Callus induction and Organogenesis in an Indian Box-wood (*Gardenia latifolia* Ait.)

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ABSTRACT

An efficient method to evaluate the most suitable concentration of growth regulators for callus induction and subsequent organogenesis in *Gardenia latifolia* has been studied. The best callus induction was found in half cut seeds cultured on MS medium fortified with 2, 4-D (2mg/l) concentration produced white callus. Combination of 2, 4-D (2mg/l) + BAP (0.1mg/l) produced 80% of brownish white colour callus which gave the most effective for callus induction. Internodal callus showed maximum percentage of response (55%) in the MS medium containing NAA (0.1mg/l) + BAP (1.0mg/l) produced yellowish and jelly nodular soft callus. Half cut seed callus showed 55% of response observed in the MS medium containing IAA (0.1mg/l) + BAP (0.5mg/l) which was compact and smooth in nature and not shown any response for shoot formation except appearance of shoot buds.

Key words: Callus induction, *Gardenia latifolia*, Meristemoids, Organogenesis.

INTRODUCTION

Medicinal plants are of great interest to the researchers in the field of biotechnology as most of the drug industries depend, in part, on plants for the production of pharmaceutical compounds (Chand *et al*, 1997). Herbal medicines are the precursors of many common drugs prescribed in clinical practice in many countries today. Furthermore, herbs and herbal products are still an important part of the primary health care systems in many parts of the world (Jawahar *et al*, 2008). *In vitro* culture techniques offer a viable system for true-to-type rapid mass multiplication and germplasm conservation of rare, endangered, aromatic and medicinal plants (Arora and Bhojwani, 1989; Sharma *et al*, 1991; Sudha and Seeni, 1994; Sahoo & Chand, 1998; Karuppusamy and Pullaiah, 2007; Mallon *et al*, 2010).

Gardenia latifolia is a small deciduous tree or large shrub. Root used as a remedy for indigestion in children. Fruits used in affections of the mammary glands. Pounded pulp is applied to forehead in fever. Stem and fruit used for stomach pain. Fruit extract is used in treating snake-bite, sores of hand and feet, stomach ache and wounds. To treat caries, stem bark crushed and boiled in water is applied to affected areas. Bark is used in skin diseases (Iwu *et al*., 1999).

The aim of the present study was to establish an effective protocol for callus induction and organogenesis from *G.latifolia.*

MATERIALS AND METHODS

The explants material (Shoot tip, node, internode, leaf and immature fruit segments) were collected from Kakatiya arboretum, Warangal district, Andhra Pradesh. Fruits were procured from Tirumala Foot hills, Thirupathi, Chittor district, A.P. The healthy seeds were separated and stored for the experimental work. Presoaking of explants in anti oxidant solution (100mg/l PVP + 100-500mg/l Ascorbic acid + 50-100mg/l Citric acid) for 30 minutes for removing gum substances and phenolic compounds. The explants were taken in a 500ml clean sterile Erlenmeyer flask and washed with 1% (v/v) tween-20 solution for 20 minutes and then washed with tap water thoroughly. Further operations were carried out in the laminar flow chamber. Seeds were subjected to 70% alcohol for 30 seconds and washed with sterile distilled water. Then the explants were disinfected with 0.1% (w/v) mercuric chloride ($HgCl_2$) for different time intervals (2 or 3 min) followed by thorough washing with sterile distilled water.

The surface sterilized explants were then aseptically inoculated on sterile MS medium having a wide range and combinations of plant growth regulators (2, 4-D, NAA, BAP, KN and TDZ). The medium pH was adjusted to 5.8 before autoclaving at 121^0C for 15 minutes. 0.5-3% of Activated Charcoal added to the culture medium to remove Phenolic compounds from the explants produced during the culture period. Apart from this, all the cultures were initially kept in dark for 2 days to avoid the problem of releasing phenolic compounds and gum resins from explants inoculated. These were maintained in growth chamber at 25 ± 2^0C 16h photo period provided by cool white florescent tubes (Philips, India) (Ca.2000-2500 lux) for further observations.

Fig. 1: 2, 4-D (2mg/l) produced white callus

Fig. 2: 2, 4-D (2mg/l) + BAP (0.1 mg/l) white brwnish color callus

Fig. 3: 2, 4-D (2mg/l) +BAP (0.1 mg/l) produced white friable callus

Fig. 4: 2, 4-D (2mg/l) + BAP (0.1 mg/l) dark brownish white nodular callus.

