



***In-vitro* protocol for mass multiplication in bougainvillea (*Bougainvillea* sp) cv. Mahatma Gandhi and Refulgens**

PRATIKSHA KUMARI¹, KISHAN SWAROOP², T JANAKIRAM³, S K SINGH⁴, K V PRASAD⁵ and RITU JAIN⁶

ICAR–Indian Agricultural Research Institute, New Delhi 110 012

Received: 29 January 2016; Accepted: 3 June 2016

ABSTRACT

Bougainvillea (*Bougainvillea* sp) is recognized as a high value landscape plant. It is usually propagated by hardwood cuttings, but in certain varieties, rooting per cent is very low, ultimately the rate of multiplication is less. Therefore, the present investigation was carried with an objective to standardize the protocol for quick, easy and mass multiplication of two difficult-to-root cultivars Mahatma Gandhi and Refulgens, using *in vitro* techniques. Nodal sections with axillary buds were excised, surface-sterilized and cultured on MS medium supplemented with plant growth regulators. Pre-treatment agitation of explants in carbendazim (0.1%) + Metalaxyl (0.1%) + 8-HQC (200 mg/l) for 3 hr followed by quick dip in ethyl alcohol (70%; v/v) for 30 sec and surface sterilization in HgCl₂ (0.1%) for 5 min was used for reducing contamination. MS medium with BAP (5 mg/l) was found to be the best with highest percentage of culture establishment (81.13) and the fastest bud sprout (8.18 days). MS medium supplemented with BAP (4.0 mg/l) and kinetin (0.5 mg/l) resulted in highest shoot proliferation in both the cultivars. The best treatment for micro-shoot elongation was the one where MS medium was supplemented with 0.5 mg/l GA₃ giving the highest elongation (3.89 cm). *In vitro* rooting of micro-shoots was done in half-strength MS medium supplemented with 1.0 mg/l IBA. Hardening was done in glass jar with polypropylene cap.

Key words: *Bougainvillea*, Cultivars, Difficult-to-root, *In vitro*

Bougainvillea, a member of family Nyctaginaceae, is a beautiful plant having different uses, viz. climber, shrub, hedge, potted plant, bonsai, arches, pergola etc. Propagation of *bougainvillea* is a challenge in many cultivars as like Sweat Heart, Refulgens, Dr H B Singh, Mahara, Shubhra, Mahatma Gandhi, Mary Palmer, Los Banos Beauty and Thimma because they do not produce seeds in north Indian conditions and are difficult to root through hardwood cuttings (Jain *et al.* 2016). Therefore, tissue culture is the best method for the rapid propagation and multiplication of clones of desirable plants. To overcome such problems, the present research was undertaken with an objective to provide an easy and quick method for *in vitro* propagation of two difficult-to-root *bougainvillea* cultivars, Mahatma Gandhi and Refulgens.

¹Ph D Scholar (e mail: prattil311@gmail.com), Division of Floriculture and Landscaping, IARI, New Delhi stationed at ICAR-IIHR, Bengaluru. ²Principal Scientist (e mail: krishan.swaroop@rediffmail.com), ³Assistant Director General (Hort) (e mail: tolety07@gmail.com) KAB-II, New Delhi 110 012. ⁴Principal Scientist (e mail: sanjaydr2@gmail.com), NRL, ⁵Principal Scientist (e mail: kvprasad66@gmail.com), ⁶Scientist (e mail: ritujain.uhf@gmail.com), Division of Floriculture and Landscaping.

MATERIALS AND METHODS

The experiment was conducted at the Central Tissue Culture Laboratory, NRC on Plant Biotechnology, IARI, New Delhi. Two cultivars, viz. *Bougainvillea peruviana* cv. Mahatma Gandhi (syn Mrs HC Buck) and *B. spectabilis* cv. Refulgens maintained at the International *Bougainvillea* Registration Authority's Repository at IARI, New Delhi were used for the study. In this research work, *in vitro* growth and development of *bougainvillea* was done in five stages as suggested by Debergh and Read (1991).

