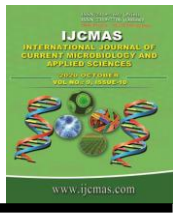
Int.J.Curr.Microbiol.App.Sci (2020) *9*(10): 3756-3763

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 10 (2020)**
Journal homepage: http://www.ijcmas.com



Original Research Article https://doi.org/10.20546/ijcmas.2020.910.432

Studies on Callus Induction and *in Vitro* Production of Secondary Metabolite in *Andrographis echioides* (L.) Nees

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Keywords

MS medium, Explant, Callus, Proliferation, Andrographolide

Article Info

Accepted:
26 September 2020
Available Online:
10 October 2020

A B S T R A C T

The present research was focused in standardizing the callus induction technique and studies the behaviour of callus in secondary metabolite production in *Andrographis echioides* (L.) Nees. In this study, the leaf bits of *Andrographis echioides* responded positively for callus induction. Earliest (21.00 days) and profuse callusing was observed on MS medium supplemented with 2, 4-D (2.0 mgl^{-1}) + NAA (2.0 mgl^{-1}) + BAP (2.0 mgl^{-1}). The same treatment combination responded highly for callus proliferation also. Highest andrographolide content of 1.31 per cent was observed in callus grown in media supplemented with 2, 4-D (2.0 mgl^{-1}) + NAA (2.0 mgl^{-1}) + BAP (2.0 mgl^{-1}) combination. The alkaloid content registered an increasing trend from 45 days to 60 days of culturing.

Introduction

Plant-derived chemicals are valuable sources for a variety of pharmaceuticals, flavors, dyes, oils and resins (Parr, 1988). Many of these commercially valuable phytochemicals are secondary metabolites that are not essential to plant growth, but are produced in small amounts, and often accumulate in specialized tissues, e.g. Trichomes. *Andrographis echioides* (L.) Nees (Gopuram thanki) is one of the important medicinal plant species belonging to the family Acanthaceae. *Justicia echioides* L. and *Indoneesiella echioides* (L.) Sreemadh. are the synonyms of this plant. In the Indian Systems of Medicine predominantly, it 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. *Andrographis echioides* (L.) Nees. is a rare exception in the genus *Andrographis* where limited investigations are available on account of *in vitro* production when compared to *Andrographis paniculata* which has been extensively studied on micropropagation, *in vitro* production, pharmacological and phytochemical compositions. Hence the present research work has been carried out in *Andrographis echioides* to standardize the procedure for callus induction and *in vitro* production of secondary metabolite viz., andrographolide in *Andrographis echioides*.

Materials and Methods

Callus induction

The present investigation on callus induction and *in vitro* production of *Andrographis echioides* (L.) Nees was carried out in the Tissue Culture Laboratory, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. For callus induction, the experiment was laid out in Completely Randomized Design and the explants of stem bits (1 – 1.5 cm), leaf bits (0.5 – 1.0 cm^2), root bits (1.0 – 2.0 cm) and nodal segments (2 – 2.5 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. The nutrient media chosen for callus induction study was MS medium (Murashige and Skoog, 1962) containing sucrose (3.0 %), agar (0.8 %) and with different growth regulator combinations (2, 4-D 1 to 2.5 mgl^{-1}, BAP 1 to 2.5 mgl^{-1} and NAA 1 to 2.5 mgl^{-1}). The cultures were maintained in dark and were incubated at 25 ± 2˚ C temperature and observations are made.

Quantification of secondary metabolites

The concentration of andrographolide in *Andrographis echioides* was estimated by following the standard protocol as determined by Gained *et al*. (1963). A solution of known concentration (10 – 100 μgml^{-1}) of andrographolide obtained from Natural Remedies Private Ltd., Bangalore containing more than 95% purity was prepared using methanol for standard. A known volume (10 µl) of standard and samples prepared were injected to HPLC in triplicate by means of a suitable syringe and the chromatogram was recorded.

Results and Discussion

Callus induction

1. Explant Standardization

The per cent response was significantly higher in leaf bits (89.32 %) (Plate 1) followed by root bits (15.34 %) (Plate 2) whereas, the stem bits showed no response (Table 1). Among all the explants, early callusing was observed in leaf bits (15.00 days) followed by root bits (21.00 days) and nodal segment (25.58 days). The variation in the performance of the explants could possibly be due to the variation in ratio of endogenous phytohormones present in the explant system, age of the explant, position from where the explant was taken, types of cells present and their physiological and developmental stage (Baskarrajan, 1994).

