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High frequency somatic embryogenesis and plant regeneration in tissue cultures of *Codonopsis lanceolata*

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ABSTRACT

Culture conditions for high frequency somatic embryogenesis and plant regeneration from cotyledonary explants of *Codonopsis lanceolata* are described. The maximum induction frequency of somatic embryos from cotyledonary explants was 80% on Murashige and Skoog (MS) medium containing 6% sucrose with 1 mg/l 2,4-dichlorophenoxyacetic acid and 10% coconut water. Upon transfer onto MS basal medium containing 3% sucrose, most somatic embryos developed into plantlets.

Key words : *Codonopsis lanceolata* - Plant regeneration - Plant tissue culture - Somatic embryogenesis

Abbreviations : 2,4-D, 2,4-dichlorophenoxy-acetic acid ; GA_3, gibberellin A_3 ; MS, Murashige and Skoog

INTRODUCTION

Codonopsis lanceolata is a perennial herb belonging to the family Campanulaceae. Its taproot is used in medicine or as a wild vegetable in Korea, Japan and China. Recently, it has been adopted as an ornamental crop. Hence, the demand for such a herb is increasing considerably. In the Japanese bellflower (*Platycodon grandiflorum*), which belongs to the same family, somatic embryos can be obtained from cultured anthers (Harn and Lee, 1976). However, no morphogenesis could be induced in callus obtained from anthers of *C. lanceolata* (Lee et al., 1980). Likewise, protoplasts isolated from stem segment-derived callus form cell colonies without producing any organs (Ahn et al., 1986). In this communication we report the culture conditions for high frequency somatic embryogenesis and plant regeneration from cotyledonary explants of *C. lanceolata*.

MATERIALS AND METHODS

Seeds of *C. lanceolata* (S. et Z.) Trautv. were disinfested with 70% ethanol for 5 min and subsequently, in 25% Clorox solution for 20 min. They were rinsed three times with sterile double-distilled water and placed onto a nutrient agar medium supplemented with 1 mg/l GA_3 to promote germination. After 4 weeks of incubation, 50% of the seeds germinated (without GA_3, 25% germinated). Unless mentioned otherwise, incubation was at 25°C in the dark. Two x 3-mm cotyledonary explants were excised and placed onto media to induce somatic embryos. The basal culture medium consisted of Murashige and Skoog's (1962) inorganic salts, 100 mg /l myo-inositol, 0.4 mg/l thiamine·HCl, 3% sucrose, and 0.8% Bacto-agar (MS basal medium). The medium was supplemented with factorial combinations of either 0, 0.1, 1, or 3 mg/l 2,4-D and either 0 or 10% coconut water (GIBCO). In addition, a medium containing 6% sucrose was also supplemented with the same factorial combinations. All media were adjusted to pH 5.8 before autoclaving 150-ml-aliquots for 15 min at 121°C. Twenty-five-ml of medium was dispensed into 87 x 15-mm plastic Petri dishes. Ten explants were placed in each Petri dish and sealed with Parafilm. Three replicates were prepared for each treatment. Periodically,

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cultures were observed under a dissecting microscope. For histological studies, heart-shaped somatic embryos induced from the explant were fixed in formalin-acetic acid-alcohol, dehydrated in a tertiary-butanol series and embedded in paraffin. Serial sections were cut at 10 μm and stained with 0.5% Hematoxylin and 1% Safranin (Sass, 1971). To regenerate plantlets, cotyledonary somatic embryos were transferred onto MS basal medium at a 16-hr photoperiod with cool-white fluorescent tubes (5 W/m^2).

RESULTS AND DISCUSSION

During the second week of culture, both compact and friable calli developed from the cut edges of the explants cultured on media with 2,4-D, with or without coconut water. Few calli formed without 2,4-D. After 4 weeks of culture, the two types of calli and numerous organized structures proliferated on the surface of explants on media with 2,4-D. The organized structures were induced either directly from explants or from intervening compact callus. Up to 20% of the organized structures obtained from explants on media with 0.1 mg/l 2,4-D arose without an intervening callus (only 10% at 1 mg/l 2,4-D). However, at 3 mg/l 2,4-D, most of the organized structures developed from intervening callus. No organized structures were observed on explants on media without 2,4-D. The friable callus maintained mitotic divisions without producing organized structures. The organized structures were identified as somatic embryos on the basis of their globular, heart-shaped, and torpedo-shaped morphology (Fig. 1A). A histological examination of one of the heart-shaped structures exhibited a bipolar organization with future shoot and root apices (Fig. 1B). Hence, the compact and friable calli were recognized as embryogenic and nonembryogenic calli, respectively. The optimum concentration of 2,4-D for the induction of

