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Original Article

STANDARDIZATION OF FRIABLE CALLUS DEVELOPMENT IN *CATHARANTHUS ROSEUS* (LINN.) G. DON

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ABSTRACT

Objective: The objective of the study was to develop an effective hormonal combination for the maximum growth of callus and development of friable calli using the same medium with reduced concentration of agar.

Methods: The percentage responses of five varied growth hormonal combinations and concentrations, supplemented with Murashige and Skoog (MS)medium were recorded. The effect of casein hydrolysate on callus induction was also studied. The nature of friable calli obtained from best responsive media fortified with 0.7% and 0.6% agar was observed.

Results: The present study revealed that, three media *viz.,* MS + 1.0 mg/L BAP + 1.0 mg/L NAA, MS + 1.5 mg/L 2,4-D + 1.0 mg/L Kin and MS + 1.5 mg/L 2,4-D + 0.5 mg/L BAP, as the best responsive media in the descending order. The effect of casein hydrolysate supplemented along with the above three media revealed MS + 1.0 mg/LBAP + 1.0 mg/L NAA + 1.0 gm/L casein hydrolysate as the best responsive media. Also, the above media supplemented with 0.6% agar was found to be the effective in terms of nature and amount of friable callus obtained.

Conclusion: The results indicated MS + 1.0 mg/L BAP + 1.0 mg/L NAA + 1.0 gm/L casein hydrolysate + 0.6% agar (85% response) as the best media for the growth and development of both callus and friable callus.

Keywords: Callus induction, Friable callus, *Catharanthus roseus*.

INTRODUCTION

Herbal plants are known to produce a wide range of secondary metabolites, also referred to as natural products [1], bioactive compounds or compatible solutes that are used to cure contagious diseases [2-4]. Despite of their usage for food and shelter, they are also cultured on a large scale to obtain valuable compounds which are used in pharmaceuticals, agrochemicals, flavors, fragrances, colors, biopesticides and food additives [5]. *Catharanthus roseus* (Linn.) G. Don. is one such ornamental plant, also commonly known as Sadabahar (or) Periwinkle [6]. It is a perennial tropical plant, that belongs to the family Apocynaceae and to the place Madagascar [7]. This herbal plant has always gained the nuclear place in the traditional plant system [8]. It has the ability of producing wide range of indole alkaloids which are known to have anti-cancer [9], anti-dysenteric, anti-septic [10], anti-hypertensive, anti-oxidant, anti-malarial, anti-mitotic, anti-fertility, anti-hypercholesterolemic, anti-diabetic, anti-mutagenic, diuretic, anti-inflammation, anti-fungal, anti-spasmodic, anti-viral and anti-tumour properties [11]. *C. roseus* produces more than 100 mono-terpenoid indole alkaloids (MIAs) [12-15], such as vinblastine, ajmalicine, serpentine [16] *etc*. that is found in various sections of the plant. Due to its valuable pharmaceutical properties, *C. roseus* have been the object of various biotechnological studies [17].

Tissue culture technique has immense advantages in culturing medicinal plants to overproduce secondary metabolites, as a resource for herbal and pharmaceutical industries [18]. Cell suspension cultures have been utilized for deriving such plant metabolites of wide medicinal value. The procedure of standardizing friable calli development becomes a necessary step in various plants to manufacture valuable plant metabolites. Therefore, the present study was undertaken to derive callus and friable calli, by optimizing the growth hormones in combination with MS medium.

MATERIALS AND METHODS

Surface sterilization of explants

The explant was derived from *C. roseus* plants, identified at VIT University, Vellore, Tamil Nadu, India. The leaves were initially surface sterilized by subjecting under running tap water followed by 0.1% bavistin treatment for 30 min. Then these leaves were subjected to detergent wash using 3 drops of Tween-20 for 15 min. These semi-sterilized leaves were then transferred to a laminar air flow chamber and washed with autoclaved distilled water for 4 times. It was further surface sterilized with 75% ethanol for 3 min, and then with 0.1% $HgCl_2$ for 3 min. Washing with autoclaved distilled water in between the treatments, for 4 times were carried out. The sterilized leaves were then cut into bits of 0.5 cm length (explant) and inoculated in MS media along with varied concentrations of growth hormones.

Culture media

Different growth hormonal concentrations and combinations with MS media supplemented with 3% sucrose were prepared *viz*., BAP (0.5-2.0 mg/l) + NAA (1.0 mg/l), 2,4-D (1.5–2.0 mg/l) + Kin (0.1-1.0 mg/l), 2,4-D (1.5–2.0 mg/l) + BAP (0.1-0.5 mg/l), Kin (0.5–1.0 mg/l) + NAA (1.0–1.5 mg/l) and Kin (0.5–1.0 mg/l) + BAP (1.0–1.5 mg/l) for callus development. The media was supplemented with 0.8% agar and the pH was adjusted to 5.8 prior to autoclaving at 121ºC under 15 lb $inch^{-2}$ for 15 min.

