IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372.Volume 6, Issue 6 (Jan. 2014), PP 01-06
www.iosrjournals.org

In vitro Micropropagation of Selected *Bougainvillea sp.* through callus induction

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Abstract: *This study was conducted to develop a protocol for rapid callus induction and subsequent shoot regeneration in Bougainvillea (B. ×buttiana, B. spectabilis* and *B. glabra). The best results of callus induction response (92.86%) in whole area of node explant was observed on WPM medium of B. × buttiana compared with B. × buttiana when cultured on MS medium gave (85.71). The lowest callus induction response (71.00%) was observed on WPM medium from B. glabra followed by MS medium (71.43%) callus was formed from B. glabra. While the maximum number of shoot induced from callus, maximum shoots length and highest number of leaves per culture were obtained on WPM was 12.14 shoots/ culture, 2.14 cm and 20.71 leaves/ culture respectively from B.× buttiana after 6 weeks of culture inoculation compared with all characterized for B.× buttiana when cultured on MS medium was give number of shoot induced from callus, maximum shoots length and highest number of leaves per culture (11.43shoot induced/ callus, 17.14 cm, 1.96number of leaves/ culture) respectively.*

The best response was observed when B. ×buttiana cultured on ½ strength WPM medium which was (89.29%) while82.14% of the shoots were rooted when B. × buttiana cultured on ½ strength MS medium. The lowest root induction response (50.00%) was observed on ½ strength MS medium from B. glabra explant. A large number of roots, root length and root percentage (2.57roots /explant, 3.41cm and 89.29%) respectively, was observed when B. ×buttiana cultured on ½ strength WPM medium. While a large number of roots, root length (2.43 roots /explant and 2.73cm) respectively, was observed when B. ×buttiana cultured on ½ strength MS medium.

Key word*: In vitro, Bougainvillea, callus induction, different media, WPM and MS medium*

I. Introduction

The Bougainvillea (*B.×buttiana, B. spectabilis and B. glabra*), of the Nyctaginaceae. Plants (*Bougainvillea sp.*) are appraised as decorative plants because of their lovely blossoms that bloom several times throughout the year. It is believed to have originated from South America, but widely cultivated in the tropical and sub-tropical areas of the world. Bougainvillea, because of its special characteristics, like; high variation in type of foliage, production of many flowering inflorescence on one plant and continuous blooming of flowers with short production cycle had been very useful in the ornamental industry [1].

They belong to the class of economic woody ornamental plants. They are widely cultivated as porch, adornments, arbour and ornaments. Their growth habits and beautiful showing bracts make them popular for landscapes. They are also used in mass planting, as shrubs or bushes, ground covers, as hedge plants, barrier plants and slope coverings, in hanging baskets, and in containers for Bonsai.

Bougainvilleas are primarily propagated by stem cuttings, but lack of competence to form adventitious roots by cuttings occurs routinely and is an obstacle for the vegetative propagation [2]. Adventitious root formation is a key step in vegetative propagation of woody or horticultural species, and problems associated with rooting of cuttings frequently result in significant economic losses [3].

The propagation of *Bougainvillea* is difficult. In our climatic conditions it does not produce seeds while success percentage from cutting is very low. There is thus a need to propagate the plant through in vitro culture. Tissue culture techniques have successfully been employed to produce lager number of difficult–to- propagate plants [4]; [5]. Information on micropropagation of *Bougainvillea* became scarce in our region. Keeping in view the economical, aesthetic and ornamental value, the present report describes the propagation of *Bougainvillea* through nodal explants culture.

The main objective in plant cultures is to regenerate a plant or plant organ from the callus culture. The regeneration of plant or plant organs only taken place by the expression of cellular totipotancy of the callus tissues. Scattered areas of actively dividing cells, known as meristematic centers, develop as a result of differentiation and their further activity results in the production of root and shoot primordial. These processes can be controlled by adjusting the cytokinins: auxin ratio in culture medium

II. Material and Method

The experiment was designed to study the effect of two different types of culture media, WPM and MS media, on callus development using node explants. The nodal explants from *B. ×buttiana, B. spectabilis*and *B. glabra* were cultured on WPM [6] and MS [7] media at full strength. All the culture media were amended with a fixed concentration of $2mgl^{-1}$BA (6-Benzylamino purine) + $0.2mgl^{-1}$NAA (Naphthalene acetic acid). Thus, the experiment included 6 treatments [3 species X 2 culture media] during the initiation stage, with 7 replicates per treatment each replication contain 4 explant. The experiments were designed according to the factorial completely randomized design (CRD). The comparison between means was done according to the Duncan multiple range tests at $P \leq 0.05$ [8].

