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Original Article

Micropropagation from cultured nodal explants of rose (*Rosa hybrida* L. cv. 'Perfume Delight')

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Abstract

A method for the micropropagation of rose (*Rosa hybrida* L. cv. 'Perfume Delight') was developed. First to fifth nodal explants from young healthy shoots were excised and cultured on basal medium of Murashige and Skoog (1962, MS) containing several concentrations of BA and NAA. Multiple shoot formation of up to 3 shoots was obtained on MS medium supplemented with 3 mg/l BA and 0.003 mg/l NAA. Shoot readily rooted on ¼MS medium devoid of growth regulators. Rooted plantlets were hardened and established in pots at 100% survival. *In vitro* flowering was observed on rose plants cultured on MS medium containing 3 mg/l BA and 0.003 mg/l NAA.

Keywords: nodal culture, organogenesis, *Rosa hybrida*, rose

1. Introduction

Roses have been one of the world's most popular ornamental plants for a long time. They belong to the Rosaceae and are grown worldwide as cut flowers and potted plants and in home gardens. The flowers vary greatly in size, shape and color. Tissue culture system in roses has been established (Hsia and Korban, 1996; Kintzios *et al.*, 1999; Ibrahim and Debergh, 2001; Kim *et al.*, 2003; Rout *et al.*, 2006; Hameed *et al.*, 2006; Drefahl *et al.*, 2007; Previati *et al.*, 2008). Recently, *in vitro* flower induction in roses was demonstrated (Wang *et al.*, 2002; Vu *et al.*, 2006). To establish an *in vitro* flowering research system, it is necessary to develop a reliable and rapid shoot organogenesis protocol. In this context, we describe an efficient tissue culture technique to yield large number of shoots from nodal explants of rose. This study is part of a larger program designed to investigate *in vitro* flowering of *Rosa* species.

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2. Materials and Methods

2.1 Plant materials

Nodal explants containing lateral buds of actively field-grown 'Perfume Delight' rose were used for multiplication experiments. They were cut in 3-4 cm length segments and surface-disinfested using 70% ethanol for 10-20 s and then immersed in 40% (v/v) Clorox™ solution of commercial laundry bleach (5.25% NaOCl) containing 2 drops of Tween-20 emulsifier to aid wetting for 20 min. The sterilized explants were washed 2-3 times with sterile distilled water to remove disinfecting solution. They were trimmed down to 1 cm long prior to transferring to shoot multiplication medium.

2.2 Micropropagation

The basal nutrient medium containing MS (Murashige and Skoog, 1962) salts and vitamins was used with NAA and BA. In the first experiment, NAA at the concentration of 0.3 mg/l was combined with BA at the concentrations of 0, 1, 3, 6 or 9 mg/l. In the second experiment, the effects of NAA were examined individually at the concentrations of 0, 0.003, 0.03 or 3 mg/l in the presence of 3 mg/l BA. Explants were subcultured to fresh medium every 4 weeks. After this period excised single shoot from multiple shoots were transferred to fresh medium for root induction. To establish root proliferation, green and normal adventitious shoots from shoot multiplication cultures were excised and placed on ¼MS, ½MS and MS medium supplemented with 0, 1, 2, or 4 mg/l NAA.

2.3 *In vitro* conditions

All media were supplemented with 3% sucrose and 8.2 g/l of Mermaid^{TM} agar. The pH of all media was adjusted to 5.8 with 1 N NaOH or 1 N HCl prior to autoclaving at 1.05 kg/cm^2, 121^{o}C for 20 min. Cultures were maintained at 25±1^{o}C air temperatures in a culture room with a 16-h photoperiod under an illumination of 20 mmol $m^{-2}s^{-1}$ photosynthetic photon flux density provided by cool-white fluorescent light. Plant materials were stored in glass-capped culture jars (115 ml capacity) each containing 20 ml of medium.

2.4 Statistical analysis

Number of shoots and roots were evaluated after each culture period. A culture cycle was 4 weeks. One explant was implanted per culture and 20 cultures were raised for each treatment unless otherwise stated. All experiments were conducted on two different days. Shoot and root numbers were submitted to analyses of variance and mean of numbers were compared using Duncan's multiple range test at $p \leq 0.05$.

3. Results and Discussion

After 4 weeks of initial culture, nodal explants containing lateral buds cultured on MS medium in the first experiment developed multiple shoots at a high frequency of 100% with 2.6±0.5 shoots on 3 mg/l BA and 0.3 mg/l NAA. There was no significant difference in shoot number per nodal explants; however, regeneration frequency differed (Table 1). In the present study, multiplication occurred in all BA- containing media this may be attributed to the presence of NAA. An experiment was subsequently conducted to determine the optimal concentrations of NAA (0, 0.003, 0.03, 0.3 or 3 mg/l) with BA held constant at 3 mg/l. Results revealed that low level of NAA (0.003 mg/l) gave the highest number of 3.2±1.0 shoots ($p \leq 0.05$). Higher levels of NAA (0.3 or 3 mg/l) were significantly less effective ($p \leq 0.05$) in shoot multiplication when compared to 0, 0.003 or 0.03 mg/l (Table 2). At all concentrations of BA and NAA tested, multiple shoots developed without the intervening callus stage. BA or NAA have been used for most experiments on shoot multiplication of a number of rose species (Wang *et al.*, 2002; Vu *et al.*, 2006; Drefahl *et al.*, 2007). Some other research obtained different results for other rose species with the combination of BA and IBA (Kumar *et al.*, 2001; Khosh-Khui and Jabbarzadeh, 2007). These multiple shoots with green expanded leaves and single main stem (Figure 1) continued to proliferate after several subcultures with an average of 3 shoots per cycle. Clonal propagation of shoots was achieved by repeating subculture at 4 weeks intervals. Each nodal explant provided 27 shoots in 12 weeks, for a total production of 55 plantlets per six months.

