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Studies on influence of growth regulators on *in vitro* propagation of *Andrographis echioides* (L.) Nees

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Abstract
Andrographis echioides is given importance recently among all the *Andrographis* species due to its excellent medicinal properties. The major objective of this study is to study the influence of growth regulators on micro propagation of this plant. Among the various explants, shoot tips responded positively for shoot induction. MS medium fortified with BAP (2.5 mgl^{-1}) was found highly responsive for shoot induction. The multiple shoot induction was achieved in MS medium + BAP (3.0 mgl^{-1}) and was maintained upto third subculturing. For shoot elongation, BAP (2.0 mgl^{-1}) + GA_3 (1.0 mgl^{-1}) was found better. Rooting was best (94.85%) in ½ MS + IAA 0.5 mgl^{-1} + IBA 1.0 mgl^{-1}.

Keywords: Explant – MS medium – growth regulators - shoot induction – root induction

Introduction
Andrographis echioides (L.) Nees (Gopuram thanki) is one of the important medicinal plant species belonging to the family Acanthaceae. *Justicia echioides* L. and I*ndoneesiella echioides* (L.) Sreemadh. are the synonyms of this plant. The plant is known by various vernacular names *viz*., Kalu kariyatu (Gujarathi), Birbhubat (Hindi), Banchimani (Marathi), Gopuramthangi (Malayalam and Tamil) and False water willow (English). The plant is common in all the dry districts of Tamil Nadu (Tadulingam *et al*., 1985) [14]. The plant is an erect, annual herb, simple or slightly branched, growing up to a height of 20 to 60 cm. In the Indian Systems of Medicine, predominantly *Andrographis echioides* is used against blood cancer. The leaf extract is recommended for oral consumption. Traditionally, the plant has been used as febrifuge, bitter tonic, astringent, anodyne and also for dysentery, cholera and diabetes. The ethanol extract of this plant used as diuretic and in sluggishness of liver and jaundice has been reported as the modern use of this plant. The chemical constituents of this plant are echioidin and echioidinin (Guhabakshi *et al*., 1999) [5]. The research works on tissue culture aspect in this important medicinal plant are very meager. Hence, the present research work on studying the influence of growth regulators on *in vitro* propagation of *Andrographis echioides* (L.) Nees has been conducted at the Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore.

Materials and Method
For micropropagation, the explants of shoot tips (1.5 – 2 cm), nodal segments (2 – 2.5 cm), leaf bits (0.5 – 1.0 cm^2), root bits (1.0 – 2.0 cm) and stem bits (1 – 1.5 cm) were collected from healthy mother plants and trimmed off to required sizes with a sterilized knife before inoculation. The explants were rinsed with liquid detergent for five minutes and then rinsed with distilled water for three to four times. Prior to inoculation, explants were sterilized with ethyl alcohol (70%) for 25 seconds and were rinsed with 0.1 per cent mercuric chloride for different durations (2-6 minutes), depending upon the type and physiological status of the explants.

For shoot formation, the explants were cultured in MS basal medium alone and in combination with BAP (0.5 to 4.0 mgl^{-1}). For multiple shoot production, the explants were inoculated in MS basal medium supplemented with BAP (1.0-5.0 mgl^{-1}). The shoot elongation was tried in MS basal medium alone and with BAP (1.0-4.0 mgl^{-1}) and GA_3 (0.5 – 1.0 mgl^{-1}) combinations. The individual microshoots obtained from the shoot induction media were transferred to ½ MS basal medium (Control), IAA (0.5 to 1.0 mgl^{-1}), IBA (0.5 to 1.0 mgl^{-1}) and their combinations for root induction. Activated charcoal (200 mgl^{-1}) was added to all the treatment combinations involved for rooting.

The experiments were laid out in CRD design and the results were interpreted. The data from various experiments conducted for the study were analysed statistically following the procedures developed by Panse and Sukhatme (1995).

Results and Discussion

Ex-plant Standardization

The explants like shoot tips, nodal segments, stem bits, leaf bits and root bits were sourced from the stock plant that were maintained for the purpose of micropropagation. Among the various explants used, shoot tips gave significantly highest culture response (85.72%). The highest percentage of survival (68.00%) with lowest contamination percentage (19.42%) was also recorded in the similar treatment (Table 1). Explants that have a rudimentary or organized structure (shoot tip, nodal segment) are the most responsive and pose the least problems in respect of an organized structure which it will suffice to reveal in an appropriate medium (Haripriya, 2003) [6]. This response might be also due to the higher meristematic activity (Suryanarmada, 2000) [13]. The endogenous auxin content in both these explants was high, which promotes cell division and thereby good regeneration.

