Multiple Shoot Induction and Plant Regeneration of *Rosa damascena* Mill.

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Abstract

An *in vitro* regeneration system was developed for propagation of *Rosa damascena* Mill. Axillary shoot proliferation and root induction were induced in the nodal segments on Murashige and Skoog (MS) medium and Quoirin and Lepoivre (QL) medium supplemented with benzyladenine (BA) or á-napthalene acetic acid (NAA) or in combination within 4 weeks. The highest number of root per explant was obtained from MS medium supplemented with 0.5 mg/l NAA and 87.5 % of the explants exhibited root development, 2.71 roots per explant. The best response in terms of frequency of shoot regeneration (100%) and maximum number of shoots (8.27 shoots per explant) were developed on QL medium containing a combination of 0.1 mg/l NAA and 4.0 mg/l BA. A proliferating shoot culture was established by repeatedly subculturing newly formed shoots at 4 week intervals. Thus, 59.8 shoots were obtained in 8 weeks from a single nodal explant. Rooting of the regenerated shoots was induced on MS medium supplemented with 0.5 mg/l NAA and 85% of the shoots developed roots at an average of 2.3 per shoot within 4 weeks. Ninty percent of plantlets were acclimatized and established in soil.

Introduction

Rosa damascena Mill. (Rosaceae) is commonly known as Damask rose and Kulaap mon in Thai [Fig. 1A]. Damask rose has a deep, rosy and fresh aroma. The essential oil of this plant soothes the mind and helps to relief depression, grief, nervous tension and stress, and is used in poor circulation, asthma and coughs, irregular menstruation, leucorrhea, and uterine disorders. On the skin it can be used for dry skin, eczema and sensitive skin. (Smittinan, 2001). It is usually propagated by grafting, seeding, cutting and budding. (Nakkawatchara, 2001).

Since the reports of Skirvin and Chu (1979) on the micropropagation of *Rosa hybrida* L. 'Forever Yours' by proliferation of axillary buds and Hasegawa (1979) on the *in vitro* propagation of *Rosa hybrida* L. cv. Improved Blaze, several studies concerning different aspects of commercial rose multiplication have been published such as Carelli and Echeverrigaray (2002) on the *in vitro* propagation of *Rosa hybrida* cv. Baronesse and other nine cultivars. However, there are contradictory results and the low multiplication rate achieved with the most important rose cultivars. *In vitro* propagation of *Rosa damascena* Mill. was not reported by any paper. This paper describes an efficient and rapid propagation of *Rosa damascena* Mill. using nodal explants from natural grown plants.

Materials and Methods

Plant material

The middle part including axillary buds of *R.damascena* Mill. from vegetative shoots were excised and collected. The explants were surface sterilized by dipping in 70 % ethanol for 30 s, then incubated in 10% Clorox (sodium hypochlorite 5.25%) with 3 drops of tween-20 for 10 min, followed by 5% Clorox for 5 min and subsequently rinsed three to four times with sterile distilled water. Axillary node (1 cm long) sections were excised and transferred to glass bottles with plastic closures (1 explant per flask).

Culture medium and conditions

In the first experiment, the regeneration medium, cytokinin (benzyladenine, BA) at the concentrations of 1.0, 2.0, 3.0 and 4.0 mg/l or auxin (á-napthalene acetic acid, NAA) at 0.1, 0.3, 0.5 and 0.7 mg/l were tested on basal Murashige and Skoog (MS, 1962) medium with 3% (w/v) sucrose and 0.8% agar and Quoirin and Lepoivre (QL, 1977) medium with 2% (w/v) sucrose and 0.8% agar [Table 1]. The second experiment, the shoot proliferation medium was based on the best result of the previous experiment with the combinations of BA and NAA at the same concentrations of the first experiment [Table 2]. The pH was adjusted to 5.6-5.8 before agar was added. Culture media was autoclaved at 121˚C for 20 min. The cultures were incubated at 25±2˚C with 16 h photoperiod using white fluorescent light at 2,000 lux. Regenerating explants were separated individually and subcultured to fresh medium of the same composition at an interval of 4 weeks.

