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ROOT FORMATION ON *IN VITRO* MICROPROPAGATED SHOOTS OF *CAMELLIA SINENSIS* L

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Root development on *in vitro* proliferated shoots of clones CY 9 and DG 7, was studied. Shoots were obtained from meristem culture of axillary buds. Several media compositions and culture conditions were tested on 1/2 MS. It was observed that the presence of higher amounts of auxins (IBA and NAA) enhanced root formation in tea. A root system was established when shoots were dipped in IBA prior to inoculation to the rooting medium. However, dipping in IBA was not effective when the cultures were supplemented with auxin/s. A protocol for enhanced root formation on *in vitro* shoots is suggested.

INTRODUCTION

Micropropagation through shoot tip culture of *Camellia sinensis* (L). O. Kuntze, has been reported (Phukan and Mitra, 1984; Seneviratne, Lattiff and Arulpragasam, 1988; Nakamura, 1991; Sarathchandra, Upali and Arulpragasam, 1990; Samartin, 1991; Manivel, 1993). However, one of the major problems associated with micropropagation of tea is the difficulty of rooting of *in vitro* shoots. This hinders its potential application in breeding programmes etc. Reports on rooting of *in vitro* shoots suggest several possibilities for different species (Kato, 1985; Nakamura, 1991; Samartin, 1991).

Experiments have been conducted on rooting of shoots regenerated directly from cotyledon cultures of tea. Filter paper bridge techniques has been adopted and only 20-25% success is reported. Also this technique is cumbersome and a clonal variation of rooting is reported (Arulpragasam and Lattiff, 1986). The effectiveness of the technique on the rooting of *in vitro* shoots cultured from axillary buds on solid medium is not reported. The present study was aimed at increasing the precentage of rooting of *in vitro* shoots of clone CY 9 and DG 7.

MATERIALS AND METHODS

Shoots, 2.5 to 3.0 cm long. were excised from established cultures (6th multiplication cycle) of clones CY 9 and DG 7 grown in Hantane. *In vitro* shoots were obtained according to the method of Arulpragasam and Lattiff (1986).

The culture media for testing of root formation was basic Murashige and skoog (1962) (MS) but at 1/2 macro nutrients, supplemented either with IBA alone or in combination with NAA (Table 1). The media also contained 3% sucrose, 0.7% agar and the ph was adjusted to 5.6 - 5.7. A similar experiment was conducted simultaneously where the same number of explants were initially dipped in IBA solution (1.0 mg l^{-1}) for 5 min prior to inoculating on respective culture media as given in Table 1. Each treatment had 25 replicates. The cultures

were grown in dark for 10 days and subsequently in a growth room over a 16 h photoperiod and 3000 lux light intensity at about 25 c.

The precentage of rooting of shoots in each media was recorded after 3 months. The number of roots and their lengths per shoot were counted every month. Data were satistically analyzed for their significance, using Tukey's HSD test.

Table 1.- *Root development on shoots produced in vitro of clones CY 9 and DG 7 (3 months in culture)*

Medium mg l^{-1} hormone	*% rooting without dipping in IBA*	*% rooting with dipping in IBA*
1. 1/2 MS	0	50 - 60
2. 1/2 MS + 0.5 IBA	0	0
3. 1/2 MS + 1.0 IBA	0	10
4. 1/2 MS + 1.0 IBA + 0.5 NAA	40 - 50	0

Mean value of both clones is given.

RESULTS AND DISCUSSION

Root formation was observed only on certain media compositions. The most suitable composition, out of those tested was half MS with 1.0 mg l^{-1} IBA and 0.5 mg l^{-1} NAA (Table 1). The observations support the fact that a combination of IBA and NAA enhances root formation and that auxin concentration determines root initiation and their development (Salisbury and Ross, 1993; Seneviratne *et al,* 1988).

Even though IBA alone was not effective in the present study, the *in vitro* shoots of clone TRI 2025 and CY 9, in a reported study had produced roots on a medium with IBA alone. However in the latter, shoots have been developed from cotyledon callus (Seneviratne *et al,* 1988). Thus it appears that rooting of *in vitro* shoots is affected by several factors one being the mode of regeneration. It as also been reported that plant regeneration depends on the culture condition (Nabors Kroskey and D.M. Mchugh, 1982). This can be justified as the hormones of culture media on which the plant had been growing would affect its subsequent plant growth and development.

