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In-vitro callogenesis of medicinal important plant *Andrographis paniculata* (Burm. f) Nees

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ABSTRACT

Medicinally important secondary metabolites gained much attention these days, owing to the world's inclination towards herbal medicines. The practice of in vitro culturing of medicinally important plants has helped in increasing their production quantitatively and qualitatively. Considering the medicinal importance of Andrographis paniculata, the present investigation was undertaken to develop a rapid and reliable method for in vitro callogenesis of this species using different explants. Among the various explants used, leaf and nodal explants showed the better response for callus induction on Murashige and Skoog's medium supplemented with various combinations of growth regulators. Best callus induction from leaf explants was obtained on MS medium containing 2,4-D (1.0mg/lit) + Kinetin (0.5mg/lit) followed by 2,4-D (1.5mg/lit)+Kinetin (1.0mg/lit). It was observed that nodal explants proved better for callogenesis on MS medium supplemented with 2, 4-D + Kinetin (1.5 + 1.0mg/lit). Out of total explants used for callogenic induction, internodal explants showed less callogenic induction than other explants under investigation.

Key words: Andrographis paniculata, callogenic induction, callus, growth regulators, MS Medium.

I INTRODUCTION

The annual herbaceous medicinal plant *Andrographis paniculata* Nees., commonly known as Kalmegh or "King of bitters" belongs to family Acanthaceae, is found in India, China and Sri Lanka [1-2]. Traditionally, the leaves and other aerial parts of this medicinal plant are used for the treatment of hepatitis, bronchitis, cough, fever, mouth ulcers, colitis, sores, tuberculosis, bacillary dysentery, venomous snake bites, common cold, urinary tract infections and acute diarrhoea [3]. The plant is also reported to have immune enhancement and anti-HIV activities [4], hypoglycaemic effect [5], hepatoprotective properties [6], cardio-protective activity [7], antihypertensive activity [8], antifungal [9], antioxidant [10], anticancer and immunostimulatory properties [11]. Andrographolide, a diterpene lactone, is a major constituent of *A. Paniculata* [12], mostly extracted from leaves. Due to its high medicinal value, it is having high market demand in Homeopathy as well as in Ayurveda [13].

Biotechnological approaches, specifically, plant tissue cultures, are found to have potential as a supplement to traditional agriculture in the industrial production of desirable bioactive plant metabolites [14-15]. There are successful examples of tissue cultures for several medicinally important plants where an increased content of the bioactive secondary metabolites has been achieved compared to that from wild plants [16]. Taking into account the increasing demand for these products, plant cell culture provides an alternative source that can overcome the limitations of extracting useful metabolites from limited natural resources. Currently, *in vitro* culture technique is being widely employed as a novel system to examine the production of specific secondary metabolites as it offers experimental advantages to both basic and applied research and to the development of models with scale-up potential [17-19]. The aim of the present study was to establish a reliable protocol for callus production of *Andrographis paniculata* which could be used for the extraction of valuable secondary metabolites of immense therapeutic importance.

II MATERIALS AND METHODS

2.1. Collection of plant material

In the present investigation the seeds as well as plants of *Andrographis paniculata* were collected from Nagarjun nursery, Dr. Panjabrao Deshmukh Krishi Vidhyapith, Akola (India). The plants were potted and seeds were sown in the Botanical garden of Department of Botany Sant Gadge Baba Amravati University, Amravati for future use in various *in vitro* experiments.

2.2. Media preparation and its sterilization

During the present investigation, MS media was used as a basal medium. It was supplemented with 3% (w/v) sucrose and 0.7% (w/v) agar. Then pH of the medium was adjusted to 5.6-5.8. Different combinations of growth regulators were added and mixed thoroughly in the growing media. Homogenous solution was transferred to various culture tubes with autoclavable caps and then sterilized in autoclave at 121° C, 15 lb psi for 15 to 20 minutes. Medium was allowed to solidify under aseptic conditions once after autoclaving.

2.3. Sterilization and inoculation of explants

Different explants such as nodal, internodal, and leaves from healthy and disease free plants were washed thoroughly with autoclaved double distilled water. These explants were then surface sterilized with 70% ethanol for 30 to 40 seconds, followed by rinsing with sterile double distilled water 3-4 times. The various explants were then surface sterilized with 0.1% mercuric chloride ($HgCl_2$) for 2-3 minutes, then rinsed with sterile double distilled water 3-4 times to remove the effect of surface sterilizing agents. All this procedure was carried out under Laminar Air Flow. The surface sterilized explants were then cut into small pieces, inoculated in MS [20] medium supplemented with different concentrations and combinations of growth regulators and were maintained in culture room at 25 ± 2^{0}C temperature and 16 hours light and 8 hours dark period. The cultures were observed at regular intervals for callus initiation and results were recorded regularly.

