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In vitro micropropagation of miniature rose cv. Candy Sunblaze

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ABSTRACT

A study was conducted to standardize micropropagation protocol for a popular miniature rose cv. Candy Sunblaze using nodal segment from filed grown plants. Of the different pre-treatments tried, the maximum response (82.3%) was obtained with 1,000 mg L^{-1} Ridomil + 200 mg L^{-1} 8-HQC for 2 h agitation. Cultures were initiated on Murashige and Skoog (8) medium supplemented with some plant growth regulators. MS basal medium fortified with 4.0 mg L^{-1} BAP + 2.0 mg L^{-1} kinetin and 0.1 mg L^{-1} NAA, gave the highest shoot multiplication rate (6.9 shoots/ explant). *In vitro* rooting percentage was enhanced by use of half-strength MS basal medium fortified with 1.0 mg L^{-1} IBA + 1.0 mg L^{-1} NAA. The regenerated plantlets were best acclimatized in glass jar filled with peat: soilrite (1:1) with PC cap. After acclimatization the plants were successfully transferred to the glasshouse.

Key words: Miniature rose, micropropagation, protocol.

INTRODUCTION

Miniature roses form an interesting group of rose cherished for compact growing habit, small flowers, long flowering period, etc. *In vitro* propagated miniature roses are more compact, better branched and bloom early (Singh *et al.*, 14; Bharadwaj *et al*., 14; Madhubala *et al*., 7). The *in vitro* responses vary considerably with regard to genotype and hence, specific protocols need to be standardized for each of them. In the present investigation, an attempt was made to develop a protocol for micropropagation of miniature cv. Candy Sunblaze, which is popular amongst deep pink petaled cultivars.

MATERIALS AND METHODS

The study was carried out at the Central Tissue Culture Laboratory, LBS Centre, IARI, New Delhi during 2007-08. Newly emerged twigs were collected from just mature stems, during November from Germplasm Block of IARI, New Delhi. The twigs were collected and dipped in water. Nodal segments were excised from 4th to 6th node position from the tip. The segments were agitated in 0.1% Tween-20® solution for 10 min. followed by washing under running tap water (30 min.). The explants were then pre-treated with different solutions for 2h (Table 1) followed by three rinsings with double-distilled water. The nodal segments were then cultured onto Murashige and Skoog (8) medium (MS) fortified with different growth regulator combinations (Table 2) along with 3% sucrose and media solidified with 0.8% agar-agar. The pH of medium was adjusted to 5.7-5.8 before autoclaving. After surface sterilization with 0.1% $HgCl_2$ for 4 min. followed by three rinsings with sterile double-distilled water the explants were inoculated individually in test tubes.

The *in vitro* induced shoots were excised and cultured individually onto MS medium supplemented with different growth regulator combinations (Table 3). The cultures were maintained in a growth chamber (26 ± 1^{0}C) under 16/8 h of light (57 µmol $m^{-2}s^{-1}$) and dark cycle. For rooting of micro-shoots, 2-4 cm long individual micro-shoots were cultured 100 ml flask containing half-strength MS medium supplemented with different levels and combinations of IBA and NAA (Table 4). Sucrose was supplemented at a constant dose of 20 g/l. The rooted plantlets (30-day-old) were taken out from the conical flask and washed thoroughly with autoclaved distilled water for removal of adhering agar. The plantlets were then acclimatized using three different hardening strategies such as (i) plastic pot-filled with peat: soilrite (1:1) along with plastic cover; and (ii) glass jar filled with peat: soilrite (1:1) with ploy carbonate (PC) cap. The jars were maintained in growth chamber for three weeks for acclimatization before shifting to glasshouse. The experiments were carried using factorial randomized block design with approx 15 units per replication per treatment. The percent data were subjected to Arc Sin "% transformation before carrying out the ANOVA.

RESULTS AND DISCUSSION

As evident data presented in Table 1, the explant pre-treatments had significantly improved the culture

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Table 1. Effect of different pre-treatments on culture initiation in miniature rose.