It was clearly seen that dipping of the shoot base prior to inoculation for rooting has a positive effect on root initiation. The shoots in 1/2 MS alone produced a profuse root system when dipped in IBA (Table 1 and 2 ; Figs 1 and 2)

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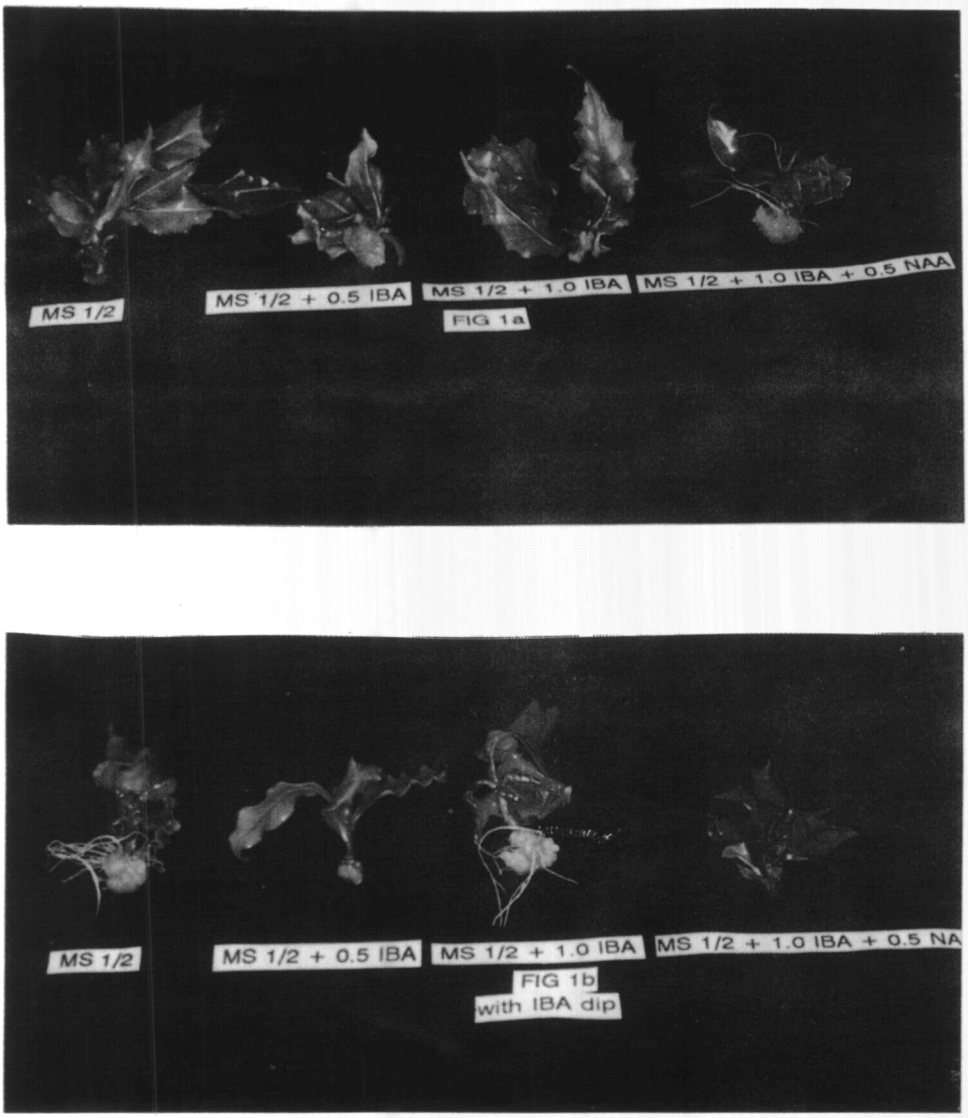

Fig. 1 – *The effect of culture composition and dipping in IBA on root formation. a: without dipping, b: with dipping. (1) MS ½ (2) MS ½ + 0.5 mg/L IBA, (3) MS ½ + 1.0 mg/L IBA, (4) MS ½ + 1.0 mg/L IBA,+0.5mg/L NAA.*

