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Research Article

EFFECT OF GROWTH REGULATORS ON CALLUS INDUCTION FROM *CYCLEA PELTATA* (LAM.) HOOK. F. THOMS.

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ABSTRACT

Objective: To evaluate the effect of nutrient media and growth regulators on the stem explants of *Cyclea peltata* followed by the histological observations for the induction of callus.
Methods: The stem explants were inoculated on to MS, B5 and Nitsch media to check the suitable nutrient medium for the *in vitro* culture. Growth regulators such as 2, 4 – D, NAA, IAA, BAP and Kn were added to MS medium prior to the inoculation of stem explants and observed for the induction of callus, nature and color/pigmentation of the callus and initiation of roots and shoots. Callus induction was confirmed by histological observations.
Results: The young stem explants of *Cyclea peltata* cultured on MS medium supplemented with different concentrations of growth regulators showed the induction of callus. The callus obtained on the medium supplemented with either 2, 4 –D alone or in combination with BAP/Kn was yellowish cream and friable whereas, the callus obtained in the medium fortified with NAA/IAA alone or with BAP/Kn was compact and light to dark green in color. NAA (2mg/l), 2, 4 – D + BAP (1 + 3mg/l), 2, 4 – D + Kn (1 + 2, 2 + 0.1mg/l), NAA + BAP and IAA + BAP (3 + 2mg/l and 0.1 + 0.5mg/l) of concentrations induced significantly a higher number of calli. The histological study revealed the induction of callus from the cambial region of the explants.
Conclusion: The study revealed the induction of callus from the cambial cells of stem explants of *Cyclea peltata* when cultured on MS medium supplemented with combinations of auxin and cytokinins.

Keywords: *Cyclea peltata*, auxin, cytokinin, callus

INTRODUCTION

Cyclea peltata (Lam.) Hook. f. Thoms. (Menispermaceae) is a medicinally important plant used in the treatment of various health problems. *C. peltata* is reported to have anti-toxin function besides playing a role in diabetic disorders of skin, like boils and carbuncle and antidiabetic, anti-inflammatory activities, protection against nephrotoxicity and oxidative damage [1-5]. Jain [6] reported the use of *Cyclea peltata* for small pox and stomach ache.

The *in vitro* culturing of cells, tissues or organs depends on various physico-chemical factors such as light, temperature, humidity, nutrient supply, pH conditions, growth regulators, season for explant collection, type/age of explant and genotype. Nurazah et al. [7] studied the role of explant age on *in vitro* culturing in cotton at weekly intervals. The best result for the multiple shoot induction from the nodal explants of *Madhuca latifolia* Macb. was observed when the explants were collected in the month of May [8].

The role of different growth regulators on the induction of callus with varying colors were reported in *Ruta graveolens* with the induction of green to yellow colored callus in the medium with NAA [9], *Phyllanthus amarus* with the induction of dark green, nodular callus in the medium supplemented with BAP/TDZ [10] and *Pulsatilla koreana* with the induction of soft, light green callus in the medium with Kn [11]. In the present study an attempt was made to check the type of nutrient medium, age of the explant and type of suitable growth regulators to induce the optimum callus from different explants of *C. peltata*.

MATERIALS AND METHODS

Plant material and surface sterilization

The stem of *C. peltata* (Menispermaceae) were collected from natural forests of Dakshina Kannada District, Karnataka, India, and identified following the Flora of Udupi and Dakshin Kannada [12] and the voucher specimen (MU/AB/BN-02) were deposited at the herbarium of Department of Applied Botany.

The mature, young and youngest explants were collected to find out the suitable explant for *in vitro* culture. The collected plant materials were washed thoroughly under running tap water for 20-30min to remove the surface debris [13]. The explants were treated with Bavistine (30-45min), 70% alcohol (30sec) and 0.1% $HgCl_2$ (10min). After each step, the explants were washed in sterile distilled water. Later, the explants were cut into ~0.5cm and inoculated on to nutrient medium.

