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Effect of plant growth regulators on growth of callus of *Decalepis hamiltonii*

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Abstract
Decalepis hamiltonii is an medicinal plant with different biological activities. Present study involves callus growth in this plant using different plant growth regulators. Producing callus cultures is a very important for production of secondary metabolites. Callus cultures were developed by using the media Murashige and Skoog (MS), supplemented with 2, 4-D, BAP and combination of 2, 4-D+Kn, BAP+GA_3, NAA + Kn, 2, 4-D+IBA. The callus development was estimated by its fresh, weight, dry weight and the morphology.

Keywords: *Decalepis hamiltonii*, plant growth regulators, explants, callus culture

Introduction
Decalepis hamiltonii Wight & Arn, commonly known as Swallow root, belongs to the family Apocynaceae [1]. It grows mainly in dry and moist deciduous forests, peninsular region and Western Ghats. Alkaloids, Saponins, Tannins, Glycosides were present the extracts of root and Tannins and Glycosides were not present in the extracts of stem and leaf. It is an perennial plant with articulated stem and a 5cm girth [2]. The roots of *Decalepis hamiltonii* are believed to have biological activities, the roots of *Decalepis hamiltonii* are consumed as juice and pickles [3]. In this present study different plant growth regulators are used to identify the better growth regulator for the growth of callus. Secondary metabolites which are used for the drug formation can be acquired from callus [4].

Fig 1: Picture of *Decalepis hamiltonii*

Materials and Methods
Collection and Authentication of plant material
Plant material was obtained from the Rajiv Gandhi Institute of I.T and Biotechnology in Pune. It was authenticated by Dr. Digambar Mokat, Principal Investigator & Regional

Director, Regional-Cum-Facilitation Centre, Western Region-National Medicinal Plants Board, Ministry of AYUSH, Department of Botany, Savitribai Phule Pune University.

Surface sterilization of explants

Tuberous roots of *Decalepis hamiltonii* were washed using tap water for 30 minutes before being treated with 10% Dettol soap solution for 10 minutes. Explants were rinsed with water to remove the remaining soap solution. The explants were then transferred to Laminar Air Flow; explants were treated with 70% alcohol for 2 to 3 minutes and washed with distilled water, then treated with freshly prepared 0.1% mercuric chloride solution for 1 to 2 minutes, followed by frequent washing of explants with distilled water for 5 to 6 times. Explants which are surface sterilized are ready to transfer [5].

Media Preparation

Stock solutions of MS (Murashige and Skoog) medium were prepared and stored separately in a refrigerator at 100°C to ensure consistent medium composition, convenience, and time savings. Pant Growth Regulator was prepared and stored as well.0.01gm of PGR was weighed and dissolved in 0.5ml of ethanol before being adjusted to the desired volume with distilled water. Below table shows the composition of MS medium stock solution [6].

Table 1: Composition of stock solution for Murashige and Skoog medium

Constituents	Composition(mg/l)	For 1lit. MS stock medium
Macronutrients		Stock Solution **A** 50ml/l
$CaCl_2.H_2O$	440	
KNO_3	1900	
KH_2PO_4	170	
$MgSO_4.7H_2O$	370	
NH_4NO_3	1650	
Micronutrients		Stock Solution **B** 5ml/l
$CuSO_4.5H_2O$	0.025	
$CoCl_2.6H_2O$	0.025	
H_3BO_3	6.2	
KI	0.83	
$MnSO_4.H_2O$	22.3	
Na_2MoO_4	0.25	
$ZnSO_4.7H_2O$	8.6	
Iron Supplements		Stock Solution **C** 5ml/l
$FeSO_4.7H_2O$	27.8	
$Na_2EDTA.H_2O$	37.3	
Vitamins		Stock Solution **D** 5ml/l
Nicotinic acid	0.5	
Myoinositol	100	
Pyridoxine hydrochloride	0.5	
Thiamine hydrochloride	0.5	
Sucrose	30gm	
pH	5.8	
Agar-agar	6gm	

Media for static culture

The required growth hormones were added to MS medium that contained 3% sucrose, and the final volume was adjusted to 1 litre using distilled water. The pH was changed to 5.5±0.02 using either 0.1N HCL or 0.1N NaOH. The Agar-Agar 6g/l solidifying agent was mixed into the medium by heating it. After cooling, the medium was poured into culture jars. At 121°C temperature and 15 pressure, the medium was sterilized for 20 minutes. Before being aseptically transferred into culture jars explants were surface sterilized and incubated at 25±2ºC under white light (1600 lux) for 16 hours [7].

Initiation of callus:

For the purpose of callus initiation, explants were surface sterilized, cut, and aseptically placed into MS media containing different combinations of growth hormones. The explant was transferred in front of laminar air flow and incubated at 25±2ºC with a 16 hour photoperiod. Table 2 lists the growth hormones and their combinations that were used for the roots of *Decalepis hamiltonii* to start the production of calluses [8].

