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IN-VITRO PROPAGATION OF (*BOUGAINVILLEA SPECTABILIS*) THROUGH SHOOT APEX CULTURE

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Abstract

Shoot apices of *Bougainvillea spectabilis* cv. Texas Dawn were excised and cultured on MS modified medium containing different concentrations and combinations of plant growth regulators for shoot growth, multiplication and root induction. Shoot development was observed where BAP 0.25 + NAA 0.25 mg/L were used. Medium containing NAA 0.1 + BAP 2.0 + glutamine 250 mg/l induced maximum number of multiple shoots with higher percentage (70%) of rooting where IBA 5.0 + NAA 5.0 mg/l were added to the medium. The system will provide a means for rapid clonal propagation future.

Introduction

Bougainvillea (*Bougainvillea spectabilis*), of the family Nyctaginaceae, a native of Brazil, South America, is commonly grown in gardens, porches, boundary walls, lawns and road median steps. It has attractive flowers which remain throughout the year particularly from April to August. The propagation of Bougainvillea is difficult. In our climatic conditions it does not produce seeds while success percentage from cuttings 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 large number of difficult-to-propagate plants (Jones, 1976; Barlass & Skene, 1978; Khan *et al.*, 1985). Information on micropropagation of Bougainvillea in Pakistan is rather scarce. Keeping in view the economical, aesthetic and ornamental value, the present report describes the propagation ofBougainvillea through shoot apex culture.

Materials and Methods

One cm long shoot tips of Bougainvillea var. 'Texas Dawn' were cut from the plant growing outside the lawn at Nuclear Institute for Food and Agriculture, Tarnab, Peshawar. The pieces thoroughly washed in distilled water containing 1-2 drops of detergent (Zip) to remove dust then surface sterilized with 70% ethanol for one minute and in 2% sodium hypochlorite having 1-2 drops of Tween-20 for 20 minutes on a shaker followed by washing in sterilized distilled water under Laminar flow bench to remove sterilants.

Murashige & Skoog (1962) basal medium (MS) modified with B_5 vitamins of Gamborg *et al.*, (1986) supplemented with 3% sucrose as a carbon source and 0.8% agar was used. The medium was enriched with different combinations and concentrations of phytohormones for shoot development, multiplication and rooting.

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Table 1. Effect of different concentrations and combination of NAA and BAP on shoot apex development of *Bougainvillea spectabilis* supplemented to MS medium.

Concentration mg/l NAA BAP	No. of shoots cultured	No. of shoots developed into plantlets	Average shoot length (mm)
.01.+0.25	48	35b	19.5b
0.25+0.25	48	45a	24.0a
0.5+0.25	48	22cd	8.3c
0.1+0.5	48	21cd	20.0b
0.25+0.5	48	34b	16.6b
0.5+0.5	48	14de	17.6b
0.1+1.0	48	28bc	17.0b
0.25+1.0	48	23cd	8.6c
0.5+1.0	48	24cd	17.3b
MS alone	48	8e	6.5c

Means of the same category followed by different letters are statistically different at 5% level of probability.

The pH was adjusted to 5.8 before adding agar and autoclaved at 121°C, 15 psi for 15-20 minutes. Sterilized shoot tips were excised 0.5 cm in size and incubated under light (4000 lux) at 25±2°C with a photoperiod of 16h light/8h dark. All the treatments were replicated 4 times using Randomized Complete Block Design. The data recorded after 4-6 weeks were statistically analyzed by using analysis of variance and Duncan Multiple Range Test (Steel & Torrie, 1980) to check the level of significance between the treatments.

