Plant Cell, Tissue and Organ Culture **28**: 115–117, 1992.

43

Micropropagation note

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

Pramod Tandon & Tilok S Rathore
Plant Biotechnology Laboratory, Department of Botany, North-Eastern Hill University, Shillong 793 014, India

Received 8 January 1991; accepted in revised form 16 August 1991

Key words: callus, differentiation, medicinal plant, micropropagation

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, backaches 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 μM indole-3-acetic acid (IAA) + 0.5 μM kinetin at 10°C under 20 μmol m^{-2} 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 μmol m^{-2} 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 μM 2,4-D and 0.5 μM kinetin at 10°C. The hypocotyl segments were completely covered by a mass of yellowish-white friable

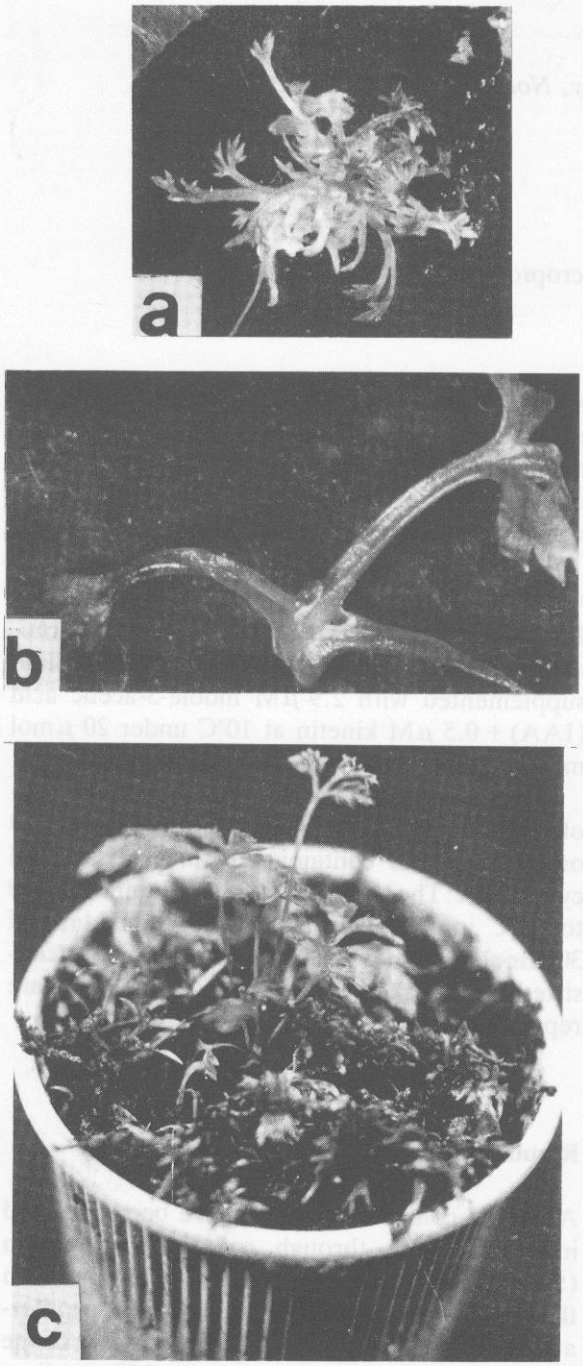

Fig. 1. Micropropagation of *Coptis teeta*. (*a*) Differentiation of multiple shoots in the subcultured callus, (*b*) An excised microshoot, (*c*) A complete plantlet hardened in soil.

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 μM kinetin (Fig. 1a). Excised microshoots (Fig. 1b) were rooted on 1/2 MS nutrient medium containing 4.9 μM 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° and 15°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 25–30°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.

Acknowledgement

This study was supported by a research grant to PT from Ministry of Environment and Forests, Government of India under All India Co-ordinated Project on Conservation of Plants.

References

Borkowska B & Opilowska M (1988) Influence of BA and other cytokinins on proliferation and metabolic status of sour cherry cultures cultivated in vitro. Fruit Sci Rep. 15: 147–156

Hegde SN (1988) *Coptis teeta* Wall. (*Mishmi teeta*) – A rare medicinal plant from Arunachal Pradesh. Arunachal For. News. 6: 27–29

Jain SK & Sastry ARK (1980) Threatened Plants of India, A State of the Art Report (pp 10 & 19). Botanical Survey of India, Howrah

Mudgal V & Jain SK (1980) *Coptis teeta* Wall. local uses, distribution and cultivation. BOBSI 22: 1–4

Murashige T & Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473–497

Nakagawa K, Miura Y, Fukin H & Tabata M (1982) Clonal propagation of medicinal plants through the induction of somatic embryogenesis from the cultured plant cell. In: Fujiwara A (Ed) Plant Tissue Culture (pp 701–702). Abe Photo Printing Co Ltd, Tokyo, Japan

Sato F & Yamada Y (1984) High berberine producing cultures of *Coptis japonica* cells. Phytochemistry 23: 281–285

Staba EJ (1977) Tissue culture and pharmacy. In: Reinert J & Bajaj YPS (Eds) Applied and Fundamental Aspects of Plant Cell Tissue and Organ Culture (pp 699–715). Springer-Verlag, Berlin, New York

Syôno K & Furuya T (1972) The differentiation of *Coptis* plants *in vitro* from callus cultures. Experientia 28: 286