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Standardization of protocol for *in vitro* multiplication of bougainvillea

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ABSTRACT

Bougainvillea is commonly propagated by hardwood cuttings but this method is tedious and time consuming. Moreover, there are certain varieties where the rooting percentage is very low. For easy, quick and mass multiplication of such cultivars, tissue culture technique can be put to use. Tissue culture has been proved to be useful for successful multiplication in case of number of vegetatively propagated shrubs. Present investigation was carried out in order to standardize a protocol for *in vitro* multiplication of bougainvillea cultivar Mahara through axillary bud induction of nodal explants. Shoot tips and nodal sections with axillary buds were excised, surface-sterilized and then cultured on MS medium supplemented with plant growth regulators. Pre-treatment agitation of explants in Carbendazim (0.2%) + Diathane M-45 (0.2%) + 8-HQC (200 mg/l) for 2 hr followed by quick dip in ethyl alcohol (70%; v/v) for 30 sec and surface sterilization with $HgCl_2$ (0.1%) for 5 min was found to be most effective in culture initiation with only 16.48% microbial contamination. For culture establishment, MS medium supplemented with BAP (5mg/L)+ NAA (0.5mg/l) was found to be the best with highest percentage of culture establishment (100%) and the fastest bud sprout (4.50 days). MS medium supplemented with BAP (5.0 mg/l)+ NAA (1.0mg/l) and GA_3 (0.5 mg/l) gave the highest shoot proliferation. The best treatment for micro-shoot elongation was MS medium was supplemented with 1.5 mg/l GA_3 which gave the highest elongation (2.34 cm). Highest *in vitro* rooting (70.52%) of micro-shoots was observed in the treatment where, half-strength MS medium was supplemented with IBA (1.0 mg/l)+ NAA (1.0 mg/l). Hardening of *in vitro* propagated plants of bougainvillea rooted plantlets was done for 21 days in glass jars filled with agro peat medium {A mixture of soilrite (1) + coco peat (1) + perlite (1)} supplemented with 1/2 strength liquid inorganic MS medium and covered with polypropylene lids. The hardened plantlets were successfully transferred to the glasshouse after a short period of *in vitro* acclimatization.

Key words: Axillary bud, Bougainvillea, Cultivar, Difficult-to-root, *In vitro* propagation, Multiplication

Bougainvillea is a beautiful, versatile plant belonging to the family Nyctaginaceae. It has varied uses including shrub, hedge, potted plant, bonsai, climber, etc. Its unique capacity of absorbing pollutants and tolerating environmental fluctuations to a great extent makes it desirable flowering plant in cities. Propagation of bougainvillea is a challenge as it does not produce seeds in most north Indian conditions and low rooting through cuttings and air layering. Therefore, tissue culture is the best method for the rapid propagation and multiplication of clones of desirable plants, which are otherwise difficult to multiply by conventional methods. Bougainvillea elite cultivars like Sweat Heart, Refulgens, Dr. H.B. Singh, Mahara, Shubhra, Mahatma Gandhi, Mary Palmer, Los Banos Beauty and Thimma can be multiplied *en mass* using tissue culture. *In vitro* mass multiplication of bougainvillea cultivars can be exploited as an important and advantageous technique with potential for production of uniform, true-to-type, disease-free planting material of elite cultivars. Mahara is a very prominent cultivar of bougainvillea having vigorous growth habit and excellent flowering with beautiful persistent magenta bracts. It is a very important plant for landscape purpose as it requires minimum maintenance. Keeping in view the above, the present research were undertaken with an objective to provide an easy, viable and reproducible protocol for *in vitro* multiplication of bougainvillea cultivar Mahara.

MATERIALS AND METHODS

The experiment was carried out at the Division of Floriculture and Landscaping and Central Tissue Culture Laboratory, LBS Centre, Indian Agricultural Research Institute, New Delhi from 2011-2014. For carrying out this study, explants were obtained from cv. Mahara, maintained at the 'International Bougainvillea Registration Authority' Repository at IARI, New Delhi. The bud wood having 4-5 matured axillary buds were selected from the middle portion

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of current season flowering shoots. The bud wood was excised during morning hours with secateurs. The leaves and thorns of explants were excised with the help of sterilized secateurs and small section of explants each containing a dormant bud was taken.

