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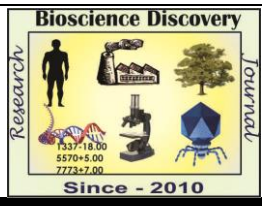

Somatic embryogenesis of medicinally important herb *Centella asiatica* L.

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Abstract

Centella asiatica is a small herbaceous plant belonging to Apiaceae (Umbelliferae) family. This paper reports the induction of somatic embryogenesis. An efficient protocol was designed for successful regeneration of *Centella asiatica* (L.) from *In vitro* derived callus through somatic embryogenesis. Leaf explants were isolated and cultured on MS medium fortified with 0.5,1.0,1.5 & 2.0 mg/l 2,4- D. The callus formed, compact, light green to greenish coloured. It was observed that the increased concentration of 2,4- D induced the formation of embryoids. Some differences in the morphology of callus such as changes in the colour and texture was also observed with increasing the concentration of 2,4- D. Maximum frequency of callus induction was noticed on 2.0 mg/l 2,4- D. The calli were separated and further cultured on fresh media containing BAP alone and in combination with auxins such as NAA and IAA. The maximum shoots were recorded on KIN & BA 1.5, 2.0mg/L with combination of 0.2 mg/l IAA and NAA. The well regenerated healthy micro shoots were separated and transferred to rooting medium for rooting. MS medium supplemented with IAA 2.0, mg/L & NAA 2.0 showed maximum rooting frequency. The well rooted plants were transferred to field conditions.

INTRODUCTION

Centella asiatica is a small herbaceous plant belonging to Apiaceae (Umbelliferae) family. Centella comprises some 50 species (James and Dubery, 2009), inhabiting tropical and sub-tropical regions. This perennial creeper flourishes abundantly in moist areas and is in the subfamily Mackinlaya (Liu *et al.*, 2003) previously included in hydrocotyle (Brinkhaus *et al.*, 2000), occurring in swampy areas of India, Sri Lanka, Madagascar, Africa, Australia (Schaneberg *et al.*, 2003)

Centella asiatica has originated in the wetlands of Asia (China, India, and Malaya). It apparently spread through the South Pacific and to Mauritius, Madagascar, East and South Africa, Turkey, and the southeastern United States many centuries (or perhaps millennia) ago. Centella grows in India up to an altitude of 600 –1800 meters above sea level (Tiwari *et al.*, 2000; Patra *et al.*, 1998) on moist, sandy or clayey soils, forming a dense green carpet.

The plant is prostrate, faintly aromatic stoloniferous perennial herb, with a glabrous stem and long petiolated fleshy leaves rooting at the nodes. The flowers are greenish to pinkish-white and are borne in dense umbels (clusters in which all the flower stalks arise from the same point) on separate stems in the summer. The seeds are pumpkin-shaped nutlets, 0.1-0.2 in (3-5 mm) long.

C. asiatica possesses a wide range of pharmacological effects, being used for wound healing, mental disorders, antibacterial, antioxidant and anticancer purposes. The plant is highly effective in ulcerpreventive (Cho, 1981), anti-depressive sedative and ability to improve the venomous insufficiency (Zheng and Qin, 2007). The plant is found to improve the power concentration, general ability and behavior of mentally retarded in children (Appa Rao *et al.,*1973) and to treat rheumatic disorders (Howes and Houghton, 2003). Asiaticoside is one of the prime triterpene saponin found in leaves in large amount is utilized commercially as a wound healing agent due to its potent anti-inflammatory effect (Pointel *et al.,*1987; Shukla *et al.,*1999) and showed the potential use as anti -gastric ulcers drugs (Cheng *et al.,* 2004).

Somatic embryogenesis offers an alternative and efficient protocol for plant regeneration. The technique of somatic embryogenesis has also contributed for the genetic, morphological and physiological manipulation (Sharma *et al.,* 2010). Embryogenic tissue was identified as a target tissue for transformation by many researchers (Steward *et al.,* 1996; Maughan *et al.,* 1999). Somatic embryogenesis offers several advantages in crop improvement, as cost-effective and large-scale clonal propagation is possible using bioreactors, ultimately leading to automation of somatic seed production and development of artificial seeds. Besides, such a system could also provide a new source for use in genetic transformation.