Rooting of shoot and transferring plantlets to soil

Shoot (3-4 cm long) was separated individually and transferred to rooting medium obtained from the first experiment [Table 1]. After 4 weeks, the number of rooted shoots, and number of roots per shoot were recorded. The plantlets (6-7 cm in length) were washed in sterile distilled water to remove traces of medium and then transferred to plastic pots (5 cm in diameter) containing soil (soil : meal of coconut fruit 1:1) under controlled indoor conditions (temperature 25±2˚C, 16 h photoperiod and 2,000 lux light intensity). After 1 week the plants were transferred to grow in outdoor conditions.

Experimental design, data collection and analysis

Each treatment had ten replications and there was one explant per culture bottle. The experiment was Completely Randomized Design. Number of explants forming shoots, roots, number of shoots/ roots per responding explants were recorded. Data were subjected to ANOVA test.

Results and Discussion

Multiple shoot and root induction from nodal explants

Nodal explants enlarged in size after 2 weeks of culture and they differentiated axillary shoots on both MS and QL media giving best results when 1.0-3.0 mg/l BA were used [Table 1]. The highest number of shoot per explant (6.75 shoots) and 100% of the explants was obtained from the QL medium supplemented with 2.0 mg/l BA. Rooting was obtained only on MS medium supplemented with 0.1-0.7 mg/l NAA and the highest number of root per explant was obtained from MS medium supplemented with 0.5 mg/l NAA providing 87.5% rooting [Fig. 1B]. The QL medium was not beneficial to root induction. BA provided higher number of shoots per explant compared to NAA. The QL medium positively affected the shoot multiplication of *R.damascena* Mill. compared to MS medium, leading to overall increase in the number of developing shoots [Table 1].

Table 1 Effect of MS medium and QL medium supplemented with BA or NAA on multiple shoot and root induction of *Rosa damascena* Mill.*

Growth regulator (mg/l)	Percentage of explants producing shoots	Mean number of shoots	Percentage of explants producing roots	Mean number of roots
MS control (basal medium)	60	1.17 gh	0	0 c
NAA 0.1	80	1.25 gh	70	2.14 ab
NAA 0.3	0	0 h	75	1.83 ab
NAA 0.5	0	0 h	87.5	2.71 a
NAA 0.7	0	0 h	71.4	1.40 b
BA 1.0	88.8	4.63 bcd	0	0 c
BA 2.0	100	6.30 ab	0	0 c
BA 3.0	88.8	5.50 abc	0	0 c
BA 4.0	100	2.60 efg	0	0 c
QL control	80	1.75 fgh	0	0 c
NAA 0.1	88.8	3.00 defg	0	0 c
NAA 0.3	0	0 h	0	0 c
NAA 0.5	90	4.00 cde	0	0 c
NAA 0.7	0	0 h	0	0 c
BA 1.0	90	5.00 abcd	0	0 c
BA 2.0	100	6.75 a	0	0 c
BA 3.0	88.8	4.88 abcd	0	0 c
BA 4.0	90	3.56 cdef	0	0 c

*Data were collected after 4 weeks of culture. Means within a column followed by the same letter are not significantly different at 5% level according to Duncan's multiple range test.

