

IN VITRO RESPONSE OF VARIOUS GROWTH REGULATORS ON THE REGENERATION OF *BOUGAINVILLEA SPECTABILIS* WILLD

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Received: Oct 2, 2006; Revised: Jan 15, 2007; Accepted: Feb 9, 2007

Abstract

Bougainvillea is commonly propagated by cuttings but this method is tedious and time consuming with very little success. Tissue culture technique has been proved as a potential tool for quick and mass propagation of several ornamental species. The research was carried out to investigate optimum levels of BAP, glutamine, IAA and IBA supplemented to MS medium for micro propagation of *Bougainvillea spectabilis* Willd. Shoot apices were excised, sterilized and then cultured on MS medium supplemented with plant regulators for callus induction and plant regeneration. Maximum number of plantlets (81.25%) with conspicuous callus formation was observed with BAP 1.0 mg/l + Glutamine 500 mg/l followed by BAP 1.0 mg/l + 250 mg/l Glutamine where 64.58% plantlets have been developed. The minimum number of plantlets developed on control MS basic medium was 12.5%. Plantlets produced were then re-cultured in full and half strength MS medium supplemented with different concentrations and combinations of IAA and IBA. It was found that half strength MS medium supplemented with IAA 0.5 mg/l and IBA 0.5 mg/l, proved the best medium where 79.16% of plantlet rooted. Higher average number of roots per plant (30.0) was obtained on IBA 5 mg/l. The lowest number of plantlets (25.0%) rooted when IAA 5.0 mg/l was used only. The lowest average number (2.7) of roots per plant was obtained on MS medium with IAA 2.5 mg/l. The maximum number of plantlets (58.33%) rooted when full strength MS medium was supplemented with higher concentrations (5.0 mg/l) each of IAA and IBA. The maximum number of average roots per plant (15.4) were obtained when full strength MS medium was supplemented with IAA 2.5 mg/l. Half strength MS medium supplemented with IAA and IBA 0.5 mg/l each gave maximum root induction while full strength MS medium with IAA and IBA 5.0 mg/l each proved best for root initiation.

Keywords: *Bougainvillea*, shoot tips culture, callus induction, *in vitro* regeneration and rooting

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Introduction

Bougainvillea (*Bougainvillea spectabilis* Willd) belongs to the Dicot family, Nyctaginaceae. The plant is a shrub, tree or vine, which can reach over 30 feet high. Its leaves are large, simple and mostly ovate. They may show rippling along the edges and hair can be seen underneath. Bracts are usually in shades of red, shades of dark pink or shades of purple. Thorns are large and may be curved. Flowers are cream coloured. Its habitat is dense and the bracts appear up and down the branches. During the dry season or in a cool spell, flowering is triggered. The root system is very fragile and doesn't form a good root ball.

Bougainvillea has many adaptations to its climate and environment. It lines in a climate from subtropical to dense forests where it can cling and grab onto others plants to reach the sunlight. It can tolerate semi-shaded areas and can also tolerate full sun. It thrives in full sun. At least 5 h a day of full sunlight is the minimum light required for a good bloom. More hours of direct sun is better, the plant does not like to live with a lot of water.

The plant has earned a reputation as to be the pride of gardens among the naturalists and ornamentalists due to its wide range of habitats, prolonged flowering seasons and variety of flower colours. It also has a wide range of uses in landscaping. The traditional multiplication of *Bougainvillea* is very difficult because in our climatic conditions it does not produce seeds while from cutting the percentage of success is very low. The plants which are produced by air layering are in small quantity, need more skill and labour. So there is a dire need to adopt a technique known as Tissue culture which has recently emerged as a potential tool for quick and mass regeneration of several cultivated plant species (Javed *et al.*, 1996).

The current micropropagation of plants has allowed for strong and continued growth within the micro propagation industry. Tissue culture techniques have been successfully employed to produce large number of plant which are difficult to propagate plants as reported by Khan *et al.* (1985) and Javed *et al.*

(1996). Micropropagation is rapid, efficient and plants can be produced year round for the market by using of this technique. The vegetative axillary buds of *Bougainvillea* have exhibited good subsequent in vitro growth (Sharma and Dhir, 1985). Micropropagation of *Bougainvillea* by shoot tip culture has been successful (Chaturvedi *et al.*, 1978). Keeping in view the economical, aesthetic and ornamental value these studies were carried out to improve the micropropagation protocol of *Bougainvillea* through shoot tip culture for commercialization.

Materials and Methods

Bougainvillea spectabilis Willd the Texas Dawn variety was selected to study. One cm long shoot tips of vigorously plants were used as explants. The surface sterilized according to Zamir *et al.* (2004). The MS (Murashige and Skoog, 1962) basal medium supplemented with cytokinins and auxins and 3% sucrose was used. The medium was solidified with 0.7% plant agar. The pH of the medium was adjusted to 5.7 before adding agar and autoclaved at 121°C, 1.1 kg/cm² for 25 min. The cultures were kept in growth chambers at 26 ± 1°C under a 16 h light/8 h dark with a light intensity of 2,000 - 3,000 lux. Experiment was randomized complete block design with six treatments and three replications, 16 explants per treatment. The data were recorded after 5 - 7 weeks and statistically analyzed by using Duncan's Multiple Range Test (Steel and Torrie, 1980).