Fig. 5: NAA (0.1mg/l) + BAP (1.0mg/l) yellowish and jelly nodular soft callus

Fig. 6: IAA (0.1mg/l) + BAP (1.0mg/l) yellowish soft jelly like callus

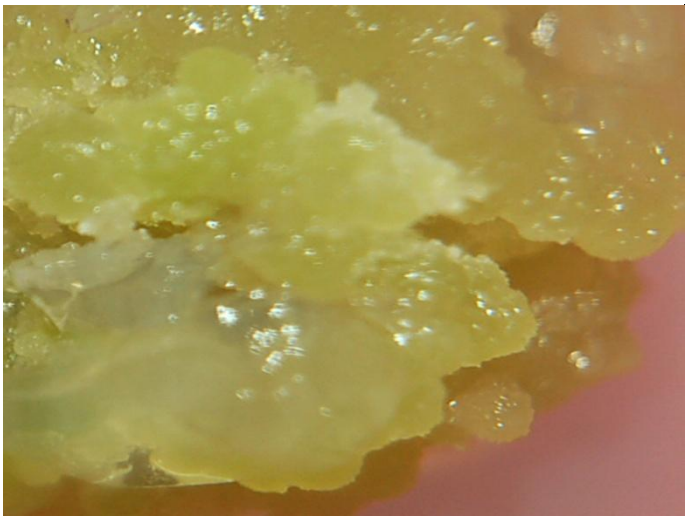

Fig. 7 : NAA (0.1mg/l) + BAP (0.5mg/l) white yellowish color callus

Fig. 8: IAA (0.1mg/l) + BAP (0.5mg/l) compact and smooth callus

Frequent observations were noted and contaminated tubes were removed carefully. For callus studies, observations were recorded after 30 days of incubation and 45 days in shoot multiplication experiments. For each experiment minimum of 50 tubes were maintained and all experiments were repeated thrice. After 30 days of incubation the callus induction frequency was estimated. After callus induction from the explants, the calli were transferred into the fresh medium for further proliferation and maintenance.

The average data for all the above experiments were tabulated and was analyzed statistically by Analysis of variance (ANOVA) was applied to signifying the results.

RESULTS AND DISCUSSION

A) Medium evaluation

Different media such as MS, B_5 and WPM supplemented with effective concentration of 2, 4-D (2mg/l) were tested for callus initiation from nodal segments and shoot tip. Based on the percentage of response, fresh and dry weight, the best medium was selected.

Among the three media tested, MS medium was found to be effective by producing fresh and dry weight 393.38mg and 74.30 respectively with 80% of response followed by B_5. However, WPM did not show any response (Table 1).

B) Effect of hormones on morphogenic response

i) Explant and callus induction

Callus is an unorganized mass of plant cells and its formation is controlled by growth regulating substances present in the medium (auxins and cytokinins) (Shah *et al*., 2003). The specific concentration of plant regulators needed to induce callus, varies from species to species and even depends on the source of explant (Charriere *et al*., 1999).

Half cut seeds cultured on MS medium fortified with different levels of lower concentrations (0.5 and 1.0 mg/l) of 2, 4-D produced white spongy and tiny callus with shoot respectively. And in 2mg/l also produced white callus (Figure 1), however at higher concentration (4 mg/l) responded interestly by initiating root like structure which later produced abundant calli from their ends.

Internodal segments cultured on MS medium fortified 0.5, 1.0 and 2.5 mg/l) of 2, 4-D produced white callus at the basal regions of internodes. At higher concentrations 3-4 mg/l produced green colour proliferations at the tip regions of cut ends.

Leaf segments cultured on MS medium fortified with lower concentrations 0.5-2mg/l of 2, 4-D produced nodular calli around the cut regions. Root segments cultured on medium fortified with 2mg/l of 2, 4-D initiated white callus at lower and upper tip ends. However, there was no response at all on medium devoid of hormones in all explants experimented.

Table 1: Effect of different media on percentage of callus initiation, fresh and dry weight of calli in *Gardenia latifolia*

Medium	% of Response	Mean Fresh weight (mg)	Mean Dry Weight (mg)
MS	80	393.38 ± 25.49	74.3 ± 18.77
B_5	55	251.10 ± 15.23	61.8 ± 10.33
WPM	-	-	-

- No response

*While looking at the results of ANOVA, it can be said that the F-value is found to statistically significant at the 1% level. Therefore it can be concluded that the null hypothesis is rejected and the alternative hypothesis of significant response is accepted.

Half cut seeds cultured on MS medium fortified with 2,4-D (2mg/l) + BAP(0.1 mg/l) produced 80% of brownish white colour callus (Figure 2) where as in 2,4-D (2mg/l) +BAP (0.5mg/l) produced 65% of brownish white with green pigmented callus. 60% of white callus with green shoot buds were observed in 2, 4-D (2mg/l) + BAP (1.0mg/l). MS medium fortified with 2, 4-D (2mg/l) + KN (0.1mg/l) produced 75% of white callus and in 2, 4-D (2mg/l) + KN (0.5 mg/l) produced 70% callus. Whereas 60% of callus was recorded in 2, 4-D (2mg/l) + KN (1.0 mg/l).

Internodal segments cultured on MS medium fortified with 2, 4-D (2mg/l) +BAP (0.1 and 0.5 mg/l) produced 75 and 70% of white friable callus at the basal region respectively (Figure 3).

