Optimization of MS media for Callus and Suspension Culture of *Costus pictus*

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Abstract-- *Costus pictus* is vulnerable species in India and threatened to extinction due to its indiscriminate collection. Tissue culture seems to be the way to preserve, protect and multiply this rare species for large scale multiplication. In the present study, protocol for callus culture was standardized using various concentrations of hormones (2, 4-D, Kinetin, IAA, BAP), MS medium (full strength, half strength, one-fourth strength), pH **(**4.5 – 7**)** and carbon sources (sucrose, fructose, glucose, galactose, sorbitol, mannitol at 3% and 6% concentration). The highest callus growth was observed on half strength MS medium supplemented with 2,4-D/ Kin (1/0.5 mg/L) and IAA/BAP (1 mg/L).Maximum callus growth was observed with glucose (3% and 6%) and the pH value5.5. This is the first report of successful callus culture of *Costus pictus* leaves and also its suspension culture. The growth pattern of cell suspension culture was examined over a period of 10 days.

Keywords: Callus culture, *Costus pictus*, Glucose, MS medium, Suspension culture, 2, 4-D, Kin.

1 INTRODUCTION

Costus pictus D.Don commonly known as Spiral ginger, Step ladder is a member of *Zingiberaceae* family and is newly introduced plant in India originated in Mexico [1], [2]. It is a potent antidiabetic plant [3] and used in folk medicine, ayurvedic and homeopathic system of medicine. It is also used in asthma, eye complaints and snake bites. It is considered as purgative, astringent, expectorant and useful in burning sensation, constipation, leprosy, skin disease, inflammation, renal disorder and anemia. Its natural strands are fast disappearing and threatened with extinction due to its indiscriminate collection and over exploitation to meet the requirements of pharmaceutical industries. Conventional propagation is hampered due to low rate of germination and poor rooting ability of vegetative cuttings. Hence, plant tissue culture has proved to be helpful in conserving threatened plant species.

The manipulation of medium components has proven to be an important strategy for improvement of secondary metabolites yield by callus and cell suspension culture [4]. In general to obtain maximum yield of callus it is necessary to fine-tune the type and level of growth regulators required in cultured medium [5].To maximize the formation of a particular compound, it is desirable to initiate the callus from the plant part that is known to be a high producer [6].The callus tissues can be extracted by suitable solvents to isolate the desired compound. However, from an engineering perspective, cell suspension cultures have more immediate potential for industrial application [7].When it is introduced into liquid medium and agitated, the cells disperse throughout the liquid to form a cell suspension culture [8]. Such cells are totipotent and should also have a potential to synthesize any of the compounds normally associated with intact plants. Cells in suspension can exhibit much higher rates of cell division than cells in callus culture [1].

Considering the above mentioned facts, the present study was aimed at optimizing media composition to obtain the maximum callus growth and its cell suspension culture. In this work callus of *Costus pictus* was established and grown in different media formulations to determine the individual and interaction effect of medium components, media strength, plant growth regulators, various carbon sources and different pH values on growth of callus culture.

2 MATERIALS AND METHODS

2.0 Plant material

Healthy and young plants of *Costus pictus* (3- 6 months old) were purchased from Kerala and maintained in Paradise nursery, Nashik.

2.1 Explant preparation

The plants parts viz. rhizome, stem and leaves were washed thoroughly under running tap water for 2 h. The explants were then sterilized with 0.1%$HgCl_2$ for 10 min. It was then given treatment with 70% ethanol for 10 seconds for stem and rhizome explants and for 2 seconds for leaf explants. The explants were rinsed with sterile distilled water for 5-6times and used for

inoculation. Rhizome (1cm), nodal segment (1cm), intermodal segment (1cm) and leaf (10-12mm^2) were cut with the help of sterile forceps and scalpel. The explants were aseptically transferred into the culture medium and incubated.

2.2 Preparation of media with different hormonal combinations

The explants were then inoculated on full, half and one fourth strengths of MS medium (Murashige and Skoog [9]) supplemented with auxins (2, 4-D, IAA) and cytokinins (BAP, Kin) ranging from 0.5 to 2mg/L individually and in combination [12]. The pH of the medium was adjusted to 5.7 with NaOH or HCl before autoclaving at 121°C for 15 min. [3]. The explants were incubated under 16 h photoperiod of 2000-3000 lux at 25±2°C, until callus was induced [10],[11]. The callus tissues, formed were subcultured onto fresh medium every three weeks. Data were recorded and were presented as mean of 20 explants per treatment and repeated thrice.

