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Research Article

IN VITRO MORPHOGENIC RESPONSE OF LEAF AND SHOOT TIP EXPLANTS OF GARDENIA GUMMIFERA, (L.) f.-A MEDICINAL PLANT

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Abstract

In vitro morphogenic response of leaf and shoot tip explants of *Gardenia gummifera*, (L.) f. medicinal plant of Rubiaceae has been reported. Multiple shoots were developed directly from shoot tip explants. In case of leaf explants, multiple shoots were developed through the callus on MS medium supplemented with different concentrations of BAP, Kn individually and in combination with different concentrations of IBA, IAA and NAA. Efficient regeneration of maximum number of adventitious shoots from the shoot tip was obtained on MS medium augmented with 2.0 mg/L NAA and 2.0 mg/L IBA. High frequency shoots from leaf explants were observed on the MS media supplemented with 2.0 mg/L of Kn and 5.0 mg/L BAP and 2.0 mg/L NAA.

Key words: *Gardenia gummifera, Growth regulators, In vitro response, multiple shoots.*

Introduction

Gardenia gummifera, (L.) f. is one among the red listed plants belongs to the family Rubiaceae. The plant is endemic to peninsular India. It is found in dry forests of Karnataka, Tamil Nadu, Andhra Pradesh and Kerala. *Gardenia gummifera* is one of the endangered plant species of India.[1] It is a small tree or large woody bush, about 3-7 m tall, wood yellowish white, hard. Lateral nerves of leaves 10-16 pairs, distinct, with a dot like gland known as 'Domatia' at the axils of each nerve. Flowers bisexual, petals white and turns yellow. Fruits berry, seeds many. Resin from the leaf buds is used in healing wounds, indigestion, gas trouble, ulcer and cardiac problems. Certain chemicals such as triterpenoides, aldehydes, mannitol and sitosterols are found in the plants [2]. Paste obtained from the bark is used as an antiplasmodic and expectorant. *In vitro* techniques offer the powerful tool for germplasm conservation, multiplication and introduction of new species. It also facilitates the engineering and selection

of elite superior genotypes and serves as a vehicle for depth investigation of physiological and biochemical processes.[3],[4] Hence, the present work is undertaken to develop an efficient regeneration system via multiple shoot induction from shoot tip explants and highest frequency multiple shoots from leaf explant derived callus of *Gardenia gummifera*. Histological examination is also conducted to determine the origin of root and shoot from the callus.

Materials and Methods

MS medium.[5] with 3% of sucrose and 0.8% of dextrose agar served as the best basal medium for the experiment, which were prepared in glass double distilled water. The pH of the medium was adjusted to 5.5-5.7 with 0.1 N NaOH or 0.1N HCl by using pH meter. 15 ml of the medium was dispensed to each culture tube and are plugged with non-absorbent cotton wrapped with cheese cloth which are autoclaved and are solidified as slants. For *in vitro* studies, the shoot tip and the leaf explants were collected from the plants grown in Botanical garden of Karnatak University campus Dharwad. The collected explants were washed under running tap water and are surface sterilized by dipping in 70% (v/v) alcohol followed by 0.1-0.15% (w/v) mercuric chloride for 10-15 minutes. For exudation of phenolic compound the explants were kept in 3 % (w/v) citric acid for 10-15 minutes and are incubated in dark by dipping in sterile distilled water for overnight. The further conventional processes were carried out in laminar air flow. Repeated washing was done before inoculation and are cut into desirable size. For each experiment a set of 24 cultures were repeated twice. The cultured materials are periodicaly fixed in Cornoys fluid.[6] for 24 hours, then washed with running tap water to remove the traces of fixative and stored in 70 % alcohol, then dehydrated through alcohol- xylol series and were infiltered and embeded in paraffin wax. The sections were cut into 8-10µ thickness by using Leica microtome and paraffin ribbons containing sections mounted on micro glass slides by using gelatin adhesive. These microslides were further processed and stained in 1% (v/v) Safranin to study organogenesis. Axiostar plus (Carl Zeiss) microscope with Canon powershot G2 digital camera is used for Microphotography.

