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In vitro Root Induction of *Centella asiatica* (L.) in Different Concentrations of Indol 3-Butyric Acid (IBA)

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Abstract: ***Centella asiatica L. is an important herbal medicinal plant, abundant in Jharkhand. It is considered a brain tonic and also a good wound healer. The study was conducted to determine the most suitable explant and different concentrations of IBA for adventitious root induction. The detailed study of adventitious root induction has been performed by varying the concentration of IBA in the range of (0, 0.5, 1.0, 1.5 2.0) mg/l. The highest percentage of explants forming adventitious roots, number of roots per explant, and longest roots generated from leaf explant were observed in the concentration of 1.5 mg /l IBA, Tap roots were also observed in nodal explant.***

Keywords: Explant, adventitious roots, IBA, *Centella asiatica*, tap roots etc

1. Introduction

In traditional medicine, the roots of many plants are used and harvested by local people for the production of herbal medicine. Roots are an abundant source of much-valued secondary metabolites, which can be beneficial to mankind for combating various ailments. The rootstock of *Centella aciatica* is consists of rhizomes, a growing horizontal underground stem that puts out lateral shoots and adventitious roots at intervals. An adventitious root formation is a key step in the vegetative propagation of many crops and therefore of at most economic importance (Scheres 2000)**.** They are pale yellow in color and covered with root hairs. They are capable of producing some economically important compounds such as asiaticoside and madecassoside which are active triterpene compounds produced in insignificant amounts in the root of *Centella asiatica* (L.) Urban. The asiaticoside has wound curative ability by increasing angiogenesis and collagen formation. It enhances the stretching strength of newly formed skin and promotes the wound's healing. Madecassoside is an active antioxidant that has the ability to scavenge free radicals. Therefore, the root cultures can display high biosynthetic capabilities that are often comparable to those of normal roots (Keveres et al 1999). The culture of adventitious roots is a potential source for the production of valuable plant secondary metabolites on a commercial scale (Min et al, 2007) Roots are the principal material for the preparation of drugs from the traditional system of medicine.

The present study is based on the regeneration of adventitious roots in response to different concentrations of IBA. Phytohormones play a significant role in the formation of adventitious roots. Adventitious roots are a special type of root system that arise from an organ other than the root, usually from non-root parts of plants such as leaves, petioles, nodes, and internodes, and help plants to survive in environmentally adverse conditions. Several complex molecular processes such as endogenous and exogenous physiological factors like stress and wounding are responsible for the formation of adventitious roots. These roots are formed during normal growth and development or either in the reaction to wounding, nutrient deficiency, or various kinds of environmental stresses. Adventitious roots (AR) also facilitate gas and water transport and uptake of minerals and nutrients and ensure plant survival (Sauter, 2013). Well- organized root regeneration was observed on an MS medium containing different concentrations of IBA on different explants of *Centella asiatica*.

2. Materials and Methods

Sampling
Mature and healthy branches of *Centella asiatica* with no visible symptoms of the disease were carefully selected were collected from the herbal garden of Ranchi Women's College, brought to the laboratory in sterile bags, and processed within 24 hr after sampling. These plantlets were identified by Prof. Kunul Kandir, Taxonomist of the University Department of Botany, Ranchi University

Surface sterilization of the explant
The surface sterilization was done as per the procedure of Tejovathi et al. (1996). Initially, the plant samples were washed for 5-10 minutes thoroughly under running tap water to remove adhered dust and debris. It was then washed with teepol and was cut into small pieces with 1.5 mm segments which were surface sterilized with 70% ethyl alcohol for 1 min. The segments were soaked in 0.2% (w/v) mercuric chloride solution for 1 min and then rinsed with 70% ethyl alcohol for 1 min, and finally rinsed thrice with sterile distilled water.

Media preparation:
1 L of Murashige and Skoogs media were prepared by using various inorganic nutrients, it includes macro and micronutrients along with vitamins and carbon sources in 400 ml double distilled water. The pH was adjusted to 5.8

and finally, 0.8% Agar-Agar (Hi Media, India) was dissolved as gelling agent to provide support to the culture for their establishment. Agar was melted in a water bath until a clear solution will appear and the final volume was made up to 1L. The media was supplemented with various concentrations of IBA in the range of (0.5, 1.0, 1.5, and 2.0mg /l) to support the growth of the explant. In each culture tube, 20 ml culture medium was dispensed carefully, and covered with a cotton plug. Autoclaving was done at 15 lb/inch^2 pressures so that the temperature became 121ºC for 15-20 minutes. Each treatment with six replicates was repeated thrice.