Pre-treatment	Explant survival (%)	Microbial contamination (%)	Days to shoot bud emergence	No. of shoots/ explant
Control (distilled water)	49.73(44.72)*	34.10(35.73)*	8.83	0.70
Carbendazim (0.2%)	77.60(61.75)	13.57(20.70)	5.33	0.90
8-HQC (200 mg/l)	63.20(52.65)	18.53(25.49)	6.60	1.23
Carbendazim (0.2%) + 8-HQC (200 mg/l)	74.90(59.93)	8.67(17.07)	4.33	1.07
Carbendazim (0.2%) + Ridomil (0.2%)	80.90(64.08)	5.47(13.44)	4.56	1.20
Carbendazim (0.2%) + Ridomil (0.2%) + 8-HQC (200 mg/l)	90.13(71.78)	3.30(10.49)	3.70	1.50
CD at 5%	9.58	5.25	0.97	0.35

*Arc Sin "% transformed data

initiation by minimizing the microbial contamination. The treatment comprising Bacistin (carbendazim) (0.2%) + Ridomil (0.2%) + 8-HQC (200 mg/l) agitation for 2 h resulted in the highest culture initiation (90.13%), which was also significantly superior over control (49.73%). This pretreatment gave the earliest bud sprouting (3.7 days) compared to control (8.83 days). Use of dual fungicide along with 8-HQC was shown to give beneficial response with minimal contamination. Pre-treatments are known to improve *in vitro* culture initiation. Bavistin is a systemic fungicide, while Ridomil has both systemic and contact fungicides. Similarly, 8-HQC has the proven strong bactericidal activity. These compounds when used as explant pre-treatment shake lead to considerable reduction in microbial load. Efficacy of these compounds has earlier been demonstrated by Prasad (10), Salehi and Khosh-Khui (12), Machado *et al.* (6), Bharadwaj *et al*. (2). However, delayed response with the use of antibiotics is proposed due to their phyto-toxic effects (Pollack *et al.*, 9).

Significant effect of growth regulator treatments was noted for culture initiation and also for days to bud sprouting (Table 2). The maximum (92.2%) culture initiation was recorded for the treatment MS + NAA (0.2 mg/l) + BAP (2 mg/l) + GA_3 (0.5 mg/l) followed by MS + NAA (0.2 mg/l) + BAP (1 mg/l) + GA_3 (0.5 mg/l) (86.13%). The above treatment gave the earliest (3.1 days) bud sprouting compared to hormone-free control (8.93 days). Use of cytokinins along with GA has been found beneficial as these growth regulators at optimum concentration trigger the dormant buds on the nodal segments. Earlier, Douglas *et al.* (5), Arnold *et al.* (1), Chu *et al.* (4), and Syamal and Singh (19) also suggested the efficacy of cytokinin in combination with an auxin or together with GA_3.

Addition of two cytokinins along with an auxin had marked influence on shoot multiplication and to improve the shoot quality (Table 3). The maximum shoot proliferation (94.4%) with maximum number of micro-shoots (4.8 shoots/ explant) was registered with MS + BAP (4.0 mg/l) + Kin (2.0 mg/l) + GA_3 (0.5 mg/l). The above treatment also lead to formation of micro-shoots

Table 2. Effect of growth regulators on *in vitro* culture initiation in miniature rose.

Growth regulator (mg/l)	Explant survival (%)	Microbial contamination (%)	Days to shoot bud emergence	No. of shoots/ explant
MS + no hormone	40.60(39.58)*	6.77(15.03)*	8.93	0.87
MS + NAA (0.2 mg/l) + BAP (1 mg/l) + GA_3 (0.5 mg/l)	86.13(68.36)	5.17(15.04)	3.23	1.60
MS + NAA (0.2 mg/l) + BAP (2 mg/l) + GA_3 (0.5 mg/l)	92.20(73.78)	5.47(13.47)	3.10	1.67
CD at 5%	6.14	0.76	1.07	0.32

*Arc Sin "% transformed data

Table 3. Effect of growth regulators on shoot multiplication in miniature rose.

Treatment	Shoot multiplication (%)	Days to shoot initiation	No. of shoots/ explant	Mean shoot length (cm)
MS + BAP (1.0 mg/l) + Kin (0.5 mg/l) + GA_3 (0.5 mg/l)	75.97(60.61)*	7.70	3.38	2.8
MS + BAP (2.0 mg/l) + Kin (1.0 mg/l) + GA_3 (0.5 mg/l)	82.40(65.20)	7.40	4.07	3.1
MS + BAP (4.0 mg/l) + Kin (2.0 mg/l) + GA_3 (0.5 mg/l)	94.23(76.08)	7.36	4.80	3.3
CD at 5%	4.89	0.87	0.01	0.21

*Arc Sin "% transformed data.

of longer length (3.3 cm). The multiplication rate of the cultivar could be rated moderate. Superiority of BAP in shoot multiplication has earlier been shown by Scotti Campos and Pais (13). It is opined that multiple shoots arise as the result of axillary bud proliferation owing to the loss in apical dominance (Bresan *et al.*, 3; Douglas *et al.*, 5; Sahoo and Debata, 11; Singh and Syamal, 14; Bharadwaj *et al*., 2).

