

CALLUS BIOMASS STUDIES OF *CENTELLA ASIATICA* (L.)

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ABSTRACT

Centella asiatica L. (Apiaceae) is an important medicinal plant. Callus biomass initiated from leaf, leaf with petiole and petiole explants using 0.5 to 3.0 mg l⁻¹ Kn and 1.5 to 3.0 mg l⁻¹ 2,4-D in different combinations. In leaf explant higher biomass was noticed in 2.0 : 2.5 mg l⁻¹ followed by 3.0 : 3.0 mg l⁻¹ combination of Kn and 2,4-D. While 2.5 (Kn) : 2.0 (2,4-D) mg l⁻¹ gave maximum biomass in leaf with petiole and petiole explants.

Keywords : Biomass, Callus, *Centella asiatica*

INTRODUCTION

Plant-based remedies have always been an integral part of traditional medicine throughout the world. The increasing demand for herbal medicines in recent years due to their fewer side effects in comparison to synthetic drugs and antibiotics, has highlighted the need for conservation and propagation of medicinal plant. Plant tissue culture provides efficient techniques for rapid and large scale propagation of medicinal plants and their *in vitro* conservation as germ - plasms. Plant tissue culture also represents one way of possible recovery of some endangered and over harvested medicinal plants as well as a method for providing plant materials for extraction of medicinally important plants. Considerable attention has now been given to the conservation and multiplication of Himalayan threatened species of medicinal importance (Arumugam and Bhojwani, 1990; Giri *et al.*, 1993; Mathur, 1993; Sulaiman 1994).

Centella asiatica L. belonging to family Apiaceae is a weakly scented medicinal plant occurring in parts of India, Sri-Lanka, China, Indonesia, Malasia, Australia and Southern and Central Africa. *C. asiatica* is found throught India in marshy places up to 6,000 ft.. Plant used in several ayurvedic preparations and is reported to possess antileprotic, antifilarial, antifeedant, adaptogenic, antiviral and antibacterial properties (Welth of India, 1950). It is also reported to possess anti – epileptic activity (Moharana and Moharana, 1994). Clinical trials have shown that extract of the plant heals wounds,

burns and ulcerous abnormalities of the skin, cure stomach and duodenal ulcers, and are effective in the treatment of leprosy, lupus, scleroderma and diseases of the veins (Kartnig, 1998). Asiaticoside, a trisaccharide triterpene, has been identified as the most active compound in the plant associated with the healing of wounds and duodenal ulcers, whilst the triterpene saponins are also reported to possess immunomodulatory properties (Plohmann *et al.*, 1994).

Callus biomass studies of *C. asiatica* has not been previously reported. Due to high medicinal importance the plant its population is declining as a result of its over-exploitation. Therefore, the development of a protocol for obtaining the maximum biomass is pre-requisite for tissue culture which is being tried in the present investigation.

MATERIAL AND METHODS

C. asiatica explants (leaf, leaf with petiole and petiole) collected from nature (Oak pine forest of Garhwal) are initially washed with tap water followed by a wash with 1% (v/v) Labolene detergent for 15 minutes and then in running tap water for 30 minutes. The explants are then surface sterilized with an aqueous solution of 0.1% HgCl_2 (w/v) for 2-3 minute. The explants are then rinsed several times with sterilized double distilled water. The cut surfaces exhibiting mercuric chloride damage were aseptically trimmed with sharp, sterile surgical blade. The petiole explants are cultured separately in the medium. The leaf is placed dorsally or ventrally in the medium. Explants are inoculated in conical flasks/test tubes containing MS basal medium (Murashige and Skoog, 1962) supplemented with 3% sucrose and 0.8% agar. The medium is fortified with auxin (2,4-D) and cytokinin (Kn) in varying concentration and combinations as indicated in the results.

The pH of all the media is adjusted to 5.8 ± 0.1 using 0.1 N NaOH or 0.1 N HCl. The medium is autoclaved at 1.06 kg cm^{-2} at 121°C for 25 to 30 minutes. All the culture are incubated at $24^\circ\text{C} \pm 2^\circ\text{C}$ temperature and 60% relative humidity with 16:8 hours light : dark photoperiod.

The fresh callus is weighed and then dried in an oven at 40°C for one week for obtaining dry weight values. Initially fresh weight (FW) and dry weight (DW) values were measured after 30 days and then at the interval of 25 days onwards upto 100 days after inoculation. Three replicates are tried for each combination and the data are tested statistically.

have been depicted in *Psoralea corylifolia* leaves and stem explants (Saxena *et al.* 1997). Higher concentration of Kn (1.5 : 2.5 mg^l⁻¹) in combination with higher concentration of 2,4-D (1.5 : 2.5 mg^l⁻¹) responded towards best callusing in the present study.

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