Callus initiation

The explants after surface sterilization was inoculated in MS media supplemented with varied growth hormonal combinations as mentioned above (with and without casein hydrolysate) (fig. 1a). The cultures were maintained under dark conditions. The date of initiation of callogenesis (fig. 1b), and percentage of responses of callus were recorded in five replicates (table 1). Based on the percentage responses of the callus, the best responsive MS media was chosen and considered for the further experiments.

Callus culture

Thirty days old callus obtained from best responsive media, were further inoculated in the same MS media supplemented with the same hormonal combinations as that of the parent culture. Uniform weights of callus were used for subculturing. Five subsequent passages were done using the similar media (fig. 1c, 1d, 1e), at 20 days of interval.

Friable callus culture

After five subcultures the callus were further transferred into MS media with reduced agar concentrations (0.7% and 0.6%) to obtain friable callus (fig. 1f). These partially friable calli obtained after 20 days of its inoculation (table 3), was further subcultured into fresh medium. Finally, the amount (dry weight) of friable callus obtained from the second subculture was recorded (table 4).

RESULTS AND DISCUSSION

Effect of growth hormones

MS media fortified separately with different growth hormones in combinations using leaf bits as explants, were examined to determine the optimum growth hormone requirement for callus initiation and multiplication of callus(table 1).

Table 1: Effect of MS medium supplemented with growth hormones on callus initiation in *C. roseus*

Plant growth hormones (mg/l)	Mean days to callus induction	Percentage response
BAP + NAA		
0.5 +1.0	24	40
1.0 + 1.0	20	70
2.0 + 1.0	21	60
2,4-D + Kin		
1.5 + 0.1	-	-
1.5 + 1.0	21	65
2.0 + 0.5	-	-
2,4-D + BAP		
1.5 + 0.1	-	-
1.5 + 0.5	21	65.5
2.0 + 0.5	20	40
Kin + NAA		
0.5 + 1.0	21	60
1.0 + 1.0	24	50
1.0 + 1.5	21	55
Kin + BAP		
0.5 + 1.0	24	40
1.0 + 1.0	-	-
1.0 + 1.5	25	45

The inoculated explants responded partially to the varied growth hormones supplemented along with MS medium at different rates. Based on maximum percentage of responses, three media combinations *viz*., MS + 1.0 mg/l BAP + 1.0 mg/l NAA, MS + 1.5 mg/l 2,4-D + 1.0 mg/l Kin and MS + 1.5 mg/l 2,4-D + 0.5 mg/l BAP were chosen and considered for further experiments. The maximum percentage of response by a calli was shown in MS + 1.0 mg/l BAP + 1.0 mg/l NAA, after 20 days of its inoculation.

In harmony, Renu Singh *et al*. (2011) reported that, leaf (explant) inoculated in MS + 3.0 mg/l BAP + 2.0 mg/l NAA subjected to light (13 days to callus induction) and dark (12 days to callus induction) conditions separately, proved to be the best responsive media (92.2 and 90.2% respectively). In contrast, Rukhama Haq *et al*. (2013) [19], reported that MS + 1.0 mg/l 2,4-D + 1.0 mg/l Kin as the best responsive media (95%). Also, Ashutosh Verma*et al*. (2012) and Taha *et al*. (2008) [20], reported that MS media supplemented with 1.0 mg/l 2,4-D + 0.5 mg/l BA and 1.0 mg/l 2,4-D + 1.0 mg/l Kin respectively, produced the highest mass of callus with leaf as an explant.

Effect of casein hydrolysate

Effect of casein hydrolysate supplemented along with MS media and three best responsive growth hormones obtained, was examined. The nature of the callus produced and days to callus induction were recorded (table 2).

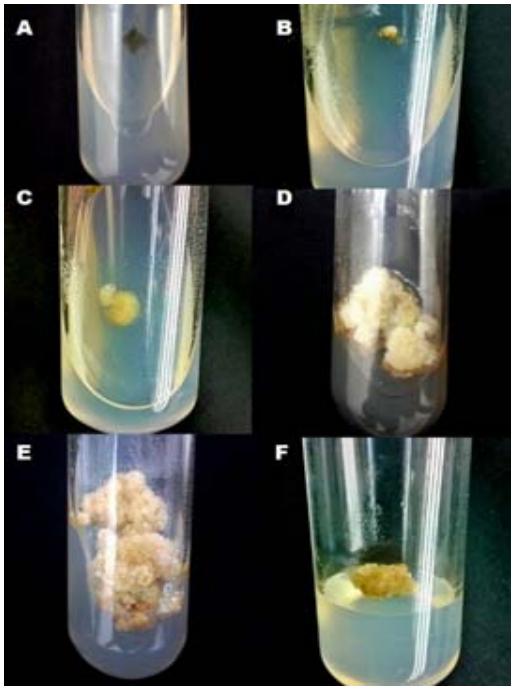

Fig. 1: Development of friable callus
A: leaf bit (explant) inoculation, B: initial growth of callus, C: third subculture, D: fourth subculture, E: fifth subculture, F: friable callus