The explants were immediately put under running water for about half an hour and thereafter, washed in Teepol (0.1%) solution and rinsed 5-6 times with tap water. The explants were then pre-treated with different agitation treatments such as; (i) Carbendazim (0.2%) + 8-HQC (200 mg/l) for 2 hr, (ii) Carbendazim (0.2%) + Diathane M-45 (0.2%) + 8-HQC (200 mg/l) for 2 hr, (iii) Control (distilled water) for 2 hr on horizontal shaker at 200 rpm. These pre-treated explants were then surface sterilized under laminar flow with ethyl alcohol (70%, v/v) for 30 sec then with 0.1% mercuric chloride for 5 min followed by two-three washings with sterile autoclaved distilled water. The surface sterilized explants were cultured on MS medium supplemented with BAP (0.50, 0.25, 0.125 and 5.0 mg/l) along with NAA (0.50, 0.25 and 0.125 mg/l) in different combination.

For the induction of multiple shoots, sprouted buds were cultured onto MS medium (containing double strength of EDTA, organic components and 40 g/l sucrose) supplemented with different concentrations of BAP, NAA, and GA_3 to find out the best treatment combination for shoot proliferation. After initial shoot proliferation, the cultures were further sub-cultured by separating the individual microshoot and were transferred on media containing BAP (2.0, 2.5, 3.0, 3.5, 4.0 and 5 mg/l) along with GA_3 (0.25 and 0.50 mg/l) and NAA (0.1 and 1.0 mg/l) in different combinations.

The multiplied shoots were separated and individual micro shoots were transferred onto elongation media comprising of basal MS medium supplemented with concentrations of GA_3 (0.5, 1.0, 1.5 and 2.0 mg/l) and NAA(0.2 mg/l). A constant dose of sucrose, i.e. 40 g/l was used for culture establishment, microshoot proliferation and elongation.

For *in vitro* rhizogenesis, elongated micro-shoots were then excised individually and sub-cultured onto half-strength MS medium fortified with different concentrations of NAA (1.0 and 2.5 mg/l) and IBA (1.0 and 2.5 mg/l) individually or in combination supplemented with 60 g/l sucrose for rooting.

In vitro rooted plantlets were carefully removed from flasks, washed thoroughly with autoclaved distilled water to remove the agar sticking with roots. The rooted plantlets were then treated with carbendazim (0.1%) for 15 sec and thereafter, hardened in glass jars on agro peat medium {A mixture of soilrite + coco peat + perlite (1:1:1)} supplemented with 1/2 strength liquid inorganic plain MS medium devoid of calcium, sucrose and organic components. These glass jars were covered with polypropylene lids and were kept in culture room for 21 days before transferring to green house for further growth and development. For culture initiation, 30 explants were inoculated per treatment in three replications. The cultures were maintained at 25 ± 1°C under fluorescent white light (47 Imol/m^2/S) at a photoperiod of 16/8 hr light and dark cycles.

The data was analyzed employing Factorial Completely Randomized Block Design (FCRD) and the percent data was subjected to Arc Sin transformation before carrying out ANOVA by using OPSTAT statistical software.

RESULTS AND DISCUSSION

Explant sterilization

It is evident from Table 1 that pre-treatment of explants with different fungicidal and bactericidal treatments had significant effect on explant survival, microbial contamination, bud sprouting and days to bud sprouting. The treatment comprising of carbendazim (0.2%) + diathane M-45 (0.2%) + 8-hydroxy quinoline citrate (200 mg/l) for 2 hr gave the highest explant survival (74.16%) and bud sprouting (70.09%),whereas, the minimum explant survival (17.25%) and bud sprouting (36.79%) was recorded in control. The pre-treatment of explants with different chemicals significantly reduced the microbial contamination and improved the survival and showed early bud sprouting as compared to control (distilled water). The pre-treatment of axillary bud explants of cultivar Mahara with carbendazim (0.2%) + diathane M-45 (0.2%) + 8-hydroxy quinnoline citrate (200 mg/l) for 2 hr resulted in minimum microbial contamination (16.48%) as compared to control (42.50%), treatment T_2 (carbendazim (0.2%) + diathane M-45 (0.2%) + 8-hydroxy quinnoline citrate (200 mg/l) for 2hr) also had a marked influence on days to bud sprouting resulting in earliest bud sprouting (7.05 days). Pre-treatments are known to improve *in vitro* culture initiation. Since, the fungicides used in the treatment were both systemic and contact types, thus, they gave efficient control over microbial

Table 1 Effect of different pre-treatments on *in vitro* culture initiation in Bougainvillea cv. Mahara.

