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Morphogenetic responses of explant types in *Hypericum hookerianum*

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Abstract
The *Hypericum hookerianum* Wight and Arnn, (Hypericaceae), a shrub of Palni and Nilgris hills of the Western Ghats, reported to have a range of antimicrobial, antiviral and antitumor properties, was selected for *In vitro* studies. Seeds of different accessions collected from natural areas were surface decontaminated and cultivated on Murashige and Skoog (1962) solid medium to produce seedlings at 60-80% efficiency. After 8 weeks, the seedlings were transferred to nutrient medium containing 0.5mg/l Kinetin (KN) for shoot multiplication. Number and length of the shoots proliferated upon the seedlings in presence of this cytokinin were significantly higher those obtained on basal medium. After 4 weeks, shoots raised from seedlings of different accessions were uniform except in the case of Bryant Park accession where the shoots regenerated into green (97%) and red (3%) types. Nodal explants (0.5cm) of the green plants were subcultured in nutrient medium containing different concentrations of cytokinin (KN, BAP) to obtain maximum number (9.6) of shoots per node at 0.5mg/l BAP. The shoots raised in presence of 0.5mg/l KN were relatively fewer but longer than those obtained on BAP. Comparative analysis of the responses of the nodal explants cultured in medium supplemented with the individual cytokinins (KN, BAP, TDZ) revealed that TDZ 1mg/l was the best to induce maximum number (3.4) of shoots compared to 2.0- 2.4 shoots obtained with other cytokinins in 4 weeks. Shoots transferred to rooting medium containing the auxins (IAA, IBA) formed upto 9.5 roots/ shoot in presence of 0.5mg/l IBA in 8 weeks. Isolated leaves of the shoots were induced to form roots. Roots were best formed in presence of IBA in 4 weeks, without callus formation. The 20+ roots obtained are being subcultured to establish exclusive root suspension culture in MS liquid medium. For want of time, logical end of obtaining such a culture for metabolic studies could not be reached.

Keywords: Hypericum hookerianum, IAA, IBA

1. Introduction
Species of the genus *Hypericum* (*Hypericaceae*) are medicinal plant naturally occurring in dry temperate zones of Europe and North America. In India, it is distributed in the temperate western Himalayan region between 3000 and 9000 ft altitude [1]. The plant is mostly found in Sikkim, Khari and Jaintia hills and also in Nilgris and palanis of Southern India [2]. The plants consist of herbs, shrubs, or small trees, and are distributed chiefly in the temperate regions of the world. *Hypericum hookerianum* is a round –topped shrub with weakly spading, non-erect branches with golden yellow flowers. *Hypericum hookerianum* is aterete shrub 2-2.5m in height, with stout stems and rathe flaccid ovate leaves, set bifarious, mucronate to mucronulate at apex [3]. Different phytochemical constituents of this plant such as hypericin, hyperforin, xanthones and flavonoids have been shown to be effective antimicrobials, antivirals, and antibacterials against gram-positive bacteria, and to possess wound healing potential and antidepression qualities [4]. The chemical constituents, hypericine and hyperforin posses and has become popular medicinal plant due to its anti (retro) viral activity, anticancer, bactericidal, anti-inflammatory and sedative properties [5]. *In vitro* culture is an attractive tool for multiplication and production of secondary metabolites from *H. perforatum* [6, 7]. Growth, multiplication and production of phytochemicals in cultures are governed by factors such as explant selection, its age and plant regulators. *In vitro* system have been reported as an effective tool for obtaining genetically uniform plants for making less variable pharmaceutical preparations. Plant regeneration of *Hypericum* species has been achieved using as explants whole seedlings or their excised parts hypocotyl

sections [8] and leaves using various types and concentration of cytokinins and auxins. *H. perforatum* callus formation has been obtained from leaf explants, stamens and calli were the intermediate step to regenerate shoots. Callus obtained from anthers of *H. perforatum* were induced to shoot formation using BAP and NAA [9].The higher level of IAA induces cellular growth by increasing RNA synthesis, and consequently, protein synthesis, resulting in an increase of the callus proliferation [10]. The shoot induction and multiplication of *Hypericum* species have reported to yield an average of 2-5.5 shoots, depending on the explant used [11, 12]. IBA, IAA, and NAA have been the auxins used to induce root formation in several species of *Hypericum* [13].

2.0 Materials and Methods

2.1 Plant materials:

Seeds of *H. hookerianum* were collected from different locations of Palni hills (Kodaikanal) and Nilgiris (Ootacamund) of Tamil Nadu, India. Seeds collected from different locations were treated as different accession. Explants (nodes, leaf) for regeneration and root culture were collected from three month old *In vitro* raised plants. For subculturing, the nodes of *In vitro* raised seedlings (10 weak old) served as explants to induce shoot multiplication in the presence of 0.5 mg/l kn. The *In vitro* raised shoots were used for root initiation with different auxins. Leaf explants of the shoot culture were used for root initiation and establishment of root culture in presence of IAA and IBA.