Effect of growth regulators

Callus induction and callus response

Highest callus induction percentage of 77.45 per cent was registered in T_{14} (2, 4-D 2.0 mgl^{-1} + NAA 2.0 mgl^{-1} + BAP 2.0 mgl^{-1}) followed by T_{13} (2, 4-D 1.5 mgl^{-1} + NAA 1.5 mgl^{-1} + BAP 1.5 mgl^{-1}) (73.23 %). However, poor callus induction was observed in MS basal medium and in combinations of 2, 4-D with NAA (Table 2). The explants and growth regulators combinations for callusability was scored at 0-3 scale. Profuse callusing was recorded in the treatment T_{14} (2, 4-D 2.0 mgl^{-1} + NAA 2.0 mgl^{-1} + BAP 2.0 mgl^{-1}) and T_{13} (2, 4-D 1.5 mgl^{-1} + NAA 1.5 mgl^{-1} + BAP 1.5 mgl^{-1}). Among the auxins, 2, 4-D was in general more efficient and addition of low concentration of cytokinin will improve the callusing ability of auxin (Bajaj, 2002).

Callus growth

Significant difference was observed for days to callusing in 2, 4-D, NAA and BAP combinations. Earliest response was registered in T_7 (2, 4-D 2.0 mgl^{-1} + NAA 2.0 mgl^{-1} + BAP 2.0 mgl^{-1}) when callus could be observed in 21.00 days itself which was on par with T_6 (2, 4-D 1.5 mgl^{-1} + NAA 1.5 mgl^{-1} + BAP 1.5 mgl^{-1}) which induced callusing in 23.50 days after inoculation. Relative growth rate of callus ranged from 0.14 to 1.52 (Table 3). The highest relative growth of 1.52 was observed in T_7 (2, 4-D 2.0 mgl^{-1} + NAA 2.0 mgl^{-1} + BAP 2.0 mgl^{-1}) treatment (Plate 3). Similarly, the highest callus index of 125.79 was registered in T_7 (2, 4-D 2.0 mgl^{-1} + NAA 2.0 mgl^{-1}+ BAP 2.0 mgl^{-1}) combination. Fresh weight of callus was also high (1.23 g) in the similar treatment.

Table.1 Standardization of explants for callus induction in *Andrographis echioides* (L.) Nees

xplants	Culture response (%)	Days taken for callusing
Stem bit	0.00 (0.64)	**45.00**
Leaf bit	**89.32 (70.93)**	**15.00**
Root bit	15.34 (23.06)	**21.00**
Nodal segment	5.13 (13.07)	**25.58**
Mean	27.45 (26.92)	**26.64**
SEd	0.385	**0.678**
CD (0.05)	0.838	**1.478**
CD (0.01)	**1.175**	**2.072**

Values in parentheses are arcsine-transformed.

Table.2 Effect of growth regulators on callus induction and callus response in *Andrographis echioides* (L.) Nees leaf bits

Treatments	Growth regulators (mgl^{-1})			Callus induction (%)	Callus response
	2,4-D	NAA	BAP		
T_1	MS basal	-	-	0.00 (0.64)	**0**
T_2	1.0	-	-	10.48 (18.88)	**1**
T_3	2.0	-	-	11.05 (19.42)	**1**
T_4	1.0	-	1.0	20.13 (26.66)	**1**
T_5	1.5	-	1.5	50.13 (45.07)	**2**
T_6	2.0	-	2.0	55.45 (48.13)	**2**
T_7	2.5	-	2.5	40.28 (39.39)	**2**
T_8	1.0	1.0	-	0.00 (0.64)	**0**
T_9	1.5	1.5	-	0.00 (0.64)	**0**
T_{10}	2.0	2.0	-	0.00 (0.64)	**0**
T_{11}	2.5	2.5	-	0.00 (0.64)	**0**
T_{12}	1.0	1.0	1.0	60.75 (51.21)	**2**
T_{13}	1.5	1.5	1.5	73.23 (58.84)	**3**
T_{14}	**2.0**	**2.0**	**2.0**	**77.45 (61.67)**	**3**
T_{15}	2.5	2.5	2.5	65.15 (53.82)	**2**
Mean				30.94 (28.42)	
SEd				1.184	
CD (0.05)				2.523	
CD (0.01)				3.489	

Values in parentheses are arcsine-transformed.
1. Poor callusing 2. Slight callusing 3. Moderate callusing 4.Profuse callusing