Treatment	Explant survival (%)	Bud sprouting (%)	Microbial contamination (%)	Days to bud sprouting
Carbendazim (0.2%) + 8-HQC (200 mg/L) (T_1)	52.75 (46.56)	45.64 (42.48)	34.31 (35.84)	7.98
Carbendazim (0.2%) + Diathane M-45 (0.2%) + 8-HQC (200 mg/L) (T_2)	74.16 (59.49)	70.09 (56.83)	16.48 (23.94)	7.05
Control (distilled water) (T_3)	17.25 (24.53)	36.79 (37.31)	42.50 (40.67)	9.24
Mean	48.05 (43.53)	50.84 (45.54)	31.10 (33.48)	8.09
CD (P=0.05)	6.33 (4.06)	5.45 (3.24)	1.98 (1.31)	1.02

The values given in parentheses denote the Arc sin value of per cent data.

contamination. Similarly, 8-HQC was effective due to its bactericidal activities. Efficacy of these compounds has earlier been reported by Machado *et al.* (1991), Bharadwaj *et al.* (2006), Singh *et al.* (2013) and Kumari *et al.* (2013) in rose.

Culture establishment

Data presented in Table 2 indicates that MS medium supplemented with different concentrations of growth regulators resulted in maximum per cent culture establishment, number of microshoots/explants, minimum days required to shoot emergence and minimum per cent infection. 100% aseptic culture establishment was observed in all the treatment combinations except control (54.00%) and no infection was observed in treatments T_2, T_3, T_4, T_7, T_9 and T_{10}. Maximum infected cultures (33.31%) were observed in treatment T_8, i.e. MS media + BAP 0.25 mg/l + 0.125mg/l NAA and was at par with control (T_{11}).

The data presented in Table 2 shows that average number of microshoots per plants were 3.55, however, maximum average number of microshoots/explants (5.00) were obtained with T_{10}, i.e. MS+BAP 5 mg/l + NAA 0.5mg/l and it was statistically at par with T_2, T_4, T_6, T_7 and T_9 whereas, minimum number of microshoots (2.00) were observed with control (T_{11}) and was at par with T_1, T_3, T_5 and T_8. Minimum number of days for shoot emergence (4.50 days) were taken by T_{10}, i.e. MS + BAP 5 mg/l + NAA 0.5 mg/l and it was at par with T_1 and T_3, whereas, maximum time for shoot emergence (12.00 days) was taken by control (T_{11}). The better results obtained might be due to the role of most effective growth regulator, i.e. BAP in stimulating shoot proliferation, similar results were reported by Vijaya *et al.* (1991) and Peng *et al.* (2010). Furthermore, they also suggested the role of auxin in effective culture initiation and found NAA to be more effective than IAA or IBA in shoot multiplication. BAP have been proved to be more effective than other cytokinins in inducing the shoot buds in gerbera and other floriculture crops as earlier reported by Parathasarathy and Nagaraju (2001) and Kumar and Pratheesh (2004). Earlier Douglas *et al.* (1989); Arnold *et al.* (1992); Chu *et al.* (1993) and Habib *et al.* (1996) also suggested the efficacy of cytokinins in combination with an auxin or GA_3 in obtaining good culture initiation.

Table 2 Effect of plant growth regulators on *in vitro* aseptic culture establishment in Bougainvillea cv. Mahara

