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Effects of IAA, NAA and IBA on the vegetative propagation of *Centella asiatica* (L.) Urban

Mohammed Sala Uddin, Tanjina Islam, Abu Saleh Musa, K M Mesbaul Alam
Professor, Department of Botany, University of Chittagong, Chattogram, Bangladesh

Abstract
Centella asiatica (L.) Urban is a prostrate, herbaceous, perennial plant of Apiaceae family that obtain various medicinal activities. The quality of the germination of seeds is poor. Therefore, the effects of IAA, NAA and IBA on the vegetative propagation by nodal or stem cuttings of the plant was analyzed. A total of 13 treatments were performed on the cuttings following the quick dip technique and transplant in sandy polybag. The whole evaluation was conducted following completely randomized design. Data were recorded at 30, 60 and 90 days after planting. The maximum number of roots, length of roots, length of petiole, number of leaves, length of leaf, width of leaf, number of stolons and total fresh biomass were observed to be 14, 15.87 cm, 6.83 cm, 46, 2.63 cm, 3.28 cm, 12 and 14.66 g respectively at 90 days after planting in the cuttings treated with IBA- 750 ppm solution (T_{12}) whereas control (T_1) showed minimum rooting and shooting performance. The present study concludes that IBA- 750 ppm solution would be considered as the most suitable for producing maximum growth performance of *Centella asiatica* at 90 days after planting.

Keywords: *Centella asiatica*, vegetative propagation, stem cuttings, rooting hormone, auxins

Introduction

Centella asiatica (L.) Urban is a small edible, perennial, prostrate, herbaceous traditional medicinal plant belonging to the Apiaceae family commonly known as Gotu kola, Indian pennywort and Thankuni in Bengali. It is generally found in the paddy fields, riverbanks, marshy areas and forest undergrowth of Bangladesh, India, Sri Lanka, Indonesia, Malaysia and South Africa (Hein *et al.* 2025, Uddin and Alam 2022) [1, 2]. It is also indigenous in China, the western South Sea Island, Australia, Madagascar, Southern United State and tropical America. It is treated as Chinese herbal around two thousand years ago and Indian Ayurvedic medicine over three thousand years ago. It has an aromatic fragrance (Hein *et al.* 2025, Rahman *et al.* 2013) [1, 3]. The Chinese, Indian and Malays utilize this plant for multi problems ranging from treatment of skin problem, asthma, brain tonic, tetanus, Immune system deficiency, hair loss, liver aliments epilepsy, Circulatory problems and mental disorder (Hein *et al.* 2025) [1]. In addition, this medicinal herb is also known for neuroprotective, antidiabetic, cardioprotective, anti-inflammatory and antioxidant activities (Chunthawodtiporn *et al.* 2025 and Singh *et al.* 2023) [4, 5]. Nodal or stem cutting is a common vegetative propagation of *Centella asiatica*. Low percentage of the germination of seeds is one of the barriers for its propagation. Due to storage seeds lose their viability and capacity to germinate (Gandi and Giri 2014) [6].

Literature review reveals that no specific study has yet been done on the study of vegetative propagation in Bangladesh. The present study was under taken to evaluate the effects of IAA (Indole-3-acetic acid), IBA (Indole-3-butyric acid) and NAA (α-Naphthalene acetic acid) on the vegetative propagation by nodal or stem cuttings of *Centella asiatica* (L.) Urban.

Materials and Methods

The stem of *Centella* plant was used as plant material. Cuttings were prepared 5-8 cm in length having 1-2 nodes. All the leaves were cut off. IAA/NAA/IBA solution was made by suspending 0.1g analytical auxin hormone into 20 ml alcohol. Subsequently a stock solution of 1000 ppm IAA/NAA/IBA was prepared by including 80 ml purified water to the solution. Finally, 250 ppm, 500 ppm, 750 ppm and 1000 ppm IAA/ NAA /IBA were prepared by including 75 ml, 50 ml, 25 ml and 0 ml, water to the 25 ml, 50 ml, 75 ml and 100 ml of stock solution respectively. A total of 13 treatments were used, they were: T_1- Control, T_2- 250ppm IAA, T_3- 500ppm IAA, T_4- 750ppm IAA, T_5-1000ppm IAA, T_6- 250ppm NAA, T_7- 500ppm NAA, T_8- 750ppm NAA, T_9- 1000ppm NAA, T_{10}- 250ppm IBA, T_{11}- 500ppm IBA, T_{12}- 750ppm IBA and T_{13}- 1000ppm IBA. Cuttings were treated following the quick dip method (Bhagya and Sreeramu 2013) [7]. Basal ends of the cuttings were dipped in treatment solution for ten seconds and then air-dried and lastly treated cuttings were planted in sandy polybag.