***Plant Material*:**

juvenal shoots 5-7 cm of different three species of *Bougainvillea (B.× buttiana, B. spectabilis* and *B. glabra*) were collected in April with 2-4 nodal explants for all species and used in this study. **Establishment Stag**e, the explants were excised and cultured on MS solidified medium and woody plant medium (WPM) supplemented with 3% sucrose,0.7% agar and supplemented with $2mgl^{-1}$ BA and $0.2mgl^{-1}$ NAA. After the removal leaves the explants were surface sterilized with 70% ethanol for 30 seconds followed by 0.05% Mercuric Chloride ($HgCl_2$) for 5 minutes [9]. Then the explants were rinsed three times with sterile distilled water under laminar air flow bench to remove sterilants. Then they were cut on nodal segment (1cm) and cultured on WPM [6] and MS [7] media. The cultures were incubated in the culture room under 25± 2°C temperature and 16 h daily exposure to 1000 Lux cool white light, followed by 8 h of darkness. After four weeks of culture, callus formations from nodal explants in each species were recorded. Then after 6 weeks, the following data were recorded: percentage of callus induction, average callus fresh weight (mg) and organogenesis induction percentage, Number of shoot development, Number of leaves per culture, Number of node per culture and average of shoots length (cm).

After four weeks of incubation in initiation medium in establishment stage, shoots >1.5 cm long were harvested for rooting experiment. Shoots>1.5 cm were excised from explants of initiation medium and inoculated on (½ and ¼) strength MS medium or WPM medium containing one concentration of NAA at $1mgl^{-1}$. *Bougainvillea* explants were incubated for six weeks in the growth room to determine the rooting data as: Percentage of root formation, number of roots/explants, and Root length in cm.

Data Statistics and Analysis

Frequency of callus induction from node explants, the morphology and color of callus, degree of callus formation, frequency of callus differentiation, and mean number of induced shoots per explants were recorded after 30 days of culture. One or two-way analysis of variance (ANOVA) and Duncan's multiple range tests ($P<0.05$) were employed to determine the significant differences among means of the recorded parameters.

IV. Results and Discussion:

***Effect of culture media and fixed concentration of BA and NAA in callus induction*:**

The most important factors contributing the induction of somatic embryo from the callus, plant growth regulators and medium formulation [10]. Among the plant growth regulators, generally auxin is known to be essential for the induction of somatic embryogenesis and 2,4-D is the most commonly used auxin [11]. Moreover, a combinations of 2,4-D or NAA with cytokinine was reported to be essential for induction of callus and somatic embryos [12]. Certain cells may need simple MS medium for the induction of somatic embryos and further development [13].

To determine the best callus induction response from node explant of different types of culture media, WPM and MS media, fixed concentration and combinations of plant growth regulators ($2mgl^{-1}$ BA + $0.2mgl^{-1}$ NAA)and three genotypes of *Bougainvillea(B.× buttiana, B. spectabilis* and *B. glabra*) were tried (Table 1, Fig. 1). The best results of callus induction response (92.86%) in whole area of node explant was observed on WPM medium of *B. ×buttiana* compared with *B. ×buttiana* when cultured on MS medium gave (85.71). The lowest callus induction response (71.00%) was observed on WPM medium from *B. glabra* followed by MS medium (71.43%) callus was formed from *B. glabra*. [14] reported callus development from *Zizyphus jujuba Mill* leaves cut cultured on MS media supplemented with 0.5 mg/L benzyl amino purine (6-BA), 2.0 mg/L 2,4-dichlorophenoxyacetic acid (2, 4-D), and 0.5 mg/L silver nitrate (AgNO3). [15] and [16] obtained callus in *Chimonanthus praecox* and *Croton uruerana* using different levels of 2 mgl^{-1}BA or 3 mgl^{-1} 2,4-D from leave segments respectively.