Treatment	Per cent aseptic cultures established	Per cent infected cultures	Number of micro-shoot/ explants	Number of days for shoot emergence
MS + BAP 0.5 mg/l+ NAA 0.5 mg/l (T_1)	100.00 (90.00)	7.15 (15.53)	3.00	5.00
MS + BAP 0.25 mg/l+ NAA 0.5 mg/l (T_2)	100.00 (90.00)	0.00 (0.00)	4.00	9.00
MS + BAP 0.125 mg/ L+NAA 0.5 mg/l (T_3)	100.00 (90.00)	0.00 (0.00)	3.00	5.00
MS + BAP 0.5 mg/l+ NAA 0.25 mg/l (T_4)	100.00 (90.00)	0.00 (0.00)	4.00	9.00
MS + BAP 0.25 mg/l+ NAA 0.25 mg/l (T_5)	100.00 (90.00)	16.67 (19.78)	3.00	8.00
MS + BAP 0.125 mg/l+ NAA 0.25 mg/l (T_6)	100.00 (90.00)	16.67 (24.09)	4.00	8.00
MS + BAP 0.5 mg/l+ NAA 0.125 mg/l (T_7)	100.00 (90.00)	0.00 (0.00)	4.00	9.00
MS + BAP 0.25 mg/l+ NAA 0.125 mg/l (T_8)	100.00 (90.00)	33.31 (34.90)	3.00	9.00
MS + BAP 0.125 mg/l+ NAA 0.125 mg/l (T_9)	100.00 (90.00)	0.00 (0.00)	4.00	9.00
MS + BAP 5 mg/l+ NAA 0.5 mg/l (T_{10})	100.00 (90.00)	0.00 (0.00)	5.00	4.50
MS media (Control) (T_{11})	54.00 (47.29)	30.15 (33.09)	2.00	12.00
Mean	95.82 (86.11)	9.45 (11.58)	3.55	7.95
CD (P=0.05)	2.14	11.25	1.05	1.54

Shoot proliferation

Data presented in Table 3 depicts marked influence of cytokinins along with auxins and gibberellic acid on multiplication of shoots. The maximum initial number of shoot sprouted per explant (6.87) were induced with treatment T_7 (MS + 5.0 mg/l BAP + 1.0 mg/l NAA + 0.5 mg/l GA_3) followed by T_6 (MS + 5.0 mg/l BAP + 0.1 mg/l NAA + 0.5 mg/l GA_3), whereas the lowest number of initial shoots sprouted per explant (2.00) were recorded under treatments, i.e. MS + BAP (2.0 mg/l) + NAA (0.1 mg/l) + GA_3 (0.25 mg/l) and MS + BAP (2.5 mg/l) + NAA (0.1 mg/l) + GA_3 (0.25 mg/l). After first sub-culture cultivar Mahara gave maximum shoots per explant (16.25) under the treatment T_7, i.e. MS + BAP (5.0 mg/l) + NAA (1.0 mg/l) + GA_3 (0.5 mg/l) followed by MS + BAP (5.0 mg/l) + NAA (0.1 mg/l) + GA_3 (0.5 mg/l) with 10.43 shoots per explant.The least number of shoots per explant (4.00) were recorded in T_1, i.e. MS + BAP (2.0 mg/l) + NAA (0.1 mg/l) + GA_3 (0.25 mg/l). Marked differnce was also noticed in number of shoots sprouted per explant after second sub-culture. Maximum number of shoot (26.50) was obtained with treatment MS + BAP (5.0 mg/l) + NAA (1.0 mg/l) + GA_3 (0.5 mg/l). Finally after third sub-culture, the maximum number of shoots per explant (39.63) were also recorded under the treatment T_7, whereas the least number of shoots per explant (9.5) were observed with treatment MS + BAP (2.0 mg/l) + NAA (0.1 mg/l) + GA_3 (0.25 mg/l). Growth regulators at an optimum dose lead to maximum shoot proliferation after each sub-culture. To obtain good shoot proliferation, an optimum balance is essential between cytokinin and auxin. The favourable influence of BAP and NAA may be related to the fact that BAP influences many metabolic processes (Kulaeva 1980) and NAA is known to effect plant metabolism (Church and Galston, 1988 and Vozquez *et al.* 1989).The better results regarding shoot proliferation in tissue cultures might be due to the role of optimum BAP dose, which enhanced axillary branching and

Table 3 Effect of plant growth regulators on microshoots proliferation in Bougainvillea cv. Mahara

Treatment	Average number of microshoots/explant/subculture				General plantlet growth
	First	Second	Third	Fourth	
MS + BAP 2.0 mg/l + NAA 0.1 mg/l + GA_3 0.25 mg/l (T_1)	2.00	4.00	7.00	9.50	Poor
MS + BAP 2.50 mg/l + NAA 0.1 mg/l + GA_3 0.25 mg/l (T_2)	2.00	5.50	8.50	10.00	Average
MS + BAP 3.00 mg/l + NAA 0.1 mg/l + GA_3 0.25 mg/l (T_3)	2.50	6.00	10.25	10.80	Good
MS + BAP 3.50 mg/l + NAA 0.1 mg/l + GA_3 0.25 mg/l (T_4)	3.00	8.00	12.50	15.50	Very good
MS + BAP 4.00 mg/l + NAA 0.1 mg/l + GA_3 0.25 mg/l (T_5)	2.75	7.50	10.80	13.50	Good
MS + BAP 5.00 mg/l + NAA 0.1 mg/l + GA_3 0. 5 mg/l (T_6)	6.71	10.43	16.57	24.57	Very good
MS + BAP 5.00 mg/l + NAA 1.0 mg/l + GA_3 0. 5 mg/l (T_7)	6.87	16.25	26.50	39.63	Good
Ms Media (Control) (T_8)	3.17	7.00	7.67	11.0	Poor
Mean	3.63	8.09	12.47	16.81	
CD (P=0.05)	0.74	1.34	1.34	1.29	

