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Somatic Embryogenesis and Plant Regeneration from Embryonic Axes and Cotyledons Explants of Tea (*Camellia sinenesis* L.)

B. Kaviani
Department of Horticultural Science, Rasht Branch, Islamic Azad University, Rasht, Iran.

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*Corresponding author's email: b.kaviani@yahoo.com

Abstract

In the present study, 2,4-dichlorophenoxyacetic acid (2,4-D) was assessed individually for its effectiveness to induce somatic embryogenesis in tea (*Camellia sinenesis* L.). Embryonic axes and cotyledons explants were dissected from the seeds. Explants were cultured on Murashige and Skoog (MS) medium containing 0, 1 and 5 µM 2,4-D alone for embryonic axes and 0, 1 and 5 µM 2,4-D along with 0 and 0.5 µM IBA for cotyledons. Embryos were observed in embryonic axes explants cultured on MS medium containing 1 µM 2,4-D. No somatic embryos were seen on cotyledons explants.

Keywords: *In vitro* culture, 2,4-D, Plant growth regulators, Medicinal plants, Micropropagation.

INTRODUCTION

Tea (*Camellia sinenesis* L.) is an important species; especially its leaves are used in medicinal and pharmaceutical, also as a drink everywhere around the world. It is proven that biomolecules like flavonoids and antioxidants in tea possess medicinal properties (Ghanati and Ragmati Ishka, 2009). Reports on tea tissue culture including somatic embryogenesis are available, however, rapid multiplication is very poor as the plant is relatively recalcitrant (Akula *et al*., 2000a; Mondal *et al*., 2001a; Suganthi *et al*., 2012). Plant regeneration through embryogenesis often gave variant plants (Diettrich *et al*., 1991). Somatic embryogenesis is considered profitable over other *in vitro* propagation systems as it curtails the proliferation time duration and proves to be potential as an efficient regeneration system with high genetic integrity (Kothari *et al*., 2010). Somatic embryogenesis acts as a key component of *in vitro* propagation. Somatic embryogenesis provides a precious implement to boost the pace of genetic enhancement of commercial crop species (Stasolla and Yeung, 2003). The induction of direct and indirect somatic embryogenesis for *in vitro* plant regeneration provides several advantages over the traditional organogenesis (Wang and Bhalla, 2004). Somatic embryogenesis is a proper morphogenetic system for investigating the cellular and molecular process, also growth and differentiation (Benelli *et al*., 2001). In addition, somatic embryogenesis also provides the possibility to produce artificial seeds and valuable tools for genetic engineering and germplasm conservation by cryopreservation (Litz and Gray, 1995; Merkle, 1997). Direct somatic embryogenesis has a lower probability of genetic variation than other propagation methods (Merkle, 1997) and is the most desirable approach because of genetic stability of regenerated plantlets (Pedroso and Pais, 1995). Auxins especially 2,4-D play the most important role in induction of somatic embryogenesis (Pierik *et al*., 1987; Jain and Ochatt, 2010). Induced somatic embryogenesis from callus (indirect somatic embryogenesis) (often in suspension cultures) can result in variation among seedlings. Thus, the present study was carried out to obtain indirect somatic embryos from embryonic axes and cotyledons explants of tea (*Camellia sinenesis* L.) cultured in media containing 2,4-D and BAP.

MATERIALS AND METHODS

Plant Materials

Seeds of tea (*Camellia sinenesis* L.) were prepared from a tea field in Lahijan city, Guilan province in the northern part of Iran. Seeds of *C. sinensis* were obtained from ripe capsules and embryonic axes and cotyledons were isolated from the seeds and were used as explants.

Disinfection Procedure

Seeds were disinfected after removing the seed coats. Seeds without the coats were rinsed under tap water for 20 min. Then, they were disinfected in 96% ethanol (v/v) for 2 min, followed by 10% H_2O_2 for 10 min and 15% sodium hypochlorite (w/v) for 15 min and then rinsed three times with sterile water. Embryonic axes and cotyledons were excised from the seeds and used as explants. Explants were placed on culture media for experiments.