Results and Discussion

The explants grown on MS medium alone did not show any growth. While, the explants reared on MS medium supplemented with different concentrations of Auxins such as IAA, NAA, IBA (0.5, 1.0, 2.0, 5.0, 10.0 mg/L) and

cytokinins such as Kinetin and BAP (0.5, 1.0, 2.0, 5.0, 10.0 mg/L) showed the induction of callus and multiple shoots. The combination of 5.0 mg/L BAP and 1.0 mg/L Kn in MS medium induced callus (Fig 1.B), which on subculture resulted in multiple shoot formation on MS medium supplemented with 2.0 mg/L Kn and 5.0 mg/L BAP (Table 1, Fig 1.C). The maximum numbers of shoots reported were 14 per explant. The rooting was initiated on MS medium containing 0.5 mg/L IAA and 1.0 mg/L NAA (Fig 1. E).When MS medium supplemented with 2.0 mg/L BAP and 2.0 mg/L IBA induced roots in callus obtained shoot tip. While in case of leaf explants high frequency callus and roots were seen on MS medium containing 2.0 mg/L IAA and 0.5 mg/L NAA. When the higher concentrations of auxins were used (0.5 mg/L IAA and 5.0 mg/L IBA) the explants directly induced rooting without the formation of callus. Poor induction of callus was noticed from the leaf explants cultured on MS medium containing 1.0 mg/L BAP and 0.5 mg/L Kn. The callus obtained from MS medium containing 2.0 mg/L Kn, 5.0 mg/L BAP and 2.0 mg/L NAA showed maximum of 22 shoots on subsequent culture in the MS medium with same combinations and concentrations of hormones.

Phenolic compounds are one of the major problems associated with the tree species. It is also true with present study on *Gardenia gummifera*. In many cases PVP is used for exudation of phenolic compounds. In *Myrica esculanta*.[7] used 0.5 % of PVP on WPM medium with 10.0 μM of Kn and 0.1 μM NAA to remove phenolic compounds and get maximum number of shoots. Where as in apple 3.0 mg/L PVP was used to remove phenolic compound and to obtain multiple shoots on MS medium containing 1.0 mg/L thiamine, 1.0 mg/L pyridine, 1.0 mg/L niacin, 1.0 mg/L NAP and 0.5 mg/L GA_3.[8] In the present study, 1.0 % of activated charcoal helped in partial removal of phenolic compounds; however, the complete exudation of phenolic compounds was achieved by keeping the explants in 3 % (w/v) citric acid for 10-15 minutes and are incubated in dark by dipping in sterile distilled water for overnight. Multiple shoots were induced on MS medium containing 0.5 mg/L BAP and 2.0 mg/L Kn. Individually when tested for callus formation from shoot tip BAP was found to be superior than Kn, where as multiple shoots were reported in many plants such as *Crataeva magna*.[9] *Phoenix dactylifera.*[10] *Anacardium occidentale*.[11] *Holorrhena antidysentrica*.[12],[13] *Randia dumetorum.*[14] *and Tectona grandis*.[15] In case of *Tectona grandis*.[15] used 10.0 μM of BAP and 1.0 μM of NAA for multiple shoot formation and rooting in liquid medium with 15.0 μM NAA. In *Zizipus-spina-christi*.[16] multiple shoots were obtained from axillary buds on MS medium