It was done on the 1st week of June, 2023 in rainy season (June-August) which is the proper time of vegetative propagation for maximum plants (Waman *et al.* 2019) [8]. Watering and weeding were done as when required. Data for number of roots, length of roots, length of leaf petiole, number of leaves, length of leaf, width of leaf, number of stolon and total fresh biomass per cutting were collected at 30, 60 and 90 DAP (Days after planting). The whole experiment was done following complete randomized design. Three replications were used for each treatment and statistical analyses were done using MS Excel 2013.

Results and Discussion

The results (Fig. 1 & 2) reveal that the number of roots and the length of roots varied with the change of different treatments. Maximum number of roots were found to be 5, 11 and 14 at 30, 60 and 90 DAP respectively in T_{12} (IBA- 750 ppm) and minimum in T_1 (control). Highest length of roots was found to be 15.7cm, 24.23cm and 27.51cm at 30, 60 and 90 DAP respectively in T_{12} (IBA- 750 ppm) and

lowest in T_1 (control). ANOVA of number of roots and length of roots at 90 DAP indicated significant value ($P<0.01$) with treatments (Table 3).

It is reported that the auxin is used to generate root initiation for the stem cuttings (Husen and Pal 2007) [9]. It was also enumerated that increased number of roots in Mehedi (*Lawsonia inermis* L.), Guggul [*Commiphora wightii* (Arn.) Bhan.] and olive (*Elaeagnus angustifolia* L.) cuttings treated with IBA (Uddin and Musa 2024, Sure *et al.* 2018, Porghorban *et al.* 2014) [10-12] These reports are analogous to the findings of the present experiment.

The results of length of leaf petiole and number of leaves at 30, 60 and 90 DAP are presented in Table 1 & Fig. 5. Highest length of leaf petiole was found to be 3.47, 5.26 and 7.47 at 30, 60 and 90 DAP respectively in T_{12} (IBA- 750 ppm) and lowest in T_1 (control). Maximum number of leaves were found to be 14, 28 and 46 at 30, 60 and 90 DAP respectively in T_{12} (IBA- 750 ppm) and minimum in T_1 (control). ANOVA of length of leaf petiole and number of leaves at 30, 60 and 90 DAP indicated significant value ($P<0.01$) with treatments (Table 1). Higher or lower concentration of auxin did not significantly produce a better rooting result (Ling *et al.* 2013) [13]. It is recorded that the maximum number of leaves found in IBA-750 ppm in Guggul plant (Kumrawat *et al.* 2024) [14].

The results of length and width of leaves at 30, 60 and 90 DAP are displayed in Table 2 & Fig. 5. Maximum length of leaves was found to be 2.23cm, 2.57cm and 2.63cm at 30, 60 and 90 DAP respectively in T_{12} (IBA- 750 ppm) and minimum in T_1 (control). Highest width of leaves was found to be 2.55cm, 2.81cm and 3.28cm at 30, 60 and 90 DAP respectively in T_{12} (IBA- 750 ppm) and lowest in T_1 (control). ANOVA of length and width of leaves at 30, 60 and 90 DAP indicated significant value ($P<0.01$) with treatments (Table 2) It is noticed that the maximum leaf area found in IBA-750 ppm in Guggul plant (Kumrawat *et al.* 2024). Auxins initiated shoot growth which might have generated hydrolysis and transfer of carbohydrates and nitrogenous substances at the base of cuttings and consequence in improving cell expansion and cell division (Patidar *et al.* 2019) [15].

