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Invitro Induction of Shoot In Medicinally Important Plant Hybanthus Enneaspermus (Linn.)

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Abstract

In the present investigation, *Hybanthus enneaspermus* is an medicinally important plant. It is known for its therapeutic value in folkfare medicine. *H. enneaspermus* shows the presence of alkaloids, flavonoids, carbohydrates, steroids, tannins, glycosides and terpenoids. Traditionally this plant is used as an tonic, diuretic, demulcent, aphrodisiac, in dysuria, sterility, diarrhea, and in urinary infection (Yoganarasimhan, 2ooo).In an attempt was to observe effect of different growth hormones viz., BAP (Benzyl amino purine), KIN (Kinetin) on *invitro* propogation in medicinally important *Hybanthus enneaspermus* plant. The shoot and nodal region of *Hybanthus enneaspermus* used as a explants for *invitro* culture and different combinations of cytokines in the Murashige and Skoog medium (Murashige and Skoog, 1962). Shoot formation in maximum number has been resulted in the MS medium supplemented with 9.0 µM BAP in which 14 shoots and average length of the shoot measured is about 14.9 cm. and MS medium supplemented with 10.0 µM KIN in which 12 shoots and average length of the shoot measured is about 6.9 cm. The initiation of shoot takes place in 25 days after inoculation and observations were measured after 45 days after inoculation.

Keywords: *In vitro* induction, *Hybanthus enneaspermus* (Linn), *Regeneration*

Introduction

Hybanthus enneaspermus (L.) is a small Perennial herb belongs to family Violaceae. It is branching herb, (Keshava Murthy & Yoganarasimhan 1990). Distributed in different Warmer parts from Uttar Pradesh southwards to Deccan Peninsula of India, (Schippmann et.al.2002)

In ayurveda it is commonly known as “Sthalakamla”. It has common names like spade flower, pink ladies sleeper and Ratanpurus. This plant is cultivated particularly as medicine for its aphrodisiac, demulcent tonic properties and possessing biological activities like anti – cancer, anti-fungal, anti- inflammatory. In this plant presence of bioactive compounds like phenol, saponins, alkaloids, amino acids and flavonoids (Arul Doss et. al. 2012). Roots are diuretic and administrated as an infusion in gonorrhea and urinary infection. (E. prakash et.al.1999). Fruits and leaves are used as antitodes for scorpion sting and cobra bites by the Yanadi tribes (G.Sudarshanam and G.Sivaprasad1995). It increases sexual desire. This herb is good for regulating the monthly periods of women.

H. enneaspermus (L.) has lot of medicinal properties but the natural regeneration potential of this herb is very poor due to low seed viability (E.prakash et.al.1999). Plant Tissue culture is a gateway of invitro micropropogation for conservation and mass propogation of rare and endangered medicinal plant.

Materials and Methods

For this study, whole plants of *H. enneaspermus* (Linn.) was collected from the Botany Dept., Kakatiya University, Warangal, Hydrabad, Telangana and brought to the Tissue culture lab.,Dept. of Botany, S. C. S. College, Omerga (MS). These plantlets were cultivated in the pots, after settlement of the plants in the pots used as mother plant for propagation. The leaves, stems and nodal region of mother plant were used as explants for further study.

Surface sterilization

Surface sterilization of explants is necessary to disinfect tissues with a minimum damage to the host tissue. After collection of explants from mother plant the first step of surface sterilization of explants were carried out. First off all explants were rinsed with running tap water for 30mins. After that gently rinsed with 70% ethanol for 60sec. and Labolene for 10 mins, after every stage of sterilization the explants were washed with sterile double distilled water for three times. Second stage of surface sterilization of explants was carried out in laminar air flow chamber of Tissue culture laboratory. In this step the explants were rinsed in 0.1% $Hgcl_2$ solution for 5 min. Then these explants were rinsed 5 times in sterile double distilled water. After complete sterilization explants were cut in to small pieces with the sterile scissor in the laminar air flow chamber.

Inoculation

For inoculation of explants the MS media (Murashige & Skoog 1962) was prepared supplemented with auxins like BAP, KIN. PH of the medium was adjusted at 5.8 and prior to autoclaving, 0.6% agar (Himedia, Mumbai), then autoclaving at 15psi for 30mins of MS media were carried out. After complete preparation of MS medium small pieces of explants were introduced in the MS media.

For shoot induction the leaves and stem explants were punched with forceps and introduced in MS medium supplemented with BAP and KIN to obtain multiple shoot.

Result and Discussion

The explants were grows on MS basal medium supplemented with different conc. of BAP and KIN ranging from 1.0µM to 10.0µM for multiple shoot formation. Multiple shoot were initiated within 25 days. Maximum shoots was observed in 45 days. The given data of shoot regeneration frequency, number, length of shoot on different growth harmone shown in Table 1 and figures 1.1 to1.6.

