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In vitro biomass accumulation and regeneration of potential medicinal plant green chiretta-*Andrographis paniculata* (Burm.f.) nees

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Abstract
The plant *Andrographis paniculata* (Burm.f.) Nees comes under the Acanthaceae family, its widely used around the worldwide used for cold, diabetes, liver disorders, upper respiratory tract infections, snake bite, dysentery, fever, malaria, etc., An efficient protocol was established for rapid micropropagaion *A. paniculata* from nodal explants. MS medium with IAA (2 mg^{-1}) showed highest growth in terms of fresh and dry weight was observed (44.5/8.7 mg), BAP (0.5 mg^{-1}) showed (44.7/8.5 mg), NAA (1 mg^{-l}) obtained more yielded the greatest fresh/dry weight 24.8/3.5 mg from the nodal explants, 2,4-D (5 mg^{-l}) observed more yielded of fresh/dry weight 60.5/10.6 mg. The combination of IAA and BAP (2+0.5 mg^{-l}) utmost growth occurred fresh/dry weight (79.7/17.3 mg), numerous shoots (20-80%) on the medium with 2, 4-D+NAA+BAP (5+2+1 mg^{-l}) showed multiple shoot formations. The shootlets has high elongation capacity: rooting from callus was not occurred in the nodal explants but minimum rooting performance in the medium complement with IAA (1 mg^{-l}). Well growned shoots were excised from the medium supplemented with 2, 4-D+NAA+BAP (5+2+0.5 mg^{-l}) and transferred to IAA (1 mg^{-l}). After one week from the subculture, few numbers of roots initiation stared from the cut ends of transferred shoots on the medium with IAA (1 mg^{-l}), concluded that competent protocol has been developed for plant regeneration from the calli derived from nodal explants of green chiretta.

Keywords: acanthaceae, calli, green chiretta, multiple shoots, organogenesis

Introduction

The plant tissue culture by *in vitro* method based on manipulation of organic and inorganic constituents in the medium, environment of explants type, plants, and species (Sri Devi *et al*., 2015) [1]. Biotechnological tools are very important for medicinal plants genetic enhancement and multiplication, carried out through techniques such as *in vitro* regeneration of plants in different methods based on explants (Tripathi and Tripathi, 2003) [2]. The tissue culture process is measured to be extremely important in terms of marketable medicinal plant productions, in which the sertile technique (free from microbes) of organs and cells preserve be achieved underneath *in vitro* conditions (Arjun, 2011) [3].
The precedent combine of decades encompass exaggerated the function of *in vitro* techniques in conservation plant efforts, it's mostly owing to decline rapidly and threats of biodiversity worldwide. The most important objectives of plant tissue culture are for increase conservation, food productivity, plants commercialization, promotion of sustainable plant parts, secondary metabolites production, understanding the growth and development requirements of plants through molecular markers (Hariprasath *et al*., 2015) [4].
Andrographis paniculata (Burm. f.) Wall. ex Nees essential medicinal plant belongs to Acanthaceae family, its extensively used around the worldwide, used as a herbal medicine traditionally in India, China, Malaysia, Bangladesh, Philippines, Pakistan, Hong Kong, Thailand and Indonesia (Kabir *et al*., 2014) [5]. It's a medicinal herbal plant with an tremendously bitter taste used for general childrens cold, liver disorders, bowel complaints, upper respiratory tract infections and colic pain (Roxas and Jurenka, 2007) [6].
The ethnobotanically plant used for the snake bite treatment, bug bite, dysentery, fever, diabetes, and malaria, Ayurvedic and Unani medicines, it's one of the typically used medicinal plants (Akbar, 2011) [7]. In current period, marketable arrangements of the plant extracts are also used in convinced countries. Mainly used in this plant extracts contains phenolic compounds, flavonoids, diterpene, diterpenoids, lactones, and flavonoid glycosides. Entire plant leaves and roots are used as a tradition medicine for different diseases in Asia and Europe Countries (Jarukamjorn and Nemoto 2008) [8]. *A. paniculata* has been reported to have a extensive assortment of pharmacological effects including anti-inflammatory, antidiarrheal, antihyperglycemic, anticancer (Chao *et al*., 2010: Arjun *et al*., 2015) [9, 10], anti-HIV, antihepatitis antimicrobial, antioxidant, antimalarial, cytotoxic, cardiovascular (Radhika *et al*., 2010) [11], hepatoprotective, immune stimulatory and sexual dysfunctions (Sheeja *et al*., 2006) [12]. *A. paniculata* 'cools' and inflammation relieves internal heat, pain and used as detoxication (Chao *et al*., 2009) [13]. Based on the important medicinal properties and pharmaceutical difficulty the *Andrographis paniculata* (Burm. f.) Nees plant selected for plant tissue culture techniques, reading reports the *in vitro* propagation and proportional fresh and dry

weight of calli obtained from *A. paniculata*.