Based on these results, the combinations of BA (1.0, 2.0, 3.0 and 4.0 mg/l) and NAA (0.1, 0.3, 0.5 and 0.7 mg/l) on QL medium was adopted in the following experiments [Table 2]. The combination of 4.0 mg/l BA and 0.1 mg/l NAA gave the highest number of shoots per explant and 100% of the explants exhibited axillary shoot development providing the average of 8.27 shoots in 4 weeks [Fig. 1C]. The results are in accordance with those obtained from other rose cultivars. Skirvin and Chu (1979) reported shoot proliferation of *Rosa hybrida* L.'Forever Yours', achieving 16 shoots per shoot tip after 5 weeks by using MS medium with combination of 2.0 mg/l BA and 0.1 mg/l NAA. Hasegawa (1979) cultured *R.hybrida* L. cv.Improved Blaze, proliferated multiple shoots were obtained on MS medium supplemented with 3.0 mg/l BA and 0.3 mg/l NAA giving 5.9 shoots per nodal explant in 8 weeks, and Carelli and Echeverrigaray (2002) on *R.hybrida* cv. Baronesse obtained 30.3 shoots per nodal explant on QL medium supplemented with combination of 3.0 mg/l BA and 0.5 mg/l NAA after 24 weeks.

Table 2 Effect of QL medium supplemented with NAA and BA on multiple shoot induction of *Rosa damascena* Mill.*

Growth regulator (mg/l)	Percentage of explants producing shoots	Mean number of shoots
QL 1 (BA 1.0 + NAA 0.1)	88.8	4.57 cde
QL 2 (BA 1.0 + NAA 0.3)	88.8	5.25 cd
QL 3 (BA 1.0 + NAA 0.5)	88.8	4.63 cde
QL 4 (BA 1.0 + NAA 0.7)	0	0 g
QL 5 (BA 2.0 + NAA 0.1)	90	7.22 ab
QL 6 (BA 2.0 + NAA 0.3)	81.8	4.22 cde
QL 7 (BA 2.0+ NAA 0.5)	70	1.57 fg
QL 8 (BA 2.0 + NAA 0.7)	90	4.89 cde
QL 9 (BA 3.0 + NAA 0.1)	77.7	4.00 cde
QL 10 (BA 3.0 + NAA 0.3)	90	5.00 cde
QL 11 (BA 3.0 + NAA 0.5)	87.5	1.86 fg
QL 12 (BA 3.0 + NAA 0.7)	90	3.22 def
QL 13 (BA 4.0 + NAA 0.1)	100	8.27 a
QL 14 (BA 4.0 + NAA 0.3)	80	5.50 bc
QL 15 (BA 4.0 + NAA 0.5)	88.8	4.13 cde
QL 16 (BA 4.0 + NAA 0.7)	80	3.00 ef
QL 17 (control)	70	1.57 fg

*Data were collected after 4 weeks of culture. Means within a column followed by the same letter are not significantly different at 5% level according to Duncan's multiple range test.