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

Treatments	Growth regulators (mgl^{-1})			Days taken for callusing	Relative growth	Callus index	Fresh weight of callus (g)
	2,4-D	NAA	BAP				
T_1	1.0	-	1.0	37.50	0.14	2.71	**0.18**
T_2	1.5	-	1.5	30.50	0.28	14.52	**0.30**
T_3	2.0	-	2.0	25.67	0.90	50.27	**0.75**
T_4	2.5	-	2.5	35.50	0.51	20.40	**0.61**
T_5	1.0	1.0	1.0	33.50	0.75	45.20	**0.54**
T_6	1.5	1.5	1.5	23.50	1.34	98.49	**0.91**
T_7	**2.0**	**2.0**	**2.0**	**21.00**	**1.52**	**125.79**	**1.23**
T_8	2.5	2.5	2.5	27.83	1.10	71.69	**1.03**
Mean				29.38	0.82	53.64	**0.69**
SEd				1.221	0.030	1.640	**0.032**
CD (0.05)				2.588	0.064	3.477	**0.067**
CD (0.01)				**3.565**	**0.088**	**4.790**	**0.092**

Table.4 Estimation of andrographolide content (%) of callus in *Andrographis echioides* (L.) Nees.

Treatments	Growth regulators (mgl^{-1})			Andrographolide (%) Days		T- Mean
	2,4-D	NAA	BAP	45	60	
T_1	MS basal	-	-	0.07	0.09	**0.08**
T_2	1.0	1.0	-	0.07	0.11	**0.09**
T_3	1.5	1.5	-	0.15	0.33	**0.24**
T_4	2.0	2.0	-	0.72	0.86	**0.79**
T_5	2.5	2.5	-	0.12	0.18	**0.15**
T_6	1.0	1.0	1.0	0.09	0.12	**0.11**
T_7	1.5	1.5	1.5	0.28	0.29	**0.29**
T_8	**2.0**	**2.0**	**2.0**	**1.30**	**1.31**	**1.31**
T_9	2.5	2.5	2.5	0.20	0.23	**0.22**
D-Mean				**0.33**	**0.39**	**0.36**

	SEd	CD (0.05)	CD (0.01)
T	0.008	0.015	**0.021**
D	0.004	0.007	**0.010**
TD	0.011	0.022	**0.029**
T – Treatment		**D - Mean**	

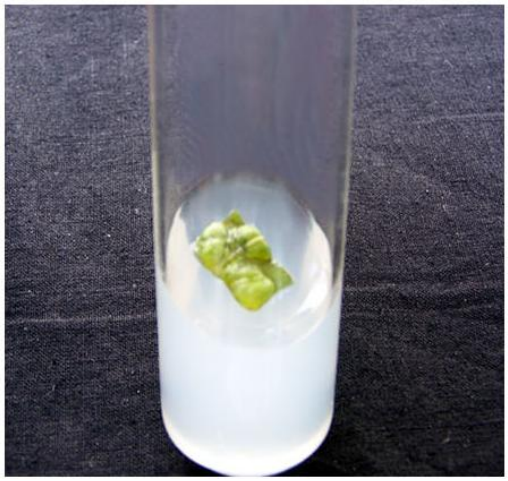

Plate.1 Callus initiation from *Andrographis echioides* leaf bit (MS + with 2, 4-D 2.0 mgl^{-1} + NAA 2.0 mgl^{-1} + BAP 2.0 mgl^{-1})

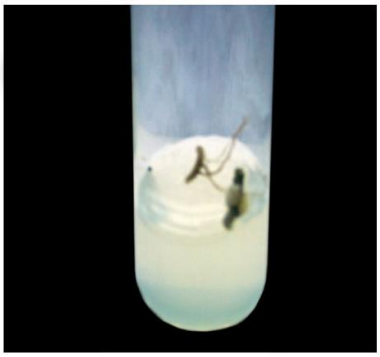

Plate.2 Callus initiation from *Andrographis echioides* root bit (MS + with 2, 4-D 2.0 mgl^{-1} + NAA 2.0 mgl^{-1} + BAP 2.0 mgl^{-1})

Plate.3 Callus proliferation from *Andrographis echioides* leaf bit (MS + with 2, 4-D 2.0 mgl^{-1} + NAA 2.0 mgl^{-1} + BAP 2.0 mgl^{-1})

This result was in accordance with the observation of Anand and Hariharan (1997) who reported that elicited callus growth was observed when 2, 4-D (1.5 mgl^{-1}), NAA (1.0 mgl^{-1}) and BAP (0.5 mgl^{-1}) was used together in MS medium in *Curcuma aromatica.* The addition of auxin was essential to induce and maintain callus growth in cultured explant. 2, 4-D and NAA, both synthetic auxins were used widely since they were found more stable than IAA (Indole Acetic Acid) which was a natural auxin that would get easily degraded by peroxidase (Keisarlourdusamy, 2002).