2.2 Media and culture condition:-

Murashing and Skoog,(1962) nutrient formulation was used uniformly for all the culture experiment (Table 3).The medium supplemented with 3% sucrose as carbon source. The plant growth regulators were added to the medium prior to autoclaving. The pH of the medium was adjusted to 5.8 before 1.5 g Gelzan (Sigma Chemical Co. USA) was added to the medium followed by boiling to dissolve the same. The medium was dispensed into the culture vessels viz. 25x150mm culture tube and 100 ml/250 ml Erlenmeyer flasks as the case may be, and sterilized by autoclaving at 15 psi and 121 ºC for 20 minutes. Unless otherwise stated all the cultures were maintained in a culture room kept at 25±2 ºC and 12 h photoperiod. A photon flux density of 1500 flux at the level of the culture was provided by a bank of white fluorescent tubes (Philips. India).

2.3 Surface sterilization:

The seeds were collected from natural stands of H. hookerianum were expected to have a high microbial load of bacteria and fungi. Therefore, suitable multiple surface sterilization procedure was adopted to decontaminate them.The seeds were transferred to 250 ml of Erlenmeyer flask having 100 ml of distilled water and 1 drop of Labolene detergent (Glaxo India Pvt. Ltd., Bombay) for 6-8 minutes. Then the solution was carefully drained and the seeds are washed 4 to 5 times with distilled water to remove the traces of Labolene. Then, the seeds were transferred to a sterile 250 ml Erlenmeyer flask under laminar air flow and were treated with 0.1% $HgCl_2$ solution for 4 to 5 minutes followed by 3 to 4 rinses in sterile distilled water. The surface decontaminated seeds were transferred to a sterile petridish.

2.4 Seed germination

After surface sterilization, seeds of the 8 accession were sown on hormone free MS medium (Murashige and Skoog, 1962) in petridishes containing 0.6% agar and 3% sucrose. The petridishes were sealed with parafilm, and seeds of different accessions were incubated at 25± 2ºC under 12h photoperiod for germination. After 8 weeks, seedlings emerged on the germination medium.

2.5 Multiplication of plantlets

Eight weeks old seedlings were carefully introduced into Erlenmeyer flasks each containing 50 ml MS agar medium supplemented with 3% sucrose and 0.5mg/l kinetin for shoot multiplication. Each flask contained 5 seedlings. Hormone free medium was used as the control. All the culture were maintained at 27 ±4ºC under cool white fluorescent light (4000 \lux) with a 16h photo period and were monitored for shoot production at regular intervals. Shoot proliferated upon each seedlings were counted and were used for subcultures and further multiplication.

2.6 Nodal explants culture

The isolated nodes of *H. hookerianum* were collected from three month old *In vitro* raised plants. Nodel segments (0.5 cm) were dissected and the leaves are discarded. The basal medium for shoot initiation contained MS salts and vitamins (Murashige and skoog 1962) which served as the control. In order to test different cytokinin on shoot induction, 6-benzylaminopurine (BAP) and Kinetin (Kn) indifferent concentration (0.5, 1.2 mg/l) were used.

2.7 Comparision of three different hormones for shoot induction

Nodal explants of H. *hookerianum* were collected from three month old *In vitro* shoot culture under aseptic conditions, after discarding of leaves. For shoot initiation, the explants (nodes) were transferred to nutrient medium containing MS salts, vitamins, and cytokinins viz.(6-benzylaminopurine(BAP) ,Kinetin(Kn) and Thidiazuron(TDZ) at varied concentration (0.5,1.0,2 mg/l). Five explants were used for each concentration. Observations were made at regular intervals and data on the frequency of shoot initiation, mean number of shoots, mean length of shoots was collected after 30 days of culture.

2.8 Rooting of shoots

For root induction, medium hormone free MS medium and medium containing IAA and IBA were tested. Elongated shoots (0.5-1.0 cm) were harvested from medium containing 0.5 mg/l kn medium and transferred to MS medium supplemented with IAA and IBA at different concentrations (0.5, 1.0). Hormone free medium served as a control. Each culture tube containing 20 ml of agar medium, one shoot were placed into culture tube containing. Data were recorded after 6 weeks of culture. Frequency of rooting and average number of roots per shoot were recorded.

2.9 Root culture

The leaf explants of *H. hookerianum* were collected from three-month old *In vitro* plants under aseptic conditions. The basal medium for root initiation contained MS salts and vitamins (Murashige and sk00g, 1962). In order to test the effect of auxins on root initiation both Indole-3-acetic acid (IAA) and Indole -3-butyric acid (IBA) as different concentrations (0.5, 1.2 mg/l) were used. Hormone free medium served as control.

3.0 Results & Discussion

3.1 Seed germination

Seeds of different accessions/collections were cultured on MS basal medium in 9 cm dia petridishes. The data on germination are presented in (Table 5). After 8 weeks, the percentage seed germination varied marginally between the accessions. So also the shoot and root lengths of the seedlings showed marginal variation. Particularly, seeds of Fairy fall germinated into seedlings having short (0.5 cm) shoot and long (1.5 cm) root. Seeds of Rose Garden, Ooty germinated into long seedlings having 1.4cm shoot and short 0.7 cm root (Plate 1). By and large, seeds drawn from the fruits of different accessions were fertile as more than 80% of them produced large number of seedlings, no infertility was observed in any of the accessions. (Plate 1), Table 5.