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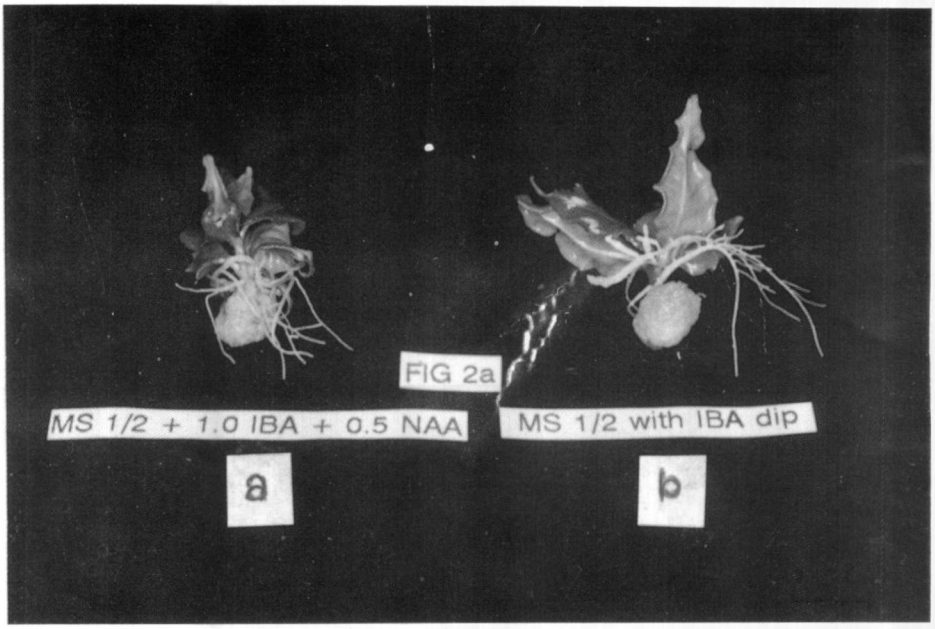

Fig. 2 – *Enhanced rooting on (a) MS ½ + 1.0 mg/L IBA,+ 0.5 mg/L NAA, (b) MS at ½ with dipping in IBA.*

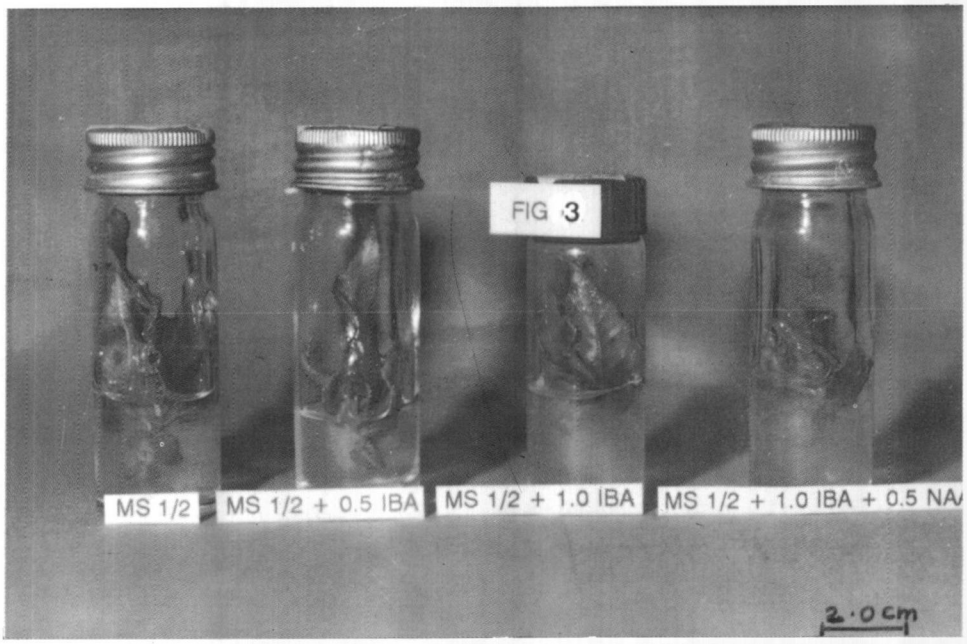

Fig. 3 – *Shoot development under tested conditions. (1,2,3,4 represent the same culture composition given in Fig 1)*