Culture Media and Treatments

Excised embryonic axes and cotyledons of *C. sinensis* were placed on MS (Murashige and Skoog, 1962) medium (basal salt mixture and vitamins) supplemented with 3% sucrose, 0.7% Agar-agar, 3% mannitol and 200 mg l^{-1} casein hydrolysate. 2,4-D (0, 1 and 5 µM) and BAP (0 and 0.5 µM) were added to the media.

Data Analysis

Analysis of variance (ANOVA) was done using SPSS statistical software and means were compared using Duncan's test at 0.05 level of probability.

RESULTS AND DISCUSSION

After 2 months of incubation of explants on the medium containing 1 µM 2,4-D, somatic embryos emerged around embryonic axes explants without apparent callus formation (Fig. 1). However, the medium including 5 µM 2,4-D induced callus tissues on the explants surface without somatic embryos. Embryos were found mature, with different developmental stages, and germinated with shoots and roots after 3 months, resulting in plantlets after 4 months (Fig. 1, Table 1). Embryonic axes germinated, and developed into plantlets on the initial culture medium. Embryonic axes developed into plantlets after 45 days on culture medium without any plant growth regulators. Explants of cotyledons failed to produce somatic embryos under the same culture conditions. The medium containing 5 µM 2,4-D induced more callus tissues on the explants surface than that of 1 µM 2,4-D. No callus was formed on cotyledons explants cultured on medium without 2,4-D and BAP.

The above results show that somatic embryogenesis occurs *in vitro* on the embryonic axes of Camellia sinensis L., but not on the cotyledons. Thus, success depends on the kind of initial explants used (embryonic axes or cotyledons). Vieitez and Barciela (1990) showed somatic embryogenesis on the cotyledon explants of *Camellia japonica* L. using BAP and IBA. But, they demonstrated that the kind of explants influences significantly the overall percentage of somatic embryogenesis with embryonic axes the most responsive initial explants. Current study revealed that somatic embryogenesis did not occurred on medium without growth regulators. Contrary to this study, Vieitez and Barciela (1990) showed somatic embryogenesis in media without growth regulators. Akula and Dodd (1998) showed direct somatic embryogenesis in *Camellia sinenesis* (L.) O. Kuntze from nodal explants. Study of Ponsamuel *et al*. (1996) on *Camellia sinenesis* (L.) O. Kuntze showed that the highest somatic embryo induction frequency was obtained using phenylboronic acid along with BA or KIN treatments. Most auxin treatments promoted somatic embryogenesis when used in combination with cytokinins. Current study showed that somatic embryogenesis was not formed on medium containing high concentration of 2,4-D. Study of Vieitez and Barciela (1990) and Ponsamuel *et al*. (1996) on *Camellia japonica* L. and *Camellia sinenesis* (L.) O. Kuntze confirmed this finding. Vieitez and Barciela (1990) demonstrated that high concentrations of BAP and IBA were definitely inhibitory. Also, Ponsamuel *et al*. (1996) showed that auxins tested at higher levels produced only nonembryogenic callus from cotyledon explants and at lower levels did not show any morphogenetic response. Vieitez and Barciela (1990) revealed that 2,4-D, the auxin most widely used for embryonic cultures, induced callus but completely inhibited embryogenesis. This finding is not agreement with current study. In current study the somatic embryos were generally formed by direct embryogenesis. Direct embryogenesis on embryonic axes and cotyledons explants of *Camellia japonica* L. were shown by Vieitez and Barciela (1990). Direct embryogenesis differentiation theoretically seems to ensure genetic stability, while capacity for indirect somatic embryogenesis is of interest for the production of somaclonal variations or enabling genetic manipulation (Vieitez and Barciela, 1990). Current study showed induction of somatic embryogenesis and germination on the same medium. Suganthi *et al*. (2012) induced somatic embryos using mature cotyledons obtained from open pollinated flowers of cultivated Camellia species using abscisic acid (ABA) alone and its combination with osmotic. But germination of embryos required a new combination of nutrient medium containing BAP and gibberellic acid (GA3).