The growth regulator treatments had significant effect on rooting of individual microshoot (Table 3). The maximum (93.8%) rooting was observed for the treatment Half-MS + IBA (1.0 mg/l) + NAA (1.0 mg/l) + activated charcoal (200 mg/l) followed by Half-MS + NAA (2.0 mg/l) + activated charcoal (200 mg/l) (85.3%). The maximum root number per micro-cutting was noted when NAA was supplemented. Number of roots per plantlet (7.03) was significantly higher in half-MS + IBA (1.0 mg/l) + NAA (1.0 mg/l) + activated charcoal (200 mg/l).The mean root length was noted higher (4.63 cm) in the above treatment compared to control (2.30 cm). These findings are in agreement with those of Salehi and Khosh-Khui (12), Singh and Syamal (14). It was evident that there has been a synergistic effect, when the two auxins were employed together. IBA alone could strike fewer rootlets (1.8/plantlet). Optimum role of two auxins has earlier been reported by Syamal and Singh (16), Singh and Syamal (14), and Bharadwaj *et al*. (2).

Table 4. Effect of different auxins with or without activated charcoal on *in vitro* rooting of micro-shoots in miniature rose.

Treatment (mg/l)	Rooting (%)	Days to root initiation	No of roots /shoot	Mean root length (cm)
Control	58.00 (49.60)*	14.00	1.20	2.30
Half-MS +IBA(1.0 mg/l)	67.47(55.12)	13.43	2.70	3.20
Half-MS + IBA (2.0 mg/l)	71.73 (57.87)	13.67	3.13	4.13
Half-MS + NAA (1.0 mg / l)	65.17(53.79)	14.87	4.23	2.86
Half-MS + NAA (2.0 mg / l)	73.13(58.76)	14.63	4.67	2.63
Half-MS + IBA (1.0 mg/l) + activated charcoal (200 mg/l)	80.07(63.44)	13.80	3.60	4.90
Half-MS + IBA (2.0 mg/l) + activated charcoal (200 mg/l)	82.53(65.28)	13.90	4.10	5.63
Half-MS + NAA (1.0 mg/l) + activated charcoal (200 mg/l)	74.37(59.56)	12.83	6.40	3.80
Half-MS + NAA (2.0 mg/l) + activated charcoal (200 mg/l)	85.30(67.45)	11.73	5.50	3.53
Half-MS + IBA (1.0 mg/l) + NAA (1.0 mg/l) + activated charcoal (200 mg/l)	93.83(76.82)	11.60	7.03	4.63
CD at 5%	5.06	2.95	1.25	0.75

*Arc Sin "% transformed data.

Significant effect of different hardening strategies and the genotype was recorded for plantlet survival and days to acclimatization (Table 5). The highest survival rate (85.8%) was recorded for the treatment where glass jar filled with peat: soilrite (1:1) with PC cap followed by plastic pot filled with peat: soilrite with inverted beaker (75.8%). Minimum (69.2%) survival was noted where plastic pot covered with plastic. Glass jar with PC caps resulted in earliest acclimatization (18.7 days) followed by plastic pot with inverted beaker (22.3 days). Efficacy of glass jar had earlier been shown in *Rosa hybrida* (Singh and Syamal, 14). Prior to their *ex vitro* transfer two-week-old hardened plantlets were transferred to greenhouse for further acclimatization.

Table 5. Effect of different hardening strategies miniature rose plantlets.

Treatment	Plant survival (%)	Day taken for *ex vitro* transfer
Glass jar with PP cap	88.10(69.82)*	18.03
Plastic rots with polythene cover	70.30(56.98)	24.43
CD at 5%	3.17	1.58

*Arc Sin "% transformed data.