In the same table, the average callus fresh weight was obtained on WPM medium an average of (667.29) mg/ culture form *B. ×buttiana*. While MS medium gave (631.71) fresh weight of callus / culture from *B. ×buttiana* . However, the lowest fresh weight of callus / culture observed when *B. glabra* culture on WPM and MS medium (339.43, 336.29 mg/ culture) respectively, and the highest percentage of organogenesis was (96.43%, 89.29%) in the WPM medium from *B. × buttiana, B. spectabilis* respectively, and (85.71%)

organogenesis was observed from *B. × buttiana* when cultured on MS medium. While the lowest percentage of organogenesis induction (64.2%) was obtained in MS medium from *B. glabra* [17] reported somatic embryo from *Codiaeum variegatum* L. leaves explant cultured on MS medium containing 0.2mgl^{-1} 2, 4-D + 2mgl^{-1} BA., [18] concluded that in leaf explant of *Begonia rex and Begonia Semperflorens*, the callus induction was achieved on MS medium with combinations of NAA and BAP. The results of this work support those found by other authors. For instance, [19] did not observed callus formation in leaf explant of *Morus alba* L. in the absence of 2,4-D and [20] did not obtained callus in leaf explant of *Fagus silvatica* L. in the presence of BA.

Table (1):Effects of plant growth regulators on callus induction and Organogenesis from single node of *Bougainvillea sp.* after 6weeks of culture in WPM and MS medium.

Genotype	WPM			MS		
	Callus induction %	Average callus fresh weight (mg)	Organogenesis induction %	Callus induction %	Average callus fresh weight (mg)	Organogenesis induction %
B.× buttiana	**92.86a**	**667.29a**	**96.43a**	**85.71a**	**631.71a**	**85.71ab**
B. spectabilis	**82.14a**	**522.86a**	**89.29a**	**75.00a**	**510.29a**	**78.57ab**
B. glabra	**71.00a**	**339.43b**	**78.57ab**	**71.43a**	**336.29b**	**64.29b**

* Means followed by the same letter within each character (column) do not differ significantly (P≤0.05) according to Duncan's Multiple Range Test [8].

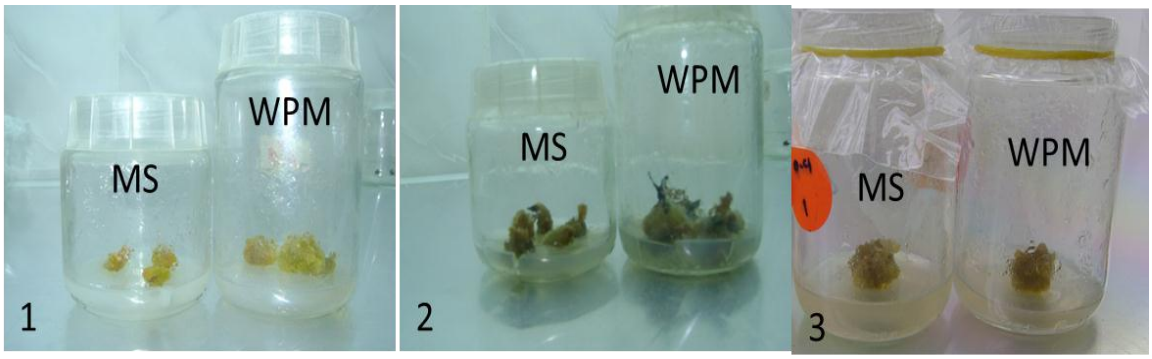

Fig 1 . Callus induction from node explants of *Bougainvillea sp.* on different media MS and WPM supplemented with 2mgl^{-1}BA + 0.2mgl^{-1}NAA . *(1)B.× buttiana. (2) B. spectabilis (3) B. glabra.*

***Effect of different types of culture medium and different genotypes of Bougainvillea on shoot formation from callus induction*:**

Data on shoot formation revealed that when the fixed concentration of BA combination with NAA (2mgl^{-1}BA+0.2mgl^{-1}NAA), the rate of shoot formation was increased. Maximum number of shoot induced from callus, maximum shoots length and highest number of leaves per culture were obtained on WPM was 12.14 shoots/ culture, 2.14 cm and 20.71 leaves/ culture respectively from *B.× buttiana* after 6weeks of culture inoculation (Table 2, Fig. 2).compared with all characterized for *B.× buttiana* when cultured on MS medium was give number of shoot induced from callus, maximum shoots length and highest number of leaves per culture (11.43shoot induced/ callus, 17.14 cm, 1.96number of leaves/ culture) respectively. While the lowest number of shoot induced from callus, maximum shoots length and highest number of leaves per culture were obtained on WPM and MS medium was observed from *B. glabra in* all characterized (9.14 shoot induced/ callus, 11.29 cm, 1.51number of leaves/ culture) respectively, observed on WPM and (8.29 shoot induced/ callus, 8.43 cm, 1.09 number of leaves/ culture) respectively, observed on MS medium. Finally, all genotypes when cultured on WPM were give the best characterized (number of shoot induced from callus, maximum shoots length and highest number of leaves per culture) compared with all genotypes when cultured on MS medium.

