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STUDIES ON THE TISSUE CULTURE OF TEA *(CAMELLIA SINENSIS* (L.) O. KUNTZE*)*.1. DEVELOPMENT OF A CULTURE METHOD FOR THE MULTIPLICATION OF SHOOTS

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Investigations were carried out for evolving a culture technique for the rapid propagation of tea by tissue culture. *In vitro* proliferation of shoots of tea by axillary branching was achieved using shoot tips and nodal segments as explants. Shoot multiplication was successful in two media, both consisting of Murashige and Skoog salts supplemented with (1) 0.4 mg thiamine-HCl, 100 mg m-inositol, 0.01 mg IBA, 1.0 mg BA and 0.1 mg GA_3 (2) 1.0 mg thiamine-HCl, 100 mg m-inositol, 1.0 mg pyridoxine-HCl, 0.01 mg biotine, 2.0 mg ascorbic acid, 1.0 mg nicotinic acid, 1.0 mg calcium pantothenate, 20.0 mg adenine sulphate, 0.1 mg IAA and 1.0 mg BA. Better shoot proliferation occurred when the concentration of BA was increased to 2.0 mg l^{-1}. Results obtained so far clearly indicate that propagation of tea by tissue culture is a distinct possibility in the near future.

INTRODUCTION

A number of papers have been published in recent years on the propagation of woody plants by tissue culture. Micropropagation or *in vitro* propagation of woody plants, specially the fruit trees, has progressed from a theoretical possibility to a commercial reality during the last decade. However, only a few attempts have been made in the propagation of tea plants using tissue culture techniques. Wu (1976) obtained plants from cotyledon callus while Wu *et al.* (1981) have obtained new clones derived from cotyledon callus. Kato (1982, 1985) obtained embryoids directly from slices of cotyledons of *Camellia sinensis* and *C. japonica* and successfully produced plantlets from tea stem callus. Samartin, Vieitez and Vieitez (1984) were successful in producing plantlets by culturing shoot tips from 4-5-month-old seedlings of *C. japonica*. The possibility of regeneration of shoots from nodal explants of tea has been reported by Phukan and Mitra (1984). Growth of buds and callus formation on explanted organs of tea was reported by Sarwar (1985).

Work on the micropropagation of tea was initiated at the Tea Research Institute of Sri Lanka during the latter part of 1984. Our immediate objective was to investigate the possibility of propagation of tea by tissue culture and to develop a culture technique for its successful implementation. Shoot tips and nodal segments with axillary buds were used in our present study for the production of shoots because of the increased likelihood of producing phenotypically uniform and genetically stable plants as opposed to plants produced from callus tissue which may not be phenotypically uniform.

MATERIALS AND METHODS

Shoot tips were collected from actively growing shoots of tea plants in the field, mostly from those recovering from pruning. Initially we worked with clone TRI 2025 and later with clones CY 9 and PK 2. Shoot tips 4 – 5 cm in length were collected in polythene bags and transported to the laboratory. To prepare the shoot tips for culturing, the leaves with the petioles were snapped off as close as possible to the stem without damaging the axillary buds. As many as possible of the tightly furled leaves around the shoot apex were loosened and removed. The shoot tips were then placed in a jar with a wire mesh lid and washed in running tap water for 15 - 30 min. They were then surface sterilized by agitating for 15 min. in 10 – 15% chlorox solution to which 0.01% Tween 20 had been added. The shoot tips were then rinsed in three changes of sterile distilled water and prepared for explanting by trimming them to 10 - 15 mm in length. The shoot tips were again surface sterilized, this time in 10% chlorox for 10 min. and rinsed three times in sterile distilled water. For the nodal explants, nodal segments (10 – 15 mm) with axillary buds from the third to the fifth visible nodes, counting downwards from the shoot apex, were used. The explants were placed vertically in universal containers each containing 10 ml of the medium.

Many media, based on MS mineral formula (Murashige and Skoog, 1962), were tried out and the following two media (Table 1) were found to be suitable. MS 1 was found to be more suitable for clone TRI 2025 and MS 2 for clones CY 9 and PK 2. Once established however, the three clones grew equally well on both media.

The pH was adjusted to 5.6 – 5.8 before autoclaving and the cultures were incubated in a growth room at 26 – 28°C under 16 h photoperiod of 2,000 lux light intensity (from fluorescent lamps) at the level of the cultures.

