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IN VITRO PROPAGATION OF *CENTELLA ASIATICA* L. BY USING COCONUT WATER AND HOUSE HOLD SUGAR

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ABSTRACT
The present investigation aimed at developing in-vitro propagation protocol, which can be used for conservation of *Centella asiatica.* The MS medium supplemented with house hold sugar i.e. 30gm /L and coconut water with various concentration instead of phytohormones. Various concentration of coconut water i.e. 2.5%, 5%, 10% and 15% were used. Ms medium supplemented with 5% and 10 % shows the maximum percentage of shooting and rooting. Micro- propagated plantlets were hardened, acclimatized and transferred to the field. This micro propagation procedure could be useful for mass multiplication of superior plant material for field cultivation, as well as, research purpose.

KEY WORDS: Coconut water, Household sugar, Micro propagation.

INTRODUCTION

The major application of plant tissue culture lies in the production of true-to-type high quality planting material that can be multiplied under aseptic conditions on a year round basis any where irrespective of season and weather. Micropropagation is a capital-intensive technology involving energy and labour. This problem has been addressed by inventing reliable cost effective tissue culture methods without compromising on quality of plants. Cost of chemical inputs, media, energy, labour and capital counts on production cost. The cost of medium preparation can account for 30-35% of the micropropagated plant production. Therefore, low cost alternatives are needed to reduce cost of production of tissue-cultured plants (George, 1993; Anonymous, 2004). Low cost technology means an advanced generation technology in which cost reduction is achieved by improving process efficiency and better utilization of resources (Savangikar, 2002). Low cost options should lower the cost of production without compromising the quality of the micropropagules and plants (Anonymous, 2004). The present study aims at developing a cost effective protocol for *in vitro* clonal propagation of *C. asiatica*.

Centella asiatica (L.) Urban, synonym *Hydrocotyle asiatica* (Family: Apiaceae) is a small perennial herb, commonly known as Mandukparni. In India, this species is mostly found in the swampy areas up to an altitude of 600 to 1800 m asl (Patra et al., 1998). Medicinally, *C. asiatica* used as memory enhancer and in the treatment of chronic diseases, mental disorders and neuropharma-cological disorders like insomnia, insanity, depression, psychosis, epilepsy and stress (Chopra et al., 1980). The major bio-active ingredients in the plant are the triterpenes, asiatic acid, madecassic acid and their glycosides such as asiaticoside and madecassoside (Zheng and Qin, 2007). Due to the presence of these active ingredients, it possesses antileprotic, antifilarial, antibacterial, antifeedant, adaptogenic and antiviral properties (Warrier et al., 1994). The roots contain many polyacetylenic compounds, the major compound being 8-acetoxyfalcarinol (Loc and Tam, 2010).

In addition, triterpene madasiatic acid, madecassoide and asiaticoside, active in treatment of leprosy, as well as 3-glycosyl quercetin, 3-glycosyl kaempferol and 7-glycosyl kaempferol were also isolated from leaves of *Centella* (Rastogi and Mehrotra, 1993). Due to its high medicinal value this plant has high demand for pharmaceutical industries. The requirement of *C. asiatica* is now being met from the natural population leading to their depletion. Developing low cost tissue culture techniques can play an important role in the rapid multiplication of elite clones, *in vitro* biomass production and germplasm conservation of *Centella asiatica*.

The present investigation aims at developing a viable cost effective protocol, which can be used for the true to type mass propagation, *in vitro* biomass production and conservation of *C. asiatica* to meet the pharmaceutical demand. In this investigation house hold sugar were instead of sucrose and coconut water instead of phytohormones.

MATERIALS AND METHODS

Collection and surface sterilization of explants

The explants were collected from Amrutkund Tq. Basavkalyan, Dist. Bidar near Maharashtra-Karnataka border. The plant material were washed carefully in running tap water for 10 minute and followed by distilled water for 5 minutes. For surface sterilization, chemical such as 70% ethanol and Hgcl2 (0.1 %) were used. Explants kept for 1 minute in 70% ethanol after the 1 minute they are also sterilizing with 0.1% mercuric chloride for 3 minute followed by three subsequent rinses with sterilized double distilled water in a laminar flow. All these explants were dissected very carefully. Explants were cut into small pieces and aseptically inoculated in test tube as well as culture vessels containing MS medium with various concentration of growth hormones.

Media Preparation

Throughout the study, different strengths of Murashige and Skoog (1962) medium were used for experiment for the in vitro multiplication. Different concentration of coconut water was used as low cost alternative. The household sugar i.e. 30 gm / lit was used instead of sucrose as a carbon source in the all combination. All media combination were solidified by adding 1.5 gm / lit clerigel. The pH of of the medium was adjusted between 5.6 to 5.8 use in 0.1 N HCL or 0.1 N NaOH solution prior to the autoclaving of the medium. Sterilization of the medium was done at a pressure of 15 psi for 20 mins and was allowed to cool at room temperature. After inoculation all culture tubes were inoculated at 25$\pm$ 2^0 C under cold white florescent tubes.