Andrographolide content in callus

The treatment containing 2, 4-D 2.0 mgl^{-1} + NAA 2.0 mgl^{-1} + BAP 2.0 mgl^{-1} during 60 days of culturing recorded the highest andrographolide content (1.31 %) which was on par with 45 days of culturing (1.30 %) in the same growth regulators combination (Table 4). Growth regulators combination indicated that MS basal medium recorded lower level of andrographolide followed by 2, 4-D and NAA combinations. 2, 4-D can stimulate both cell division and cell expansion, but it can also bring about dramatic suppression of secondary metabolite synthesis. The lower secondary metabolites in growth regulator free medium was due to increased cell death and low absorption of nutrients (Aneesarani, 2002).

The promotion of secondary metabolite production by cytokinin has been reported by Zenk *et al*. (1975) and Lemenager *et al*. (2004). Accordingly the treatment with optimum auxin and cytokinin combination produced higher alkaloid content.

Considering the duration of culture initiation, highest andrographolide content was recorded in 60 days of culturing. The secondary metabolite production was a dynamic balance between biosynthesis and biodegradation metabolites. At 60^{th} day of culturing, substantial decrease in cell growth and primary metabolite content which indicates the transforming stages of cells for secondary metabolite synthesis (Chawla, 2003).

Hence concluded that the investigations were undertaken to standardize the *in vitro* production of secondary metabolites in *Andrographis echioides* (L.) Nees. at the Tissue Culture Laboratory of Horticultural College and Research Institute Tamil Nadu Agricultural University, Coimbatore. In this study, leaf bits were considered as ideal explants for the highest (89.32 %) and earliest (15.00 days) callus induction. MS medium supplemented with 2, 4-D (2.0 mgl^{-1}) + NAA (2.0 mgl^{-1}) + BAP (2.0 mgl^{-1}) combination served as the best culture medium for earliest initiation (21.00 days) of profuse callus. Relative growth (1.52), callus index (125.79) and fresh weight of callus (1.23 g) were also high in the above treatment combination. Highest andrographolide content of 1.31 per cent was observed in callus grown in media supplemented with 2, 4-D (2.0 mgl^{-1}) + NAA (2.0 mgl^{-1}) + BAP (2.0 mgl^{-1}) combination. The alkaloid content registered an increasing trend from 45 days to 60 days callus.

References

Anand, P.H.M. and M. Hariharan. 1997. *In vitro* plant regeneration from rhizome bud-derived callus in yellow zedoary (*Curcuma aromatica* Salisb.) – a medicinal plant. In: Plant tissue culture and biotechnology: Emerging trends. Proceedings of a symposium held at Hyderabad, India, Jan, 29-31.

Aneesarani, A.M.S. 2002. Evaluation of ecotypes and production of secondary metabolites through cell suspension culture in *Gymnema sylvestre*. Ph.D., (Hort.) Thesis submitted to

Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore-3.

Bajaj, Y.P.S. 2002. Micropropagation of *Aegle marmelos*. In: High tech and Micropropagation. Springer, p. 281.

Baskarrajan, G. 1994. Studies on *in vitro* multiplication of patchouli (*Pogostemon patchouli* Hook.). M.Sc., (Hort.) Thesis, submitted to Annamalai University, India.

Chawla, H.S. 2003. Cell suspension and secondary metabolites. In: Introduction to Plant Biotechnology. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, pp. 57-73.

Gained, K.N., R.N. Dar and R.N. Kaul. 1963. Spectrophotometer estimation of andrographolide in Kalmegh. The Indian Journal of Pharmacy, 25: 225-226.

Keisarlourdusamy, K. 2002. Investigations on *in vitro* techniques for biosynthesis of secondary metabolites from high value medicinal plants. Ph.D., (Hort.) Thesis submitted to Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore – 3.

Lemenager, P., M. Clastre, M. Rideau and J. Aguirreolea. 2004. Hormone mediated alkaloids accumulation: Implication of redox state in cytokinin signaling. Univ-tours.fr/ed/edsst/comm.2004/ lemenager.pdf.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15: 437-497.

Parr, A.J. 1988. Secondary products from plant cell culture. In: Advances in biotechnology progress – Biotechnology in agriculture. (Eds.) Mizrahi, A. and Alan R. Liss, New York, 9: 1-34.

Zenk, M.H., H. El-Shagi and U. Schulte. 1975. Anthraquinone production by cell suspension cultures of *Morinda citrifolia*. Planta Medica Supplement: 79-101.

How to cite this article:

Hemalatha, P., V. Sivakumar and Rajamani, K. 2020 Studies on Callus Induction and *in Vitro* Production of Secondary Metabolite in *Andrographis echioides* (L.) Nees. *Int.J.Curr.Microbiol.App.Sci.* 9(10):3756-3763.doi: https://doi.org/10.20546/ijcmas.2020.910.432