Inoculation

Inoculation was done in the aseptic chamber under Laminar Air Flow Cabinet. Different explants were taken as an inoculum such as leaves and the nodal part. After surface sterilization the explants were cut into small pieces and the intact leaves were inoculated in the Media containing different concentrations of IBA. With the help of the sterile forceps, one explant was selected and placed vertically on the culture medium. After inoculation, the culture tubes were capped with sterile cotton plugs.

Incubation:

All the cultures were placed aseptically in an incubator under white fluorescent light (2000 Lux.) with 24 hours photoperiod at 25±1°C temperature. The observation was made on an alternate day to note if there was any contamination. Contaminated cultures were discarded.

3. Observations

Roots were observed after 20 days of incubation. The maximum number of roots per leaf explant and nodal explant culture was observed and carefully studied. Basal media supplemented with 1.5 mg/l IBA showed a best result than the media containing 2.0 mg/l IBA. Further, it was also observed that both the leaf and nodal part (fig 2A-2B) is best suited for rooting. Tap roots (Fig.2B) were observed in nodal explant along with primary and secondary roots. The morphology of the leaf was also changed during root induction. In leaves, hypertrophication (Fig 3A-3C) was observed. Approximately 1.0 -3 cm long roots were observed in the basal media supplemented with 0.5 mg/l IBA. Longest root observed from petiole base and the junction of lamina and petiole (Fig.1 D) approx. 6-7 cm in MS media containing 1.5 mg/l IBA.

Here **Graph 1**. represents no. of day vs. no. of roots. The roots were observed after 20 days and the no increases in as the days increase. After 40 days a stationary period was reached when the increase in length of roots were stopped this is probably due to the limitation of nutrient. Maximum nutrients were consumed by the roots after 40 days. **Graph 2.** shows Avg. no. of Roots vs conc. of IBA in mg/l, again 1.5 mg/l of IBA proved to be best and shows maximum no. of roots.

Graph 3 shows the maximum length of root 6.8 cm was observed in 1.5mg/l of IBA, it was in the range of 1- 7 cm. in length. Graph 4. Shows maximum no. of roots emerged from the lamina of the leaves rather than petiole base in the concentration 1.5 mg/l of IBA. And the longest roots were also observed in the explant containing 1.5mg/l IBA. Clustered fibrous roots were observed from the lamina of the leaf (Fig.4A)

Effect of various concentrations of auxin on adventitious roots induction from leaf explant of *Centella aciatica* after 20 days of culture.