To establish a rooting protocol, regenerated shoots were excised and transferred to rooting medium. Up to 75% rooting was achieved on ¼MS medium without growth regulators (Table 3). Rooting ability was affected by different salt concentrations and this is in accordance with the results of Douglas *et al.* (1998) and Ibrahim and Debergh (2001). Roots that developed on this medium were thick, long and fibrous. Two weeks of rooting incubation was adequate

Table 1. Effect of different BA concentrations with 0.3 mg/l NAA on shoot induction in the rose cultivar 'Perfume Delight'

BA (mg/l)	Number of cultures	Number explants forming shoot (%)	Number of shoots (Mean ± SE)
0	20	44	1.7±1.2^a
1	20	67	2.0±0.8^a
3	20	100	2.6±0.5^a
6	20	89	2.7±0.5^a
9	20	78	2.8±1.0^a

Values of mean number of shoots not having a superscript in common differ significantly using Duncan's multiple range test at $p \leq 0.05$.

Table 2. Effect of different NAA concentrations with 3 mg/l BA on shoot induction in the rose cultivar 'Perfume Delight'

NAA (mg/l)	Number of cultures	Number explants forming shoot (%)	Number of shoots (Mean ± SE)
0	22	91.6	2.8±1.2[a]
0.003	22	100	3.2±1.0[a]
0.03	22	91.6	2.6±1.4[a]
0.3	22	83.3	1.9±0.7[b]
3	22	50	1.7±0.8[b]

Values of mean number of shoots not having a superscript in common differ significantly using Duncan's multiple range test at $p \leq 0.05$.

Table 3. Effect of different concentrations of MS salt and NAA on root induction in the rose cultivar 'Perfume Delight'

MS medium + NAA (mg/l)	Number of cultures	Number explants forming root (%)	Number of roots (Mean ± SE)
¼MS + (0)	20	75	6.5±0.4[a]
½MS + (0)	10	50	3.6±1.0[b]
MS + (0)	26	62	3.1±1.4[b]
MS + (1)	8	13	1.9±0.7[c]
MS + (2)	9	0	0[d]
MS + (4)	8	0	0[d]

Values of mean number of roots not having a superscript in common differ significantly using Duncan's multiple range test at *p*d"0.05.

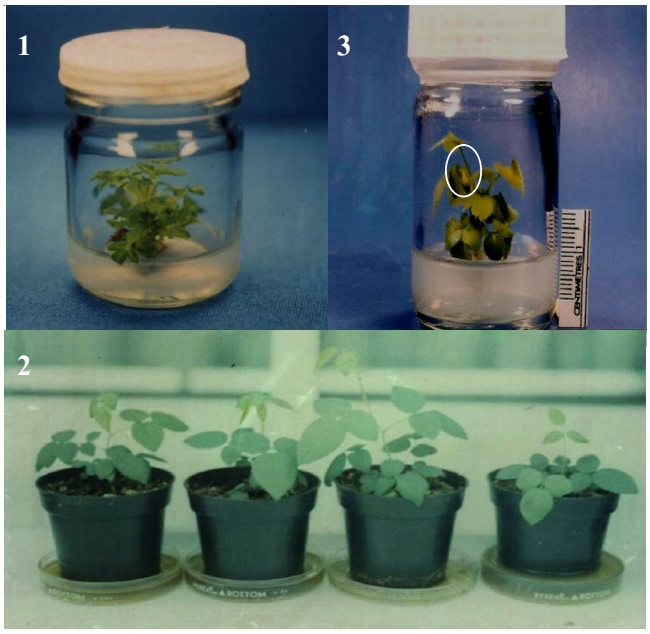

Figure 1-3. Micropropagation of *Rosa hybrida* L. cv. 'Perfume Delight'. (1) Adventitious shoot formation from nodal culture. (2) Complete plantlets in soil. (3) Acclimatized rose plantlet with *in vitro* flowering.

before transplanting to polystyrene pots containing soil mixture (1 sand: 1 manure: 1 decayed leaves) (Figure 2). *In vitro*-derived plants did not display any phenotypic variation during subsequent vegetative development. It is interesting to note that *in vitro* flowering was observed after transferring regenerated shoot cultured on MS medium containing 0.003 mg/l NAA and 3 mg/l BA to rooting medium. This flower had normal petals and sepals and proceeded to open (Figure 3). BA has been used for most experiments on *in vitro* flowering of a number of plants (Wang et al., 2002). In *Rosa hybrida* cultivar 'Meirutral' and cultivar 'Fairy' could flower in propagation medium containing BA (Dobres *et al.*, 1998).

In conclusion, a micropropagation system for *Rosa hybrida* cultivar 'Perfume Delight' has been worked out utilizing nodal explants. Micropropagated plants were rooted and established in soil successfully. The preliminary result in this system enables *in vitro* flowering but requires further improvement.

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