3.2 Multiplication of plantlets from seedlings

Eight week- old seedlings so raised were transferred to nutrient medium containing 0.5 mg/l KN for shoot multiplication (Table 6). After 4 weeks of culture, maximum number of shoots were proliferated on the seedlings of Bryant Park followed by Pillar Rock, Fairy Falls, Botanical Garden, Ooty and so on. The number and length of the shoots proliferated upon the seedlings were always significantly higher than those obtained in basal medium. The number of shoots per seedling varied between 11 and 24 (Plate 2). It was of interest to note that a few shoots proliferated upon the seedlings of accession Bryant Park were somewhat red coloured and could be discriminated from all other green seedlings. The differential Pigmentation of the seedlings could not be observed in any other accession/collection. The total number of harvestable shoots obtained in presence of the cytokinin was adjudged as high. (Plate 2)

3.3 Nodal explants culture

Plants already raised *In vitro* (Plate 3) in presence of 0.5 mg/l KN served as source of nodal explants for further investigations. Nodes dissected out of these plants were cultured in basal and cytokinin supplemented media. The number of shoots proliferated upon the nodes in presence of both the cytokinins (KN, BAP) were marginally higher than the basal medium after 4 weeks of observation (Plate 4). The difference became obvious with enhanced shoot multiplication rate after this period (Table 7). However increase in shoot elongation was not corresponding to the shoot multiplication after 8 weeks of culture (Plate 5). Between the two cytokinins, BAP was far superior to kinetin in inducing rapid proliferation of shoots. Maximum number of shoots was harvested in the lowest concentration (0.5 mg/l) of BAP (9.6 shoots per node). Even in kinetin, the lowest concentration (0.5 mg/l) induced maximum number (5.2) of shoots per node (Table 8). In general shoots obtained in presence of kinetin were longer than those induced by BAP. Even within the concentrations of both cytokinins, increasing concentration had inhibitory effect on the length of the shoots (Plate 3).

3.4 Rooting of shoots

Shoots obtained in presence of the cytokinins (0.5-1cm) were transferred to nutrient medium containing auxins for root initiation. Basal medium was no good for rooting. Among the two auxins IBA at the lowest concentrations (0.5mg/l) induced maximum number (9.5) of roots per shoot without affecting the growth of the shoots (Table 10). After 8 weeks of culture, the shoots had attained a length of 2.4cm in the lowest concentrations of the auxins tested (Plate 7).

3.5 Root culture

In order to raise possible root cultures rich in root specific compounds, isolated leaves of *In vitro* derived plants were cultured in MS medium supplemented with different concentrations of auxins (Table 11). There was no root initiation in the basal medium devoid of auxins. Among the two auxin types (IAA, IBA), IBA was found to be better than IAA to induce upto 22 roots per leaf within a period of 4 weeks. There was no significant difference in the length of the roots produced in different concentrations of the auxins (Plate 8).

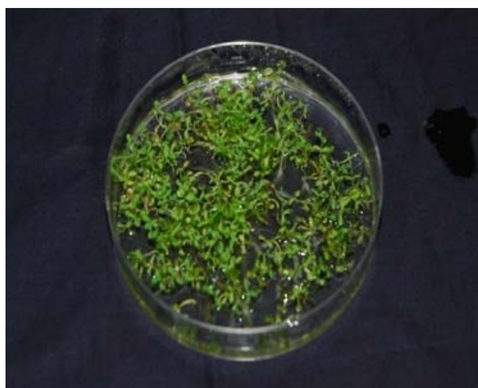
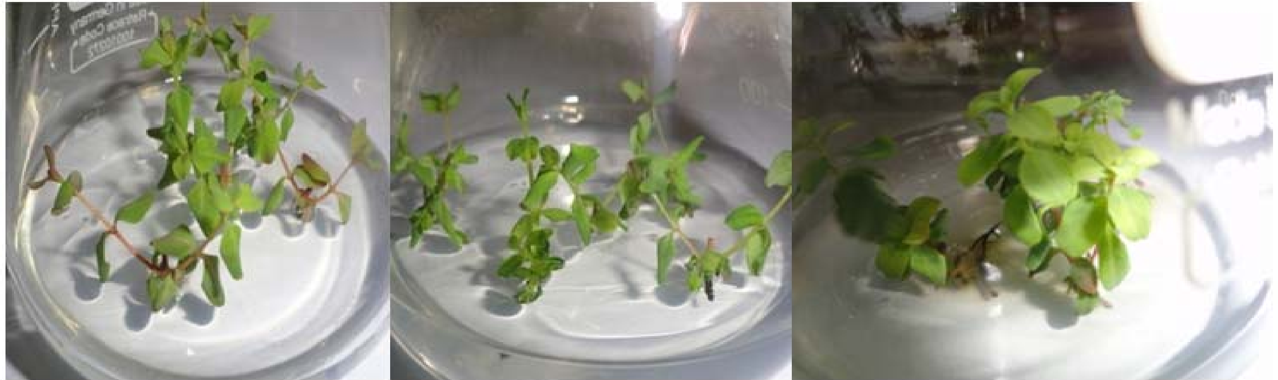