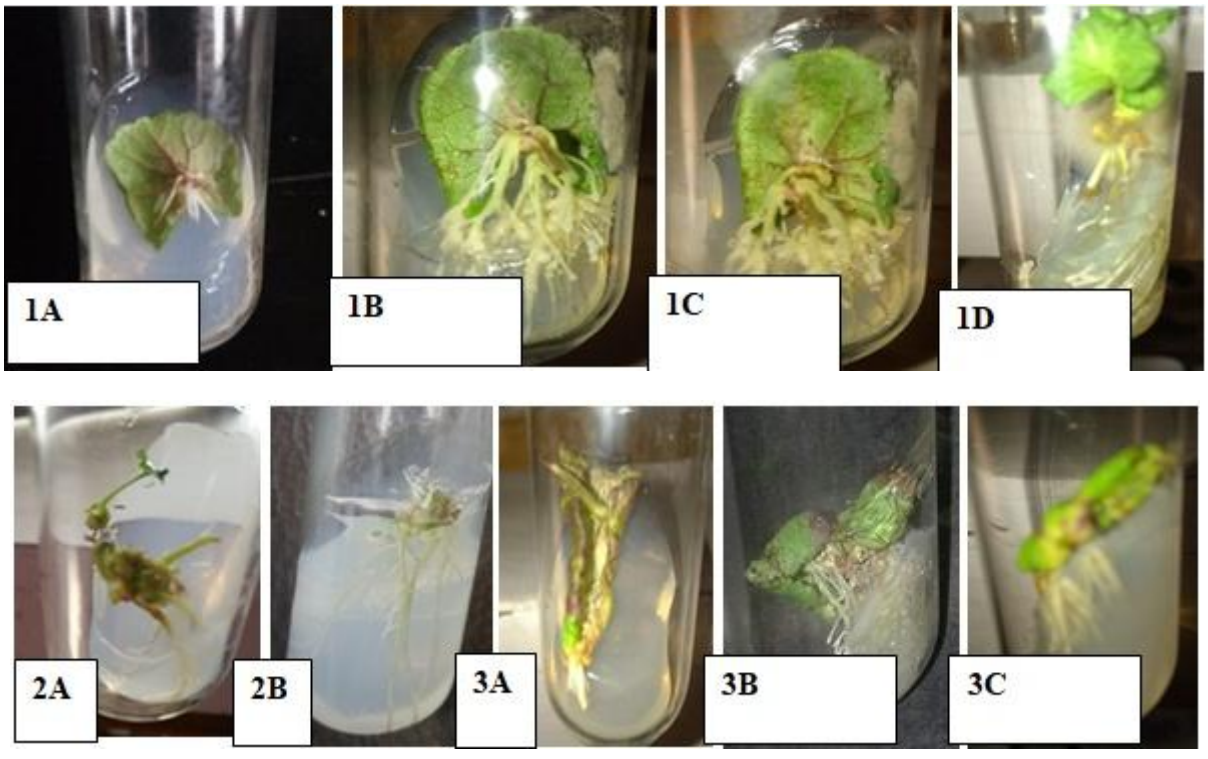
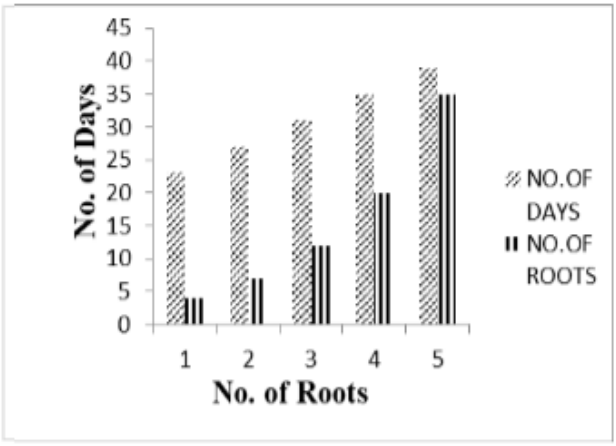
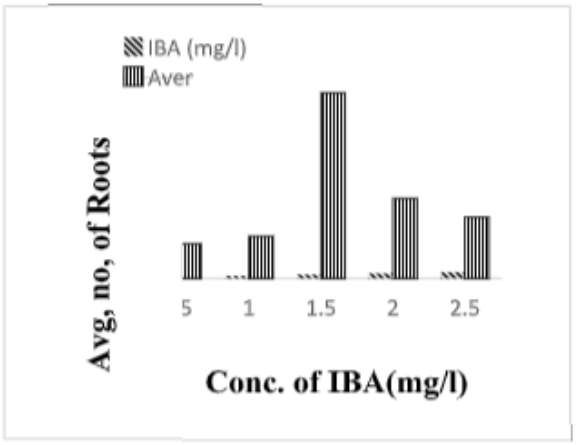


