

*Micropropagation note*

## Regeneration of plantlets from hypocotyl-derived callus of *Coptis teeta*

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### Abstract

Callus cultures of *Coptis teeta* were established from hypocotyl segments (excised from aseptically germinating seeds) on Murashige and Skoog (MS) medium containing 2,4-dichlorophenoxyacetic acid (2,4-D) and kinetin. Microshoots were produced within 6–7 weeks of subculturing this callus in 1/2 MS nutrient medium supplemented with kinetin alone. Excised microshoots were rooted in 1/2 MS nutrient medium containing indolebutyric acid (IBA). The complete plantlets were hardened and established.

### Introduction

*Coptis teeta* Wall. (family: *Ranunculaceae*) is found in the temperate zone of Mishmi Hills of Arunachal Pradesh in North-East India. The rhizome of this plant contains berberine and is used in the treatment of fever, malaria, bac-kaches and also as a tonic (Mudgal & Jain 1980; Hegde 1988). *C. teeta* has become rare in its natural habitat due to excessive collection for trade (Jain & Sastry 1980). In nature, its reproduction is quite poor. Several attempts have been made to micropropagate medicinal plants including *C. japonica* (Syôno & Furuya 1972; Staba 1977; Nakagawa et al. 1982; Sato & Yamada 1984). Here we report successful micropropagation of *C. teeta* by shoot induction from callus cultures.

### Materials and methods

Plants of *Coptis teeta* Wall. were collected from Mayodia (altitude 2800 m), Arunachal Pradesh, India and maintained in the University Botanical Garden, Shillong (altitude 1500 m). The seeds were disinfested with sodium hypochlorite (1 –

1.2% available chlorine) for 10 min and germinated on MS (Murashige & Skoog 1962) medium supplemented with 2.9  $\mu\text{M}$  indole-3-acetic acid (IAA) + 0.5  $\mu\text{M}$  kinetin at 10°C under 20  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light for 10 h.

Hypocotyl segments (ca 1 mm) from 20-day-old germinating seeds were aseptically cultured on MS medium containing various auxins and cytokinins. The cultures were maintained at 10 to 25°C in 5°C intervals under illumination of 30  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for 10 h. Each treatment consisted of 10 replicates and experiments were repeated thrice.

### Results and discussion

Although complete plantlets have been obtained in *C. japonica* through callus differentiation (Syôno & Furuya 1972), this is the first report on the micropropagation of *C. teeta*. Callus proliferation was observed within 15 days in 65% of the hypocotyl segments cultured on MS medium containing 4.5  $\mu\text{M}$  2,4-D and 0.5  $\mu\text{M}$  kinetin at 10°C. The hypocotyl segments were completely covered by a mass of yellowish-white friable

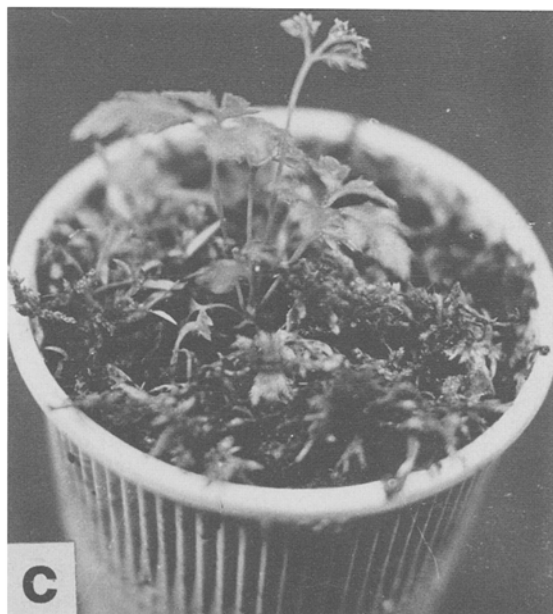
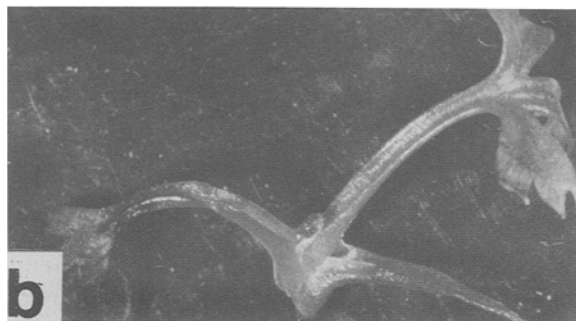


Fig. 1. Micropropagation of *Coptis teeta*. (a) Differentiation of multiple shoots in the subcultured callus, (b) An excised microshoot, (c) A complete plantlet hardened in soil.

callus in 6 weeks, which was subsequently subcultured in the same medium for almost 2 years. Microshoots, 7–8 per culture, were produced within 6–7 weeks in 55% of calluses subcultured on 1/2 MS nutrient medium containing  $4.6 \mu\text{M}$  kinetin (Fig. 1a). Excised microshoots (Fig. 1b) were rooted on 1/2 MS nutrient medium containing  $4.9 \mu\text{M}$  IBA. Roots emerged directly from the base of 65% of the isolated shoots within 5–6 weeks. In this study, optimum temperatures for callus initiation and differentiation were  $10^\circ$  and  $15^\circ\text{C}$ , respectively. The superiority of 2,4-D for callus induction has been reported in *C. japonica* (Syôno & Furuya 1972). Further, the optimum temperatures for callus induction and subsequent differentiation in *C. japonica* were between  $25\text{--}30^\circ\text{C}$ . However, in the present study, lower temperatures were required. The complete plantlets of *C. teeta* (4–5 cm in height) were hardened in plastic pots containing soil rich in humus and covered with a layer of moss (Fig. 1c). About 40% plants survived under glasshouse conditions. Plants were morphologically similar. Attempts for the mass multiplication and transfer to field conditions of *C. teeta* are underway.

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