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In vitro flowering and regeneration of *Centella asiatica*

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ABSTRACT

In vitro plant regeneration was achieved from explants of nodal segments on MS medium containing different concentrations and combinations of auxins and cytokinins. *In vitro* flowering was noticed from nodal explants cultured on MS medium supplemented with BAP 2 mg/L + NAA 1mg/L. Multiple shoot induction was observed on MS medium supplemented with BAP + NAA (0.5 - 4 mg/L). The regenerated shoots were transferred onto MS Medium fortified with IBA for root induction. The regenerated plantlets have been successfully established in soil and they were shifted to field condition.

Key words: Plant regeneration, auxins, Multiple shoot induction and MS Medium.

INTRODUCTION

Today plant based drugs plays as important role in traditional as well as 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 materials procured from naturally occurring stands causing rapid depletion of this important source of medicinal herb. Hence, it has become imperative to establish a suitable protocol to generate enough materials to ensure its supply for pharmaceutical industries without further depleting this species.

This clearly high lightened and pointed out the need for conservation and propagation of medicinal plants through tissue cutter techniques for rapid and large-scale proposition of medicinal plants and their *in vitro* conservation of germplasm for future purposes.

C. asiatica belongs to the family apiaceae and vernacular name is gotu-kola., Petioles of Centella asiatica are 1-15 cm. long. The flowers are pinkish purple in color, 1mm wide, stamen ; the flowers are bisexual and self-fertile.

C.asiatica in an important medicinal plant in several ayurvedic preparations. It is reported to possess antipyloric, antileprotic, antibacterial and wound healing properties. The species, however, in rapidly getting depleted from natural habitat due to over exploitation by the pharmaceutical companies. This has also been listed as threatened species by the International Union for Conservation of Nature and Natural Resources (IUCN) and as an endangered species (Sharma *et al.*, 1998) [1].

In the present studies we have developed as efficient protocol for in vitro propagation and *in vitro* flowering of *C.asiatica.*

MATERIAL AND METHODS

C.asiatica plants were collected from CMR gardens situated at Boyapalem, Visakhapatnam, and Andhra Pradesh, India. Healthy explants like nodal segments, leaf petiole 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 wasted with sterile distilled water finally. After surface sterilization the explants were cultured on MS Medium (Murashige and Skoog , 1962)[2] containing 3% sucrose and supplement with various concentrations and combination of cytokinins (BAP, KN) auxins (2,4D, NAA, IBA, IAA) and Gibberellins (GA3) and PH of media adjusted to 5.7 before adding 0.8% agar (W/V) and autoclaved at 1200c for 15 min under 15 LB pressure conditions.

The Cultures were inoculated under laminar air flow cabinet and further cultured in the culture room at 25 0± 20c under 16 hrs. Photoperiod and with a light intensity 1000 lux (white fluorescent light).

RESULTS AND DISCUSSION

C. asiatica nodal explants are existed and inoculated on MS medium containing BAP and NAA. Multiple shoots were induced from the nodal explants .The percentage of response varied with the type of growth regulator, concentration and the nature of explants used. Shoot induction was also observed on nodal explants. BAP and KN showed variable responses and BAP was more effective than KN in induction of multiple shoots.

Plate : 1 (A, B, C, D) A shows maximum percentage of shoot proliferation was 90% observed on a MS medium containing BAP (4 mg/L) and NAA (2 mg/L) and minimum 40% . Similar result was reported in *vitex negundo* (Jayabalan *et al.*, 2001)[3] with BAP and NAA but with different concentration. (Fig: 1) Number of shoots per explants 8 shoots observed and length of the shoots were measured to be 7-8 cm after 3 weeks of inoculation of explant. The superior activity of BAP compared to other cytokinins were reported in many plants *Gymnena sylvestra* (Komalavalli and Rao., 2000)[4]. Among the two explants the nodal segments responded well than the petiole explants for multiple shoot induction on MS medium containing BAP + NAA, different concentration (0.4-4 mg/L) BAP + NAA (0.5-2 mg/L) were tested.

Multiple shoots which are produced on BAP (4 mg + NAA 2 mg/L) per explants were excised carefully and with different concentrations of NAA, IBA separately out of which IBA induced maximum number roots with concentration IBA (1mg/L) and percentage of response is 90%. Table (IBA 3mg/l also induced 90% of response but with intervening callus was observed. The medium supplemented with IAA (0.5-2 mg/L) induced poor rooting with intervening callus. Similar reports of IAA inducing rooting were previously reported in *plumbago rosea* on MS media containing NAA (0.5-2 mg/L) also

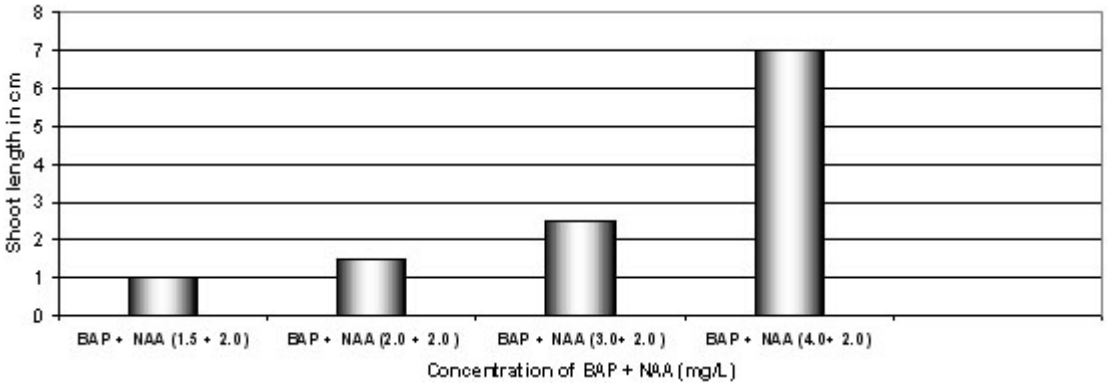

Fig. 1: Effect of growth regulators on shoot length from nodal segements of *Centella asiatica* using BAP + NAA