Effect of nutrient media and growth regulators on the stem explants of *C. peltata*

Three different types of culture media *viz*: MS, B5 and Nitsch were used in the present study to find out the suitable medium for callus induction. The stem explants with ~5cm size were inoculated on to MS medium with varying concentrations of growth regulators either singly or in combinations. The selected growth regulators include 2, 4 – D, NAA, IAA, BAP and Kn (0.1- 3mg/l). All the cultures were incubated at 25 ± 2°C with 16hr of photoperiod and 40.0 ± 3.0µmol m^{-2} s^{-1} light intensity unless specified. The explants were observed at the end of 25-30d of incubation for various details such as development of callus from mature or immature regions of the explants, nature of callus, color/pigmentation of the callus, initiation of roots and shoots. The histological studies of the Carnoy's B fluid fixed cultures were carried out to observe the induction of callus from the explants.

Statistical analysis of the data

The data presented are the average of three replicates and expressed as Mean ± SD. The statistical analysis of the data was carried out using SAS package (version 9.0) and the treatment means were compared using Duncun's Multiple Range Test (DMRT) at a level of 5% significance.

RESULTS AND DISCUSSION

Effect of nutrient media on the stem explants of *C. peltata*

There was no significant variation between the callus induced in three types of media by the explants of *C. peltata*. Therefore, the most commonly used MS medium was selected for further experiments. However, there was a significant variation in callus induction with respect to the concentrations of growth regulators used in the study and age of the explants taken. MS medium is the most common and suitable nutrient medium used for the *in vitro* culturing of many plant species. The culturing of four genotypes of rice in MS, N6 and R media showed a better callus induction on MS medium compared to N6 and Regeneration (R) media [14]. Similarly, a fairly good callus was observed in the present study irrespective of the media used. Thus, MS medium was used in all further experiments as this is the versatile medium used in culture studies.

The young explants of *C. peltata* responded well with the initial swelling followed by the emergence of callus from the explants after 10d of incubation. In three different tomato cultivars, Hana, Premium and Money maker, the *in vitro* regeneration of shoots was observed better when 8-10d old cotyledon and hypocotyl explants were used for the culturing [15]. The *in vitro* culturing of lettuce for callus induction showed better result with 3d old explants indicating the age as the critical factor for callus induction [16].

Effect of growth regulators on the stem explants of *C. peltata*

The stem explants of *C. peltata* cultured on medium supplemented with auxin (2, 4 –D, NAA, IAA) or cytokinin (BAP, Kn) or combinations of auxin with cytokinin showed morphologically different types of callus irrespective of the concentrations of growth regulators used in the study (Table 1). The explants showed a twist and swelling (Fig. 1a) soon after a week of incubation followed by the emergence of callus all over the explants. The twisting and swelling of the explant observed in *C. peltata* may be due to the internal pressure developed during the incubation. Similar to this result, the swelling of explants during the induction of callus was reported in the hypocotyl and leaf explants of *Meconopsis simpficifofia* [17], nodal explants of *Dendrocalamus hamiltonii* Nees et Arn. Ex Munro [18] and leaf explants of *Mentha piperita* (L.) [19].

Table 1: Effect of growth regulators on callus, root and shoot induction from *C. peltata* stem explants

Growth regulators	Callus texture	Callus color	Root induction	Shoot induction
2, 4 – D, 2, 4 – D + BAP/ Kn	Friable	Yellowish cream	Nil	Nil
NAA, NAA+ BAP/Kn	Compact	Light to dark green	Nil	Nil
IAA, IAA + BAP/Kn	Compact	Cream to green	Nil	Nil
BAP, Kn	Hard	Light green	Nil	Yes

The medium supplemented with either 2, 4 –D alone or in combination with BAP/Kn induced yellowish cream, friable callus (Fig. 1b). The callus obtained in the medium fortified with NAA/IAA or NAA/IAA + BAP/Kn was compact and light to dark green in color (Fig. 1c). This result is in agreement with the report of Shirin et al. [20], where in, they have observed the formation of green colored calli of *Solanum tuberosum* in the medium supplemented with NAA and BAP and yellow colored calli in 2, 4 – D and green colored calli in the medium supplemented with NAA alone or in combination with BAP. The induction of callus from other members of Menispermaceae viz. *Stephania cepharantha* Hayata [21], *Coscinium fenestratum* [22] and *Tinospora cordifolia* [23] were reported when cultured on MS medium supplemented with 2, 4 – D + Kn/BAP. Based on the results obtained in different plant species and from the present study, it is clear that the callus nature, texture and color are highly species specific.