Results and Discussion

Effect of BAP and NAA on shoot development: Significant differences among the various treatments were observed when different combinations and concentrations of BAP (0.25, 0.5 and 1.0 mg/l) and NAA (0.1, 0.25 and 0.5 mg/l) were added to MS medium for shoot development of Bougainvillea. Best results were found with BAP 0.25 + NAA 0.25 mg/l followed by either BAP 0.25 + NAA 0.1 mg/l or BAP 0.5 + NAA 0.25 mg/l where respectively, 45,35 and 34 plants were produced (Table 1). Only 8% plants developed in basal medium having no phytohormone. A maximum increase of 14 time in plant height was obtainedas compared to the original shoot length where a combination of BAP 0.25 + NAA 0.25 mg/l was used (Fig.1). These findings are similar to the reports of Chaturvedi *et al.*, (1978) where an increase of 4-6 fold in length have been reported.

Induction of Multiple Shoots: After development of shoot apices into plantlets, these were sub-cultured for induction of multiple shoots on MS medium supplemented with different concentrations and combinations of NAA 0.1+BAP (0.5, 1.0 and 2.0 mg/l) + glutamine (250 and 500 mg/l). The cultures incubated for a period of 6-8 weeks that NAA 0.1+BAP 2.0 + glutamine 250 mg/l induced significantly higher number of multiple shoots (156) where on an average of 4.33 new shoots per plant were produced (Table 2). MS medium containing NAA 0.1 + BAP 0.5 + glutamine 250 mg/l produced 123 shoots having an average of 3.42 shoots/plant. Increasing the concentration of glutamine from 250 to 500 mg/l and that of BAP from 0.5 to 2.0 mg/l gave no significant enhancement in shoot multiplication and number of shoots per plant. The are reports by (Swamy & Sahijram, 1988) when maximum number of shoots (5.6) were produced MS medium containing BA 8.0 + NAA 4.0 mg/l. It was also found that BA alone at a concentration of 2 mg/l proved effective in producing an average of 4.9 shoots. Chaturvedi *et al.*, (1978) found an average of 10 shoots on MS medium containing BA 0.5 + IAA 1.5 mg/l BA was effective in inducing multiple shoots in Bougainvillea (Swamy & Sahijram, 1988).

Effect of different auxins on root development: In order to induce rooting, shoots with excellent growth, having 3-4 leaves were transferred to MS medium with different combinations and concentrations of NAA, IAA and IBA. Significant differences among the various treatments in response to rooting were observed. A combination of IBA 5.0 + NAA 5.0 and either NAA 2.5 or 5.0 mg/l alone when added to the medium, showed significantly higher percentages (70.0, 60.0 and 67.5) of plantlets formed roots (Table 3, Fig.2). Higher concentration of NAA (5 mg/l) when used alone, produced a heavy

Fig.1. Development of better shoot of *Bougainvillea spectabilis* on MS medium supplemented with NAA 0.25 + BAP 0.25 mg/l.

Table 2. *In vitro* multiple shoot induction on *Bougainvillea spectabilis* when cultured on Ms medium containing different concentrations and combinations of plant growth regulators for a period of 6 weeks.

Concentration mg/l NAA + BAP + Glutamine	No. of plant let cultured	Total no. of shoots developed	Av. no. of shoots/ plant	Range of shoots/ per plantlet
0.1+0.5+250	36	123b	3.42	1-6
0.1+1.0+250	36	84c	2.33	2-3
0.1+2.0+250	36	156a	4.33	2-6
0.1+0.5+500	36	83c	2.30	1-3
0.1+1.0+500	36	90c	2.50	2-4
0.1+2.0+500	36	72c	2.0	1-3

Means of the same category followed by different letters are statistially different at 5% level of probability.

mass of callus at the base alongwith shortening of root length (5.8 mm). A combination of IBA 5.0 + NAA 5.0 mg/l proved superior for root induction on Bougainvillea. Shoots of *Lagerstromia speciosa* cultured on MS medium supplemented with 5-10 mg/l IAA produced abundant roots (Lim & Lee, 1986). Rooting was induced on MS medium containing IBA 6 mg/l. Swamy & Sahijram (1988) obtained 47.6% rooting when IBA 5 mg/l was added to MS medium. In the present study a combination of IBA 5.0 + NAA 5.0 gave good response for *in vitro* root development of Bougainvillea. After 6 weeks,