Inoculation of explants

Apical shoot was used as explant. The explants were inoculated on MS (Murashige and Skoog 1962) medium supplemented with different concentration of coconut water istead of phytohormones eg, BA, KIN, NAA for studying the *in vitro* multiplication responses. **The house hold sugar (30gm/L) was used in all the concentration.**

RESULTS AND DISCUSSION

Explants inoculated on MS medium supplemented with different concentration of coconut water instead of phytohormones as a low cost alternative. In the present study coconut water was taken with different concentration i.e. 2.5 %, 5.0 %, 10.0%, 15.0% and household sugar was added i.e. 30 gm / lit. The treatment of coconut water resulted in shoot as well as root induction (Table 1; Figure 1 and 2). Maximum shoot numbers per explants (17. 66 $\pm$ 1.45) was recorded. when MS media supplemented with 10 % coconut water followed by 15 % coconut water which were significantly higher than others (Table 2). While maximum shoot length (1.33 $\pm$ 0.37) was observed on MS Media supplemented with 10% coconut water.

Root initiation was achieved from the bases of excised shoots in the presence of coconut water (2.5 $\pm$ 15 %) After 3 to 4 weeks of transfer among all concentration tested maximum root proliferation recorded on MS medium with 10% coconut water, with maximum number of roots (15 $\pm$ 3.60) and the root length (6.7 $\pm$ 1.14) as shown in table no.2.

Table 1:- Response of *Centella asiatica* L. to various concentration of coconut water

Percentage of Coconut water	2.5%	5%	10%	15%
Coconut water Response	+	+++	+++	++

Table 2:- Effect of different concentration of coconut water on in vitro shooting and rooting.

Concentration of coconut water	No. of shoots	No. of roots	Length of shoots in cm	Length of roots
2.5%	5:6 $\pm$ 0.33	2.33 $\pm$ 0.87	3.4 $\pm$ 0.73	1.8$\pm$ 0.35
5%	13.33 $\pm$ 1.32	12 $\pm$ 1.52	5.83 $\pm$ 0.39	4.39 $\pm$ 1.45
10%	17.66 $\pm$ 1.45	15 $\pm$ 3.60	7.33 $\pm$ 0.37	6.73 $\pm$ 1.14
15%	16 $\pm$ 1.15	10.33 $\pm$ 0.32	6.23 $\pm$ 0.40	4.46 $\pm$ 1.22

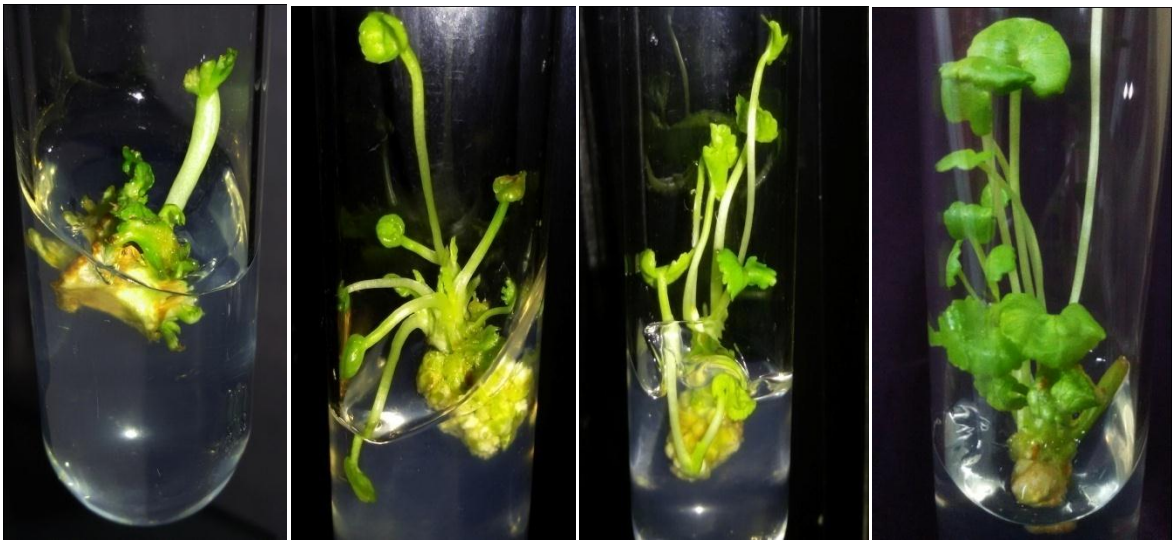

Fig: A **Fig:B** **Fig:C** **Fig:D**

Fig:E **Fig:F** **Fig:G** **Fig:H**

Figure: 1.
A-H –Showing shoot and root initiation at 10% concentration of Coconut water. A. initiation of shoot from the explant. B. multiplication of shoots. C-F. multiplication of shoot and basal callus formation. G. initiation of roots from regenerated shoots. E&F. multiplication of roots.

Fig: i **Fig:ii** **Fig:iii** **Fig:iv**

Figure: 2.
i- iv –Showing shoot initiation at 5 % (1&2) & 15% (3&4) concentrations of Coconut water. 1. Initiation of shoot from the explants. 2. Multiplication of shoots at 5% Coconut water. 3 & 4. Multiplication of shoot and basal callus formation at 15% coconut water.

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