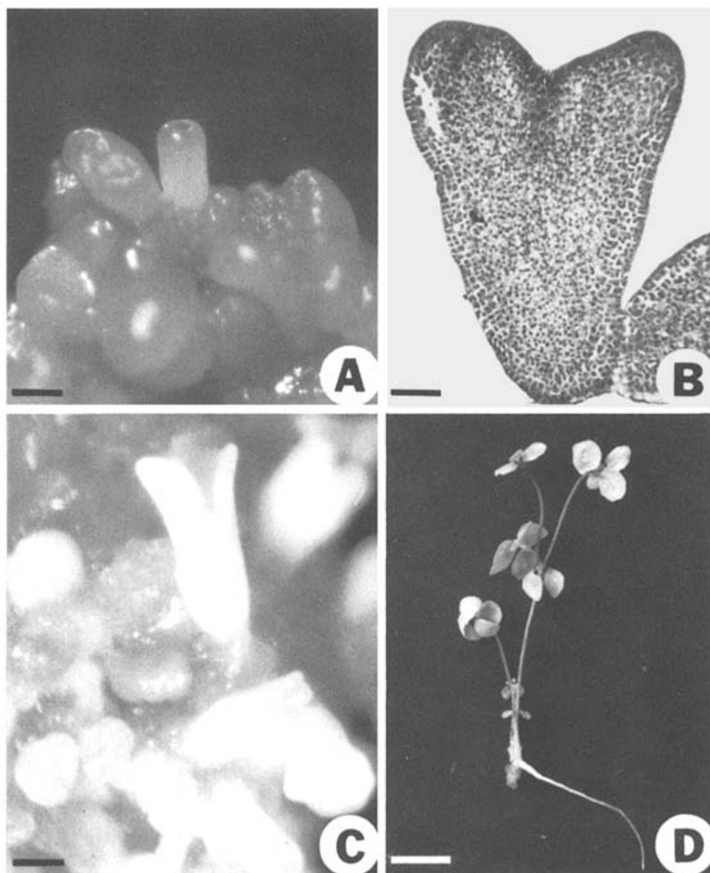

Fig. 1. Somatic embryogenesis and plant regeneration of *C. lanceolata*.
A: Globular to torpedo-shaped somatic embryos derived from cotyledonary explants (scale bar = 900 μm); B: Longitudinal section of a heart-shaped somatic embryo (scale bar = 50 μm). C: Cotyledonary somatic embryos (scale bar = 900 μm) ; D: Plantlet regenerated from somatic embryo (scale bar = 1 cm).

somatic embryos from the explants was 1 mg/l in all treatments except for the medium containing 3% sucrose without coconut water, wherein the highest frequency of embryogenic callus was obtained at 0.1 mg/l 2,4-D. In general, the inclusion of 10% coconut water in the medium promoted the induction of embryogenic callus in the range of 0.1 to 3 mg/l 2,4-D. However, a double level (6%) of sucrose in the medium enhanced the induction of the callus at only 1 mg/l 2,4-D. The maximum induction frequency of somatic embryos from explants reached as high as 80% on the medium containing 6% sucrose with 1 mg/l 2,4-D and 10% coconut water (Fig. 2). Many of the somatic embryos had more than two cotyledons (Fig. 1C). Cotyledonary somatic embryos grew into plantlets (Fig. 1D) when transferred onto MS basal medium. Finally 30 plants were prepared for transplantation to potting soil.

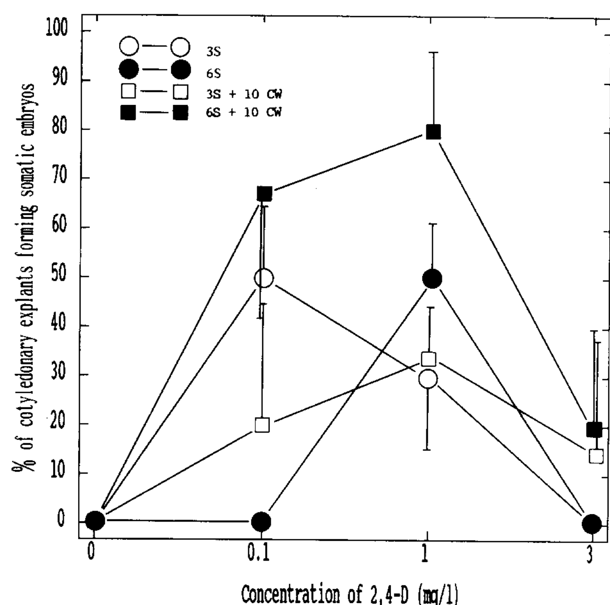

Fig. 2. Effects of various concentrations of 2,4-D, sucrose, and coconut water on the formation of somatic embryos from cotyledonary explants of *C. lanceolata*. 3S: MS medium containing 3% sucrose ; 6S: MS medium containing 6% sucrose ; 10 CW: 10% coconut water. Data were collected after 4 weeks of culture. Vertical bars represent ±SD.

Prior to this communication, the description of somatic embryogenesis in the Campanulaceae (Harn and Lee, 1976) has been reported for only one species, the Japanese bellflower. Most species in this family are widely used as medicinal herbs in Korea, Japan and China, and due to the recent increase in demand for these species, propagation by somatic embryogenesis can be an attractive alternative to the current practice of propagation by root cuttings. Somatic embryogenesis described in this communication will contribute to the practice of micropropagation.

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