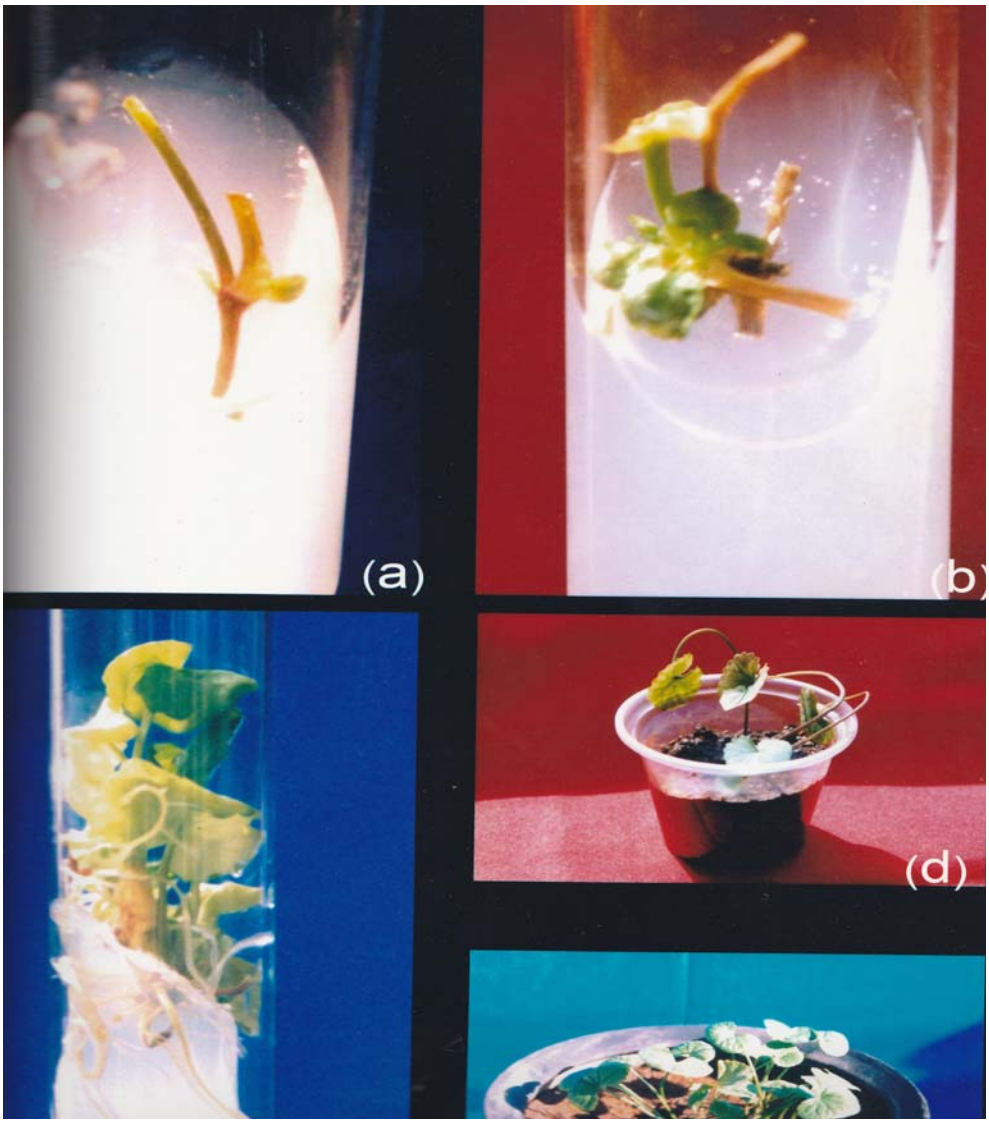

Plate 1

induced rooting and the response was similar to that of IAA. IBA found to be superior in inducing root induction when compared to other auxins used. Sufficiently grown plantlets were transferred to the plastic cups containing sterile soil and were kept under room condition. Humidity was maintained by covering each plastic cups with transparent polythene bags. The bags were removed after 3 weeks of transfer to soil conditions and the plantlets in the pats were transplanted in the fields 85% of survival was noticed in the transplanted plants as *c. asiatica* shoots wide range of variation in growth accordingly to season even seeds produced only in winter our present work tried could be useful to mass propagate the *centella asiatica* which has medicinal values noted is history.

***In vitro* flowering**

Flowering was considered to be a complex process regulated by both internal and external factors and its induction of *in vitro* flowering under this type of culture conditions is extensively rare

under *in vitro* flowering was observed within 45 days of inoculation of nodal explants containing BAP (2mg/L) + NAA (1mg/L). After flowering successful seed set formation occurred in the same conditions i.e. MS medium containing BAP (2 mg/L) + NAA (1mg/L).

CONCLUSION

Since dawn of the civilization a large number of plants have been used for treatments of various ailments. Ayurveda, unani and homeopathic systems of medicine are based on plants or plant products .As the wild resource of medicinal plants has already been depleted due to unscientific collection practices, extinction of rare species and adulteration of plant materials are the problems now faced by the phyto based pharmaceutical industry. To overcome these problems the propagation and conservation of medicinal plants by tissue culture was found to be novel method. *Centella asiatica* was selected for conservation, the above medium found to be very effective for large-scale production of this important medicinal plant.

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