containing 7.5 mg/L BAP, 7.5 mg/L Kn and 0.01 mg/L IAA and rooting was induced on White's semi-solid medium with 123 μM IBA and activated charcoal. In *Gardenia gummifera,* multiple shoots were obtained from nodal explants on MS media supplemented with 2 mg/L BAP and 0.5 mg/L NAA and for rooting half strength of MS media with 1 mg/L IAA.[17a] Subsequently, MS basal medium with 1 mg/L BAP and 0.5 mg/L IBA yielded the highest number of shoots from leaf and internode explants of *Gardenia gummifera*.[18b] In *Morinda citrifolia*.[19] shoot tip was used to culture on MS medium supplemented with 5.0 mg/L BAP and 2.0 mg/L Kn for induction of high frequency multiple shoots and 5.0 mg/L IBA for rooting. However, in the present study on *Gardenia gummifera* 5.0 mg/L IBA and 5.0 mg/L IAA is used for the induction of roots.

Table 1.Morphogenic response of shoot tip explants of *Gardenia gummifera,* (L.) f. cultured on MS medium with cytokinins.

Sl. No	Medium	Cytokinin concentration in mg/L	*Percentage of explants showing response
1	MS	-	-
2	MS+Kn	0.5	-
3	MS+Kn	1.0	1.3
4	MS+Kn	2.0	12.8
5	MS+Kn	5.0	14.5
6	MS+Kn	10.0	3.5
7	MS+BAP	0.5	-
8	MS+BAP	1.0	8.1
9	MS+BAP	2.0	28
10	MS+BAP	5.0	34.9
11	MS+BAP	10.0	7.8
12	MS+Kn+BAP	1.0+1.0	8.4
13	MS+Kn+BAP	1.0+2.0	15.3
14	MS+Kn+BAP	1.0+5.0	28.9
15	MS+Kn+BAP	2.0+1.0	32.8
16	MS+Kn+BAP	2.0+2.0	25.1
17	**MS+Kn+BAP**	**2.0+5.0**	**72.1**
18	MS+Kn+BAP	5.0+1.0	38.2
19	MS+Kn+BAP	5.0+2.0	52.6
20	MS+Kn+BAP	5.0+5.0	26.4

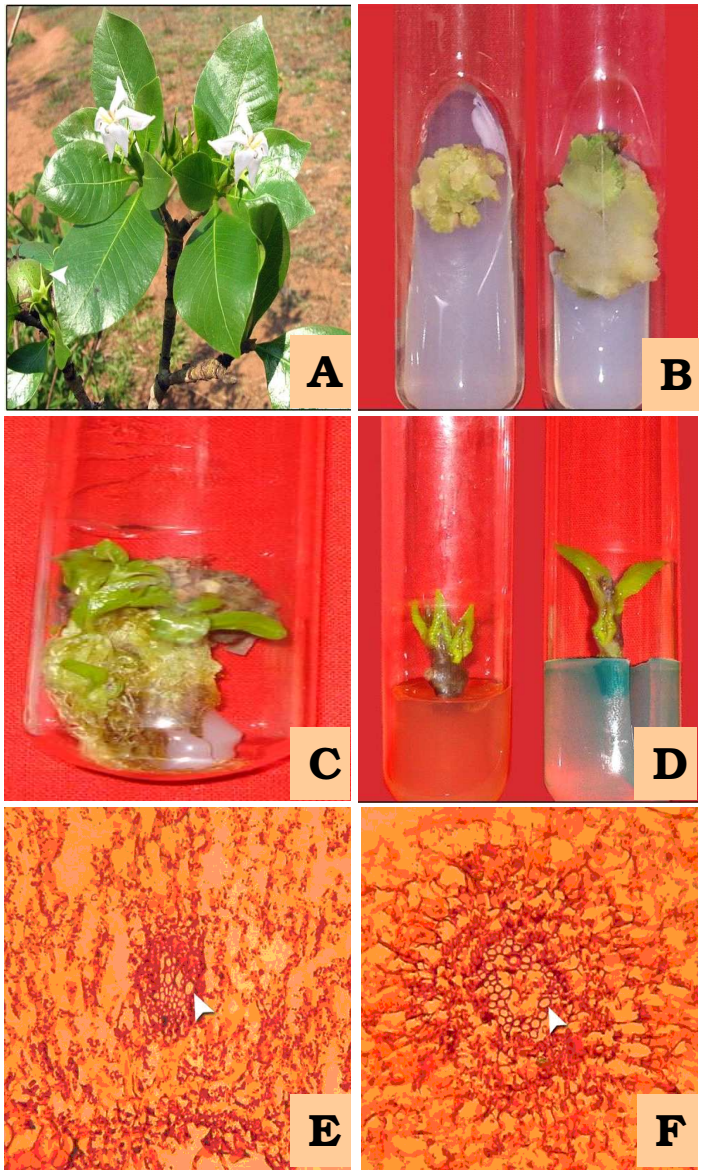
*Culture reports after 8 weeks of culture

