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Standardization of *in vitro* mass multiplication protocol for hybrid tea rose cv. Pusa Mohit

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ABSTRACT

An efficient protocol for *in vitro* multiplication of hybrid tea rose cv. Pusa Mohit was developed using axillary bud explant. Out of different pre-treatments of explants with fungicides and bactericides tried, the highest explant survival (62.47%) was obtained with carbendazim (0.2%) + diathane M-45 (0.2%) + 8-HQC (200 mg/l) for 3 h on a horizontal shaker (120 rpm). The effect of different combinations of BAP (2.5, 3.0 and 3.5 mg/ l), NAA (0.1 and 0.2 mg/l), and GA_3 (0.3 and 0.5 mg/l) on culture establishment and shoot proliferation was studied. MS medium supplemented with 3.5 mg /l BAP + 0.1 mg/l NAA + 0.5 mg/l GA_3 was found most effective for culture establishment and shoot proliferation with highest number of micro-shoots (5.30 shoots per explant). Rooting on micro-shoots was induced on half-strength MS basal medium supplemented with NAA (0.5 mg/l) + IBA (0.5 mg/l). The regenerated plantlets were efficiently hardened in glass jars filled with vermiculite + agropeat (1:2) moistened with one-quarter strength MS medium salts and covered with polypropylene lids. After hardening the plants were successfully transferred to the glasshouse with good survival.

Key words: Rose, micropropagation, *in vitro* regeneration.

INTRODUCTION

Rose (*Rosa hybrida* L.) is a favourite plant cultivated for its beautiful flowers and is universally claimed as the "Queen of Flowers". It is important not only for its outstanding aesthetic beauty but also for its potential marketing as cut flowers and potted plants to many countries of the world. As cut flower, it occupies top position in acreage, production and consumption. Roses are generally multiplied vegetatively by grafting and budding that are very slow and time consuming methods. Moreover, diseases and environmental hazards make the cultivar degenerate gradually. Micropropagation techniques allow producing roses with higher quality under a virus indexing programme, attending in this way the market demand. Keeping this in view, the present investigation was carried out to establish an efficient and reproducible protocol for rapid and large scale propagation of rose.

MATERIALS AND METHODS

The present study was carried out at the Central Tissue Culture Laboratory, L.B.S. Centre, IARI, New Delhi during 2005-2008. Rose cultivar Pusa Mohit maintained at Research Farm of Division of Floriculture and Landscaping, IARI, New Delhi was used for this experiment. The budsticks having 3 to 4 matured axillary buds were selected from the middle portion of current season flowering shoots. With secateurs they were excised during morning hours and cut into individual axillary buds (≥1.5 cm). The explants were washed with Teepol (0.1%) solution for 5 min. followed by washing under running tap water for 15 min. The nodal segments were then treated with different pre-treatments such as: (i) bavistin (carbendazim) (0.2%) + 8-HQC (200 mg/l), (ii) bavistin (0.2%) + diathane m-45 indofil (0.2%) + 8-HQC (200 mg/l) along with control (distilled water) for 3 h on horizontal shaker (120 rpm). The pre-treated explants were then surface sterilized with 0.1% mercuric chloride for 5 min. followed by two-three rinsing with autoclaved distilled water. The surface sterilized explants were cultured on MS medium supplemented with different concentrations of BAP (2.5, 3.0 and 3.5 mg/l), NAA (0.1 and 0.2 mg/l), and GA_3 (0.3 and 0.5 mg/l) to find out the best treatment combination for culture establishment. The sprouted shoots were then sub-cultured onto MS medium supplemented with different concentrations of BAP (2.5, 3.0 and 3.5 mg/l), NAA (0.1 and 0.2 mg/l), and GA_3 (0.3 and 0.5 mg/ l) to find out the best treatment combination for shoot proliferation. The multiplied shoots on proliferation media were separated and individual micro-shoots were transferred onto elongation media comprising basal MS medium with various concentrations of GA_3 (0.5, 1.0 and 1.5 mg/l) to standardize its optimum dose for micro-shoots elongation. Elongated shoots were then transferred individually in cultured vessels containing full- and half-strength of MS medium fortified with different concentrations of auxins like NAA and IBA individually or in combination for rooting. A constant dose of sucrose, *i.e.* 40 g/l was used in all the combinations.