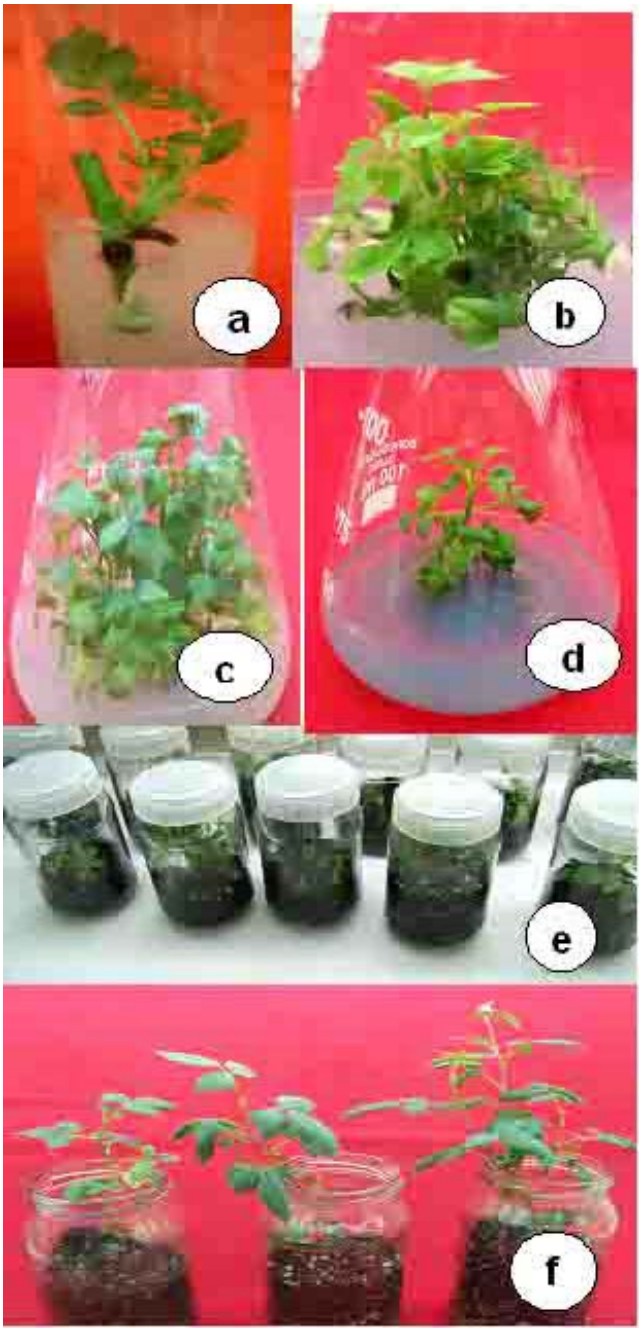

Fig. 1. Micropropagation stages in miniature rose cv. Candy Sunblaze.

REFERENCES

1. 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. *J. Hort. Sci.* **67**: 727-35.
2. Bharadwaj, Ruchika, Singh, S.K., Pal, Surindra and Kumar, Surendra 2006. An improved protocol for micropropagation of miniature rose (*Rosa chinensis* Jacq.). *J. Orn. Hort.* **9**: 238-42.
3. Bressan, P.H., Kim, Y.J., Hyndman, S.E., Hasegawa, P.M. and Bressan, R.A. 1982. Factors affecting *in vitro* propagation of roses. *J. American Soc. Hort. Sci.* **107**: 979-90.
4. Chu, C.V., Knight, S.L. and Smith, M.A.L. 1993. Effect of liquid culture on the growth and development of miniature rose (*Rosa chinensis* Jacq.) 'Minima'. *Plant Cell Tissue Organ Cult.* **32**: 329-34.
5. 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 Organ Cult.* **19**: 55-64.
6. 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 Organ. Cult.* **22**: 155-60.

7. Madhubala, Singh, K.P. and Prasad, K.V. 2010. Standardization of *in vitro* mass multiplication protocol for hybrid tea rose cv. Pusa Mohit. *Indian J. Hort*. **67**: 198-201.

8. Murashige, T. and Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant,* **15**: 473-97.

9. Pollack, K. Barfield, D.J. and Shields, R. 1983. The toxicity of antibiotics to plant cell cultures. *Plant Cell Rep.* **2**: 36-39.

10. Prasad K.V. 1995. Studies on *in vitro* regeneration of roses. Ph.D. thesis submitted to P.G. School, Indian Agricultural Research Institute, New Delhi.

11. Sahoo, S. and Debata, B.K. 1997. A note on *in vitro* micropropagation and induction of flowering in the miniature rose - The Fairy. *Orissa J. Hort.* **25**: 87-89.

12. Salehi, H. and Khos-khui, M. 1997. A simple procedure for disinfection of 'Baby Masquerade' miniature rose explants. *Scientia Hort.* **68**: 145-48.

13. Scotti Campos, P. and Pais, M.S. 1990. Mass propagation of the dwarf rose cultivar 'Rosamini'. *Scientia Hort.* **43**: 321-30.

14. 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 Hort.* **91**: 169-77.

15. Singh, S.K., Raghava, S.P.S. and Kumar, S. 1999. Rose tissue culture: Potentials and Achievements. *Souv.First Regional Rose Conf.*, Jaipur, India, pp. 44-47.

16. Syamal, M.M. and Singh, S.K. 1996. *In vitro* propagation of rose. *Hort. J.* **9**: 57-62.

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