Many researchers have also reported positive effect of BA on shoot multiplication and proliferation. [21] Cultured leaf segments of *Torenia fournieri* L. on different media and reported a number of adventitious shoots/ leaf formation (50.9±9.0) on MS medium containing 0.05 mg/l NAA and 3 mg/l BA. Similarity, [22] found mass prolific growth of *Anthemisxylopoda* plants using 0.5mgl^{-1} BA. During this investigation, it was also observed that 0.5 mgl^{-1} of BA was the best concentration for shoot development whereas higher concentrations inhibited it. This was in accordance with the study conducted by [23] in which they reported that lower concentration of BA (1 mgl^{-1}) stimulated shoots growth in *Gerbera jamesoni*, but the higher concentration of BA (5 mgl^{-1}) inhibited shoot proliferation.

[24] also reported the best shoot response (81.25%) of *B. spectabilis* on MS medium supplemented with 1mgL^{-1}BA+500mgL^{-1}L-glutamin. However, [4] reported best shoot development in MS medium containing (0.25 mgl^{-1} BAP+ 0.25 mgl^{-1}NAA) and [25] used MS medium supplemented with 0.5 mgL-1 6-BA, 0.1 mgL-1 NAA, 3% sucrose and 0.72% agar for adventitious shoot number per callus from leaf explants.

Table (2): Effects of plant growth regulators on callusinduction and Organogenesis from single node of *Bougainvillea sp.* after 6weeks of culture in WPM and MS medium

Genotype	WPM			MS		
	No. of shoots/culture	No. of Leaves/culture	average Length of Shoots (cm)	No. of shoots/culture	No. of Leaves/culture	average Length of Shoots (cm)
B.× buttiana	**12.14a**	**20.71a**	**2.14a**	**11.43a**	**17.14ab**	**1.96ab**
B. spectabilis	**11.57a**	**14.86bc**	**1.59abc**	**8.86b**	**14.14bc**	**1.80ab**
B. glabra	**9.14b**	**11.29cd**	**1.51bc**	**8.29b**	**8.43d**	**1.09c**

* Means followed by the same letter within each character (column) do not differ significantly (P≤0.05) according to Duncan's Multiple Range Test [8].

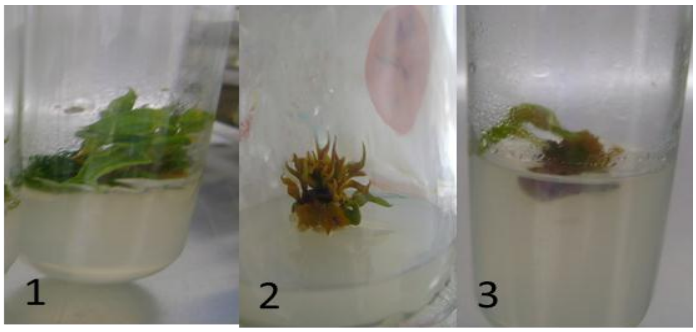

Fig 2. Adventitious shoots on WPM medium supplemented with 2mgl^{-1}BA + 0.2mgl^{-1}NAA after 6 weeks in induction *(1)B.×buttiana. (2) B. spectabilis (3) B. glabra*

***Effects of fixed concentration of NAA with (½ strength) WPM and MS medium on in vitro rooting*:**

Fixed concentrations of auxin (1 mgl^{-1}) of NAA at two type of media WPM and MS medium at (½ strength) were used for *in vitro* roots formation. The best response was observed when ***B.× buttiana*** cultured on ½ strength WPM medium which was (**89.29**%) while **82.14**% of the shoots were rooted when ***B.× buttiana*** cultured on ½ strength MS medium (Table 3). The lowest root induction response (**50.00**%) was observed on ½ strength MS medium from ***B. glabra*** explant. A large number of roots, root length and root percentage (**2.57**roots /explant, **3.41**cm and **89.29**%) respectively, was observed when ***B. ×buttiana*** cultured on ½ strength WPM medium. While a large number of roots, root length (**2.43** roots /explant and **2.73**cm) respectively, was observed when 1 mgl^{-1} NAA were used in ½ strength MS medium and 60% root percentage was observed when ***B.× buttiana*** cultured on ½ strength MS medium . In the same table, minimum number of roots per shoot and root length were formed (**1.43**roots/ shoot and **1.81**cm) when ***B.× glabra*** cultured on media supplemented with 1 mgl^{-1} NAA was added to ½ strength MS medium respectively. Also minimum number of roots per shoot and root length were formed (1.86roots/ shoot and 1.83cm)when ***B.× glabra*** cultured on media supplemented with 1 mgl^{-1} NAA was added to ½ strength WPM medium respectively. IBA has been reported to have stimulatory effect on root induction in many plant species including *Ruscus hypoglossum L* [26], *Jojoba* [27], *bitter almond* [28] *and Bambusa vulgaris* [29].