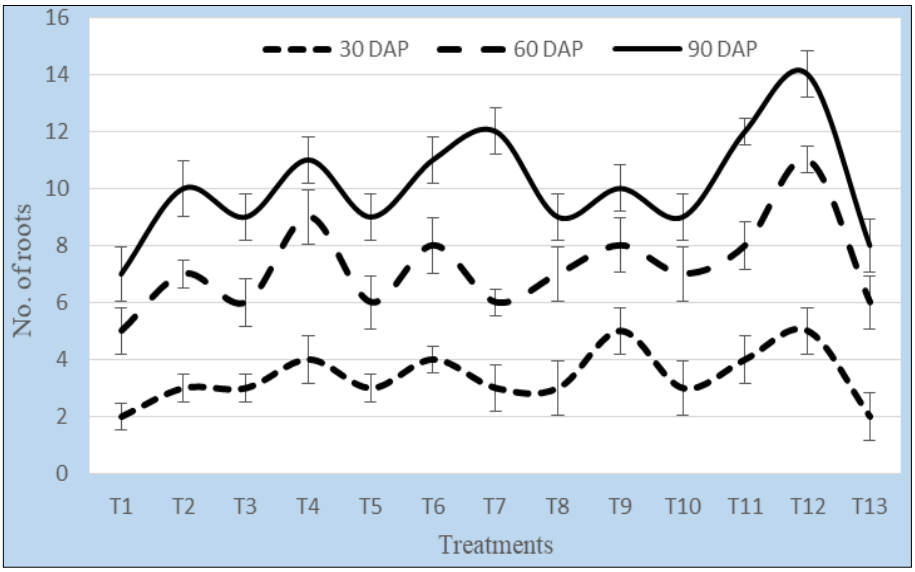

Fig. 1: Change of the number of roots in the stem cuttings of *Centella asiatica* at 30, 60 and 90 DAP on the effect of IAA, NAA and IBA.

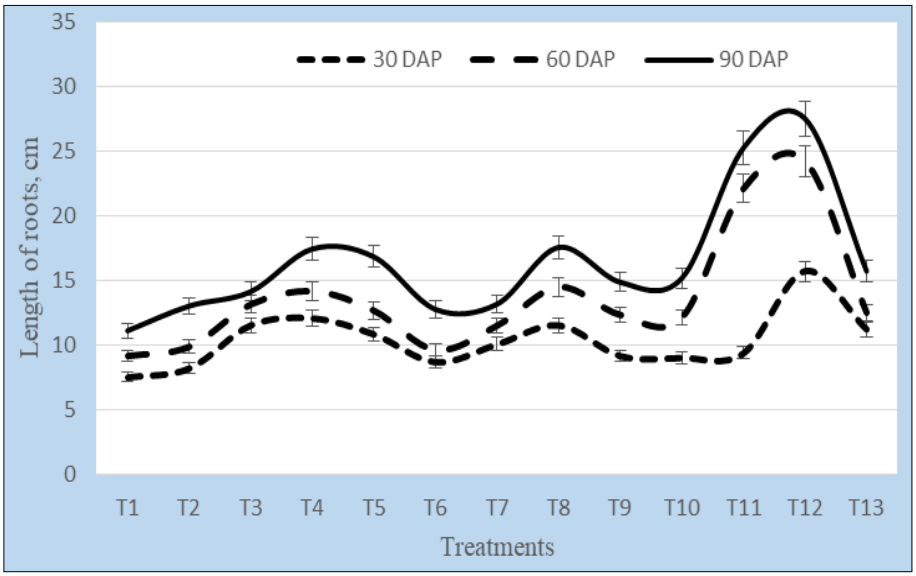

Fig. 2: Change of the length of roots in the stem cuttings of *Centella asiatica* at 30, 60 and 90 DAP on the effect of IAA, NAA and IBA.

Table 1: Effects of auxins on the length of leaf petiole and number of leaves on the stem cuttings of *Centella asiatica*.

Treatment	Length of leaf petiole (cm)			Number of leaves		
	30 DAP	60 DAP	90 DAP	30 DAP	60 DAP	90 DAP
T_1	2.03	3.57	4.93	6	16	25
T_2	3.03	4.43	5.64	8	20	30
T_3	3.22	5.03	6.29	9	24	32
T_4	3.38	5.11	6.68	12	26	35
T_5	3.07	4.66	5.75	8	23	33
T_6	2.60	4.02	5.22	7	21	31
T_7	3.20	4.84	5.88	9	23	33
T_8	3.27	5.07	6.55	11	25	38
T_9	2.87	4.21	5.47	10	23	35
T_{10}	2.56	3.89	5.18	11	22	33
T_{11}	3.40	5.18	6.83	12	26	39
T_{12}	3.47	5.26	7.47	14	28	46
T_{13}	2.40	3.73	5.08	9	20	28
F	58*	37*	185*	9*	16*	95*

Legend: T_1- Control, T_2- 250ppm IAA, T_3- 500ppm IAA, T_4- 750ppm IAA, T_5-1000ppm IAA, T_6- 250ppm NAA, T_7- 500ppm NAA, T_8- 750ppm NAA, T_9- 1000ppm NAA, T_{10}- 250ppm IBA, T_{11}- 500ppm IBA, T_{12}- 750ppm IBA and T_{13}- 1000ppm IBA, DAP= days after planting, F= F value, *denotes significant at 1% level.