The nodal explants showed the development of dark green colored callus (Fig. 1d). However, a few of the nodal explants showed the induction of single axillary shoot (Fig. 1e) and these shoots failed to induce roots upon transfer to medium with IBA or IAA. The direct organogenesis in *Tinospora cordifolia* from the nodal explants [24] and *Psoralea corylifolia* [25] from the shoot tips were reported when cultured on MS medium fortified with varying concentration of growth regulators. The *in vitro* regeneration of *C. peltata* using nodal explants was reported by Abraham et al. [26] where, the explants were cultured on MS medium supplemented with 3mg/l of BAP and 0.5mg/l of IAA to induce the highest regeneration. However, in the present work, the use of same growth regulators at the same concentration induced only 1-2 axillary shoots and these failed to induce roots. This may be due to various factors like environmental conditions, genotype of the explant, culture conditions, and concentration of endogenous growth regulators.

The initiation of callus in the stem explants of *C. peltata* was observed from the cambial region (Fig. 1f). The cells were arranged one above the other with a prominent nucleus. The induction of callus from the cambial cells, phloem and cortical cells was reported in tea [27] and black pepper [28]. In *Vigna radiata* W. the induction of callus started from the outer layer of cambial cells [29]. Similarly, in *C. peltata* the histological studies confirmed the induction of callus from the cambial region of the explants.

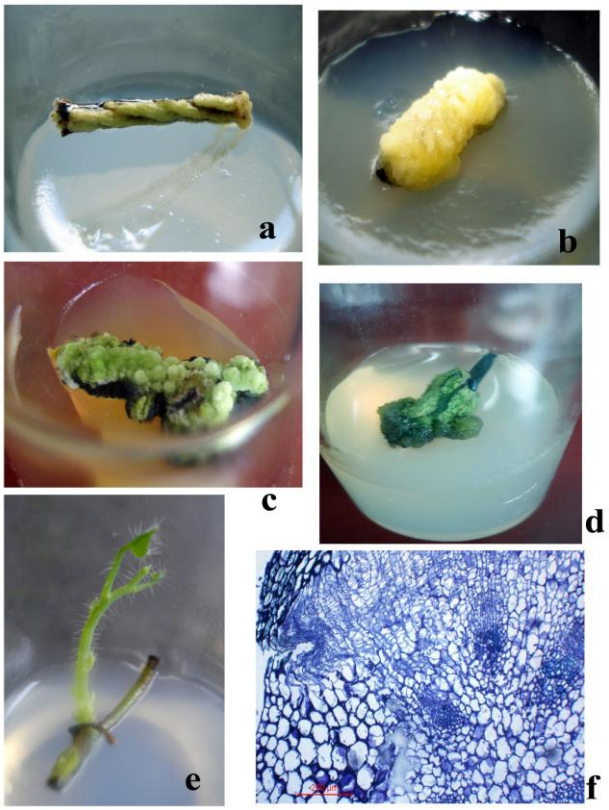

Fig. 1: a Twisted explant showing the induction of callus; **b** Callus induction on medium supplemented with 2, 4 – D; **c** Callus induction on medium supplemented with NAA; **d** Callus induction from nodal explant; **e** Emergence of single axillary shoot from nodal explant; **f** Stem explant showing callus induction from the cambial region (bar= 0.2mm)

The stem explants responded positively by inducing the callus when cultured on medium supplemented with either auxin or cytokinin with a highest mean number of 6.66 ± 0.57 explants in the medium with NAA at the concentration of 2mg/l (Table 2). The stem explants showed a highest callus induction (8.50 ± 0.71) when cultured on medium supplemented with 2, 4 – D + BAP at the concentrations of 1 + 3mg/l (Table 3).