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 medium were used for experiments. Full strength MS basal medium and modified MS medium with different concentrations and combinations of BA, KIN, 2,4-D IAA, NAA for studying the in vitro multiplication responses were used as the initiation medium. Sucrose (30%) was used as the carbon source in all the combinations. All media combinations were solidified by adding 0.4% clerigel instead of agar.

The pH of the medium was adjusted between 5.6 and 5.8 using 0.1N HCl or 0.1N NaOH solutions prior to the autoclaving of the medium. Sterilization of the medium was done at a pressure of 15 psi for 20 min and was allowed to cool at room temperature. After inoculation all culture tubes were incubated at 25 ± 2^0 C under cool white fluorescent tubes.

Induction of SE:

The SE induction medium used in this study was based on MS (1962) medium. This medium was supplemented with several concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D). Four concentrations of 2,4-D (0.5, 1.0,1.5, and 2.0 mg/L) were used. Cultures were maintained at $25\ ^0C \pm 2\ ^0C$ in the dark for 3 to 4 weeks until nodular callus with early stages of somatic embryos was seen.

Development of SE:-

Once the induction of embryogenic callus and somatic embryos was evident, these calli were subcultured every 3 to 4 weeks to the same medium for the production of SEs and were kept in the dark. For further development and their germination, SEs was subcultured into hormone-free MS medium. Somatic embryos were cultured for 4 weeks under a 14-h photoperiod at $25\ ^0C \pm 2\ ^0C$ and observed periodically to record various stages in the development.

Shoot formation

MS medium supplemented with various concentrations of KIN & BA 0.5, 1.0, 1.5, 2.0mg/L with combination of 0.2 mg/l IAA gives average percentage of shoot multiplication.

Root formation

The formed shoots were transferred on MS medium supplemented with various concentrations of IAA 1.5, 2.0; mg/L & NAA 2.0 mg/L gives average percentage of root multiplication.

Acclimatization and hardening

Well developed rooted plants were carefully removed from culture tubes and washed to remove traces of clerigel. These healthy shoots were transferred to tray containing soil and vermiculate in 1:1 ratio for acclimatization. For a period of week the plants were kept in polythene membrane. After that the surviving plants were transferred to pots and maintained under greenhouse for hardening

RESULT AND DISCUSSION

Somatic embryos were induced on leaf explants when cultured on MS basal medium supplemented with 2,4-D. Onset of nodular callus and development of SEs was observed on explants after 3 to 4 weeks of culturing in the dark. Younger unfurled leaves showed