Table 2: Morphogenetic response of 5 week old calli derived from internode and half cut seed subjected to various concentrations and combinations of auxins and cytokinins on MS medium in *Gardenia latifolia*

Auxin (0.1mg/l)	Cytokinins (mg/l)			% of Response		Morphogenic Response	
	BAP	KN	TDZ	I.N	H.C.S	I.N	H.C.S
NAA	0			-	-	-	-
NAA	0.1			35	45	YC	YC
NAA	0.5			40	60	WYC	WYC
NAA	1.0			55	50	YJNSC	YC
NAA	2.0			-	-	-	-
NAA		0.1		45	65	WYC	YWC
NAA		0.5		50	-	YC	-
NAA		1.0		-	60	-	YC
NAA		2.0		-	-	-	-
NAA			0.05	-	-	-	-
NAA			0.5	-	-	-	-
NAA			1.0	-	-	-	-
IAA	0.1			20	-	WYC	-
IAA	0.5			-	55	-	CSC
IAA	1.0			35	40	YC	YC
IAA	2.0			-	-	-	-
IAA		0.1		25	60	WYC	YC
IAA		0.5		45	40	YC	YC
IAA		1.0		-	-	-	-
IAA		2.0		-	-	-	-
IAA			0.05	-	-	-	-
IAA			0.5	-	-	-	-
IAA			1.0	-	-	-	-
IBA	0.1			-	-	-	-
IBA	0.5			-	-	-	-
IBA	1.0			-	-	-	-
IBA	2.0			-	-	-	-
IBA		0.1		-	-	-	-
IBA		0.5		-	-	-	-
IBA		1.0		-	-	-	-
IBA		2.0		-	-	-	-
IBA			0.05	-	-	-	-
IBA			0.5	-	-	-	-
IBA			1.0	-	-	-	-

-No response I.N- Internode; H.C.S-Half Cut Seed YJNSC-Yellowish and Jelly Nodular soft callus; YC-Yellow colour callus WYC-White yellowish colour callus YWC-Yellowish White colour callus ; CSC- Compact and Smooth Callus

65% of white cloudy callus was observed in 2, 4-D (2mg/l) +KN (0.1mg/l). Leaf segments cultured on MS medium fortified with 2, 4-D (2mg/l) + BAP (0.1 mg/l) produced 60% of dark brownish white nodular callus (Figure 4). In 2, 4-D (2mg/l) + BAP (0.5 mg/l) observed 50% of white colour callus at the cut ends. 55% of callus was observed in 2, 4-D (2mg/l) + KN (0.1 mg/l). Root segments showed poor response (30%) of white colour callus fortified with 2, 4-D (2mg/l) + TDZ (0.5mg/l).

These results are in support of earlier investigations carried out for callus induction in *Cichorium intybus* (Velayutham, 2006), *Actinidia deliciosa* (Filiz Akbas, 2008), *Arnica Montana* (Mariya petrova, 2010), *Nigella sativa* (Suresh chand, 1981), *Pelargonium* (Jinsong, 2007), *Hipericum triquetrifolium* (Esim Akcam, 2010), *Clematis gouriana* (Hanumanaika, 2008), *Lycopercicum esculentum* (Magdoleen, 2010), *Celastrus paniculatus* (De silva, 2009).

ii) Organogenesis

Internodal callus showed maximum percentage of response (55%) was in the MS medium containing NAA (0.1mg/l) + BAP (1.0mg/l) produced yellowish and jelly nodular soft callus (Figure 5). 50% of response was observed in the combination of NAA (0.1) mg/l) + KN (0.5mg/l) and it was yellowish callus. TDZ showed no response in all combinations.

Internodal callus exhibited yellowish soft jelly like callus, this callus further not responded towards organogenesis in the MS medium containing IAA (0.1mg/l) + BAP (1.0mg/l) (Figure 6). Maximum percentage of response (45%) was observed in the combination of IAA again further studies not shown any response for shooting. TDZ showed no response in all combinations.

Half cut seed callus responded 60% proliferation of white yellowish colour callus observed in the MS medium containing NAA (0.1mg/l) + BAP (0.5mg/l) (Figure 7). 65% was observed in the combination of NAA (0.1mg/l) + KN (0.1mg/l) which was again yellowish white colour callus. TDZ showed no response in all combinations.

Half cut seed callus showed 55% of response observed in the MS medium containing IAA (0.1mg/l) + BAP (0.5mg/l) which was as compact and smooth in nature without any response for shoot initiation in further experiments (Figure 8). 60% response was observed in the combination of IAA (0.1mg/l) + KN (0.1mg/l) again it was similar in nature not giving shoot buds. TDZ showed no response in all combinations among all cytokinins. Among all auxins IBA showed no response in all combinations. All the observations were tabulated (Table 2). Regarding the organogenesis/plant regeneration it needs still further work for induction of organogenesis either from calli or from any explant directly.

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