Choice of the explant is critical for efficient plant regeneration (Ebrahimie *et al.* 2006). Tissue culture of *bougainvillea* using nodal segments, has previously been reported by Rajan *et al.* (1998), Nagarajan *et al.* (2006), Datta and Mandal (2012) and Jain *et al.* (2016). The middle portion of the new growing stems, were selected and cuttings were taken in the early hours. Small sections each containing a dormant bud of about 2.0-2.5 cm length were made, and were put under running water for about half an hour and thereafter washed in Teepol® (0.1%) solution and rinsed 3-4 times with tap water. The explants were agitated for 3 hr in 0.1% carbendazim + 0.1% Metalaxyl + 8-hydroxy quinoline citrate (200 mg/l) followed by surface sterilization using 70% ethanol (30 sec) followed by agitation in 0.1% HgCl₂ (5 min.). The above treatment was used on the basis of initial experiments conducted by using different treatment

combinations of above mentioned chemicals.

For the culture establishment, the surface sterilized explants were cultured on MS basal medium supplemented with BAP (1, 3, 5, 5, 5 mg/l) along with GA₃ (0.1, 0.1, 0.1, 0.1 and 0 mg/l) and NAA (0.01, 0.01, 0.01 and 0 mg/l) in different combinations with 3% sucrose.

After initial establishment, the cultures were further sub-cultured at 15 and 30 days interval on proliferation media containing BAP (1, 2, 3, 4 and 5 mg/l) along with kinetin (0.5 mg/l).

The multiplied shoots on proliferation media were separated and individual micro-shoots were transferred onto different elongation medium comprising basal MS medium supplemented with various concentrations of GA₃ (0.0, 0.5, 0.75, 1.0 and 1.5 mg/l) to standardize the optimum dose required for micro-shoot elongation. Elongated micro-shoots were then excised individually and sub-cultured onto half-strength MS medium with 1.0 mg/l IBA supplemented with 60 g/l sucrose for rooting.

Elongated micro-shoots were then excised individually and sub-cultured onto half-strength MS medium fortified with different concentrations of NAA and IBA individually (or in combination) supplemented with 60 g/l sucrose for rooting.

For hardening of rooted plantlets, two types of strategies were tried, i.e. glass jars (with polypropylene caps) and plastic pot (4.5') with polythene cover. Before transferring the rooted plantlets to for hardening, they were washed with sterile distilled water followed dip in carbendazim (0.2%). The potting media used for hardening comprised of cocopeat + perlite (1:1) moistened with 1/4th strength Ms basal salts devoid of organics. For initial 15 days, the plantlets were kept in culture room (white fluorescent light 57 u mol/m²/s

with 16/8 hr light and dark photoperiod, 65-70% RH, 26±1⁰C), thereafter the lid was loosened for the next 15 days. The polythene cover was punctured after 15 days to reduce moisture levels. After complete hardening, the plants were transferred to shade net house for further growth and maintenance.

The data was analyzed employing Factorial Completely Randomized Block Design (FCRD) and the per cent data were subjected to Arc Sin% transformation before ANOVA.

RESULTS AND DISCUSSION

It is clear from Table 1 that the treatment in which MS medium was supplemented with BAP (5 mg/l); earliest bud sprout (8.18 days) and highest percentage (81.13) of culture establishment was recorded. Irrespective of the genotype, higher callusing was recorded in the treatments where exogenous auxin (NAA) was supplemented. Genotype Mahatma Gandhi showed higher callusing than Refulgens. The treatment where MS medium was supplemented by BAP (1 mg/l) along with NAA (0.01 mg/l) and GA₃ (0.1 mg/l) resulted in delayed bud sprout (9.72 days). A comparison amongst the two cultivars for the percentage culture establishment was found to be non-significant (Table1) while, for the no. of days for bud sprouting the difference was significant. The genotype 'Mahatma Gandhi' was faster (7.39 days) than Refulgens (10.40 days) in sprouting. The treatment × genotype interaction was also significant for both the traits. Overall in the genotype Mahatma Gandhi a maximum of 83.59% culture establishment was observed in the treatment in which MS medium was supplemented with BAP (5 mg/l), while a minimum of 66.67% culture establishment was noted with BAP (1.0 mg/l) along with NAA (0.01 mg/l) and GA₃ (0.1