Plate1: Germination of seeds after 8 weeks in MS medium	**Plate 2:** Pigmentation of shoots in an accession in medium containing 0.5mg/l kinetin

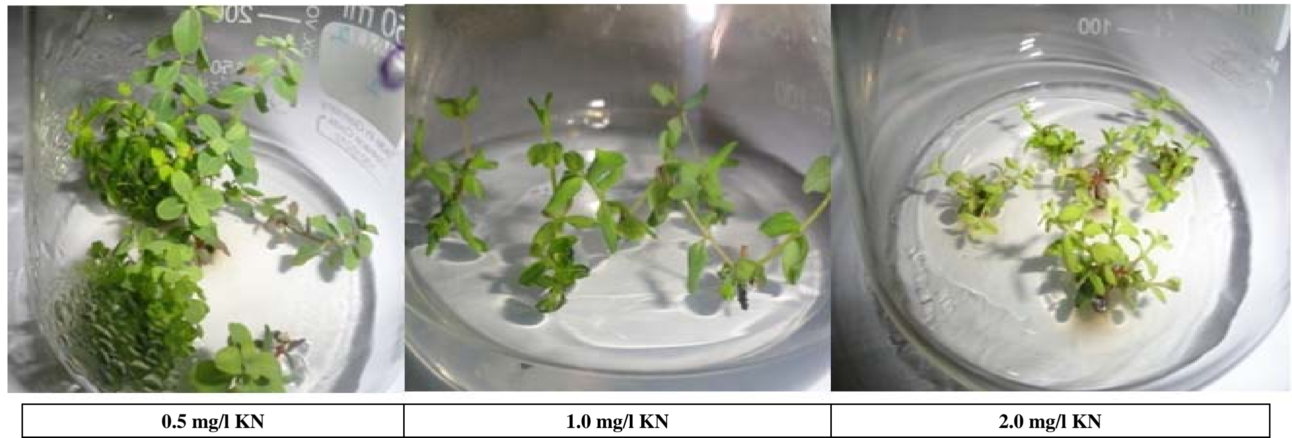
Plate 3: Three months old *In vitro* plants raised from seedlings



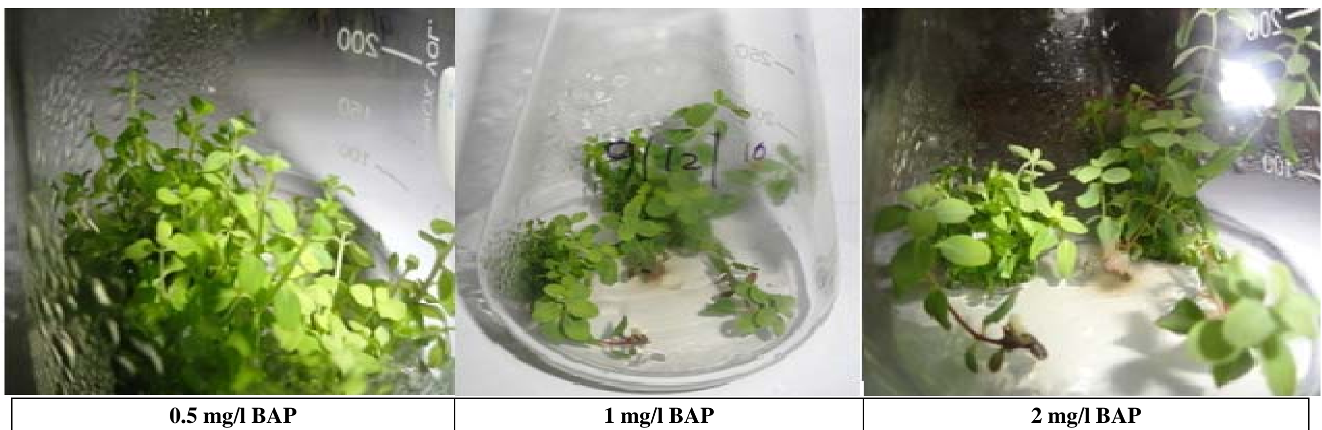
0.5 mg/l KN	1 mg/lKN	2 mg/l KN

0.5mg/l KN	1.0mg/l KN	2.0mg/l KN

Plate 4: Nodal explants of *In vitro* raised plants producing shoot after 4 weeks in   in MS medium containing various concentrations of cytokinin