When shoot tips developed, they were sub-cultured on MS medium supplemented with different concentrations of BAP or BAP in combination with glutamine. The cultures were incubated for 7 weeks. Shoots with excellent growth, having 4 - 5 leaves were transferred to MS medium with different concentrations and combinations of IAA and IBA to induce roots.

The plantlets were transferred into pots containing a mixture of sand: silt (1:1) and compost after washing the roots thoroughly to remove any remains of the medium. The potted

plants were covered with a glass beaker for one week for their establishment.

Results and Discussion

Effect of Different Concentrations and Combinations of BAP and Glutamine on the Regeneration of *Bougainvillea Spectabilis* from Shoot Tips

The effect of different concentrations and combinations of BAP and glutamine on the regeneration of shoots indicates that BAP and glutamine significantly affected shoot tips developed (Table 1). The maximum number of shoot tips (81.25%) developed when MS was supplemented with BAP 1.0 mg/l

combined with glutamine 500 mg/l followed by BAP 1 mg/l + 250 mg/l glutamine where 64.58% shoots were vigorous (Figure 1(a-b)). The lowest number of shoots (12.5%) were obtained on MS control medium, while BAP alone in concentration of 0.5, 1.5, and 2.0 mg/l obtained 39.58%, 36.25%, and 41.66% respectively. The decrease or increase in BAP concentration from 1.5 mg/l negatively affects the development of shoot tips. The increase in Glutamine concentration positively affects the development of shoots. Javed *et al.* (1996) also induced maximum number of shoots (70%) when MS medium was supplemented with NAA 0.1 mg/l + BAP 2.0 mg/l + L-glutamine 250 mg/l. Less number of percent shoots may be due to

Table 1. Effect of different concentration and combinations of BAP and glutamine on the regeneration of *Bougainvillea spectabilis* Willd from shoot tips

Media Concentration	No. of shoots cultured	No. of shoots developed into plantlets	Percentage of shoot development
MS (Basic) Control	48	6 d	12.50
BAP 0.5 mg/l	48	19 c	39.58
BAP 1.5 mg/l	48	27 b	56.25
BAP 2.0 mg/l	48	20 c	41.66
1.0 + 250 mg/l glutamine	48	31 b	64.58
1.0 + 500 mg/l glutamine	48	39 a	81.25

Means followed by different letter are significantly different at $P = 0.05$ level by DMRT

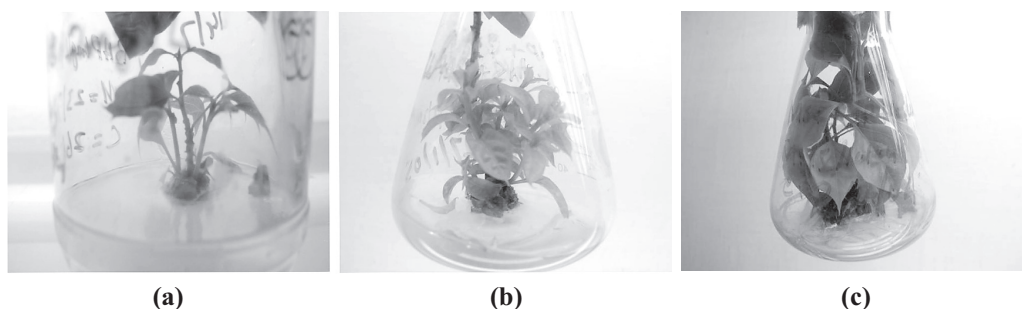


Figure 1. A. Shoot proliferation in *Bougainvillea* on BAP 1.0 mg/l + glutamine 500 mg/l; B. A clump of *in vitro* multiple shoots with callus at the basal part on 1 mg/l BAP and 250 mg/l glutamine; C. *In vitro* root induction on medium containing 5.0 mg/l IBA and 5.0 mg/l IAA

NAA which may have suppressed the multiple shoots per explants. The results of Zamir *et al.* (2003) who used 1.0 mg/l BAP + 250 mg/l L-glutamine supplemented to MS medium resulted in the maximum number of shoots per explants development in guava Cv. Safeda.

The regeneration and proliferation of *Bougainvillea* occur on almost all concentration of BAP alone or in combination with L-glutamine. Higher concentration of auxin like NAA can suppress the growth. Khan *et al.* (2004) cultured *Ixora coccinea* plants for 12 weeks on BAP with Peptone. But the concentration of BAP used was very low i.e. 0.005, 0.05, and 0.5 mg/l as compared to our concentration of BAP. Sajid *et al.* (2006) also observed poor shoot proliferations in grapes germplasm when low levels of BAP and auxins were used. However, when the BAP concentration was increased beyond 0.3 mg/l there was an increase in the number of shoots per explants.