Influence of BAP on shoot induction

In the present study, the best response (83.22%) to direct shoot regeneration from shoot tips was observed on MS medium supplemented with BAP (2.5 mgl^{-1}) (Plate 1). The response to shoot induction decreased as the concentration of BAP increased (Table 2). Similar results were evident in *Coleus forskohlii* (Jayanthi and Sharma, 1991) [7]. The decrease in shoot production at higher concentration of BAP may be due to the inhibition of shoot initiation or induction of calli. The advantage of direct organogenesis helps to retain clonal fidelity (Broertjes and Keen, 1980) [1] than that of shoot production through callus.

Performance of BAP on multiple shoot induction

The multiple shoots were observed more (78.62%) in media composition containing BAP (3.0 mgl^{-1}) (Plate 2). Days taken for multiple shoot induction were lower and number of multiple shoots was higher at higher concentration of BAP (Table 2). However, shoot length was not correlated with shoot proliferation at higher concentration of BAP. Such reduction in shoot length due to higher concentration of BAP was reported in *Wedelia calendulaceae* by Emmanuvel *et al*. (2000) [2], in *Rhinacanthus nastus* by Johnson *et al*. (2002) [8] and in *Rauvolfia tetraphylla* by Ghosh and Banerjee (2003) [4].

Effect of BAP and GA_3 upon shoot elongation

The effect of GA_3 in combination with BAP was studied for shoot elongation in which BAP (2.0 mgl^{-1}) + GA_3 (1.0 mgl^{-1}) was found to be better (Plate 3). The longest shoots were produced in BAP (2.0 mgl^{-1}) + GA_3 (1.0 mgl^{-1}) treatment in 8.97 days (Table 3). GA_3 stimulates cell elongation and cell wall plasticity. Cell division was stimulated in the shoot apex especially in the more basal meristematic cells, from which develops the long files of cortex and pith cells. GA_3 treatment helps in both the transport of potassium ions and increase in the number of mitotic figures throughout the meristematic zone (Sachs, 1965) [11]. They also promote cell growth because they increase hydrolysis of starch and sucrose into glucose and fructose molecules (Salisbury and Ross, 1986) [12]. The similar trend of shoot elongation was found in *Centella asiatica* (Panimalar, 2002) [9] and *Andrographis paniculata* (Haripriya, 2003) [6].

Role of IAA and IBA on rooting

The media composition containing ½ MS + IAA 0.5 mgl^{-1} + IBA 1.0 mgl^{-1} gave highest (94.85%) and earliest (11.45 days) rooting (Plate 4). The same treatment composition was found better for producing longer (3.73 cm) and more number of roots (10.75) (Table 4). A mixture of more than one auxin can particularly be effective for root induction, since auxin was implicated in vascular differentiation (George and Sherrington, 1984). This report was supported by the results observed in *Rauvolfia serpentina* (Roy *et al*., 1995) [10].

Table 1: Standardization of explants for direct regeneration in *Andrographis echioides* (L.) Nees.

Explants	Culture response (%)	Contamination (%)	Culture survival (%)	Dead explants (%)
Shoot tip	85.72 (67.78)	19.42 (26.14)	68.00 (55.61)	14.26 (22.17)
Nodal segment	70.63 (57.19)	31.17 (33.94)	60.48 (51.05)	29.20 (32.71)
Stem bit	0.00 (0.64)	64.05 (53.26)	0.00 (0.64)	35.98 (36.82)
Leaf bit	1.76 (7.44)	40.77 (39.68)	45.66 (42.51)	20.44 (26.88)
Root bit	0.00 (0.64)	72.39 (58.30)	0.00 (0.64)	27.61 (31.70)
Mean	31.62 (26.74)	45.56 (42.26)	34.83 (30.09)	25.50 (30.06)
SEd	0.710	1.547	1.128	1.014
CD (0.05)	1.514	3.298	2.405	2.162
CD (0.01)	2.093	4.559	3.325	2.989

Values in parentheses are arcsine-transformed.

Table 2: Effect of BAP on shoot induction and proliferation from shoot tip explants in *Andrographis echioides* (L.) Nees.

Treatments	BAP (mgl^{-1})	Shoot induction (%)	Multiple shoot induction (%)
T_1	MS basal	0.00 (0.64)	0.00 (0.64)
T_2	0.5	20.21 (26.70)	-
T_3	1.0	37.45 (37.73)	0.00 (0.64)
T_4	1.5	61.65 (51.76)	-
T_5	2.0	63.67 (52.96)	65.12 (53.82)
T_6	2.5	83.22 (66.10)	-
T_7	3.0	74.56 (59.81)	78.62 (62.52)
T_8	3.5	67.18 (55.10)	-
T_9	4.0	43.33 (41.16)	67.64 (55.35)
T_{10}	4.5	-	-
T_{11}	5.0	-	60.33 (50.97)

Mean	50.14 (43.55)	45.29 (37.32)
SEd	2.431	1.448
CD (0.05)	5.107	3.155
CD (0.01)	6.998	4.423

Values in parentheses are arcsine-transformed.