Table 2: Growth hormones and their concentrations used for initiation of callus from *D. hamiltonii* roots

S. No.	Media	Growth hormones	Concentration in ppm
1.	MS	2,4-D	2
2.	MS	2,4-D+Kn	1+1
3.	MS	BAP	1
4.	MS	BAP+GA_3	2+3
5.	MS	NAA+Kn	1+2
6.	MS	2,4-D+IBA	2+1

Estimation of callus growth:

Weekly observations of callus appearance were made, and growth was determined by qualitative (morphological changes) and quantitative (changes in dry weight) means. The grown callus was dried to a consistent weight in a 60 °C oven to determine the dry weight (mg) of the callus [8].

Results and Discussion

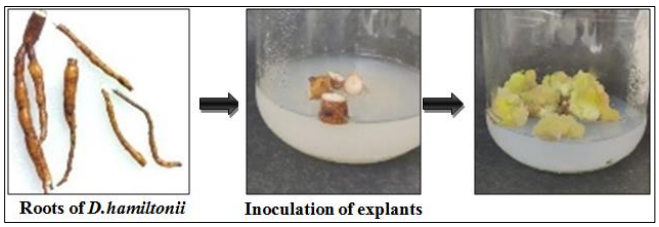

Fig 2: Initiation of callus

Figure 2 shows the initiation of callus from the *D. hamiltonii* roots. The explant was cut cylindrically approx 1mm and was inoculated to the media. After 3 weeks green and high mass callus was observed.

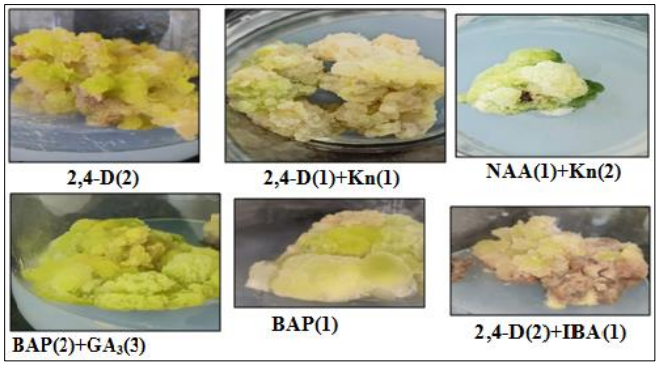

Fig 3: Effect of growth hormone on morphology of callus

Figure 3 shows the change in morphology of callus, when supplemented to different growth hormones. Highest mass and friable callus was observed in media concentrated with 2, 4-D(2).

Table 3: Effect of growth hormone on morphology of callus

Media	Growth hormone	Conc.(ppm)	Morphology
MS	2, 4-D	2	Green, friable, high mass
MS	2, 4-D + Kn	1+1	Green, high mass, friable
MS	BAP	1	Green, Compact callus, less mass
MS	BAP+GA_3	2+3	Compact, green callus
MS	NAA + Kn	1+2	Greenish base and upper whitish
MS	2,4-D+IBA	2+1	Brown, green, high mass

Table 3 shows the effect of growth hormone on morphology of callus. Combination of both auxin and cytokinin which is 2, 4-D and kinetin shows friable and green callus. While, combination of GA_3 and BAP shows compact callus, NAA and IBA shows brown, creamy whitish callus with combination of kinetin and 2,4-D. No differentiation was observed in any of the media combination.

Table 4: Effect of growth hormone on callus growth

Media	Growth hormone	Conc. (ppm)	FW(gm)	DW(gm)
MS	2,4-D	2	12.23	0.55
MS	2,4-D+Kn	1+1	8.44	0.38
MS	BAP	1	4.46	0.22
MS	BAP+GA3	2+3	5.31	0.29
MS	NAA+ Kn	1+2	5.55	0.31
MS	2,4-D+IBA	2+1	7.45	0.37

Table 4shows effect of growth hormones on callus growth. Maximum fresh weight of callus (12.23gm) and dry weight(0.55gm) was obtained on MS medium supplemented with 2,4-D(2).This observation was done in the callus culture of 3weeks old.Very less growth was observed in the callus supplemented with NAA (1)+Kn(1) FW(5.55gm), DW (0.31gm).

Conclusion

In the present study, *in vitro* callus initiation was studied on root explants of *Decalepis hamiltonii*.

- Maximum callus growth was observed in the MS media supplemented with 2,4-D(2), 2,4-D(1)+Kn(1),
- Friable and green and high mass of callus was observed the in media supplemented with 2,4-D(2).
- Maximum fresh weight of callus (12.23gm) and dry weight(0.55gm) was obtained on MS medium supplemented with 2,4-D(2).This observation was done in the callus culture of 3weeks old.
- Very less growth was observed in the callus supplemented with BAP(1) FW(4.46gm), DW(0.22gm)

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