Table 2: Effect of growth regulators on callus induction from *C. peltata* stem explants: Auxin and cytokinin

Growth regulators	Number of explants showing callus (Mean±SD)				
	0.1	0.5	1	2	3
2, 4- D	1.33±0.57 bcd	2.66±2.08 bcd	5.33±0.57 a	3.66±1.15 b	1.33±0.57 bcd
NAA	1.00±0.00 bcd	4.00±1.00 a	6.33±1.15 a	6.66±0.57 a	2.33±1.52 bcd
IAA	5.00±0.00a	3.66±1.52 b	2.33±0.57 bcd	2.00±2.00 bcd	3.00±1.00 bc
BAP	2.33±1.15 bcd	4.33±0.57 a	4.33±0.57 a	4.00±2.64 a	0.33±0.57 bcd
Kn	2.66±2.30 bcd	5.33±0.57 a	5.66±1.52 a	1.66±1.52 bcd	0.66±0.57 bcd
SE/plot = 1.25, CV (%) = 38.24					

Values with the different letters indicate significant difference at 5% level.

Table 3: Effect of growth regulators on callus induction from *C. peltata* stem explants: 2, 4 – D + BAP and 2, 4 - D + Kn

2, 4-D	Number of explants showing callus (Mean±SD)				
	0.1	0.5	1	2	3
			BAP		
0.1	5.00±0.00 b	4.50±0.71 b	5.00±1.41 b	7.00±1.41 a	6.50±0.71 a
0.5	5.50±0.71 b	6.00±0.00 a	5.50±0.71 b	6.00±1.41 b	7.50±0.70 a
1	7.50±0.70 a	4.50±0.71 b	8.50±0.70 a	7.00±0.00 a	8.50±0.71 a
2	6.50±0.70 a	7.50±0.70 a	6.00±1.41 b	4.50±0.71 b	5.50±0.71 b
3	5.00±1.41 b	5.50±2.12 b	5.50±0.71 b	4.50±2.12 b	5.50±0.71 b
SE/plot=1.03, CV (%) = 17.10					
			Kn		
0.1	3.50±0.71 b-l	6.00±1.41 b-i	4.00±1.41 b-l	5.50±0.71 b-k	5.00±0.00 b-k
0.5	3.50±0.70 b-l	2.50±0.70 b-l	2.50±0.70 b-l	3.50±0.70 b-l	2.50±0.71 b-l
1	3.00±1.41 b-l	6.50±0.71 b-g	8.00±1.41 a	9.50±0.71 a	8.50±0.71 a
2	9.50±0.71 a	8.00±0.00 a	9.00±0.00 a	7.50±0.71 bc	7.50±0.71 bc
3	5.50±0.70 b-k	8.50±0.71 a	6.50±0.70 b-g	6.00±0.00 b-i	8.50±0.70 a
SE/plot = 0.81, CV (%) = 13.49					

Values with the different letters indicate significant difference at 5% level.

The stem explants showed a highest mean number of 9.50 ± 0.71 explants for callus induction when cultured on medium supplemented with at 2, 4 – D + Kn at the concentration of 1 + 2 and 2 + 0.1mg/l (Table 3). The stem explants showed a highest mean number of 9.50 ± 0.71 and 8.50 ± 2.12 explants for callus induction when cultured on medium supplemented with NAA + BAP and IAA + BAP at the concentrations of 3 + 2mg/l and 0.1 + 0.5mg/l respectively (Table 4 and 5).