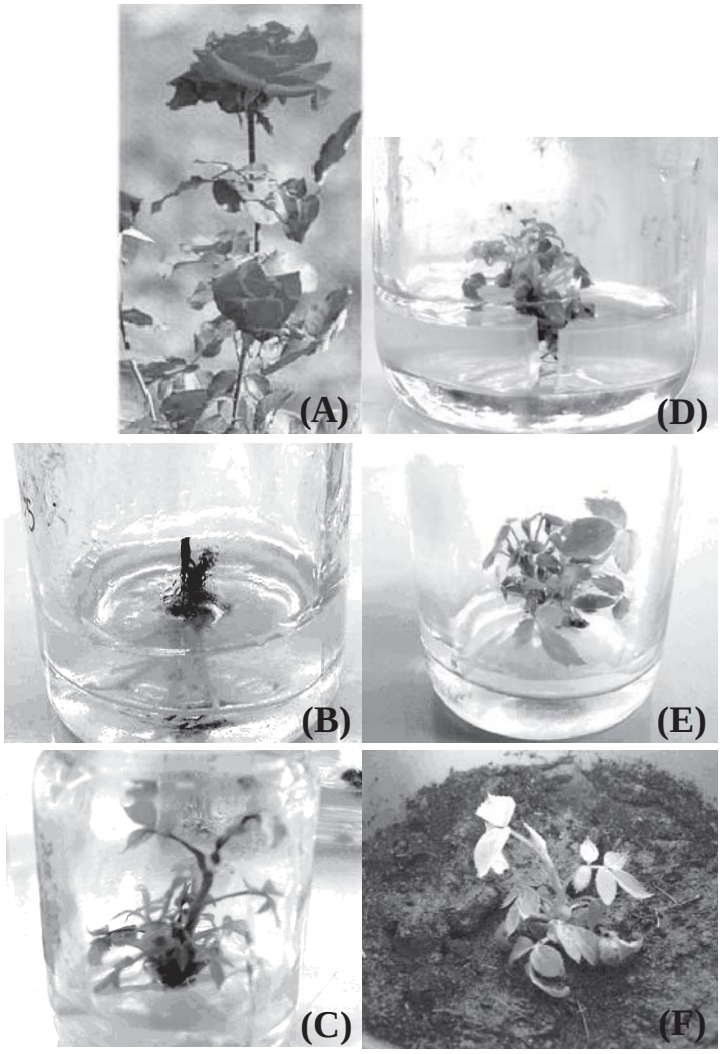

Fig.1 *In vitro* induction of multiple shoots and plant regeneration of *R.damascena* Mill. (A) Damask rose (Kulaap mon) in nature . (B) Root induction from nodal explant on MS + 0.5 mg/l NAA . (C) Shoot proliferation from nodal explant on QL medium + 0.1 mg/l NAA + 4.0 mg/ l BA. (D) Rooting of *in vitro*-derived shoots after 4 weeks of culture on MS + 0.5 mg/l NAA. (E) Multiple shoots developed on newly formed shoot on QL + 0.1 mg/l NAA + 4.0 mg/l BA. (F) Regenerated *R.damascena* Mill. in soil.

Shoot proliferation

Based on the above observations, the shoot cultures were multiplied by repeatedly subculturing the individual newly formed shoots on shoot multiplication medium (QL medium supplemented with 4.0 mg/l BA and 0.1 mg/l NAA). At the end of 8 weeks, a total of 59.8 shoots was obtained from a single initial nodal explant, subculturing at 4 week intervals. [Fig. 1E] This multiplication rate is better than that obtained by Carelli and Echeverrigaray (2002) on *R.hybrida* cv. Baronesse, giving 30.3 plantlets per explant on QL medium supplemented with combination of 3.0 mg/l BA and 0.5 mg/l NAA in 24 weeks.

Rooting

Axillary/adventitious shoots (3-4 cm long) were excised from parent culture and grown on a rooting medium (MS medium supplemented with 0.5 mg/l NAA), 85% of the cultured shoots developed root at an average of 2.3 roots per shoot within 4 weeks [Fig. 1D]

Transplantation

The rooted plantlets (6-7 cm in length) were taken out from the culture flasks and washed to remove adhered agar and traces of medium. The plantlets were then transferred to soil (soil : meal of coconut fruit 1:1). Hardening of potted plants for 1 week under indoor conditions was found to be essential. The survival percentage of the plantlets was 90%, and plants were established under outdoor conditions. [Fig. 1F]

Summary

The multiple shoot induction of *Rosa damascena* Mill. was obtained from culturing a single nodal explant on QL medium supplemented with 4.0 mg/l BA and 0.1 mg/l NAA, giving 8.27 shoots per explant in 4 weeks which could then be repeatedly subcultured the individual newly formed shoot on the same medium. At the end of 8 weeks, a multiplication rate of 59.8 shoots from each initial explant was obtained. Rooting was induced by

subculturing the individual new shoot on MS medium supplemented with 0.5 mg/l NAA in 4 weeks, giving 2.3 roots per shoot. After 12 weeks of culture, 90% of the plantlets obtained were established under outdoor conditions. Thus, the procedure could be used for the commercial propagation of *Rosa damascena* Mill.

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