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Effect of Hormones and Sucrose on Maturation of Somatic Embryos in *Centella Asiatica* And it's Qualitative Analysis.

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Abstract
Somatic embryos were obtained from explants of *Centella asiatica* cultured on MS medium containing different concentrations and combinations of auxins and cytokinins. Somatic embryos upon maturation on MS basal medium 2,4D (2mg/L) + IAA (3mg/L) and shoot regeneration was obtained on MS medium containing BAP (2mg/l) + IBA (1mg/L). Regenerated shoots with rooting transferred to field conditions grew normally.

Key Words
Centella asiatica, Qualitative Analysis.

Introduction
Plant based drugs plays important role in traditional and conventional medicine throughout the world. The demand for herbal medicines continuously increased due to their lesser side effects when compared with synthetic drugs. Pharmaceutical companies largely depend upon plant bulk material procured from natural habitats causing depletion of this important plant. Hence it has become imperative to establish a protocol to regenerate this plant using tissue culture techniques in large scale proportion of this important medicnal plant and invitro conservation of germplasm of this plant as the plant has been listed in International Union for Conservation of Nature and Natural Resource (IUCN) and as an endangered species (Sharma etal., 1998). *Centella asiatica* belongs to Apiaceace family is prostrate, stolon runner and perennial herb that grow well in damp conditions. The plant contains triterpenoid saponins such as asiaticoids triterpenic acids and brahmoside. The plant is also known as Gotu kola.. Gotukola has been used as a medicinal herb for thousands of years in India, China Indonesia *Centella asiatica* is an important herb in Ayurvedic medicine. The plant was first used in India where it is a part of Ayurvedic medicine and is popular as a nerve tonic to promote relaxation and to enhance memory in present work emphasized on somatic embyoids production which proved powerful tool for genetic manipulation due to somaclonal variation in somatic embryogenesis. Herbal Medicine is the major component in all traditional medicine system in Siddha, Ayurveda, and Homeopathy in countries like China, India and African. The present day environmental crisis, over exploitation of certain plants for their medicinal application lead to the severe threat to the biodiversity and necessitated the implementation of modern conservation steps, in order to protect from extinction.

Materials and Methods
Centella asiatica plants were collected nearby fields of Visakhapatnam District Andhra Pradesh India. Healthy explants like nodal segments, petioles and leaves were collected, washed thoroughly under tap water for several times, then the explants were surface sterilized with 0.1% aqueous mercuric chloride solution for 3 min and washed with sterile distilled water finally. After surface sterilization the explants were cultured on MS medium (Murashige and Skoog 1962) containing 3% sucrose and supplement with various concentrations and combinations of auxin (2,4D,NAA,IBA,IAA) and cytokinin (BAP and Kn) and PH of media adjusted to 5.7 before adding 0.8% agar (W/V)and autoclaved at 120 ^{o}C for 15min under LB pressure conditions. The cultures were inoculated under laminar air flow cabinet and further cultured in the culture room at 25^{+}_2^{o}C under 16hrs Photoperiod and with a light intensity 1000lux (white fluorescent light).

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TLC

Somatic embryoids taken and subjected to drying at 60^{o}C and macerated with methanol for 10 hrs and filtered under vacuum and concentrated, this residue was taken and coated on precoated gel GF254 TLC (E. Merck)and developed using n-Butanol: Glacial acetic acid :water(5:1:4) as a mobile phase. The spots were visualized by spraying with Anisaldehdye-Sulphuric acid reagent and RF values were compared with reference standard.

Results and Discussion

Various explants like leaf, nodal segments, and petioles were used. Different concentrations and combinations of auxins and cytokinins were tried. 2, 4D+Kn, 2, 4D+IAA were found effective in induction of Somatic embryos. Higher concentrations Kn of (3 mg/l) in combination with 2,4D (2mg/l) resulted in high embryogenic calli is 84%. Different concentrations of Kn (0.5-4mg/l) were tried in combination with auxin 2,4D of these concentrations 2, 4D (2mg/l) + Kn(0.5mg/l) the frequency of embryogenesis is 28 % 2,4D(2mg/l) + Kn(1mg/l) is 48%, 2,4D (2mg/l) + Kn(2mg/l) is 60%, 2,4D (2mg/l) +Kn(4mg/l) is 80%. (Table1). In the first week of culture highly granular and shiny masses of calli were produced in small groups from the abaxial surface of leaf, leaf petiole region, nodal segments (Plate a,b,c,d,e fig). Later they turned into globular structure and light green in color in the second week. During the third week of culture these structures started to turn dark green color. At first embryo like cell cluster appeared from the surface cells and grew into heart shaped structure observed microscopically. In the fourth week of culture some heart shaped embryos differentiated into torpedo and finally to cotyledonary stage. This change of stages of Somatic embryos is called maturation, this occurred on special medium, maturation medium MS basal medium without growth regulators where somatic embryos matured when transferred to BAP (2mg/L)+IAA(1mgL) . After 4 weeks of culture on germination medium, somatic embryoids transferred to BAP (2mg/l) +IAA (1mg/l) where plantlets were raised physiologicaly there was no difference between *invitro* raised plants and *invivo* plants. Sucrose plays an important role as carbon source in maturation of somatic embryos sucrose(1-4%) were tested, out of which 4% found very effective in germination of somatic embryos.