Table 1 Effect of BAP, NAA and GA₃ on *in vitro* culture establishment (%), days to bud sprouting and av. shoot length after 20 days in bougainvillea

Treatment	Culture establishment (%)		Mean	Days to bud sprouting		Mean	Av. shoot length after 20 days (cm)		Mean
	Refulgens	Mahatma Gandhi		Refulgens	Mahatma Gandhi		Refulgens	Mahatma Gandhi	
BAP (1 mg/l) + NAA (0.01 mg/l) + GA ₃ (0.1 mg/l)	66.67 (54.76)	70.55 (57.10)	68.61	10.60	8.84	9.72	1.87	1.93	1.90
BAP (3 mg/l) + NAA (0.01 mg/l) + GA ₃ (0.1 mg/l)	66.67 (54.76)	73.33 (58.89)	70.1	10.44	8.08	9.26	1.56	1.76	1.66
BAP (5 mg/l) + NAA (0.01 mg/l) + GA ₃ (0.1 mg/l)	76.67 (61.14)	70.60 (57.17)	73.63	10.61	7.10	8.85	1.48	1.38	1.43
BAP (5 mg/l) + GA ₃ (0.1 mg/l)	73.33 (58.89)	76.64 (61.07)	75.0	10.27	6.66	8.46	1.53	1.52	1.52
BAP (5 mg/l)	79.67 (63.22)	83.59 (65.35)	81.63	10.08	6.29	8.18	1.58	1.66	1.62
Mean	72.60 (46.49)	74.75 (59.80)		10.40	7.39		1.604	1.65	
CD (P=0.05)									
Treatment			13.0			0.45			0.25
Genotype			NS			0.63			NS
T × G			14.66			0.76			0.28

mg/l) in 'Refulgens'. For the number of days required for bud sprouting, only 6.29 days were required when MS medium was supplemented with BAP (5 mg/l) only and maximum of 10.61 days were required in Refulgens when the MS medium was supplemented with BAP (5.0 mg/l) + NAA (0.01 mg/l) and GA₃ (0.1 mg/l).

The average micro-shoot length after 20 days of sub culturing showed significant variation due to different growth regulator treatments (Table 1). The longest micro-shoots (1.90 cm) were observed in the treatment where MS medium was supplemented by BAP (1.0 mg/l) + NAA (0.01 mg/l) + GA₃ (0.1 mg/l), while shortest micro-shoots (1.43 cm) were recorded in the treatment, where MS medium was supplemented by BAP (5.0 mg/l) along with NAA (0.01 mg/l) and GA₃ (0.1 mg/l). Similarly, maximum shoot length in bougainvillea cv. Mahara was recorded in MS medium supplemented with 1.5 mg/l GA₃. Gibberellins are known to induce stem elongation in number of crops due to rapid elongation of internodes, caused by both cell division and cell elongation (Krishnamoorthy 1981). The application of gibberellic acid into shoot elongation medium resulted in rapid growth due to the increased activity of the endogenous auxin in the presence of GA like substance (Michiewicz 1962). The shoot elongation stage is also considered as the preparatory stage for root induction. The present findings lend support from the previous work done by Datta and Mandal (2012) and Jain *et al.* (2016). The response of two cultivars in interaction with various growth regulators was found to be non-significant. The interaction effect between treatment x genotype was found to be significant. The longest (1.93 cm) micro-shoots were observed in Mahatma Gandhi in the treatment, where MS medium was supplemented with BAP (1.0 mg/l) + NAA (0.01 mg/l) + GA₃ (0.1 mg/l). The shortest micro-shoots (1.52 cm) were noted in 'Mahatma Gandhi' in the treatment, where MS medium supplemented with BAP (5.0 mg/l) + NAA (0.01 mg/l) + GA₃ (0.1 mg/l).