Effect of Different Auxins (1AA and IBA) on the Development of Roots Supplemented to MS Medium

The data regarding the effect of different concentrations and combinations of 1AA and IBA on the development of roots when supplemented to MS medium indicates that maximum number of shoots (58.33%) developed into rooted when MS was supplemented with 1AA 5 mg/l combined with IBA 5.0 mg/l

followed by 1AA 2.5 mg/l + IBA 2.5 mg/l where (45.83%) rooted plantlets were obtained (Table 2). The lowest number of shoots developed into root (25%) when MS medium was supplemented with 2.5 mg/l IBA while IBA 5/mg/l produced (37.5%) rooted shoots as in Table 2. Javed *et al.* (1996) also found significantly higher percentage of root development (70.0, 60.0, and 67.5) when 5 mg/l IBA and 5 mg/l NAA and either 2.5 mg/l NAA or 5 mg/l alone was supplemented to the medium.

Effect of Different Auxins (1AA and IBA) on the Average Number of Roots Supplemented to MS Medium

The average number of roots per shoot as affected by different concentrations and combinations of 1AA and IBA indicates that the highest average number of roots (10.0) per shoot were obtained when MS medium was enriched with 1AA 2.5 mg/l + IBA 2.5 mg/l followed by IBA 2.5 mg/l where (9.8) average number of root/shoot were obtained (Table 2). The lowest average number of roots/shoot of (4.2%) were obtained when MS was supplemented with 1AA 5 mg/l + IBA 5 mg/l. Singh *et al.* (2002) got maximum number of roots per shoot in guava seedlings when 4.90 µM/l IBA was combined with 100 mg/l activated charcoal.

Table 2. Effect of different auxins (1AA and IBA) on the Rooting of *Bougainvillea spectabilis* Willd cultured on MS medium

Auxins (mg/l)	No. of shoots cultured	No. of shoots developed in to roots	Percent of shoots rooted	Average number of roots/plants
IBA 2.5	48	12 cd	25.00	9.8 cd
IBA 5.0	48	18 bc	37.50	7.3 de
IAA 2.5	48	12 cd	25.00	15.4 b
IAA 5.0	48	11 d	22.90	5.8 e
IAA 2.5 + IBA 2.5	48	22 ab	45.83	10.0 cd
IAA 5.0 + IBA 5.0	48	28 a	58.33	4.2 de

Means followed by different letter are significantly different at $P = 0.05$ level by DMRT

Table 3. Effect of different auxins (IAA and IBA) on the rooting of *Bougainvillea spectabilis* Willd cultured on ½ strength MS medium

Auxins (mg/l)	No. of shoots cultured	No. of shoots developed roots	Percent of shoots rooted	Average number of roots/shoots
IBA 2.5	48	36 a	75.00	15.5 c
IBA 5.0	48	26 b	54.16	30.0 a
IAA 2.5	48	16 bc	33.33	2.7 e
IAA 5.0	48	12 c	25.00	22.9 b
IAA 2.5 + IBA 2.5	48	24 b	50.00	5.0 de
IAA 5.0 + IBA 5.0	48	38 a	79.16	12.5 cd

Means followed by different letter are significantly different at $P=0.05$ level by DMRT

Effect of Different Concentrations and Combinations of IAA and IBA on the Rooting Supplemented to ½ Strength MS Medium

The response of different concentrations and combinations of IAA and IBA to half strength MS medium is given in Table 3. The highest number of shoots were rooted (79.16%) when half strength MS medium was supplemented with IAA 5 mg/l + IBA 5 mg/l followed by IBA 2.5 mg/l where 75% shoots were rooted (Figure 1(c)). The lowest number of shoots were rooted when ½ strength MS medium was supplemented with IAA 5 mg/l only. Misra and Datta (1999) suggested half strength medium for root induction in *Ixora*. Similar observations found in *Ixora singaporensis* by Malathy and Pai (1998) in which roots were induced by transferring the shoots previously cultured in MS basal to B5, a lower salt medium.

Effect of Different Auxins (IAA and IBA) on the Average Number of Roots/Plant Supplemented to ½ Strength MS Medium

In case of half strength MS medium the highest average number of roots/shoots (30) were obtained when MS was supplemented with IBA 5 mg/l. followed by (22.9) at IAA 5 mg/l (Table 3). The lowest number of roots/shoots 2.7 were obtained when MS was supplemented with IAA 2.5 mg/l. Zamir *et al.*

(2003) also got highest number of roots (3.5) per plant in guava cultured on half strength MS medium supplemented with IAA + IBA 2.5 mg/l.

The *in vitro* growth response of *Bougainvillea* under the study has strengthen our understanding on culture establishment, rooting and shoot proliferation which are vital in the commercial propagation. From this protocol, the supply of both rooted plantlets and proliferating culture of shoots will be available continuously, which are essential requirements for mass propagation (Rajaseger *et al.*, 1999).

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