Table 2: Effects of auxins on the length and width of leaf on the stem cuttings of *Centella asiatica*.

Treatment	Length of leaf (cm)			Width of leaf (cm)		
	30 DAP	60 DAP	90 DAP	30 DAP	60 DAP	90 DAP
T_1	1.51	1.83	2.07	2.1	2.3	2.6
T_2	1.88	2.22	2.17	2.2	2.4	3.1
T_3	2.00	2.33	2.36	2.4	2.7	3.1
T_4	2.10	2.44	2.43	2.6	2.9	3.2
T_5	1.97	2.28	2.40	2.4	2.7	3.1
T_6	1.78	2.11	2.20	2.2	2.4	3.1
T_7	2.03	2.37	2.30	2.3	2.6	3.1
T_8	2.13	2.56	2.51	2.5	2.8	3.2
T_9	1.84	2.18	2.24	2.2	2.5	3.1
T_{10}	1.72	2.13	2.25	2.2	2.5	3.1
T_{11}	2.06	2.48	2.55	2.4	2.7	3.2
T_{12}	2.23	2.57	2.63	2.6	2.8	3.3
T_{13}	1.66	1.91	2.10	2.1	2.2	2.8
F	24*	4*	26*	5*	3**	11*

Legend: DAP= days after planting, F= F value, *denotes significant at 1% level, ** denotes significant at 5% level.

Table 3: Analyses of variance of number of stolon and roots, length of roots and total fresh biomass on the effects of auxins in the stem cutting of *Centella asiatica*.

SV	DF	F values											
		Number of roots			Length of roots			Number of stolon			Total fresh Biomass		
13	12	30 DAP	60 DAP	90 DAP	30 DAP	60 DAP	90 DAP	30 DAP	60 DAP	90 DAP	30 DAP	60 DAP	90 DAP
		0.4^{NS}	2.1^{NS}	8.7*	44*	54*	112*	2.5^{NS}	0.9^{NS}	6.8*	85*	216*	242*

Legend: SV= source of variance, DF= Degree of freedom, DAP= days after planting, *denotes significant at 1% level and NS= non-significant.

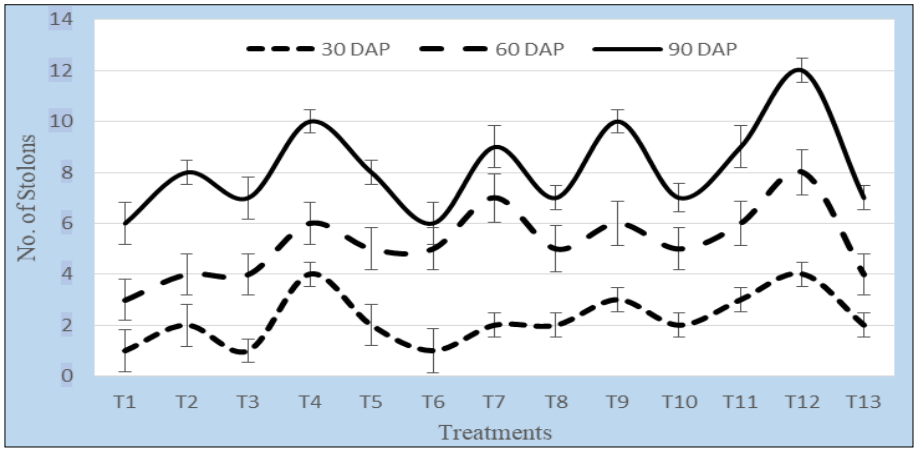

Fig. 3: Change of the number of stolons in the stem cuttings of *Centella asiatica* at 30, 60 and 90 DAP on the effect of IAA, NAA and IBA.