Table (3): *Effects of fixed concentration of NAA with (½ strength) WPM and MS medium* on shoot rooting of *Bougainvillea sp.* after 4 weeks of culture

Genotype	½WPM			½MS		
	% root/culture	No. of root/culture	average Length of roots (cm)	% shoots/culture	No. of root/culture	average Length of roots (cm)
B.× buttiana	**89.29a**	**2.57a**	**3.41a**	**82.14a**	**2.43a**	**2.73b**
B. spectabilis	**78.57a**	**2.14a**	**2.39bc**	**75.00ab**	**1.86ab**	**2.14bc**
B. glabra	**60.71ab**	**1.86ab**	**1.83c**	**50.00b**	**1.43c**	**1.81c**

* Means followed by the same letter within each character (column) do not differ significantly (P≤0.05) according to Duncan's Multiple Range Test [8].

Effects of fixed concentration of NAA with (½ strength) WPM and MS medium on in vitro rooting:

Fixed concentrations of auxin (1 mgl-1) of NAA at two type of media WPM and MS medium at (¼ strength) were used for in vitro roots formation. The best response was observed when *B.× buttiana* cultured on ¼ strength WPM medium which was (100%) while 100% of the shoots were rooted when *B.× buttiana* cultured on ¼ strength MS medium (Table 4). The lowest root induction response (**82.14**%) was observed on ½ strength MS medium from *B. glabra* explant. A large number of roots and root percentage (3.43 roots /explant, and 100%) respectively, was observed when *B. ×buttiana* cultured on ¼ strength WPM medium. While the root length (**4.26**cm) was observed when *B. ×buttiana* cultured on ¼ strength WPM medium. A large number of roots, root length (**3.00** roots /explant and **3.16** cm) respectively, was observed when 1 mgl^{-1} NAA were used in ½ strength MS medium and 100% root percentage was observed when *B.× buttiana* cultured on ¼ strength MS medium . In the same table, minimum number of roots per shoot and root length were formed (**1.57** roots/ shoot and **2.06**cm) when *B.× glabra* cultured on media supplemented with 1 mgl^{-1} NAA was added to ¼ strength MS medium respectively. Also minimum number of roots per shoot and root length were formed (**2.29** roots/ shoot and **2.51**cm) when *B.× glabra* cultured on media supplemented with 1 mgl^{-1} NAA was added to ¼ strength WPM medium respectively. IBA has been reported to have stimulatory effect on root induction in many plant species including *Ruscus hypoglossum L* [26], *Jojoba* [27], *bitter almond* [28] and *Bambusa vulgaris* [29].

Table (4): Effects of fixed concentration of NAA with (¼ strength) WPM and MS medium on shoot rooting of *Bougainvillea sp.* after 4 weeks of culture

Genotype	¼WPM			¼MS		
	% root/culture	No. of root/culture	average Length of roots (cm)	% shoots/culture	No. of root/culture	average Length of roots (cm)
B.× buttiana	**100.00a**	**3.43a**	**4.26a**	**100.00a**	**3.00a**	**3.16a**
B. spectabilis	**96.43a**	**3.00a**	**3.11a**	**85.71a**	**2.14bc**	**2.41c**
B. glabra	**89.29a**	**2.29b**	**2.51c**	**82.14a**	**1.57c**	**2.06c**

* Means followed by the same letter within each character (column) do not differ significantly (P≤0.05) according to Duncan's Multiple Range Test [8].

Figure (3): shoots >1.5 cm were excised from explants of initiation medium and inoculated on (½ and ¼) strength MS medium or WPM medium containing NAA (1) mgl^{-1}. *1) B.× buttiana 2) B. spectabilis 3) B. glabra.* 4) Root formation in regenerated shoots in ¼ strength WPM.

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