Fig-1.1

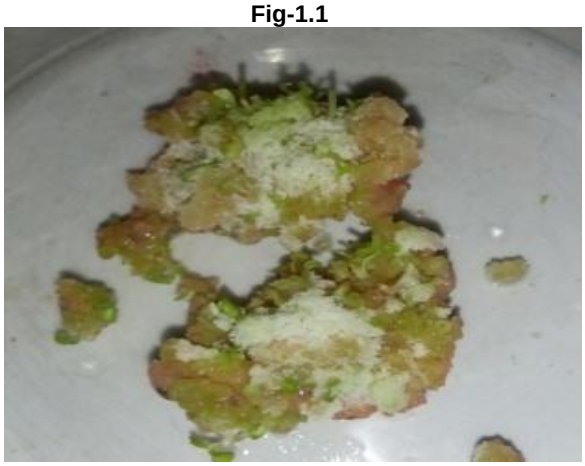

Fig-1.2

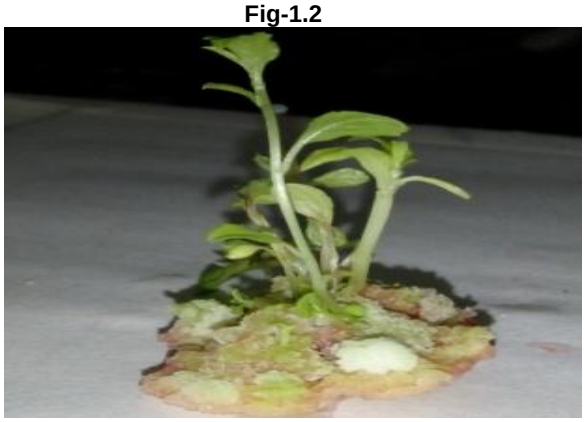

Fig-1.3

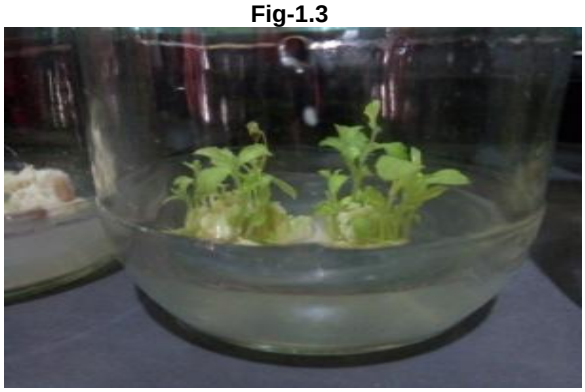

Fig-1.4

Fig-1.5

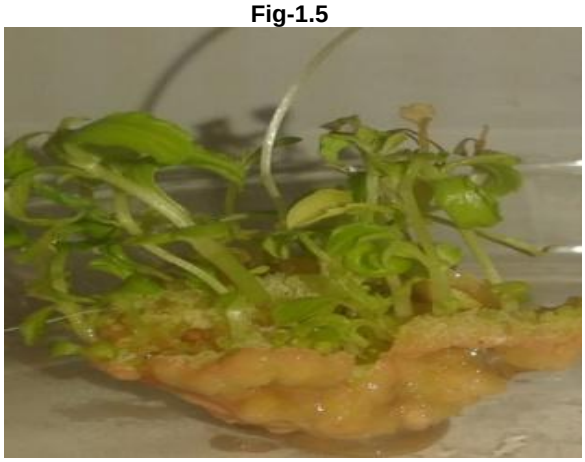

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Fig-1.6

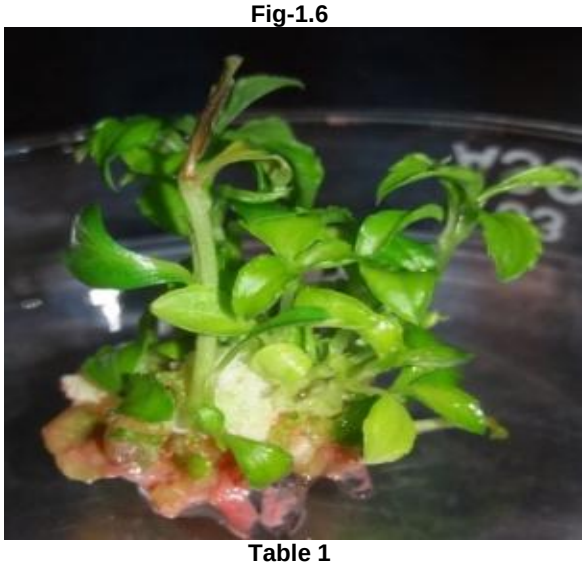

Table 1
Effect of growth hormone i.e. cytokinins on shoot multiplication from the explants of *Hybanthus enneaspermus* (Linn.)

Sr. No	BAP	KIN	Responce (%)	Number Of Shoot	Length Of ShoOT
1.	1.0 µM	- -	60	8.0±1.01	3.87±0.25
2.	2.0 µM	- -	80	10.2±1.30	4.94±0.32
3.	3.0 µM	- -	86	10.9±1.26	4.5±0.36
4.	4.0 µM	- -	90	11.4±1.14	4.82±0.25
5.	5.0 µM	- -	94	09.9±1.23	4.2±0.27
6.	6.0 µM	- -	98	10.4±1.14	5.06±0.23
7.	7.0 µM	- -	89	9.4±1.15	4.1±0.19
8.	8.0 µM	- -	100	14.6±1.14	5.64±0.27
9.	9.0 µM	- -	96	14.9±1.12	4.3±0.22
10.	10.0 µM	- -	90	11.8±0.83	4.88±0.14
11.	- -	1.0 µM	45	5.6±1.22	3.56±1.01
12.	- -	2.0 µM	65	6.5±1.16	3.85±1.21
13.	- -	3.0 µM	95	11.2±1.30	5.04±0.28
14.	- -	4.0 µM	88	9.5±1.25	6.04±1.26
15.	- -	5.0 µM	86	9.0±1.11	5.5±2.32
16.	- -	6.0 µM	90	10.4±1.14	5.02±0.17
17.	- -	7.0 µM	89	9.6±1.25	5.23±0.27
18.	- -	8.0 µM	92	9.1±112	6.11±0.31
19.	- -	9.0 µM	95	10.8±0.83	5.38±0.34
20.	- -	10.0 µM	100	12.8±1.30	6.98±1.34

Conclusion

The present investigation on Invitro multiplication was carried in *Hybanthus enneaspermus* (Linn.).Explant was grown on MS medium and various concentration of cytokinins sviz ; BAP and KIN. The result shows multiple shoots were found in BAP than KIN concentration.

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