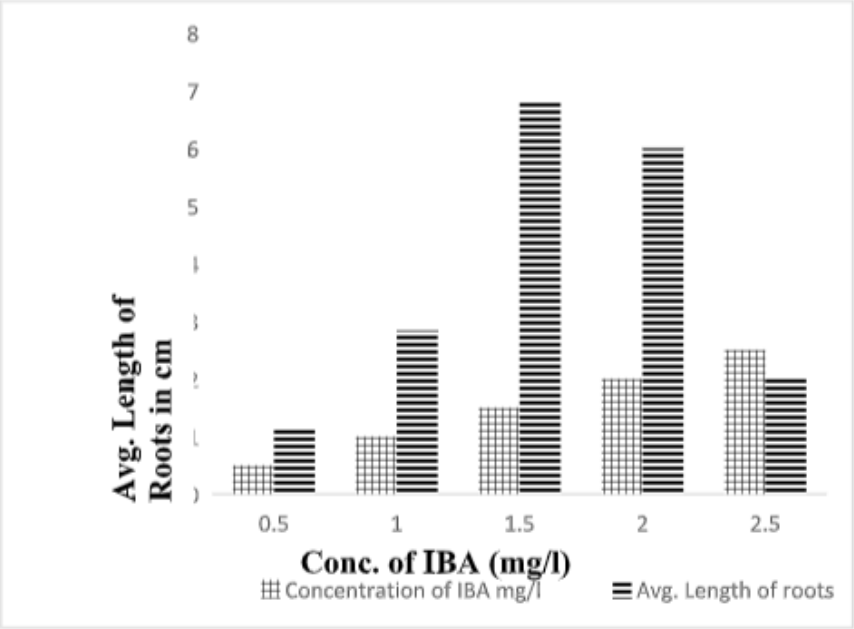
Figure 1A-1C: Rooting from petiole base and the junction of lamina and petiole
Figure 2A-2B: Tap Roots from nodal part along with leaf
Figure 3A-3C: Rooting from Mid rib of the leaf
Figure 4A-4B: Roots emerging from lamina (dorsal surface of the leaf



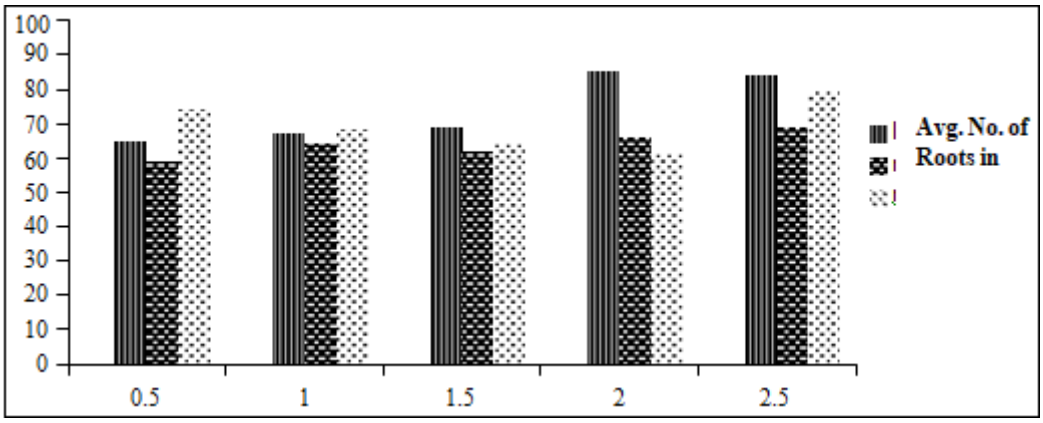
Graph 1: No. of Days vs. No. of Roots



Graph 2: Avg. no. of Roots vs Conc. of IBA in mg/l



Graph 3: Avg. length of roots vs. Conc. Of IBA (mg/l)



Graph 4: Avg. no. of Roots vs Conc. of IBA

4. Result and Discussion

IBA-derived auxin has a strong effect in various aspects of root development, including regulation of root apical meristem size, root hair elongation, lateral root development, and formation of adventitious roots. From this experimental study it was seen that 1.5mg/l of IBA is best for root regeneration from various parts of leaf and the nodal parts of plant, and it was also seen that 1.5mg/l IBA is best suited in all aspects including no. of roots and root length.

We achieved an efficient rooting when, 1 to 7 cm long creamish roots were observed after 20 to 40 days duration in MS media supplemented with 0.5mg/l to 2.5mg/l IBA. It was observed that minor changes in the IBA concentration can markedly alter the number of adventitious roots produced. Mohapatra13 reported rooting of in vitro raised shoots was best induced on half strength MS supplemented with 0.5 mg dm-3 indole-3-butyric acid (IBA). In the present study, petiole derived roots generally have higher rooting percentages and produce more adventitious roots than nodal explant-derived roots.

5. Conclusions

The present study proves that different concentrations of Indol -3-butyric acid are best suited for the regeneration of roots in vitro conditions. And it was seen that with the age of the culture, the nutrients also consumed fast and the length of the roots attains its maximum length. Rooting was highest (90%) on full- strength MS medium containing 1.5mg/l IBA. It was also seen that no. of roots e merging from the petiole is highest in comparison to other parts of the plant.

Conflicts of Interest

The authors declare no conflict of interest.

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