Materials and Methods

Plant collection

The plant *Andrographis paniculata* (Burm.f.) Nees materials were collected from a small village Pillayarpatti, and kept at Herbal Garden of Tamil University, Thanjavur (DT), for *in vitro* studies explants were collected from garden.

Surface sterilization of the explants

The explants were treated with mercuric chloride (0.1%: 1 to 2 min), washed double with sterilized distilled water, ethanol (50% 2 to 3 min), explants were systematically washed twice with sterilized distilled water Murashige and Skoog (1962) [14]. The MS medium and freshly prepared sucrose (30 g^{-l}: 3%), mesoinositol (100 mg^{-l}: 0.1%), adjusted the pH 5.6-5.8 through HCl (0.1N) or NaOH (0.1N) after adjusted agar (8 gm^{-l}) was added, subsequently, the medium was reserved beneath refrigerator condition pending further use. Autoclaving at 121ºC and 1.06 kg/cm^2 pressure - 20 min, the cultures were incubated at 25±2ºC with a relative humidity (70-80%) and 16/8 hours (Light/Dark) photoperiod beneath photon flux thickness (Vinothini *et al*., 2017) [15].

For callus and calli induction, the explants were surface sterilized and placed on MS basal medium incubated under dark for 5 days, well grown callus pieces were transferred to MS medium with plant growth hormones: auxin and cytokinins are 2,4-Dichlorophenoxy Acetic Acid (2,4-D), Indole-3-acetic acid (IAA), Naphthalene Acetic Acid (NAA) and Benzyladenine (BA) (Arjun, 2011) [3]. The organogenic calluses were sub-cultured at an interval of every 20 day with the same treatment as applied for fresh medium until shoot regeneration began. The cultures were incubated at 25±2ºC in photoperiod (16/8 Light/Dark) for four weeks and calluses were harvested for further studies (Nagarajan *et al*., 2009) [16].

Growth measurements

Fresh and dry weights of the explants were measured before inoculation Fresh and dry weights of the explants were measured after 5 weeks of culture. Regular observation at an interval of two days was made for the formation of calli, change of colour and initiation of the root or shoots.

Statistical analysis

One-way analysis of variance (ANOVA) was used to estimate the implication of the dissimilarity of means of data from assorted experiments by using SPSS arithmetical software package (version: 16). The principles are offered as mean±SD and $P < 0.05$ is measured as noteworthy (Daoud *et al*., 2021) [17].

Results and Discussion

The *A. paniculata* nodal and leaf explants cultured on MS medium supplements with different concentrations of plant growth hormones: 2, 4-D, IAA, NAA and BAP, tissue culture responses was observed only from nodal explants, it showed high growth responses. Therefore only nodal explants of *A. paniculata* were used throughout the present study.

Out of the different concentrations of the IAA (0.5-5 mg^{-1}) used, the maximal growth in terms of fresh and dry weight was observed (2 mg^{-l}), fresh/dry weight 44.5/8.7 mg was obtained, the above 2 mg^{-1} of IAA was found to be inhibitory (Table 1), 5 mg^{-l} showed fresh/dry weight 18.7/3.9 mg. Among different concentrations of BAP (0.5-5 mg^{-l}) used, was establish to be supplementary successful in promoting growth (0.5 mg^{-l}) from the nodal explants and minimal growth was observed at BAP (5 mg^{-l}) (Table 2). Uppermost fresh/dry weight 44.7/8.5 mg notice, when increase the hormone concentrations it decrease the growth, fewest fresh/dry weight 30.9/2.8 mg was obtained.

