in shoot multiplication medium after 30 days*

Fig. 3 – *A rapidly proliferating shoot clump from shoot tip culture, 120 days after culturing in shoot proliferation medium*

Fig. 4 – *A proliferating shoot mass after 150 days*

RESULTS

In the initial rooting experiment, shoots were placed in semi – solid rooting medium supplemented with various concentrations: 0.1, 0.5, 1.0, 1.5 or 2.0 mg l^{-1} of IBA and NAA. A minimum concentration of IBA (0.1 mg l^{-1} would be sufficient for the basal cut end to swell but with no symptoms of root formation (Table 1). At 0.5 mgl^{-1} IBA, root initiation occurred within 20 to 25 days but the rooting rate was only 5 to 10%. The rest of the explants produced microcalli at the rim of the cut ends. The callus production was found to be directly proportional to the IBA content. There were no symptoms of root initiation at the concentrations of NAA studied in the experiment. The callus production showed a hierarchial increment with the addition of NAA from 0.1 to 1.5 mg l^{-1} and then it decreased at 2 mg l^{-1}.

Rooting under *in vitro* conditions

In view of low percentage of rooting and defoliation of leaves observed in the previous experiment, a new experiment involving "dipping technique" was employed. Since IBA alone helped for root initiation, only IBA was used in the "dipping technique". The basal cut end of shoots were dipped for 20 to 30 min. in 1000 mg l^{-1} w/v IBA solution. The micro-cuttings were then immediately transferred to the rooting medium and then the cultures were incubated in dark. The percentage of rooting was increased in all clones by incubating the cultures in dark at 25^{0}C for the first week of the rooting stage. Rooting percentage was more in UPASI–9 (Table 2). Dark treatment resulted in better rooting percentages than in light. In light, 82 to 100% of the shoots produced callus instead of root initials. The minimum length of dark period necessary to increase the rooting percentage above the light treatment varied with clone (Table 2). Three to six days of dark treatment appeared to be optimum in UPASI-3, UPASI-10 and UPASI-17. Incubation of shoots in the dark for more than 9 days resulted in shoot tip necrosis in about 12% of cultures. The percentage of necrosis increased with the increase of duration of dark incubation. UPASI–10 required a minimum of 10 days of dark incubation for better root induction. In this case, shoot tip necrosis was avoided by incubating the cultures in the incubator with reduced light.

Dipping the cut ends of the shoots in 1000 mg l^{-1} IBA solution for 20 min. and then transferring to hormone free rooting medium resulted in basal swelling and root induction in 30% of the shoots. In the other shoots only basal swelling was observed. However, the shoots dipped for 30 minutes in IBA solution resulted in 94 to 99.2% rooting (Table 2). Within a week after inoculation, swelling of the shoot base and root initiation occurred in the cultures of all the clones. The root initials grew out by bursting the bark of the stem (Fig. 5) in 20 days. In 20 to 25 days, roots continued to elongate attaining a length of about 1 cm. The roots also emerged near the basal-most axillary bud region. Though many roots were induced from the aerial part of the shoot, they penetrated into the medium.

TABLE 1 – *Effect of IBA and NAA on the morphogenetic behaviour of isolated shoots proliferated from shoot tip and nodal culture of 4 clones (observations taken 20 days after culturing; n=50)*

Concentration of auxin (mg l^{-1})	Response and Remarks	
	IBA	NAA
0.1	Slight basal swelling, no root initiation and no callus formation	No response
0.5	Root initiation and root growth at the basal cut end. No callus formation	No root initiation. Formation of pink and pale yellow callus (+++).
1.0	Only root initiation. Formation of green callus (+).	No root initiation. Formation of pink and yellow callus and bulged base (+++).
1.5	No root initiation. Formation of pink callus (++).	No root initiation. Formation of pink and pale yellow callus and bulged base (+++).
2.0	No root initiation. Formation of pale yellow and pink callus (+++).	No root initiation. Formation of pink and green callus (++).

* Plus (+) marks in parenthesis denote intensity of callus formation.

+ = below 25% ++ = 25 – 50% +++ = above 50%

TABLE 2 –*Effect of dark vs light on root induction of in vitro propagated shoots of 4 tea clones in ex vitro rooting*

Clone	*No. of cuttings/ treatment*	*Rooting percentage*			
		Light	*Dark* *No. of days in dark*		
			3	*6*	*9*
UPASI – 3	100	3	30	33	97
UPASI – 9	100	18	42	96	99.2
UPASI – 10	100	4.8	37	39	68.7
UPASI – 17	100	0	13	60	94

Rooting under *ex vitro* conditions

As in *in vitro* rooting, after dipping in 1000 mg l^{-1} IBA solution for 30 min. the shoots were directly transferred to soil medium in polythene sleeves. Root initials were observed after 25 days of incubation in about 95% of the shoots. Within 43 days, 15 to 21 roots (1 to 2 cm long), were formed (Fig 6). After 45 days, the humidity was reduced by gradually removing the polythene tent and the plants were kept under overhead shade provided by a coir mat cover.