III RESULTS AND DISCUSSIONS

In the present study different explants of *Andrographis paniculata* were inoculated which produced callus in varying responses. Leaf explants showed varied levels of callogenesis with different hormonal supplements.

Among the different concentrations of auxins and cytokinins, better callogenesis was shown by 2, 4-D (1.0 and 1.5mg/lit) in combination with kinetin (0.5 and 1.0mg/lit) respectively. Ample amount of callus from the leaf explants were obtained when medium was supplemented with 2, 4-D (1.0mg/lit) in combination with Kinetin (0.5mg/lit) (Table 1). These findings support the work of Jindal *et. al*., 2016 [21] who induced callus from leaf explants on MS medium supplemented with various concentrations of 2, 4-D and TDZ alone, and in combinations (2, 4-D+NAA; 2, 4-D+Kn; BAP+NAA). Growth regulators such as 2, 4-D, NAA, BAP and Kinetin are frequently used to induce callus tissue in many plant species [22]. Hence, several concentrations and combinations of growth regulators were used to initiate and establish callus cultures of *Andrographis paniculata.* The present investigator reported that 2, 4-D (0.5mg/lit) in combination with Kinetin (1.0mg/lit) showed less amount of callus formation, while as 2, 4-D (2.0mg/lit) alone does not respond callus formation at all. The callus was compact, hard, regenerative and yellowish. It was observed that the callus obtained from the leaf explants took nearly about 25-28 days after inoculation.

Table 1: Callogenic response of leaf explants at different combinations and concentrations of growth regulators.

Sr. No.	Concentration of plant growth regulator		Callogenic response*	No of days required to induce callus	Texture and colour of callus
	2,4-D (mg/lit)	Kinetin (mg/lit)			
1	0.5	1.0	+	25-28	Compact, hard, regenerative and yellow
2	1.0	0.5	++	25-28	Compact, hard, regenerative and yellow
3	1.5	1.0	++	25-28	Compact, hard, regenerative and yellow
4	2.0	0.0	-	-	No callus

Where, - = No callus, + =Average callus induction and ++ = Good callus induction.
***Each value is the mean of three replicates**

During the study, it was also observed that nodal explants responded efficiently towards callus formation on MS media supplemented with different concentrations of auxins (2, 4-D and IBA) and cytokinins (Kinetin and BAP). However, maximum callus was produced on MS medium containing 2, 4-D + Kinetin (1.5+1.0mg/lit respectively) which got support from the studies made by Bidari *et. al*., 2012 [23]. In combination of 2, 4-D (0.5, 2.0mg/lit) and Kinetin (1.0, 0.0mg/lit), no callus induction occurred at all. Combination of IBA (1.0, 1.5mg/lit) and BAP (1.5, 2.0mg/lit) produce small amount of callus (Table 2). The callus was compact and light yellowish in colour after 25-30 days of inoculation.

Table 2: Callogenic response of nodal explants at different combinations and concentrations of growth regulators.

Sr. No.	Concentration of plant growth regulator				Callogenic response*	No of days required to induce callus	Texture and colour of callus
	2,4-D (mg/lit)	Kinetin (mg/lit)	IBA (mg/lit)	BAP (mg/lit)			
1	0.5	1.0	-	-	-	-	No callus
2	1.0	0.5	-	-	+	25-30	Compact, fragile and light yellowish
3	1.5	1.0	-	-	++	25-30	Compact, fragile and light yellowish
4	2.0	0.0	-	-	-	25-30	No callus
5	-	-	1.0	1.5	+	25-30	Compact, fragile and light yellowish
6	-	-	1.5	2.0	+	25-30	Compact, fragile and light yellowish

Where, - = No callus, + =Average callus induction and ++ = Good callus induction.
***Each value is the mean of three replicates.**

The inter-nodal explants gave less callus formation than the other explants under investigation. The callus was obtained within 25-30 days in combination of IBA (1.0, 1.5mg/lit) and BAP (1.5, 2.0mg/lit) respectively. However no callus induction occurred in combination of 2, 4-D (0.5, 2.0mg/lit) and Kinetin (1.0, 0.0mg/lit) respectively (Table 3).