Table 1: Growth effect of IAA on *A. paniculata* nodal explants

S. No	Concentrations (mg^{-l})	Weight at Harvest (mg)	
		Fresh weight	Dry weight
1	0.5	19.4±1.97	4.2±0.06
2	1	31.6±2.61	5.4±0.15
3	2	44.5±4.45	8.7±0.48
4	5	18.7±3.54	3.9±0.21

Table 2: Growth effect of BAP on *A. paniculata* nodal explants

S. No	Concentrations (mg^{-l})	Weight at Harvest (mg)	
		Fresh weight	Dry weight
1	0.5	44.7±3.92	8.5±1.49
2	1	37.4±3.23	3.6±0.24
3	2	33.5±3.87	3.2±0.19
4	5	30.9±2.98	2.8±0.34

The various concentrations of NAA (0.5-5 mg^{-l}) was used and it was found that concentration of NAA (1 mg^{-l}) obtained more yielded the greatest fresh/dry weight 24.8/3.5 mg from the nodal explants (Table 3), lowest was noticed on NAA (5 mg^{-l}) showed fresh/dry weight 17.3/2.1 mg. The dissimilar concentrations of 2,4-D (0.5-5 mg^{-l}) used, among them 5 mg^{-1} showed more yielded of fresh/dry weight 60.5/10.6 mg the plant growth response from the nodal explants, when increase the concentration of 2,4-D showed more yield (Table 4).

Table 3: Growth effect of NAA on *A. paniculata* nodal explants

S. No	Concentrations (mg^{-l})	Weight at Harvest (mg)	
		Fresh weight	Dry weight
1	0.5	17.5±1.37	2.3±0.06
2	1	24.8±0.98	3.5±0.28
3	2	20.5±1.67	2.9±0.16
4	5	17.3±1.52	2.1±0.12

Table 4: Growth outcome of 2, 4-D on *A. paniculata* nodal explants

S. No	Concentrations (mg^{-l})	Weight at Harvest (mg)	
		Fresh weight	Dry weight
1	0.5	38.1±3.18	4.4±0.14
2	1	47.4±2.76	6.9±1.21
3	2	52.7±3.23	0.8±0.67
4	5	60.5±4.61	10.6±1.1

The combination of IAA and BAP in different concentrations were used utmost growth occurred on the medium supplemented with IAA+BAP (2+0.5 mg^{-l}) showed fresh/dry weight (79.7/17.3 mg), followed by IAA+BAP (1+0.5 mg^{-l}) obtained that fresh/dry weight (55.6/11.9 mg). In the IAA+BAP (1+0.5 mg^{-l}) combination lowest amount of fresh/dry weight (23.4/4.3 mg) was obtained. The BAP+NAA combination was noticed, the maximum growth response fresh/dry weight (95.4/19.1 mg) was obtained from

BAP+NAA (2+0.5 mg^{-l}), followed by response fresh/dry weight (70.7/14.5 mg) was obtained, least response was observed on BAP+NAA (0.5+1 mg^{-l}) fresh/dry weight (34.4/6.1 mg) (Table 5).

Table 5: Plant growth hormone (PGR) combination and growth effects of *A. paniculata* nodal explants

S. No	Different concentrations of PGR (mg^{-l})				Weight at Harvest (mg)	
	IAA	BAP	NAA	2,4-D	Fresh weight	Dry weight
1	0.5	0.5	-	-	39.9±3.2	8.1±0.81
2	0.5	1	-	-	24.1±2.63	4.9±0.42
4	0.5	2	-	-	23.4±3.35	4.3±0.86
5	0.5	5	-	-	28.3±2.77	5.2±0.77
6	1	0.5	-	-	55.6±4.29	11.9±1.91
7	1	1	-	-	37.2±3.32	6.3±0.66
8	1	2	-	-	31.3±2.14	5.6±0.93
9	1	5	-	-	41.4±3.66	9.1±1.39
10	2	0.5	-	-	79.7±5.98	17.3±2.87
11	2	1	-	-	35.5±1.31	11.1±1.19
12	2	2	-	-	37.3±2.12	7.2±0.28
13	2	5	-	-	42.2±3.72	6.1±0.93
14	5	0.5	-	-	38.6±2.73	5.7±0.76
15	5	1	-	-	35.7±2.76	3.8±0.61
16	5	2	-	-	34.8±1.28	3.5±0.39
17	5	5	-	-	37.6±3.15	4.1±0.87
18	-	0.5	0.5	-	54.8±3.24	11.5±1.22
19	-	0.5	1	-	80.2±4.37	18.1±1.91
20	-	0.5	2	-	95.4±5.11	19.1±2.17
21	-	1	0.5	-	37.9±2.87	7.5±0.67
22	-	1	1	-	69.4±3.94	13.2±1.87
23	-	1	2	-	70.7±4.13	14.5±2.47
24	-	2	0.5	-	34.4±2.29	6.1±0.21
25	-	2	1	-	36.2±2.18	8.2±0.80
26	-	2	2	-	39.1±4.26	7.1±1.08
27	-	5	0.5	-	36.7±2.57	6.8±0.03
28	-	5	1	-	51.1±3.18	9.7±1.21
29	-	5	2	-	54.3±4.26	10.0±2.52
30	-	0.5	0.5	5	66.5±3.93	6.3±0.91
31	-	0.5	1	5	87.2±4.41	8.1±0.29
32	-	0.5	2	5	107.3±6.25	9.9±0.71
33	-	1	0.5	5	47.1±3.17	8.1±0.49
34	-	1	1	5	66.4±3.97	11.3±1.61
35	-	1	2	5	81.3±4.36	13.8±1.90