TABLE 2.- *Effect of culture composition and dipping in IBA, on root development.*

Treatment	*Average number* *Time (month)*			*Average length (cm)* *Time (month)*		
	1	2	3	1	2	3
1/2 MS with IBA dipped	2.0a	4.0a	5.0b	0.25a	1.0b	1.5b
1/2 MS + 1.0 mgl^{-1} IBA with IBA dipping	1.0a	3.0a	4.0b	0.25a	1.0b	1.7b

Mean of 25 replicates of each clone is given. Values followed by the same letter are not significant at P=0.05

It was also observed that in the media where the root production was highest, there was successful shoot development (Fig. 3).

The results suggest that IBA in combination with NAA enhances rooting of *in vitro* shoots. It is also clear that dipping in IBA is more effective than adding to the culture medium. The possible reason is that dipping would result in a higher amount of auxin at the base which would induce cell division and root formation. This may be more effective than having a uniform, lower amount being spread throughout the base of the shoot. Further, high auxin would enhance ethylene induced rooting of the shoots. However, the latter phenomenon is reported only in a particular plant species (Salisbury and Ross, 1993).

The study suggests that dipping in IBA prior to transfer of shoots to 1/2 MS can be practised to enhance rooting of *in vitro* shoots. However, more work with several other clones is needed before generalizing on this fact for *in vitro* shoots of tea obtained through meristem culture.

REFERENCES

ARULPRAGASAM, P.V. and LATTIFF, R. (1986). Studies on the tissue culture of tea *(Camellia sinensis* (L) O. Kuntze). 1. Development of a culture method for the multiplication of shoots. *S.L.J. Tea Sci.* **55**, 44-47.

ARULPRAGASAM, P.V. LATTIF, R and SENEVIRATNE, P. (1988). Studies on the tissue culture of tea *(Camellia sinensis* (L) O. Kuntze). 3. Regeneration of plants from cotyledon callus culture. *S.L.J. Tea Sci.* **57**, 20-23.

KATO, M (1985). Regeneration of plantlets from tea stem callus. *Japan J. Breed* **35**, 317-322.

MANIVEL, L. (1993). Micropropagation of tea clones through tissue culture. *The Planters Chronical.* **88**, 483-484.

MURASHIGE, T and SKOOG, F. (1962). A revised medium for rapid growth and bioassay with tobacco tissue callus. *Physiol plant.* **15,** 473-497.

NABORS, M.W., KROSKEY, S.C. and McHUGH, D.M. (1982). Green spots are predictors of high callus growth rates and shoot formation in normal and salt stressed tissue cultures of oats (Avena sativa L.) Z. Pflanzenp. 105, 341-349.

NAKAMURA, Y. (1991). *In vitro* propagation techniques of tea plant. **Proc. Int. Symp.** on *Tea Science.* Shizuoka, Japan. 436-441.

PHUKAN, M.K. and MITRA, G.C. (1984). Regeneration of tea shoots from nodai explants in tissue culture. *Curr. Sci.* **53 (16),** 874-76.

SALISBURY, F.B. and ROSS. C.W. (1993) *Plant Physiol.* 3rd Edition. Wadsworth Publishing company. USA.

SARATHCHANDRA, T.M. UPALI, P.D. and WIJEWARDENA, R.G.A. (1988) Studies on the tissue culture of tea *(Camellia sinensis (L) O. Kuntze).* 4. Somatic embryogenesis in stem and leaf callus cultures. *S.L.J. Tea Sci.* **57 (2)** 50-54.

SARATHCHANDRA, T.M., UPALI, P.D. and ARULPRAGASAM, P.V. (1990). Progress towards the commercial propagation of tea by tissue culture techniques, *S.L.J. Tea / Sci.* **59,** 62-64.

SENEVIRATNE, P., LATIFF, R and ARULPRAGASAM, P.V. (1988). Studies on the tissue culture of tea *(Camellia sinensis* (L) O. Kuntze). 2. Rooting of shoots produced in culture. *S.L.J. Tea Sci.* **57.** 16-19.

SAMARTIN, A. (1991). Potential for large scale *in vitro* propagation of *Camellia* sasanqua. *Hot Sci.* **67 (2),** 211-217.