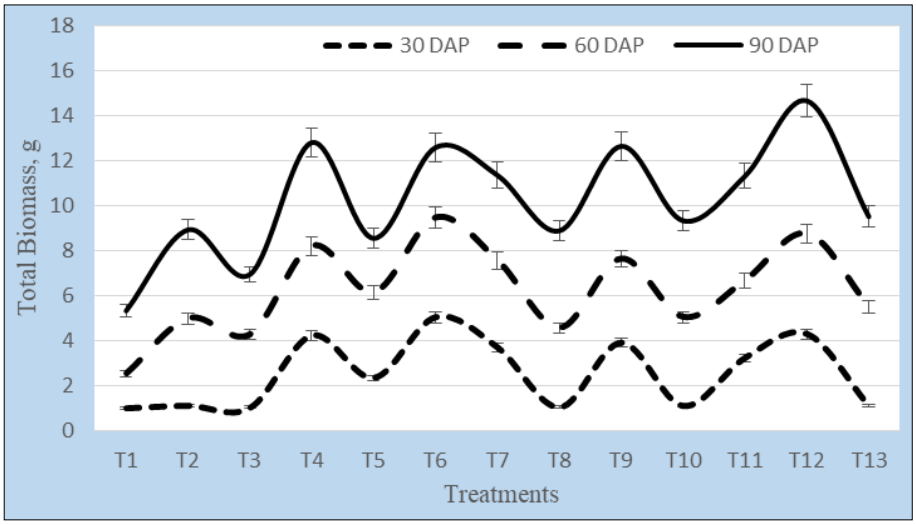

Fig. 4: Change of the total fresh biomass in the stem cuttings of *Centella asiatica* at 30, 60 and 90 DAP on the effect of IAA, NAA and IBA.

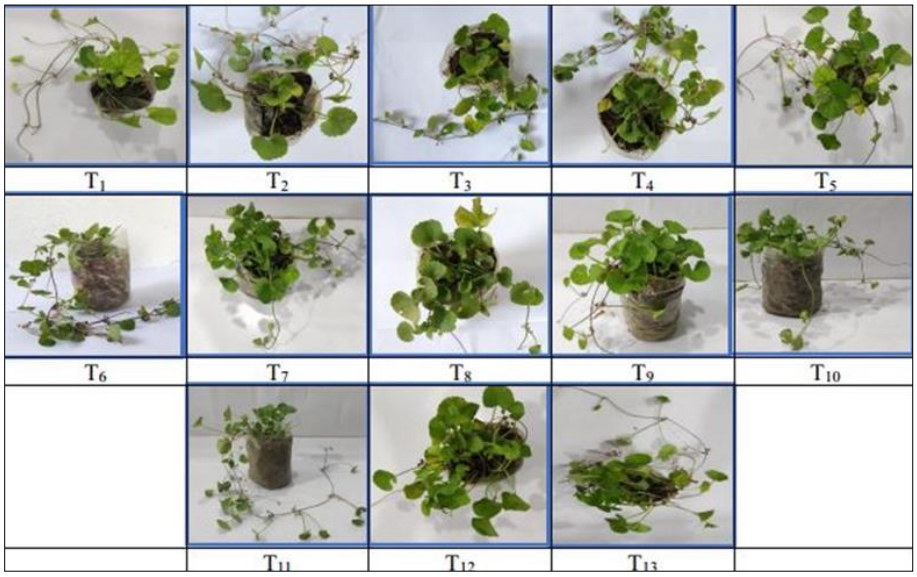

Fig. 5: Change of the vegetative growth behavior of *Centella asitica* (Photographs) on the effect of auxins (T_1-T_{13}) at 90 DAP.

The results of the number of stolons and total fresh biomass at 30, 60 and 90 DAP are presented in Fig. 3 & 5. Highest number of stolons was found to be 4, 8 and 12 at 30, 60 and 90 DAP respectively in T_{12} (IBA- 750 ppm) and lowest in T_1 (control). Maximum total fresh biomass was found to be 4.27g, 8.75g and 14.66g at 30, 60 and 90 DAP respectively in T_{12} (IBA- 750 ppm) and minimum in T_1 (control). ANOVA of number of stolon and total fresh biomass at 30, 60 and 90 DAP exhibited significant value ($P<0.01$) with treatments (Table 3).

It is noticed that the maximum biomass found in IBA- 750 ppm in Guggul plant (Kumrawat *et al*. 2024) [13]. It is reported that cuttings fresh weight or biomass have been increased in higher concentrations of IBA level (Uddin and Rashid 2023, Chinapolaiah 2019, Babu *et al.* 2018 and Tiwari *et al*. 2018) [16-19]. These observations are consistent with the present study.

Conclusion

The present study concludes that IBA- 750 ppm solution is suitable for the maximum number of roots, length of roots, length of petiole, number of leaves, length of leaf, width of leaf, number of stolons and total fresh biomass in the nodal or stem cutting of *Centella*. Considering the all studied elements IBA- 750 ppm solution is the best concentration for the vegetative propagation of *Centella asiatica* (L.) Urban.

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