Data presented in Table 2 shows that after 15 days of

inoculation, the treatment, where MS medium was supplemented with BAP (5.0 mg/l) formed the highest number of micro-shoots/explant (1.22), and after 30 days MS medium was supplemented with BAP (5.0 mg/l) + GA₃ (0.1 mg/l) showed the higher number of micro-shoots (1.68), which was at par with MS medium was supplemented with BAP (5 mg/l). The two cultivars differed significantly for the number of micro-shoots formation/explant both at 15 and 30 days interval, Cultivar Refulgens proliferated more and produced more number of micro-shoots (1.20 and 1.68/ explant than Mahatma Gandhi (1.08 and 1.18/ explant). The interaction effect of the treatment × genotype was also found to be significant. Overall in case of Refulgens, after 15 days of culture where MS medium was supplemented with BAP (5 mg/l) and was found best (1.3) among the treatments tried (Fig 1). After 30 days the treatment where MS medium was supplemented with BAP (5.0 mg/l) + GA₃ (0.1 mg/l) formed the highest number (2.1) of micro-shoots/explant. Similarly, MS medium supplemented with BAP 5.00 mg/l+ NAA (1.0 mg/l) and GA₃ (0.5 mg/L) resulted in highest number of microshoots (Jain *et al.* 2016)

It is evident from the Table 3 that MS medium supplemented with BAP (4.0 mg/l) + kinetin (0.5 mg/l) showed the highest multiple shoot formation (4.11 and 2.30) after 15 and 30 days. The lowest shoot proliferation (2.81 and 1.57) was noticed (after 15 and 30 days) in the treatment where MS medium supplemented with BAP (5 mg/l) only. Amongst the two cultivars, Mahatma Gandhi showed higher shoot proliferation both after 15 and 30 days (1.97 and 3.30/ explant) in comparison to Refulgens (1.79 and 3.09/ explant). The interaction effect of the treatment × genotype on both 15 and 30 days of inoculation were found to be significant (Table 2). Among all treatment combinations, Mahatma Gandhi genotype where MS medium supplemented with BAP (4.0 mg/l) + kinetin (0.5 mg/l) was found to produce highest number of multiple shoots (2.33 and 4.75/explant). The minimum shoot proliferation

Table 2 Effect of BAP, NAA and GA₃ on micro-shoot proliferation in Bougainvillea.

Treatment	15 day		Mean	30 day		Mean	Refulgens	Mahatma Gandhi	30 day
	Refulgens	Mahatama Gandhi		Mahatama Gandhi	Refulgens				
BAP (1 mg/l) + NAA (0.01 mg/l) + GA ₃ (0.1 mg/l)	1.20	1.06	1.13	1.36	1.10	1.23	++	++	Too much callusing
BAP (3mg/l) + NAA (0.01 mg/l) + GA ₃ (0.1 mg/l)	1.19	1.06	1.12	1.54	1.096	1.31	++	++	-do-
BAP (5 mg/l) + NAA (0.01 mg/l) + GA ₃ (0.1 mg/l)	1.12	1.09	1.10	1.6	1.08	1.34	++	++	-do-
BAP (5 mg/l) + GA ₃ (0.1 mg/l)	1.22	1.04	1.13	2.10	1.27	1.68	+	+	Less callusing
BAP (5 mg/l)	1.30	1.14	1.22	1.80	1.36	1.58	+	+	-do-
Mean	1.20	1.08		1.68	1.18				
CD (P=0.05)									
Treatment			0.13			0.24			
Genotype			0.12			0.26			
T × G			0.18			0.37			