MS basal salts with double strength of Fe EDTA and organic components and sucrose @ 40 g/l.

multiple shoot formation. Superiority of BAP in shoot multiplication has earlier been shown by Scotti and Pias (1990). It is opined that multiple shoots arise as the result of axillary bud proliferation owing to the loss in apical dominance (Douglas *et al.* 1989, Singh and Syamal 2001, Verma *et al.* 2012).

Data on the effect of gibberellic acid on shoot length and numbers of shoot of bougainvillea cultivar Mahara were presented in Table 4. It is evident that maximum average shoot length (2.34 cm) was obtained with treatment T_3, i.e. MS + GA_3 (1.5 mg/l). After 15 days of transfer on elongation medium, the maximum shoot length (1.48 cm) was recorded with the same treatment, i.e. MS + GA_3 (1.5 mg/l) and minimum shoot length (0.75 cm) with control, i.e. MS devoid of hormones. After 30 days, 45 days and 60 days of transfer, the highest shoot length 1.86, 2.69 and 3.32 cm, respectively, was observed under the treatment MS + GA_3 (1.5 mg/l) followed by 1.52, 1.80 and 2.85 cm with MS + GA_3 (1.0 mg/l). Maximum number of shoots recorded highest (2.55) with MS + GA_3 (1.0 mg/l) followed by 2.40 under MS + GA_3 (2.0 mg/l) + NAA (0.2 mg/l). Gibberellins are known to induce stem elongation in number of crops. The stem elongation is not due to increased formation of nodes and internodes but results from rapid elongation of internodes, which is due to both cell division and cell elongation (Krishnamoorthy 1981). The application of gibbrellic 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 (Michiewiez, 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 Choudhary (1991) and Singh *et al.* (2013).

Table 4 Effect of gibberellic acid (GA_3) on microshoot elongation and number of shoots in Bougainvillea cv. Mahara

Treatments	Shoot length (cm) after duration of				No. of shoots
	15 days	30 days	45 days	60 days	
MS media + 0.5 mg/l GA_3	0.87	1.15	1.97	2.85	1.97
MS media + 1.0 mg/l GA_3	1.32	1.52	1.80	2.85	2.55
MS media + 1.5 mg/l GA_3	1.48	1.86	2.69	3.32	2.31
MS media + 2.0 mg/l GA_3 + 0.2 mg/l NAA	1.35	1.75	2.10	2.17	2.40
MS media (Control)	0.75	1.75	1.47	2.35	0.80
Mean	1.15	1.61	2.01	2.71	2.01
CD (P=0.05)					
Media (A)		0.11			0.24
Duration (B)		0.10			
Media × Duration (A×B)		0.22			

Rhizogenesis

Data of rhizogene is presented in Table 5, depicts the significant effect of ½ MS and different concentrations of auxins on days to root initiation, rooting percentage, number of roots per micro-shoot and root length. The earliest root initiation (16.20 days) was observed in ½ MS + NAA 2.5 mg/l as compared to control (25.15 days). The highest percentage rooting (70.52%) was observed in ½ MS + NAA (1.0 mg/l) + IBA (1.0 mg/l) and lowest (27.86%) in control. The maximum numbers of roots per micro-shoot (7.33) were induced by T_5 (basal medium of ½ MS + NAA (1.0 mg/l) + IBA(1.0 mg/l) followed by 6.89 roots per micro-shoot on basal medium of ½ MS + NAA (1.0 mg/l). Number of roots per shoot induced on all basal media used was significantly higher than that on control (2.85). The longest roots (6.16 cm) were induced with ½ MS + NAA (1.0 mg/l) + IBA (1.0 mg/l) compared to control (2.96 cm). Visual observations of the cultures growing on different media indicated that the T_5 treatment, i.e. ½ MS + NAA (1.0 mg/l) + IBA (1.0 mg/l) produced very long and thin roots and was proved to be the best medium for success during hardening of the plantlets. Datta and Mandal (2012) reported