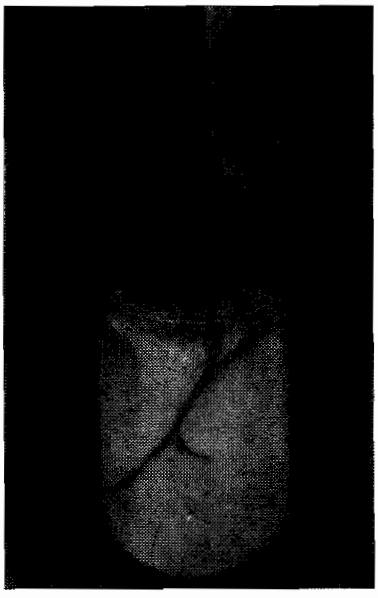

Fig.2. A treatment of IBA 5.0 + NAA 5.0 + MS showing better root induction.

Table 3. Effect of different auxins alone and in combination on rooting of *Bougainvillea spectabilis* when added to MS medium.

Auxins (mg/l)	No. of plantlet cultured	No. of plantlet rooted	Percent of plantlet rooted	Av. root no. per plantlet	Av. root length (mm)
NAA 2.5	40	24a	60.0	15.5b	8.3cde
IBA 2.5	40	12b	30.0	9.8cd	14.8b
IAA 2.5	40	8b	20.0	6.9de	15.4b
NAA 5.0	40	27a	67.5	12.4bc	5.8e
IBA 5.0	40	11b	27.5	7.3de	11.9bc
IAA 5.0	40	9b	22.5	5.8e	9.2cde
NAA 5+IAA5.0	40	10b	25.0	18.6a	6.7de
IBA 5.0+NAA5.0	40	28a	70.0	10.0cd	12.5bc
IAA 5.0+IBA5.0	40	6b	15.0	4.2e	9.7cd
MS alone	40	3b	7.5	4.7e	22.2a

Means of the same category followed by different letters are statistically different at 5% level of probability.

the complete rooted plants were transferred into pots containing a mixture of sand + silt (1:1) covering them with glass jars to avoid excessive transpiration. The plant started growth as they were watered with Hoagland solution and most of them flowered within 2-4 months. Micropropagation of Bougainvillea *in vitro* may provide a means of rapid increase of new plants.

References

Barlass, M. and K.Q.M. Skene 1978. *In vitro* propagation of grape vine (*Vitis vinifera* L.) from fragmented shoot apices. *Vitis*, 17: 335-340.

Chaturvedi, H.C., A. K. Sharma and R.N. Prasad. 1978. Shoot apex culture of *Bougainvillea glabra* 'Magnifica'. *Hort. Sci.*, 13: 36.

Gamborg, O.L., R.A. Miller and K.O. Ojema. 1986. Nutrient requirements for suspension culture of soybean root cells. *Expt. Cell. Res.*, 50: 151-158.

Jones, O.P. 1876. Effect of phloridizin and phloroglucinol on apple shoot. *Nature* UK, 262-392. Erratum, Ld, 262,724.

Khan, M.A,, M. Ghamdi and M.Jahjh. 1985. *In vitro* propagation of date palm (*Phoenix dactylifera* L.). *Pak. J. Agri. Sci.*, 22: 177-180.

Lim-Ho, C.L. and S.K. Lee. 1986. Micropropagation of *Lagerstromia speciosa. Hort. Abst.*, 56: 681.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.*, 15: 473-497.

Steel, R.G.D. and J.H. Torrie. 1980. *Principles and procedures of Statistics*. McGraw Hill, New York.

Swamy, D.R. and L. Sahijram, 1988. Tissue culture propagation of *Bougainvillea. Gartenbauwissenschaft*, 53: 174-176.

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