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MULTIPLE SHOOT REGENERATION FROM THE CALLUS CULTURE OF *CENTELLA ASIATICA* UNDER THE INFLUENCE OF VARIOUS CONCENTRATIONS OF PGRS

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ABSTRACT

Plants have been important sources of medicine for thousands of years. It is estimated that approximately one quarter of prescribed drugs contain plant extracts or active ingredients obtained from plants. It is important to select, multiply and conserve the critical genotypes of medicinal plants. *In vitro* regeneration holds tremendous potential for the production of high quality plant based medicine. Multiple shoots were regenerated from callus obtained from node as an explant of *Centella asiatica* L. Urban on Murashige and Skoog's medium supplemented with Kinetin and Indole-3-butyric acid (IBA). The callus was further subcultured on MS medium supplemented with IBA and various concentrations of kinetin to obtain the optimum shoot regeneration. The callus production was maximum on basal medium supplemented with 3 mg/l IBA + 3 mg/l kinetin and the maximum shoots were obtained on MS + 1 mg/l IBA + 3 mg/l kinetin media. MS medium supplemented with 1mg/l to 2 mg/l IBA gave maximum root initiation in excised shoots.

Key words: *Centella asiatica, callus, multiple shoots, rooting, plantlets*

INTRODUCTION

Centella asiatica L. Urban (Syn. *Hydrocotyle asiatica* L.) of the Apiaceae family commonly known as Indian Pennywort or Mandukparni is a small creeping herb (Fig. A), common all over India growing plentifully in moist localities [8]. *Centella asiatica* is widely used as nervine tonic to increase the mental ability in the traditional system of Indian medicine Ayurveda. The leaves of the plant are considered beneficial in improving memory [1], however the whole plant is used as drug. An oleaginous white crystalline substance Vellarin is the active principle of the leaves [4]. The plant is also very effectively used in the treatment of eczema, epilepsy, insanity, in chronic rheumatism. It is one of the recognized drugs used for Rasayana purpose [4]. Cultivation of wild stock of medicinally important *C. asiatica* has been remarkably depleted and listed as threatened species [5] and an endangered species [7]. Hence there is a need to develop alternative approaches for ensuring the availability of raw material of a consistent quality from regular and viable sources. In order to increase its production, a standard protocol was developed to regenerate the plant through tissue culture.

MATERIALS AND METHODS

Young leaves and nodes were collected from *C. asiatica* plants grown in Botanical Garden, Gujarat University Campus. It was thoroughly washed with running tap water followed by double distilled water. The surface of the explant was sterilized with 0.1% (w/v) systemic fungicide - bavistin and then the explant was washed with 0.1 % (w/v) $HgCl_2$, 5% (v/v) sodium hypochlorite (NaOCl) and 5% (v/v) Tween-20 solution respectively. Finally it was rinsed 4-5 times with sterile double distilled water and used as explant for culture initiation.

Culture Media and Conditions: MS media described by Murashige and Skoog, [3] was used as basal medium throughout this investigation. According to the need of the different experiments, the formulations of MS were suitably modified. 3% Sucrose and 0.8% agar-agar were used. The culture were incubated at 25 ± 2°C under the cool white florescent tubes. MS media was supplemented with the different concentrations (1, 2, 3, 4 mg/l) of 2, 4-D, IBA individually and in combination of 2,4-D + kinetin (in concentration of 1:1, 2:2, 3:3, 4:4 mg/l 2,4-D : kinetin) and IBA + kinetin (in concentration of 1:1, 2:2, 3:3, 4:4 mg/l IBA : kinetin) for the callus culture. After the callus initiation it was further sub-cultured on the MS media supplemented with the various combinations of 1 mg/l IBA and (1, 2, 3, 4 mg/l) kinetin in combination for the shoot regeneration. The regenerated shoots were separated from the clump of the shoots and the excised shoots were cultured on rooting media supplemented with different concentrations of IBA (1, 2, 3, 4 and 5 mg/l) for rooting. Plants with well developed roots were removed from culture medium and roots were washed with double distilled water to remove the trace of agar medium. Plantlets were transferred to plastic bags containing sterilized garden soil. The potted plants were covered with a polythin sheet to maintain the relative humidity. The potted plants were maintained inside a culture room at 25 ± 2°C for 16 hr/day illumination with cool white fluorescent light. After a week, the polythene bags were gradually removed. The plants were kept in the culture room for 2 weeks without polythin sheet cover before transferring outside into the field.

RESULTS AND DISCUSSION

Profuse and rapid callus initiation was obtained from the nodal explant of *C. asiatica* grown on MS media supplemented with 3 mg/l IBA + 3 mg/l kinetin (Table - 1). Surprisingly 2, 4-D, which is generally helpful in initiating callus, did not respond at all for callus initiation. The proliferated callus obtained from the nodal segment was subcultured on MS medium supplemented with 1mg/l IBA + 3mg/l kinetin, 1mg/l IBA + 2mg/l kinetin, 1mg/l IBA + 3mg/l kinetin, 1mg/l IBA + 4mg/l kinetin and 1mg/l IBA + 5mg/l kinetin for the shoot initiation (Figs. B, C & Table - 2). Maximum number of the shoot formation was obtained on MS media supplemented with 1mg/l IBA + 3mg/l kinetin but higher concentration of kinetin i.e. MS + 1mg/l IBA + 5mg/l kinetin was not promoting the shoot initiation. MS media supplemented with 1mg/l to 2 mg/l IBA gave maximum root initiation in excised shoots (Fig. D, Table - 3).