The 2-4-D and BAP alone showed callus and calli formation in different combinations, combination NAA+BAP, 2,4-D+NAA+BAP observed abundant growth in callus, calli formations, In all combinations callus and calli was formed of NAA and BAP different concentrations were obtained like readily formed and more proliferative, green and compact calli were formed (Table 6: Figure 1).

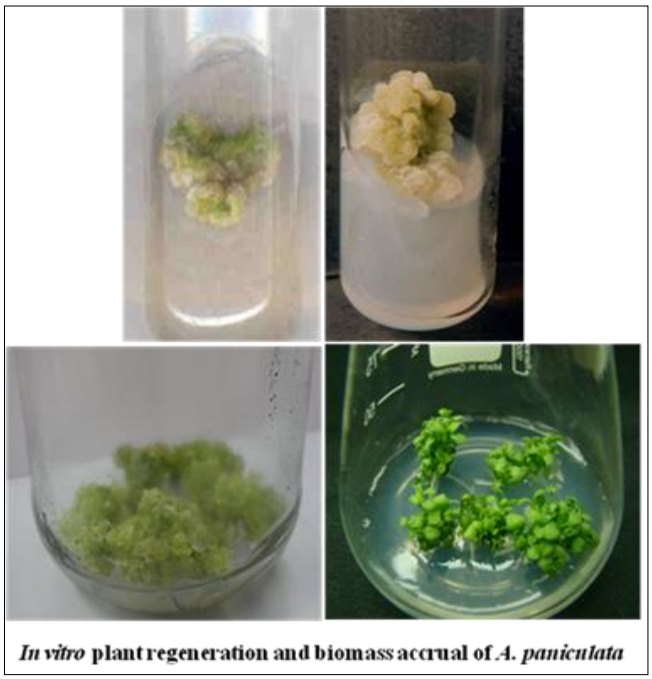
Table 6: Calli, root, shoot formation from different PGR combination and growth effects of *A. paniculata* nodal explants.

S. No	Different concentrations of PGR (mg^{-l})				Organogenesis		
	IAA	IAA	IAA	2,4-D	Inductions		
					Calli	Root	Shoot
1	0.5	-	-	-	-	-	-
2	1	-	-	-	-	++	-
3	2	-	-	-	-	-	++
4	5	-	-	-	-	-	-
5	-	0.5	-	-	++	-	-
6	-	1	-	-	+	-	-
7	-	2	-	-	+	-	-
8	-	5		-	-	-	-
9	-	-	0.5	-	-	-	-
10	-	-	1	-	-	-	-
11	-	-	2	-	-	-	+
12	-	-	5	-	-	-	-
13	-	-	-	0.5	+	-	-
14	-	-	-	1	+	-	-
15	-	-	-	2	++	-	-
16	-	-	-	5	+++	-	-

17	-	0.5	0.5	-	++	-	-
18	-	1	1	-	++	-	+++
19	-	2	2	-	+	-	-
20	-	1	0.5	-	+	-	-
21	-	1	1	-	++	-	-
22	-	1	2	-	++	-	-
23	-	2	0.5	-	-	-	-
24	-	2	1	-	+	-	-
25	-	2	2	-	+	-	-
26	-	5	0.5	-	+	-	-
27	-	5	1	-	+	-	-
28	-	5	2	-	++	-	-
29	-	0.5	0.5	5	+	-	++++
30	-	0.5	1	5	++	-	-
31	-	0.5	2	5	-	-	-
32	-	1	0.5	5	++	-	-
33	-	1	1	5	++	-	-
34	-	1	2	5	-	-	-

Note:- Morphogenesis absent, + above 20%, ++ above 40%, +++ above 60%, ++++ above 80%.