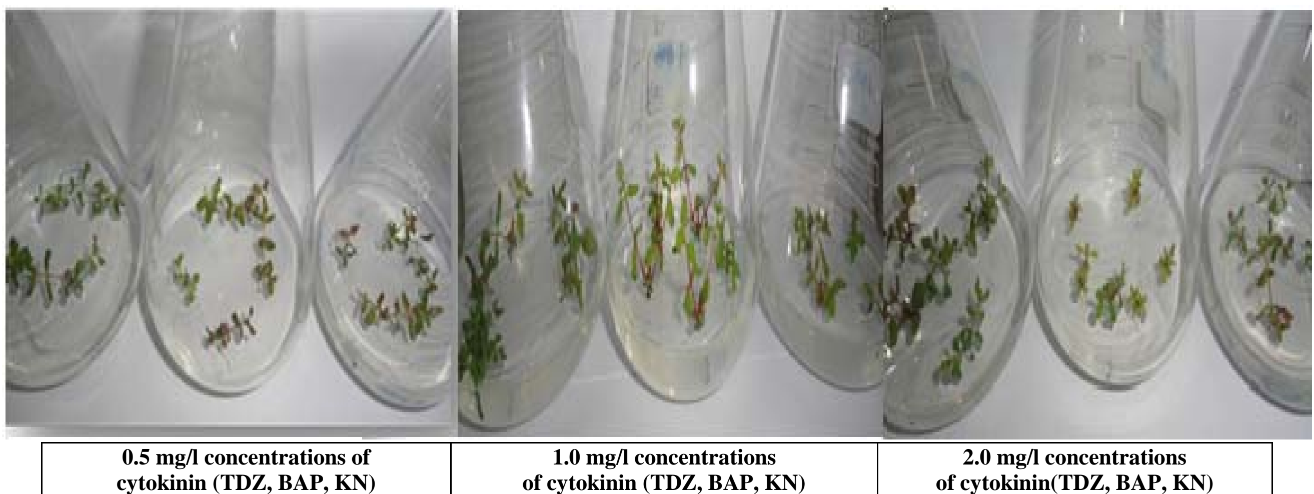


0.5 mg/l KN	1.0 mg/l KN	2.0 mg/l KN



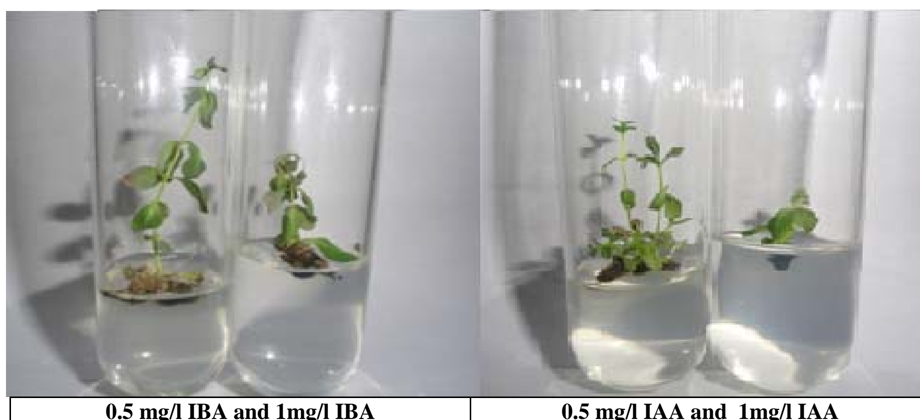
0.5 mg/l BAP	1 mg/l BAP	2 mg/l BAP

Plate 5: Response of shoots after 8 weeks



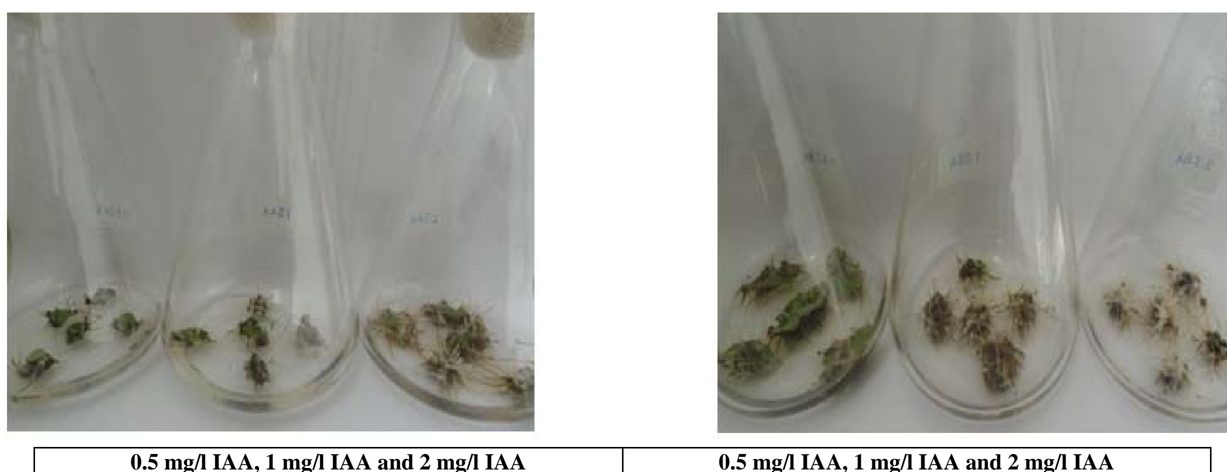
0.5 mg/l concentrations of cytokinin (TDZ, BAP, KN)	1.0 mg/l concentrations of cytokinin (TDZ, BAP, KN)	2.0 mg/l concentrations of cytokinin(TDZ, BAP, KN)

Plate 6: Shoots developed from Nodes.