Table 3 Effect of BAP and kinetin on micro-shoot multiplication in bougainvillea

Treatment	15 day			30 day		
	Refulgens	Mahatama Gandhi	Mean	Refulgens	Mahatama Gandhi	Mean
BAP (1 mg/l) + kinetin (0.5 mg/l)	1.56	1.97	1.76	3.18	2.92	3.05
BAP (2 mg/l) + kinetin (0.5 mg/l)	2.08	1.93	2.00	2.78	2.96	2.87
BAP (3 mg/l) + kinetin (0.5 mg/l)	1.55	2.00	1.77	3.06	3.18	3.12
BAP (4 mg/l) + kinetin (0.5 mg/l)	2.28	2.33	2.30	3.46	4.75	4.11
BAP (5 mg/l)	1.54	1.61	1.57	2.95	2.66	2.81
Mean	1.79	1.97		3.09	3.30	
CD (P=0.05)						
Treatment			0.304			0.42
Genotype			NS			0.17
T × G			0.33			0.52

was observed in Refulgens cultured on MS medium supplemented with BAP (5 mg/l) after 15 days of treatment and Refulgens × BAP (2 mg/l) + kinetin (0.5 mg/l) after 30 days (1.54 and 2.78). Similarly, Poudel *et al.* (2005) reported that cytokinin supplementation was essential for culture initiation in grape cultivars. The superiority of BA, followed by kinetin over different cytokinins used, was reported by Rana *et al.* (2001) in *Dahlia variabilis*. The efficacy of BAP in stimulating shoot proliferation was already reported by Javed *et al.* (1996) in *B. spectabilis* and Syamal and Singh (1996) in rose. Shah *et al.* (2006), worked on mass propagation of *B. spectabilis* using shoot tip culture observed that culturing in MS medium supplemented with 0.25 mg/l BA + 0.1 mg/l NAA had resulted in the development of 90% of the shoots to plantlets.

To study the effect of growth regulators on elongation of micro-shoots, five treatments were attempted. The effect of these treatments was found to be significant in terms of micro-shoot elongation, but non-significant to the number of micro-shoots formed (Table 4).

The best treatment was the one where MS medium supplemented with 0.5 mg/l GA₃ where highest elongation (3.89 cm) was noted. Lowest micro-shoot elongation was observed in untreated. There was a significant difference in the response by the two cultivars for micro-shoot elongation.

Table 4 Effect of Gibberellic acid levels on micro-shoot elongation in bougainvillea.

GA ₃ (mg/l)	Shoot length (cm) after 20 days		
	Refulgens	Mahatama Gandhi	Mean
0.00	1.56	1.79	1.67
0.50	3.96	3.82	3.89
0.75	2.91	3.84	3.36
1.00	4.12	3.65	3.37
1.50	2.84	3.26	3.06
Mean	3.07	3.27	
CD (P=0.05)			
Treatment			0.54
Genotype			0.16
T × G			0.60

Among the two cultivars, Mahatma Gandhi had resulted in more micro-shoot elongation (3.27 cm) than Refulgens (3.07 cm). The interaction effect of the treatment × genotype was found to be significant. Irrespective of the treatment and genotype, the most responsive (4.12 cm) combination was that of Refulgens with 1.0 mg/l GA₃. The least (1.56) elongation was noted in Refulgens where basal MS medium was used. Singh *et al.* (2013) also reported that MS basal medium supplemented with GA₃ (0.5 mg/l) had marked influence on the shoot length (2.77 cm) after 20 days of transfer to elongation medium. The application of gibberellic acid into culture medium resulted in rapid elongation, which might be due to the increased activity of the endogenous auxin in the presence of GA₃. Gibberellic acid is involved in several important biochemical and morphogenetic responses, which includes promotion of elongation in axial organs, such as stems and flower pedicels, along with stimulation of root growth (Srivastava 2005).