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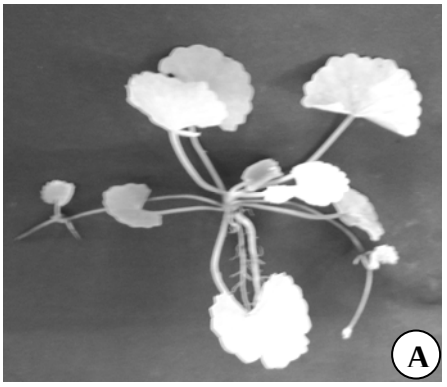
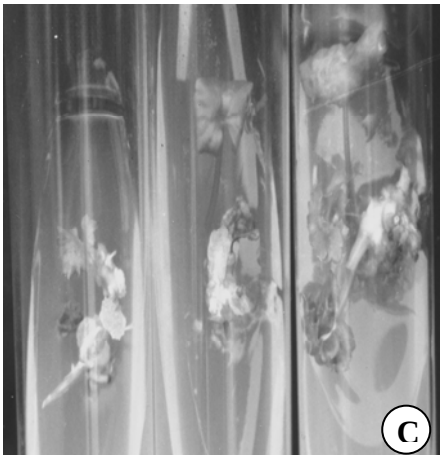


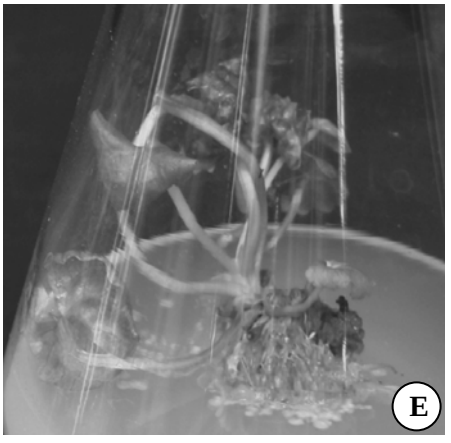
C. asiatica

Emergence of multiple
Shoots from node



Shoot proliferation

Rooted plantlets



Profusely Rooted plantlets

Acclimatized plantlets

100 % rooting frequency was obtained on medium supplemented with 2 mg/l IBA. The rooted plants were transferred to plastic cups (Figs. E, F) and polybags containing sterile peat and soil mixture (1:1). With 90% survival the acclimatized plantlets were successfully established in the field with only 10% mortality.

Tiwari et al., [9] developed a protocol for rapid and large-scale *in vitro* clonal propagation of the valuable medicinal herb *Centella asiatica* by enhanced axillary bud proliferation in nodal segments isolated from mature plants and could induce the optimum frequency (91%) of shoot formation as well as shoot number 4 to 5 only on synergistic combination of 22.2 μM BA and 2.68 μM NAA. However Patra et al., [6] succeeded plant regeneration from callus cultures of *C. asiatica* while Banerjee et al., 1999 [2] reported multiplication of *C. asiatica* from leaf explant. Banerjee et al., [2] also found promontory effect of IBA in rooting in *C. asiatica*, while IAA and lower level of sucrose was reported optimum by Patra et al., [6]. He achieved 55 to 65% survival on transfer of rooted plantlets into pots containing soil: sand: well rooted cow dung manure in the ratio of 1:1:1.

Table - 1 Media for callus initiation

Basal Media	Plant Growth Regulators			Rate of callus initiation
	2,4-D (mg/l)	IBA (mg/l)	Kinetin (mg/l)	
MS	-	-	-	-
	1	-	1	-
	2	-	2	-
	3	-	3	-
	4	-	4	-
	-	1	1	-
	-	2	2	++
	-	3	3	++++
	-	4	4	+

- = No callus; + = Very less callus; ++ = Less callus; +++ = Moderate callus; ++++ = Optimum callus

Table - 2 Media for shoot regeneration

Basal Media	Plant Growth Regulators		Rate of shoot regeneration
	IBA (mg/l)	Kinetin (mg/l)	
MS	-	-	-
	1	1	-
	1	2	+++
	1	3	++++
	1	4	++
	1	5	+

- = No shoots; + = Very less shoots; ++ = Less shoots; +++ = Moderate shoots; ++++ = Optimum shoots

Table - 3 Rooting response of *Centella asiatica*

Basal Media	Plant Growth Regulators IBA (mg/l)	Rate of root formation
MS	1	++++
	2	++++
	3	++
	4	+
	5	-

- = No root; + = Very less root; ++ = Less root; +++ = Moderate root; ++++ = Optimum root

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