Table 2: Effect of casein hydrolysateon callus initiation

Key component	Media composition	Mean days to callus induction	Nature of the callus (colour)	Percentage of responses (%)
With casein hydrolysate (1.0 gm/l)	MS + 1.0 mg l^{-1} BAP + 1.0 mg l^{-1} NAA	16	White	85
	MS + 1.5 mg l^{-1} 2,4-D + 1.0 mg l^{-1} Kin	14	White	80
	MS + 1.5 mg l^{-1} 2,4-D + 0.5 mg l^{-1} BAP	18	White	80
Without casein hydrolysate	MS + 1.0 mg l^{-1} BAP + 1.0 mg l^{-1} NAA	20	Brown	70
	MS + 1.5 mg l^{-1} 2,4-D + 1.0 mg l^{-1} Kin	21	White	65
	MS + 1.5 mg l^{-1} 2,4-D + 0.5 mg l^{-1} BAP	21	Green	65.5

It was observed that the callus derived were whiter and took lesser days to callus induction in the presence of the casein hydrolysate compared to the callus obtained on the same media without casein hydrolysate. The maximum response by a friable calli was observed in MS media supplemented with 1.0 mg/l BAP + 1.0 mg/l NAA + 1.0 gm/l casein hydrolysate (table 2). The callus obtained from the best responsive media were subcultured upto five passages, after every 20 days.

Table 3: Mass of friable callus obtained from first passage (gm)

Replicates MS + 1.0 mg/L BAP + 1.0 mg/L NAA + 1.0 gm/Lcasein hydrolysate	0.7% agar	0.6% agar
R_1	0.11	0.49
R_2	0.10	0.38
R_3	0.11	0.29
R_4	0.15	0.57
R_5	0.08	0.87
R_6	0.12	0.25
R_7	0.12	0.22
R_8	0.15	0.20
R_9	0.13	0.23
R_{10}	0.10	0.26
Mean	0.117	0.376
SD	0.073	0.204
SE	0.023	0.064

It was observed that the amount of friable calli derived from the second passage particularly from the media supplemented with 0.6% agar was comparatively more (table 4) and loose.

Table 4: Mass of friable callus obtained from second passage (gm)

Replicates MS + 1.0 mg/L BAP + 1.0 mg/L NAA + 1.0 gm/Lcasein hydrolysate	0.7% agar	0.6% agar
R_1	0.16	0.45
R_2	0.15	0.40
R_3	0.13	0.46
R_4	0.19	0.43
R_5	0.19	0.41
R_6	0.13	0.40
R_7	0.13	0.42
R_8	0.11	0.40
R_9	0.12	0.43
R_{10}	0.13	0.44
Mean	0.144	0.424
SD	0.026	0.02
SE	0.008	0.006

Mass of friable callus

The friable callus obtained from the best responsive media were taken into consideration. The mass of that particular callus after 20 days of inoculation into a fresh media, was recorded (table 3). Also, it was again transferred onto a same media as that of the parent media. The weight (grams) of the friable callus obtained after 20 days of inoculation were recorded (table 4).

CONCLUSION

In the present investigation of developing the callus and friable callus in *Catharanthus roseus*, we found that the MS basal media fortified with growth hormones, BAP (1.0 mg/L) and NAA (1.0 mg/L) was the best responsive (70%) media for callogenesis. We have also studied the effect of casein hydrolysate(1.0 gm/L) supplemented along with MS media and the best responsive growth hormones, on callus induction and development. Casein hydrolysate was found to be a positive regulator (85% response) and it induced growth in lesser days. To obtain the friable callus, best responsive media were supplemented along with reduced agar concentrations of 0.7% and 0.6%. Media with 0.6% agar resulted to be the best in terms of the nature and mass of the callus obtained. Finally, we can conclude that the MS + 1.0 mg/L BAP + 1.0 mg/L NAA + 1.0 gm/L casein hydrolysate + 0.6% agar as the best media for the growth and development of both callus and friable callus.

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CONFLICT OF INTERESTS

Declared None

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