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The *in vitro* rooted plantlets were removed from flasks, washed thoroughly with autoclaved distilled water to remove the sticking agar-agar to roots. The roots were then dipped in carbebdazim (0.1%) for few 10 sec. The plantlets were then acclimatized using two different strategies like: (i) plastic pots (4") filled with vermiculite + agropeat (1:2) moistened with ¼th strength of MS medium salts (macro + micro) and covered with polythene bags, (ii) glass jars filled above potting mixture and covered with polypropylene lids. The plantlets were kept in culture room (15 days) before transferring to greenhouse. For cultured initiation, 20-25 explants were inoculated per treatment in three replications. The cultures were maintained at 25±1°C under fluorescent white light (47 µmol $m^{-2}s^{-1}$) at a photoperiod of 16/8 h light and dark cycles. The data was analyzed employing completely randomized design (CRD) and the percent data was subjected to Arc Sin √% transformation before ANOVA.

RESULTS AND DISCUSSION

As evident from the data presented in Table 1, pre-treatment of explants with different fungicidal and bactericidal treatments had significant effect on survival of explant, microbial contamination, bud sprouting and days to bud sprouting. The treatment comprising carbendazim (0.2%) + diathane M-45 Indofil® (0.2%) + 8-hydroxy quinnoline citrate (200 mg/l) for 3 h agitation gave the highest explant survival (62.47%) and bud sprouting (56.63%), which were significantly superior compared to the other treatments. Whereas, the minimum explant survival and bud sprouting were recorded with distilled water control. The pre-treatment of axillary bud explants with bavistin (0.2%) + indofil (0.2%) + 8-HQC (200 mg/l) for 3 h minimized microbial contamination (22.36%) as compared to control (46.33%). This pre-treatment also gave the earliest bud sprouting (10.79 days) as compared to control (12.44 days). It is obvious that the fungicides used had both systemic and contact fungicides, thus gave efficient control of microbial infection. Similarly, 8-HQC was effective due to its bactericidal activities. Efficacy of these compounds has earlier been demonstrated by Machado *et al.* (8), Prasad (9), and Bharadwaj *et al.* (2) in rose.

The maximum survival of explant (75.07%) and bud sprouting (70.63%) was recorded for the treatment MS + BAP (3.5 mg /l) + NAA (0.1 mg /l) + GA_3 (0.5 mg /l) (Table 2). The minimum response was noted with hormone-free MS medium. The above treatment also gave the earliest (10.80 days) bud sprouting when comparing with other treatments, which was maximum delayed (15.77) in control. The efficacy of BAP in stimulating shoot proliferation has earlier been reported by Vijaya *et al.* (12), and Kumar and Pratheesh (7). Earlier, Douglas *et al.* (5) and Arnold *et al.* (1) reported the efficacy of cytokinins in combination with an auxin or together with GA_3.

The maximum number of shoot sprouted on initial explant (1.96 per explant) was noted with treatment MS + BAP (3.5 mg /l) + NAA (0.1 mg /l) + GA_3 (0.5 mg /l). After first sub-culturing maximum shoots per explant (3.56) were recorded was noted with the same treatment. The least number of shoots per explant (1.56) were recorded under control, *i.e.* MS without any hormone. Shoot multiplication rate estimated revealed that maximum number of shoots per explant (5.30) were also recorded in the treatment, *i.e.* MS + BAP (3.5 mg /l) + NAA (0.1 mg /l) + GA_3 (0.5 mg /l) compared to least (2.16) in control. Growth regulators at an optimum dose lead to good shoot proliferation and the same is observed in each sub-culture. The favourable influence of BAP in different metabolic processes (Kulaeva, 6) and NAA is known to effect plant metabolism. The better results regarding shoot proliferation in tissue culture might be due to the role of optimum dose of BAP which enhances axillary

Table 1. Effect of different pre-treatments on *in vitro* culture initiation in hybrid tea rose.

Pre-treatment	Explant survival(%)	Bud sprouting (%)	Microbial contamination (%)	Days to bud sprouting
Control (distilled water) - 3 h	11.90 (20.19)*	25.33 (30.24)*	46.33 (42.92)	12.44
Bavistin (0.2%) + 8-HQC (200 ppm) - 3 h	47.07 (43.34)	37.40 (37.72)	32.43 (34.73)	12.13
Bavistin (0.2%) + Indofil (0.2%) + 8-HQC (200 mg/l) - 3 h	62.47 (52.25)	56.63 (48.84)	22.36 (28.23)	10.79
CD at 5%	1.59	1.52	1.42	0.90

*The values given in parentheses denote the Arc Sin √% value.

Table 2. Effect of growth regulators on *in vitro* culture establishment in hybrid tea rose.