Table 5 Effect of auxins on *in vitro* rooting of micro-shoots in Bougainvillea cv. Mahara

Treatments	Days to root initiation	Rooting (%)	Number of roots/ microshoot	Root length (cm)
½ MS + NAA (1.0 mg/l) (T_1)	17.56	68.35 (55.76)	6.89	3.79
½ MS + NAA (2.5 mg/l) (T_2)	16.20	66.79 (54.81)	6.14	4.37
½ MS + IBA (1.0 mg/l) (T_3)	22.40	53.16 (46.79)	5.26	4.73
½ MS + IBA (2.5 mg/l) (T_4)	25.08	47.99 (43.83)	4.98	5.10
½ MS + NAA (1.0 mg/l) + IBA (1.0 mg/l) (T_5)	19.65	70.52 (57.10)	7.33	6.16
½ MS + NAA (2.5 mg/l) + IBA (2.5 mg/l) (T_6)	26.15	64.27 (53.27)	6.25	5.38
½ MS (control) (T_7)	25.15	27.86 (31.84)	2.85	2.96
Mean	21.74	56.99 (49.06)	5.67	4.64
CD (P=0.05)	1.75	2.26	0.79	0.88

The values given in parentheses denote the Arc sin value of percent data.

that neither NAA nor BAP alone but lower BAP concentration in combination with all tested concentrations of NAA was effective. Similarly, growth of cultures in terms of leaf appearance, greenness, leaf retention and growth of plantlets, etc. was also observed to be in good condition on the same medium. The qualitative data suggest that roots were not only few but had stunted growth in medium devoid of auxins. However, supplementing the medium with NAA alone resulted in small, stunted and thick roots while those cultured on medium supplemented with IBA had thin and long roots. Interestingly, half strength medium supplemented with the dual auxin were of medium length and thin. The successful root induction on elongated shoots might be due to the strength of basal medium salts, sucrose level and auxin supplements. Furthermore, addition of auxins in combination to the culture medium also resulted in early and better quality roots. It was evident that there was a synergistic effect, when the two auxins were employed together. Optimum role of two auxins has earlier been reported by Singh and Syamal (2001), Jang *et al.* (2003), Bharadwaj *et al.* (2006) and Singh *et al.* (2013).

Plantlet acclimatization

The rooted plantlets were acclimatized in glass jars filled with agropeat medium moistened with 1/2 strength liquid inorganic MS medium devoid of calcium, growth regulators, sucrose and organic components and covered with polypropylene lids had better survival and general growth. Efficacy of glass jar covered with polypropylene lids was also reported earlier by Bala *et al.* (2010) and Kumari *et al.* (2013). After complete hardening for 21 days, the plants were transferred to glass house and established well under glass house conditions. The result of present investigation demonstrates a reproducible and efficient regeneration protocol of *in vitro* multiplication of bougainvillea cultivar Mahara.

It is concluded from the present study that the bougainvillea cv. Mahara can be mass multiplied easily by following the protocol standardized. Firstly, pretreat the explants (axillary bud) with carbendazim (0.2%) + diathane M-45 (0.2%) + 8-hydroxy quinoline citrate (200 mg/l) for 2 hr on 200 rpm and culture them on MS media supplemented with BAP 5 mg/l + NAA 0.5 mg/l; followed by *microshoot proliferation* on MS + BAP (5.0 mg/l) + NAA (1.0 mg/l) + GA_3 (0.5 mg/l) and shoot elongation by adding 1.5 mg/l GA_3 to MS media. For rooting culture the elongated plants on ½ MS + NAA (1.0 mg/l) + IBA(1.0 mg/l) and acclimatized the rooted plantlets for 21 days in glass jars (with polypropylene lids) filled with agropeat and moistened with MS medium devoid of sucrose, growth regulators and organics.

REFERENCES

Arnold N P, Binns M R, Barthakur N N and Cloutier D C. 1992. A study on the effect of growth regulators and time of plantlet harvest on the *in vitro* multiplication rate of hardy and hybrid tea roses. *Journal of Horticulture Science* **67**(6): 727–35.