0.5 mg/l IBA and 1mg/l IBA	0.5 mg/l IAA and 1mg/l IAA

Plate 7: Rooting of shoots



0.5 mg/l IAA, 1 mg/l IAA and 2 mg/l IAA	0.5 mg/l IAA, 1 mg/l IAA and 2 mg/l IAA

Plate 8: Rooting responses of leaf explants in medium containing IAA and IBA

Table3: Revised MS (Murashige and Skoog) media and its composition for plant tissue culture

STOCKS	COMPONENTS	REQUIRED AMOUNT (mg/l)
A	NH_4NO_3	*1650*
B	KNO_3	*1900*
C	$Cacl_2.2H_20$	440
D	$MgSO_4.7H_2O$	370
E	KH_2PO_4	170
F	NA2 EDTA	37.3
	$FeSO_4.\ 7H_2O$	27.8
G	H_3BO_3	22.3
	$MnSO_4.4H_2O$	8.6
	$ZnSO_4.7H_2O$	0.83
	KI	0.25
	Na_2MoO_4	0.025
	$CuSO_4.5H_2O$	0.025
	$COCl_2$	0.5
H	Nicotinic acid	0.5
	Pyridonic-HCl	0.1
	Thiamine HCl	100
Add directly	Myoinositol	30,000
	Sucrose	1500
pH -- 5.8	Gelzan	

Table 4: surface sterilization procedure tested for *Hypericum hookerianum*

Plant materials	Cleaning		Decontamination	
Seeds	Labolene	Time (min)	$HgCl_2$	Time (min)
	1 drop	6-8 mins	0.1	5

Table 5: Seeds were collected from different locations of Kodaikanal and Ootacamund and germinated on MS basal agar medium. Observations were

Accession/location	No. of seeds transferred	No. of seeds germinated *	Mean length of shoot/ seedlings (cm)	Mean length of root/seedling (cm)
Bryant Park, Kodaikanal	60	48 (80)	1.3±0.1	0.8±0.2
Bear Shola, Kodaikanal	60	40 (66)	1.1±0.2	0.7±0.1
Botanical Garden, Ooty	60	43 (71.6)	1.3±0.1	1.2±0.2
Rose Garden, Ooty	60	40 (66)	1.4±0.1	0.7±0.2
Pampar Shoal, Kodaikanal	60	39 (65)	0.7±0.2	0.5±0.2
Fairy Falls, Kodaikanal	60	41 (68.3)	0.5±0.1	1.5±0.2
Suicide Point, Kodaikanal	60	39 (65)	0.8±0.2	0.5±0.3
Pillar Rock, Kodaikanal	60	46 (76.6)	1.5±0.1	1.2±0.2

*Figures in parenthesis indicate percentage response.

Table 6: Eight week- old seedlings were transferred to MS medium containing 0.5mg/l KN. Observations on shoot multiplication were made after 4 weeks of culture.

Accession	No. of seedlings transferred	No. of seedlings infected	No. of seedlings responded	Total no. of shoots	Mean no. of shoots per seedling	Mean length of the shoot (cm)	Pigmentation
Control	5	-	5	55	12	0.5	-
Bryant Park	5	-	5	120	24	0.8±0.2	Red
Bear Shola	5	-	5	80	16	0.9±0.2	-
BotanicGarden	5	-	5	95	19	0.9±0.2	-
Rose Garden	5	-	5	90	18	1.5±0.5	-
Pampar Shola	5	-	5	80	16	1.5±0.5	-
Fairy falls	5	-	5	95	19	0.8±0.2	-
Suicide Point	5	-	5	90	18	1.5±0.5	-
Pillar Rock	5	-	5	105	21	0.8±0.2	-

Table 7: Nodal explants of *In vitro* raised plants were cultured in MS solid medium containing different concentrations of cytokinins. Observations were made after 4 and 8 weeks of culture.

Cytokinin (mg/l)	No. of nodes transferred	No. of nodes infected	No. of nodes responded	Total no. of shoots	Mean no. of shoots/ node	Mean length of the shoot (cm)	Mean no. of nodes per shoot
After 4 weeks							
Basal	5	-	5	8	1.65	0.3±0.2	1
BAP							
0.5	5	-	5	12	2.4	1±0.5	3
1.0	5	-	5	10	2	0.6±0.2	3
2.0	5	-	5	10	2	0.6±0.2	3
Kinetin							
0.5	5	-	5	10	2		4
1.0	5	-	5	10	2		4
2.0	5	-	5	10	2		3

Table 8: After 8 weeks, different concentration of cytokinins induce shoot formation on nodal segments

Cytokinin (mg/l)	No. of nodes transferred	No. of nodes infected	No. of nodes responded	Total no. of shoots	Mean no. of shoots/ node	Mean length of the shoot (cm)	Mean no. of nodes per shoot
After 4 weeks							
Basal	5	-	5	8	1.6	0.3±0.2	1
BAP							
0.5	5	-	5	48	9.6	1.5±0.5	4
1.0	5	-	5	24	4.8	1±0.5	3
2.0	5	-	5	20	4	0.6±0.2	3
Kinetin							
0.5	5	-	5	26	5.2	3.5±0.5	6
1.0	5	-	5	14	2.8	2.5±0.5	5
2.0	5	-	5	13	2.6	2±0.5	4