TABLE 1—*Composition of media used for the establishment and proliferation of shoot tips and nodal segments of tea* (*mg* l^{-1})

Component	*MS* 1	*MS* 2
Major elements	MS*	MS*
Minor elements	MS*	MS*
Thiamine-HCl	0·4	1·0
myo-Inositol	100	100
Pyridoxine-HCl	—	1·0
Biotine	—	0·01
Ascorbic acid	—	2·0
Nicotinic acid	—	1·0
Calcium pantothenate	—	1·0
Adenine sulphate	—	20·0
Indoleacetic acid	—	0·1
Indolebutric acid	0·01	—
Benzyladenine	1·0	1·0
Gibberellic acid	0·1	—
Sucrose	30,000	30,000
Phytagar	8,000	8,000
pH	5·6 – 5·8	5·6 – 5·8

* Murashige and Skoog mineral salts.

RESULTS

The major problem encountered in Stage I was contamination of the explant. Several types of surface sterilants were tried out but we found that it was virtually impossible to culture tea shoot tips without contamination. We were able to reduce the level of contamination from 100% to about 25% by following the techniques described under materials and methods.

Browning of the explant was a problem to start with but it was overcome to a great extent by using the mineral salts at half strength in Stage I and by adjusting the strength of the sterilant and the sterilization time. The incorporation of activated charcoal in the medium did not have any effect on browning. Optimum establishment and growth of the explant with minimum of necrosis and callus formation occurred in MS 1 for the clone TRI 2025 and in MS 2 for the clones CY 9 and PK 2. The three clones were found to grow equally well in both media once they were established.

The shoot tip explants begin to grow within one week of culture, when the tightly furled leaves enclosing the shoot tip start to elongate and unfurl. The axillary bud of the nodal explants begin to show signs of growth by the second week (Figs 1 and 2). After two weeks the explants are transferred to fresh media with full strength mineral salts. The axillary buds of the shoot tips begin to grow about the fourth week from explanting (Fig. 3). These showed further growth when subcultured in fresh media. These were transferred to Stage II media six weeks after explanting. In Stage II media the BA concentration was increased to 2.0 mg l^{-1}. Proliferation of shoots occurred after about two weeks in the Stage II media (Fig. 4). The shoots produced were of uneven size, usually one of the shoots would be large measuring about 2.0 cm while the others would be about 1.0 cm in height (Fig. 5). Two to four shoots were generally produced after four weeks in the proliferation media. The shoots produced from the nodal explants were found to be more uniform and arise from the base of the axillary bud (Fig. 6). Cuttings were taken from the larger shoots produced *in vitro* and are being cultured in Stage II media for further multiplication (Fig. 7).

No attempt is being made at the moment to induce rooting in the shoots obtained by multiplication *in vitro*. Studies on rooting will be undertaken once sufficient number of shoots are obtained in culture.

DISCUSSION

There are no reports, to date, in the literature on the successful micropropagation of tea using shoot tips or nodal explants. Sarwar (1985) reported that in his experiments all shoot tips turned brown and died within ten days. Phukan and Mitra (1984) were able to induce shoot formation on nodal segments of seedlings, but were unable to grow shoot tips in any of the media they tested. Other reports have been on the regeneration of plantlets from cotyledon and stem callus.

In our studies which commenced an year ago we have been able to develop a culture method for the rapid propagation of tea by tissue culture. The rooting of the shoots multiplied *in vitro* and acclimatization would not present any problems since we have already rooted and acclimatized plants regenerated from cotyledon pieces (unpublished). Besides, it is known that shoots that have gone through a series of subcultures lend themselves more easily to rooting than those which have not. The problem of contamination with bacteria has been overcome to a great extent by the use of a cocktail of antibiotics (unpublished). We are at present working on refinements to the methods reported here, which would enable us to obtain the maximum number of plants within an optimum period of time.

We conclude that tea responds well to propagation by tissue culture and that micropropagation of tea is a distinct possibility in the near future. Using this technique it would be possible to produce hundreds of thousands of clonal plants. This technique would be extremely useful for the rapid and large scale propagation of our new clones and the existing clones to meet the increasing demands for clonal plants in our replanting programme.

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