Effect of synthetic auxins on callus induction from tea stem tissue*

CARL H. FRISCH** and N.D. CAMPER**

Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634-0377, USA

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Abstract. A study was initiated to establish an in vitro culture protocol for tea (Camellia sinensis). Explant sources, disinfection methods and culture media were examined. Segments (divots) were dissected from greenwood stem (current year growth) internodes of field grown plants. Disinfection was achieved by separate treatments of 3.75% sodium hypochlorite and 7.5% CaCl₂. MS medium with sucrose (30 g/L), inositol (100 mg/L) and thiamine-HCl (1.3 mg/L) and kinetin was used with combinations of the auxins: (2,4-dichlorophenoxy) acetic acid (2,4-D), (2,4,5-trichlorophenoxy) acetic acid (2,4,5-T), (naphthalene) acetic acid (NAA) and 4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid (Picloram). Picloram (10⁻⁷ M) induced the most callus proliferation without kinetin. At a constant level of kinetin (10⁻⁸ M), the concentrations inducing the most callus growth were 10⁻⁷ M for 2,4-D, 10⁻⁶ M for 2,4,5-T, 10⁻⁷ M for Picloram and 10⁻⁶ M for NAA. A factorial test of 2,4,5-T and kinetin concentrations showed the optimum for callus growth was 10⁻⁷ M and 10⁻⁸ M, respectively.

Introduction

Cultivated tea (Camellia sinensis) has been maintained for centuries by vegetative propagation. In recent years in vitro culture techniques have been developed for many plant species. However, compared to other woody crops of economic importance such as coffee [11] and citrus [13], little in vitro technology has been applied to tea. Most studies used tissue culture of tea to investigate secondary product biosynthesis [2, 6, 10, 14, 15, 16, 17]. In attempts to culture tea several explants have been examined: cotyledons [5, 7, 12], leaves [5], stems [1, 2, 5, 9], apical buds [2], flower buds [2] and anthers [1]. Contamination has been a major problem [2, 5, 12]. Media used for callus initiation included amended basal media of Heller [4] and more recently Murashige and Skoog [8]. Callus initiation was most successful in the dark, but the growth rate was slow with all media modifications reported.

This study was undertaken to determine the most appropriate explant source, and to compare the effects of four synthetic auxins and auxin/cytokinin ratios on callus growth.

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** Graduate Research Assistant and Professor, respectively.
Figure 1. Selected stages of in vitro culture of tea. A. Divot explant dissected from greenwood stem; B. Callus development on divot explant around cambial cells; C. Apparent polar growth of callus on divot explant; D. Callus development showing nodular growth pattern.