In order to assess the comparative efficiency of the 3 cytokinins (Kinetin, BAP, In order to assess the comparative efficiency of the 3 cytokinins (Kinetin, BAP, TDZ), specific concentrations of the cytokinins were tested on shoot initiation in nodal explants (Table 9). The differences between the cytokinin types were marginal. Among the three cytokinins, maximum number of 3.4 shoots per node was obtained in medium containing 1mg/l TDZ (Plate 6). At this concentration there was no inhibitory influence of the cytokinin on shoot length. In other concentrations, there was no pronounced difference on length of the shoots produced between the cytokinin types.

Table 9: Comparative influences of specific concentrations (0.5- 2.0 mg/l) of the three cytokinins on shoot initiation in nodal explant cultures. Observations were made after 4 weeks of culture.

Cytokinin (mg/l)	Total no. of nodes transferred	Total no. of nodes responded	Mean no. of shoots per node	Total no. of shoots	Mean length of shoot(cm)
Conc. 0.5mg/l					
0.5 kn	5	5	2	10	1.0±0.2
0.5 BAP	5	5	2	10	0.8±0.1
0.5 TDZ	5	5	2	10	1.0±0.2
Conc. 1.0 mg/l					
1.0 Kn	5	5	2	10	1±0.2
1.0 BAP	5	5	2	10	1.5±0.2
2.0 TDZ	5	5	3.4	17	1.3±0.2
Conc. 2.0 mg/l					
2.0 kn	5	5	2.2	11	1.5±0.2
2.0 BAP	5	5	2	10	0.5±0.2
2.0 TDZ	5	5	2.6	13	1.0±0.2

Table 10: Rooting of shoots (0.5-1.0 cm) transferred to MS medium containing the auxins, IAA and IBA. Observations were made after 8 weeks of culture.

Auxin	No. of shoots transferred	Total no. of roots	Mean no. of roots/shoot	Initial length of shoot(cm)	Mean length of shoot after 8 weeks (cm)
Basal	4	-	-	-	-
IAA (mg/l)					
	4	7	1.75	0.5	2.47
	4	12	3	0.5	1.4
IBA (mg/l)					
	4			0.5	2.1
	4	12	3	0.5	2.4

Table 11: *In vitro* root initiation in isolated leaves of *H. hookerianum* dissected out of shoot cultures. The explants were cultured **in MS** medium containing different concentrations of the auxins for 4 weeks.

Auxins mg/l	No. of explants transferred	No. of explants responded	Total no. of roots	Mean no. of roots per explants	Mean length of the roots (cm)
Control	5	5 (100%)	-	-	-
IAA					
0.5	5	5 (100%)	24	4.8	0.3±0.1
1.0	5	5 (100%)	42	8.4	0.3±0.1
2.0	5	5 (100%)	90	18	0.5±0.2
IBA					
0.5	5	5 (100%)	38	7.6	0.3±0.1
1.0	5	5 (100%)	72	114.4	0.3±0.1
2.0	5	5 (100%)	110	22	0.5±0.2

4. Discussion

Hypericum hookerianum, the Hooker's Wort is a perennial shrub growing wild in damp forested sites at high altitude (>2500 MSL) of Palni (Kodaikanal) and Nilgris (Ooty) hills of southern India [14]. Otherwise an ethnomedicinal plant used by the local Toda tribes of Nilgris, the plant is already pharmacologically evaluated and demonstrated to posses significantly antimicrobial, antitumor and antiviral properties [15]. Hundred or more fold synthesis of hypericin in presence of the auxin indicated possible commercial application of the system, provided aspects of regeneration, production, biomass production through subcultures and scale up are worked out. *In vitro* organogenesis and multiplication of species for possible planting, cultivation and variant isolation have not yet been studied in *H. hookerianum.* Supplementation of the medium with BAP resulted in reduced percentage (21.7%) of germination. In the present study frequency of germination varied from 65-80% which broadly agrees with the result of others. In all the accessions emergence of plumule was invariably observed after 4 weeks ultimately leading to the formation of 0.5-1.5cm seedlings after 8 weeks (Table 5). Since seeds of all accessions germinated at reasonable frequencies and no infertility was observed in any of the accessions, it is assumed that differential environmental parameters operating in Kodaikanal and Ooty did not have any overriding influence on seed germination. Similar events of seed germination may take place under favourable natural conditions. Although reasonable frequency of seed germination was obtained in all the accessions, requirements of a cytokinin (KN) at a concentration of 0.5mg/l was seriously felt for subsequent proliferation of shoots and seedlings. This was evident from the less number of shoots obtained in basal medium. Maximum number of shoots were proliferated upon the seedlings of Bryant Park. The seedlings so obtained in Bryant Park accession also got discriminated into green (95%) and red types (5%) (Table 6). The observation that this kind of differential pigmentation was not encountered in any of the other accessions suggest that at least some of the seedlings may be the result of sexual fusion and consequent variation. Both cytokinin types (BAP and KN) were used for multiplication of shoots using nodal explants of *In vitro* plants. Shoot multiplication occured in both the cytokinins. Between the two cytokinin, BAP was far more superior to KN in inducing maximum number (9.6) of shoots than KN (5.2) after 8 weeks of culture. As a synthetic cytokinin, BAP is stronger and more effective than KN in inducing shoot multiplication in several species of plants (Pretto and Santarem, 2000). However, BAP is known to induce shoots with shorter internodes, thereby reducing the length of the shoots as also observed in the present study. As expected increasing concentrations of BAP and also KN had corresponding inhibitory effects on shoot length. The calorigenic influences of BAP and KN were compared with TDZ (Table 9). The differences between the influences of TDZ and BAP were marginal, However, while TDZ (1mg/l) induced the formation of (3.4) shoots per node, only 2.4 shoots were obtained in BAP (0.5mg/l). As such, TDZ had more favourable effect on shoot initiation. It should be noted that thidiazuron induced micropropagation is better than other cytokinins in *H. triquetrifolium* (Oluke and Orhan). In a few other systems as also, TDZ is preferred more than the less effective KN and BAP for enhanced shoot initiation. Shoot induced by the cytokinin are normally rooted in presence any of the three auxin types (IAA, IBA and NAA). Since there was no root initiation in the shoots transferred to basal medium, it is possible that the shoots are deficient in endogenous concentrations of the auxin. The results presented in Table 10 indicate that IBA at 0.5 mg/l is far better than natural auxin, IAA for inducing maximum number of the roots per shoot (9.5). Among auxin types IBA is preferred over IAA in inducing root formation in several species of plants.