Table 3: Callogenic response of inter-nodal explants at different concentrations and combinations of growth regulators

Sr. No.	Concentration of plant growth regulator				Callogenic response*	No of days required to induce callus	Texture and colour of callus
	2,4-D (mg/lit)	Kinetin (mg/lit)	IBA (mg/lit)	BAP (mg/lit)			
1	0.5	1.0	-	-	-	-	No callus
2	2.0	0.0	-	-	-	-	No callus
3	-	-	1.0	1.5	+	25-30	Compact, hard and yellowish
4	-	-	1.5	2.0	+	25-30	Compact, hard and yellowish

Where, - = No callus, + =Average callus induction and ++ = Good callus induction.
***Each value is the mean of three replicates**

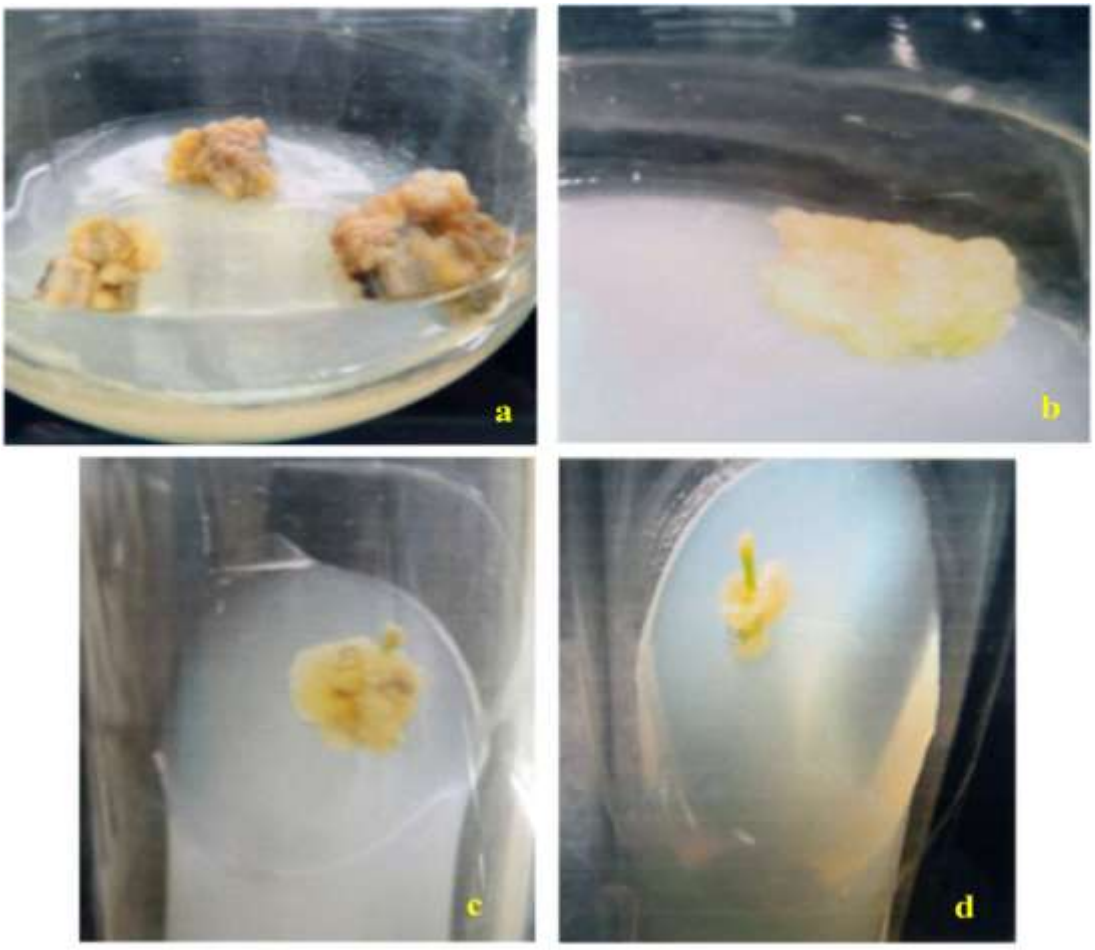

Fig. 1: Callogenic induction from *Andrographis paniculata.*
a: Callus formation from leaf explants at 2,4-D (1.0 mg/l)+kinetin(0.5 mg/l)
b:Callus formation from leaf explants at 2,4-D (1.5 mg/l) +kinetin(1.0 mg/l)
c: Callus formation from nodal explants at 2,4-D (1.5 mg/l)+kinetin(1.0 mg/l)
d: Callus formation from inter-nodal explants at BAP (1.5 mg/l)+kinetin(2.0 mg/l)

IV CONCLUSION

From the present study, it is concluded that leaf explants have good initiative activity for callus induction than other explants. The protocol for *in vitro* callogenesis of *Andrographis paniculata* is efficient and cost effective and may be applied for the extraction and estimation of valuable secondary metabolites like andrographolide having a lot of medicinal importance.

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