Treatment (mg/l)	Explant survival (%)	Bud sprouting (%)	Days to bud sprouting
MS devoid of hormones (Control)	34.03 (35.67)*	30.70 (33.65)*	15.77
MS + BAP (2.5) + NAA (0.1) + GA_3 (0.3)	55.83 (48.33)	53.20 (46.86)	12.07
MS + BAP (2.5) + NAA (0.1) + GA_3 (0.5)	63.27 (52.65)	55.27 (48.05)	12.30
M S + BAP (3.0) + NAA (0.1) + GA_3 (0.3)	60.97 (51.30)	56.83 (48.97)	12.50
MS + BAP (3.0) + NAA (0.1) + GA_3 (0.5)	64.93 (53.67)	60.57 (51.15)	11.73
MS + BAP (3.5) + NAA (0.1) + GA_3 (0.3)	68.23 (55.67)	64.57 (53.50)	11.70
MS + BAP (3.5) + NAA (0.1) + GA_3 (0.5)	75.07 (60.00)	70.63 (57.22)	10.80
M S + BAP (2.5) + NAA (0.2) + GA_3 (0.3)	59.57 (50.48)	54.60 (47.66)	12.40
MS + BAP (2.5) + NAA (0.2) + GA_3 (0.5)	58.13 (49.66)	54.43 (47.57)	12.37
MS + BAP (3.0) + NAA (0.2l) + GA_3 (0.3)	60.97 (51.30)	56.63 (48.84)	12.63
M S + BAP (3.0) + NAA (0.2) + GA_3 (0.5)	53.17 (46.78)	47.17 (43.40)	13.27
MS + BAP (3.5) + NAA (0.2) + GA_3 (0.3)	47.23 (43.39)	43.73 (41.41)	12.37
MS + BAP (3.5) + NAA (0.2) + GA_3 (0.5)	46.80 (43.17)	43.00 (40.99)	12.70
CD at 5%	4.78	4.65	0.79

*The values given in parentheses denote the Arc Sin √% values.

Table 3. Effect of growth regulators on *in vitro* shoot multiplication in hybrid tea rose.

Treatment	No. of shoots proliferated/explant			
	Initial culture	First sub-culture	Second sub-culture	Third sub-culture
MS devoid of hormones (Control)	0.73	1.56	2.00	2.16
M S + BAP (2.5) + NAA (0.1) + GA_3 (0.3)	1.13	1.76	2.23	2.80
MS + BAP (2.5) + NAA (0.1) + GA_3 (0.5)	1.43	1.86	2.13	2.36
M S + BAP (3.0) + NAA (0.1) + GA_3 (0.3)	1.56	2.23	2.56	3.70
MS + BAP (3.0l) + NAA (0.1) + GA_3 (0.5)	1.86	2.40	2.73	3.83
MS + BAP (3.5) + NAA (0.1) + GA_3 (0.3)	1.93	3.30	3.63	4.90
MS + BAP (3.5) + NAA (0.1) + GA_3 (0.5)	1.96	3.56	3.66	5.30
M S + BAP (2.5) + NAA (0.2) + GA_3 (0.3)	1.60	1.93	2.10	3.93
MS + BAP (2.5) + NAA (0.2) + GA_3 (0.5)	1.30	2.16	2.20	2.96
MS + BAP (3.0) + NAA (0.2) + GA_3 (0.3)	1.77	1.93	2.40	3.03
M S + BAP (3.0) + NAA (0.2) + GA_3 (0.5)	1.43	1.83	2.23	3.47
MS + BAP (3.5) + NAA (0.2) + GA_3 (0.3)	1.60	2.03	2.36	2.87
MS + BAP (3.5) + NAA (0.2) + GA_3 (0.5)	1.23	1.90	2.46	2.50
CD at 5%	0.25	0.71	0.65	0.67

branching and multiple shoot formation. Superiority of BAP in shoot multiplication has earlier been shown by Scotti Compos and Pais (10). It is also opined that in multiple shoots a proliferation may be due to loss of apical dominance (Bressan *et al.*, 4; Douglas *et al.,* 5 Singh and Syamal, 11).

It is evident from the data that treatment of MS + GA_3 (0.5 mg/l) had a marked influence on the shoot length (1.70 cm) after 20 days of transfer on elongation medium which was drastically low in control (Table 4). After 40 days of transfer, highest shoot length (2.40 cm) was registered in the above treatment MS + GA_3 (0.5 mg/l). Gibberellins are known for inducing stem elongation in a number of crops. The application of gibberellic acid into shoot elongation medium resulted in rapid growth which might be due to the increased activity of the endogenous auxin in the presence of GA_3. The present findings lend support

from the previous work done by Bhat (3), Prasad (9) and Kumar (7).