2.3 Preparation of media with different carbon sources and pH values

Different carbon sources (sucrose, fructose, glucose, galactose, mannitol and sorbitol), each at 3 and 6% also were added to the medium to study the effect on callus growth. To optimize the medium pH for callus growth, the pH of the media was adjusted at the range of 4.5 to 7 (prior to autoclaving), using either NaOH or HCL. The selected carbon source and pH value was individually tested at a concentration of 2, 4-D and Kinetin ranging from 0.5 to 2 mg/L [8].

2.4 Suspension culture of *Costus pictus*

Cells in suspension can exhibit much higher rates of cell division [12], [13], [14]. Liquid MS media with half strength was prepared for suspension culture containing appropriate growth regulators. Media was autoclaved at 121°C for 15 min and used for suspension culture. Callus obtained from leaves of *Costus pictus* was aseptically inoculated in½ strength liquid MS media with IAA +BAP for *Costus pictus*. It was incubated at room temperature on rotary shaker (55 rpm) for 7 days and the growth curve was prepared. [15]

3.0 RESULTS AND DISSCUSSION

3.1 Effect of plant growth regulators and media strength on callus induction and callus growth.

The type and concentration of auxins and cytokinins alone or in combination has been known to strongly influence the growth of callus. Maximum Callus growth was observed only for the leaf explant within 20 days of culture in treatments containing IAA + BAP (1.5mg/L) and 2,4-D + KIN (1mg/L)on 1/2 and 1/4 strength MS media (Table 1&2). In the study by S.Biradar et al, micropropagation studies in *Costus pictus* using different growth hormones viz. BAP, KIN, IBA, IAA, and NAA have been carried out. Suitable combinations of growth regulators recorded by them for micropropagation were 2.7 mg/L BAP, 0.2 mg/L IAA from the nodal region. In present study, we have observed good callus growth not only with IAA and BAP but also with hormones like Kin and 2, 4- D.[16] No response was observed on full strength MS media supplemented with growth regulators indicating that explants are sensitive to high nutrient concentration. Also there was no effect of 2, 4-D and kinetin when used individually in the MS medium for callus initiation. All other growth regulator combinations were ineffective. These results show that *Costus pictus* is a low nutrient requiring plant. Masoumian et al [8] reported the effect of media strength on callus growth for the production of Flavonoids in *Hydrocotylebonariens.*

3.2 Effect of different carbon sources.

Callus culture with different carbon sources i.e. sucrose, fructose, glucose, galactose, sorbitol, mannitol at 3% and 6% were tested with different hormonal combinations of 2,4-D and Kinetin. Among the carbon sources used, sucrose, glucose, fructose each at 3% and 6% showed the callus growth.(see table 3,4,5) In this study however, glucose at 3% and 6% yielded highest callus growth followed by sucrose and fructose respectively. These results may be due to the easy metabolism of glucose in combination with different hormonal combinations and media strength as compared to other carbon source used.

O.L.Gamborg, *et al* has stated that glucose can be alternatively used with sucrose as a medium for plant tissue culture. [17] Generally in plant tissue, sucrose is used as a carbon source for growth of callus however in this study glucose and sucrose showed almost similar results for callus growth.

3.3 Effect of pH value

pH is extremely important as it influences the uptake of nutrient and plant growth regulator by regulating their solubility in culture medium. It also regulates wide range of biochemical reaction occurring in plant tissue. The range of pH at 4.5-7 was tested in the medium using sucrose as a carbon source. It was found that the pH more than 6 made the medium very hard on the contrary pH less than 5 did not allow satisfactory gelling. Optimum callus growth was observed at pH 5.5. (see table 6). Masoumian et al [8] also checked the effect of pH value on growth of callus for accumulation of flavonoids in *Hydrocotylebonariensis.*

3.4 Cell Suspension culture of *Costus pictus*

Cell Suspension culture of *Costus pictus* callus obtained from leaves was successfully established. The cell number (1, 80,000 cells/ml) of *Costus pictus* increased till 9th day.

Thus instead of furnessing medical components directly from plants, suspension culture can be established which will aid in plant conservation as well as lead the growing need of pharmaceutical industries.[18] (see fig. 1)

Table 1 - Callus growth on 1/2 strength / 1/4 strength MS media respectively with IAA + BAP after 20 days of culture initiation from leaf explant.