In conclusion, in the present study, 2,4-D successfully induced somatic embryogenesis with a minimum concentration range. The most effective concentration of 2,4-D for somatic embryo initiation was 1 µM individually. Embryonic axis was better explants than cotyledon for somatic embryogenesis.

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Tables

Table 1. The mean comparisons for the effect of different concentrations of 2,4-D on induction of somatic embryogenesis in callus of tea (*Camellia sinenesis* L.) formed on embryo axes.

2,4-D Concentrations (µM)	Somatic embryogenesis (%)
0	0b
1	100a
5	0a

Means in a column followed by the same letter are not significantly different at p≤0.05.

Figures

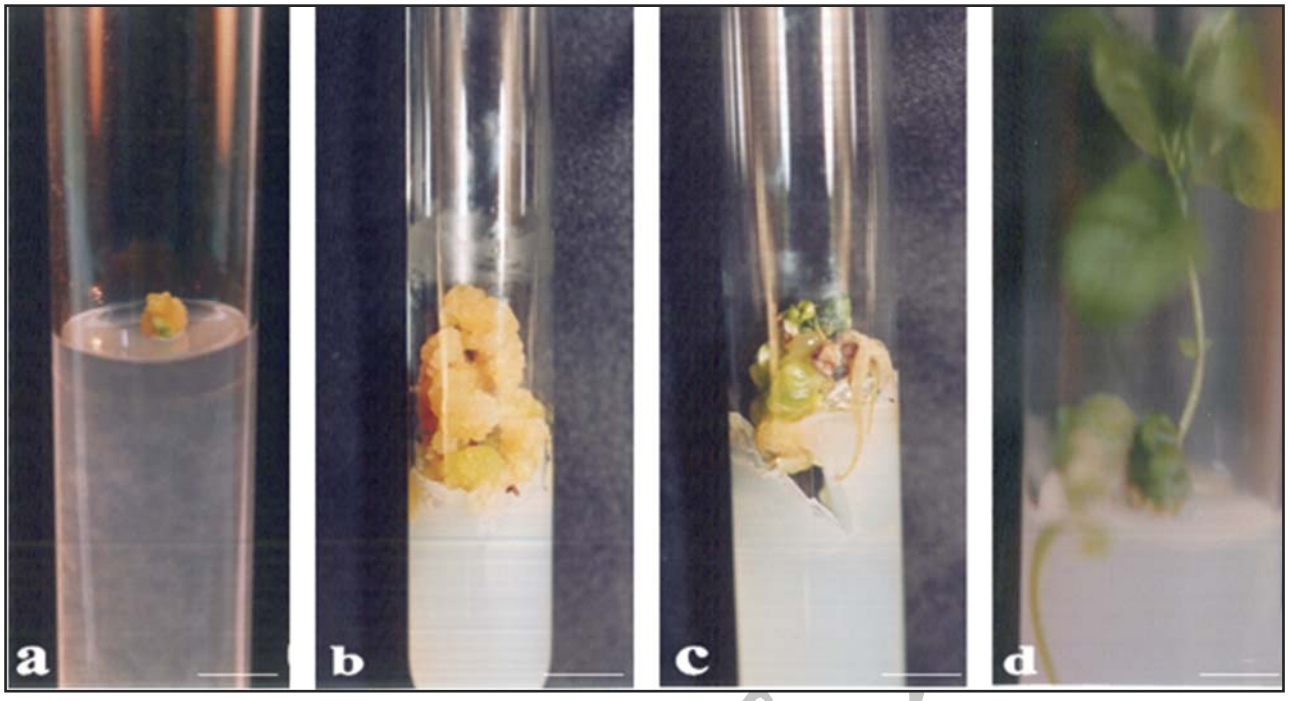

Fig.1. The process of direct somatic embryogenesis and plant regeneration from embryonic axes explants of tea (*Camellia sinenesis* L.). a) callus forming in MS medium containing 2,4-D, b) Callus formation in medium including 5 µM 2,4-D, c) induction of somatic embryos on callus formed on MS medium containing 1 µM 2,4-D and d) plant regeneration from somatic embryos (scale bar = 10 mm).