Table-2: Morphogenic response of Leaf explants of *Gardenia gummifera,* (L) f. cultured on MS medium with Auxins.

Sl. No	Medium	Auxin concentration in mg/L	*Percentage of explants showing response
1	MS	-	-
2	MS+IAA	0.5	9.3
3	MS+IAA	1.0	10.2
4	MS+IAA	2.0	21.6
5	MS+IAA	5.0	58.6
6	MS+IAA	10.0	15.4
7	MS+IBA	0.5	8.3
8	MS+IBA	1.0	11.5
9	MS+IBA	2.0	12.1
10	MS+IBA	5.0	36.7
11	MS+IBA	10.0	11.8
12	MS+NAA	0.5	7.6
13	MS+NAA	1.0	8.2
14	MS+NAA	2.0	16.4
15	**MS+NAA**	**5.0**	**61.4**
16	MS+NAA	10.0	20.1

*Culture reports after 8 weeks of culture.

Fig.1. *In vitro* morphogenic response of Leaf and Shoot tip explants.



A. *In vivo* flowering shoot of *Gardenia gummifera*, (L) f.

B. Callus obtained from MS medium with 1.0mg/L BAP+2.0 mg/L IAA.

C. Multiple shoots from leaf callus on MS medium with 5.0 mg/l BAP +2.0 mg/L Kn.

D. Multiple shoots from shoot tip explant and shoot tip showing phenolic exudates with charcoal.

E. Section of the callus showing differentiation into root as the setion showing exarch condition.

F. Section of the callus showing differentiations of multiple shoots as section showing many endarchs.

Fig.2.Morphogenic response of shoot tip explants of *Gardenia gummifera*, (L) f. cultured on MS with Cytokinins after 8 weeks of culture.