It is clear from the data presented in Table 5 that maximum rooting (64.99%) was noted in the treatment where half-strength MS medium was supplemented with IBA (1.0 mg/l). Amongst the cultivars, there was significant response for rhizogenesis. Refulgens showed higher rooting (64.81%) compared to Mahatma Gandhi (35.03%). The interaction effect of the treatment × genotype was found to be significant. Irrespective of auxin levels and cultivars, the highest (94.44%) rooting was observed in Mahatma Gandhi in the treatment where MS medium was supplemented with IBA (1.0 mg/l). However, poorest rooting (10.56%) was observed in Refulgens in the treatment, where MS medium was supplemented with NAA (1.0 mg/l). Earliest (10.61 days) root initiation was observed in case of the treatment where MS medium was supplemented with 4.0 mg/l NAA, whereas medium supplemented with IBA (2.0 mg/l) took maximum number of days (23.77) to induce rooting. The rooting response of the two cultivars was found to be non-significant. Interaction data shows that irrespective of the auxin treatments and genotype combinations, earliest (9.35 days) rooting was observed in Mahatma Gandhi with MS medium fortified by NAA (4.0 mg/l) and delayed rooting (24.45 days) in Refulgens with IBA (1.0 mg/l).

Table 5 Effect of auxin(s) on *in vitro* rooting of bougainvillea micro-shoots

Treatment	Rooting (%)			No. of days required for root initiation		
	Refulgens	Mahatama Gandhi	Mean	Refulgens	Mahatama Gandhi	Mean
NAA (0.5 mg/l)	14.25 (22.14)	16.66 (24.04)	15.45	18.45	18.33	18.39
NAA (1.0 mg/l)	10.56 (19.00)	16.66 (24.06)	13.61	22.55	19.66	21.10
NAA (2.0 mg/l)	44.44 (41.78)	38.45 (38.29)	41.45	12.83	17.45	15.14
IBA (0.5 mg/l)	24.45 (29.60)	46.66 (41.90)	35.55	24.57	21.0	22.7
IBA (1.0 mg/l)	35.55 (36.57)	94.44 (76.31)	64.99	23.45	15.6	19.52
IBA (2.0 mg/l)	30.45 (33.45)	49.99 (44.94)	40.22	24.45	23.1	23.77
IBA (2 mg/l) + NAA (2 mg/l)	29.45 (32.83)	16.66 (24.06)	23.05	21.35	24.25	22.80
NAA (3.0 mg/l)	72.22 (58.18)	15.42 (23.11)	43.82	12.75	20.35	16.55
NAA (4.0 mg/l)	77.77 (61.82)	20.35 (26.78)	49.06	11.86	9.35	10.61
Mean	64.81	35.03		19.14	18.78	
CD (P=0.05)						
Treatment			8.56			7.32
Genotype			10.05			NS
T × G			13.65			14.18

Table 6 Effect of different hardening strategies on the survival of *in vitro* raised bougainvillea plantlets.

GA ₃ (mg/l)	Survival (%)			Shoot length (cm)		
	Refulgens	Mahatama Gandhi	Mean	Refulgens	Mahatama Gandhi	Mean
Glass jar with PP cap	45.50 (42.42)	85.50 (67.86)	65.5	4.25	5.50	4.87
Plastic pot (4.5')	30.75 (33.65)	62.50 (52.24)	46.62	3.55	4.25	3.90
Mean	38.12	74.00		3.90	4.90	
CD (P=0.05)						
Treatment			5.99			0.32
Genotype			12.00			0.37
T × G			17.10			0.82

It is evident from Table 6 that use of glass jar with polypropylene cap was found to be better, where the survival rate was 65.5% with mean shoot length was 4.87 cm. similarly, Singh *et al.* (2011) while comparing different hardening strategies, glass jar with polypropylene cap (PP) found to be most effective as far as hardening success (66.67%) was concerned in grape. The genotype effect was also significant. The plants of cv. Mahatma Gandhi showed higher survival (74.00%) with longer shoots (4.90 cm) in comparison to Refulgens plantlets.

From the present studies it is concluded that both the difficult-to-root cultivars (Mahatma Gandhi and Refulgens) rooted well under *in vitro* conditions. They can be successfully multiplied under *in vitro* conditions, with moderate multiplication rate.