The physiological state of shoots plays a major role in acclimatizing unrooted micro-cuttings in *ex vitro* condition. Juvenile shoots with actively growing shoot tips, resulted in less than 80% survival of shoots, whereas fully elongated dormant shoots, similar to the "banji" shoots in the field, resulted in 95% of survival during acclimatization. The type of soil too had an influence on inducing roots after transferring to soil. The treated shoots transferred to polythene sleeves filled with red soil at the top and the black soil and sand mixture at the bottom, showed root induction in 79.8% of shoots, while the mixture of peat moss: black soil: sand induced roots in 97.3% of shoots.

DISCUSSION

Shoots which were inoculated in MS medium (half strength macro nutrients) supplemented with IBA at 0.5 mg l^{-1} were rooted but the number of roots and percentage of rooting were very low. It appears that the type of auxin and its concentration plays a major role in induction of rooting in tea plants. NAA had no effect on rooting except callus formation whereas lower concentration of IBA (0.5 mg l^{-1}) induced roots in 5 to 10% of the shoots. Zimmerman (1988) suggested a two step procedure (quick-dip in concentrated auxin solution and transfer to auxin free medium) for improving rooting. The same method had been adopted by many researchers (Vicitez *et al.* 1983., Zimmerman and Fordham, 1985., Samartin, Vieitez and Vieitez, 1986). Vieitez, Sanjose and Ballester (1989) induced roots in *Camellia japonica* by dipping the base of

Fig. 5 – *Rooted shoots after 25 days in in vitro rooting medium*

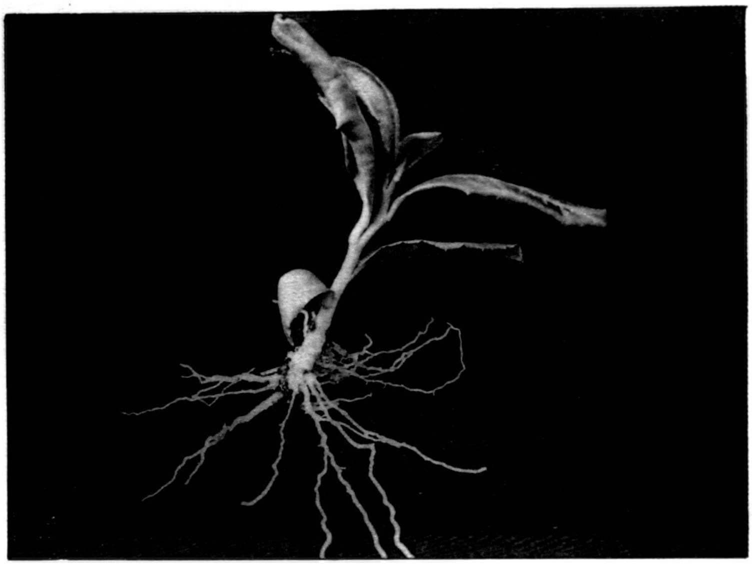

Fig. 6 – *Rooted shoot after 50 days in ex vitro condition*

the shoots in a solution of 1000 mg l^{-1} of IBA for 15 min. and then incubating for 12 days in dark. Dark incubation had helped to induce roots. But, when the cultures were incubated in the dark for longer time (more than 9 days), abscission of leaves and necrosis of shoot tips had occurred. Samartin, Vieitez and Vieitez, (1986) observed similar phenomena in rooting of *in vitro* produced Camellias.

The four clones used in the present investigation differed in their morphological characters (Mohanan and Sharma, 1981), genetic character and in their performance both in nursery and field (Venkataramani and Sharma, 1975). For example, UPASI–9 has a good rooting capacity while UPASI–17 is a shy rooter in vegetative propagation. The other two clones possess medium rooting capability. Further, UPASI–3 is a triploid and possesses high yield potential (Venkataramani and Sharma, 1974). Despite these variations, these clones responded well to the rooting procedure (both in *in vitro* and *ex vitro*).

Seneviratne *et al.* (1988) reported that the *in vitro* rooting response in Sri Lankan tea clones varied depending on the nature of the clone. The clone CY 9 needed IBA at 1 mg l^{-1} whereas TRI 2025 required IBA at 0.1 to 0.2 mg l^{-1}. The shoots regenerated directly from the cotyledon required IBA at 1.0 to 2.0 mg l^{-1}. Further, in their study, out of the four clones investigated only two had responded to the rooting medium. Recently Agarwal *et al.* (1992) reported that inspite of giving rigid temperature, relative humidity and sterile conditions acclimatization of micropropagated tea shoots was very difficult. In this study, by selecting sizeable mature *in vitro* shoots, 95% of shoots survived during acclimatization and they have completed their second year in the field.

Rooting of micropropagated shoots under *ex vitro* conditions helps in reducing in manipulation costs and the time taken for rooting and acclimatization (Zimmerman, 1988). From the results of rooting and transplanting in the field, it can be concluded that, (a) both *in vitro* and *ex vitro* rooting methods are suitable for the different genotypes, and (b) establishment of both rooted (*in vitro* and *ex vitro*) shoots in the field can be achieved with a high rate of success.

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