Bala M, Singh K P and Prasad K V. 2010. Standardization of in vitro mass multiplication protocol for hybrid tea rose cv. Pusa Mohit. *Indian Journal of Horticulture* **67**: 225–9.

Bharadwaj R, Singh S K, Pal S and Kumar S. 2006. An improved protocol for micropropagtion of miniature rose (*Rosa chinensis* Jacq.var.*minima*) cultivars. *Journal of Ornamental Horticulture* **9**(4): 238–42.

Choudhary M L. 1991. Effect of medium components and explant position on *in vitro* shoot proliferation of Indian rose. *National Academy Science Letters* **14**(11): 439–41.

Chu C Y, Knight S L and Smith M A L. 1993. Effect of liquid culture on the growth and development of miniature rose (*Rosa chinensis*). *Plant Cell Tissue and Organ Culture* **32**: 329–34.

Church D L and Galston A W. 1988. 4-Coumarate:Coenzyme A ligase and isoperoxidase expression in Zinnia mesophyll cells induced to differentiate into tracheary elements. *Plant Physiology* **88**: 679–84.

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.

Douglas G C, Rutledge C B, Casey A D and Richardson D H S. 1989. Micropropagation of floribunda, ground cover and miniature roses. *Plant Cell Tissue and Organ Culture* **19**: 55–64.

Habib A, Jahan M A A and Hossain M Y. 1996. *In vitro* regeration and rapid clonal propagation in eight elite "Hybrid Tea" Roses. *Plant Cell Tissue and Organ Culture 3***6**: 83–88.

Jang G W, Kim K S and Park R D. 2013. Micropropagation of Venus fly trap by shoot culture. *Plant Cell Tissue and Organ Culture* **72**(1): 95–8.

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

Kulaeva O N. 1980. Cytokinins action on enzyme activity in plants. (*In*) *Plant growth substances*. Skoog, F (Ed). Proceedings of the 10th International Conference on Plant Growth substanced, Modison, Wisconsin, July 1979, Springes-Verlag, New York, pp 119–28.

Kumar M A and Pratheesh P T. 2004. *In vitro* shoot multiplication in *Rosa indica* L. *Plant Cell, Biotechnology and Molecular Biology* **5**(1/2): 73–6.

Kumari S, Singh K P, Janakiram T and Raju D V S. 2013. Standardization of *in vitro* mass multiplication protocol for hybrid tea rose cv. Grand Gala. *Indian Journal of Horticulture* **1:** 91–3.

Machado M L C, Machado A C, Hanzer V, Kalthoff B, Weib H, Mattanovich D, Regner F and Katinger H. 1991. A new efficient method using 8-hydroxy quinolinol sulphate for the initiation and establishment of tissue cultures of apples from adult materials. *Plant Cell Tissue and Organ Culture* **27**: 155–60.

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

Murashige T and Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15**: 473–97.

Parathasarathy V A and Nagaraju V. 2001. Morphogenetic response of gerbera shoots to benzyl amino purine. *Annals of Plant Physiol*ogy **9**(1): 10–2.

Peng C, Ru L, Rui Yang and Yang Z. 2010. Direct shoot organogenesis and plant regeneration from cotyledonary node of kenaf (*Hibiscus cannabinus* L.). *African Journal of Biotechnology* **9**(50): 8 693–7.

Scotti C P and Pias M S. 1990. Mass propagation of the dwarf rose cultivar Rosamini. *Scientia Horticulturae* **43**: 321–30.

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.

Singh S K and Syamal M M. 2001. A short pre-culture soak in thidiazuron or forchlorfenuron improves axillary shoot proliferation in rose micropropagation. *Scientia Horticulturae* **91**: 169–77.

Verma A K, Prasad K V, Janakiram T and Kumar S. 2012. Standardization of protocol for pre-treatment, surface sterilization, regeneration, elongation and acclimatization of C*hrysanthemum morifolium* Ramat. *International Journal of Hort*iculture **2**: 7–12.

Vijaya N, Satyanarayana G, Prakash J and Pierik R L M. 1991. Effect of culture media and growth regulators on *in vitro* propagation of rose. *Curr. Plant Sci. Biotech. Agric.* **12**: 209–14.

Vozquez F F,Quiroz J, Scores K N and Loyola V V M. 1989. Effct of the auxin cytokinin ratio on the enzymes of nitrogen metabolism in *Canavalia ensiformis* L. *Tissue cultures. Journal of Plant Physiology* **135**: 57–62.