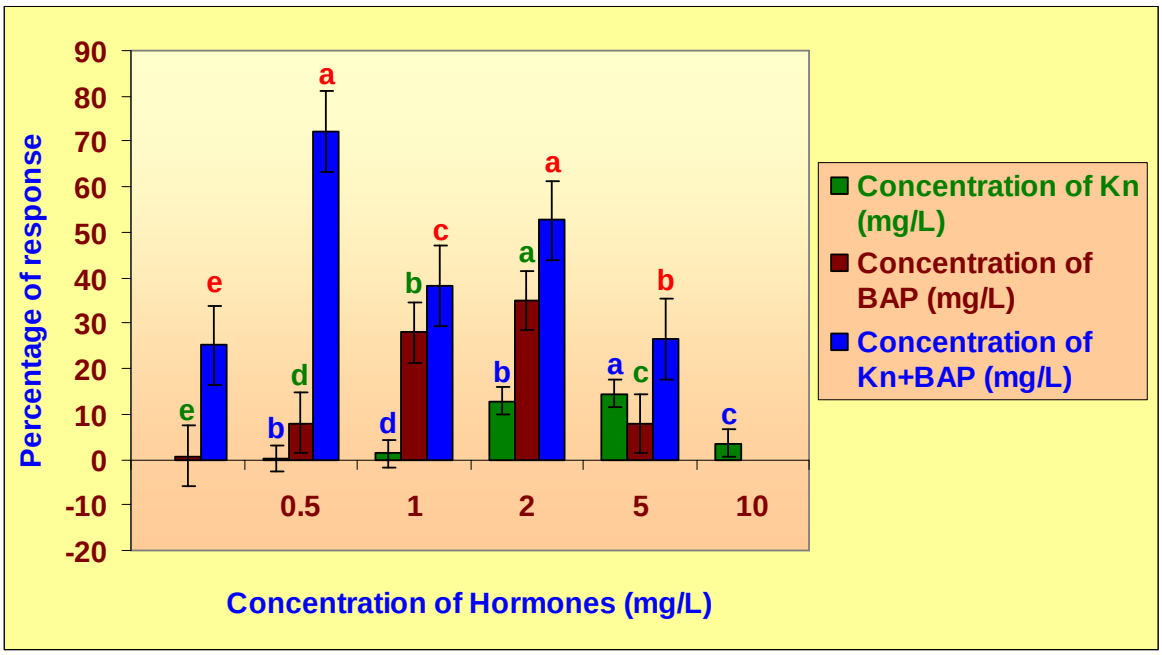
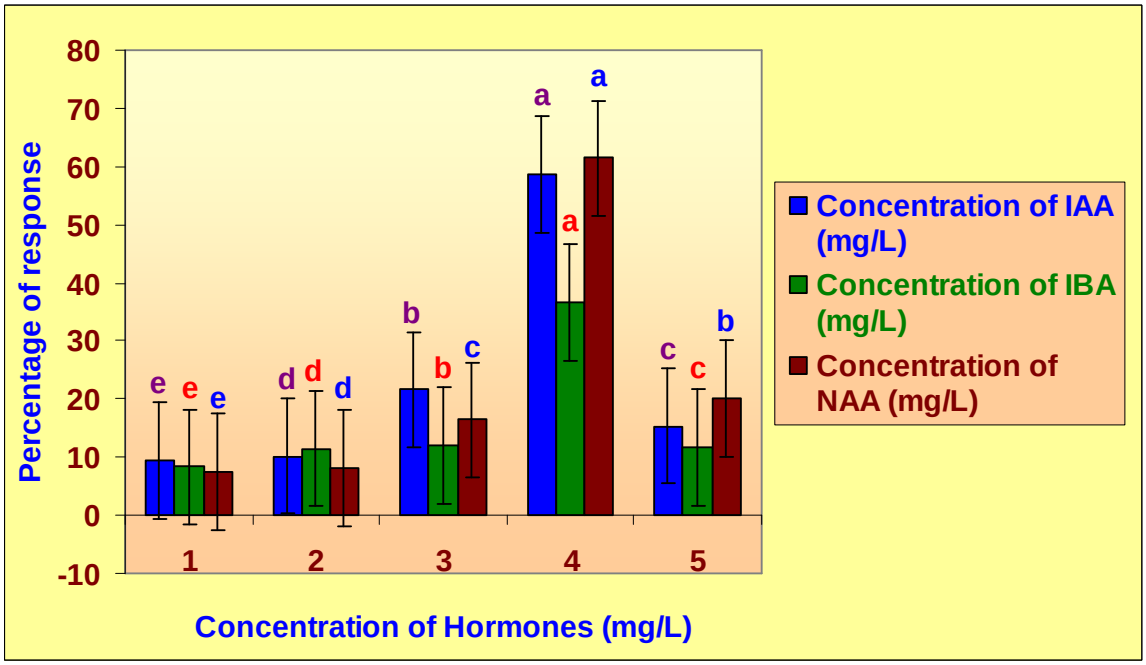


Fig.3. Morphogenic response of leaf explants of *Gardenia gummifera*,(L) f. cultured on MS with Auxins after 8 weeks of culture.



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