ACKNOWLEDGEMENT

The first author is thankful to ICAR, New Delhi for providing the Junior research fellowship to undertake this study.

REFERENCES

Datta S K and Mandal A K A. 2012. Standardization of *in vitro* multiplication of two difficult-to-root bougainvillea cultivars

for commercial exploitation. *Science and Culture* **78**(5/6): 251–4.

Debergh P C and Read P E. 1991. Micropropagation. (*In*) *Micropropagation Technology and Application*, pp 1–13. Debergh P C and Zimmerman R H (Eds). Kluwer Academic Publishers, Boston-London. Method and

Ebrahimie E, Habashi A A, Mohammadi-Dehcheshmeh M, Ghannadha M R, Ghareyazie B and Yazdi-Amadi B. 2006. Direct shoot regeneration from mature embryo as a rapid and genotype-independent pathway in tissue culture of heterogeneous diverse sets of cumin (*Cuminum cyminum* L.) genotypes. *In Vitro Cellular and Developmental Biology - Plant* **42**(5): 455–60.

Jain Ritu, Janakiram T, Kishan Swaroop, Kumar Surendra and Kumawat G L. 2016. Standardization of protocol for *in vitro* multiplication of bougainvillea. *Indian Journal of Agricultural Sciences* **86**(4): 516–21

Javed M A, Hassan S and Nazir S. 1996. *In-vitro* propagation of *Bougainvillea spectabilis* through shoot apex culture. *Pakistan Journal of Botany* **28**(2): 207–11.

Krishnamoorthy H N. 1981. Gibberellins. (*In*) *Plant Growth Substances including Applications in Agriculture*, pp 60–79. Tata McGraw Hill Publishing Company, New Delhi.

Michiewie J M. 1962. Chromatographic analysis of growth regulators from winter wheat plants treated with gibberellins. *Acta Agrobot* **11**: 197–211.

- Nagarajan S M, Rajasekaran S, Kannan T M S and Sundaramoorthy P. 2006. Direct shoot regeneration from nodal explants of *Bougainvillea spectabilis* Willd. *Plant Archives* **6**(2): 537–9.
- Poudel P R, Kataoka I and Mochioka R. 2005. Effect of plant growth regulators on *in vitro* propagation of *Vitis ficifolia* var. gane bound its interspecific hybrid grape. *Asian Journal of Plant Science* **4**(5): 466–71.
- Rajan G B, Rani G J and Balakrishnamurthy G. 1998. Plant regeneration in bougainvillea cv. Shubra through direct and indirect adventitious shoot development. *South Indian Horticulture* **46**(3/6): 214–5.
- Rana P, Varghese T M, Yadav N R, Chowdhury V K and Chowdhury J B. 2001. (*In vitro*) studies in *Dahlia variabilis* Cav. *Annals of Agriculture Biology Research* **6**(1): 9–13.
- Shah S T, Zamir R, Muhammad T and Ali H. 2006. Mass propagation of *Bougainvillea spectabilis* through shoot tip culture. *Pakistan Journal of Botany* **38**(4): 953–9.
- Singh N V, Singh S K and Singh A K. 2011. Standardization of embryo rescue technique and bio-hardening of grape hybrids (*Vitis vinifera* L.) using arbuscular mycorrhizal fungi (AMF) under sub-tropical conditions. *Vitis* **50**(3): 115–8.
- Singh M, Singh K P, Prasad K V and Singh S K. 2013. Standardization of an efficient protocol for *in vitro* mass multiplication of hybrid tea rose cv. Raktima. *Indian Journal of Horticulture* **70**(3): 404–10.
- Srivastava L M. 2005. *Plant Growth and Development: Hormones and Environment*, pp 172–8. Academic Press, An imprint of Elsevier, San Diego, USA.
- Syamal M M and Singh S K. 1996. *In vitro* propagation of rose. *Horticultural Journal* **9**(1): 57–62.