Qualitative analysis

TLC studies of the somatic embryoids showed blue spots having Rf values 0.41 and 0.39 respectively. The Rf value 0.39 very near to value of reference standard. Thus the compound asiaticosides is a major active component.

Somatic embryogenesis has proved to be useful for micro propagation and production of mutants; artificial seeds for use in plant genetic engineering Tanava et.al (2000). The pattern of development of embryoids was found from intervening callus but in the present study direct development embryoids was observed in auxin-cytoxinin (2,4D+Kn) and auxin – auxin (2,4D+IAA) combination induced somatic embryogenesis Centella our present plant similar reports were reported earlier by Chengalray as et.,al (1999) in peanut. The auxin – cytoxinin combination found very effective in induction of somatic embryoids, these results were substantiated by the similar type of observation made by many earlier Prasad babu et.,al (1997). Higher concentration of cytoxinin (Kn) in combination with 2,4D in our study resulted in production of high quantity of embryogenenic Calli is 84%.

Sucrose 4% effective where as 2% effective in Eucalyptus by Chowdary et. al (2004). It was clear that depending on plant species percentage of carbon source varies in somatic embryoid maturation.

Conclusion

Since several years from time of civilization a large number of medicinal plants have been used for treatment of several diseases. As Ayurveda Unani, homeopathic systems of medicine are based on plants or plant products. To overcome over exploitation of plants with medicinal value conservation is taken up by tissue culture was found to be novel method. *Centella asiatica* was selected for conservation as the plant have potent medicinal compounds in it, the above medium found to be very effective for large scale propagation.

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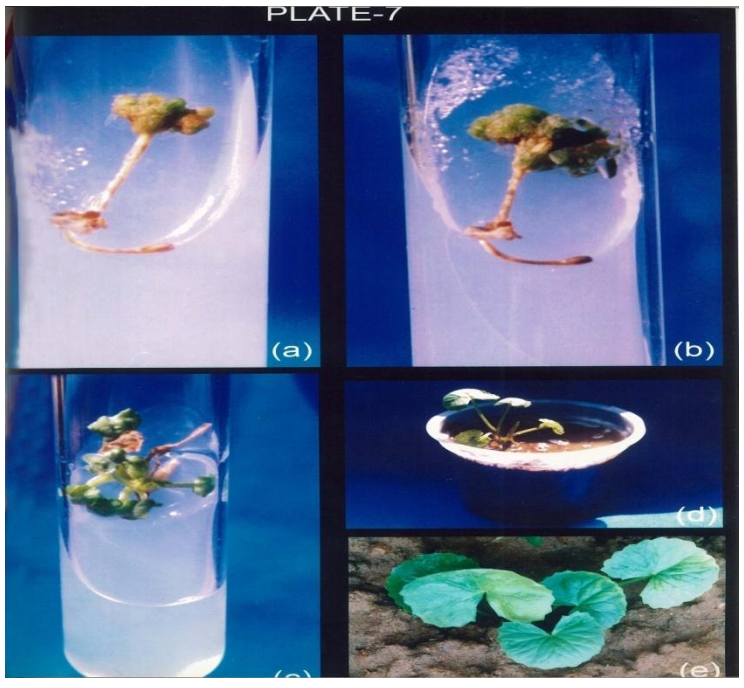

Fig. 1: Explants of *Centella asiatica* cultured on MS media.

Table 1: Effect of hormones 2,4D AND IAA on somatic embryoids induction.

S. No.	Plant hormones		Total number of cultures	Number of cultures responded	Frequency percentage (%)
	2,4D mg	Kn Mg			
1	2	0.5	25	7	28
2	2	1	25	12	48
3	2	2	25	15	60
4	2	3	25	21	84
5	2	4	25	20	80

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