(05-2-26)

Plant regeneration via organogenesis in *C. asiatica*

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Objectives

Regeneration of whole plants of *C.asiatica* was achieved by organogenesis using petiole explants. Optimum culture conditions for shoot organogenesis were determined. As reported earlier, *Centella* micropropagation were experimented in using nodal segments with multiplication(Kavindra et al, 2000), somatic embryogenesis using leaf segments(Ch, Paramageetham, 2004) and regeneration from callus culture(Patra A et al, 1998). All these reports described was useful for producing large scale propagation and conservation of this important neutraceutical herb. However, the aim of the study reported here was to establish an efficient shoot regeneration and then to make successful transformants.

Materials and Methods

1. Material

Seeds of *C. asiatica* obtained from Jeju island of Korea were sterilized with 3% sodium hypochlorite solution containing 0.1% Tween 20 for 10 min and then rinsed twice with sterile distilled water. The culture medium used for the present work was Murashige and skoog(1962) basal medium with 3%(w/v) sucrose and 0.3%(w/v) gellan gum.

2. Methods:

Petiole explants were excised 2.0cm up from the base of leaves produced on 28-day-old shoot culture. MS medium was further augmented with different concentrations of 0.0-13.3μM BA, 2.68-26.85μM NAA, 2.26-22.62μM 2,4-D, 2.32-23.23μM Kinetin and 2.86-28.54μM IAA combinations. Regeneration shoots were separated from petiole explants and sampled at 28 and 30 days after culture, fixed and embedded in paraplast . Plantlets with well-developed roots were removed from culture medium and after washing the roots under running tap water to remove phytagel, plantlets were transferred to plastic cups containning sterilized garden soil and were covered with polythene bags.

Results and Discussion

A whole plant regeneration system was developed for *Centella* utilizing petiole explants, using MS media containing BA(8.9μM) and IAA(17.13μM) incubating for 28 days. Formation of adventitious shoots by indirect shoot organogenesis from the petiole explant was verified by histological sectioning plantlets. The explants were then transplanted to hormone-free MS medium for elongation and rooting. Four weeks later, approximately, the plantlets were able to be transferred to sterile soil for acclimation and to be fully developed in the greenhouse.

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