In a preliminary experiment conducted to induce root culture as source of bioactive metabolites, leaf explants were cultured in presence of the auxins. The auxin IBA was more efficient to induce the formation upto14.4 roots compared to 8.4 roots obtained with 1mg/l IAA. For want of time more experiment could not be conducted to transfer these roots to liquid media so as to establish root suspension culture. It could be our endeavor to establish root suspension culture as source of metabolites for comparative studies with the shoot cultures already established.

5. References

1. Anonymous (1962) Wealth of India-Raw Mayterials, Vol.9, Publication and Information Directorate, CSIR, New Delhi, pp.393-394.
2. Ayan AK, Cirak C. *In vitro* multiplication *of Hypericum helerophyllum*, an endemic Turkish species. Amer J Plant Physiol 2006; 1:76-81
3. Bais HP, Vepachedu R, Lawrence CB, Stermitz FR, Vivanco JM. Molecular and biochemical characterization of an enzyme responsible for the formation of hypericin in St. John's Wort *Hypericum perforatum L.* J Biol Chem 2003; 278(34):32413-22.
4. Bais HP, Walker TS, MC-Grew JJ, Vivanco JM Factors affecting growth of suspension culture of *Hypericum perforatum L.* & production of hypericin. *In vitro* Cell Dev –Plant 2002; 38:58-65.
5. Bhat SR, Chitralekha P, Chandel KPS, Regeneration of plants from long- term root culture of lime, *Citrus aurantifolia* (christm) Swing. Plant cell tissue organ Cult 1992; 29:19-25.
6. Bhattacharya SK, Chakrabarti, S.S Chatterjee Pharmacopsychiatry 1998; 31(22).
7. Briskin DP. Medicinal plants and phytomedicines. Linking plant biochemistry and physiology to human health. Plant Physiol 2000; 124:507-514.
8. Cellarova E, Kimakova K, Brutovska R. Multiple shoot formation in *Hypericum perforatum* L. and variability of R_0. Biol Plant 1992; 34 (suppl):536.
9. DeSmet P, Nolen WA. St. John's Wort as an antidepressant. Br J Med 1996; 313:241-242.
10. Ernst E. In: Hypericum: The genus *Hypericum.* Medicinal and Aromatic Plants-industrial Profiles series. Taylor & Francis, London & Newyork, 2003.
11. Fehr MJ, McCloskey MA, Petrich JW. Light induces acidification by the antiviral hypericin. J of the Am Chem Soc 1995; 117:1833-1836.
12. Fyson PF. The flora of the Nilgiris and Pulney Hill Tops. Dehradun: Bishen Singh & Mahendra Pal Singh, 1974; 1:37-39.
13. Gadzovska S, Maury S, Ounnar S, Righezza M, Kascakova S, Joseph C *et al*. Identification and quantification of hypericin and pseudohypericin in different *Hypericum perforatum L. In vitro* cultures Plant Physiol Biochem 2005; 43(6):591-601.
14. Goel MK, Kukreja AK, Khanuja SPS. Cost effective approaches for *In vitro* mass propagation of Rauwolfia serpentine Benth. Ex Kurz Asian J Plant Sci 1995; 6:957-961.
15. Holzl J, Ostrowiski E. St. John's Wort (*Hypericum perforatum L.).* HPLC analysis of the main components and their variability in a population. Deuts. Apothekerztg 1987; 127:1227-1230.