Table 4: Effect of growth regulators on callus induction from *C. peltata* stem explants: NAA+BAP and NAA + Kn

NAA	Number of explants showing callus (Mean±SD)				
	0.1	0.5	1	2	3
			BAP		
0.1	8.50±0.71 a	6.00±0.00 b-e	6.50±0.71 b-d	7.00±1.41 b	7.00±0.00 b
0.5	6.00±0.00 b-e	5.50±0.71 b-e	5.00±1.41 b-h	7.00±1.41 b	6.00±1.41 b-e
1	7.50±0.70 a	6.00±0.00 b-e	5.00±1.41 b-h	7.00±1.41 b	3.50±2.12 b-i
2	4.00±0.00 b-i	4.50±0.71 b-i	9.00±1.41 a	5.00±1.41 b-h	3.00±1.41 b-i
3	7.50±0.71 a	7.50±0.70 a	4.00±1.41 b-i	9.50±0.71 a	9.00±1.41 a
SE/plot =1.10, CV (%) = 17.64					
			Kn		
0.1	6.00±0.00	8.00±1.41	9.50±0.71	9.00±1.41	9.00±1.41
0.5	7.50±0.71	6.50±0.71	8.50±2.12	8.00±0.00	6.00±1.41
1	7.00±1.41	6.50±0.71	8.00±2.83	7.00±1.41	5.50±2.12
2	9.50±0.70	7.50±0.70	9.00±1.41	8.00±2.83	6.50±2.12
3	10.00±0.00	10.00±0.00	9.00±0.00	10.00±0.00	9.50±0.70
SE/plot =1.37, CV (%) = 17.05					

Values with the different letters indicate significant difference at 5% level.

Table 5: Effect of growth regulators on callus induction from *C. peltata* stem explants: IAA+BAP and IAA + Kn

IAA	Number of explants showing callus (Mean±SD)				
	0.1	0.5	1	2	3
			BAP		
0.1	1.00±1.41 b-l	8.50±2.12 a	6.50±0.71 b-d	8.00±1.41 a	7.00±2.83 b
0.5	3.50±0.71 b-k	7.00±1.41 b	8.00±1.41 a	5.50±0.71 b-f	5.00±1.41 b-g
1	5.00±0.00 b-g	4.50±0.71 b-h	2.50±0.70 b-l	3.50±0.70 b-k	1.00±0.00 b-l
2	1.50±2.12 b-l	2.00±1.41 b-l	1.00±1.41 b-l	6.00±1.41 b-d	4.00±0.00 b-i
3	4.00±1.41 b-i	2.50±0.71 b-l	2.00±0.00 b-l	2.50±0.71 b-l	1.50±2.12 b-l
SE/plot =1.32, CV (%) = 31.86					
			Kn		
0.1	3.00±1.41	3.00±0.00	2.50±0.71	1.00±1.41	1.00±1.41
0.5	5.50±0.71	3.00±1.41	3.50±0.70	1.00±1.41	2.50±3.55
1	3.50±2.12	4.50±0.71	2.50±0.71	2.50±0.71	4.00±0.00
2	5.50±0.71	2.00±0.00	3.00±1.41	5.00±0.00	2.50±0.71
3	4.00±1.41	4.50±0.71	4.00±1.41	4.00±1.41	3.00±1.41
SE/plot = 1.29, CV (%) = 40.01					

Values with the different letters indicate significant difference at 5% level.

The influence of different concentrations and combinations of growth regulators on the induction of callus from the nodal explants of *Rauwolfia serpentina* L. Benth showed a better response in the medium supplemented with BAP + NAA at the concentration of 0.5 + 2mg/l [30]. Nurazah et al.[7] studied the effect of growth regulators on callus induction from the flower petal explants of *Cananga odorata* and observed a better response in the medium supplemented with NAA + BAP (3 + 0.5mg/l). The induction of callus using auxin alone or in combination with cytokinin in MS medium was reported in different explants of various plant species viz. cotyledon explants of *Mucuna pruriens* (L.) DC. in the medium with 6.7µM of 2, 4 – D [31] and leaf explants of *Phyllanthus amarus* in the medium with 2.26µM of 2, 4 - D and 2.32µM of Kn [10]. In the present study, the stem explants of *C. peltata* responded well when cultured on the medium supplemented with combinations of auxin and cytokinins selected in the study. This suggests that the callus induction and adventitious shoot regeneration from the selected explants depended on the genotype and combinations of growth regulators as reported by Zhang et al. [32]. The callus obtained may be maintained on suitable culture condition either to regenerate the plantlets or to produce pharmaceutically important bioactive compounds.

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