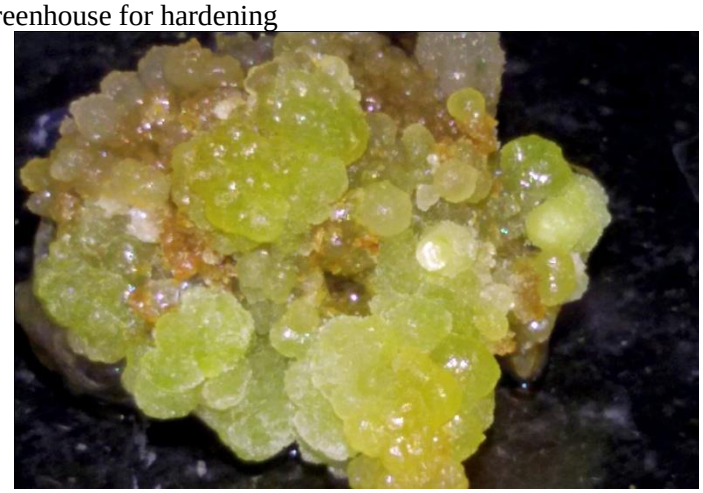

Photo plate: 1 Callus formation

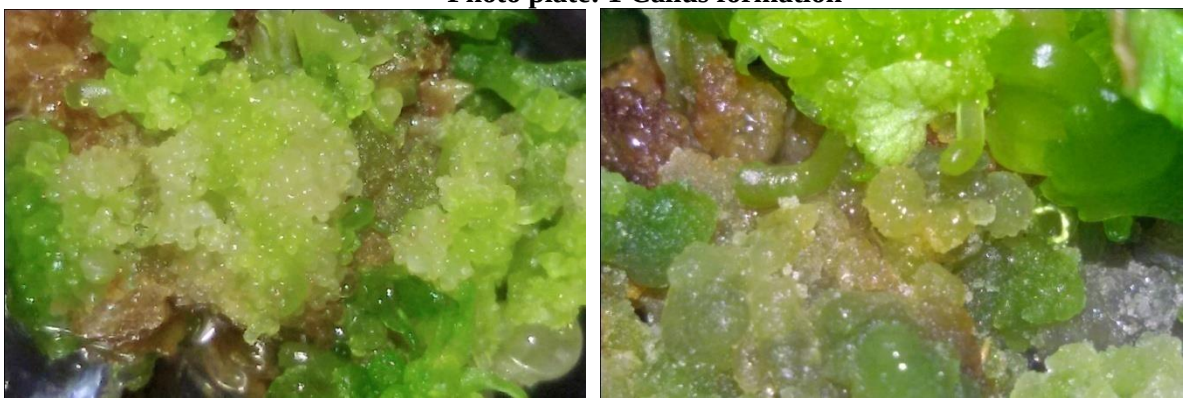

Photo plate: 2 Induction of somatic embryogenesis

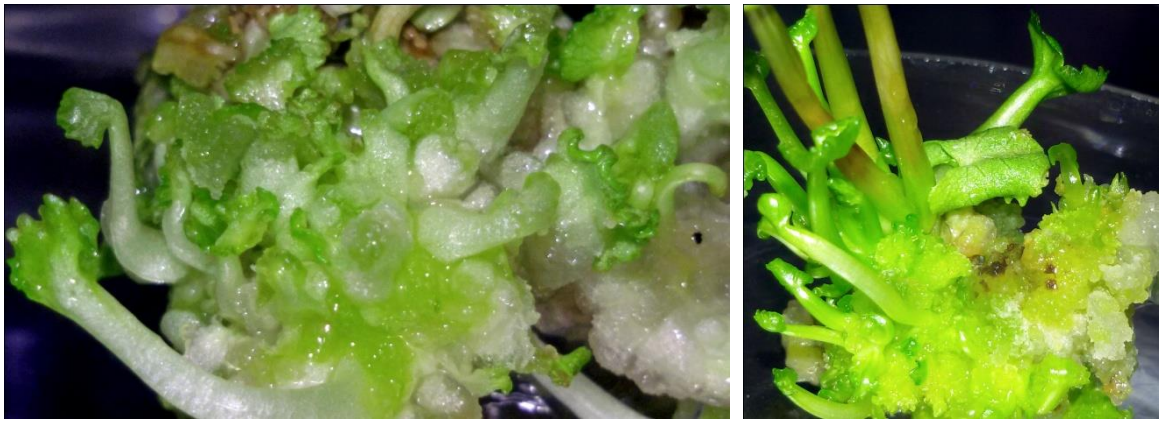

Photo plate: 3 & 4 Regeneration from somatic embryoids

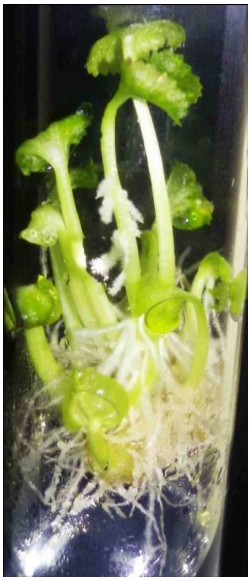

Photo plate: 5 & 6 Regeneration of plant

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