The data presented in Table 5 depicts the effect of basal medium strength and auxin on days to root initiation, rooting, number of roots per shoot, root length and root quality. The earliest root initiation (13.86 days) was noted on ½MS + NAA (0.5 mg /l) + IBA (0.5 mg /l) followed by ½ MS + NAA (1.0 mg /l) + IBA (1.0 mg /l) (14.20 days). The time taken for root initiation was most delayed in control (32.30 days). The highest (86.97%) rooting was observed for the treatment ½ MS + NAA (0.5 mg /l) + IBA (0.5 mg /l). The rooting on reduced

Table 4. Effect of gibberellic acid on micro-shoot length and internodal length in hybrid tea rose cv. Pusa Mohit.

Treatment (mg/l)	Shoot length (cm)		Internodal length (cm)
	After 20 days	After 40 days	After 40 days
MS devoid of hormones (Control)	0.80	1.40	0.56
M S + GA_3 (0.5 mg/ l)	1.70	2.40	0.92
MS + GA_3 (1.0 mg/ l)	1.33	1.93	0.84
MS + GA_3 (1.5 mg/ l)	0.93	1.73	0.67
CD at 5%	0.91	0.56	0.07

Table 5. Effect of basal medium strength and auxins on rooting of hybrid-tea rose micro-shoots.

Treatment (mg/l)	Days to root initiation	Rooting (%)	No. of roots per shoot	Root length (cm)
MS devoid of hormones (Control)	32.30	14.63 (22.44)*	0.97	1.10
MS + NAA (0.5)	17.93	19.50 (26.22)	2.37	1.50
MS + NAA (1.0)	18.63	25.20 (30.15)	2.27	1.80
MS + IBA (0.5)	22.10	56.40 (48.73)	2.10	2.57
MS + IBA (1.0)	25.00	51.97 (46.15)	1.97	2.87
½ MS devoid of hormones	25.67	24.90 (29.94)	1.77	2.27
½ MS + NAA (0.5)	14.50	78.17 (62.29)	3.37	2.60
½ MS + NAA (1.0)	15.00	45.03 (42.17)	3.30	2.50
½ MS + IBA (0.5)	17.36	59.97 (50.79)	3.27	3.13
½ MS + IBA (1.0)	17.46	44.70 (41.98)	3.23	3.37
½ MS + NAA (0.5) + IBA (0.5 mg/l)	13.86	86.97 (68.91)	3.50	4.20
½ MS + NAA (1.0) + IBA (1.0)	14.20	81.33 (64.54)	3.40	3.83
CD at 5%	2.36	6.00	0.27	0.30

*The values given in parentheses denote the Arc Sin √% value.

Table 6. Effect of different acclimatization strategies on survival of *in vitro* raised plantlets.

Treatment	Survival (%)	Plantlets height (cm)	No. of leaves per plantlet
Plastic pots with polythene bags	77.54 (61.68)*	5.74	3.80
Plantlets in glass jars with polypropylene lids	85.13 (67.29)	7.37	4.38
CD at 5%	4.82	1.93	0.01

*The values given in parentheses denote the Arc Sin √% value.

basal salt strength was significantly higher as compared to full strength medium. The maximum number of roots per shoot (3.50) was induced on ½MS + NAA (0.5 mg /l) + IBA (0.5 mg /l) followed by ½MS + IBA (1.0 mg /l) as compared to minimum in control (0.97). The longest root length (4.20 cm) was induced with ½ MS + NAA (0.5 mg /l) + IBA (0.5 mg /l). The qualitative data suggest that roots were not only few but stunted in medium devoid of auxins. When NAA was supplemented individually, roots were small stunted and thick while those cultured on medium supplemented with IBA had thin and long roots. Interestingly, roots on half- strength medium supplemented with the dual auxins were of medium length and thin. It is evident from the study that there has been synergistic effect, when the two auxins were employed together. Optimum role of two auxins has been reported earlier also by Singh and Syamal (11), and Bharadwaj *et al.* (2).

The highest plantlet survival (85.13%), the plant height (7.37cm) with good number of branches (1.43 per plant) and leaves (4.38 per plant) were recorded for the treatment where plantlets were acclimatized in glass jars filled with vermiculite + agropeat supplemented with one quarter strength MS medium and covered with polypropylene lids, the plantlet survival in plastic plastic pots covered with polythene bags. The better results obtained in glass jars might be due to less open space but appropriate relative humidity as compared to those hardened in plastic pots covered with polythene bags. Efficacy of glass jars for *Rosa hybrida* has been earlier been reported by Singh and Syamal (22), and Bharadwaj *et al.* (2). The result of present investigation demonstrates that rose cultivar Pusa Mohit can be multiplied *in vitro* employing the above protocol.

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