Table 3: Effect of growth regulators on shoot elongation in *Andrographis echioides* (L.) Nees

Treatments	Growth regulators (mgl^{-1})		Shoot length (cm)	Days taken for elongation
	BAP	GA_3		
T_1	MS basal	-	2.08	16.10
T_2	1.0	0.5	2.83	11.40
T_3	1.0	1.0	4.08	10.92
T_4	2.0	0.5	5.30	10.75
T_5	2.0	1.0	7.00	8.97
T_6	3.0	0.5	3.97	12.53
T_7	3.0	1.0	4.55	12.22
T_8	4.0	0.5	3.13	14.55
T_9	4.0	1.0	3.75	14.00
Mean			4.08	12.38
SEd			0.070	0.513
CD (0.05)			0.148	1.077
CD (0.01)			0.202	1.476

Table 4: Effect of growth regulators on rooting percentage and days taken for rooting in *Andrographis echioides* (L.) Nees

Treatments	Growth regulators (mgl^{-1})		Rooting (%)	Days taken for rooting	Number of roots /plant	Root length (cm)
	IAA	IBA				
T_1	½ MS basal	-	0.00 (0.64)	0.00 (0.64)	0.00 (0.64)	0.00 (0.64)
T_2	0.5	-	20.50 (26.92)	3.85 (11.31)	3.85 (11.31)	*1.50 (7.03)*
T_3	1.0	-	46.43 (42.95)	4.45 (12.17)	4.45 (12.17)	1.98 (8.09)
T_4	-	0.5	70.37 (57.04)	6.45 (14.71)	6.45 (14.71)	2.10 (8.33)
T_5	-	1.0	88.55 (70.37)	6.90 (15.22)	6.90 (15.22)	2.70 (9.45)
T_6	0.5	0.5	90.50 (72.26)	8.02 (16.44)	8.02 (16.44)	3.07 (10.08)
T_7	0.5	1.0	94.85 (77.60)	10.75 (19.13)	10.75 (19.13)	3.73 (11.13)
T_8	1.0	0.5	88.52 (70.34)	7.05 (15.39)	7.05 (15.39)	2.70 (9.45)
T_9	1.0	1.0	90.83 (72.60)	13.05	8.43 (16.87)	3.45 (10.70)
Mean			65.62 (54.52)	16.05	6.21 (13.54)	2.36 (8.32)
SEd			2.448	0.551	0.425	0.292
CD (0.05)			5.144	1.157	0.893	0.613
CD (0.01)			7.049	1.586	1.223	0.840

Values in parentheses are arcsine-transformed.

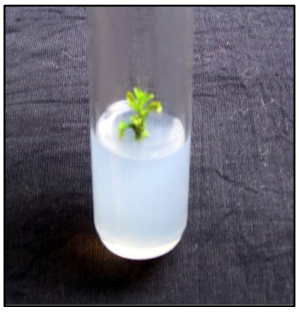

Plate 1: Shoot induction (BAP 2.5 mgl^{-1})

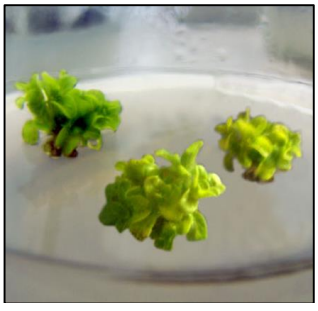

Plate 2: Multiple shoot induction (BAP 3.0 mgl^{-1})

Plate 3: Shoot elongation (BAP 2.0 mgl^{-1} + GA_3 1.0 mgl^{-1})

Plate 4: *In vitro* rooting (1/2 MS + IAA 0.5 mgl^{-1} + IBA 1.0 mgl^{-1})

Conclusion

The study was conducted to investigate the influence of various growth regulators on *in vitro* propagation of *Andrographis echioides* (L.) Nees at Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. Among the various explants, shoot tips responded positively for shoot induction. MS medium fortified with BAP (2.5 mgl^{-1}) was found highly responsive for shoot induction. The multiple shoot induction was achieved in MS medium + BAP (3.0 mgl^{-1}). For shoot elongation, BAP (2.0 mgl^{-1}) + GA_3 (1.0 mgl^{-1}) was found better. Rooting was best (94.85%) in ½ MS + IAA 0.5 mgl^{-1} + IBA 1.0 mgl^{-1}. Pot mixture containing vermiculite + red earth + sand (1:1:1) was found optimum for hardening.

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