BAP / IAA	0.5 mg/L	1 mg/L	1.5 mg/L	2 mg/L
0.5 mg/L	EC / -	C / C	S / C	-/ C
1 mg/L	C / C	EC /-	-/ C	C /-
1.5 mg/L	- / C	C / C	EC / EC	EC / EC
2 mg/L	S / -	EC / C	C /-	C / C

EC= Extensive callus growth; C= moderate callus growth; S= callus initiation

Table 2- Callus growth on 1/2 strength / 1/4 strength MS media **respectively** with Kin + 2, 4-D after 20 days of initiation of culture from leaf explant.

Kin / 2,4-D	0.5 mg/L	1 mg/L	1.5 mg/L	2 mg/L
0.5 mg/L	EC / C	EC / C	-/-	-/ C
1 mg/L	-/-	-/ C	-/-	C / C
1.5 mg/L	-/-	C /-	C / C	C / C
2 mg/L	-/-	EC / C	-/ C	C / C

EC= Extensive callus growth; C= moderate callus growth

Table 3 - Effect of 3% / 6% sucrose concentration respectively on ½ strength MS media with 2,4-D + Kin on growth of callus from leaf explant.

Kin / 2,4-D	0.5 mg/L	1 mg/L	1.5 mg/L	2 mg/L
0.5 mg/L	- / -	- / C	C / C	- / C
1 mg/L	C / -	C / C	- / C	- / C
1.5 mg/L	C / C	C / -	EC / C	C / -
2 mg/L	- / -	- / -	C / EC	C / -

EC= Extensive callus; C= moderate callus

Table 4 - Effect of 3% / 6% glucose concentration respectively on ½ strength MS media with 2,4-D + Kin on growth of callus from leaf explant

Kin / 2,4-D	0.5 mg/L	1 mg/L	1.5 mg/L	2 mg/L
0.5 mg/L	EC / C	EC / EC	-/-	C /-
1 mg/L	-/-	C / C	C /-	C /C
1.5 mg/L	-/-	-/-	-/ C	C / S
2 mg/L	-/-	C / C	C / C	-/ C

EC= Extensive callus; C= moderate callus; S= callus initiation

Table 5 - Effect of 3% / 6% fructose concentration respectively on 1/2 strength MS media with 2, 4-D + Kin on growth of callus from leaf explant.

Kin / 2,4-D	0.5 mg/L	1 mg/L	1.5 mg/L	2 mg/L
0.5 mg/L	-/-	-/-	-/-	-/-
1 mg/L	-/-	C /-	-/-	C / C
1.5 mg/L	-/-	-/-	-/ C	C /-
2 mg/L	-/-	C / C	C / C	-/-

C = Moderate callus

Table 6- Effect of pH 5.5 on ½ strength MS media + 2,4-D + kin on growth of callus.

Kin / 2,4-D	0.5 mg/L	1 mg/L	1.5 mg/L	2 mg/L
0.5 mg/L	C	C	C	C
1 mg/L	-	-	-	C
1.5 mg/L	-	C	EC	-
2 mg/L	C	C	C	-

EC= Extensive callus; C= moderate callus

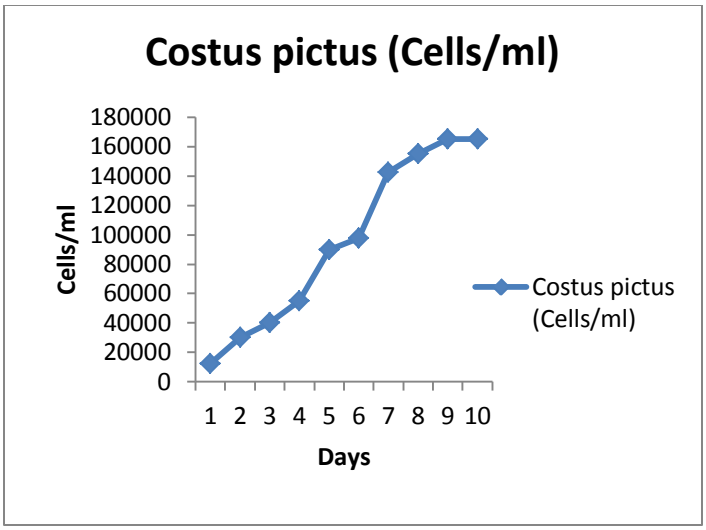

Fig. 1- Growth curve of suspension culture of *Costus pictus*.

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