Maximum callus proliferation was observed on medium contain 2, 4-D (0.5 mg^{-1}), callus proliferation was observed both on the cut ends of the laminal and midrib region of the leaf explants. The callus was friable and honey in colour turning into brown as the culture period proceeded. Generally in all the leaf explants cultures, callusing occurred on second week of culture period. The callus was compact and light brown in colour callus also formed on the media supplemented with BAP (0.5-5 mg^{-l}). However maximum callus proliferation was observed on medium contain BAP (0.5 mg^{-l}) but lower then 2, 4-D induced callus. In this medium callus was formed after 15 days from the date of inoculation (Table 6: Figure 1).



In vitro plant regeneration and biomass accrual of *A. paniculata*

Fig 1

High frequency of morphogenetic potentiality was occurred on the medium contain higher in lower concentration of BAP with one or two fold increasing volume of NAA. The effective concentration of 2, 4-D (5 mg^{-l}) was supplemented with BAP and NAA in factorial combination. The selective combination was used for the investigation purpose of the hormonal responses. The highest growth of the nodal explants was found in the medium supplemented with BAP+NAA+2, 4-D (0.5+2+5 mg^{-l}) and also maximum growth was occurred (Table 6: Figure 1).

Callus was initiated on the midrib and vein ending of the leaf explants the callus was compact and water in colour changing into brown. In medium supplemented with BAP (1-5 mg^{-l}) also induced small amount of callus on the out ends and midrib region. The portion of leaf explants plays a vital role in the morphogenesis. The adaxial surface of leaf was response in medium. Nodal explants of *A. paniculata* showed different organogenetic response to different response to different factorial combination of hormones (Table 6: Figure 1).

Shoots developed from IAA developed shoots or induced shoots were transfer to the medium with NAA+BAP (1+1 mg^{-l}). After seven days from the date of subculture, shoots were elongated. Multiple shoots were formed on nodal explants of *A. paniculata* after to 4 weeks of culture period. MS medium with the factorial combination of NAA, BAP and 2, 4-D favored multiple shoot formation (0.5+0.5+5 mg^{-1}). In these moderate green and compact callus formed after two weeks, after two weeks from the inoculation, the certain explants was enlarged in green colour and friable callus developed around the explants. Later it proliferated into numerous shoots (20-80%) on the medium supplemented with NAA+BAP+2, 4-D (2+1+5 mg^{-l}) showed multiple shoot formations (Table 6). The shootlets has high elongation capacity: rooting from callus was not occurred in the nodal explants but minimum rooting performance in the medium supplement with IAA (1 mg^{-l}).

Well growned shoots were excised from the medium supplemented with NAA+ BAP 2, 4-D (2+0.5+5 mg^{-l}) and transferred to IAA (1 mg^{-l}). After one week from the subculture, few numbers of roots stared to grow from the cut ends of transferred shoots on the medium with IAA (1 mg^{-l}) (Table 6: Figure 1) Kumud *et al*. (2015a) [18], was reported that the shoot development utmost was obtained in MS medium with BA and KN, to augment the number of shoots by adding Casein hydrolysate (100 mg/L) (Rahman *et al*., 2004a: Chauhan *et al*., 2015: Vinothini *et al*., 2018: Sri Devi *et al*., 2018: Priya Prasannan *et al*., 2020) [19-23].

Conclusion

Morphogenesis induction from leaf and nodal explants of *A. paniculata* focused on MS medium with different

concentration and combination of auxin and cytokinins: 2, 4-D, IAA, NAA, BAP were attempted in the current investigation study. Different morphogentic responses was obtained from nodal explants, leaf explants showed only callus formation and also percentage limited. Our findings are highest amount of callus, calli formation, callus proliferation, green compact calli, shootlets has high elongation capacity, and root growth responses were obtained. Concluded that an efficient protocol has been developed for plant regeneration from nodal explants, we can conserve the plants via plant tissue culture techniques and without disturbing the plants in nature also we can focuses compound isolation through calli, secondary metabolites are used in medical, food, pharmaceutical industries, useful for human societies.

Conflict of Interest

There is no conflict of interest among authors

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